July 15, 2009

Delivered by Hand and Via E-mail

Joan E. Denton, Ph.D., Director
Office of Environmental Health Hazard Assessment
1001 "I" Street
PO Box 4010
Sacramento, CA 95812
jdenton@oehha.ca.gov

Re: Petition for Listing of Bisphenol A Pursuant to Authoritative Bodies Mechanism of Safe Drinking Water and Toxic Enforcement Act of 1986

Dear Dr. Denton:

We write on behalf of the Natural Resources Defense Council – an environmental and public health organization which has 1.2 million members and activists, 250,000 of whom are Californians – to ask that OEHHA move forward immediately to list bisphenol A [CAS # 80-05-7] under Proposition 65 as a chemical that is "known to the state to cause reproductive toxicity" because it has been identified as a reproductive toxicant by an authoritative body. California Health and Safety Code § 25249.8(b); 27 Cal. Code Regs. § 25306.

Under section 25249.8(b) of the Act, and 27 Cal. Code Reg. § 25306, a chemical is known to the State to cause reproductive toxicity if OEHHA determines that an authoritative body has formally identified the chemical as causing reproductive toxicity. OEHHA's implementing regulations, set out at 27 Cal. Code Reg. § 25306 (attached as Appendix A), provide criteria for such a determination. These criteria are met for bisphenol A. Specifically:

- The National Toxicology Program, as to final reports of the National Toxicology Program's Center for Evaluation of Risks to Human Reproduction ("NTP-CERHR"), is an authoritative body for purposes of the identification of chemicals as causing reproductive toxicity. 27 Cal. Code Regs. § 25306(l)(3).
- Bisphenol A is the subject of a final report which concludes that the chemical causes reproductive toxicity; the report identifies bisphenol A as causing reproductive toxicity in a document that indicates that such identification is a final action following peer review and public comment. 27 Cal. Code Regs. § 25306(d)(1); Center for the Evaluation of Risks to Human Reproduction, National Toxicology Program, U.S. Department of Health and Human Services, *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A*, NIH Publication No. 08-5994 (September 2008) ("NTP Monograph").[1] BPA is used in the manufacture of polycarbonate and polyvinyl chloride plastics and epoxy resins and found in the urine of more than 90% of

[1] The NTP Monograph is available at http://cerhr.niehs.nih.gov/chemicals/bisphenol/bisphenol.pdf, and a copy of relevant portions of the report is enclosed as Appendix B.

Americans. NTP Monograph, NTP Brief on Bisphenol A, at 1, 4. The NTP Monograph concluded that humans are exposed to bisphenol A and that the primary route of exposure is contaminated food. *Id.* at 1. They also concluded that bisphenol A can affect human development or reproduction, stating that "studies with laboratory rodents show that exposure to high dose levels of bisphenol A during pregnancy and/or lactation can reduce survival, birth weight, and growth of offspring early in life, and delay the onset of puberty in males and females" and that these effects are "not considered scientifically controversial and provide clear evidence of adverse effects" for developmental toxicity. *Id.* at 6-8. OEHHA regulations recognize chemicals as causing reproductive toxicity if required labeling or identification for the chemical uses words or phrases intended to communicate a risk of reproductive harm to men and women, or a risk of birth defects or other developmental harm. 27 Cal. Code Regs. § 25902.

- The NTP Monograph "specifically and accurately" identifies bisphenol A. NTP Monograph, NTP Brief on Bisphenol A, at *i,* 1; 27 Cal. Code Regs. § 25306(d)(2). The NTP Monograph was reviewed by a scientific advisory committee, the NTP Board of Scientific Advisors, in a public meeting and was made subject to multiple rounds of public review and comment prior to its issuance. NTP Monograph, Preface, at *v*; *see* the Peer Review Report and Public Comment History at http://cerhr.niehs.nih.gov/chemicals/bisphenol/bisphenol.html (last updated Sept. 3, 2008); 27 Cal. Code Regs. § 25306(d)(2)(A)-(B). The NTP Monograph was also published in a publication – not only in NIH Publication No. 08-5994, but also in *Birth Defects Research Part B: Developmental and Reproductive Toxicology*. Chapin, R. E., J. Adams, et al., *NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A*, Birth Defects Res B Dev Reprod Toxicol 83(3): 157-395 (2008); 27 Cal. Code Regs. § 25306(d)(2)(C).

For these reasons, bisphenol A should be listed as a reproductive toxin pursuant to Proposition 65.

Please do not hesitate to contact us should you have any questions. We look forward to prompt action on this petition.

Sincerely,

Gina M. Solomon, MD, MPH
Senior Scientist

Avinash Kar
Staff Attorney

cc: Cynthia Oshita (by email to coshita@oehha.ca.gov) (without enclosure)

BARCLAYS OFFICIAL CALIFORNIA CODE OF REGULATIONS
TITLE 27. ENVIRONMENTAL PROTECTION
DIVISION 4. OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT
CHAPTER 1. SAFE DRINKING WATER AND TOXIC ENFORCEMENT ACT OF 1986
ARTICLE 3. SCIENCE ADVISORY BOARD: CARCINOGEN IDENTIFICATION COMMITTEE AND DEVELOPMENTAL AND REPRODUCTIVE TOXICANT (DART) IDENTIFICATION COMMITTEE

This database is current through 7/3/09, Register 2009, No. 27

§ 25306. Chemicals Formally Identified by Authoritative Bodies.

(a) Pursuant to section 25249.8(b) of the Act, a chemical is known to the state to cause cancer or reproductive toxicity if the lead agency determines that an authoritative body has formally identified the chemical as causing cancer or reproductive toxicity, as specified in this section.

(b) A "body considered to be authoritative" is an agency or formally organized program or group which utilizes one of the methods set forth in subsection (d), for the identification of chemicals, and which the Carcinogen Identification Committee has identified as having expertise in the identification of chemicals as causing cancer or the DART Identification Committee has identified as having expertise in the identification of chemicals as causing reproductive toxicity. For purposes of this section, "authoritative body" means either a "body considered to be authoritative" in the identification of chemicals as causing cancer by the Carcinogen Identification Committee or a "body considered to be authoritative" in the identification of chemicals as causing reproductive toxicity by the DART Identification Committee. The Carcinogen Identification Committee and the DART Identification Committee shall have the authority to revoke or rescind any determination that a body is authoritative on the grounds that the respective Committee no longer considers the body to have expertise in the identification of chemicals as causing cancer or reproductive toxicity, respectively, in which case chemicals listed pursuant to this section prior to the effective date of the revocation shall remain on the list. Nothing in this section shall be construed to limit or otherwise interfere with such authority.

(c) The lead agency shall determine which chemicals have been formally identified by an authoritative body as causing cancer or reproductive toxicity.

(d) For purposes of this section a chemical is "formally identified" by an authoritative body when the lead agency determines that:

(1) the chemical has been included on a list of chemicals causing cancer or reproductive toxicity issued by the authoritative body; or is the subject of a report which is published by the authoritative body and which concludes that the chemical causes cancer or reproductive toxicity; or has otherwise been identified as causing cancer or reproductive toxicity by the authoritative body in a document that indicates that such identification is a final action; and

(2) the list, report, or document specifically and accurately identifies the chemical, and has been:

(A) Reviewed by an advisory committee in a public meeting, if a public meeting is required, or

(B) Made subject to public review and comment prior to its issuance, or

(C) Published by the authoritative body in a publication, such as, but not limited to, the federal register for an authoritative body which is a federal agency, or

(D) Signed, where required, by the chief administrative officer of the authoritative body or a designee, or

(E) Adopted as a final rule by the authoritative body, or

(F) Otherwise set forth in an official document utilized by the authoritative body for regulatory purposes.

(e) For purposes of this section, "as causing cancer" means that either of the following criteria has been satisfied:

(1) Sufficient evidence of carcinogenicity exists from studies in humans. For purposes of this paragraph, "sufficient evidence" means studies in humans indicate that there is a causal relationship between the chemical and cancer.

(2) Sufficient evidence of carcinogenicity exists from studies in experimental animals. For purposes of this paragraph, "sufficient evidence" means studies in experimental animals indicate that there is an increased incidence of malignant tumors or combined malignant and benign tumors in multiple species or strains, in multiple experiments (e.g., with different routes of administration or using different dose levels), or, to an unusual degree, in a single experiment with regard to high incidence, site or type of tumor, or age at onset.

(f) The lead agency shall find that a chemical does not satisfy the definition of "as causing cancer" if scientifically valid data which were not considered by the authoritative body clearly establish that the chemical does not satisfy the criteria of subsection (e), paragraph (1) or subsection (e), paragraph (2).

(g) For purposes of this section, "as causing reproductive toxicity" means that either of the following criteria have been satisfied:

(1) Studies in humans indicate that there is a causal relationship between the chemical and reproductive toxicity, or

(2) Studies in experimental animals indicate that there are sufficient data, taking into account the adequacy of the experimental design and other parameters such as, but not limited to, route of administration, frequency and duration of exposure, numbers of test animals, choice of species, choice of dosage levels, and consideration of maternal toxicity, indicating that an association between adverse reproductive effects in humans and the toxic agent in question is biologically plausible.

(h) The lead agency shall find that a chemical does not satisfy the definition of "as causing reproductive toxicity" if scientifically valid data which were not considered by the authoritative body clearly establish that the chemical does not satisfy the criteria of subsection (g), paragraph (1) or subsection (g), paragraph (2).

(i) At least 60 days prior to adding a chemical determined to have been formally identified by an authoritative body as causing cancer or reproductive toxicity to the list of chemicals known to the state to cause cancer or reproductive toxicity, the lead agency shall cause to be published in the California Regulatory Notice Register a notice identifying the authoritative body and the chemical, and stating the lead agency's intention to cause the chemical to be added to the list. Copies of the notice shall be provided to the Carcinogen Identification Committee or the DART Identification Committee, as appropriate, to permit the appropriate Committee at least 30 days to review and comment on the proposed action. Within 30 days following the publication of the notice, interested parties, including any member of the appropriate Committee, shall submit to the lead agency their written objections to the addition of the chemical to the list of chemicals known to the state to cause cancer or reproductive toxicity, along with any supporting documentation. Objections shall be made on the basis that there is no substantial evidence that the criteria identified in subsection (e) or in subsection (g) have been satisfied. The lead agency shall review such objections. If the lead agency finds that there is no substantial evidence that the criteria identified in subsection (e) or in subsection (g) have been satisfied, the lead agency shall refer the chemical to the appropriate Committee to determine whether, in the Committee's opinion, the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity.

(j) Subsequent to the addition of a chemical determined to have been formally identified by an authoritative body as causing cancer or reproductive toxicity to the list of chemicals known to the state to cause cancer or reproductive toxicity, the lead agency shall reconsider its determination that the chemical has been formally identified as causing cancer or reproductive toxicity if the lead agency finds:

(1) there is no substantial evidence that the criteria

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identified in subsection (e) or subsection (g) have been satisfied, or

(2) the chemical is no longer identified as causing cancer or reproductive toxicity by the authoritative body.

Reconsideration may be initiated by the lead agency on its own motion, or on a request from an interested party, including any member of the appropriate Committee. The lead agency shall refer chemicals under reconsideration pursuant to this subsection to the appropriate Committee for a recommendation concerning whether the chemical should continue to be included on the list of chemicals known to the state to cause cancer or reproductive toxicity. Pending such reconsideration, the chemical shall remain on the list.

(k) The Carcinogen Identification Committee or the DART Identification Committee may condition any determination that a body is considered to be authoritative upon the subsequent application of the controls set forth in this section to the determination of which chemicals have been formally identified by the body as causing cancer or reproductive toxicity. In the event that this section or any portion thereof is found to be invalid by any court of competent jurisdiction, the Carcinogen Identification Committee or the DART Identification Committee may determine that such invalidation constitutes a failure of the condition. Upon finding such failure of condition, the determination that the body is authoritative shall be deemed to be revoked. Chemicals which the lead agency has determined have been formally identified by the body as causing cancer or reproductive toxicity pursuant to the controls set forth in this section and which have been placed upon the list of chemicals known to the state to cause cancer or reproductive toxicity prior to such revocation shall remain on the list.

(l) The following have been identified as authoritative bodies for purposes of this section for the identification of chemicals as causing reproductive toxicity:

(1) International Agency for Research on Cancer solely as to transplacental carcinogenicity

(2) National Institute for Occupational Safety and Health

(3) National Toxicology Program solely as to final reports of the National Toxicology Program's Center for Evaluation of Risks to Human Reproduction

(4) U. S. Environmental Protection Agency

(5) U. S. Food and Drug Administration

(m) The following have been identified as authoritative bodies for the identification of chemicals as causing cancer:

(1) International Agency for Research on Cancer

(2) National Institute for Occupational Safety and Health

(3) National Toxicology Program

(4) U. S. Environmental Protection Agency

(5) U. S. Food and Drug Administration

Note: Authority cited: Section 25249.12, Health and Safety Code. Reference: Sections 25249.8 and 25249.12, Health and Safety Code.

HISTORY

1. New section filed 10-30-89 as an emergency; operative 10-30-89 (Register 89, No. 44). A Certificate of Compliance must be transmitted to OAL within 120 days or emergency language will be repealed on 2-27-90.

2. Change without regulatory effect filed 10-31-89 pursuant to section 100,

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Title 1, California Code of Regulations (Register 89, No. 44).

3. Certificate of Compliance as to 10-30-89 order including amendment of subsections (c) - (l) transmitted to OAL 2-27-90 and filed 3-29-90 (Register 90, No. 16).

4. Amendment of subsection (l) submitted to OAL for printing only pursuant to Government Code section 11343.8 (Register 90, No. 28).

5. Editorial correction of printing error relettering subsection (d) (Register 90, No. 45).

6. Certificate of Compliance as to 10-24-94 order including amendment of section transmitted to OAL 2-22-95 and filed 4-5-95 (Register 95, No. 14).

7. Amendment of subsections (l)-(l)(1), repealer of subsection (l)(3), subsection renumbering, and new subsections (m)-(m)(5) filed 4-9-99; adopted and effective 7-27-98; changes not considered to be a regulation for the purposes of the Administrative Procedure Act pursuant to Health and Safety Code section 25249.8(e); printed pursuant to Government Code section 11343.8 (Register 99, No. 15).

8. Amendment of subsections (a), (b), (i) and (k) filed 1-7-2003; operative 2-6-2003 (Register 2003, No. 2).

9. Change without regulatory effect adopting new subsection (l)(3) and renumbering subsections filed 2-19-2003 pursuant to section 100, title 1, California Code of Regulations (Register 2003, No. 8).

10. Change without regulatory effect renumbering title 22, section 12306 to title 27, section 25306 filed 6-18-2008 pursuant to section 100, title 1, California Code of Regulations (Register 2008, No. 25).

27 CCR § 25306, 27 CA ADC § 25306

1CAC

27 CA ADC § 25306
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National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation of Risks To Human Reproduction

NTP-CERHR MONOGRAPH ON THE POTENTIAL HUMAN REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF BISPHENOL A

September 2008 *NIH Publication No. 08–5994*

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PREFACE

The National Toxicology Program (NTP)[1] established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the CERHR is to provide timely, unbiased, scientifically sound evaluations of the potential for adverse effects on reproduction or development resulting from human exposures to substances in the environment. The NTP-CERHR is headquartered at the National Institute of Environmental Health Sciences (NIEHS) and Dr. Michael Shelby is the director.[2]

CERHR broadly solicits nominations of chemicals for evaluation from the public and private sectors. Chemicals are selected for evaluation based on several factors including the following:

- potential for human exposure from use and occurrence in the environment
- extent of public concern
- production volume
- extent of database on reproductive and developmental toxicity studies

CERHR follows a formal process for review and evaluation of nominated chemicals that includes multiple opportunities for public comment. Briefly, CERHR convenes a scientific expert panel that meets in a public forum to review, discuss, and evaluate the scientific literature on the selected chemical. Public comment is invited prior to and during the meeting. The expert panel produces a report on the chemical's reproductive and developmental toxicities and provides its opinion of the degree to which exposure to the chemical is hazardous to humans. The panel also identifies areas of uncertainty and where additional data are needed. Expert panel reports are made public and comments are solicited.

Next, CERHR prepares the NTP Brief. The goal of the NTP Brief is to provide the public, as well as government health, regulatory, and research agencies, with the NTP's conclusions regarding the potential for the chemical to adversely affect human reproductive health or children's development. CERHR then prepares the NTP-CERHR Monograph, which includes the NTP Brief and the Expert Panel Report. The NTP-CERHR Monograph is made publicly available on the CERHR website and in hardcopy or CD from CERHR.

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[1]NTP is an interagency program headquartered in Research Triangle Park, NC at the National Institute of Environmental Health Sciences, a component of the National Institutes of Health.

[2]Information about the CERHR is available on the web at *http://cerhr.niehs.nih.gov* or by contacting:

Michael Shelby, Ph.D.
Director, CERHR
NIEHS, P.O. Box 12233, MD EC-32
Research Triangle Park, NC 27709
919-541-3455 [phone]
919-316-4511 [fax]
shelby@niehs.nih.gov [email]

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ABSTRACT

NTP-CERHR MONOGRAPH ON THE POTENTIAL HUMAN REPRODUCTIVE AND DEVELOPMENTAL EFFECTS OF BISPHENOL A

The National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) conducted an evaluation of the potential for bisphenol A to cause adverse effects on reproduction and development in humans. The CERHR Expert Panel on Bisphenol A completed its evaluation in August 2007.

CERHR selected bisphenol A for evaluation because of the:

- Widespread human exposure
- Public concern for possible health effects from human exposures
- High production volume
- Evidence of reproductive and developmental toxicity in laboratory animal studies

Bisphenol A (CAS RN: 80 – 05 – 7) is a high production volume chemical used primarily in the production of polycarbonate plastics and epoxy resins. Polycarbonate plastics are used in some food and drink containers; the resins are used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. To a lesser extent bisphenol A is used in the production of polyester resins, polysulfone resins, polyacrylate resins, and flame retardants. In addition, bisphenol A is used in the processing of polyvinyl chloride plastic and in the recycling of thermal paper. Some polymers used in dental sealants and tooth coatings contain bisphenol A. The primary source of exposure to bisphenol A for most people is assumed to occur through the diet. While air, dust, and water (including skin contact during bathing and swimming) are other possible sources of exposure, bisphenol A in food and beverages accounts for the majority of daily human exposure. The highest estimated daily intakes of bisphenol A in the general population occur in infants and children.

The results of this bisphenol A evaluation are published in an NTP-CERHR Monograph that includes the (1) NTP Brief and (2) Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A. Additional information related to the evaluation process, including the peer review report for the NTP Brief and public comments received on the draft NTP Brief and the final expert panel report, are available on the CERHR website (*http://cerhr.niehs.nih.gov/)*. See bisphenol A under "CERHR Chemicals" on the homepage or go directly to *http://cerhr.niehs.nih.gov/chemicals/bisphenol/bisphenol.html*).

The NTP reached the following conclusions on the possible effects of exposure to bisphenol A on human development and reproduction. Note that the possible levels of concern, from lowest to highest, are negligible concern, minimal concern, some concern, concern, and serious concern.

The NTP has *some concern* for effects on the brain, behavior, and prostate gland in fetuses, infants, and children at current human exposures to bisphenol A.

The NTP has *minimal concern* for effects on the mammary gland and an earlier age for puberty for females in fetuses, infants, and children at current human exposures to bisphenol A.

The NTP has *negligible concern* that exposure of pregnant women to bisphenol A will result in fetal or neonatal mortality, birth defects, or reduced birth weight and growth in their offspring.

The NTP has *negligible concern* that exposure to bisphenol A will cause reproductive effects in non-occupationally exposed adults and *minimal concern* for workers exposed to higher levels in occupational settings.

NTP will transmit the NTP-CERHR Monograph on Bisphenol A to federal and state agencies, interested parties, and the public and make it available in electronic PDF format on the CERHR web site *(http://cerhr.niehs.nih.gov)* and in printed text or CD from CERHR:

Dr. Michael D. Shelby
Director, CERHR
NIEHS, P.O. Box 12233, MD EC-32
Research Triangle Park, NC 27709
919-541-3455 [phone]
919-316-4511 [fax]
shelby@niehs.nih.gov [email]

INTRODUCTION

Bisphenol A (CAS RN: 80–05–7) is a high production volume chemical used primarily in the production of polycarbonate plastics and epoxy resins. Polycarbonate plastics are used in food and drink packaging; the resins are used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. To a lesser extent bisphenol A is used in the production of polyester resins, polysulfone resins, polyacrylate resins, and flame retardants. In addition, bisphenol A is used in the processing of polyvinyl chloride plastic and in the recycling of thermal paper. Some polymers used in dental sealants and tooth coatings contain bisphenol A.

In 2007, the CERHR Expert Panel on Bisphenol A evaluated bisphenol A for reproductive and developmental toxicity. Because most people in the United States are exposed to bisphenol A and a number of studies have reported effects on reproduction and development in laboratory animals, there is considerable interest in its possible health effects on people. For these reasons, the CERHR convened an expert panel to conduct an evaluation of the potential reproductive and developmental toxicities of bisphenol A.

This monograph includes the NTP Brief on Bisphenol A, a list of the expert panel members (Appendix I), and the Expert Panel Report on bisphenol A (Appendix II). The monograph is intended to serve as a single, collective source of information on the potential for bisphenol A to adversely affect human reproduction or development.

The NTP Brief on Bisphenol A presents the NTP's opinion on the potential for exposure to bisphenol A to cause adverse reproductive or developmental effects in people. The NTP Brief is intended to provide clear, balanced, scientifically sound information. It is based on information about bisphenol A provided in the expert panel report, public comments, comments from peer reviewers[3] and additional scientific information available since the expert panel meeting.

[3]Peer review of this brief was conducted by the NTP Board of Scientific Counselors (supplemented with eight non-voting *ad hoc* reviewers) on June 11, 2008. The peer report is available at *http://cerhr.niehs.nih.gov/chemicals/bisphenol/bisphenol.html.*

National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation of Risks To Human Reproduction

NTP BRIEF ON BISPHENOL A

[CAS NO. 80–05–07]

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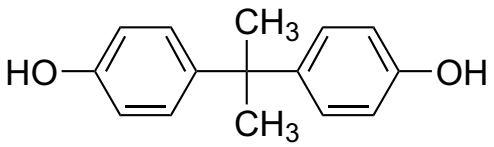
NTP BRIEF ON BISPHENOL A

WHAT IS BISPHENOL A?

Bisphenol A (BPA) is a chemical produced in large quantities for use primarily in the production of polycarbonate plastics and epoxy resins (Figure 1).

Figure 1.
Chemical structure of Bisphenol A
($C_{15}H_{16}O_2$; molecular weight 228.29)



It exists at room temperature as a white solid and has a mild "phenolic" or hospital odor. Polycarbonate plastics have many applications including use in certain food and drink packaging, e.g., water and infant bottles, compact discs, impact-resistant safety equipment, and medical devices. Polycarbonate plastics are typically clear and hard and marked with the recycle symbol "7" or may contain the letters "PC" near the recycle symbol. Polycarbonate plastic can also be blended with other materials to create molded parts for use in mobile phone housings, household items, and automobiles. Epoxy resins are used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. Some polymers used in dental sealants or composites contain bisphenol A-derived materials. In 2004, the estimated production of bisphenol A in the United States was approximately 2.3 billion pounds, most of which was used in polycarbonate plastics and resins.

CERHR selected bisphenol A for evaluation because it has received considerable attention in recent years due to widespread human exposures and concern for reproductive and developmental effects reported in laboratory animal studies. Bisphenol A is most commonly described as being "weakly" estrogenic; however, an emerging body of molecular and cellular studies indicate the potential for a number of additional biological activities. These range from interactions with cellular receptors that have unknown biological function to demonstrated effects on receptor signaling systems known to be involved in development.

The NTP Brief on Bisphenol A is intended to be an environmental health resource for the public and regulatory and health agencies. It is not a quantitative risk assessment nor is it intended to supersede risk assessments conducted by regulatory agencies. The NTP Brief on Bisphenol A does not present a comprehensive review of the health-related literature or controversies related to this chemical. Only key issues and study findings considered most relevant for developing the NTP conclusions on concerns for potential reproductive and developmental human health effects of bisphenol A are discussed. Literature cited includes the most relevant studies reviewed in the CERHR Expert Panel Report on Bisphenol A and relevant research articles published in the peer-reviewed literature subsequent to the deliberations of the expert panel.

ARE PEOPLE EXPOSED TO BISPHENOL A?[4]

Yes. Based on the available data the primary source of exposure to bisphenol A for most people is through the diet. While air, dust, and water (including skin contact during bathing and swimming) are other possible sources of exposure, bisphenol A in food and beverages accounts for the majority of daily human exposure [(*1*); reviewed in *(2, 3)*]. Bisphenol A can migrate into food from food and beverage con-

[4]Answers to this and subsequent questions may be: *Yes, Probably, Possibly, Probably Not, No or Unknown*

tainers with internal epoxy resin coatings and from consumer products made of polycarbonate plastic such as baby bottles, tableware, food containers, and water bottles. The degree to which bisphenol A migrates from polycarbonate containers into liquid appears to depend more on the temperature of the liquid than the age of the container, i.e., more migration with higher temperatures *(4)*. Bisphenol A can also be found in breast milk *(5)*. Short–term exposure can occur following application of certain dental sealants or composites made with bisphenol A-derived material such as bisphenol A dimethacrylate (bis-DMA). In addition, bisphenol A is used in the processing of polyvinyl chloride plastic and in the recycling of thermal paper, the type of paper used in some purchase receipts, self-adhesive labels, and fax paper *(6, 7)*. Bisphenol A can also be found as a residue in paper and cardboard food packaging materials *(7)*. Workers may be exposed by inhalation or skin contact during the manufacture of bisphenol A and bisphenol A-containing products, e.g., polycarbonate and polyvinyl plastics, thermal paper, epoxy or epoxy-based paints and lacquers and tetrabrominated flame retardants *(6)*.

Estimating human exposure to bisphenol A is generally done in one of two ways. Concentrations of bisphenol A can be measured directly in human blood, urine, breast milk, and other fluids or tissues ("biomonitoring"). Researchers can use biomonitoring information, such as the concentration of bisphenol A in urine, to estimate ("back calculate") a total intake that reflects all sources of exposure, both known and unknown. Scientists can also add, or aggregate, the amounts of bisphenol A detected in various sources, i.e., food and beverage, air, water, dust. The approach of aggregating exposure to estimate daily intake requires sources of exposure to be known and measured. In general, estimates based on biomonitoring are preferred for calculating total intake because all sources of exposure are integrated into the fluid or tissue measurement and do not have to be identified in advance. Estimates based on sources of exposure are useful to help discern the relative contributions of various exposure pathways to total intake.

The highest estimated daily intake of bisphenol A in the general population occur in infants and children (Table 1).

Table 1.
Summary of Ranges of Estimated Daily Intakes in People Based on Sources of Exposure

Population		*Bisphenol A µg/kg bw/day*	*Assumptions*	*References*
Infant	0–6 months Formula-fed	1–11*	1 assumes body weight of 4.5 kg and formula intake of 700 ml/day with 6.6 µg/L [maximum concentration detected in U.S. canned formula *(23, 24)*] *(2)* 11 assumes body weight of 6.1 kg and formula intake of 1060 ml/day with (1) 50 µg/L bisphenol A/day migrating into formula from polycarbonate bottles (8.7 µg/kg bw/day); and (2) 14.3 µg bisphenol A/day ingested from powdered infant formula packed in food cans with epoxy linings (2.3 µg/kg bw/day) [0.143 kg powder/day (the amount of powder required to reconstitute a volume of formula of 1060 ml/day) containing 14.3 µg bisphenol A (100 µg bisphenol A/kg powder)]. 8.7+2.3=11 µg/kg bw/day *(25)*	*(2, 25–27)*

(continued on next page)

Population		Bisphenol A µg/kg bw/day	Assumptions	References
Infant	Breast-fed	0.2 – 1*	0.2 assumes body weight of 6.1 kg and breast milk intake of 1060 ml/day with 0.97 µg/L bisphenol A [maximum concentration of bisphenol A detected in Japanese breast milk samples *(28)](25)* 1 assumes body weight of 4.5 kg and breast milk intake of 700 ml/day with 6.3 µg/L free bisphenol A [maximum concentration of free bisphenol A detected in U.S. breast milk samples *(5)](2)*	*(2, 25)*
	6 – 12 months	1.65 – 13*	1.65 assumes body weight of 8.8 kg with (1) 7 µg/L bisphenol A/day from formula intake of 700 ml/day with 10 µg/L (0.8 µg/kg bw/day); and (2) 7.6 µg/kg bisphenol A/day from ingestion of 0.38 kg canned food/day with 20 µg/kg (~0.85 µg/kg bw/day). 0.8 + 0.85 = 1.65 *(26)* 13 assumes body weight of 7.8 kg, formula intake of 920 ml/day, and food consumption of 0.407 kg/day with (1) 50 µg/L bisphenol A migrating into formula from polycarbonate bottles (5.9 µg/kg bw/day); (2) 12.4 µg bisphenol A/day ingested from powdered infant formula packed in food cans with epoxy linings (1.6 µg/kg bw/day) [0.124 kg powder/day (the amount of powder required to reconstitute a volume of formula of 920 ml/day) containing 12.4 µg bisphenol (100 µg bisphenol A/kg powder)]; (3) 40.7 µg bisphenol A/day ingested from canned food (5.2 µg/kg bw/day) [0.407 kg food/day containing 40.7 µg bisphenol A (100 µg bisphenol A/kg food)]; and (4) 2.04 µg bisphenol A/day migration from polycarbonate tableware (0.26, or ~ 0.3 µg/kg bw/day)[0.407 kg food/day containing 2.04 µg bisphenol A (5 µg bisphenol A/kg food)] 5.9 + 1.6 + 5.2 + 0.3 = 13.0 µg/kg bw/day *(25)*	*(24 – 27)*
Child	1.5 – 6 years	0.043 – 14.7	0.043 is the mean (range: 0.018 – 0.071 µg/kg bw/day) based on individual body weight and measured concentrations of bisphenol in indoor and outdoor air, dust, soil, and liquid and solid food from day care and home and the assumption of 100% absorption *(29)* 14.7 assumes body weight of 14.5 kg and consumption of 2 kg canned food/day with (1) 200 µg bisphenol A/day ingested from canned food (~14 µg/kg bw/day) [2 kg food/day containing 200 µg bisphenol A (100 µg bisphenol A/kg food)]; and (2) 10 µg bisphenol A/day migration from polycarbonate tableware (~ 0.7 µg/kg bw/day) [2 kg food/day containing 10 µg bisphenol A (5 µg bisphenol A/kg food)] 14 + 0.7 = 14.7*(27)*	*(1, 25 – 27, 29, 30)*

(continued on next page)

Population		Bisphenol A µg/kg bw/day	Assumptions	References
Adult	General Population	0.008 – 1.5**	0.008 assumes body weight of 74.8 kg and is based on measured concentrations of bisphenol A in 80 canned and bottled food items and a 24 – hour dietary recall in ~4400 New Zealanders *(31)* 1.5 assumes body weight of 60 kg and (1) 70 µg bisphenol A/day from canned food (1.2 µg/kg bw/day) [3 kg/day total consumption (1 kg solid food with 50 µg bisphenol A/kg and 2 L beverage with 10 µg bisphenol A /L)]; and 15 µg bisphenol A/day migration from polycarbonate tableware (0.25, or ~ 0.3 µg/kg bw/day) [3 kg food/day containing 15 µg bisphenol A (5 µg bisphenol A/kg food)] 1.2 + 0.3 = 1.5 µg/kg bw/day *(25)*	*(24 – 27, 30, 31)*
	Occupational	0.043 – 100	0.043 is based on back calculating from a median urinary bisphenol A concentration of 1.06 µmol/mol creatinine (2.14 µg/g creatinine) from Hanaoka *et al. (32)*. A daily intake of 0.043 µg/kg bw/day is based on the assumption of 1200 mg/day creatinine excretion (2.57 µg/day bisphenol excreted) and a body weight of 60 kg *(2)*. 100 is the maximal estimated exposures in U.S. powder paint workers based on time weighted averages of 0.001–1.063 mg/m^3, an inhalation factor of 0.29 m^3/kg day *(33)*, 100% absorption from the respiratory system, and 8 hours worked per day *(2)*.	*(2, 27, 33)*

*A study by Miyamoto *et al. (30)* reported much lower estimated intakes for infants (0.028 to 0.18 µg/kg bw/day); however, these estimates were excluded from the summary table because (1) insufficient detail was presented in the study to understand the assumptions used to derive these values, and (2) the authors assumed no bisphenol A in breast milk, an assumption not supported by data from the CDC *(5)* and Sun *et al. (28)*.

**In 2003, the European Union *(27)* calculated an extreme worst – case scenario of ~ 9 µg/kg bw/day based on 1.4 µg/kg bw/day from food plus ~ 7 µg/kg bw/day from wine. The high estimated intake from wine (0.75 L wine/day with 650 µg bisphenol A /L = 325 µg bisphenol A/day, or ~7 µg/kg bw/day, from wine) was based on an extraction study conducted with an epoxy resin that is sometimes used to line wine vats. A study published subsequent to the evaluation by the European Union identified a maximum concentration of 2.1 µg bisphenol A/L in wine *(34)*.

Infants and children have higher intakes of many widely detected environmental chemicals because they eat, drink, and breathe more than adults on a pound for pound basis. In addition, infants and children spend more time on the floor than adults and may engage in certain behaviors, such as dirt ingestion or mouthing of plastic items that can increase the potential for exposure.

Biomonitoring studies show that human exposure to bisphenol A is widespread (Table 2). The National Health and Nutrition Examination Survey (NHANES) 2003 – 2004 conducted by the Centers for Disease Control and Prevention (CDC) found detectable levels of bisphenol A in 93% of 2517 urine samples from people 6 years and older *(8)*. This study did not include children younger than 6 years of age. The CDC measured the "total" amount of bisphenol A in urine, a value that includes both bisphenol A and its metabolites. The CDC NHANES data are considered representative of exposures in

Table 2. Urinary Concentrations and Corresponding "Back Calculated" Daily Intakes of bisphenol A in People (United States)

Population	*Urinary Concentration of Total bisphenol A [µg/L]* (8)*	*Estimated Intake of bisphenol A [µg/kg bw/day]**(35)*
All	2.7 (1.3 – 15.9/149)	0.0505 (0.0235 – 0.2742/3.47)
6 – 11 years	3.7 (1.7 – 16.0/46.1)	0.0674 (0.0310 – 0.3105/0.55)
12 – 19 years	4.2 (1.9 – 16.5/149)	0.0773 (0.0378 – 0.3476/3.47)
20 – 39 years	3.1 (1.5 – 15.4/61.4)	0.0563 (0.0272 – 0.289./0.84)
40 – 59 years	2.4 (1.1 – 15.5/75.2)	0.0415 (0.0179 – 0.2335/0.88)
60+ years	1.9 (0.8 – 13.3/52.4)	0.0334 (0.0163 – 0.2331/0.88)
Female	2.4 (1.2 – 15.7/80.1)	0.0443 (0.0190 – 0.2705/1.40)
Male	3.2 (1.4 – 16.0/149)	0.0572 (0.0269 – 0.2778/3.47)

Data is shown as median (25th – 95th percentile range/maximum)

*The CDC data for ages 20 – 39 and 40 – 59 years were not presented in the study by Calafat *et al. (8)*. Lakind *et al. (35)* obtained these values from data files available on the CDC website (*http://www.cdc.gov/nchs/about/major/nhanes/nhanes2003 – 2004/lab03_04.htm*). Lakind *et al. (35)* conducted a separate analysis of the CDC data and calculated mean and percentile values within 0.2 µg/L of those presented by Calafat *et al. (8)*. The NTP obtained maximum urine concentrations for each category from the CDC data files. The highest urinary concentrations and estimated intakes in Table 2 represent data from the same individual.

** Lakind *et al. (35)* assumed that daily intake of bisphenol A was equivalent to daily excretion. Daily excretion was calculated by multiplying the urine concentration of bisphenol A (µg/L) by 24 – hour urinary output volume. Daily urinary volume was assumed to be 600 ml for children aged 6 – 11 years, 1200 for males and females aged 12 – 19, 1200 for adult females, and 1600 for adult males. Body weight data from the 2003 – 2004 NHANES database was used to calculate daily intake adjusted for body weight. The NTP calculated the maximum estimated daily intakes by multiplying the maximum detected urine concentration for each category by the corresponding default urine output volume used by Lakind *et al.* and then dividing this number by the individual's body weight provided in the CDC data files.

the United States because of the large number of people included in the survey and the process used to select participants. In addition, the analytical techniques used by the CDC to measure bisphenol A are considered very accurate by the scientific community. There is some indication that exposure to bisphenol A may be increasing. The median levels of bisphenol A in human urine doubled (from 1.3 µg/L to 2.7 µg/L) and the 95th percentile values tripled (from 5.2 µg/L to 15.9 µg/L) between NHANES III (1988 – 1994) and NHANES 2003 – 2004. Many smaller studies also report detection of bisphenol A in urine, blood, and other body fluids and tissues from people in the United States, Europe, and Asia [(*9 – 12*); studies published prior to mid-2007 are reviewed in (*2, 3, 13*)]. Because bisphenol A does not persist for long periods of time in the body, its widespread detection in people indicates that exposures occur frequently.

Bisphenol A can be detected in the blood of pregnant women, amniotic fluid, placental tissue, and umbilical cord blood indicating some degree of fetal exposure (*12, 14 – 17*). Concentrations of bisphenol A measured in breast milk and the blood of pregnant women in the United States are presented in Table 3.

Table 3. Blood and Breast Milk Biomonitoring of bisphenol A in People (United States)

Biological Medium	Population (sample size)	Free bisphenol A (µg/L) Mean or Median [range]	Total bisphenol A (µg/L) Mean or Median [range]	Reference
Blood	Pregnant women (40)	*Mean:* 5.9 [0.5–22.4]		*(12)*
Breast milk	Lactating women (20)	*Mean:* 1.3; *Median:* 0.4 [<0.3 (LOD)–6.3]	*Mean:* 1.3; *Median:* 1.1 [<0.3 (LOD)–7.3]	*(5)*

LOD = limit of detection

It is helpful in interpreting the biomonitoring data for bisphenol A to understand how the body processes and excretes it once exposure occurs. Following ingestion, the majority of bisphenol A is quickly bound to glucuronic acid to produce bisphenol A-glucuronide, a metabolic process called glucuronidation that is carried out by enzymes primarily in the liver [reviewed in *(2)]*. Glucuronidation makes bisphenol A more soluble in water and, therefore, easier to eliminate in the urine and also minimizes its ability to interact with biological processes in the body. To a lesser extent, unconjugated parent (commonly referred to as "free")[5] bisphenol A is converted to other metabolites, primarily bisphenol A sulfate. Understanding the degree to which bisphenol A is metabolized is very important in determining whether bisphenol A poses a potential risk to human reproduction and development. While free bisphenol A and its major metabolites (bisphenol A-glucuronide and bisphenol A-sulfate) can all be measured in humans, only free bisphenol A is considered to be biologically active. Bisphenol A is metabolized more quickly following oral exposure compared to non-oral exposures such as inhalation because of "first pass effects" (see below).

[5] Unmetabolized bisphenol A is commonly referred to as "free"; however, the majority of "free" bisphenol A circulating in human blood is bound to plasma proteins.

There is evidence in laboratory rodents that very young animals metabolize bisphenol A less efficiently than adult animals *(18–20)*. Neonatal rats have higher circulating concentrations of free bisphenol A in their blood compared to older animals given an equal exposure, presumably due to an underdeveloped ability to glucuronidate early in life *(18)*. However, neonatal rats do have the capacity to metabolize and eliminate bisphenol A. The specific enzymes that glucuronidate bisphenol A have not been identified in people, but there is evidence of postnatal maturation for a number of glucuronidation enzymes in humans. For this reason, a reduced ability or efficiency to glucuronidate is generally predicted for human fetuses and infants [reviewed in *(2)]*. However, a number of the enzymes involved in metabolizing bisphenol A to bisphenol A sulfate in humans are known and have been shown to be active in fetal and neonatal life *(21, 22)*, suggesting that this metabolic pathway may be more important than glucuronidate early in life relative to adulthood.

CAN BISPHENOL A AFFECT HUMAN DEVELOPMENT OR REPRODUCTION?

Possibly. Although there is no direct evidence that exposure of people to bisphenol A adversely affects reproduction or development, studies with laboratory rodents show that exposure to high dose levels of bisphenol A during pregnancy and/or lactation can reduce survival, birth weight,

and growth of offspring early in life, and delay the onset of puberty in males and females. These effects were seen at the same dose levels that also produced some weight loss in pregnant animals ("dams"). These "high" dose effects of bisphenol A are not considered scientifically controversial and provide clear evidence of adverse effects on development in laboratory animals. However, the administered dose levels associated with delayed puberty (≥ 50 mg/kg bw/day), growth reductions (≥ 300 mg/kg bw/day), or survival (≥ 500 mg/kg bw/day) are far in excess of the highest estimated daily intake of bisphenol A in children (< 0.0147 mg/kg bw/day), adults (< 0.0015 mg/kg bw/day), or workers (0.100 mg/kg bw/day) (Table 1).

In addition to effects on survival and growth seen at high dose levels of bisphenol A, a variety of effects related to neural and behavior alterations, potentially precancerous lesions in the prostate and mammary glands, altered prostate gland and urinary tract development, and early onset of puberty in females have been reported in laboratory rodents exposed during development to much lower doses of bisphenol A (≥ 0.0024 mg/kg bw/day) that are more similar to human exposures. In contrast to the "high" dose developmental effects of bisphenol A, there is scientific controversy over the interpretation of the "low" dose findings. When considered together, the results of "low" dose studies of bisphenol A provide limited evidence for adverse effects on development in laboratory animals (see Figures 2a & 2b).

Recognizing the lack of data on the effects of bisphenol A in humans and despite the limitations in the evidence for "low" dose effects in laboratory animals discussed in more detail below, the possibility that bisphenol A may alter human development cannot be dismissed (see Figure 3).

SUPPORTING EVIDENCE

The NTP finds that there is clear evidence of adverse developmental effects at "high" doses of bisphenol A in the form of fetal death, decreased litter size, or decreased number of live pups per litter in rats (≥ 500 mg/kg bw/day) *(36, 37)* and mice (≥ 875 mg/kg bw/day) *(38–40)*, reduced growth in rats (≥ 300 mg/kg bw/day) *(36, 37)* and mice (≥ 600 mg/kg bw/day) *(38, 39, 41)*, and delayed puberty in male mice (600 mg/kg bw/day) *(41),* male rats (≥ 50 mg/kg bw/day) *(37, 42)* and female rats (≥ 50 mg/kg bw/day) *(37, 43)*.

In addition to these "high" dose effects on survival and growth, the NTP recognizes that there are studies that provide evidence for a variety of effects at much lower dose levels of bisphenol

Figure 2a. *The weight of evidence that bisphenol A causes adverse developmental or reproductive effects in humans*

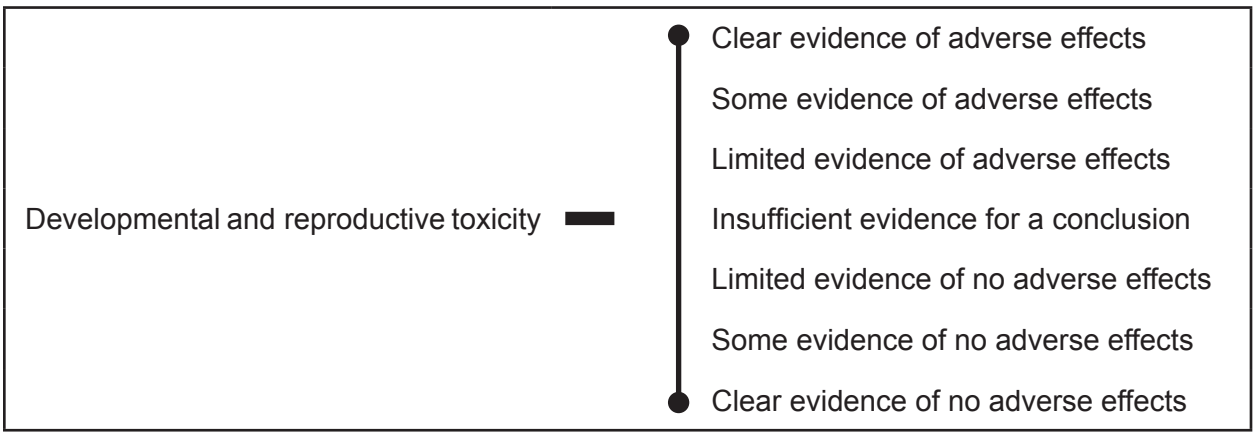
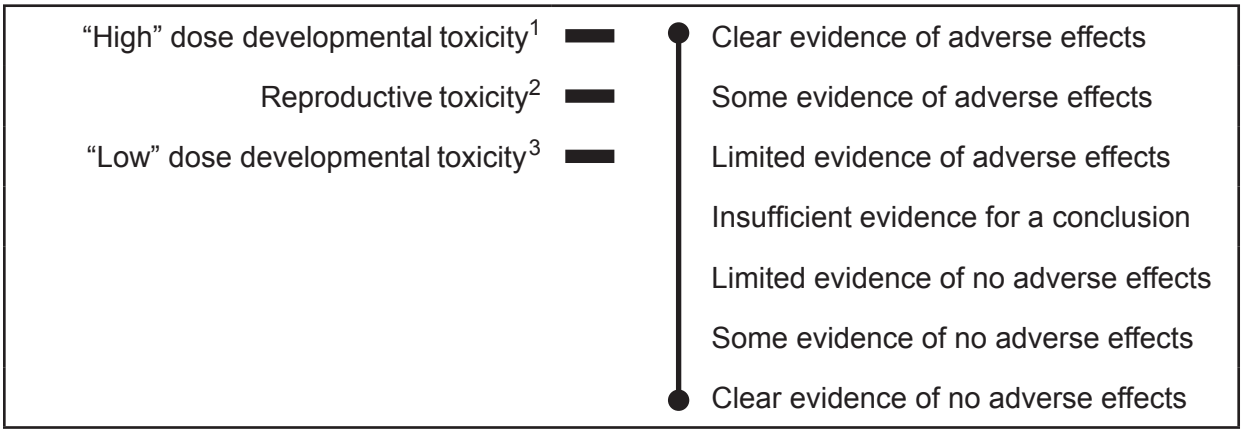


Figure 2b. *The weight of evidence that bisphenol A causes adverse developmental or reproductive effects in laboratory animals*

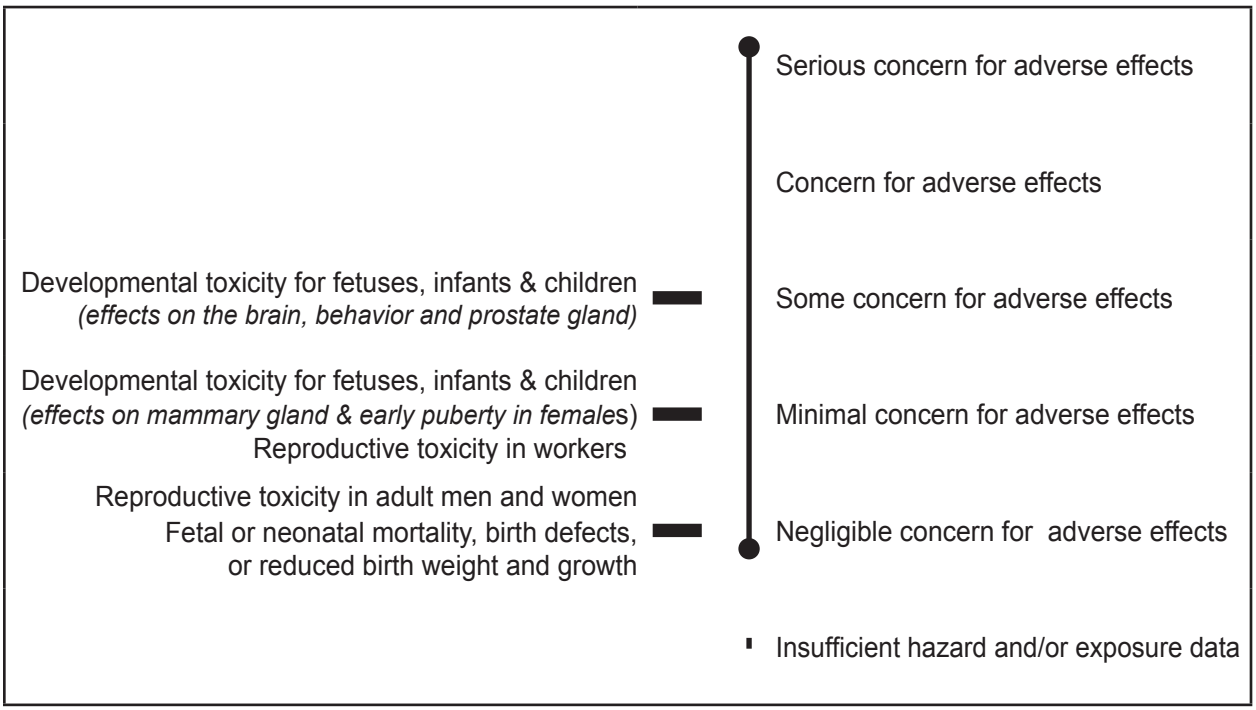


[1]Based on reduced survival in fetuses or newborns (≥500 mg/kg bw/day) *(36–40)*, reduced fetal or birth weight or growth of offspring early in life (≥300 mg/kg bw/day) *(36, 37, 41)*, and delayed puberty in female rats (≥50 mg/kg bw/day) and male rats and mice (≥50 mg/kg bw/day) (*37, 41–43*).

[2]Based on possible decreased fertility in mice (≥875 mg/kg bw/day) (*40*); altered estrous cycling in female rats (≥600 mg/kg bw/day) *(110),* and cellular effects on the testis of male rats (235 mg/kg bw/day) *(111).*

[3]Based a variety of effects related to neural and behavior alterations (≥10 µg/kg bw/day) *(44–50)*, lesions in the prostate (10 µg/kg bw/day) (*51*) and mammary glands (0.0025–1 mg/kg bw/day) *(52, 53)*; altered prostate gland and urinary tract development (10 µg/kg bw/day) *(54),* and early onset of puberty (2.4 and 200 µg/kg bw/day) *(48, 55)*.

Figure 3. *NTP conclusions regarding the possibilities that human development or reproduction might be effected by exposure to bisphenol A*



A related to neural and behavioral alterations in rats and mice (≥ 0.010 mg/kg bw/day) *(44 – 50)*, preneoplastic lesions in the prostate and mammary gland in rats (0.010 mg/kg bw/day and 0.0025 mg/kg bw/day, respectively) *(51 – 53)*, altered prostate and urinary tract development in mice (0.010 mg/kg bw/day) *(54),* and early onset of puberty in female mice (0.0024 and 0.200 mg/kg bw/day) *(48, 55)*.

These "low" dose findings in laboratory animals have proven to be controversial for a variety of reasons including concern for insufficient replication by independent investigators, questions on the suitability of various experimental approaches, relevance of the specific animal model used for evaluating potential human risks, and incomplete understanding or agreement on the potential adverse nature of reported effects. These issues have been extensively addressed elsewhere *(2, 56 – 60)* and were considered by the NTP when evaluating the bisphenol A literature.

HOW WAS THIS CONCLUSION REACHED?

Scientific decisions concerning health risks are generally based on what is known as the "weight-of-evidence." In the case of bisphenol A, evidence from the limited number of studies in humans exposed to bisphenol A is not sufficient to reach conclusions regarding possible developmental or reproductive hazard. In contrast, there is a large literature of laboratory animal studies. These include studies of traditional designs carried out to assess the toxicity of bisphenol A, as well as a wide variety of studies examining the possibility that exposure to "low" doses of bisphenol A, defined in the NTP Brief on Bisphenol A as ≤ 5 mg/kg bw/day *(61),* during critical periods of development might result in adverse health outcomes later in life due to its estrogenic or other biological properties. Many of these latter studies were designed not as toxicology studies but rather to probe very specific experimental questions, and their results are not always easily interpreted with regard to how they contribute to the weight-of-evidence for human health risks.

Many of the laboratory animal studies of bisphenol A have technical or design shortcomings or their reports do not provide sufficient experimental details to permit an assessment of technical adequacy *(2)*. As discussed in more detail below, the NTP did not establish strict criteria for determining which studies from the bisphenol A literature to consider for the evaluation. Rather, in an effort to glean information that might contribute to understanding the numerous reported effects of bisphenol A, NTP evaluated many individual study reports. Attention was paid to issues of sample size, control for litter effects, and various other aspects of experimental design; however, experimental findings were initially evaluated in relation to their biological plausibility and consistency across studies by multiple investigators. Studies were then evaluated as to their adequacy of experimental design and the likelihood that any inconsistent outcomes resulted from differences or shortcomings in experimental design. The NTP considered several overarching issues when evaluating the bisphenol A literature:

Are the *in vivo* effects biologically plausible?

Historically, bisphenol A has been characterized as being weakly estrogenic. For this reason the most common type of positive control compounds used in bisphenol A studies are potent estrogens. There is wide variability in *in vitro* estrogenic potency estimates for bisphenol A, although the mean estimate is ~1,000 to 10,000 times less potent than positive control compounds *(2)*. However, a number of the "low" dose studies suggest that bisphenol A has a higher *in vivo* potency than would be predicted based on binding to estrogen receptor alpha. The lack of concordance in potency estimates based on estrogen receptor binding and *in vivo* biological activity has been a point of debate

in considering the biological plausibility of a number of the reported low dose effects. The NTP does not necessarily consider it appropriate to consider the reported biological effects of bisphenol A exclusively within the context of estrogen receptor α or β binding. An increasing number of molecular or cell-based ("*in vitro*") studies suggest that attributing the effects of bisphenol A solely to a classic estrogenic mechanism of action, or even as a selective estrogen receptor modulator (SERM)[6], is overly simplistic. In addition to binding to the nuclear estrogen receptors ERα and ERβ, bisphenol A has been reported to interact with a variety of other cellular targets [reviewed in *(2, 62)*] including binding to a non-classical membrane-bound form of the estrogen receptor (ncmER) *(63–65)*, a recently identified orphan nuclear receptor called estrogen-related receptor gamma ERR-γ *(66–70)*, a seven-transmembrane estrogen receptor called GPR30 *(71),* and the aryl hydrocarbon receptor (AhR) *(72, 73)*.

Several *in vitro* studies show that bisphenol A can act as an androgen receptor antagonist *(72, 74–80)* and is reportedly mitogenic in a human prostate carcinoma cell line through interactions with a mutant tumor-derived form of the androgen receptor *(81)*. Bisphenol A also interacts with thyroid hormone receptors (TRs) and, based on *in vitro* studies, is reported to either inhibit TR-mediated transcription *(82),* inhibit the actions of triiodothyronine (T3) or its binding to TRs *(83, 84)*, or stimulate cell proliferation in a thyroid hormone responsive cell line *(85)*. One *in vivo* study suggests that bisphenol A acts as a selective TRβ antagonist *(86)*. Bisphenol A may also inhibit activity of aromatase, the enzyme that converts testosterone to estradiol *(72, 87)*.

[6]A selective estrogen receptor modulator (SERM) is a compound that binds nuclear estrogen receptors and acts as an estrogen agonist in some tissues and as an estrogen antagonist in other tissues.

The toxicological consequences of the non-nuclear estrogen receptor interactions identified so far are unclear. In some instances, the physiologic role of the receptor is unknown or not well characterized, i.e., ERR-γ, GPR30, which makes interpreting the consistency of the data impossible with respect to the implicated mechanism based on the cellular or molecular studies and the observed *in vivo* toxicology. In other instances, the binding affinity of bisphenol A for the receptor is sufficiently low that no or minimal influences on biological processes *in vivo* would be expected. However, even when the physiological effects are generally understood, e.g., AR binding, aromatase function, scientists can only speculate as to the possible *in vivo* impacts when multiple receptor or other cellular interactions are considered together. Nevertheless, the identification of a growing number of cellular targets for bisphenol A may help explain toxicological effects that are not considered estrogenic or predicted simply based on the lower potency of bisphenol A compared to estradiol. Effects mediated through the ncmER are of interest because of its role in regulating pancreatic hormone release and because bisphenol A has been shown to activate this receptor *in vitro* at a concentration of 1 nM, which is similar to the active concentration of the potent estrogen diethylstilbestrol *(63, 65)*.

Are the *in vivo* effects reproducible?

Two issues become evident when considering the topic of reproducibility of effects in the bisphenol A literature. In some cases, the reproducibility of certain effects has been questioned because attempts at replication by other researchers using similar experimental designs did not necessarily produce consistent findings. This leads to reduced confidence in the utility of the effect for identifying a hazard. Numerous reasons have been suggested to explain the inconsistent findings including differences in sensitivity of the rodent model, i.e., species, strain, breeding stock, the author's funding source, the degree

of laboratory expertise, and variations in diet,[7] husbandry and route of administration. However, it is not known if these factors account for the inconsistencies. In other cases, particularly for findings based on studies with very specific experimental questions, variations in experimental design are large enough to conclude that the reproducibility of the finding is essentially unknown. A number of these effects have not been addressed in traditional toxicity studies carried out to assess the toxicity of bisphenol A. Typically, the safety studies do not probe for potential organ effects with the same degree of specificity or detail as those studies with specific experimental questions. The NTP evaluated the biological plausibility of findings with unknown reproducibility in light of supporting data at the mechanistic, cellular, or tissue level.

Another issue is that the "low" dose studies generally have not tested higher dose levels of bisphenol A, i.e., >1 mg/kg. Testing over a wide range of dose levels is necessary to adequately characterize the dose-response relationship. Typically, effects are easier to interpret when the dose-response curve is monotonic and the incidence, severity, or magnitude of response increases as the dose level increases. Effects that have biphasic, or non-monotonic dose response curves, have been documented in toxicology, endocrinology and other scientific disciplines *(90, 91)*, but can be more difficult to interpret, which often limits their impact in risk assessments or other health evaluations. Testing higher dose levels may also identify additional effects that aid in interpreting the "low" dose finding with respect to potential health risk.

Do the in vivo effects represent adverse health findings in laboratory animals and/or humans?

A general limitation in the "low" dose literature for bisphenol A is that many studies have addressed very specific experimental questions and not necessarily established a clear linkage between the "low" dose finding and a subsequent adverse health impact. For example, when an effect is observed in fetal, neonatal, or pubertal animals, investigations may not have been conducted to determine if the effect persists or manifests as a clear health effect later in life. Establishing a linkage to an adverse health impact is important because many of the "low" dose findings can be described as subtle, which can make them difficult to utilize for risk assessment purposes. An additional factor in considering the adversity of a finding is determining if the experimental model is adequate for predicting potential human health outcomes.

How should studies that use a non-oral route of administration be interpreted?

Because the majority of exposure to bisphenol A occurs through the diet *(1),* laboratory animal studies that use the oral route of administration are considered the most useful to assess potential effects in humans. However, a large number of the laboratory animal studies of bisphenol A have used a subcutaneous route of administration to deliver the chemical, either by injection or mini-pumps that are implanted under the skin. The consideration of these studies in health evaluations of bisphenol A has proven controversial *(2, 92)*. There is scientific consensus that doses of bisphenol A administered orally and subcutaneously cannot be directly compared in adult laboratory animals because the rate of metabolism of bisphenol A differs following oral and non-oral administration. There is also consensus that fetal and neonatal rats do not metabolize

[7] Understanding the impact of variations in dietary phytoestrogen content in laboratory animal studies of estrogenic compounds, including bisphenol A, is an active area of inquiry *(88).* Recent research suggests that bisphenol A may alter DNA methylation (an epigenetic mechanism to alter phenotype) following exposure during development and that this effect may be offset by dietary exposure to methyl donors or the phytoestrogen genistein *(89).*

bisphenol A as efficiently as adult rats at a given dose because the enzyme systems that are responsible for the metabolism of bisphenol A are not fully mature during fetal or neonatal life. However, there is scientific debate on whether the reduced metabolic capability of neonatal rats is sufficient to adequately metabolize low doses of bisphenol A.

In adult rats and monkeys, bisphenol A is metabolized to its biologically inactive form, or glucuronidated, more quickly when administered orally than by a non-oral route, e.g., subcutaneously, intraperitoneally, or intravenously *(93–95)*. This is because bisphenol A administered orally first passes from the intestine to the liver where it undergoes extensive conjugation primarily with glucuronic acid before reaching the systemic circulation ("first pass metabolism"). Because non-oral administration bypasses the liver, and therefore first pass metabolism, these routes of dosing in adult rats and monkeys result in higher circulating concentrations of biologically active, free bisphenol A compared to oral administration. Although not tested directly in adult laboratory mice, the impact of first pass metabolism is predicted to be similar. Thus, a subcutaneous dose is expected to have a greater biological effect than the same dose delivered by mouth in adult laboratory animals, including in the offspring of dams treated with bisphenol A during pregnancy.

Studies that administer bisphenol A through non-oral routes are most useful for human health evaluations when information on the fate, e.g., half-life, and concentration of free bisphenol A in the blood or other tissue is also available. For example, if the peak and average daily concentrations of free bisphenol A in blood were measured following non-oral administration, these values could then be compared to levels of free bisphenol measured in rodent studies where bisphenol A is administered orally or to levels measured in humans. However, none of the reproductive and developmental toxicity studies that treated animals by non-oral routes of administration determined the circulating levels of free bisphenol A or its metabolites. As a result, studies that treat laboratory animals using non-oral routes of administration have often been considered of no or of limited relevance for estimating potential risk to humans *(2, 27, 56)*.

As discussed previously (see "Are People Exposed to Bisphenol A?"), fetal and neonatal rats do not metabolize bisphenol A as efficiently as the adult and, as a result, have higher circulating concentrations of free bisphenol A for some period of time compared to adults receiving the same dose *(18–20)*. The peak concentrations of free bisphenol A in the blood of 4-day old male and female rat pups orally dosed with 10 mg/kg are 2013 and 162-times higher than the peak blood levels measured in male and female adult rats treated with the same mg/kg dose *(18)*. A measure of how long it takes the body to eliminate free bisphenol A, referred to as "half-life," was also slower at this dose in neonatal rats: >6.7 hours in male or female pups compared to well under an 1 hour in adult animals *(18)*. Thus, for a given administered dose, blood levels of bisphenol A are higher in neonatal rats than in adults, and remain so longer following exposure. However, neonatal rats do have the ability to metabolize bisphenol A as indicated by the presence of bisphenol A glucuronide in the blood and the inability to detect the free form within the measurement sensitivity of the assay by 12 to 24-hours after treatment in females and males respectively *(18)*.

Neonatal rats appear to be able to more efficiently metabolize bisphenol A when given at lower dose levels than at higher dose levels. Although Domoradzki *et al. (18)* also treated neonatal and adult animals with a lower dose level of bisphenol A, 1 mg/kg, making a direct comparisons based on age at exposure was not possible at that dose because free bisphenol A was too low

to be quantified in the blood of adults. However, in 4-day old male and female rats treated with 1 mg/kg of bisphenol A, 98 – 100% of administered bisphenol A was detected as bisphenol A-glucuronide[8] compared to 71 – 82% at 10 mg/kg, i.e., a smaller proportion of administered bisphenol A is glucuronidated at 10 mg/kg compared to 1 mg/kg. This would be expected when the limited capacity of young animals to metabolize bisphenol A is overwhelmed by higher dose levels of the compound. These data suggest more efficient metabolism by neonatal rats at 1 mg/kg compared to 10 mg/kg and imply that the age at exposure differences described above may be less profound in the "low" dose range (≤ 5 mg/kg bw/day).

Taken together these data indicate that, compared to adults at a given dose, neonatal rats (and presumably mice) metabolize bisphenol A more slowly and suggest that differences in circulating levels of free bisphenol A arising from oral and subcutaneous routes of administration as a result of "first-pass metabolism" are reduced in fetal or infant animals compared to adults. This prediction is supported by a recent study that did not detect differences in the blood concentration of free bisphenol A as a function of route of administration (oral versus subcutaneous injection) in 3-day old female mice following treatment with either 0.035 or 0.395 mg/kg of bisphenol A *(92)*.

Additional research is needed to understand the metabolism of bisphenol A in both laboratory animals and humans. For example, a complete assessment of the UDP-glucuronosyltransferase (UGT) and sulfotransferase (SULT) isoforms involved in the glucuronidation and sulfation of bisphenol A is needed for both rodents and humans. UGT2B1 has been identified as the principle UGT isoform that metabolizes bisphenol A to bisphenol A glucuronide in the rat *(20)*. This isoform shows low expression and activity during development. However, it is important to note that the Matsumoto *et al.* study only characterized UGT2B1 activity during development and did not include other members of the UGT2B family. Thus, the understanding of bisphenol A metabolism during development in the rat is still incomplete. In addition, it is difficult to translate the rat findings to humans because the UGT isoform(s) that metabolize bisphenol A in humans have not been identified. Humans have 7 members of the UGT2 family that have functional activity, 1 UGT2A and 6 UGT2B isoforms.

In contrast, there is information on the SULT isoforms that metabolize bisphenol A in humans. In humans, SULT1A1 has been identified as the SULT with the highest catalytic activity towards bisphenol A, although SULT1E1, SULT2A1 and a SULT1C isoforms are also capable of catalyzing bisphenol A-sulfate formation *(21)*. In humans, SULT1A1 activity is comparable in fetal and postnatal liver although there are differences in localization (hematopoietic stem cells during fetal life and hepatocytes after birth). Characterizing the ontogeny of individual UGT and SULT enzymes is complex as specific isoforms show unique patterns of expression during development and also vary with respect to preferred substrates and associated catalytic activity. As a result, it is unknown if the various metabolic pathways provide for "sufficient" metabolism of low doses of bisphenol A in humans exposed during fetal life and infancy. Although infants can metabolize bisphenol A, it is likely that significant variation in the developmental profile, e.g., rate and extent of metabolic capacity, would be observed at the population level. The issue of sulfation is also important given the role of sulfation pathways in regulating endogenous compounds that are involved in controlling the growth and function of some of the reproductive tissues identified as targets of

[8]Based on percentage of plasma area under the curve (AUC) for radioactivity that was bisphenol A glucuronide.

bisphenol A. For example, this raises the possibility that bisphenol A-sulfate conjugates may interfere with estriol biosynthesis during fetal development *(96)*.

While more research in this area is warranted, data from studies where bisphenol A was given by subcutaneous injection were considered as useful in the NTP evaluation as oral administration when treatment occurred during infancy when the capacity to metabolize bisphenol A is low. Studies in adult animals, including pregnant dams, that administered bisphenol A by subcutaneous injection or by a subcutaneous mini-pump were considered informative for identifying biological effects of bisphenol A but not for quantitatively comparing exposures in laboratory animals and humans.

What is the impact of limitations in experimental design and how should studies with these limitations be interpreted?

The impact on study interpretation due to limitations in experimental design has been a significant point of discussion for bisphenol A, especially for the issues of (1) small sample size, (2) a lack of experimental or statistical control for litter effects, and (3) failure to use a positive control *(2, 97)*.

In general, studies with larger sample sizes will have more power to detect an effect due to bisphenol A exposure than studies with small sample sizes. For this reason, "negative" results from small sample size studies are viewed with caution. On the other hand, "negative" results from studies with larger sample sizes are usually considered more credible *(98)*. However, there is no single sample size that can be identified as appropriate for all endpoints. The ability to detect an effect is affected by the background incidence, e.g., tumor or malformation rates in control animals, variability of a particular endpoint, and the magnitude of the effect. A sample size of at least six may be reasonable for many endpoints with low or moderate degrees of variability, such as body weight, but could be insufficient to detect statistically significant differences in endpoints with a higher degree of variability such as hormone level or sperm count, or that occur infrequently such as malformations or tumor formation. These factors can make consistent detection of relatively small changes especially difficult on endpoints that have a high degree of inherent variability.

Lack of statistical or experimental control for litter effects was perhaps the single most common technical shortcoming noted in the developmental toxicity studies evaluated by the CERHR Expert Panel for Bisphenol A *(2)*. Adequate control for litter effects when littermates are used in an experiment is considered essential in developmental toxicology. In 2000, the NTP co-sponsored a workshop with the U.S. Environmental Protection Agency referred to as the "Low Dose Endocrine Disruptors Peer Review." As part of the peer review, a group of statisticians reanalyzed a number of "low" dose studies *(98)*. Based on studies that used littermates, they determined that litter or dam effects were generally present such that pups within a litter were found to respond more similarly than pups from different litters. The overall conclusion on this issue was that "[f]ailure to adjust for litter effects (e.g., to regard littermates as independent observations and thus the individual pup as the experimental unit) can greatly exaggerate the statistical significance of experimental findings." Studies that did not adequately control for litter effects were given less weight in the NTP evaluation and were generally only used as supportive material.

The NTP concurs with the opinion of several scientific panels that positive control groups can be very useful to evaluate the sensitivity and performance of a given experimental model *(2, 60, 98)*. However, the NTP does not consider

use of a positive control to be a required study design component particularly in animal model systems that are well characterized regarding the background incidence of "effects" and their variability. For bisphenol A studies, potent estrogens, such as diethylstilbestrol, ethinyl estradiol, 17β-estradiol, and estradiol benzoate, are the most commonly used positive control chemicals given bisphenol A's historical classification as a weak estrogen. Failure to obtain predicted responses with these chemicals is generally interpreted as a "failed" experiment, perhaps reflecting the selection of a relatively insensitive animal or experimental model or insufficient chemical challenge. Studies where no responses are observed in the positive control group have generally contributed less weight to evaluations of bisphenol A *(2, 60)*. The significance of a "failed" positive control for bisphenol A varies from endpoint to endpoint and reflected more negatively on a study in the NTP evaluation when the predicted effect on reproductive tissue or function was not observed at dose levels that should be sufficiently high to produce an effect. In addition, although potent estrogens are used as positive controls for bisphenol A, as discussed earlier an increasing number of molecular or cell-based studies suggest that interpreting the toxicological effects of bisphenol A solely within the context of their consistency with a classic estrogenic mechanism of action is overly simplistic.

HUMAN STUDIES

Only a very small number of studies have looked at associations between bisphenol A exposure and disorders of reproduction or developmental effects in humans [*(12, 99, 100)*, studies prior to mid-2007 reviewed in *(2, 3)*]. The human studies have looked at the relationship between urine or blood concentrations of total or free bisphenol A and a variety of health measures including levels of certain hormones that help regulate reproduction *(32, 101)*, markers of DNA damage *(102),* miscarriage *(103),* chromosomal defects in fetuses *(104),* fertility and obesity in women *(16, 99, 105)*, effects on the tissue that lines the uterus ("endometrium") *(99, 106)*, polycystic ovary syndrome *(101, 105)*, and birth outcomes and length of gestation *(12, 100)*.

In these studies, there are reports of associations between higher urine or blood concentrations of bisphenol A and lower levels of follicle–stimulating hormone in occupationally exposed men *(32),* higher levels of testosterone in men and women *(101, 105)*, polycystic ovary syndrome *(101, 105)*, recurrent miscarriage *(103),* and chromosomal defects in fetuses *(104).* In addition, one study reported that patients with endometrial cancer and complex endometrial hyperplasia had lower blood levels of bisphenol A than healthy women and women with simple endometrial hyperplasia *(106).* Bisphenol A was not associated with decreased birth weight or several other measures of birth outcome in two recent studies *(12, 100)*. Drawing firm conclusions about potential reproductive or developmental effects of bisphenol A in humans from these studies is difficult because of factors such as small sample size, cross-sectional design, lack of large variations in exposure, or lack of adjustment for potential confounders. However, the CERHR Expert Panel on Bisphenol A *(2)* concluded that several studies collectively suggest hormonal effects of bisphenol A exposure *(32, 101, 105)* including one in occupationally exposed male workers likely exposed through multiple routes including inhalation *(32).*

The NTP concurs with findings of the recent evaluations *(2, 3)* that while these studies may suggest directions for future research, there is currently insufficient evidence to determine if bisphenol A causes or does not cause reproductive toxicity in exposed adults. There is also insufficient evidence from studies in humans to determine if bisphenol A does or does not cause developmental toxicity when exposure occurs prenatally or during infancy and childhood.

LABORATORY ANIMAL STUDIES

In contrast to the limited literature evaluating possible effects of bisphenol A in humans, the scientific literature on the toxic effects of bisphenol A in laboratory animals is extensive and expanding. For example, between February 2007 (the cut-off date for literature included in the CERHR Expert Panel Report on Bisphenol A) and April 11, 2008, more than 400 new articles related to bisphenol A were identified by PubMed search. All new studies related to the potential reproductive and developmental effects of bisphenol A were considered during preparation of the NTP Brief on Bisphenol A. However, only those studies that were considered the most informative for developing NTP conclusions are cited in the Brief. In addition to the new literature cited, many key studies reviewed in the expert panel report are cited herein.

Reproductive Toxicity Studies

The reproductive toxicity studies of bisphenol A include assessment of fertility, sperm counts, estrous cycling, and growth or cellular damage in reproductive tissues. Reproductive toxicity can be studied in animals exposed during adulthood, during development, or both. Conclusions on reproductive toxicity presented in this section of the NTP Brief on Bisphenol A are limited to the assessment of fertility in laboratory animals, regardless of when exposure occurred, and other indicators of reproductive effects in animals exposed only during adulthood. Assessments of aspects of the reproductive system other than fertility in animals exposed during development are discussed under the headings of "High" Dose and "Low" Dose Developmental Toxicity Studies below.

Studies show that bisphenol A does not reduce fertility in laboratory animals exposed in adulthood and/or during development at dose levels up to 500 mg/kg bw/day in rats *(37, 107)*. Fertility may be negatively impacted at higher dietary doses (≥875 mg/kg bw/day) in mice exposed as adults as indicated by a decreased number of litters per breeding pair *(40),* although two multigenerational reproductive toxicity studies did not report effects on fertility in mice at doses up to 1669–1988 mg/kg bw/day *(39, 41)*. There are occasional reports of decreased fertility in smaller sample size studies of rodents exposed to much lower dose levels of bisphenol A during adulthood, such as oral treatment with 0.025 and 0.100 mg/kg bw/day in male mice *(108).* In the Al-Hiyasat *et al.* study, decreased pregnancy rates and increased incidence of resorptions in untreated female mice were attributed to effects in treated adult males, i.e., reductions in the number of testicular or epididymal sperm and hypothesized impaired sperm quality. However, the magnitude of the impact on weight–corrected testicular or epididymal sperm number, ~16 to 37%, is not generally considered severe enough to account for the observed pregnancy rate decrease of ~33 to 40%.[9]

At high oral dose levels, adult exposure to bisphenol A caused reproductive toxicity in the form of altered estrous cycling in female rats (≥600 mg/kg bw/day)[10] *(110)* and cellular effects on the testis of male rats (235 mg/kg bw/day) *(111).* In addition, more subtle effects on maternal behavior, i.e., decreased duration of licking and grooming of pups, are reported at a lower oral dose in treated adult female rats (0.04 mg/kg bw/day) *(112).*

"High" Dose Developmental Toxicity Studies (> 5 mg/kg bw/day)

Results from developmental toxicity studies in mice and rats show adverse effects on pup

[9]Sperm counts in laboratory rodents and rabbits generally have to be severely impacted to cause infertility. Rats may still be fertile with a 90% reduction in sperm count *(109).*

[10]Animals were treated with 1000 mg/kg bw/day for 1-week and then the dose was reduced to 600 mg/kg for 22–25 additional days.

survival and growth following maternal exposure to dose levels of bisphenol A defined by the NTP as "high" (>5 mg/kg bw/day). In rats, a ~20–36% decrease in the number of pups per litter is reported following maternal dosing with ≥500 mg/kg bw/day *(36, 37)*. Increases in fetal death and post-implantation loss are seen in rats treated with 1000 mg/kg bw/day during pregnancy *(36)*. Reductions in fetal weight or growth during postnatal life occur at oral dose levels of ≥300 mg/kg bw/day in rats *(36, 37)*. In mice, developmental toxicity is generally reported at higher oral doses in the form of fetal death, decreased number of live pups, reduced fetal or pup body weight at ≥875 mg/kg bw/day *(38–40)*, and reductions in body weight during postnatal life in the F1 generation (but not the F2 generation) at 600 mg/kg bw/day *(41)*. Fetal death in mice has also been observed in a recent study that reported embryo lethality following subcutaneous dosing with 10 mg/kg bw/day bisphenol A to pregnant mice *(113)*. Occasionally, decreases in pup survival have been reported at much lower oral dose levels, such as 0.0024 mg/kg bw/day in mice *(114)*. However, this effect is not typically reported at oral doses in this range even in studies from the same laboratory using a similar dosing regimen and the same source of mice *(115)*.

Delayed onset of puberty (assessed by day of vaginal opening) has been reported in the female offspring of rats orally treated with bisphenol A at 50 mg/kg bw/day during gestation *(43)* or 500 mg/kg bw/day during gestation and lactation *(37)*. In the study by Tyl *et al. (37),* this effect has been attributed to a decrease in body weight also observed at that dose and has not necessarily been considered a direct developmental effect *(27)*. However, decreased body weight was not observed in females at the dose where delayed vaginal opening was reported by Tinwell *et al. (43)*. This high dose effect of delayed vaginal opening is not the predicted effect of exposure to an estrogenic compound. It is worth noting that Tinwell *et al. (43)* did not detect any difference in onset of puberty in female rats when age at first estrus assessed by vaginal smear was used as the marker of puberty. Other "high" dose studies report no effect on onset of puberty in female rats exposed during gestation and lactation at maternal oral doses ranging from 3.2 to ~1000 mg/kg bw/day *(116–119)*. One "high" dose study reported an accelerated onset of puberty in female rats following subcutaneous injection of bisphenol A during early postnatal life at 105 and 427 mg/kg bw/day *(120)*. Delayed puberty in male rats treated during development has also been reported at oral doses of ≥50 mg/kg bw/day *(37, 42)*. This effect was associated with decreased body weight in the study by Tyl *et al. (37),* but not in the study by Tan *et al. (42)*. A delay in puberty of 1.8 days has also been reported in male mice at 600 mg/kg bw/day in a 2-generation reproductive toxicity study *(41)*.

With the exception of a possible morphological alteration of the urethra (discussed below) *(54),* bisphenol A has not been shown to cause malformations, such as skeletal birth defects or abnormally shaped or absent organs, in rats or mice at oral doses up to 1000 and 1250 mg/kg bw/day, respectively *(36, 38)*. An indication of a possible developmental delay, apparent delayed bone formation ("ossification"), was reported at an oral dose level of 1000 mg/kg bw/day *(36)*. A more subtle effect, cellular changes in the liver, in developmentally exposed animals has been reported at ≥50 mg/kg bw/day *(41)*.

"Low" Dose Developmental Effects (≤5 mg/kg bw/day)

Neural and Behavioral Alterations

The NTP concurs with the CERHR Expert Panel on Bisphenol A that there is a sufficiently consistent body of literature to suggest that perinatal or pubertal exposure to "low" doses of bisphenol A causes neural and behavioral alterations in rats and mice, especially related to the development

of normal sex-based differences between males and females ("sexual dimorphisms" or "sexually dimorphic").

Research on the effects of bisphenol A on the brain and behavior does not have as long a history as the assessment of reproductive tissues, but is now an active area of study that has been growing quickly in the past few years. Currently, the literature is composed of a collection of findings based on behavioral assessments, morphometric and cell-based measurements of the brain of laboratory animals, and *in vitro* studies to identify molecular and cellular targets and mechanisms of action. From these studies, themes are emerging that suggest exposures to bisphenol A can produce a loss or reduction of sexual dimorphisms in non-reproductive behaviors and in certain regions of the brain as well as effects on the dopaminergic system. Neural effects are also implicated from mechanistic studies that show bisphenol A can interfere with thyroid hormone signaling.

Sexual dimorphisms include differences in the size, cellular composition, or molecular expression patterns of specific regions or structures in the brain. The studies detecting bisphenol A-induced changes in sexually dimorphic brain structures generally report a reduction or loss of sexual dimorphisms, for example, in the locus ceruleus (LC; a brain region involved in mediating responses to stress) *(121, 122)*, and the bed nucleus of the stria terminalis (involved in regulating emotional behavior) *(123).* Similar effects are reported in some, but not all, studies *(124–126)* of the anteroventral periventricular nucleus (AVPV), a brain region that provides input to gonadotropin-releasing hormone neurons involved in regulating ovulation. The lowest administered doses delivered to either pregnant dams or neonatal animals associated with these effects range from ~0.03 mg/kg bw/day (oral) *(122),* 0.000025 mg/kg bw/day (subcutaneous mini-pump) *(125)* to ~100 mg/kg bw/day (subcutaneous injection) *(124).* Changes are not reported for all sexually dimorphic structures. One well-known sexually dimorphic structure reportedly not affected even at doses up to 320 mg/kg bw/day in rats is the sexually dimorphic nucleus in the preoptic area (SDN-POA), a brain region that has a homologue in humans and is known to be modified by gonadal hormones during perinatal life *(116, 118, 121, 122, 126, 127)*. Interpreting the potential human health or behavioral significance of effects on sexually dimorphic brain regions can be difficult. For example, the bed nucleus of the stria terminalis is described as being responsive to reproductive hormones and generally involved in regulating emotional behavior *(128),* but the specific functions of this brain region in rats, and therefore the impact of loss of sexual dimorphism, remain unclear.

Effects on behavior have been assessed by a wide variety of experimental tests. Reported behavioral changes in rats or mice relate to play *(129),* maternal behavior *(44, 112)*, aggression *(130, 131)*, cognitive function *(132),* motor activity *(133, 134)*, exploration *(46),* novelty-seeking *(45, 46,135)*, impulsivity *(135),* reward response *(45, 135–137)*, pain response *(138),* anxiety and fear *(46, 48, 50, 139)*, and social interactions *(140).* Many of these behaviors, including activity, anxiety, exploration, and novelty seeking are sexually dimorphic to some degree. The lowest oral dose associated with behavioral changes is 0.01 mg/kg bw/day (via treatment to the pregnant dam) *(44–46)* and a number of behavioral changes have been reported following developmental exposure to oral doses between 0.01 and 1 mg/kg bw/day *(48, 50, 112, 129–132, 135, 138, 140–142).*

With the exception of a study that showed a slight increase in receptive behavior in females and an impairment of sexual performance in males *(130),* the loss of behavioral sexual dimorphisms does not relate to reproductive

behavior *(116, 122, 143)*. For instance, responses to novelty and exploratory behavior are sexually dimorphic behaviors where female mice tend to display more of these behaviors than males *(46, 135)*. Bisphenol A seems to dampen this sex-difference by reducing the expression of these behaviors in female mice ("defeminization" or "masculinization") exposed during development, either through gestation via the dam with oral doses of 0.01 mg/kg bw/day or through gestation until weaning at 0.04 mg/kg bw/day *(46, 135)*.

While a loss of sexual dimorphism seems to be one general trend observed in the behavior literature, findings for other effects can be more difficult to interpret. A number of studies have looked at the relationship between developmental exposure to bisphenol A and increased activity. The studies that most directly support an effect of increased activity administered bisphenol A directly into the brain *(133, 134, 144, 145)*. This route of administration limits the ability to interpret these studies in relation to human exposure levels as well as to compare the findings to results from other studies that use more typical routes of administration. Other studies using similar behavior assessments have not reported differences in spontaneous motor activity in the offspring of dams orally treated with a range of doses from 0.1–400 mg/kg bw/day *(50, 146)*. Indications of increased activity based on other types of behavioral tests are also mixed. Some studies report no impact of bisphenol A treatment on activity *(107, 142, 147)*, increased morphine-induced locomotion in animals treated during development with bisphenol A *(136, 148)*, no difference between control and bisphenol A treated animals in response to methylphenidate, a drug used to treat attention deficit hyperactivity disorder (ADHD) *(147),* and decreased amphetamine-induced activity in bisphenol A-treated male rats *(46)*. The literature provides more consistent support for a loss of sexual dimorphism in locomotor activity. Bisphenol A exposure during development eliminated statistically significant sex differences observed in control animals where females are more active than males *(122, 125)*, or caused significant differences in activity consistent with a loss of sexual dimorphism, i.e., increased activity in male, but not female rats *(149)*.

Certain behavioral effects such as alterations in locomotor activity, reward behavior, response to novelty, motivation, cognition, and attention can display some degree of sexual dimorphism but also implicate involvement of the dopaminergic system, a monoaminergic neurotransmitter. Interactions with the dopaminergic system are supported by findings that bisphenol A can alter the gene expression of D1, D3, and D4 dopamine receptors *(137, 145, 150)* and dopamine transporters *(145, 151, 152)*. In addition, several studies report that perinatal exposure to bisphenol A can alter (usually decrease) expression of the rate–limiting enzyme for dopamine synthesis, tyrosine hydroxylase (TH), that catalyzes the conversion of tyrosine to a pre-cursor of dopamine, dihydroxyphenylalanine (DOPA), in several regions of the brain including the substantia nigra *(145, 153)*, the anteroventral periventricular nucleus of the hypothalamus (AVPV) *(124),* midbrain *(151),* limbic area *(152),* and rostral periventricular preoptic area *(125)*.

Additional support for the brain as a target of bisphenol A is provided by a number of studies that report neural alterations at the cellular level including interactions with or changes in measures of expression of a number of receptors involved in brain function, such as estrogen receptors ERα and ERβ *(47, 154–156)*, gamma-aminobutyric acid type A ($GABA_A$) *(157, 158)*, progesterone *(159, 160)*, aryl hydrocarbon receptor (AhR), retinoic acid receptor (RAR) alpha, retinoid X receptor (RXR) alpha *(161–163)*, and thyroid receptors *(82–86)*. Other studies report effects on neuronal migration or organization *(164, 165)*, synaptogenesis *(166, 167)*, GABA-induced currents *(158),* neuronal cell

death *(168),* synaptic plasticity *(169)*; thyroid receptor-mediated differentiation of oligodendrocytes *(170),* and reduced proliferation of neural progenitor cells *(171).*

The NTP concurs with the CERHR Expert Panel on Bisphenol A that the results of neurological and behavioral studies of exposures of laboratory animals to bisphenol A during development raise questions about possible risks to human development. Although the technical merit of the scientific literature on brain and behavior varies, a number of the "low" dose studies have been considered by various evaluation groups to be experimentally well-conducted.[11] For example, the CERHR Expert Panel on Bisphenol A classified the studies by Kwon *et al. (116),* Negishi *et al. (50),* Della Seta *et al. (49),* Palanza *et al. (44),* and Ryan and Vandenbergh *(48)* as having "high utility" in its evaluation.

The NTP also concurs with the CERHR Expert Panel on Bisphenol A that additional research is needed to more fully assess the functional, long-term impacts of exposures to bisphenol A on the developing brain and behavior. Overall, the current literature cannot yet be fully interpreted for biological or experimental consistency or for relevance to human health.[12] Part of the difficulty for evaluating consistency lies in reconciling findings of different studies that use different experimental designs and different specific behavioral tests to measure the same dimension of behavior.

For some of the reported effects, there is some degree of consistency in the rodent studies *(96).* For example, Rubin *et al. (125)* reported that perinatal exposure to low doses of bisphenol A significantly reduced the number of tyrosine hydroxylase (dopaminergic) neurons in the AVPV of female mice to the values observed in control and bisphenol-treated males. Because the AVPV is involved in regulating ovulation in rodents this finding is consistent with an earlier study by this same group that reported disrupted estrous cyclicity in adult female mice following perinatal exposure to bisphenol A *(173).* However, the manifestation or translation of these effects to primates or humans is unclear because there is no homologous hypothalamic structure to the AVPV in humans.

Another issue that complicates translation of the rodent findings to primates and humans is species differences in the role of estradiol in regulating sexual differentiation of the brain

[11]The studies by Negishi 2004 *et al. (50),* Carr *et al. (132),* Ryan and Vandenberg *(48),* and Adriani *et al. (135)* were described as sufficiently reliable for regulatory use in a minority opinion expressed by Denmark, Sweden, and Norway in the latest European Union risk assessment *(6).* Dr. Michael Baum, an *ad hoc* reviewer in the NTP Board of Scientific Counselors peer review, considered the studies by Ryan and Vandenberg *(48),* Gioiosa *et al. (46),* and Rubin *et al. (125)* to be exceptionally well-conducted.

[12]The following "low" dose studies were cited in the "Characterization of Risk to Human Health" section of the Health Canada Draft Screening Assessment for Bisphenol A *(172)*: Palanza *et al.* 2002 *(44),* Laviola *et al.* 2005 *(45),* Gioiosa *et al.* 2007 *(46),* Farabollini *et al.* 2002 *(130),* Della Seta *et al.* 2005 *(112),* Adriani *et al.* 2003 *(135),* Negishi *et al.* 2004 *(50),* and Carr *et al.* 2003 *(132).* From these studies, Health Canada concluded that "While collectively these studies provide evidence that exposure to bisphenol A during gestation and early postnatal life may be affecting neural development and some aspects of behaviour in rodents, the overall weight of evidence was considered limited from the perspective of rigour (e.g., study design limitations such as conduct of behavioural assessments at a single time point); power (e.g., limited number of animals per test group), corroboration/consistency (limited consistency of studies) and biological plausibility (e.g., certain studies involve use of a single dose, lack of dose response relationship). These limitations make it difficult to determine actual significance of findings to human health risk assessment."

(96). In brief, estradiol has a clearer role in regulating male-typical brain and behavioral sexual differentiation in rodents compared to primates and humans. The sexual dimorphism of a number of the neural and behavioral endpoints affected by bisphenol A exposure, i.e., AVPV and LC volume/cell number, locomotor activity, exploration in the plus maze, have been shown to depend on estradiol formed perinatally in the male rodent brain via aromatization of testosterone secreted from the fetal and neonatal testes. To the extent that these effects of bisphenol A are due to its interactions with the estrogen receptor the translation of these findings to humans is not clear because there is currently no evidence that estrogen receptor signaling plays an essential role in male-typical brain and behavioral sexual differentiation in primates including humans *(96)*.

However, as discussed previously a number of studies suggest that bisphenol A may also exert biological effects by mechanisms that do not involve estrogen receptor binding. For this reason, future neural and behavioral studies of bisphenol A should not focus exclusively on estrogen receptor-mediated endpoints. For example, a number of male-typical brain and behavioral sexual dimorphisms in rodents depend on androgen receptor signaling, i.e., spinal nucleus of the bulbocavernosus, the posterodorsal medial amygdalar nucleus, the ventromedial hypothalamic nucleus, the suprachiasmatic nucleus, forebrain response to pheromones, play fighting and the preference of males to seek out female vs same-sex (male) urinary odors. These androgen receptor-mediated behaviors are considered to have more direct relevance to humans and should be more thoroughly assessed following bisphenol A exposure given suggestions that bisphenol A can modulate androgen receptor activity or expression levels.

In addition, several studies suggest that bisphenol A may interfere with the dopaminergic system. The CERHR Expert Panel on Bisphenol A did not consider most of these studies useful in the evaluation by due to experimental design limitations and/or use of a non-oral route of administration. Additional research that includes assessment of dopaminergic-related endpoints to address the limitations in the current literature would be helpful.

Future studies should also take precautions to distinguish between "organizational" and "activational" effects of hormones. Organizational effects are permanent and induced by hormones during perinatal life whereas activational effects are acute, generally reversible, and occur throughout life *(174)*. Many sexual and other behaviors reflect both organizational and activational influences of hormones *(175)*. Observed behavioral effects of perinatal bisphenol A could reflect organizational changes in the brain accomplished during the normal perinatal period of brain sexual differentiation or, alternatively, they could simply reflect group differences in plasma levels of circulating sex hormones at the time of adult testing. Only a small number of the studies in the bisphenol A literature controlled for the influence of differences in plasma levels of circulating sex hormones at the time of testing, for example by testing ovarectomized females, using females at the same stage of the estrous-cycle, or conducting assessments on pre-pubertal animals *(46, 48, 125)*.

Mammary Gland

There is evidence from rodent studies suggesting that perinatal exposure to bisphenol A via subcutaneous mini-pump at administered doses of 0.0025 to 1 mg/kg bw/day causes tissue changes ("lesions") in the mammary gland that may signal an increased susceptibility to develop mammary gland tumors later in life *(52, 53)*. Although these lesions have been described as preneoplastic, currently no data are available that assess whether the reported lesions progress to invasive carcinoma. For this reason, the evidence is not sufficient to conclude that bisphenol A is a rodent mammary

gland carcinogen or that bisphenol A presents a breast cancer hazard to humans.

While bisphenol A has not been shown to cause cellular changes or cancer of the mammary gland in female rats and mice exposed as adults *(176),* two recent studies suggest that exposure of rats to bisphenol A during gestation may lead to the development of "preneoplastic" lesions in adulthood, ductal hyperplasia and carcinoma *in situ*, that may potentially progress to tumors *(52, 53)*.

[*Technical comment:* During preparation and peer review of the draft NTP Brief on Bisphenol A a number of pathologists questioned the classification of the lesions with cribriform-like structures described as carcinoma *in situ* in the studies by Murray *et al.* and Durando *et al.* In addition, the degree of hyperplasia reported in these studies was described during the peer review as being of a relatively mild form and not necessarily of the type that presents the most concern for development of invasive breast cancer in women, i.e., focal areas of atypical hyperplasia (see the Peer Review Report for Bisphenol A *(96)* for additional discussion)].

In the study by Murray *et al. (53),* rats were treated with 0.0025 – 1 mg/kg bw/day bisphenol A during pregnancy by subcutaneous mini-pump. Significant increases in the incidence of hyperplastic ducts were reported in all dose groups of female offspring on postnatal day 50 and only in the lowest dose group of 0.0025 mg/kg bw/day on postnatal day 95 (sample sizes range from 4 – 6). Lesions described as carcinoma *in situ* were reported in female offspring in the 0.25 and 1 mg/kg bw/day groups on postnatal day 50 (25% incidence for both treatment groups) and postnatal day 95 (33% incidence for both treatment groups). These findings are supported by a study by Durando *et al. (52)*[13] where pregnant rats were treated with 0.025 mg/kg bw/day, again using a subcutaneous mini-pump. In this study, the percent of hyperplastic ducts was significantly increased in the female offspring at both postnatal days 110 and 180 (~2 – 5-fold). A non-significant increase in the incidence of ductal carcinoma *in situ* was noted following adult treatment with a subcarcinogenic dose of N-nitroso-N-methylurea, a chemical used in cancer research to assess susceptibility to carcinogens (2/15 compared to 0/10 in control animals).

These findings are generally consistent with other reports of changes in mammary gland growth and development following perinatal exposure to bisphenol A that are related to an altered rate of maturation, e.g., advanced fat pad maturation, delayed lumen formation, enhanced duct growth, adoption of a pregnancy-like state, enhanced responsiveness to secondary estrogenic exposures, and potentially increased susceptibility to carcinogenesis, e.g., increased number or density of terminal end buds and ducts *(52, 53, 177 – 183)*. These findings have been interpreted by some authors as indicating that developmental exposure to bisphenol A causes effects on breast tissue maturation that may lead to a predisposition to disease onset later in life *(52, 53, 181 – 183, 191)*.

[13]The study by Durando *et al. (52)* implied that 99.9% DMSO was used in the mini-pump ["Pumps are designed to deliver 25 bisphenol A (Sigma-Aldrich de Argentina S.A., Buenos Aires, Argentina) or only DMSO (99.9% molecular biology grade, Sigma-Aldrich de Argentina S.A.)"]. The manufacturer of the mini-pump does not recommend use of DMSO concentrations greater than 50% because it can degrade the pump reservoir material and potentially result in tissue inflammation and edema. For this reason, the CERHR Expert Panel on Bisphenol A considered this study critically flawed *(2).* The NTP concurs that use of a high concentration of DMSO is a technical short-coming, but is not convinced that this factor could account for the observed results. The NTP also considered the possibility that potential pump degradation could result in variations in administered dose, but concluded that the study was still useful to consider in the context of other findings.

With the exception of an oral dosing study conducted by Moral *et al. (183)* that reported an increased number of mammary gland terminal ducts in the female offspring of rats treated during gestation with 0.250 mg/kg/day, the cellular and tissue-level effects on the mammary gland occurred in studies where bisphenol A was administered by subcutaneous treatment via mini-pump at doses of 0.000025 to 10 mg/kg/day *(52, 53, 177, 179–182)*.

Certain aspects of mammary gland cancer differ between rats and humans, e.g., metastases are uncommon in rodents, but ductal hyperplasia and carcinoma *in situ*, are generally recognized as intermediary steps in chemical-induced mammary gland cancer in the rat and as preneoplastic lesions in the human *(184–187)*. The appearance of ductal hyperplasia and carcinoma *in situ* are similar enough between rats and humans that these findings in the rat are considered relevant to humans *(185)*. In humans, a greater than mild degree of ductal hyperplasia and ductal carcinoma *in situ* are associated with increased relative risk of developing invasive breast carcinoma. It is important to note that the development of these lesions does not guarantee the formation of tumors or cancer in rats or humans and they are most appropriately interpreted as risk factors. If similar changes occur in women, the increased relative risks for developing invasive breast cancer range from 1.5 to 5-fold for moderate and atypical ductal hyperplasia and 8.0 to 10.0-fold for ductal carcinoma *in situ (188)*. The relative risk is based on a comparison to women of the same age in the general population. For example, a 50-year old woman has a 1 in 39 chance of developing invasive breast cancer in the next 10 years. If a 50-year woman has atypical ductal hyperplasia, a form of ductal hyperplasia associated with a moderate level of increased relative risk (4 to 5-fold), then her chance of developing invasive breast cancer in the next 10 years increases to approximately 1 in 10 to 1 in 8.

The current literature is not sufficient to establish the reproducibility of the ductal lesion findings by multiple independent investigators. Bisphenol A was not shown to induce neoplastic or non-neoplastic lesions in the mammary gland of female rats (~74 and 135 mg/kg bw/day) or mice (650 and 1300 mg/kg bw/day) in two-year dietary cancer bioassays where exposure was initiated in young adult animals (5-weeks of age) *(176)*. However, these studies did not include perinatal exposure and the NTP recognizes that adult-only exposure may not be sufficient to detect chemical carcinogens in hormonally-responsive tissues such as the mammary gland *(187)*. Most of the toxicology studies of bisphenol A that included assessment of females following developmental exposure either (1) did not report examination of the mammary gland *(37, 43, 120, 189, 190)*, or (2) collected mammary gland tissue but did not prepare the tissue in a manner that would readily reveal these changes, i.e., whole mounts *(41, 107)*. The limited assessment of the mammary gland in these studies is critical because it is not clear that, if present, intraductal epithelial proliferations would have been detected during the routine histopathologic examinations. More severe mammary lesions were not reported in these studies. Although severe lesions or tumors could be detected during routine necropsy, the studies by Ema *et al. (107)* and Tyl *et al. (41)* were primarily designed to detect effects on reproduction and development and not tumor incidence. Animals were not followed up for a sufficiently long period of time to necessarily expect to observe tumors in control animals or differences in tumor incidence between treatment groups. In both of these studies, mammary gland tissues in the parental (F0) and F1 generations of females were only examined after weaning of their pups and the animals would have been well under one year of age at the time of tissue collection.

The NTP concurs with recent reviews *(2, 191)* that additional data are needed to more completely

understand the possible long-term consequences of disrupting mammary gland development in animals by bisphenol A exposure and its significance for human health. Namely, long-term follow-up studies with sufficient statistical power should be conducted to evaluate if the ductal hyperplasia and carcinoma *in situ* progress to mammary gland tumors, preferably without the use of a secondary chemical challenge in adulthood. In addition, conducting the appropriate pharmacokinetic studies to better understand the distribution of bisphenol A to target tissues with the subcutaneous mini-pump would aid in interpreting the results. While researchers predict that circulating levels of total and free bisphenol A in the subcutaneous mini-pump studies would be quite low based on the administered dose (≤ 1 mg/kg bw/day), the lack of supporting pharmacokinetic information limits the ability to make comparisons to human exposures.

Prostate Gland and Urinary Tract

There is some evidence that perinatal exposure to bisphenol A in rodents may alter prostate gland and urinary tract development and predispose the prostate to develop hormonally-induced pre-neoplastic lesions later in life. The evidence is not sufficient to conclude that bisphenol A is a rodent prostate gland carcinogen or that bisphenol A presents a prostate cancer hazard to humans.

In mice, exposure of pregnant dams to bisphenol A at an oral dose of 0.010 mg/kg bw/day has been shown in one study to alter prostate development in offspring by increasing the number of prostatic ducts, ductal volume, and the proliferation of a cell population implicated in the development of prostate cancer (basal epithelial cells) in one or more regions of the prostate *(54, 192)*. This study also reported a urinary tract deformation where the urethra narrows near the neck of the bladder, an effect that, if permanent, could contribute to urine flow disorders. These effects were observed in fetal mice and it is unclear if they persist into adulthood or relate to a clear adverse health outcome. It is important to note that other studies have not reported severe consequences of urinary tract constriction in adult animals exposed during development that might be predicted based on the finding by Timms *et al.* including bladder stones, hydronephrosis, hydroureter, or other indications of kidney toxicity.

In Sprague-Dawley rats, subcutaneous injection of neonates with 0.010 mg/kg bisphenol A followed by adult hormone treatment[14] was reported to cause 100% of the animals to develop "low" grade (3/10 animals) or "high" grade (7/10 animals) prostate intraepithelial neoplasia *(51)*.[15, 16] The incidence of prostate intraepithelial neoplastic (PIN) lesions in animals that did not receive the adult hormone

[14]Animals were given Silastic capsule implants packed with estradiol and testosterone that result in serum concentrations of ~75 pg/ml estradiol and 3 ng/ml testosterone. This hormone treatment is intended to mimic the ratio of estradiol to testosterone in the aging male.

[15]The classification scheme of "low" and "high" grade PIN lesions used by Ho *et al.* (2006) appears to be their own *(96)*.

[16]One other study assessed bisphenol A's ability to predispose the prostate to develop prostate intraepithelial neoplasia lesions and tumors *(193)*. In this study, female F344 rats were orally dosed with 0.05, 7.5, 30, or 120 mg/kg bw/day of bisphenol A during pregnancy and lactation. In order to induce prostate lesions and tumors, male offspring were treated with a chemical carcinogen, 3,2'-dimethyl-4-aminobiphenyl (DMAB). No statistically significant changes in prostate intraepithelial neoplasia lesions or carcinomas were observed. Differences between this study and the report of Ho *et al.* may be related to age at exposure (fetal versus neonatal and fetal), rat strain (F344 versus Sprague–Dawley), carcinogenic insult (DMAB versus estradiol+testosterone), route of administration (subcutaneous versus oral to dams), or other factor such as animal husbandry and housing.

treatment was not significantly different from controls (2/6 versus 1/9 in control animals). Proposed biological mechanisms to account for the effects of bisphenol A on the prostate include an epigenetic mode of action exemplified as altered DNA methylation patterns in genes that help regulate prostate development and growth as an epigenetic mode of action *(51, 194)*. PIN lesions in the male rodent have similar histopathology to PIN lesions in men, and evidence of high grade PIN lesions in men is considered a risk factor for developing prostate cancer *(96).* The use of adult hormone treatment to promote the development of prostate intraepithelial neoplasia lesions complicates the interpretation of this study when considering its relevance to human bisphenol A exposure. However, as discussed in more detail below, rodents are normally resistent to developing prostate cancer and the use of hormone treatment, chemical treatment, or other alternative animal model to obtain a more sensitive rodent model is considered an acceptable and recommended strategy in prostate cancer research *(187).*

The findings of Ho *et al. (51)* are consistent with a recent report of increased expression of cytokeratin 10 (CK10), a cell-marker associated with squamous differentiation, in adult male offspring of pregnant mice orally treated with 0.020 mg/kg bw/day bisphenol A during gestation *(195).* Chronic exposure to high doses of potent estrogens, such as diethylstilbestrol, leads to squamous metaplasia of the prostate, a tissue change characterized by a multilayering of prostatic basal epithelial cells. Squamous metaplasia is associated with benign prostatic hyperplasia or long-term estrogen treatment in patients with benign or malignant prostatic disease. The induction of CK10 expression in basal epithelial cells is an early indicator of changes leading to estrogen-induced squamous metaplasia. While the long-term health consequences of such an alteration are unclear, prostatic basal epithelial cells are important for normal growth and development and are implicated in the initiation and early progression of prostate cancer due to their function in maintaining ductal integrity and regulating the differentiation of luminal epithelial cell differentiation *(192).* It is important to note that prostates in the Ogural *et al.* study appeared morphologically the same as control animals based on the staining technique normally used in pathology (hematoxylin and eosin, or H&E). A stain specific for squamous keratin was required to detect the change. Thus, it is unclear whether similar changes in basal epithelial cell phenotype were present in other studies that evaluated the prostate using only an H&E stain.

The NTP concurs with the CERHR Expert Panel on Bisphenol A *(2)* and another recent evaluation *(191)* that additional studies are needed to understand the effects of bisphenol A on the development of the prostate gland and urinary tract. Studies should attempt to confirm these findings and include longer periods of follow-up to understand the significance of the structural and cellular effects observed in fetuses and to clarify the relevance of prostate intraepithelial neoplastic lesions resulting from bisphenol A exposure to the development of prostate cancer in these animals. Future research to clarify the role of bisphenol A in the development of prostate cancer presents a scientific challenge. Unlike humans where prostate cancer is common, it is the most common non-skin cancer in American men *(187),* rodents rarely develop prostate cancer. Of the almost 4,550 rats and mice used as controls in NTP 2-year inhalation or feed studies conducted during the last decade, only 1 cancerous tumor and 17 benign tumors ("adenoma") of the prostate gland were detected *(187).* No substances, including bisphenol A *(176),* have been identified as causing prostate tumors in NTP studies *(187).* The NTP has long recognized the limits of the traditional rodent cancer bioassay for detecting chemical-induced-prostate tumors and organized a workshop in May 2006 to address this issue *(187).* Suggested

strategies to improve the sensitivity of rodent models for detecting prostate cancer included using alternative models, e.g., genetically modified, and/or initiating exposure in perinatal life. In addition, NTP workshop participants suggested a more detailed histopathologic evaluation of the prostate because the assessment of human carcinogenic potential may be better determined based on chemical-induced preneoplastic changes rather than tumor incidence.

During its evaluation of bisphenol A exposure and prostate development, the NTP also considered a number of studies in rats or mice that have detected increased prostate weight at low doses *(115, 196)* or failed to detect this effect *(37, 41, 43, 107, 116, 122, 193, 197–201)*. Prostate weight effects have taken on a special significance in the controversy surrounding bisphenol A because elevated prostate weight was the first "low" dose finding reported in laboratory animals *(115)* and prompted numerous follow-up studies. Attempts to understand the basis for discordant findings has generated considerable scientific discussion and debate including their review at the NTP Low-Dose Peer Review workshop mentioned earlier *(97)*. In brief, the NTP believes that the overall conclusions of the Bisphenol A Subpanel of the NTP Low-Dose Peer Review remain valid with respect to "low" dose effects on prostate weight, i.e., increased prostate weight cannot be considered a general or reproducible finding.

More importantly, it is not clear that prostate weight should continue to be considered a critical endpoint in risk evaluations of bisphenol A given the relative crudeness of this measure. Changes in organ weight may be useful to identify potential target tissues, but become less important when additional data relating to structural, cellular, or functional integrity are available. Prostate enlargement does not correlate with the development of prostate histopathology or cancer in rodents, and the evaluation of prostate weight without corresponding assessment of histopathologic changes is not considered useful for determining carcinogenic potential *(202)*.

In addition, changes in prostate weight are not necessarily observed in the same bisphenol A studies that report prostatic cellular or tissue-level changes. For example, no effects on prostatic lobe weight were observed in studies that reported (1) increased incidence and susceptibility to develop prostate intraepithelial neoplastic lesions *(51)*, (2) changes in the prostatic periductal stroma and decreases in androgen-receptor positive stromal cells and epithelial cells positive for prostatic acid phosphatase (PAS), an enzyme produced by the prostate that can be found in higher amounts in men with prostate cancer *(203)*, and (3) increased expression of CK10 in adult mice exposed as fetuses to 0.020 mg/kg bw/day via treatment of the dam or during adulthood to high doses of bisphenol A (2–200 mg pellets implanted under the skin for 3-weeks) *(195)*.

Puberty and Sexual Maturation

NTP concurs with the CERHR Expert Panel on Bisphenol A that limited data are available at low doses to suggest an effect of accelerating the onset of puberty in female mice. In humans, early onset of puberty in girls is associated with elevated risk of developing breast cancer, early bone age maturation, and psychosocial impacts that include influencing age at first sexual intercourse and increasing risk for certain adolescent risk behaviors *(204–206)*. Depending on the magnitude of the effect, early onset of puberty in laboratory animals can be considered an "adverse" effect in reproductive toxicology *(204)*.

The consistency of the literature on "low" dose effects of bisphenol A related to puberty in female rodents is different in rats and mice. Of the eight studies in rats evaluated by the NTP, seven were interpreted as being "negative" *(37, 43, 53, 107, 122, 173, 207)* and one study was

considered "positive" *(52)*. Overall, the NTP considered the rat data to indicate that "low" doses of bisphenol A do not affect the onset of puberty in female rats. A total of six "low" dose mouse studies were identified and evaluated by the NTP. Of these, two reported effects consistent with accelerated puberty and one showed an alteration in events related to sexual maturation in females and were interpreted as "positive" and the other three were considered "negative."

The NTP strategy for evaluating the entire "low" dose literature on puberty in female rodents was to first conduct a detailed assessment of the mouse studies to determine whether any aspect of study design could account for the apparently contradictory results in this species. The most consistent difference between the "positive" and "negative" studies in mice is the approach used to measure a puberty-related event. Age at first estrus is the most accurate indicator of puberty in rodents. This occurs at the same time as vaginal opening in rats. However, in mice, vaginal opening does not correlate well with puberty and the first day of detecting cornified cells in a vaginal smear, a sign of first estrus, is the preferred measure used to indicate the onset of puberty *(208)*. Although accelerated vaginal opening is an expected response to estrogens in mice, the lack of simultaneous occurrence of vaginal opening and first estrus suggests that these events may be differentially regulated even though they are both estrogen responsive. Thus, the NTP considers vaginal opening to be a marker of sexual maturation, but not a surrogate measure of puberty, i.e., first estrus. The three mouse studies that reported effects consistent with an acceleration of a puberty-related event used first estrus as the marker of puberty *(48, 55, 189)*. In contrast, the "negative" studies used vaginal opening *(41, 178, 197)*. Each study also has its own limitation that complicates a straight-forward interpretation of the results, e.g., small sample size, positive control response, or use of subcutaneous injection to pregnant dams. Based on the analysis outlined below, the NTP concluded that the "positive" mouse studies provided limited evidence of an acceleration of puberty.

In contrast, the NTP concluded that the rat studies do not indicate an effect on puberty at "low" doses. The differences in outcomes cannot be attributed to the use of an insensitive strain or stock because a variety of rat models were used in the "negative" studies: Sprague-Dawley, Wistar, Wistar-Furth rats, Wistar-derived Alderley Park, CD, and Donryu. Moreover, three of the "negative" rat puberty studies reported other "low" dose effects *(53, 122, 173)*. The effects of bisphenol A on puberty in rats at "high" doses are more inconsistent than the "low" dose studies. Only one study has reported an effect on puberty in the predicted direction, i.e., acceleration following subcutaneous treatment on postnatal days 0 to 9 *(120)*. Other studies reported no effect *(116–119)* or a delay in puberty at ≥ 50 mg/kg bw/day *(37, 43)*. Four of these studies used a positive control group *(43, 116, 118, 120)*. In these studies, responses to potent estrogens based on age at vaginal opening ranged from no effect *(116)*, to a statistically significant small or moderate acceleration [1.7 days *(43)*; 2.4 days *(120)*; 3.6 days *(118, 119)*].

Mouse Studies

"Positive" Mouse Studies

- The largest magnitude of effect on puberty in female mice was reported by Ryan *et al.* *(48)*. In this study, age at first estrus was accelerated by 4.5 days in C57BL/6 mice whose dams were orally dosed with 0.2 mg/kg/day bisphenol A during gestation and lactation (GD3–PND21) *(48)*. Acceleration in puberty of approximately 6 days was reported in the ethinyl estradiol positive control group. The major limitation of this study was the relatively small sample sizes used for this endpoint (4–5 dams per treatment group).

- Howdeshell et *al. (55)* reported that female CF-1 offspring of dams orally-treated with 0.0024 mg/kg/day of bisphenol A during gestation (GD11–17) had a 2.5 day shorter interval between age at vaginal opening and first estrus. This study also evaluated whether fetal response to prenatal bisphenol A treatment differed based on intrauterine position (IUP). In some rodent species, females surrounded by 2 females *in utero* (0 male or 0M) have higher serum concentrations of estradiol and lower serum concentrations of testosterone compared to females surrounded by 2 males (2M). Intrauterine position effects have been reported for some behaviors or physiological characteristics, mostly in mice but also other rodents and swine *(209)*. The shorter interval of ~2.5 days between vaginal opening and first estrus in control and bisphenol A-treated animals was primarily attributed to a 5-day shortening of this interval in the 0M females leading the authors to hypothesize that animals with higher background exposures to estradiol may be more sensitive to bisphenol A exposure. The most significant limitation of this study is the interpretation of the interval between vaginal opening and first estrus. No effect was observed on age at first estrus in bisphenol A-treated mice[17] or age at vaginal opening. A shortening of the interval between these two events should not be interpreted as an acceleration in the onset of puberty and is more appropriately characterized as a alteration in the timing of events related to sexual maturation.

- The findings of Ryan *et al. (48)* and Howdeshell *et al. (55)* are supported by a subcutaneous injection study that noted a statistically significant 1-day earlier onset of first estrus and vaginal opening in female ICR/Jcl mice whose mothers were treated with 0.02 mg/kg bw/day bisphenol A during gestation (GD11–17) *(189)*. Although the reported magnitude of the effect in the bisphenol A-treated animals was small, ~1-day, the authors also reported that females in both the 0.002 and 0.02 treatment groups had significantly longer estrous cycles (a "classic" estrogenic response) compared to control animals. Accelerations in vaginal opening and puberty and lengthened estrous cycles were also observed in the diethylstilbestrol positive control groups. The interpretation of this study is limited by the small magnitude of an effect on age at first estrus and use of subcutaneous injection as the route of administration to pregnant dams.

"Negative" Mouse Studies

- Ashby *et al. (197)* did not detect an effect of bisphenol A on vaginal opening in the female offspring of CF-1 mice dosed orally with 0.002 or 0.02 mg/kg bw/day. The interpretation of this study is complicated by the response in the positive control group where diethylstilbestrol (0.0002 mg/kg bw/day) caused a significant 3.6-day delay in the age of vaginal opening compared to the vehicle control group. A delay in puberty is inconsistent with the predicted estrogenic effect of accelerated puberty for the diethylstilbestrol group.

- Markey *et al. (178)* did not report an effect of bisphenol A on age at vaginal opening in female CD-1 mice whose dams were dosed with 0.000025 or 0.00025 mg/kg bw/day via subcutaneous mini-pump. The authors noted that a portion of the mice showed partial vaginal opening approximately 4 days earlier than control animals. In addition, both bisphenol A groups had longer estrous cycles than control animals.

[17] Age at first estrus was significantly accelerated for 0M females versus controls in post hoc analyses (personal communication Frederick vom Saal, August 13, 2008)

- Tyl *et al.* (41) reported no effect of bisphenol A on age at vaginal opening in CD-1 mice using a multigenerational study design at dietary doses that ranged from 0.003 to 600 mg/kg bw/day. The positive control group used in the bisphenol A study, 17β-estradiol (0.08 mg/kg bw/day) caused the expected effect of accelerated vaginal opening. However, the experimental model used in this study did not appear to be sensitive in detecting estrogenic effects at low doses. In a separate multigenerational study designed to characterize the response in CD-1 mice to 17β-estradiol (0.0002 – 0.1 mg/kg bw/day), the authors did not report any effect on vaginal opening at doses below ~0.03 mg/kg/day *(210)*. In addition, estrous cycle length was unaffected at all doses. Thus, detecting an estrogenic effect of a weaker estrogen such as bisphenol A at very low doses (≤ ~0.03 mg/kg bw/day) would not be expected in this animal model.

Species Differences

There are other indications of species differences between rats and mice that may contribute to the inconsistent literature for bisphenol A. Research on the effects of pheromones in regulating puberty suggests that puberty may be more easily perturbed in mice compared to rats. Puberty in female mice can be accelerated when the mice are exposed to urine from a male. This effect has been reported more often and more consistently for mice than for rats *(211, 212)*. In addition, the IUP effect, which was an important factor in the Howdeshell *et al.* study, is better documented in mice compared to rats suggesting that this effect may be more robust in mice *(209)*. IUP was not considered in other studies in the bisphenol A literature, but it is worth noting that the studies by Markey *et al.* *(178)* and Ryan *et al.* *(48)* also show indications of a subpopulation of mice that may be more responsive to bisphenol A. As discussed earlier, Markey *et al.* commented that a portion of mice exhibited partial vaginal opening ~4 days earlier than control animals. In the Ryan *et al.* study, two of the five animals in the 0.200 mg/kg bw/day group that had a ~4.5 day statistically significant acceleration in puberty showed a much greater acceleration than the other animals in that group, i.e., ~10 days early onset of first estrus compared to the mean of the control group (public comment on the draft NTP Brief from Dr. Earl Gray, received May 23, 2008).

Other Effects Considered

A variety of other effects in laboratory animals have been linked to "low" dose bisphenol A exposure during development, including decreased sperm quantity or quality, obesity, disruption of meiosis, changes in reproductive hormone levels, or cellular effects in reproductive tissues. These effects had less impact in shaping NTP's conclusions on potential risks to humans from bisphenol A exposure than the developmental effects observed at "high" doses on survival and growth and the "low" dose effects on brain and behavior, mammary gland, prostate gland, and onset of puberty in females described above.

In some cases, the relationship between a specific cellular- or tissue-level finding and a potential health effect in the whole organism is unclear. This is because there is often uncertainty about the functional impact of a cellular or mechanistic finding, such as the altered level of a receptor protein or change in enzyme activity. For example, the potential health impact that may result from uterine changes characterized by altered ERα and ERβ expression and from an increase in the number and appearance of uterine epithelial cells is unclear *(213)*.

In other cases, the literature is not sufficiently developed. Newbold *et al.* *(214)* recently described a number of morphological changes in the ovaries and uteri of 18-month old mice that had received subcutaneous injections of bisphenol A at doses of 10, 100, or 1000 μg/kg on days 1 – 5 of life. Increases in cystic ovaries

and cystic endometrial hyperplasia were statistically significant in the 100 µg/kg dose group but not at 1000 µg/kg. Non-statistically significant increases in the incidence of a variety of other ovarian and uterine proliferative lesions and cysts were also reported. Replication of these findings and further study of the linkage of early and late occurring events will be important in establishing a better understanding of any long-term consequences of exposures of the developing organism to bisphenol A.

As mentioned earlier, NTP Briefs are not meant to serve as comprehensive reviews of the scientific literature. Only key study findings and issues that relate to NTP conclusions on concerns for potential reproductive and developmental health effects in humans are typically presented. However, three reported "low" dose health effects (obesity, decreased sperm count or quality, and abnormalities of meiosis) that ultimately had less impact in determining the NTP's conclusions are briefly discussed below in order to illustrate the interpretive challenges associated with this literature. Two examples of such effects, obesity and impacts on sperm, demonstrate findings that are not reported consistently enough to be considered reproducible. The third example relates to abnormalities of meiosis and is presented to demonstrate that effects predicted from *in vitro* studies are not necessarily observed in the *in vivo* studies.

Obesity

There is currently insufficient evidence to conclude that bisphenol A exposure during development predisposes laboratory animals to develop obesity or metabolic diseases such as diabetes, later in life. Obesity and metabolic disruption have become a research focus for bisphenol A based on several reports of increased postnatal growth following "low" dose exposure during development and several *in vitro* and *in vivo* studies that report effects related to altered carbohydrate and lipid regulation.

The NTP concurs with the CERHR Expert Panel on Bisphenol A that the effects of bisphenol A on body weight at "low" doses are inconsistent *(2)*. A number of studies in rats and mice report increases in postnatal growth following developmental exposure to bisphenol A at oral doses of 0.0024 – 1.2 mg/kg bw/day *(55, 155, 173, 215)* or a subcutaneous dose of 0.5 mg/kg bw/day *(179)*. Other "low" dose (≤ 5 mg/kg bw/day) studies in rats and mice have either not detected any significant effect on body weight *(43, 48, 50, 52, 107, 116, 122, 200, 214, 216)* or reported growth reductions *(37, 115, 146, 189, 217)*. Differences in study outcomes cannot easily be attributed to the use of a potentially insensitive rodent model or experimental protocol because several studies that did not detect any significant difference in body weight reported other effects at "low" dose levels *(48, 50, 52, 122, 217)*. The bases for the inconsistent findings are unclear, but may relate to factors such as diet and differences in experimental design or analysis.

The data are currently too limited to conclude that developmental exposure to bisphenol A causes diabetes or other metabolic disorders later in life. Two studies in laboratory animals have assessed endpoints related to carbohydrate or lipid regulation. In adult male mice, a single subcutaneous dose of 0.010 or 0.100 mg/kg bw/day bisphenol A caused decreased blood glucose and increased plasma insulin *(218)*. Additionally, increased pancreatic insulin content and insulin resistance were reported at 0.100 mg/kg bw/day (administered orally or by subcutaneous injection) after a slightly longer period of dosing (4-days) *(218)*. A recent study by Miyawaki *et al. (215)* assessed a variety of endpoints related to carbohydrate and lipid regulation in 1-month old mice that were exposed through maternal treatment during gestation and lactation with 0.001 or 0.010 µg/ml bisphenol A in drinking water (~0.26 and 2.42 mg/kg bw/during gestation). Endpoints included body weight, adipose tissue weight, and blood concentrations of leptin,

total cholesterol, triglycerides, non-esterified fatty acid and glucose. Body weight and total cholesterol were significantly increased in female offspring in both dose groups although adipose tissue weight and leptin levels were only significantly increased in the 1 µg/ml treatment group. Male offspring in the high dose group of 10 µg/ml were significantly heavier and had increased adipose tissue weight. Leptin levels were not associated with either of these effects in males. Significantly increased triglycerides and non-esterified fatty acid and decreased glucose were observed in male offspring in the low dose group of 1 µg/ml. Although this study addresses the hypothesis that developmental exposure to bisphenol A can affect carbohydrate and lipid metabolism in postnatal life, the inconsistent pattern of effects on serum lipid levels, leptin, and glucose and lack of control for litter effects[18] makes the study on its own insufficient to draw any conclusion.

More research in this area is warranted. Several *in vitro* studies report effects of bisphenol A related to carbohydrate and lipid regulation including effects on pancreatic cells that govern the release of insulin (β-cells) and glucagon (α-cells), altered differentiation of fibroblast cells into adipocytes, and altered glucose transport in adipocytes *(219–223)*. Some of the effects on pancreatic cells are very rapid, e.g., altered frequency of glucose-induced calcium oscillations in α- and β-cells, activation of cAMP response element binding protein, and appear to be mediated by ncmER *(63, 65, 224)*. Effects mediated through the ncmER are of interest because bisphenol A has been shown to activate this receptor *in vitro* at a concentration of 1 nM, which is similar to the active concentration of diethylstilbestrol *(63, 65)*.

[18]16–25 males or females were reported for each treatment group however these animals were derived from only 3 litters per treatment group *(215)*.

Decreased Sperm Count and Sperm Quality

There is currently insufficient evidence to conclude that bisphenol A exposure during development or adulthood causes decreased sperm count or sperm quality. A large number of studies have addressed this issue but the literature is inconsistent and not easily reconciled.

Exposure during development

There are some indications that treatment with "high" oral doses of bisphenol A during development or young adulthood can impact sperm quantity in laboratory rats *(37, 42, 43)*. Tan *et al. (42)* reported that 33% of rats did not show any evidence of having a spermatogenic cycle after treatment in young adulthood with 100 mg/kg bw/day of bisphenol A. Other reported decreases in measures of testicular or epididymal sperm count and sperm production were more modest and ranged from 10 to 19% at doses of 50 and 500 mg/kg bw/day *(37, 43)*. In addition, in the three-generation rat study conducted by Tyl *et al. (37),* significant decreases in sperm parameters were only observed in certain generations of similarly exposed males in the high dose group of 500 mg/kg bw/day: ~18% decrease in epididymal sperm concentration in F1 males, ~19% decrease in testicular daily sperm production in F3 males and no significant effects in the F0 or F2 generations. Testicular or epididymal histopathology was not detected in any treatment group *(37)*. Significantly decreased sperm motility and an increased percentage of abnormal sperm was also reported following "high" dose subcutaneous injection, ~25 mg/kg bw/day,[19] to neonatal mice in a study conducted by Aikawa *et al. (225)*. Again, these effects were not associated with testicular histological alterations.

Effects on sperm parameters have been reported at lower doses administered orally or by sub-

[19]Administered dose was 0.050 mg/pup. This is approximately equal to 25 mg/kg/day assuming that a neonatal mouse weighs 0.002 kg

cutaneous injection.[20] vom Saal *et al. (226)* reported a ~19% decrease in testicular daily sperm production in adult male mice exposed to bisphenol A as fetuses via maternal dosing with 0.02 mg/kg bw/day (higher dose levels were not tested). Toyama *et al. (227)* observed increased incidences of several measures of abnormal sperm morphology (40–80% compared to <0.3% in controls) in mice treated with >0.17 mg/kg or rats treated with >0.33 mg/kg by subcutaneous injection[21] of bisphenol A every other day during postnatal days 2 to 12.

However, a number of larger studies have not reported effects on sperm parameters following exposure during development at "high" or "low" dose levels (0.0002–600 mg/kg bw/day) *(41, 107, 199–201, 228)*.

Exposure during adulthood only

Several studies have reported effects on sperm parameters in mice or rats exposed to "low" doses of bisphenol A only during adulthood. In rats, these effects are reported following oral dosing of 0.02–200 mg/kg bw/day for six days (~24–32% decreased daily sperm production per gram tissue) *(229),* 0.0002–0.02 mg/kg bw/day for 45 days (~23–41% decrease in epididymal sperm motility; ~18–27% decrease in epidiymal sperm count at 0.002–0.02 mg/kg bw/day) *(230),* and 0.0002–0.02 mg/kg bw/day for 60 days (~30–45% decrease in epididymal sperm motility; ~12–40% decrease in epidiymal sperm count at 0.002–0.02 mg/kg bw/day) *(231)*. In adult mice, "low" dose effects on sperm are observed at oral doses of 0.025–0.1 mg/kg bw/day for 30 days (~16–37% decrease in weight corrected testicular or epididymal sperm count) (108) and subcutaneous dosing with 0.02 and 0.2 mg/kg bw/day for 6 days (abnormal sperm morphology) *(232)*.

Other larger studies have not reported effects in adult animals at these doses. The 2-generation mouse study conducted by Tyl *et al. (41)* reported a 15% decrease in epididymal sperm concentration in F0 generation animals at the highest dose tested of 600 mg/kg bw/day but not at lower doses of 0.003 to 50 mg/kg bw/day. Ema *et al. (107)* also did not detect an effect on sperm measures in the F0 generation in a rat multigeneration study at oral doses of 0.0002 to 0.2 mg/kg bw/day. The finding by Sakaue *et al. (229)* of a ~24–32% decrease in sperm production in adult Sprague-Dawley rats (obtained from CLEA Japan, Inc.) was not reproduced in a study using larger sample sizes of Sprague-Dawley rats obtained from Charles River UK *(233)*.

The basis for the inconsistent findings is not clear. One proposed explanation is that rodent species, strains, and breeding stocks differ in their responsiveness to estrogens *(59)*. Species and strain differences in response to estrogen have been documented, but animal model sensitivity varies depending upon the specific trait being assessed [discussed in *(2, 59, 200)*]. Studies that include sperm assessment in the bisphenol A literature are too varied in terms of periods of dosing, use of positive control, e.g., none used, ethinyl estradiol, or 17β-estradiol, and other aspects of experimental conduct to determine if differences in sensitivity of the animal model can account for the inconsistent findings on sperm quantity and quality.

[20] Talsness *et al. (217)* reported effects on sperm quantity in rats exposed during gestation to 0.1 and 50 mg/kg bw/day but this study is not included in the discussion because (1) reported effects included an increase in sperm number which was opposite the effect observed in the positive control group, and (2) effects on daily sperm production appeared inconsistent over time and across dose.

[21] Administered doses were ≥0.001 mg/pup in the mouse and ≥0.01 mg/pup in the rat. These doses are approximately equal to 0.17 to 0.5 mg/kg in the mouse and 0.33–1.33 mg/kg in the rat assuming that body weight between postnatal days 2 to 12 ranges from 0.002 to 0.006 kg in the mouse and 0.0075 and 0.03 kg in the rat.

Chromosome and Meiosis Abnormalities

Disruption of the processes that distribute chromosomes during meiosis or mitosis can result in aneuploid cells, i.e., germ cells that have more or fewer chromosomes than the normal haploid number or somatic cells that have more or fewer chromosomes than the normal diploid number. When this happens in eggs or sperm of humans, it can lead to such conditions as Down Syndrome in which the fetus ends up with 3 copies of chromosome 21, rather than two copies, or a range of syndromes associated with abnormal numbers of sex chromosomes (normal is XX for females, XY for males) such as Klinefelter Syndrome (XXY males) or Turner Syndrome (XO females). If a chemical exposure is capable of inducing aneuploid eggs or sperm, affected individuals would be expected to exhibit problems in achieving or maintaining pregnancy, or to produce aneuploid offspring. While the body of evidence from both *in vitro* and *in vivo* studies provides evidence that bisphenol A can disrupt certain aspects of cell division involving both mitotic and meiotic processes, breeding studies in laboratory animals exposed to bisphenol A do not present results consistent with such effects. Thus, the significance of the reported effects on meiosis and mitosis for mammalian reproduction is not yet clear.

Two *in vivo* studies *(234, 235)* reported that short-term oral exposure to low doses of bisphenol A (≥ 0.020 mg/kg bw/day) in peripubertal or pregnant mice can interfere with meiotic divisions in development of female germ cells ("egg" or "oocyte"). An increase in hyperploid (aneuploid) metaphase II oocytes was observed following treatment with 0.020 mg/kg bw/day. There was not a significant increase in aneuploid embryos. Two subsequent *in vivo* studies *(236, 237)* attempted to replicate these findings. Consistent with the previous findings, they detected no significant effects of bisphenol A exposure on the frequency of aneuploidy in "zygotes" (fertilized oocytes) produced from female mice treated before puberty or as adults with a similar range of doses. In addition, Eichenlaub-Ritter *et al. (236)* found no effects of bisphenol A exposure on aneuploid oocytes and Pacchierotti *et al. (237)* found no increase in aneuploid or diploid sperm following exposure of male mice to bisphenol A.

A number of *in vitro* studies using cultured mammalian somatic cells have also looked at the potential for bisphenol A to cause aneuploidy. Earlier studies *(238–240)* consistently reported the induction of aneuploidy in various cell lines including SHE, V79, and MCL-5 at concentrations of bisphenol A between 50 and 200 μM (14.4 and 57.6 μg/ml). Recent *in vitro* studies reported effects of bisphenol A on maturation, but not induction of aneuploidy, in mouse oocytes *(236, 241)* or cultured mammalian somatic cells *(242, 243)*, increased frequency of mitotic cells with aberrant spindles *(243),* and various effects on cellular and nuclear division in fertilized sea urchin eggs *(244)*. Although these new studies provide further evidence of bisphenol A's effects on meiotic and mitotic cell division using a variety of *in vitro* systems and treatment concentrations, no impact of such effects on reproduction is reported in animal breeding studies and the significance of these findings with regard to human health hazards is not clear. If aneuploid eggs or sperm were induced by bisphenol A, it would be expected to result in reduced litter sizes following exposure of one or both parents to bisphenol A. Such an effect is not seen in reproductive toxicity studies of bisphenol A in rats or mice except at very high exposure levels (500 mg/kg bw/day or higher) where other types of toxicities are manifest *(37, 40, 41)*, including in the F2 generation *(37, 41)*. Findings of significantly decreased litter size or pregnancy loss are reported occasionally at lower doses of bisphenol A *(114, 245)*, but in general, most "low" dose studies do not report this outcome including a number of those that report other effects of bisphenol A exposure *(44, 48, 52, 53, 115, 125, 189)*.

ARE CURRENT EXPOSURES TO BISPHENOL A HIGH ENOUGH TO CAUSE CONCERN?

Possibly. The "high" dose effects of bisphenol A in laboratory animals that provide clear evidence for adverse effects on development, i.e., reduced survival, birth weight, and growth of offspring early in life, and delayed puberty in female rats and male rats and mice, are observed at levels of exposure that far exceed those encountered by humans. However, estimated exposures in pregnant women and fetuses, infants, and children are similar to levels of bisphenol A associated with several "low" dose laboratory animal findings of effects on the brain and behavior, prostate and mammary gland development, and early onset of puberty in females. When considered together, these laboratory animal findings provide limited evidence that bisphenol A has adverse effects on development (Figure 2b).

Exposures in humans and laboratory animals can be compared using approaches based on either estimated daily intake (based on aggregating sources of exposure or back calculating from biomonitoring data) or measured blood concentrations of free bisphenol A. Each approach has a unique set of assumptions and limitations. The conclusion of similarities between exposures of certain human populations and laboratory animals treated with "low" doses of bisphenol A is supported by multiple approaches. For this reason, the possibility that human development may be altered by bisphenol A at current exposure levels cannot be dismissed.

SUPPORTING EVIDENCE

A considerable amount of research has been directed towards understanding the levels of human exposure to bisphenol A, either by estimating daily intake or by measuring bisphenol A concentrations in human blood, urine, breast milk, or other tissue. An overarching issue relevant to the bisphenol A biomonitoring studies in both humans and laboratory animals is the accuracy of the laboratory methods used to measure the compound (see Appendix A). There is concern that measurements of bisphenol A, especially free bisphenol A, may be too high due to problems related to sample preparation or storage and the analytical technique employed [reviewed in *(2, 13)*]. The NTP recognizes the possibility that the published values of free bisphenol A may, in some cases, not accurately represent the "true" concentrations of free bisphenol A in the blood or body fluids of humans or laboratory animals. However, because of the similarity among values reported with different analytical methods, with the exception of studies that use an enzyme-linked immunosorbent assay (ELISA), the NTP accepts the published values as sufficiently reliable for use in this evaluation.

DAILY INTAKE EXPOSURE ESTIMATES

The vast majority of bisphenol A exposure is through the diet, estimated at ~99% *(1)*; therefore, estimates of daily intake in humans can be compared to oral doses used in laboratory animal studies where effects considered relevant to human health were observed. Estimates of daily intake are derived using two general approaches. Researchers can use information on the amount of bisphenol A detected in various sources of exposure (i.e., food, food packaging, air, water, dust, etc.) and sum, or aggregate, the measurements to estimate a total daily intake ("aggregating sources of exposure" method). Alternatively, biomonitoring information, such as the concentration of bisphenol A in urine, can be used to estimate, or "back calculate", a total intake that reflects all sources of exposure, both known and unknown. Both approaches for estimating daily intake rely on various assumptions and default values such as average body weight, amount of food or beverage consumed, daily volume of urine output, or ability of a single measurement to characterize exposure.

Infants & children less than 6 years of age

For infants and children less than 6 years of age, estimates of daily intake were based on aggregat-

ing sources of exposure (Table 1). No biomonitoring data, i.e., blood or urine concentration of bisphenol A, are available for these life stages [reviewed in *(2)]*. An estimated daily intake of ~1 µg/kg bw/day for both breast-fed and formula-fed infants was calculated by the CERHR Expert Panel for Bisphenol A *(2)*. Higher "worst case" daily intake estimates of 11 – 13 µg/kg bw/day during the first year of life have been calculated for infants *(25)*. In children 1.5 to 6 years of age, the range of estimated daily intakes based on aggregating sources of exposure is 0.043 – 14.7 µg/kg bw/day, with 14.7 µg/kg bw/day representing a worst case scenario *(27, 29)*.

Although biomonitoring data are not available for infants and children less than 6 years of age, blood and urine levels of free bisphenol A are predicted to be higher in these age groups compared to pregnant women or other adult populations. This is based on information related to age-specific differences in daily intake of bisphenol A and in the ability to metabolize the chemical. More specifically, it is based on observations of (1) higher urinary measurements of total bisphenol A in children (6 – 11 years of age) compared to adolescents and adults *(8),* (2) higher estimated daily intakes of bisphenol A for infants and children *(2, 25, 27)* compared to estimated daily intakes for adults *(2, 25, 35)*, and (3) predicted higher blood concentrations of free bisphenol A in infants compared to adults at a given daily intake level based on less efficient metabolism of bisphenol A in rat fetuses and neonates *(18 – 20)*, and very low or absent activities in human fetuses and premature or full-term infants of the isozymes that govern glucuronidation *(246 – 248)*.

Adults and children aged 6 years and above

Daily intake estimates for adults and children aged 6 years and older are based on (1) back calculations from the most recent CDC NHANES data on urinary concentrations of total bisphenol and (2) aggregating sources of exposure (Table 1 and Table 3). Of these estimates, the NTP has more confidence in the estimates based on back calculating from urinary biomonitoring data because all sources of exposure are integrated into the fluid measurement and thus do not have to be identified in advance. However, it is worth noting that the estimates for non-occupationally exposed adults based on aggregating sources of exposure encompass the range estimated from back calculating from urine [aggregating sources of exposure: 0.008 – 1.5 µg/kg bw/day (Table 1); and back calculating based on urine: 0.233 – 0.289 µg/kg bw/day for various categories of adults ages 20+ at the 95th percentile *(35)]*. Fewer studies have estimated daily intakes for children older than 6 years of age and adolescents. In Japanese children and adolescents between the ages of 7 and 19 years, the range of estimated daily intakes based on aggregating sources of exposure is 0.36 to 0.55 µg/kg bw/day *(30),* which is only slightly higher than the estimated range of daily intakes for American children and adolescents based on back calculating from urinary concentration of total bisphenol A [0.311 – 0.348 µg/kg bw/day for children ages 6 – 11 and 12 – 19 at the 95th percentile *(35)]*.

Estimated daily intake based on blood biomonitoring

The NTP also considered the appropriateness of estimating daily intake based on back calculations from free bisphenol A measured in human blood and concluded that the scientific uncertainties are currently too large to support this exercise (see Appendix A). In brief, estimated daily intakes in adults based on this approach are much greater (~500 µg/kg – 1.54 mg/kg bw/day for a 65 kg human) *(3, 249)* than estimates of daily intake based on aggregating routes of exposure (0.008 – 1.5 µg/kg bw/day) *(25, 31)* or from back calculating from urinary data (adults aged 20 – 60+: medians 0.0563 – 0.0334 µg/kg

bw/day; 95th percentiles 0.289 – 0.233) *(35)*. In addition, data from an intentional dosing study conducted by Tsukioka *et al. (250)*[22] provides further support for daily intakes in humans of < 1 µg/kg. Several explanations have been proposed to account for the discrepancy between estimated intake based on blood and urine but they are not sufficient to fully explain it.

EXPOSURE COMPARISONS BASED ON DAILY INTAKE

The "high" dose effects of bisphenol A that represent clear evidence for adverse effects on development, i.e., reduced survival (≥ 500 mg/kg bw/day) *(36 – 40)*, reduced birth weight and growth of offspring early in life (≥ 300 mg/kg bw/day) *(36 – 39, 41)*, and delayed puberty in female rats and male rats and mice (≥ 50 mg/kg bw/day) *(37, 41 – 43)*, are observed at dose levels that are more than 3,500-times higher than "worst case" daily intakes of bisphenol A in infants and children less than 6 years of age (≥ 50 mg/kg bw/day versus 0.008 – 0.0147 mg/kg bw/day). The differences in exposures are much greater, more than 160,000-times different, when the high oral dose level is compared to estimated daily intakes for children ages 6 – 11 and adult women (as an indicator of exposure for pregnant women) at the 95th percentile of 0.311 and 0.271 µg/kg bw/day, respectively *(35)*.

However, a number of "low" dose developmental effects have been reported in mice treated orally with bisphenol A including effects on behavior (≥10 µg/kg bw/day) *(44 – 50)*, prostate gland and urinary tract development (10 µg/kg bw/day) *(54)*, and early onset of puberty (2.4 and 200 µg/kg bw/day) *(48, 55)*. In addition, subcutaneous injection with 10 µg/kg bw/day of bisphenol A during neonatal life in rats results in development of hormonally induced preneoplastic lesions in the prostate later in life *(51)*.[23] This non-oral study is considered relevant for comparing exposures because, as discussed previously, the differences in the rate of bisphenol A metabolism seen in adult rats based on route of administration (oral versus non-oral) appear to be greatly reduced in neonatal rats and mice *(18, 92)*. As stated earlier, these findings, when considered together, provide limited evidence for adverse effects of bisphenol A exposure on development in laboratory animals (Figure 2b).

In infants, the doses of 2.4 and 10 µg/kg bw/day are 2.4 – 10 times higher than the estimated daily intake of ~ 1 µg/kg bw/day calculated by the CERHR Expert Panel for Bisphenol A *(2)*. Higher "worst case" daily intakes have been calculated for infants by the European Food Safety Authority of 11 – 13 µg/kg during the first year of life *(25)*. To the extent these estimates are accurate, then dose levels of 2.4 and 10 µg/kg bw/day slightly exceed (1.1 to 5.4-times) worst case estimates. The doses of 2.4 and 10 µg/kg bw/day are approximately 7.7 – 32 and 8.9 – 37 times higher than the estimated daily intakes of 0.311 µg/kg bw/day for children (ages 6 – 11 years) and 0.271 µg/kg bw/day for adult women at the 95th percentile *(35)*.

[22]Tsukioka *et al. (250)* used GC/MS with trimethylsilyation (TMS) derivatization (LOQ 0.1 mg/L). Brock *et al. (251)* report that use of TMS may produce interfering peaks in the chromatogram. Sample workup included glucuronidase treatment, solvent extraction, and solid phase clean-up. Few details were presented in the Tsukioka *et al. (250)* study on sample preparation process, such as storage temperature.

[23]Preneoplastic lesions in the mammary gland, i.e., ductal hyperplasia and carcinoma *in situ*, have been reported in rats treated as fetuses with 2.5 µg/kg bw/day via a subcutaneous pump implanted in the dam *(52, 53)*; however, as discussed previously, studies that administer bisphenol A via subcutaneous pump are considered informative for identifying potential biological effects of bisphenol A, but not for quantitatively comparing exposures in laboratory animals and humans.

EXPOSURE COMPARISONS BASED ON BLOOD CONCENTRATIONS OF FREE BISPHENOL A

No studies in laboratory animals have measured circulating levels of free bisphenol A in the blood following a dosing schedule that mimics human exposures, i.e., long-term dietary low-dose exposure occurring numerous times during the day. However, a number of studies have detected quantifiable levels of free bisphenol A in the blood of adult rodents following a single oral administration of bisphenol A, typically at doses considered high when compared to estimated human daily intakes (500 – 1,000,000 µg/kg for rodents versus < 14.7 µg/kg bw/day for humans) *(3, 27, 35, 249)*. These studies were used by Vandenberg *et al. (3)* to estimate circulating blood levels of free bisphenol A in rodents at a lower oral dose of 50 µg/kg based on the assumption of linear proportionality between administered dose and circulating concentration of free bisphenol A. The estimated peak blood levels of free bisphenol A in the first 30 minutes after dosing at 50 µg/kg ranged from 0.01 to 1.14 µg /L (median 0.11 µg /L) *(3).* Based on this estimate, peak concentrations of free bisphenol A in mice or rats treated with 2.4 or 10 µg/kg bw/day of bisphenol A are projected to be lower than the free blood concentrations measured in humans, including pregnant women *(12, 15)*. See Appendix A for further details on these calculations.

NTP CONCLUSIONS

The NTP reached the following conclusions on the possible effects of exposure to bisphenol A on human development and reproduction. Note that the possible levels of concern, from lowest to highest, are negligible concern, minimal concern, some concern, concern, and serious concern.

The NTP has *some concern* for effects on the brain, behavior, and prostate gland in fetuses, infants, and children at current human exposures to bisphenol A.

The NTP concurs with the conclusion of the CERHR Expert Panel on Bisphenol A that the scientific evidence supports a conclusion of *some concern* for exposures in fetuses, infants, and children based on a number of laboratory animal studies reporting that "low" level exposure to bisphenol A during development can cause changes in the brain and behavior. In addition, the NTP has *some concern* for exposures to these populations based on effects on the prostate gland observed in laboratory animals. This level of concern for effects on the prostate gland is higher than that expressed by the Expert Panel and is based primarily on new supportive data related to (1) the interpretation of studies that use a non-oral route of administration in neonatal rodents, and (2) an additional publication reporting subtle cellular changes in the prostate gland. These reports were not published when the Expert Panel completed its deliberations. These studies in laboratory animals provide only limited evidence for adverse effects on development and more research is needed to better understand their implications for human health. However, because these effects in animals occur at bisphenol A exposure levels similar to those experienced by humans, the possibility that bisphenol A may alter human development cannot be dismissed.

The NTP has *minimal concern* for effects on the mammary gland and an earlier age for puberty for females in fetuses, infants, and children at current human exposures to bisphenol A.

The NTP concurs with the conclusion of the CERHR Expert Panel on Bisphenol A that the scientific evidence supports a conclusion of *minimal concern* for exposures in fetuses, infants, and children based on a number of laboratory animal studies reporting that "low" level exposure to bisphenol A during development can alter the timing of events related to sexual maturation in females. In addition, the NTP has *minimal concern* for exposures to these populations based on effects on the mammary gland observed in laboratory animals. This level of concern for effects on the mammary gland is higher than that expressed by the Expert Panel and is based primarily on (1) information received through public comments and (2) a new supportive study reporting subtle changes in the undifferentiated structures of the mammary gland. These studies in laboratory animals provide only limited evidence for adverse effects on development and more research is needed to better understand their implications for human health. However, because these effects in animals occur at bisphenol A exposure levels similar to those experienced by humans, the possibility that bisphenol A may alter human development cannot be dismissed.

The NTP has *negligible concern* that exposure of pregnant women to bisphenol A will result in fetal or neonatal mortality, birth defects, or reduced birth weight and growth in their offspring.

The NTP concurs with the conclusion of the CERHR Expert Panel on Bisphenol A that there is *negligible concern* that exposure of pregnant

women to bisphenol A will result in fetal or neonatal mortality, birth defects or reduced birth weight and growth in their offspring. In laboratory animals, exposure to very high levels of bisphenol A during pregnancy can cause fetal death and reduced birth weight and growth during infancy. These studies provide clear evidence for adverse effects on development, but occur at exposure levels far in excess of those experienced by humans. Two recent human studies have not associated bisphenol A exposure in pregnant women with decreased birth weight or several other measures of birth outcome. Results from several animal studies provide evidence that bisphenol A does not cause birth defects such as cleft palette, skeletal malformations, or grossly abnormal organs.

The NTP has *negligible concern* that exposure to bisphenol A will cause reproductive effects in non-occupationally exposed adults and *minimal concern* for workers exposed to higher levels in occupational settings.

The NTP concurs with the conclusion of the CERHR Expert Panel on Bisphenol A that there is *negligible concern* that exposure to bisphenol A causes reproductive effects in non-occupationally exposed adults and *minimal concern* for workers exposed to higher levels in occupational settings Data from studies in humans are not sufficient to determine if bisphenol A adversely affects reproduction when exposure occurs during adulthood. A number of studies, when considered together, suggest a possible effect on reproductive hormones, especially in men exposed to higher levels of bisphenol A in the workplace. Laboratory studies in adult animals show adverse effects on fertility, estrous cycling, and the testes at exposure levels far in excess of those experienced by humans. A number of other effects, such as decreased sperm counts, are reported for the reproductive system at lower doses in animals exposed only during adulthood, but these effects have not been shown to be reproducible. Laboratory animal studies consistently report that bisphenol A does not affect fertility.

These conclusions are based on information available at the time this brief was prepared. As new information on toxicity and exposure accumulates, it may form the basis for either lowering or raising the levels of concern expressed in the conclusions.

APPENDIX A: INTERPRETATION OF BLOOD BIOMONITORING STUDIES

Free bisphenol A has been measured in the blood of pregnant women at concentrations up to 22.4 µg/L *(12)*. How to account for the detection of free bisphenol A in human blood is an area of scientific debate. In a controlled and intentional dosing study in humans, free bisphenol A was not detected in the blood or urine of a small number of adult subjects (n=9) orally dosed with 5 mg/person bisphenol A, ~54–90 µg/kg *(252)*. This dose range is approximately 200 to 400-fold higher than the estimates of daily intake based on urinary biomonitoring data for adults (95th percentile of 0.233–0.289 µg/kg bw/day) *(35)*. The findings by Völkel *et al. (252)* lead to the prediction that the capacity for conjugation reactions is so large in humans that free bisphenol A should not be present in detectable concentrations in the blood of non-occupationally exposed adults. However, biomonitoring studies of the general population report detecting free bisphenol A in the blood, including from pregnant women *(12, 15)*, urine *(253),* and breast milk *(5)*. Despite the relatively high limit of detection of the analysis method for free bisphenol A of 2.28 µg/L (10 nM) for blood in the 2002 study by Völkel *et al. (252),* it is a source of scientific uncertainty why free bisphenol A was not detected in this study in light of reports of mean blood concentrations of free bisphenol A up to 4.4 µg/L *(15)* and 5.9 µg/L *(12)* in pregnant women in the general population.

This discrepancy has contributed to the concern expressed by some scientists that the reported detections of free bisphenol A are artifacts of problems related to sample preparation or storage and the analytical technique employed *(2, 13)*. Ideally, methods should measure only bisphenol A and not other compounds ("specificity"). There is scientific consensus that measurements of bisphenol A based on the enzyme-linked immunosorbent assay (ELISA) are the least reliable and non-specific due to potential cross-reactivity with structurally-similar compounds *(2, 3, 13)*.[24] Analytical methods should also be able to detect bisphenol A at low concentrations ("sensitivity"). In addition, measurements of free bisphenol A should be based on analytical methods that accurately distinguish between the concentrations of free bisphenol A and its conjugated metabolites.

There is concern that current measurements of free bisphenol A may be too high *(2, 13)*. This could occur, for example, if the method used misidentified other chemicals as bisphenol A or if there was background contamination from laboratory ware. Alternatively, the procedures used to process the samples could introduce bias in measurement even if the analytical method employed is high quality. Measurements of free bisphenol A could be overestimated if the samples were processed in a manner that allowed the conjugated metabolites to revert back to the free form of bisphenol A. For example, conjugated bisphenol A in urine only appears to be stable when stored at room temperature for ~24 hours. After 2–4 days at this temperature conjugated bisphenol A begins to degrade and the percent detected in samples decreases ~8 to 30%, i.e., higher concentrations of free bisphenol A would be detected over time *(254)*.

However, free bisphenol A has been detected in 10% of human urine samples [range=<limit of detection (0.3)–0.6 µg/L; n=30] *(253)* and in

[24]Analytical techniques used to measure bisphenol A include gas chromatography/mass spectrometry (GC/MS), liquid chromatography/mass spectrometry (LC/MS), high performance liquid chromatography (HPLC) with fluorescence or electrochemical detection, and enzyme-linked immunosorbent assay (ELISA).

60% of breast milk samples [mean = 1.3 μg/L; median = 0.4 μg/L; range = < limit of detection (0.3) – 6.3 μg/L; n = 20] *(5)* by researchers at the CDC who use analytical methods considered by many scientists to be very accurate. A recent analytical methods report by the CDC provides further support that the ratio of free to total bisphenol A may be higher in breast milk compared to other biological media. In this small study which used only 4 human milk samples, free bisphenol A was detected in all samples and ranged from 49 to 99% of the total concentration *(255)*. A proposed explanation to account for the detection of free bisphenol A in breast milk is that the free form of bisphenol A is more lipophilic than the conjugated forms and therefore more likely to sequester in breast milk *(5, 255)*.

In addition, Tsukioka *et al. (250)* were able to detect free bisphenol A in the urine of all human subjects treated with ~ 0.83 μg/kg, whereas Völkel *et al. (252)* was unable to detect any free bisphenol A in subjects treated with doses 65 – 108-times higher, ~54 – 90 μg/kg. It cannot be definitively determined if the detection of free bisphenol A in urine in the study by Tsukioka *et al. (250)* was due to the analytical method employed or partial cleavage of glucuronide during sample storage, preparation or analysis. However, Tsukioka *et al. (250)* also detected total and free bisphenol A in the urine of subjects that were not intentionally treated [total bisphenol A: 0.82 μg/L (range 0.14 – 5.47; n = 91); free bisphenol A: 0.08 μg/L (range 0.01 – 0.27 ng/m; n = 11)], and these values are lower than CDC measurements of total [2.6 μg/L for all subjects in the NHANES study *(8)]* and free bisphenol A [10 of 30 subjects at < LOD (0.3) – 0.6 μg/L *(253)*].

CDC researchers recently published an analytical methods study that reported detecting bisphenol A in only one of the 15 commercial samples tested (the concentrations of total and free in the one sample were similar, 1.5 ng/ml) *(256)*. However, caution should be exercised in interpreting this information for exposure analysis because information on sample collection, handling and storage protocols is not available for these commercial samples. The NTP considers it noteworthy that the studies reporting the highest blood concentrations of bisphenol A in humans using non-ELISA analytical methods both relied on samples collected from pregnant women in the process of delivery *(12, 15)*. The use of bisphenol A in polyvinyl chloride plastic has been documented *(6)* and, if still occurring, could result in exposures in medical settings such as in women during delivery who are often connected to an IV. If such a scenario were occurring then the reported concentrations of bisphenol A in pregnant women may be accurate but not necessarily reflective of exposures throughout gestation or to the general population.

In summary, the NTP recognizes the possibility that the published values of free bisphenol A may, in some cases, not accurately represent the "true" concentrations of free bisphenol A in the blood or body fluids of humans or laboratory animals. However, because of the similarity among values reported with different analytical methods, with the exception of ELISA-based studies, the NTP accepts the published values as sufficiently reliable for use in this evaluation.

Comparison of measured human blood concentrations of free bisphenol A with estimated concentrations in laboratory rodents at low doses

More than 10 toxicokinetic and metabolism studies have detected quantifiable levels of free bisphenol A in the blood of adult rodents, mostly rats, following oral administration of doses that are considered high when compared to estimated human daily intakes (500 – 1,000,000 μg/kg for rodents versus < 14.7 μg/kg bw/day for humans) *(3, 27, 35)* (Table 1 and Table 2). These studies were used by Vandenberg *et al. (3)* to estimate circulating blood levels of free bisphenol A in laboratory rodents at a lower oral dose of 50

μg/kg bw/day based on the assumption of linear proportionality between administered dose and circulating concentration of free bisphenol A. The estimated peak blood levels of free bisphenol A achieved in the first 30 minutes after dosing ranged from 0.01 to 1.14 μg/L *(3)*.

Using the estimates provided by Vandenberg *et al. (3)* for peak blood levels of free bisphenol A at 50 μg/kg and relying on the assumption of linear proportionality, the NTP estimated the range of peak concentrations of free bisphenol A at 10 μg/kg, a dose where a number of "low" dose effects are reported, to be five times lower, i.e., 0.002 to 0.228 μg/L. These values are 2950 to 25.9 times lower than the mean blood concentration of free bisphenol A detected in pregnant women in Michigan (5.9 ± 0.94 μg/L; range 0.5 to 22.4) *(12)*.

The appropriateness of extrapolating from higher dose studies to predict blood levels of free bisphenol A at lower dose levels rests on the validity of the assumption of proportionality. This assumption is warranted if, for example, blood levels of free bisphenol A are approximately 10 times lower following dosing with 10 mg/kg than after dosing with 100 mg/kg. Three studies are available that used non-ELISA methods to measure concentrations of free bisphenol A following oral dosing with 10 and 100 mg/kg bisphenol A in adult rats *(93, 257, 258)*. In these studies, the peak, or Cmax, blood concentrations of free bisphenol A were 4.8-times *(257),* 22.7-times *(258),* and 57-times *(93)* lower in rats treated with a 10 mg/kg dose compared to rats treated with 100 mg/kg.

Directly evaluating proportionality at lower oral doses (<10 mg/kg) has not been possible in adult animals because blood concentrations of free bisphenol A are below the limits of detection for the analytical methods employed. One strategy that can be used to address the assumption of proportionality at low doses is to rely on studies that have dosed young rodents because they have higher peak blood concentrations of free bisphenol A compared to adults treated with the same dose *(18)*. Two studies have measured concentrations of free bisphenol A in young rodents at more than one dose level *(18, 92)*. In 3-day old female mice orally treated with 0.035 and 0.395 mg/kg bisphenol A, Taylor *et al. (92)* found that the peak blood concentration of free bisphenol A at 0.035 mg/kg was 8.3-times lower than the peak concentration at 0.395 mg/kg (difference between administered does is 11.3-times). The study by Domoradzki *et al. (18)* treated neonatal rats orally with higher doses of bisphenol A than those used by Taylor *et al. (92)*. In 4-day old female and male rats, the peak concentrations of free bisphenol A were 170 to 1610-times lower at 1 mg/kg compared to 10 mg/kg bisphenol A. This finding, coupled with data for 21-day old rats presented in Domoradzki *et al. (18)* and the comparisons presented above from Tominaga *et al. (258),* and Pottenger *et al. (93),* suggest that rodents, and presumably humans, can more efficiently metabolize lower doses of bisphenol A compared to high doses. These data also suggest that extrapolating from higher dose levels in the mg/kg range may overestimate the circulating concentrations of free bisphenol A following administration of oral doses in the low μg/kg range.

Any extrapolation and use of assumptions involves some degree of uncertainty. However, the conclusion outlined above of similar blood levels in the general population and in laboratory animals at "low" doses would still hold even if the estimated blood levels of free bisphenol A in laboratory rodents were overestimated by a factor of 100 or 1000, i.e., the "real" peak blood values in laboratory animals range from 0.2 to 22.8 or 2 to 228 μg/L instead of the estimated 0.002 to 0.228 μg/L.

This possibility that blood concentrations of free bisphenol A in humans could be significantly

higher, as much as ~3000 times greater, than the estimated peak concentrations in laboratory animals where biological changes are observed is a point of intense scientific controversy. In brief, although the theoretical plausibility of receptor-mediated effects at "low" doses has been described *(259, 260)*, many scientists expect that a compound with a significant degree of biological "activity" at low doses would show more profound impacts on overall toxicity at lower doses than that observed for bisphenol A. With bisphenol A, "low" dose developmental effects can be observed at 0.0024 to 0.010 mg/kg bw/day but indications of severe developmental toxicity in rats and mice, i.e., fetal or neonatal death are not observed except when doses are used that are 50,000–200,000-times higher at ≥500 mg/kg bw/day *(36–40)*.

Estimated daily intake based on back calculating from blood and urine

Based on parameters derived from laboratory animal studies, estimated daily intakes based on back calculations from free bisphenol A measured in human blood are much greater (~500 µg/kg–1.54 mg/kg bw/day for a 65 kg human) *(3, 249)* than estimates based on any other approach. In contrast, there is a degree of concordance in estimates of daily intake based on other approaches. For these reasons, the NTP has less confidence in daily intake estimates based on blood biomonitoring data compared to other estimates, particularly those based on urine biomonitoring data.

Estimates of daily bisphenol A intake in adults based on aggregating routes of exposure fall within the range of 0.008–1.5 µg/kg bw/day *(25, 31)* (Table 1) with most estimates falling within a range that spans one order of magnitude, 0.183–1.5 µg/kg bw/day *(24–27, 30)*. Daily intakes estimated from the CDC NHANES biomonitoring data are similar and range from 0.289–0.233 µg/kg bw/day for adults aged 20–60+ years at the 95th percentile *(35)*. The NTP considered the possibility that the assumptions used to derive these intakes could underestimate human exposures. For estimates based on aggregating sources of exposure, one concern is that too much emphasis has been placed on diet as the predominant route of exposure. For estimates based on the total concentration of bisphenol A in urine, it is assumed that the daily excretion of bisphenol A is a reasonable surrogate for daily intake. Deviations from the assumptions used to derive current estimates could increase the daily intake estimates, but still result in estimated intakes in the very low µg/kg bw/day range rather than near 1 mg/kg bw/day as predicted from the blood biomonitoring data in adult humans.

Data from an intentional dosing study conducted by Tsukioka *et al. (250)* provides further support for daily intakes of <1 µg/kg. Tsukioka *et al.* gave 15 volunteers (12 men and 13 women) 50 µg of bisphenol A by mouth (~ 0.83 µg/kg for a 65 kg person) and collected urine samples for 5 hours. The average concentration of total bisphenol A was 57.2 µg/L (range 26.5–80 µg/L) and free bisphenol A was 1.13 µg/L (range 0.13–5.8 µg/L). The administered dose, ~0.83 µg/kg, and urinary concentration of total bisphenol A, 57.2 µg/L, are ~14.8-times and 18.5-times higher, than the estimated median intake of 0.056 µg/kg bw/day for adults aged 20–39 years based on a median urinary concentration of 3.1 µg/L calculated by Lakind *et al. (35)*. Extrapolating downward for administered dose and urinary concentrations of total bisphenol A from the data provided by Tsukioka *et al. (250)* would give values that are consistent with the daily intake calculated by Lakind *et al. (35)* based on the CDC urinary measurements *(8)*.

Exposure Assessment Research Needs

The NTP concurs with the CERHR Expert Panel on Bisphenol A that more measurements in humans are needed of free and total bisphenol A, its glucuronide conjugate, and other metabolite

concentrations from maternal, fetal, and neonatal tissues or fluids (i.e., placenta, amniotic fluid, breast milk, urine, serum). These data would provide further insight into the roles of metabolism and exposure route on internal dose and provide a firmer foundation for extrapolations of risks to humans from the wealth of animal studies available. Available data demonstrate that a large fraction of children and adults have detectable levels of bisphenol A, or its metabolites, in their urine. Duplicate diet studies to identify in detail the sources and routes of exposure of bisphenol A would be useful. For example, while research suggests diet is the major source of bisphenol A for infants and young children in the United States, the detailed analysis of bisphenol A levels has primarily focused on polycarbonate baby bottle leachates and canned food. The contributions of non-canned food and drinking water routes of exposure for youth and adults not occupationally-exposed to bisphenol A remain unknown and in need of further study. Levels of bisphenol A in residential drinking water wells and community water sources have not been systematically studied. Also unknown is the impact of landfill leachates on levels of bisphenol A in U.S. drinking well waters and whether chlorinated congeners of bisphenol A are found in municipal water supplies.

More research is needed to characterize the toxicokinetics of bisphenol A in developing animals under exposure scenarios that better mimic the low-level chronic exposures experienced by humans. Currently, only single or "acute" dosing kinetic studies in laboratory animals are available for predicting the metabolism and fate of bisphenol A following long-term, daily exposure, or for comparing apparent differences in the metabolism and fate of bisphenol A in laboratory rodents and humans. Repeated administration of many compounds has been shown to alter the capacity of the animal to metabolize and excrete the compound. Further characterization of the ability of repeated exposures to bisphenol A to change rates and extent of metabolism and excretion in laboratory animals and humans is a critical research need.

In addition, it is clear that there are differences in the pharmacokinetics of bisphenol A, particularly between rats and humans, which complicate using the rat data to interpret the human biomonitoring data. For example, the excretion profiles of bisphenol A differ in rodents and humans. In humans, the major route of elimination is via the urine in the form of bisphenol A glucuronide *(261)*. In contrast, the major elimination routes in rodents are as bisphenol A in the feces, as bisphenol A glucuronide in the bile, and to a lesser extent, in the urine [reviewed in *(2)]*. Also, in rats bisphenol A glucuronide can remain in the bile and be recirculated back to the liver ("enterohepatic circulation"). To address these uncertainties the NTP is pursuing studies of absorption, distribution, metabolism, and excretion in experimental animals (rodents and non human primates) as well as the kinetics associated with these processes, following exposures to bisphenol A from the perinatal period through adulthood, over a wide range of doses, by multiple routes of administration.

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APPENDIX I. NTP-CERHR BISPHENOL A EXPERT PANEL

A 12–member panel of scientists covering disciplines such as toxicology, epidemiology, and medicine was recommended by the CERHR Core Committee and approved by the Associate Director of the National Toxicology Program. Prior to the expert panel meeting, the panelists critically reviewed articles from the scientific literature, as well as a variety of other relevant documents. Based on this material, they identified key studies and issues for discussion. At public meetings held on March 5–7, 2007 and August 6–8, 2007, the expert panel discussed these studies, the adequacy of available data, and identified data needed to improve future assessments. The expert panel reached conclusions on whether exposure to bisphenol A might result in adverse effects on human reproduction or development. Panel conclusions were based on the scientific evidence available at the time of the public meeting. The NTP-CERHR released the final expert panel report for public comment on November 26, 2007 and the deadline for public comments was January 25, 2008 (*Federal Register* Vol. 72, No. 230, pp. 67730–67731, November 30, 2007). The NTP-CERHR Expert Panel Report on Bisphenol A is provided in Appendix II. The expert panel report is also available on the CERHR website (*http://cerhr.niehs.nih.gov*).

NTP-CERHR BISPHENOL A EXPERT PANEL

Robert E. Chapin, Ph.D., Chair Pfizer, Inc. Groton, CT	***Barry S. McIntyre, Ph.D.*** Schering Plough Research Institute Summit, NJ
Jane Adams, Ph.D. University of Massachusetts Boston, MA	***Kenneth M. Portier, Ph.D.*** American Cancer Society Atlanta, GA
Kim Boekelheide, M.D., Ph.D. Brown University Providence, RI	***Teresa M. Schnorr, Ph.D.*** National Institute for Occupational Safety and Health Cincinnati, OH
L. Earl Gray, Jr., Ph.D. U.S. Environmental Protection Agency Research Triangle Park, NC	***Sherry G. Selevan, Ph.D.*** U.S. Public Health Service (Ret) Silver Spring, MD
Simon W. Hayward, Ph.D. Vanderbilt University Medical Center Nashville, TN	***John G. Vandenbergh, Ph.D.*** North Carolina State University Raleigh, NC
Peter S.J. Lees, Ph.D. Johns Hopkins University Baltimore, MD	***Susan R. Woskie, Ph.D.*** University of Massachusetts Lowell, MA

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Expert Panel Report

NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Bisphenol A

Robert E. Chapin,[1] Jane Adams,[2] Kim Boekelheide,[3] L. Earl Gray Jr,[4] Simon W. Hayward,[5] Peter S.J. Lees,[6] Barry S. McIntyre,[7] Kenneth M. Portier,[8] Teresa M. Schnorr,[9] Sherry G. Selevan,[10] John G. Vandenbergh,[11] and Susan R. Woskie[12]

[1]Pfizer, Inc., Groton, CT
[2]University of Massachusetts, Boston, MA
[3]Brown University, Providence, RI
[4]U.S. Environmental Protection Agency, Research Triangle Park, NC
[5]Vanderbilt University Medical Center, Nashville, TN
[6]Johns Hopkins University, Baltimore, MD
[7]Schering Plough Research Institute, Summit, NJ
[8]American Cancer Society, Atlanta, GA
[9]National Institute for Occupational Safety and Health, Cincinnati, OH
[10]U.S. Public Health Service (Ret), Silver Spring, MD
[11]North Carolina State University, Raleigh, NC
[12]University of Massachusetts, Lowell, MA

Preface

The National Toxicology Program (NTP)[1] established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June 1998. The purpose of the CERHR is to provide timely, unbiased, scientifically sound evaluations of the potential for adverse effects on reproduction or development resulting from human exposures to substances in the environment. The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC, and is staffed and administered by scientists and support personnel at NIEHS.

Bisphenol A is a high-production volume chemical used in the production of epoxy resins, polyester resins, polysulfone resins, polyacrylate resins, polycarbonate plastics, and flame retardants. Polycarbonate plastics are used in food and drink packaging; resins are used as lacquers to coat metal products such as food cans, bottle tops, and water supply pipes. Some polymers used in dental sealants and tooth coatings contain bisphenol A. Exposure to the general population can occur through direct contact with bisphenol A or by exposure to food or drink that has been in contact with a material containing bisphenol A. CERHR selected bisphenol A for evaluation because of (1) high production volume; (2) widespread human exposure; (3) evidence of reproductive toxicity in laboratory animal studies; and (4) public concern for possible health effects from human exposures.

Relevant literature on bisphenol A was identified from searches of the PubMed (Medline) and Toxline databases through February 2007 using the term ''bisphenol'' and the bisphenol A CAS RN (80-05-7). References were also identified from databases such as REPROTOX, HSDB, IRIS, and DART, from the bibliographies of the literature reviewed, by members of the expert panel, and in public comments.

CERHR convened a 12-member, independent panel of government and non-government scientists to evaluate the scientific studies on the potential reproductive and developmental hazards of bisphenol A. The expert panel met publicly on March 5–7, 2007 and August 6–8, 2007. The Expert Panel Report on Bisphenol A is intended to (1) interpret the strength of scientific evidence that bisphenol A is a reproductive or developmental toxicant based on data from in vitro, animal, or human studies; (2) assess the extent of human exposures to include the general public, occupational groups, and other sub-populations; (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive and developmental health effects may be associated with such exposures; and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future evaluations. This report has been reviewed by members of the expert panel and by CERHR staff scientists. Copies of this report have been provided to

[1]NTP is an interagency program headquartered in Research Triangle Park, NC, at the National Institute of Environmental Health Sciences, a component of the National Institutes of Health.

*Correspondence to: Michael D. Shelby, PhD, NIEHS EC-32, PO Box 12233, Research Triangle Park, NC 27709.
E-mail: shelby@niehs.nih.gov
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the CERHR Core Committee[2] and will be made available to the public for comment.

Following the public comment period, CERHR will prepare the NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Bisphenol A. This monograph will include the NTP Brief, the Expert Panel Report, and all public comments received on the Expert Panel Report. The NTP-CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies.

Reports can be obtained from the web site (http://cerhr.niehs.nih.gov) **or from:** Michael D. Shelby, PhD, NIEHS EC-32, PO Box 12233, Research Triangle Park, NC 27709. E-mail: shelby@niehs.nih.gov

1.0 CHEMISTRY, USE AND HUMAN EXPOSURE

1.1 Chemistry

Section 1 is based initially on secondary review sources. Primary study reports are addressed by the Expert Panel if they contain information that is highly relevant for determining the effect of exposure on developmental or reproductive toxicity or if the studies were released subsequent to the reviews.

1.1.1 Nomenclature. The CAS RN for bisphenol A is 80-05-7. Synonyms for bisphenol A listed in Chem IDplus (ChemIDplus, 2006) include: 2-(4,4′-Dihydroxydiphenyl)propane; 2,2-Bis(4-hydroxyphenyl)propane; 2,2-Bis(hydroxyphenyl)propane; 2,2-Bis(p-hydroxyphenyl)propane; 2,2-Bis-4′-hydroxyfenylpropan [Czech]; 2,2-Di(4-hydroxyphenyl)propane; 2,2-Di(4-phenylol)propane; 4,4′-(1-Methylethylidene)bisphenol; 4,4′-Bisphenol A; 4,4′-Dihydroxydiphenyl-2,2-propane; 4,4′-Dihydroxydiphenyldimethylmethane; 4,4′-Dihydroxydiphenylpropane; 4,4′-Isopropylidene diphenol; 4,4′-Isopropylidenebisphenol; 4,4′-Isopropylidene diphenol; Biphenol A; Bis(4-hydroxyphenyl) dimethylmethane; Bis(4-hydroxyphenyl)dimethylmethane; Bis(4-hydroxyphenyl)propane; Bisferol A [Czech]; Bisphenol. Bisphenol A; DIAN; Diano; Dimethyl bis(p-hydroxyphenyl)methane; Dimethylbis (p-hydroxyphenyl)methane; Dimethylmethylene-p,p′-diphenol; Diphenylolpropane; Ipognox 88; Isopropylidenebis(4-hydroxybenzene); Parabis A, Phenol; (1-methylethylidene)bis-, Phenol; 4,4′-(1-methylethylidene)bis-; Phenol, 4,4′-dimethylmethylenedi-; Phenol, 4,4′-isopropylidenedi-; Pluracol 245, Propane; 2,2-bis(p-hydroxyphenyl)-; Rikabanol; Ucar bisphenol A; Ucar bisphenol HP; beta,beta′-Bis(p-hydroxyphenyl)propane; beta-Di-p-hydroxyphenylpropane; p,p′-Bisphenol A; p,p′-Dihydroxydiphenyldimethylmethane; p,p′-Dihydroxydiphenylpropane; p,p′-Isopropylidenebisphenol; and p,p′-Isopropylidenediphenol.

1.1.2 Formula and molecular mass. Bisphenol A has a molecular mass of 228.29 g/mol and a molecular formula of $C_{15}H_{16}0_2$ (European-Union, 2003). The structure for bisphenol A is shown in Figure 1.

1.1.3 Chemical and physical properties. Bisphenol A is a white solid with a mild phenolic odor (European-Union, 2003). Physicochemical properties are listed in Table 1.

1.1.4 Technical products and impurities. Purity of bisphenol A was reported at 99–99.8%, and common impurities observed were phenol and ortho and para isomers of bisphenol A [reviewed in (European-Union, 2003)]. Terasaki et al. (2004) used reversed phase chromatography and nuclear magnetic resonance spectroscopy to characterize the composition of 5 commercial bisphenol A samples. The nominal purity of the samples was 97 or 98%. Actual purities were 95.3 to >99%. Up to 15 contaminants were identified among which were: 4-hydroxyacetophenone; 4,4′-(1,3-dimethylbutylidene) bisphenol; *p*-cumylphenol; 4-hydroxyphenyl isobutyl methyl ketone; 2,4*-dibhydroxy-2,2-diphenylpropane; 2,4′-dibhydroxy-2,2-diphenylpropane; 2,4-bis(4-hydroxy-

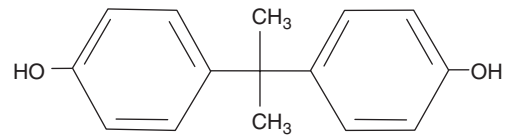

Fig. 1. Structure for bisphenol A.

Table 1
Physicochemical Properties of Bisphenol A[a]

Property	Value
Odor threshold	No data found
Boiling point	220°C at 4 mm Hg; 398°C at 760 mm Hg
Melting point	150–157°C
Specific gravity	1.060–1.195 g/mL at 20–25°C
Solubility in water	120–300 mg/L at 20–25°C
Vapor pressure	8.7×10^{-10} –3.96×10^{-7} mm Hg at 20–25°C
Stability/reactivity	No data found
Log K_{ow}	2.20–3.82
Henry constant	1.0×10^{-10} atm m^3/mol

[a]Staples et al. (1998).

[2]The Core Committee is an advisory body consisting of scientists from government agencies. Agencies currently represented are: Environmental Protection Agency, Centers for Disease Control and Prevention, Food and Drug Administration, Consumer Product Safety Commission, National Institute for Occupational Safety and Health, and National Institute of Environmental Health Sciences.

Prepared with the Support of CERHR Staff: NTP/NIEHS, Michael D. Shelby, Ph.D. (Director, CERHR), Paul M.D. Foster, Ph.D. (Deputy Director, CERHR), Kristina Thayer, Ph.D. (CERHR), Diane Spencer, M.S. (CERHR), John Bucher, Ph.D. (Associate Director, NTP), Allen Dearry, Ph.D. (Interim Associate Director, NTP), Mary Wolfe, Ph.D. (Director, NTP Office of Liaison, Policy & Review), Denise Lasko (NTP Office of Liaison, Policy & Review); Sciences International, Inc., Anthony Scialli, M.D. (Principal Scientist), Annette Iannucci, M.S. (Toxicologist), Gloria Jahnke, D.V.M. (Toxicologist), and Vera Jurgenson, M.S. (Research Assistant).

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The findings and conclusions of this report are those of the Expert Panel and should not be construed to represent the views of the National Toxicology Program. Members of this panel participated in the evaluation of bisphenol A as independent scientists. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of their employers.

cumyl)phenol; 2,3-dihydro-3-(4′-hydroxyphenyl)-1,1,3-trimethyl-1*H*-inden-5-ol; 2-(4′-hydroxyphenol)-2,2,4-trimethylchroman; and 4-(4′-hydroxyphenol)-2,2,4-trimethylchroman (Terasaki et al., 2005).

No information on trade names for bisphenol A was located.

1.1.5 Analytical considerations. Measurement of bisphenol A in environmental and biologic samples can be affected by contamination with bisphenol A in plastic laboratory ware and in reagents (Tsukioka et al., 2004; Völkel et al., 2005). Accuracy is also affected by measurement technique, particularly at the very low concentrations that can now be measured. Enzyme-linked immunosorbent assay (ELISA) has poor correlation with the LC-ECD method and also the different ELISA kits correlate poorly with each other. ELISA methods may overestimate bisphenol A in biologic samples due to lack of specificity of the antibody and effects of the biologic matrix (Inoue et al., 2002; Fukata et al., 2006). Although high performance liquid chromatography (HPLC) with ultraviolet, fluorescence, or electrochemical detection can be sensitive to concentrations <0.5 ng/ml (Sajiki et al., 1999; Inoue et al., 2000; Kuroda et al., 2003; Sun et al., 2004), these methods are unable to make definitive identification of bisphenol A or bisphenol A glucuronides, because similar retention times may occur for the metabolites of other endogenous and exogenous compounds (Völkel et al., 2005). Use of LC-mass spectrometry (MS) with and without hydrolysis of bisphenol A glucuronide permits determination of free and total bisphenol A with a limit of quantification of 0.1 for MS (Sajiki et al., 1999) and 1 μg/L for MS/MS (Völkel et al., 2005). Gas chromatography (GC)/MS has been used with solid phase extraction after treatment with glucuronidase and derivatization to measure total bisphenol A with a limit of detection of 0.05 μg/L for MS (Tan and Mohd, 2003) and 0.1 μg/L for MS/MS (Calafat et al., 2005). Some of the variability in studies cited in this and subsequent sections may be due to differences in measurement techniques and to contamination. Bisphenol A glucuronidate can be an unstable product that can be degraded in acidic and basic pH solutions and can be hydrolyzed to free bisphenol A at neutral pH and room temperature in diluted rodent urine, placental and fetal tissue homogenates at room temperature. However, conjugates in urine are stable for at least 7 days when stored at $-4°C$ and at least 180 days when stored at $-70°C$ (Waechter et al., 2007; Ye et al., 2007).

1.2 Use and Human Exposure

1.2.1 Production information. Bisphenol A is manufactured by the acid catalyzed condensation of phenol and acetone (SRI, 2004).

In 1998, members of the Society of the Plastics Industry Bisphenol A Task Group **[assumed manufacturers of bisphenol A]** included Aristech Chemical Corporation, Bayer Corporation, Dow Chemical Company, and Shell Chemical Company (Staples et al., 1998). Current manufacturers of bisphenol A in the U.S. are Bayer MaterialScience, Dow Chemical Company, General Electric, Hexion Specialty Chemicals, and Sunoco Chemicals (SRI, 2004) (S. Hentges, public comments, February 2, 2007). There are currently six bisphenol A and four polycarbonate plants in the U.S. (S. Hentges, personal communication, October 30, 2006); three of four polycarbonate plants are located within bisphenol A plants. In 2000, there were 13 epoxy plants in the U.S., but was not clear if all of the plants manufactured bisphenol A-containing epoxy resins.

In mid-2004, U.S. bisphenol A production volume was reported at 1.024 million metric tons **[∼2.3 billion pounds]** (SRI, 2004). A production volume of 7.26 billion g **[16 million pounds]** was reported for bisphenol A in 1991 (reviewed in HSDB, 2003). United States bisphenol A consumption was reported at 856,000 metric tons **[∼1.9 billion pounds]** in 2003 (SRI, 2004); 2003 consumption patterns included 619,000 metric tons **[∼1.4 billion pounds]** used in polycarbonate resins, 184,000 metric tons **[∼406 million pounds]** used in epoxy resins, and 53,000 metric tons **[∼117 million pounds]** used in other applications.

1.2.2 Use. In 1999 and 2003, it was reported that most bisphenol A produced in the U.S. was used in the manufacture of polycarbonate and epoxy resins and other products [reviewed in (Staples et al., 1998; SRI, 2004)]. Polycarbonate plastics may be used in the manufacture of compact discs, ''solid and multi wall sheet in glazing applications and film,'' food containers (e.g., milk, water, and infant bottles), and medical devices [reviewed in (European-Union, 2003)]. Bisphenol A may have been used at one time in Europe in polyvinyl chloride cling film and plastic bags, but that use is believed to have been discontinued (European Food Safety Authority, 2006). Contact with drinking water may occur through the use of polycarbonate for water pipes and epoxy-phenolic resins in surface coatings of drinking water storage tanks [reviewed by (European Food Safety Authority, 2006)].

Polycarbonate blends have been used to manufacture injected molded parts utilized in alarms, mobile phone housings, coil cores, displays, computer parts, household electrical equipment, lamp fittings, and power plugs. Automotive and related uses for polycarbonate blends include light reflectors and coverings, bumpers, radiator and ventilation grills, safety glazing, inside lights, and motorcycle shields and helmets. Epoxy resins are used in protective coatings, structural composites, electrical laminates, electrical applications, and adhesives. The European Union (2003) reported that smaller volumes of bisphenol A are used in production of phenoplast, phenolic, and unsaturated polyester resins, epoxy can coatings, polyvinyl chloride (PVC) plastic, alkoxylated bisphenol A, thermal paper, and polyols/polyurethane. Other uses reported for products manufactured from bisphenol A included protective window glazing, building materials, optical lenses, and development of dyes [reviewed in (Staples et al., 1998)]. A search of the National Library of Medicine Household Products Database (NLM, 2006) revealed that bisphenol A-based polymers are used in coatings, adhesives, and putties available to the general pubic for use in automobiles, home maintenance and repair, and hobbies, but only 3 epoxy products, used for crafts and hobbies, contain bisphenol A itself.

Some polymers manufactured with bisphenol A are Food and Drug Administration (FDA)-approved for use in direct and indirect food additives and in dental materials, as reported in the Code of Federal Regulations (CFR) (FDA, 2006). In the CFR, bisphenol A is often

referred to as 4,4′-isopropylidnediphenol. Polymers manufactured with bisphenol A are FDA-approved for use as anoxomers and in coatings, adhesives, single and repeated food contact surfaces, and tooth shade resin materials.

The European Union (2003) noted that resins, polycarbonate plastics, and other products manufactured from bisphenol A can contain trace amounts of residual monomer and additional monomer may be generated during breakdown of polymer. The American Plastics Council reports that residual bisphenol A concentrations in polycarbonate plastics and epoxy resins are generally <50 ppm (S. Hentges, personal communication, October 30, 2006). Polymer hydrolysis can occur at elevated temperature or extreme pH. An example of potential human exposure is migration of bisphenol A from a food container into the food. Exposure to bisphenol A through food is discussed in detail in Section 1.2.3.2.

1.2.3 Occurrence

1.2.3.1 Environmental fate and bisphenol A levels in environment: Bisphenol A may be present in the environment as a result of direct releases from manufacturing or processing facilities, fugitive emission during processing and handling, or release of unreacted monomer from products (European-Union, 2003). According to the Toxics Release Inventory database, total environmental release of bisphenol A in 2004 was 181,768 pounds, with releases of 132,256 pounds to air, 3533 pounds to water, 172 pounds to underground injection, and 45,807 pounds to land (TRI, 2004).

Bisphenol A released to the atmosphere is likely degraded by hydroxy radicals (European-Union, 2003). Half-life for the reaction between bisphenol A and hydroxy radicals was estimated at 0.2 days. It was also noted that photolysis and photodegradation of bisphenol A in the atmosphere is possible and photo-oxidation half-lives of 0.74–7.4 hr were estimated [reviewed in (Staples et al., 1998; European-Union, 2003)]. The European Union (2003) noted that because of its low volatility and relatively short half-life in the atmosphere, bisphenol A is not likely to enter the atmosphere in large amounts. Removal by precipitation and occurrence in rain water were thought likely to be negligible. Because of its short half-life in the atmosphere, bisphenol A is unlikely to be transported far from emission points.

Based on vapor pressure and Henry constant (Table 1), the European Union (2003) and Staples et al. (1998) concluded that bisphenol A is of low volatility and not likely to be removed from water through volatilization. Both groups concluded that hydrolysis of bisphenol A in water is unlikely. However, there was disagreement on potential for photo-oxidation of bisphenol A in water. Based on physical and chemical properties, the European Union concluded that photolysis of bisphenol A in water is unlikely. Staples et al. (1998) noted that bisphenol A is able to absorb ultraviolet light, especially in a basic solution. Therefore, it was concluded that photolysis from surface water is possible, depending on conditions such as pH, turbidity, turbulence, and sunlight. Photo-oxidation half-life of bisphenol A in water was estimated at 66 hr to 160 days [reviewed in (Staples et al., 1998)]. Rapid biodegradation of bisphenol A from water was reported in the majority of studies reviewed by the European Union (2003) and Staples et al. (1998). A biodegradation half-life of 2.5–4 days was reported in a study measuring bisphenol A concentrations in surface waters near the receiving stream of a bisphenol A manufacturer [reviewed in (Staples et al., 1998)].

When the Staples et al. (1998) review was published, soil sorption constants had not been measured but were estimated at 314–1524. Based on such data, the European Union (2003) and Staples et al. (1998) concluded that bisphenol A adsorption to soils or sediments would be ''modest'' or ''moderate.'' Based on data for degradation of bisphenol A in water, the European Union (2003) predicted that bisphenol A would be degraded in soil and estimated a half-life of 30 days for degradation of bisphenol A in soil. Subsequent to the Staples et al. (1998) and European Union (2003) reviews, a study examining fate of ^{14}C-bisphenol A in soils through laboratory soil degradation and batch adsorption tests was released by Fent et al. (2003). In that study, ^{14}C-bisphenol A was dissipated and not detectable in 4 different soil types within 3 days. Soil distribution coefficients were determined at 636–931, and based on those values, the study authors concluded that bisphenol A has low mobility in soil. The study authors concluded that bisphenol A is not expected to be stable, mobile, or bioavailable from soils.

In studies reviewed by the European Union (2003) and Staples et al. (1998), bioconcentration factors for fish were measured at 3.5–68 and were found to be lower than values estimated from the $K_{ow.}$ Both groups concluded that potential for bioconcentration of bisphenol A is low in fish. Higher bioconcentration factors (134–144) were determined for clams [reviewed in (European-Union, 2003)].

Two studies examining aggregate exposures in preschool age children in the U.S. used GC/MS to measure bisphenol A concentrations in environmental media (Wilson et al., 2003, 2006). In the first study (Wilson et al., 2003), bisphenol A concentrations were measured in air outside 2 day care centers and the homes of 9 children. Bisphenol A was detected in 9 of 13 outdoor air samples at <0.100–4.72 ng/m^3 (mean concentration = 2.53 ng/m^3 at day care centers; 1.26 ng/m^3 at home). In indoor air from day care centers and homes, bisphenol A was detected in 12 of 13 samples at <0.100–29 ng/m^3 (mean concentration = 6.38 ng/m^3 at day care centers; 11.8 ng/m^3 at home). At those same locations, bisphenol A was detected in all of 13 samples of floor dust at means (range) of 1.52–1.95 (0.567–3.26) ppm (μg/g) and play area soils at means (range) of 0.006–0.007 (0.004–0.014) ppm (μg/g). In the second study (Wilson et al., 2006), bisphenol A concentrations were measured inside and outside at least 222 homes and 29 daycare centers. Bisphenol A was detected in 31–44% of outdoor air samples from each location; concentrations ranged from <LOD (0.9) to 51.5 ng/m^3. Medians were <limit of detection (LOD). Indoor air samples (45–73%) contained detectable concentrations of bisphenol A; concentrations were reported at <LOD (0.9)–193 ng/m^3. Median values were <LOD–1.82 ng/m^3. Bisphenol A was detected in 25–70% of dust samples; concentrations were reported at <LOD (20) to 707 ng/g. Median values were <LOD–30.8 ng/g.

A second U.S. study used a GC/MS method to measure bisphenol A concentrations in dust from 1 office building and 3 homes and in air from 1 office building and 1 home (Rudel et al., 2001). Bisphenol A was detected in 3 of 6 dust samples (reporting limit >0.01 μg/extract) at concentrations of 0.25–0.48 μg/g

Table 2
Concentrations of Bisphenol A Detected in Water

Sample type	Detection method	Detection rate (%)	Concentration (μg/L) range [median]	Reference
Surface water				
German rivers	GC-MS	100	0.005–0.014 [3.8]	Kuch and Ballschmiter (2001)
Louisiana, U.S.	GC-MS	0	<MDL 0.1	Boyd et al. (2003)
U.S. streams	GC-MS	41.2	[0.14] max 12	Kolpin et al. (2002)
Netherlands	GC-MS	78–93	Max marine 0.33 Max fresh 21	Belfroid et al. (2002)
Drinking water				
Louisiana, U.S.	GC-MS	0	<MDL 0.1	Boyd et al. (2003)
Ontario, Canada	GC-MS	0	<MDL 0.1	Boyd et al. (2003)
Germany	GC-MS	100	0.005–0.002 [1.1]	Kuch and Ballschmiter (2001)
Landfill leachate				
Japan	GC-MS	100	740	Kawagoshi et al. (2003)
Japan	GC-MS	70% sites	1.3–17, 200 [269]	Yamamoto et al. (2001)
Sewage treatment works				
Germany	GC-MS	94	0.005–0.047 [10]	Kuch and Ballschmiter (2001)
Louisiana, U.S.	GC-MS	0	<MDL 0.1	Boyd et al. (2003)

Table 3
Bisphenol A Concentrations in Human Breast Milk

Source (n)	Method	LOD	Free (ng/ml) mean ±SD (range)	Total (ng/ml) mean±SD (range)	Detection rate (%)	Reference
Japanese (23)	HPLC-Fl	0.11 ng/ml	0.61±0.20 (0.28–0.97)		100	Sun et al. (2004)
Japanese (101) (colostrum 3 days after delivery)	ELISA	NA		3.41±0.13 (1–7)	100	Kuruto-Niwa et al. (2007)
United States (20)	HPLC-MS/MS	0.3 ng/ml	1.3 (<0.3–6.3)	1.9 (<0.3–7.3)	60 free 90 total	Ye et al. (2006)
Japanese (3)	GC-MS	0.09 ng/g		0.46 (<0.09–0.65)	67	Otaka et al. (2003)
U.S. (32)	NA	NA	NA	1.4[a]	NA	Calafat et al. (2006)

[a]Estimated from a graph.

dust. In indoor air samples collected from offices and residences, bisphenol A was detected in 3 of 6 samples (detection limit = ~0.5 ng/m^3) at concentrations of 0.002–0.003 μg/m^3. In another study using a GC/MS technique, bisphenol A concentrations in indoor air from 120 U.S. homes were below reporting limits (0.018 μg/m^3) (Rudel et al., 2003). Median (range) bisphenol A concentration in dust in this study was 0.821 (<0.2–17.6) μg/g, with 86% of samples above the reporting limit.

Limited information is available for bisphenol A concentrations in U.S. water (Table 2). In 1996 and/or 1997, mean bisphenol A concentrations were reported at 4–8 μg/L in surface water samples near 1 bisphenol A production site but bisphenol A was not detected (<1 μg/L) in surface water near 6 of 7 bisphenol A production sites in the U.S. (Staples et al., 2000). Bisphenol A was detected at a median concentration (in samples with detectable bisphenol A above the reporting limit of 0.09 μg/L) of 0.14 μg/L and a maximum concentration of 12 μg/L in 41.2% of 85 samples collected from U.S. streams in 1999 and 2000 (Kolpin, 2002). In 2001 and 2002, bisphenol A was not detected (<0.001 μg/L) in effluent from a wastewater treatment plant in Louisiana, and concentrations were not quantifiable **[quantification limit not defined]** in samples collected from surface waters in Louisiana and in drinking water at various stages of treatment at plants in Louisiana and Ontario, Canada (Boyd et al., 2003). In water samples collected in Europe and Japan from the 1970 s through 1989, bisphenol A concentrations were ≤1.9 μg/L and in most cases were ≤0.12 μg/L [reviewed in (European-Union, 2003)].

1.2.3.2 Potential exposures from food and water: The European Union (2003) noted that the highest potential for human exposure to bisphenol A is through products that directly contact food. Examples of food contact materials that can contain bisphenol A include food and beverage containers with internal epoxy resin coatings and polycarbonate tableware and bottles, such as those used to feed infants.

In addition to commercial food sources, infants consume breast milk. Calafat et al. (2006) reported a median bisphenol A concentration of ~1.4 μg/L **[as estimated from a graph]** in milk from 32 women (Table 3). Bisphenol A was measured after enzymatic hydrolysis of conjugates. Ye et al. (2006) found measurable concentrations of bisphenol A in milk samples from 18 of 20 lactating women. Free bisphenol A was found in samples from 12 women. The median total bisphenol concentration in milk was 1.1 μg/L (range: undetectable to 7.3 μg/L). The median free bisphenol A concentration was 0.4 μg/L (range: undetectable to 6.3 μg/L). Sun et al. (2004) used an HPLC method to measure bisphenol A

concentrations in milk from 23 healthy lactating Japanese women. Bisphenol A concentrations ranged from 0.28–0.97 μg/L, and the mean ± SD concentration was reported at 0.61 ± 0.20 μg/L. No correlations were observed between bisphenol A and triglyceride concentrations in milk. Values from 6 milk samples were compared to maternal and umbilical blood samples reported previously in a study by Kuroda et al. (2003). Bisphenol A values were higher in milk, and the milk/serum ratio was reported at 1.3. Bisphenol A values in milk were comparable to those in umbilical cord serum. **[It was not clear whether milk and serum samples were obtained from the same volunteers in the two studies.]**

Studies have measured migration of bisphenol A from polycarbonate infant bottles or containers into foods or food simulants. Results of those studies are summarized in Table 4. Analyses for bisphenol A were conducted by GC/MS or HPLC. The European Union (2003) group noted that in many cases bisphenol A concentrations were below the detection limit in food simulants. When bisphenol A was detected, concentrations were typically ≤50 μg/L in simulants exposed to infant bottles and ≤5 μg/kg in simulants exposed to polycarbonate tableware. An exception is one study that reported bisphenol A concentrations at up to ~192 μg/L in a 10% ethanol food simulant and 654 μg/L in a corn oil simulant (Onn Wong et al., 2005). In the study, cut pieces of bottles were incubated, and the study authors acknowledged that bisphenol A could have migrated from the cut edges. **[The Expert Panel notes that incubations were at 70 or 100°C for 240 hr, representing conditions not anticipated for normal use of baby bottles.]** One study conducted with actual infant food (formula and fruit juice) reported no detectable bisphenol A (Mountfort et al., 1997). Some studies examining the effects of repeated use of polycarbonate items noted increased leaching of bisphenol A with repeated use (Earls, 2000; Brede et al., 2003; CSL, 2004). It was suggested that the increase in bisphenol A migration was caused by damage to the polymer during use. Results from other reports suggested that leaching of bisphenol A decreased with repeated use, and it was speculated that available bisphenol A was present at the surface of the product and therefore removed by washing (Biles et al., 1997b;

Table 4
Examination of Bisphenol A in Polycarbonate Food Contact Surfaces

Sample (location)	Procedure	Bisphenol A concentration in simulant	Reference
Commercially available infant bottles containing residual bisphenol A concentrations of 7–46 ppm (U.S.)	Common use: bottles were boiled for 5 min, filled with water or 10% ethanol, and stored at room temperature for up to 72 hr Worst case use: bottles were boiled for 5 min, filled with water or 10% ethanol, heated to 100°C for 0.5 hr, cooled to room temperature, and refrigerated for 72 hr	ND (LOD 5 ppb **[μg/L]**; corresponding to a food concentration of 1.7 ppb) following either procedure	FDA (1996)
21 new and 12 used (1–2-year-old) infant bottles (U.K.)	Bottles were pre-washed, steam sterilized, filled with boiling water or 3% glacial acetic acid, refrigerated at 1–5°C for 24 hr, and heated to 40°C before sampling	ND (LOD 10 μg/L) **[ppb]** from new bottles; ND (<10 μg/L) to 50 μg/L from used bottles exposed to either simulant **[mean not given]**	Earls et al. (2000)
Infant bottles with residual bisphenol A concentrations of 26 mg/kg **[number tested not indicated]** (U.K.)	Bottles were sterilized with hypochlorite, in dishwasher, or by steam; filled with infant formula, fruit juice, or distilled water; microwaved for 30 sec and left to stand for 20 min (1 cycle); samples were analyzed after 3, 10, 20, or 50 cycles; other bottles were filled with distilled water and left to stand for 10 days at 40°C	ND (LOD 0.03 mg/kg) **[<30 μg/kg or ppb]** under any condition	Mountfort et al. (1997)
6 infant feeding bottles (country of purchase not known)	Bottles were filled with water at 26°C and left to stand for 5 hr or filled with water at 95°C and left to stand overnight	ND (LOD 2 ppb **[μg/L]**) in bottles filled with water at 26°C and 3.1–55 ppb **[μg/L]** in bottles filled with water at 95°C.	Hanai (1997)[a]
14 samples of new infant feeding bottles and tableware including a bowl, mug, cup, and dish recalled because residual bisphenol A and other phenol concentrations exceeded 500 ppm **[mg/kg]** (Japan)	Products were exposed to *n*-heptane, water, 4% acetic acid, or 20% ethanol; in some cases simulant was heated to 60 or 95°C; in other cases, the object was boiled for 5 min; analyses were usually conducted after a 30-min contact period	Up to 40 ppb **[μg/kg]** from recalled products and ND (LOD 0.2) to 5 μg/kg from commercially available products.	Kawamura et al. (1999)[a,b]
Discs prepared from commercial food-grade polycarbonate resins	Materials exposed to water, 10% ethanol, or Miglyol (fractionated coconut oil) at 100°C for 6 hr or water, 3% acetic acid,	ND (LOD 5 ppb **[μg/L]**) under all conditions.	Howe and Borodinsky (1998)

Table 4
Continued

Sample (location)	Procedure	Bisphenol A concentration in simulant	Reference
(residual bisphenol A at 8800 to 11,200 μg/kg) from U.S. manufacturers	10% ethanol, or Migloyl at 49°C for 6–240 hr		
2 infant bottles from Japan	In three repeated tests, boiling water was added to bottles; bottles were incubated at 95°C for 30 min and cooled to room temperature; before repeating the test a fourth time, the bottles were scrubbed with a brush	Below quantification limit (LOD 0.57 ppb **[μg/L]**) to mean concentrations of 0.75 ppb before brushing and <0.57 to 0.18 ppb after brushing.	Sun et al. (2000)
4 new different brands of infant bottles (Argentina)	Bottles were exposed to distilled water, 3% acetic acid, or 15% ethanol at 80°C for 2 min or distilled water at 100°C for 0.5 min	1.1–2.5 ppb **[μg/L]**.	D'Antuono et al. (2001)
12 infant bottles (Norway)	Bottles were tested before washing and following 51 and 169 dish washings; bottles were occasionally brushed (13 times by second test and 23 times by third test) and boiled (12 times by second testing and 25 times by third testing); unwashed bottles were rinsed with boiling water before testing; for testing, bottles were filled with hot water and incubated at 100°C for 1 hr	Mean (range) μg/L **[ppb]:** 0 washes: 0.23 (0.11–0.43) 51 washes: 8.4 (3.7–17) 169 washes: 6.7 (2.5–15)	Brede et al. (2003)
18 infant bottles (12 tested) (U.K.)	Bottles were tested before and after 20 and 50 dish washings; bottles were brushed after every 2 wash cycles; bottles were sterilized with boiling water, filled with 3% acetic acid, or 10% ethanol, and incubated at 70°C for 1 hr	Before washing: ND (LOD 1.1 ppb or μg/L) in 10% ethanol and ND (LOD 0.34 ppb or μg/L) in 3% acetic acid; 20 washes: ND to 4.5 ppb in 10% ethanol and ND to 0.51 ppb in 3% acetic acid; 50 washes: ND to 3.1 ppb in 10% ethanol and ND to 0.7 ppb in 3% acetic acid	CSL (2004)
28 brands of new infant bottles (residual bisphenol A concentrations of <3 to 141 mg/kg) manufactured in Europe or Asia (Singapore)	Bottles were cut and pieces were exposed to 10% ethanol at 70°C or corn oil at 100°C for 8–240 hr	ND (LOD 0.05) to 1.92 μg/in^2 **[<5–192 μg/L or ppb]** in 10% ethanol and ND (LOD 0.05) to 6.54 μg/in^2 **[<5–654 μg/L]** in corn oil over the 240-hr exposure period	Onn Wong et al. (2005)
22 new infant bottles and 20 used (3–36 months) bottles (Netherlands)	Bottles were immersed in boiling water for 10 min before testing and filled with distilled water or 3% acetic acid and incubated at 40 °C for 24 hr	ND in new bottles (<2.5 μg/L (LOD) **[ppb]** in distilled water and <3.9 μg/L (LOD) in 3% acetic acid) or in used bottles exposed to 3% acetic acid; not detected to non-quantifiable (<5 μg/L) in distilled water from used bottles.	FCPSA (2005)
New unwashed infant bottles (number not indicated) (Japan)	Bottles were exposed to water at 95°C for 30 min	ND (LOD 0.05 μg/L **[ppb]**) to 3.9 μg/L.	Japanese studies reviewed in Miyamoto and Kotake (2006)
5-gallon water carboys	Water was stored in the carboys for 3, 12, or 39 weeks, temperature not indicated	0.1–0.5 μg/L **[ppb]** at 3 and 12 weeks and. 4.6–4.7 μg/L at 39 weeks[c]	Biles et al. (1997b)

[a]Reviewed by European Union (2003).
[b]Reviewed by Haighton et al. (2002).
[c]The authors of this study identified an error in the units reported in their study and that the correct concentrations are 1000-fold higher than indicated in the article, the correct values are indicated in table above (T. Begley, email communication, August 6, 2007).
ND, not detected.

Kawamura et al., 1999; Haighton et al., 2002; European Union, 2003). One study (Kawamura et al., 1999) showed higher concentrations of bisphenol A in simulants exposed to products that had been recalled because of unacceptable residual concentrations of bisphenol A and other compounds. The study by Biles et al. (1997b)

Table 5
Surveys of Bisphenol A Concentrations in Canned Infant Formulas or Food

Food (no. sampled)[a]	Bisphenol A concentration μg/kg or μg/L	Country	Reference
Infant formula (14)	Mean 5 (0.1–13.2 ppb **[μg/L]**); when diluted with water to make prepared formula mean concentrations would be 2.5 (0.05–6.6)	U.S.	Biles et al. (1997a) FDA (1996)
Infant formula (4)	ND (LOD 2 μg/kg)	U.K.	Goodson et al. (2002) UKFSA (2001)
Infant formula (5)	44–113 μg/kg	Taiwan	Kuo and Ding (2004)
Infant dessert (3)	18.9–77.3 μg/kg	U.K.	Goodson et al. (2002)
Infant vegetable food (4)	<LOQ (LOQ 10 μg/kg)	New Zealand	Thomson and Grounds (2005)
Infant dessert (3)	<LOQ (LOQ 10 μg/kg)		

[a]Values before and after heating in can and from non-dented and dented cans; values did not differ under the various conditions and were presented together.
ND, not detected.

demonstrated that infant bottles exposed to 50 or 95% ethanol at 65°C for 240 hr leached bisphenol A at concentrations exceeding residual monomer concentrations, and it was suggested that hydrolysis of the polymer had occurred.

High molecular weight, heat-cured bisphenol A-based epoxy resins are used as protective linings in cans for food and beverages and may be used in wine storage vats (European-Union, 2003). Residual bisphenol A monomer can migrate from the coatings to foods or beverages contained within cans. Studies were conducted to measure actual concentrations of bisphenol A in commercially available foods or to measure concentrations of bisphenol A leaching from can linings into food simulants. Because the actual measurement of bisphenol A concentrations in canned foods represents the most realistic situation, the CERHR review will focus on those data. Studies conducted with simulants will not be reviewed, with the exception of one study by Howe et al. (1998) that was considered by the FDA (1996) in their estimates of bisphenol A intake.

Bisphenol A concentrations detected in infant foods are summarized in Table 5, and bisphenol A concentrations detected in non-infant foods are summarized in Table 6. With the exception of isolated cases in which bisphenol A concentrations were measured at up to ~0.8 mg/kg food, most measurements were below 0.1 mg/kg. The European Union also noted an extraction study conducted with an epoxy resin that is occasionally used to line wine vats. Based on that study, a worst-case scenario of 0.65 mg/L bisphenol A in wine was used. The European Union noted that the value represents a very worst-case exposure scenario but decided to use that number in risk estimates because no other value was available. **[The Expert Panel notes that a study of bisphenol A in wine (Brenn-Struckhofova and Cichna-Markl, 2006) identified a maximum concentration of 2.1 μg/L (Table 6).]**

In one study, empty cans were filled with soup, beef, evaporated milk, carrots, or 10% ethanol (Goodson et al., 2004). The cans were then sealed, processed at 5, 20, or 40°C, and sampled at 1 or 10 days or 1, 3, or 9 months. Half the cans processed according to each condition were dented. It was determined that 80–100% of the bisphenol A migrated to food immediately after processing, and that bisphenol A concentrations did not change during storage or as a result of denting. The study authors concluded that most migration occurred during can processing. Boiling the cans or heating to 230°C did not increase migration of bisphenol A, but that finding appears to contrast with findings of others. Kang et al. (2003) examined the effects of temperature, duration of heating, glucose, sodium, and oil on migration of bisphenol A from cans. In cans filled with water, heating to 121°C compared to 105°C increased migration of bisphenol A but the duration of heating had no significant effect. Compared to cans filled with water, increased amounts of bisphenol A migrated from cans filled with 1–10% sodium chloride, 5–20% glucose, or vegetable oils and heated to 121°C. Takao et al. (2002) reported increased leaching of bisphenol A from cans into water when the cans were heated to ≥80°C.

A study examining aggregate exposures of U.S. preschool age children measured bisphenol A concentrations in liquid food and solid food served to the children at home and at child care centers (Wilson et al., 2003). Duplicate plates of food served to nine children were collected over a 48-hr period. GC/MS analyses were conducted on four liquid food samples and four solid food samples from the child care center and nine liquid food samples and nine solid food samples from home. Bisphenol A was detected in all solid food samples, three liquid food samples from the child care center, and two liquid food samples from the home. Concentrations of bisphenol A ranged from <0.100–1.16 ng/g **[μg/kg]** in liquid foods and from 0.172–4.19 ng/g **[μg/kg]** in solid food.

The study examining aggregate exposures of U.S. preschool age children was repeated with a larger sample and again measured bisphenol A concentrations in liquid food and solid food served to the children at home and at child care centers (Wilson et al., 2006). Bisphenol A concentrations were measured by GC/MS in food served over a 48-hr period to at least 238 children at home and 49 children at daycare centers. Bisphenol A was detected in 83–100% of solid food samples; concentrations were reported at <LOD (0.8) to 192 ng/g **[μg/kg]**. Sixty-nine to 80% of liquid food contained detectable concentrations of bisphenol A; concentrations were reported at <LOD (0.3)–17.0 ng/mL in liquid food. Data were also collected for hand wipes of 193 children at daycare centers and 60 children at home. Bisphenol A was detected in 94–100% of

Table 6
Surveys of Bisphenol A Concentrations in Canned or Bottled Foods or Food Simulants

Food (no. sampled)	Bisphenol A concentration (range in μg/kg unless specified)	Country of purchase[a]	Reference
Vegetables with liquid (6)	Mean (range) 16 (4–39)	U.S.	FDA (1996)
Liquids from canned vegetables or mushrooms (10)	4.2 ± 4.1 (SD) –22.9 ± 8.8 μg/can [12 ± 12–76 ± 29 μg/kg]	Spain, U.S.	Brotons et al. (1995)
Coffee (13)	ND–213 [median = 11] (LOD 2)	Japan	Kawamura et al. (1999) (reviewed in European-Union, 2003; English abstract available)
Black tea (9)	ND–90 [median = <2] (LOD 2)		
Other tea (8)	ND–22 [median = 5.7] (LOD 2)		
Alcoholic beverages (10)	ND except for 1 sample with 13 (LOD 2)		
Soft drinks (7)	ND (LOD 2)		
Vegetables (10)	9–48 [median = 21]	U.K.	Goodson et al. (2002) UKFSA (2001)
Desserts (5)	ND (LOD 2)–14 [median = 10]		
Fruits (2)	19 and 38		
Pastas (5)	ND–41 [median = 11] (LOD 7)		
Meats (5)	16–422[b] [median = 52]		
Fish (10)	ND–44 [median = 16.8] (LOD 2)		
Non-alcoholic or alcoholic beverages (11)	ND except for 1 sample above LOD (LOD 2) but below LOQ (7)		
Soups (10)	ND–21 [median = <2] (LOD 2)		
Vegetables, fruits, or mushrooms (14)	ND (LOD 10)–95.3 in solid portion; ND (LOD 0.005 ug/mL)–0.004 μg/mL in liquid portion; ND–11.1 μg/can [85 μg/kg] total		Yoshida et al. (2001)
Meat products[d] (2)	8.6–25.7	U.K.	Goodson et al. (2004)
Pasta[d] (1)	67.3–129.5		
Vegetables or beans[c](2)	11.3–14.4		
Soup[c] (1)	18.5–39.1		
Pudding[c] (3)	3.8–53.2		
Pudding[d] (1)	18.5–28.1		
Grains and potatoes[e]	0[f] –75 [mean not given]	Japan	Miyamoto and Kotake (2006)
Sugar, sweets, snacks[e]	0[f]–4 [mean not given]		
Fats[e]	0[f]		
Fruits (including canned drinks), vegetables, mushrooms, seaweeds[e]	0[f]–450 [mean not given]		
Seasoning and beverages[e]	0[f]–213 [mean not given]		
Fish	9–480 [mean not given]		
Meat and eggs[e]	12.5–602 [mean not given]		
Milk and dairy products[e]	0[c]–6 [mean not given]		
"Other" [not specified further][e]	36–310 [mean not given]		
Canned fish (7)	1–23 [median = 6]	Japan	Sajiki et al. (2007)
Canned meat (5)	4–20 [median = 10]		
Canned fruit (3)	ND (LOD 0.2)		
Canned vegetables (13)	3–78 [median = 15]		
Canned soup (12)	1–156 [median = 15]		
Canned sauce (6)	ND (LOD 0.2)–842 [median = 220]		
Canned coconut milk	56–247		
Drinks in plastic containers (3)	ND (LOD 0.2) to 1 [median = 0.3]	Japan	Sajiki et al. (2007)
Cookies in plastic containers (4)	1–14 [median = 3.5]		
Soup in plastic containers (2)	ND (LOD 0.2) and 3		
Fast food sandwiches (3)	3 (all values)		
Food in paper containers (16)	ND (LOD 0.2)–1 [median = <0.2]		
Fruits and vegetables (38)	ND (LOQ 10)–24 [median = <10]	New Zealand	Thomson and Grounds (2005)
Fish (8)	ND (LOQ 20)–109 [median = <20–24]		
Soup (4)	ND (LOQ 10)–16 [median = <20]		
Sauces (4)	ND (LOQ 10) –21 [median = 16]		
Meat (6)	ND (LOQ 20)–98 [median = <20]		
Pasta (4)	ND (LOQ 10)		
Dessert (2)	ND (LOQ 20)		
Coconut cream (3)	ND (LOQ 20)–192 [median = 29]		

Table 6
Continued

Food (no. sampled)	Bisphenol A concentration (range in μg/kg unless specified)	Country of purchase[a]	Reference
Soft drinks (4)	ND (LOQ 10)		
Beverages (7)	ND (LOD 0.9)–3.4 [median = 0.4]	Austria	Braunrath et al. (2005)
Vegetables (6) (only solid portion was analyzed, with the exception of tomatoes)	8.5–35 [median = 26]		
Fruits (4)	5–24 [median = 6.6]		
Canned fat-containing products such as soups, meats, and cream (9)	2.1–37.6 [median = 20.7]		
Tuna (9)	<ND (LOQ 7.1)–102.7 [median = 11.2]	Mexico	Munguía-López et al. (2005)
Beverage/beer cans exposed to 10% ethanol at 150°F [65.6°C] for 30 min and then 120°F [48.9°C] for 10 days	ND (LOD 5)	U.S.	Howe et al. (1998) FDA (1996)
Food cans exposed to 10 or 95% ethanol at 250°F [121°C] for 2 hr and then 120°F [48.9°C] for 10 days or at 212°F [100°C] for 30 min and then 120°F [48.9°C] for 10 days	ND (LOD 5)–95 (mean = 37)[g]		
Honey (107 samples; ~90% imported in epoxy-lined drums)	ND (LOD 2)–33.3 [median = <2]	Japan	Inoue et al. (2003b)
Wine stored in steel, wood, or plastic vats, filled into glass bottles, or purchased in local markets (59)	<LOQ (0.2 ng/mL) to 2.1 μg/L; mean 0.58 in samples above the LOQ	Austria	Brenn-Struckhofova and Chichna-Markl (2006)
Solid food (309)	ND (< ~0.8)–192 [median = 3.52–4.32]	U.S.	Wilson et al. (2006)
Liquid food (287)	ND (< ~ 0.3)–17.0 [median = 0.45–0.79]	U.S.	Wilson et al. (2006)

[a]Although cans were purchased in one or two countries for each study, most studies reported that cans were packaged in various locations throughout North America, Europe, or Asia.
[b]The UKFSA noted that the higher concentrations of bisphenol A detected in one meat product likely resulted from the use of bisphenol A as a cross-linking agent in the resin at that time.
[c]Values were obtained from heated and non-heated cans but presented together because it could not be determined if heating resulted in differing extraction rates.
[d]Values were determined before and after heating in can and from non-dented and dented cans; because the values did not differ under the various conditions, they were presented together.
[e]Total number of samples analyzed was not reported.
[f]As reported by study authors; detection limits not specified.
[g]A maximum concentration of 121 ppb reported in the first phase of the study was determined to have resulted from analytical interference.
ND, not detected.

handwipe samples; concentrations ranged from <LOD **[not defined]** to 46.6 ng/cm^2. Bisphenol A was detected in 85–89% of food preparation surface wipes from homes; concentrations were reported at <LOD **[not defined]** to 0.357 ng/cm^2.

A review by Miyamoto and Kotake (2006) reported bisphenol A concentrations of 0.011–0.086 mg/kg in non-canned foods such as fats, fruits, fish, meat, and eggs. However, one study used GC-MS to examine bisphenol A in 14 types of produce purchased in southern Italy (Vivacqua et al., 2003). Bisphenol A concentrations were below the detection limit **[not reported]** in 5 produce samples. In the remaining samples, bisphenol A was detected at concentrations of 0.25 ± 0.02 (SD) to 1.11 ± 0.09 mg/kg. **[These concentrations are equal to or higher than those found in canned foods, where the presumption is that the source is the epoxy liner of the container.]**

Bisphenol A has been found in recycled paper products used for food processing at 10 or more times the concentrations found in non-recycled paper products [reviewed by the (European Food Safety Authority, 2006)]. Bisphenol A concentrations were up to 26 μg/g paper. Migration to food was not discussed.

Epoxy paints are used to coat the insides of residential drinking water storage tanks. Bisphenol A has been shown to migrate from painted concrete and stainless metallic plates; however, a water sample from a recently painted reservoir showed no detectable bisphenol A (Romero et al., 2002). When exposed to chlorine disinfectant, bisphenol A disappears within 4 hr, but the chlorinated bisphenol A congeners that are formed can

remain in solution up to 20 hr when low chlorine doses are used (Gallard et al., 2004). The toxicity of these chlorinated bisphenol A congeners is unknown; however, there is some evidence that estrogenic activity and receptor binding remains after chlorination (Hu et al., 2002).

1.2.3.3 Potential migration from dental material: Bisphenol A is used in the manufacture of materials found in dental sealants or composites (i.e., fillings) (European-Union, 2003). Examples of bisphenol A-derived materials used in dental sealants include bis-glycidyldimethacrylate and bisphenol A-dimethyl acrylate. Bisphenol A could potentially be present as an impurity or be released during degradation of the dental materials. Sealants are comprised of an organic matrix, while composites contain inorganic filler in addition to the organic matrix. According to the British Dental Association, filled composites would possibly produce lower exposure to bisphenol A than sealants, because they contain proportionately less resin than sealants [reviewed in (European-Union, 2003)]. During dental procedures, resin mixtures are applied as fluid monomers and polymerized in situ by ultraviolet or visible light. According to the European Union (2003), patients can be exposed to bisphenol A during the polymerization stage.

In a review of in vitro studies examining bisphenol A migration from dental sealants, the European Union (2003) concluded that release of bisphenol A is likely to occur only with degradation of the parent monomer. The data suggested that bis-glycidyldimethacrylate does not degrade; therefore, release of bisphenol A is only likely to occur with bisphenol A-dimethyl acrylate use. In vivo studies measuring bisphenol A in saliva following sealant application were reviewed in detail by CERHR because they provide the most relevant human exposure information.

Olea et al. (1996) measured saliva concentrations of bisphenol A for 1 hr before and 1 hr after application of 50 mg bis-glycidyldimethacrylate- and bisphenol A-dimethyl acrylate-based sealant across 12 molars of 18 patients. Concentrations of bisphenol A in saliva were measured by GC/MS and HPLC. Following treatment, saliva contained ~90–931 μg bisphenol A. Based on an assumed saliva production rate of 0.5 mL/min, a saliva concentration of 3–30 μg/mL was estimated by the study authors. With the exception of 1 patient who was excluded from the study, bisphenol A was not detected in saliva before sealant application.

Arenholt-Binslev (1999) measured bisphenol A in saliva of 8 adult patients who each had four molars treated with 38 mg of 1 of 2 sealants, Delton LC or Visio-seal. Saliva was collected before, immediately after, and at 1 or 24 hr following treatment for measurement of bisphenol A concentrations by HPLC. Bisphenol A was detected at 0.3–2.8 ppm immediately after application of Delton LC sealant [bisphenol A-dimethyl acrylate sealant according to the European Union (2003)] but was not detected 24 hr later (detection limit = 0.1 ppm **[mg/L]**). Bisphenol A was not detected in saliva of patients who received the Visio-seal sealant (bis-glycidyldimethacrylate sealant, according to the European Union). It was noted that saliva bisphenol A concentrations were much lower than those reported by Olea et al. (1996). Possible reasons for the inconsistencies in results between the 2 studies were stated to be differences in the amount of sealant used and co-elution of compounds that could have confounded bisphenol A analysis.

Fung et al. (2000) measured salivary bisphenol A concentrations in 40 patients treated with a dental sealant (Delton Opaque Light-cure Pit and Fissure Sealant) that was understood to contain bisphenol A-dimethyl acrylate, according to the European Union (2003). Eighteen patients in the low-dose group received 8 mg dental sealant on 1 tooth, and 22 patients in the high-dose group received 32 mg sealant on 4 teeth. Saliva and blood were collected for HPLC analysis before the procedure and at 1 and 3 hr and 1, 3, and 5 days after the procedure. More details of this study are included in Section 2.1.1.1. Analysis of the dental sealant revealed that bisphenol A concentrations were below the detection limit of 5 ppb. At 1 hr following treatment, Bisphenol A was detected only in saliva samples from 3 of 18 volunteers in the low-dose group and 13 of 22 samples from volunteers in the high-dose group. At 3 hr post-treatment, bisphenol A was detected in samples from 1 of 18 volunteers in the low-dose group and 7 of 22 volunteers from the high-dose group. Concentrations of bisphenol A in saliva at 1 and 3 hr following exposure were reported at 5.8–105.6 ppb **[μg/L]**. No bisphenol A was detected in saliva samples at 24 hr after treatment or in serum samples at any time point. Differences in bisphenol A concentrations and the presence of bisphenol A in saliva of the low-dose compared to the high-dose group at 1 and 3 hr achieved statistical significance. The European Union (2003) noted that the concentrations of saliva bisphenol A reported by Fung et al. (2000) were >250 times lower than those reported by Olea et al. (1996).

Sasaki et al. (2005) used ELISA to examine salivary bisphenol A concentrations in 21 patients before and after 1 cavity was filled with 0.1 g of composite resin. The resins consisted of bisphenol A diglycidylether methacrylate (i.e., bis-glycidyldimethacrylate), triethylene glycol dimethacrylate, and/or urethane dimethacrylate. Saliva was collected before treatment, during the 5 min following treatment, and then immediately after gargling with water. Following treatment, saliva bisphenol A increased **[from ≤2 to ~15–100 μg/L]**. Gargling reduced bisphenol A to near pretreatment concentrations **[≤5 μg/L]** in most patients, with the exception of 1 patient with the highest bisphenol A concentration **[reduced from ~100 to 18 μg/L]. [An increase in saliva bisphenol A concentrations was noted in 1 of 2 patients receiving a composite consisting solely of urethane dimethacrylate.]** The study authors noted that cross-reactivity is possible with the ELISA technique, but that cross reactivity between bisphenol A diglycidylether methacrylate and triethylene glycol dimethacrylate is low. Therefore, the study authors thought it possible that they were measuring only bisphenol A. **[As discussed in Section 1.1.5, ELISA may overestimate bisphenol A.]**

Joskow et al. (2006) examined bisphenol A in urine and saliva of 14 adults treated with dental sealants. The volunteers received either Helioseal F ($n = 5$) or Delton LC ($n = 9$) sealant. Only the Helioseal F sealant was noted to carry the American Dental Association Seal of Acceptance. Sealant was weighed before and after application to determine the amount applied, and the numbers of treated teeth were recorded. The mean number of teeth treated was 6/person and the mean total weight of sealant applied was 40.35 mg/person. In a

comparison of the two different sealants, no differences were reported for the number of teeth treated or amount of sealant applied. Saliva samples were collected before, immediately after, and 1 hr after sealant application. Urine samples were collected before and at 1 and 24 hr after sealant placement. A total of 14–15 saliva samples and 12–14 urine samples were collected at each time point. Samples were treated with β-glucuronidase and analyzed for bisphenol A concentrations using selective and sensitive isotope-dilution-MS-based methods. Saliva concentrations were highest immediately following treatment; mean concentrations were reported at 42.8 ng/mL in patients treated with Delton LC and 0.54 ng/mL in patients treated with Helioseal F. The highest mean urinary concentrations of bisphenol A were measured at 1 hr following exposure and were reported at 27.3 ng/mL in patients treated with Delton LC and 7.26 ng/mL in patients receiving the Helioseal F sealant. The study authors noted that saliva and urine bisphenol A concentrations after application of Helioseal F were comparable to baseline concentrations. More information on bisphenol A concentrations in saliva and urine is included in Section 2, and exposure estimates are provided in Section 1.2.4.1.2. The study authors noted that saliva concentrations detected in their study were ~1000 times lower than those reported by Olea et al. (1996) but were within the ranges reported by Fung et al. (2000) and Sasaki et al. (2005). Analytical procedures and use of a large amount of sealant were noted as possible reasons for the higher values reported by Olea et al. (1996).

The European Union noted a study by Lewis et al. (1999) that characterized materials in 28 commercial resin-based composites and sealants, including those examined by Olea et al. (1996). HPLC and infrared analysis could not verify the presence of bisphenol A in any sealant product. Lewis et al. (1999) noted that in the study by Olea et al. (1996) another component in the resin may have been misidentified as bisphenol A because of difficulties with resolution.

In their review of studies examining bisphenol A concentrations in saliva of patients treated with dental sealants, the European Union (2003) noted that the higher concentrations reported may have resulted from interference during analysis and thus may overestimate bisphenol A exposures from dental treatments. It was concluded that dental treatment would likely result in saliva bisphenol A concentrations of 0.3–3 ppm. Because bisphenol A was generally not detected in saliva at time points beyond 1 hr after treatment, it was concluded that bisphenol A exposure resulting from dental treatments is likely to be an acute event. In their 2002 position statement, the American Dental Association stated that none of the 12 dental sealants that carry the American Dental Association Seal release bisphenol A (American Dental Association, 1998). On initial analysis, one of the sealants was found to leach trace concentrations of bisphenol A, but following implementation of quality controls by the manufacturer, bisphenol A could no longer be detected in the final product.

A study on orthodontic adhesives found no bisphenol A release from these materials after simulated aging (Eliades et al., 2007). Another study found plastic orthodontic brackets in water to release bisphenol A at 0.01–0.40 mg/kg material and denture base resin in water to release bisphenol A at 0.01–0.09 mg/kg material (Suzuki et al., 2000).

1.2.3.4 Bisphenol A concentrations measured in biological samples: Bisphenol A concentrations detected in human blood are summarized in Table 7. Goodman et al. (2006) noted that although blood concentration may provide information on internal dose, it does not allow for estimates of daily intake. It was also noted that in many studies in which blood concentration of bisphenol A was measured, sample preparation and analysis methods were poorly reported. Many study groups used an ELISA method to measure blood bisphenol A concentration. As discussed in Section 1.1.5, the ELISA technique is likely to overestimate bisphenol A concentrations as a result of cross-reactivity with other substances and due to effects of biologic matrices (Inoue et al., 2002; Fukata et al., 2006; Goodman et al., 2006).

Several studies reported concentrations of bisphenol A in human urine; those studies are summarized in Table 8. As discussed in greater detail in Section 2, the majority of ingested bisphenol A is excreted in urine as bisphenol A glucuronide after acute exposure. Smaller amounts of bisphenol A are metabolized to and excreted as bisphenol A sulfate. Some of the studies determined concentrations of parent bisphenol A before and after digestion with glucuronidases. With the exception of Fujimaki et al. (2004) who used an ELISA technique to measure urinary bisphenol A, other study authors used HPLC, GC/MS, or LC/MS. Results from 394 participants of the National Health and Nutrition Examination Survey (NHANES) III survey are included in Table 8 (Calafat et al., 2005). Bisphenol A was detected in 95% of the participants, which indicated widespread exposure to bisphenol A in the U.S. Consistent with those findings, bisphenol A was detected in urine from 85 of 90 (94.4%) 6–8-year-old girls from the U.S. (Wolff et al., 2006). In a review of urinary bisphenol A data, Goodman et al. (2006) noted that in most cases, median total urinary bisphenol A concentration (the sum of parent and conjugated bisphenol A) were ~1–2 μg/L. Two studies (Yang et al., 2003; Mao et al., 2004) reported urinary bisphenol A concentrations that were orders of magnitude higher than commonly observed concentrations, despite the use of apparently reliable analytical techniques. Goodman et al. (2006) has suggested that reported hormone concentrations for the study volunteers were also higher than expected, indicating the possibility of laboratory or reporting error. The use of urinary bisphenol A concentration to estimate daily exposures appears in Section 1.2.4.1.2.

In humans, bisphenol A was measured in cord blood and amniotic fluid, demonstrating distribution to the embryo or fetus. Detailed descriptions of those studies are also presented below.

Engel et al. (2006) reported concentrations of bisphenol A in human amniotic fluid. Twenty-one samples were obtained during amniocentesis conducted before 20 weeks gestation in women who were referred to a U.S. medical center for advanced maternal age. Bisphenol A concentrations in amniotic fluid were measured using LC with electrochemical detection. Bisphenol A was detected in 10% of samples at concentrations exceeding the LOD (0.5 μg/L). Bisphenol A concentration ranges of 0.5–1.96 μg/L were reported.

Table 7
Blood Concentrations of Bisphenol A in Adults

Population (*n*)	Bisphenol A μg/L[a,c]	Method	Reference
Germany			
Men (7)	<0.5	HPLC-MS/MS	Völkel et al. (2005)
Women (12)	<0.5	HPLC-MS/MS	Völkel et al. (2005)
Pregnant Caucasian women (37; 32–41 weeks gestation)	4.4±3.9	GC-MS	Schönfelder et al. (2002a)
Japan			
Men (21; age 22–51)	"almost all" <0.2 ng/ml	HPLC-ECD	Fukata et al. (2006)
Men (9; age 30–50)	0.59±0.21 (0.38–1.0)	HPLC-MS	Sajiki et al.(1999)
Men (11)	1.49±0.11 (SEM)	ELISA[b]	Takeuchi and Tsutsumi (2002)
Women (31; age 22–51)	"almost all" <0.2 ng/ml	HPLC-ECD	Fukata et al. (2006)
Women (12; age 30–50)	0.33±0.54 (0–1.6)	HPLC-MS	Sajiki et al.(1999)
Women (14)	0.64±0.10(SEM)	ELISA[b]	Takeuchi and Tsutsumi (2002)
Pregnant women (37; late pregnancy)	1.4±0.9	ELISA[b]	Ikezuki et al.(2002)
Pregnant women with normal karyotype early 2nd trimester (200)	2.24 (0.63–14.36)	ELISA[b]	Yamada et al. (2002)
Pregnant women with abnormal karyotype early 2nd trimester (48)	2.97 (~0.0.7–18.5)[d]	ELISA[b]	Yamada et al. (2002)
Pregnant women (9)	0.43 (0.21–0.79)	HPLC-Fl	Kuroda et al. (2003)
Infertile women (21)	0.46 (0.22–0.87)	HPLC-Fl	Kuroda et al. (2003)
Women with multiple miscarriages (45; mean age 31.6 years)	2.59±5.23	ELISA[b]	Sugiura-Ogasawara et al. (2005)
Healthy woman (32; mean age 32 years)	0.77±0.38	ELISA[b]	Sugiura-Ogasawara et al. (2005)
Women with polycystic ovary syndrome (16)	1.04±0.10 (SEM)	ELISA[b]	Takeuchi and Tsutsumi (2002)
Non-obese women with polycystic ovarian syndrome (13; average age 26.5 years)	1.05±0.10 (SEM)	ELISA[b]	Takeuchi et al. (2004a)
Obese women with polycystic ovarian syndrome (6; average age 24.7 years)	1.17±0.16 (SEM)	ELISA[b]	Takeuchi et al. (2004a)
Non-obese women (19; average age 27.5 years)	0.71±0.09 (SEM)	ELISA[b]	Takeuchi and Tsutsumi (2002)
Obese women (7; average age 28.8 years)	1.04±0.09 (SEM)	ELISA[b]	Takeuchi et al. (2004a)
Hyperprolactinemic women (7; average age 27.7 years)	0.83±0.12 (SEM)	ELISA[b]	Takeuchi et al. (2004a)
Amenorrheic women (7; average age 25.1 years)	0.84±0.10 (SEM)	ELISA[b]	Takeuchi et al. (2004a)
Women with normal uterine endometrium (11; mean age 48.9 years	2.5±1.5	ELISA[b]	Hiroi et al. (2004)
Women with simple endometrium hyperplasia (10; mean age 48.4 years)	2.9±2.0	ELISA[b]	Hiroi et al. (2004)
Women with complex endometrium hyperplasia (9; mean age 48.4 years)	1.4±0.4	ELISA[b]	Hiroi et al. (2004)
Women with endometrial carcinoma (7; mean age 63.1 years)	1.4±0.5	ELISA[b]	Hiroi et al. (2004)

[a]Mean±SD or median (range).
[b]As discussed in Section 1.1.5, ELISA may overestimate bisphenol A.
[c]It is uncertain whether parent, conjugated, or total bisphenol A was measured.
[d]Estimated from a graph.

Schönfelder et al. (2002b) examined bisphenol A concentrations in maternal and fetal blood and compared bisphenol A concentrations in blood of male and female fetuses. In a study conducted at a German medical center, blood samples were obtained from 37 Caucasian women between 32 and 41 weeks gestation. At parturition, blood was collected from the umbilical vein after expulsion of the placenta. Bisphenol A concentrations in plasma were measured by GC/MS. Control experiments were conducted to verify that bisphenol A did not leach from collection, storage, or testing equipment. Bisphenol A was detected in all samples tested, and concentrations measured in maternal and fetal blood are summarized in Table 9. Mean bisphenol A concentrations were higher in maternal (4.4±3.9 [SD] μg/L) than fetal blood (2.9±2.5 μg/L). Study authors noted that in 14 cases fetal bisphenol A plasma concentrations exceeded those detected in maternal plasma. Among those 14 cases, 12 fetuses were male. Analysis by paired *t*-test revealed significantly higher mean bisphenol A concentrations in

Table 8
Urinary Concentrations of Bisphenol A and Metabolites in Adults or Children

Country	Study population	LOD (μg/L)	Urinary bisphenol A or metabolite concentrations as median (range) or mean ± SEM, μg/L[a] [detectable fraction, % > LOD]				Reference
			Free	Total	Glucuronide	Sulfate	
U.S.	30 urine samples from demographically diverse, anonymous adult volunteers	0.3	< 0.3 (<0.3–0.6) [10%]	2.12 (<LOD[b] –19.8) [97%]	1.4 (<LOD[b] –19.0) [90%]	0.3 (<LOD[b] –1.8) [47%]	Ye et al. (2005)
U.S.	394 adult volunteers (men and women; 20–59 years old) from the NHANES III survey	0.1		1.28 (10–95th percentile: 0.22–5.18) [95%][c]			Calafat et al. (2005)
U.S.	23 adults	0.5		0.47 (<1–2.24) [52%]			Liu et al. (2005)
U.S.	9 girls (9 years of age)	0.5		2.4 (0.04–16) [89%]			Liu et al. (2005)
U.S.	90 women (6–8 years of age; White, Black, Asian, or Hispanic ethnicity)	8.36		1.8 (<0.3–54.3) [85%]			Wolff et al. (2006)
Germany	7 men, 12 women	1.14 (BPA) 10.1 (BPA monoglucuronide)	<1.14 [0%]		<26.26 [LOQ]		Völkel et al. (2005)
Korea	15 men (42.6 ± 2.4 years of age)[d]	1	0.28–2.36; 0.58 ± 0.14	0.85–9.83 2.82 ± 0.73	0.16–11.67 2.34 ± 0.85	<MDL –1.03; 0.49 ± 0.27[e]	Kim et al. (2003b)
Korea	15 women (43.0 ± 2.7 years of age)[d]	0.28	0.068–1.65; 0.56 ± 0.10	1.00–7.64 2.76 ± 0.54	<MDL[c] –4.34 1.00 ± 0.34	<MDL –3.40; 1.20 ± 0.32[e]	Kim et al. (2003b)
Korea	34 men, 39 women (mean = 48.5 years of age)	0.012		Geometric mean: 9.54 (<0.012–586.14[b]) [75%]			Yang et al. (2003)
Korea	81 men not occupationally exposed to bisphenol A			Geometric mean ± SD 6.88 ± 3.72			Yang et al. (2006)
Korea	79 women not occupationally exposed to bisphenol A	0.026		Geometric mean ± SD 5.01 ± 3.16 [97.5%]			Yang et al. (2006)
Japan	48 woman college students	0.2	<0.2 [2%]		1.2 (0.2–19.1) [100%]		Ouchi and Watanabe (2002)
Japan	Pooled urine samples from at least 5 people	0.12	<0.12	0.11–0.51			Brock et al. (2001)
Japan	23 women, 46 men; in each volunteer, 2 samples per volunteer were combined		0.01–0.27	Mean = 0.81 (range: 0.14–5.47)			Tsukioka et al. (2004)
Japan	Whole-day urine samples collected from 11 men and 11 women			Mean = 0.81 (range 0.24–2.03)			Tsukioka et al. (2004)
Japan	Urine collected from 3 volunteers	0.02	<0.1	0.22, 0.41, and 0.45 [100% after deconjugation]			Kawaguchi et al. (2004)
Japan	Spot urine samples collected from 56 women who were 1–9 months pregnant; 21–43 years of age	1.1		<1.1 (<1.1–5.4)[c] (ELISA) [30%]			Fujimaki et al. (2004)
Japan	21 men, 31 women; 22–51 years of age	0.2	49/51 had <0.2 mean 0.34 ($n = 2$) [4%]	1.92 ± 0.27 [98%]			Fukata et al. (2006)

Table 8
Continued

Country	Study population	LOD (μg/L)	Urinary bisphenol A or metabolite concentrations as median (range) or mean ± SEM, μg/L[a] [detectable fraction, % > LOD]				Reference
			Free	Total	Glucuronide	Sulfate	
China	10 healthy man volunteers; 21–29 years of age	2.8		<2.7 –3950 1220 ± 1380[d] [60%]			Mao et al. (2004)
China	10 healthy woman volunteers; 21–29 years of age	2.8		30–3740 1290 ± 1220[d] [100%]			Mao et al. (2004)

[a]With the exception of the study by Fujimaki et al. (2004), which used the potentially unreliable ELISA, the studies used analytical techniques based on HPLC, GC/MS, and LC/MS.
[b]Limit of detection (LOD) for bisphenol A following digestion of conjugate was 0.3 μg/L.
[c]Samples were only digested with β-glucuronidase and do not account for bisphenol A conjugated to sulfate.
[d]Variance not indicated.
[e]Minimum detection limit based upon free bisphenol A.

Table 9
Concentrations of Bisphenol A in Maternal and Fetal Samples

Study description (analytical method)	Bisphenol A concentrations, μg/L median (range) or mean ± SD			Reference
	Serum or plasma			
	Maternal	Fetal	Amniotic fluid	
21 samples collected in women in the U.S. before 20 weeks gestation (LC with electrochemical detection)			0.5 (Non-detectable <0.5–1.96) 10% of samples detectable	Engel et al. (2006)
37 German women, 32–41 weeks gestation (GC/MS)	3.1 (0.3–18.9) 4.4 ± 3.9	2.3 (0.2–9.2) 2.9 ± 2.5		Schönfelder et al. (2002b)
37 Japanese women in early pregnancy (ELISA)[a]	1.5 ± 1.2			Ikezuki et al. (2002)
37 Japanese women in late pregnancy (ELISA)[a]	1.4 ± 0.9			Ikezuki et al. (2002)
32 Japanese infants at delivery (ELISA)[a]		2.2 ± 1.8		Ikezuki et al. (2002)
32 Japanese amniocentesis samples at 15–18 weeks gestation (ELISA)[a]			8.3 ± 8.9	Ikezuki et al. (2002)
38 samples obtained at full-term cesarean section (ELISA)[a]			1.1 ± 1.0	Ikezuki et al. (2002)
200 Japanese women carrying fetuses with normal karyotype at 16 weeks mean gestation (ELISA)	2.24 (0.63–14.36)		0.26 (0–5.62)	Yamada et al. (2002)
48 Japanese women carrying fetuses with abnormal karyotypes at a 16 weeks mean gestation (ELISA)	2.97 **[∼0.7–18.5]**[b]		0 **[∼0–7.5]**[b]	Yamada et al. (2002)
9 sets of maternal and umbilical cord blood samples obtained at birth in Japanese patients (HPLC)	0.43 (0.21–0.79) 0.46 ± 0.2	0.64 (0.45–0.76) 0.62 ± 0.13		Kuroda et al. (2003)
180 Malaysian newborns (GC/MS)		Non-detectable (<0.05) to 4.05 88% of samples detectable		Tan and Mohd (2003)

[a]As discussed in Section 1.1.5, ELISA may overestimate bisphenol A. Some samples were verified by HPLC.
[b]Estimated from a graph.

the blood of male than female fetuses (3.5±2.7 vs. 1.7±1.5 ng/mL, $P = 0.016$). Bisphenol A concentrations were measured in placental samples at 1.0–104.9 μg/kg.

Ikezuki et al. (2002) measured concentrations of bisphenol A in serum from 30 healthy premenopausal women, 37 women in early pregnancy, 37 women in late pregnancy, and 32 umbilical cord blood samples. Concentrations of bisphenol A were also measured in 32 samples of amniotic fluid obtained during weeks 15–18 of gestation, 38 samples of amniotic fluid obtained at full-term cesarean section, and 36 samples of ovarian follicular fluid collected during in vitro fertilization procedures. **[It was not stated if different sample types were obtained from the same subjects.]** An ELISA method was used to measure bisphenol A concentrations and results were verified by HPLC. The mean±SD concentration of bisphenol A in follicular fluid was reported at 2.4±0.8 μg/L. As summarized in Table 9 for maternal and fetal samples, concentrations of bisphenol A in follicular fluid were similar to those detected in the serum of fetuses and pregnant and non-pregnant women and in amniotic fluid collected in late pregnancy (~1–2 μg/L). Bisphenol A concentrations in amniotic fluid samples collected in early pregnancy were ~5-fold higher than in other samples, and the difference achieved statistical significance ($P < 0.0001$). Study authors postulated that the higher concentrations of bisphenol A in amniotic fluid collected during gestation weeks 15–18 may have resulted from immature fetal liver function. They noted that according to unpublished data from their laboratory, the percentage of glucuronidated bisphenol A in mid-term amniotic fluid was ~34%, which is much lower than reported values for other human fluids (>90%).

Yamada et al. (2002) measured bisphenol A concentrations in maternal serum and amniotic fluid from Japanese women. Samples were collected between 1989 and 1998 in women undergoing amniocentesis around gestation week 16. One group of samples was obtained from 200 women carrying fetuses with normal karyotypes, and a second group of samples was obtained from 48 women carrying fetuses with abnormal karyotypes. An ELISA method was used to measure bisphenol A concentrations. **[As discussed in Section 1.1.5, ELISA may overestimate bisphenol A.]** Concentrations of bisphenol A measured in maternal plasma and amniotic fluid are summarized in Table 9. Median concentrations of bisphenol A in maternal serum (~2–3 μg/L) were significantly higher **[~10-fold]** than concentrations in amniotic fluid (~0–0.26 μg/L) in the groups carrying fetuses with normal and abnormal karyotypes. However, in 8 samples from women carrying fetuses with normal karyotypes, high concentrations (2.80–5.62 μg/L) of bisphenol A were measured in amniotic fluid. The study authors interpreted the data as indicating that bisphenol A does not accumulate in amniotic fluid in most cases but that accumulation is possible in some individuals. Bisphenol A concentrations in maternal blood were significantly higher **[by ~33%]** in woman carrying fetuses with abnormal versus normal karyotypes. However, the study authors noted that the effect may not be related to bisphenol A exposure because there was no adjustment for maternal age, and concentrations in amniotic fluid did not differ between groups. In the group carrying fetuses with normal karyotypes, data obtained from 1989–1998 were summarized by year. Median bisphenol A concentrations in serum significantly decreased over that time from a concentration of 5.62 μg/L detected in 1989 to 0.99 μg/L in 1998.

Kuroda et al. (2003) used an HPLC method to measure bisphenol A concentrations in 9 sets of maternal and cord blood samples obtained from Japanese patients at the time of delivery. Bisphenol A concentrations were also measured in 21 sets of serum and ascitic fluid samples collected from sterile Japanese patients of unspecified sexes and ages. Results for pregnant women are summarized in Table 9. Mean±SD concentrations of bisphenol A were lower in maternal (0.46±0.20 ppb **[μg/L]**) than cord blood (0.62±0.13 ppb **[μg/L]**). There was a weak positive correlation ($r = 0.626$) between bisphenol A concentrations in maternal and cord blood. There were no differences between pregnant and non-pregnant blood levels (Kuroda et al., 2003). Mean±SD concentrations of bisphenol A were higher in ascitic fluid (0.56±0.19 ppb **[μg/L]**) than in serum (0.46±0.20 ppb **[μg/L]**). The correlation between bisphenol A concentration in serum and ascitic fluid was relatively strong ($r = 0.785$).

Tan and Mohd (2003) used a GC/MS method to measure bisphenol A concentrations in cord blood at delivery in 180 patients at a Malaysian medical center. Bisphenol A was detected in 88% of samples. As noted in Table 9, concentrations ranged from <0.10–4.05 μg/L.

Schaefer et al. (2000) measured concentrations of bisphenol A and other compounds in uterine endometrium of women undergoing hysterectomy for uterine myoma at a German medical center. Endometrial and fat samples were obtained between 1995–1998 from 23 women (34–51 years old) with no occupational exposure to bisphenol A. Samples were handled with plastic-free materials and stored in glass containers. Concentrations of environmental chemicals were measured in samples by GC/MS. None of 21 fat samples had detectable concentrations of bisphenol A. Bisphenol A was detected in 1 of 23 endometrial samples; the median concentration was reported at <1 μg/kg wet weight, and the range was reported at 0–13 μg/kg. **[It is not known why a median value and range were reported when bisphenol A was only detected in 1 sample.]**

As part of a study to compare an ELISA and an LC/MS method for biological monitoring of bisphenol A, Inoue et al. (2002) measured concentrations of bisphenol A in semen samples obtained from 41 healthy Japanese volunteers (18–38 years old). Analysis by the ELISA method indicated bisphenol A concentrations ranging from concentrations below the detection limit (2.0 μg/L) to 12.0 μg/L. The LC/MS method indicated that the bisphenol A concentration in all samples was <0.5 μg/L, the LOQ. The study authors concluded that the LC/MS method was more accurate and sensitive and that the ELISA method overestimated bisphenol A concentrations, possibly due in part to nonspecific antibody interactions.

1.2.4 Human exposure

1.2.4.1 General population exposure

1.2.4.1.1 Estimates based on bisphenol A concentrations in food or environment: Wilson et al. (2003) estimated aggregate exposures to bisphenol A in preschool aged children (2–5 years) from the U.S. In 1997, numerous chemicals were surveyed, but only bisphenol A results are reported here. Ten child care centers were surveyed

Table 10
Bisphenol A Oral Exposure Estimates by the European Union[a]

Exposure source (exposed population)	Daily food intake	Bisphenol A concentration in food	Bisphenol A intake	
			μg/day	μg/kg bw/day
Infant bottles (1–2 month-old infant)	0.699 L/day milk	50 μg/L	35	8
Infant bottles (4–6-month-old infant)	0.983 L/day milk	50 μg/L	50	7
Polycarbonate tableware (1.5–4.5-year-old child)	2 kg food/day	5 μg/kg	10	0.7
Canned food (6–12- month-old infant)	0.375 kg canned food/day	100 μg/kg	40	5
Canned food (1.5–4.5-year-old child)	2 kg canned food/day	100 μg/kg	200	14
Canned food (adult)	1.0 kg canned food/day	100 μg/kg	100	1.4
Wine (adult)	0.75 L/day	650 μg/L	500	7[b]
Canned food and wine (adult)	0.75 L/day wine 1.0 kg canned food/day	650 μg/L in wine 100 μg/kg food	600	9[b]

[a]European Union (2003).
[b]The European Union acknowledged that exposure through wine represents a very worst-case scenario.

and the 2 centers with the highest and lowest overall concentrations of target pollutants were selected for the study. Both centers were located in North Carolina. Nine children who attended one of the child care centers participated in the study. Over a 48-hr period, bisphenol A concentrations were measured in indoor and outdoor air, dust, soil, and food; the ranges detected are summarized in Sections 1.2.3.1 and 1.2.3.2. In estimating exposures, absorption was considered to be 100%. Calculations considered ventilation rates, time spent indoors and outdoors, time spent at home and in day care, the measured weight of each child, assumed ingestion of dust and soil, and total weight of foods consumed. Mean (range) bisphenol A intake was estimated at 0.042981 (0.018466–0.071124) μg/kg bw/day.

Wilson et al. (2006) conducted a second study to estimate aggregate exposures in 257 U.S. children aged 1.5–5 years. Bisphenol A was one of the compounds assessed in this study of homes and daycare centers in 6 North Carolina and 6 Ohio counties in 2000–2001. Over a 48-hr period, bisphenol A concentrations were measured in indoor and outdoor air, dust, soil, food, and surface and hand wipes; the ranges detected are summarized in Sections 1.2.3.1 and 1.2.3.2. In estimating exposures, absorption was considered to be 50%. Calculations considered ventilation rates, time spent indoors and outdoors, time spent at home and in day care, the measured weight of each child, assumed ingestion of dust and soil, and total weight of foods consumed. Median (25th percentile to maximum) bisphenol A aggregate exposures were estimated at 2.56 (1.5–57.2) μg/day for children from North Carolina and 1.88 (1.27–48.6) μg/day in children from Ohio. Median (25th percentile to maximum) potential aggregate dose, assuming 50% absorption, was estimated at 0.0714 (0.0424–1.57) μg/kg bw/day in children from North Carolina and 0.0608 (0.0341–0.775) μg/kg bw/day in children from Ohio. The study authors noted that 99% of exposure occurred through dietary ingestion.

The European Union (2003) conducted a comprehensive exposure estimate that considered exposures resulting from food and environmental sources. Oral exposure estimates for children and adults were reported and are summarized in Table 10. Estimates were based on migration studies conducted with polycarbonate and concentrations of bisphenol A measured in foods packaged in epoxy-lined cans. Assumptions used in exposure estimates included 100% oral absorption and body weights of 70 kg for adults, 14.5 kg for 1.5–4.5-year-old children, 4.5 kg for 1–2-month-old infants, 7 kg for 4–6-month-old infants, and 8.7 kg for 6–12-month-old infants. Estimated exposures for children were said to represent realistic worst-case scenarios for food and drink intake relative to body weight.

The European Union (2003) also estimated human environmental exposure to bisphenol A from sources such as drinking water, fish, plants, milk, meat, and air. The values were apparently obtained using the European Union System for the Evaluation of Substances (EUSES) model. Total regional exposure to bisphenol A was estimated at 0.0178 μg/kg bw/day. The highest local exposure was thought to occur in the vicinity of PVC-producing plants and was estimated at 59 μg/kg bw/day. Aggregate exposures in adults involving food, wine, and environmental sources were estimated at 9 μg/kg bw/day for regional scenarios and 69 μg/kg bw/day for worst-case local scenarios occurring near a PVC-manufacturing plant. However, it was noted in the European Union report that use of bisphenol A in PVC manufacture was being phased out.

The European Union (2003) noted that exposures to bisphenol A through dental sealant are single and rare events and do not lead to repeated exposure. Therefore, the issue was not considered further.

Exposures to bisphenol A from some consumer products were identified and characterized by the

Table 11
Bisphenol A Exposure Estimates by the European Commission[a]

Age (body weight)	Type of food and amount consumed	Concentration of bisphenol A in food (μg/kg)	Exposure estimate (μg/kg bw/day)
0–4-month old infant (4.5 kg)	0.7 L of formula/day	10	1.6
6–12-month old infant (8.8 kg)	0.7 L of formula/day	10	0.8
6–12-month old infant (8.8 kg)	0.38 kg canned food/day	20	0.85
4–6-year-old child (18 kg)	1.05 kg canned food/day	20	1.2
Adult (60 kg)	1.05 kg canned food/day	20	0.37
Adult (60 kg)	0.75 L wine/day	9	0.11

[a]European Commission (2002).

European Union (2003). Products included: marine antifouling agents used on boats, wood varnish, wood fillers, and adhesives. With the exception of adhesives for which frequent use was thought possible, exposure to the other products was considered to be relatively rare. Exposures were estimated based on factors such as epoxy and residual bisphenol A concentrations, exposure time, area of skin exposed, and possible generation of mists during processes such as brushing. Inhalation exposures by product were estimated at 3×10^{-4} μg for antifouling agents and 0.02 μg for wood varnish. Dermal exposure by product without protective clothing was estimated at 29 μg for antifouling agents, 3.6 μg for wood varnish, 9 μg for wood filler, and 14 μg for adhesives. **[Dermal exposure to adhesives appears to be incorrectly reported as 1 μg in Table 4.20 of the European Union review.]** Exposure was estimated to be 1–2 orders of magnitude lower when protective clothing such as gloves was used. Assuming an absorption rate of 10%, dermal exposure to bisphenol A through adhesives was estimated at 0.02 μg/kg bw/day.

The European Commission, 2002) reviewed the report by the European Union (2003) in draft and suggested alternate exposure estimates. Those estimates and the assumptions used to support those estimates are summarized in Table 11.

Miyamoto and Kotake (2006) estimated aggregate oral and inhalation exposure to bisphenol A in Japanese male children and adults. The estimates were based on unpublished Japanese data. This report is the only known study investigating potential exposure to children through mouthing of toys. Mouthing times were estimated by surveying the mothers of 50 infants and recording 25 infants on video camera. Mean ± SD daily mouthing times were reported at 41.7 ± 13.7 min for infants 0–5 months of age and 73.9 ± 32.9 min for infants 6–11 months of age. Migration rates were estimated from 0 μg/cm^2/min for toys that do not contain bisphenol A to 0.0162 μg/cm^2/min, the highest value reported in the Japanese literature. It was assumed that most toys were not manufactured with polycarbonate, epoxy resins, or grades of PVC that contain bisphenol A. Surface area of toys was assumed to be 10 cm2. In estimating oral exposures to bisphenol A, intake from food was also considered. Bisphenol A concentrations measured in migration testing of polycarbonate bottles and food surveys are summarized in Section 1.2.3.2. Volume of food consumption and frequency of article use were considered in estimates of bisphenol intake through food. Bisphenol A concentrations in drinking water were considered to be 0–0.17 μg/L, and water intake was assumed to be 2 L/day. In estimating inhalation exposures, concentrations of bisphenol A were considered to range from 0–8.1 ng/m^3 in indoor air and 0–28 ng/m^3 in outdoor air. Time spent indoors and outdoors and breathing rates were considered. Absorption from lungs was assumed at 100%. Estimated exposures from mouthing of toys, food and water intake, and inhaled air are summarized in Table 12.

Additional estimates of bisphenol A exposure through food are summarized in Table 5 and Table 6. Details of studies conducted by Earls et al. (2000) and Onn Wong et al. (2005) are presented in Section 1.2.3.2. Exposure estimates conducted by the FDA are described below. Limited details were available from the other studies that were presented in reviews.

The FDA (1996) estimated bisphenol A intake in infants and adults resulting from exposures to epoxy food-can linings and polycarbonate plastics. Exposure estimates occurring through contact of formula with polycarbonate bottles were based on results of a study conducted by the Chemistry Methods Branch of the FDA. The Chemistry Methods Branch also measured concentrations of bisphenol A in 5 brands of infant formula (14 samples total); the study is also published as Biles et al. (1997a). In estimating adult bisphenol A exposure through the consumption of canned foods, the FDA considered surveys conducted by the Chemistry Methods Branch, Brotons et al. (1995), and the Society of Plastics Industry Group. It appears that the study by the Society of Plastics Industry Group was later published by Howe et al. (1998) and included a re-analysis to correct some interferences observed in analytical methods. Exposure estimates and assumptions used to make the estimates are summarized in Table 13.

Table 14 summarizes exposure estimates for aggregate or food exposures. Studies suggest that the majority of bisphenol A exposure occurs through food and that environmental exposures do not appear to substantially affect total exposure, with the possible exception of exposure near point sources. Table 14 includes estimates that CERHR believes to represent potentially realistic exposure scenarios and does not include data from extreme worst-case scenarios such as possible point–source exposures.

1.2.4.1.2 Estimates based on biological monitoring: Goodman et al. (2006) noted that total urinary bisphenol A concentrations were useful for estimating bisphenol A intake. Because of extensive first-pass metabolism, little parent compound is systemically circulated, as discussed in more detail in Section 2. Because nearly 100% of an acute exposure to bisphenol A is excreted in urine within

Table 12
Average Estimated Exposure to Bisphenol A in Japanese Man Adults and Children[a]

Exposure source	Bisphenol A concentration (other assumptions)	Average estimated exposures in each age group[b] (μg/kg bw/day)					
		0–5 months	6–11 months	1–6 years	7–14 years	15–19 years	19 years
Human milk	Negligible	0	0				
Formula (water)	0–0.17 μg/L	0.012	0.0096				
Feeding bottle	0–3.9 μg/L	0.015	0.014				
Infant food	0–5.0 μg/kg		0.085				
Toys	0–0.0162 μg/cm^2/min (mean mouthing times of 41.7 min in 0–5-month-olds and 73.9 min in 6–11-month-olds)	0.026	0.069				
Air	0–8.1 ng/m^3 in indoor air and 0–28 ng/m^3 in outdoor air (90% indoors/10% outdoors)	0.0026	0.0024	0.0021	0.0017	0.0015	0.0015
Water	0–0.17 μg/L (intake of 2 L/day)			0.012	0.0053	0.0029	0.0027
Food and drink							
Canned	0–602 μg/kg			0.38	0.21	0.20	0.29
Non-canned	0–3 μg/kg			0.38	0.21	0.13	0.12
Tableware	0–39.4 μg/meal/utensil (3 meals/day; 1–5 types of utensils used/meal)			0.40	0.12	0.024	0.022
Total		breast-fed: 0.028 formula-fed: 0.055	breast-fed: 0.16 formula-fed: 0.18	1.2	0.55	0.36	0.43

[a]Miyamoto and Kotake (2006).
[b]Assumptions for bodyweights and most media intake levels were not provided.

24 hr (Völkel et al., 2002; Tsukioka et al., 2004), bisphenol A intake can be estimated by measuring bisphenol A in urine over a specified time interval. Arakawa et al. (2004) measured bisphenol A excretion over a 5-day period and reported intra- and inter-individual variability. As a result, caution was urged in using single time-point values to estimate long-term exposure. Typical daily intakes of bisphenol A estimated from urinary levels are <0.01–2.17íg/kg bw/day (Table 15). A Monte Carlo simulation using the urine data of Tsukioka et al. (2004) and Arakawa et al. (2004) estimated mean exposures of 0.028–0.049 ug/kg bw/day for males and 0.034–0.059 ug/kg bw/day for females (Miyamoto and Kotake, 2006). Using the U.S. NHANES data and assumptions on excretion rates and body weight a median intake of 0.026 ug/kg bw/day is estimated. An estimated median exposure based on urinary bisphenol A concentrations in 6–8-year-old girls was 0.07 μg/kg bw/day (Wolff et al., 2006).

Joskow et al. (2006) used values for total bisphenol A in urine to estimate exposure to bisphenol A following dental sealant application. Urinary concentrations of bisphenol A are reported in Table 8. Factors or assumptions used in the exposure estimates were recovery of bisphenol A in urine as its glucuronide conjugate within 24–34 hr following exposure, a 5.4-hr half-life of elimination for bisphenol A glucuronide, and a 1.5 L/day urinary excretion volume. Estimated doses of bisphenol A **[based on a 60-kg bw]** were 49–239 μg **[0.82–4.0 μg/kg bw]** following application of Delton LC and 0–9.5 μg **[0–0.16 μg/kg bw]** following application of Helioseal F. The study authors stated that the estimates were likely low because a substantial amount of bisphenol A was potentially eliminated by collection of saliva samples immediately following treatment.

1.2.4.2 Occupational exposure: Occupational exposure to bisphenol A could potentially occur during its manufacture, in the production of polycarbonate plastics, and during the manufacture or use of epoxy resins, powder coatings paints, or lacquers (European-Union, 2003). Possible exposure to bisphenol A during PVC manufacture has been considered, but the European Union (2003) stated that the application was being phased out. According to the European Union, bisphenol A is generally available as granules, flakes, or pellets, thus reducing exposure potential. Bisphenol A is manufactured in closed systems, but exposure is possible during sampling, container filling, and plant maintenance. In the manufacture of polycarbonate, bisphenol A enters the plant and remains in a closed system before extrusion. Sampling is conducted by a closed loop system. Following extrusion, the polycarbonate is

Table 13
Summaries of Studies Estimating Bisphenol A Exposures Solely from Foods

Population	Exposure source	Basis and assumptions for estimates	Exposure estimate μg/kg bw/day	Reference
Infants	Polycarbonate bottles	Bisphenol A migration concentration of 15–20 μg/L; milk consumption of up to 550 mL/day; mean body weight of 11 kg	0.75–1	Earls et al. (2000)
Infants (0–3 months old)	Polycarbonate bottles	Mean upper-bound concentration of bisphenol A migration in 10% ethanol (0.64 μg/in^2) and in corn oil (0.43 μg/in^2); body weights reported by National Center for Health Statistics, and FDA Dietary Exposure Guidelines with modifications for properties of infant formula	15–24[a]	Onn Wong et al. (2005)
Not reported	Food from epoxy-lined cans	Bisphenol A concentrations of 5 ppb [μg/L] in beverages and 37 ppb [μg/kg] in other foods; FDA Dietary Exposure Guidelines: dietary intake of 3 kg/day, body weight of 60 kg	0.105	Howe et al. (1998) Haighton et al. (2002) NAS (1999)
Adults	Cumulative exposures from food contacting cans and polycarbonate plastics	22 ppb [μg/kg] bisphenol A in vegetables, consumption factor of 0.17 for food contacting polymer-coated metal, intake of 3 kg food/bw/day, 60 kg bw, and insignificant contribution from polycarbonate	0.183	FDA (1996)
Infants	Cumulative exposures from food contacting cans and polycarbonate plastics	Bisphenol A concentration of 6.6 μg/kg in prepared infant formula, <1.7 ppb [μg/L] in infant formula from polycarbonate bottles, consumption of 820 g food/day, and 4 kg infant weight	1.75	
Adults	Canned foods	Data from survey of canned foods and food intake patterns determined from surveys	Mean = 0.0083 (0–0.29)	Thomson and Grounds (2005)
Adults	Canned foods and canned fish	Data from survey of canned foods and food intake patterns determined from surveys	0.0044 for men ≥25 0.0041 for women ≥25 0.0048 for men 19–24	Thomson et al. (2003)
Adults	Wine	Maximum bisphenol A concentration of 2.1 ng/mL in wine, consumption of 0.75 L/day, and 60 kg body weight	<0.026	Brenn-Struckhofova and Cichna-Markel (2006)
Hospital patients	Meals served at 2 hospitals	Mean intake from hospital diets was estimated at 1.3 (0.19–3.7) μg/day; [60 kg body weight was assumed]	[0.02 (0.003–0.06)]	Miyamoto and Kotake (2006) Fujimaki et al. (2004)
Japanese adults and children	~200 food items were collected in a total diet study	No details	0.00475 for children 2–6 years 0.00195 for adults	Miyamoto and Kotake (2006)

[a]The study authors acknowledged the use of aggressive migration testing conditions and conservative assumptions in calculations, thus leading to overestimated infant exposures.

chopped into granules and bagged, and it is during that stage that exposure to residual bisphenol A (reported at ≤100 ppm) through dust is possible. However, it is noted that polycarbonate is stable and that residual bisphenol A is contained within the polymer matrix. The European Union stated that exposure to bisphenol A during the manufacture of polycarbonate items is not likely to exceed values observed during the manufacture of polycarbonate. In the production of epoxy resin, bisphenol A exposure is most likely during reactor charging, but exposure during maintenance is also possible. A residual bisphenol A concentration of 300 ppm was reported for epoxy resins, but it was noted that most bisphenol A was trapped within the resin matrix. Exposure to bisphenol A during production of epoxy paints is reported to be negligible. In the manufacture of powder epoxy coatings, exposure is thought possible during weighing and milling. Exposure to bisphenol A during the use of powder paints has been documented.

There are no known regulatory limits for occupational exposure to bisphenol A in the U.S. In 2004, the

Table 14
Summary of Food or Aggregate Exposures to Bisphenol A

Population	Basis of estimates	Exposure estimate μg/kg bw/day[a]	Reference
1–2-month-old infant	Food exposure (data from migration studies of polycarbonate bottles)	8	European Union (2003)
0–4-month-old infant	Food exposure (data from migration studies of polycarbonate bottles)	1.6	European Commission (2002)
0–5-month-old infant (formula-fed)	Aggregate exposure (based on formula, environmental, and toy exposures)	0.055	Miyamoto and Kotake (2006)
0–5-month-old infant (breast fed)	Aggregate exposure (based on human milk, environmental, and toy exposures)	0.028	Miyamoto and Kotake (2006)
4–6-month-old infant	Food exposure (data from migration studies of polycarbonate bottles)	7	European Union (2003)
6–11-month-old infant (formula-fed)	Aggregate exposure (based on formula, food, environmental, and toy exposures)	0.18	Miyamoto and Kotake (2006)
6–11-month-old infant (breast-fed)	Aggregate exposure (based on human milk, food, environmental, and toy exposures)	0.16	Miyamoto and Kotake (2006)
6–12-month-old infant	Food exposure (data from survey of canned foods)	5	European Union (2003)
6–12-month-old infant	Food exposure (data from migration studies with infant bottles and canned foods)	1.65	European Commission (2002)
Infant	Food exposure (data from polycarbonate bottle leaching studies)	0.75–1	Earls (2000)
Infant	Food exposures (contact with cans and polycarbonate plastics)	1.75	FDA (1996)
1.5–4.5-year-old child	Food exposure (data from survey of canned foods and migration studies with polycarbonate tableware)	14.7	European Union (2003)
1–6-year-old child	Aggregate exposure (based on food, environmental, and tableware exposures)	1.2	Miyamoto and Kotake (2006)
1.5–5 year old child	Aggregate exposure (surveys of bisphenol in food, air, dust, soil and hand and surface wipes)	0.06-0.07 (0.03–1.57)	Wilson et al. (2006)
3–5-year-old child	Aggregate exposure (surveys of bisphenol in food, air, dust, and soil)	0.04 (0.018–0.07)	Wilson et al. (2003)
2–6-year-old child	Food exposure (collection of 200 food items)	0.004	Miyamoto and Kotake (2006)
4–6-year-old child	Food exposure (data from survey of canned foods)	1.2	European Commission (2002)
7–14-year-old child	Aggregate exposure (based on food, environmental, and tableware exposures)	0.55	Miyamoto and Kotake (2006)
15–19-year-old individual	Aggregate exposure (based on food, environmental, and tableware exposures)	0.36	Miyamoto and Kotake (2006)
Adult, ≥19 years	Aggregate exposure (based on food, environmental, and tableware exposures)	0.43	Miyamoto and Kotake (2006)
Adult	Food exposure (data from survey of canned foods not including wine)	1.4	European Union (2003)
Adult	Food exposure (data from surveys of canned food)	0.37	European Commission (2002)
Adult	Wine exposure (data from study of epoxy-lined wine drums)	0.11	European Commission (2002)
Adult	Wine exposure (data from wine samples)	<0.026	Brenn-Struckhofova and Cichna-Markel (2006)
Adult	Food exposure (from contact with epoxy-lined cans and polycarbonate)	0.183	FDA (1996)
Adults	Food exposure (survey of canned foods)	0.008	Thomson and Grounds (2005)
Adult	Food exposure (collection of 200 food items)	0.002	Miyamoto and Kotake (2006)

[a]Estimates involving extreme worst case scenarios and Japanese data with very limited information were not included in this table.

American Industrial Hygiene Association proposed a workplace environmental exposure level (WEEL) of $5\,mg/m^3$ for bisphenol A. The draft WEEL was based on irritation observed in an inhalation toxicity study (American Industrial Hygiene Association, 2004). The value is consistent with the time weighted average (TWA) exposure limits established in Germany and the Netherlands (European-Union, 2003).

The European Union (2003) summarized occupational exposure data for bisphenol A in Europe and the U.S.

Table 15
Estimates of Bisphenol A Intakes Based on Urinary Excretion

Population	Basis for estimates	Mean or median (range) of estimated intake μg/kg bw/day[a]	Reference
22 Japanese adults	Mean excretion of 1.68 μg/day (0.48–4.5 μg/day)	0.028 (0.008–0.075)	Tsukioka (2004)
36 Japanese male students	Median excretion of 1.2 μg/day (<0.21–14 μg/day)	0.02 (<0.0035–0.23)	Arakawa et al. (2004)
5 Japanese males	Median excretion of 1.3 μg/day (<0.58–13 μg/day) over a 5-day period	0.022 (<0.01–0.22)	Arakawa et al. (2004)
Data from Tsukioka (2004) and Arakawa et al. (2004)	Monte Carlo simulations	Mean exposure: 0.028–0.049 in men and 0.034–0.059 in women; low exposures (5th percentile) 0.021–0.037 in men and 0.025–0.044 in women; high exposures (95th percentile): 0.037–0.064 in men and 0.043–0.075 in women	Miyamoto and Kotake (2006)
56 pregnant Japanese females	Bisphenol A concentration in one spot sample was normalized to creatinine and exposure was estimated using average creatinine and urine volume excretion rates, which resulted in a median intake of <2 μg/day (<0.3–7.9 μg/day)	<0.04 (<0.006–0.16)[b]	Fujimaki et al. (2004)
48 Japanese female college students	Authors estimated bisphenol A intake of 0.6–71.4 μg/day, based on a median bisphenol A concentration of 0.77 ng/mg (0.1–11.9 ng/mg) creatinine in a spot urine sample, assumed creatinine excretion of 1200 mg/day and that 20% of the dose is excreted in urine. **[CERHR recalculated values using a 100% urinary excretion rate that is consistent with human data]**	0.01–1.2 based on study author assumptions **[0.015 (0.002–0.24) based on a 100% urinary excretion rate]**	Ouchi and Watanabe (2002)
7 males and 12 females without intentional exposure	**All measurements <LOD of 1.14 μg/L**	**Based on 2 L urine excreted and 60 kg adult exposure <0.038**	Völkel et al. (2005)
394 participants in the NHANES III survey (U.S.)	Median (10th–95th percentile) 1.32 (0.23–7.95) μg bisphenol A/g creatinine in a spot urine sample; **[assumed 100% urinary excretion of bisphenol A in 24 hr and creatinine excretion of 1200 mg/day]**	**[median = 0.026; 10th–95th percentile: 0.005-0.159]**	Calafat et al. (2005)
90 girls, 6–8-years-old (U.S.)	Median (range) 1.8 μg/L (<0.3–54.3) **[assumed 100% urinary excretion of bisphenol A in 24 hr; 1 L per day; 25 kg body weight]**	**[0.07 (<0.012–2.17)]**	Wolff et al. (2006)

[a]Consistent with estimates conducted by Goodman et al. (2006), body weights of 60 kg were assumed, unless otherwise indicated.
[b]A 50-kg body weight was assumed.

Only measured data for bisphenol A are summarized in this report. The European Union stated that the values reported did not account for the effects of personal protective equipment in order to avoid difficulties in attempting to quantify protection provided. TWA bisphenol A concentrations measured in occupational settings are summarized in Table 16. The limited number of values reported indicated that bisphenol A concentrations were below 5 mg/m^3. Bisphenol A exposures (>1 mg/m^3) were observed in spraying of powdered bisphenol A-containing coatings, bisphenol A manufacture and manufacture of epoxy resins. The highest daily average exposures were observed in the manufacture of bisphenol A. There is limited information on short-term exposure to bisphenol A. In manufacture of bisphenol A one facility reported short-term task exposures from 0.13–9.5 mg/m^3 (European-Union, 2003).

Data for powder paint use summarized in Table 16 were obtained from a NIOSH Health Hazard Evaluation conducted at a company that manufactured fan and ventilation equipment (NIOSH, 1979). In Plant 1 of the company, parts were coated with an epoxy-based powder paint by dipping. At Plant 2, an epoxy-based powder was applied to parts via electrostatic spraying. As evident in the data in Table 16, exposures were higher at the plant utilizing electrostatic spraying. Monitoring for bisphenol A was discussed in 2 other NIOSH Health Hazard Evaluation reports. In those reports, bisphenol A was not detected in a plant where an epoxy resin coating was used in the manufacture of electronic resistors

Table 16
TWA Measurements of Bisphenol A in the Workplace

Industry or activity	Location/year	No. of samples	Sample type	8-hr TWA (mg/m^3) mean (range)[b]
Bisphenol A manufacture				
Various	U.S./not specified	Not specified	Bisphenol A	NS (Not detected to 2.6)
Filling big bags	Europe/1998	3	Inhalable bisphenol A	0.81 (0.21-1.79)
Filling silo tankers	Europe/1998	3	Inhalable bisphenol A	0.89 (<0.5-1.61)
Various tasks	Europe/1998	8	Inhalable bisphenol A	0.3 (0.13-0.62)
Plant operator	Europe/not specified	7	Inhalable bisphenol A	NS (0.021-1.04)
Maintenance	Europe/not specified	3	Inhalable bisphenol A	NS (0.52-1.35)
Maintenance	Europe/1998–2000	8	Bisphenol A	NS (<0.05–0.62)
Charging big bags	Europe/1996–1997	5	Inhalable bisphenol A	0.35 (0.02–0.93)
Plant operator	Europe/not specified	13	Bisphenol A	0.61 (0.02–2.13)
Maintenance operator	Europe/not specified	2	Bisphenol A	1.06 (0.4–2.08)
Epoxy resin manufacture				
Loading/unloading	U.S./1970-mid 1990s	26	Bisphenol A	0.18 (<0.1-0.99)
Bagging/palletizing	U.S./1970-mid 1990s	37	Bisphenol A	0.25 (<0.1-2.8)
Process operators	U.S./1970-mid 1990s	25	Bisphenol A	0.26 (<0.1-1.1)
Equipment technician	U.S./1970-mid 1990s	6	Bisphenol A	<0.1
Maintenance	U.S./1970-mid 1990s	2	Bisphenol A	0.8 (0.37-1.2)
Bisphenol A Use				
Powder paint use[a]	U.S./~1979	7 (3 personal and 4 area samples)	Bisphenol A (plant 1)	0.005 (0.004–0.006)
		21 (15 personal and 6 area samples)	Bisphenol A (plant 2)	0.175 (0.001–1.063)

[a]NIOSH (1979). Other data are from the European Union (2003).
[b]Range given representing different occupational activities.
NS, not specified.

(NIOSH, 1984) or in a plant where an epoxy resin coating was applied to steam turbine generators (NIOSH, 1985). Rudel et al. (2001) used a GC/MS technique to measure bisphenol A concentrations at one United States workplace where plastics were melted and glued; a concentration of 0.208 $\mu g/m^3$ was reported.

[Bisphenol A exposures in U.S. powder paint workers were estimated at ~0.1–100 μg/kg bw/day based on TWA exposures of 0.001–1.063 mg/m^3, an inhalation factor of 0.29 m^3/kg day (USEPA, 1988), 100% absorption from the respiratory system, and 8 hr worked per day.]

No information was located for dermal exposure to bisphenol A in occupational settings. Using their Estimation and Assessment of Substance Exposure model, the European Union (2003) estimated that dermal exposure of workers to bisphenol A was unlikely to exceed 5 mg/cm^2/day. It was noted that the highest potential exposure to bisphenol A would occur during bag filling and maintenance work.

One study provided information on biological monitoring of bisphenol A in workers exposed to an epoxy compound. In 3 Japanese plants, exposed workers included 42 men who sprayed an epoxy hardening agent consisting of a mixture of bisphenol A diglycidyl ether (10–30%), toluene (0–30%), xylene (0–20%), 2-ethoxyethanol (0–20%), 2-butoxyethanol (0–20%), and methyl isobutyl ketone (0–30%) (Hanaoka et al., 2002). The workers wore "protection devices" during spraying. Controls consisted of 42 male assembly workers from the same plants who did not use bisphenol A diglycidyl ether. In 1999, urine samples were collected periodically, treated with β-glucuronidase, and examined for bisphenol A by HPLC. Urinary bisphenol A concentrations were significantly higher in exposed workers (median: 1.06 μmol/mol creatinine **[2.14 μg/g creatinine]**; range: <0.05 pmol to 11.2 μmol/mol creatinine **[<0.1 pg to 22.6 μg/g creatinine]**) compared to controls (median: 0.52 μmol/mol creatinine **[1.05 μg/g creatinine]**; range: <0.05 pmol to 11.0 μmol/mol creatinine **[<0.1 pg to 22.2 μg/g creatinine]**). The difference of the averages was reported as 2.5 μmol/mol creatinine **[5.05 μg/g creatinine]** (95% confidence interval [CI] 1.4–4.7 μmol/mol creatinine **[2.8–9.5]**). Bisphenol A was not detected in three exposed workers and one control. **[Assuming excretion of 1200 mg/day creatinine (Ouchi and Watanabe, 2002), mean (ranges) of bisphenol excretion in urine were 2.57 μg/day (<0.12 pg to 27.1 μg/day) in exposed workers and 1.26 μg/day (<0.12 pg to 26.6 μg/day) in unexposed workers. With an assumed body weight of 60 kg, bisphenol A occupational intake was estimated at 0.043 μg/kg bw/day (<0.002 pg to 0.45 μg/kg bw/day) in exposed workers and 0.021 μg/kg bw/day (<0.002 pg to 0.44 μg/kg bw/day) in unexposed workers.]**

1.3 Utility of Data

Numerous studies reported bisphenol A concentrations in canned foods and infant formula. Experiments examined potential concentrations of bisphenol A

Table 17
Maximum Reported Bisphenol A Concentrations in U.S. Ambient Air and Dust Samples

Sample	Bisphenol A concentration	Reference
Outdoor air	$<52\,ng/m^3$ Monthly average 0.12–1.2 ng/m^3	Wilson et al. (2003, 2006); Matsumoto et al. (2005)
Indoor air	$\leq 193\,ng/m^3$	Wilson et al. (2003, 2006); Rudel et al. (2001, 2003)
Indoor dust	$\leq 17.6\,\mu g/g$	Wilson et al. (2003, 2006); Rudel et al. (2001, 2003)
Drinking water	<0.1 (MDL) <0.005	Boyd et al. (2003); Kuch and Ballschmiter (2001)

Table 18
Maximum Reported Bisphenol A Concentrations Measured in Foods or Food Simulants

Exposure source	Bisphenol A concentration	Table reference
Polycarbonate infant bottles	$\leq 55\,\mu g/L$ ($<5\,\mu g/L$ in U.S. study)	Table 4
Polycarbonate tableware	$\leq 5\,\mu g/kg$	Table 4
Canned infant formulas	$\leq 113\,\mu g/L$ ($<6.6\,\mu g\,\mu g/kg$ in U.S. study of water mixed formula; $<13\,\mu g/kg$ in U.S. formula concentrate)	Table 5
Canned infant foods	$\leq 77.3\,\mu g/kg$	
Canned foods	$\leq 842\,\mu g/kg$ ($\leq 39\,\mu g/kg$ in U.S. studies)	Table 6

resulting from leaching of bisphenol A from polycarbonate bottles under a variety of conditions. There minimal data available for bisphenol A concentrations in drinking water but these show concentrations below the limit of detection. Bisphenol A has been detected in surface waters and solid waste landfill leachates. Bisphenol A has been detected in indoor dust samples and indoor and outdoor air samples. Data for occupational exposure to bisphenol A in the U.S. are very limited. Only 2 studies reported TWA exposures to bisphenol A in U.S. workers. Several estimates of human bisphenol A exposure were developed using bisphenol A concentrations measured in food and the environment. Although very limited for U.S. populations, there are data reporting bisphenol A concentrations in urine, breast milk, and amniotic fluid, but none for blood or fetal blood. Exposure estimates have been derived from urinary bisphenol A concentrations in multiple studies.

1.4 Summary of Human Exposure

In 1999 and 2003, it was reported that most bisphenol A produced in the U.S. was used in the manufacture of polycarbonate and epoxy resins and other products [reviewed in (Staples et al., 1998; SRI, 2004)]. Polycarbonate plastics are used in various consumer products and the products most likely to contribute to human exposure are polycarbonate food containers (e.g., milk, water, and infant bottles). Epoxy resins are used in protective coatings. Food cans lined with epoxy resin are a potential source of human exposure. Some polymers manufactured with bisphenol A are FDA-approved for use in direct and indirect food additives and in dental materials (FDA, 2006). Resins, polycarbonate plastics, and other products manufactured from bisphenol A can contain trace amounts of residual monomer and additional monomer may be generated during breakdown of the polymer (European-Union, 2003).

Bisphenol A may be present in the environment as a result of direct releases from manufacturing or processing facilities, fugitive emissions during processing and handling, or release of unreacted monomer from products (European-Union, 2003). Because of its low volatility and relatively short half-life in the atmosphere, bisphenol A is unlikely to be present in the atmosphere in high concentrations (European-Union, 2003). A study of 222 homes and 29 day care centers found bisphenol A in 31–44% of outdoor air samples with concentrations of $<$LOD (0.9) to 51.5 ng/m^3 (Wilson et al., 2006). Rapid biodegradation of bisphenol A in water was reported in the majority of studies reviewed by the European Union (2003) and Staples et al. (1998). Drinking water concentrations of bisphenol A at Louisiana and Detroit Michigan water treatment plants were below the limit of detection ($<0.1\,ng/L$). Chlorinated congeners of bisphenol A resulting from chlorination of water may be degraded less rapidly (Gallard et al., 2004). Bisphenol A is not expected to be stable, mobile, or bioavailable from soils (Fent et al., 2003). A study of 222 homes and 29 day care centers found bisphenol A in 25–70% of indoor dust samples with concentrations of $<$LOD (20) to 707 ng/g (Wilson et al., 2006). The potential for bioconcentration of bisphenol A in fish is low (Staples et al., 1998; European-Union, 2003). Table 17 summarizes concentrations of bisphenol A detected in environmental samples and drinking water.

The highest potential for human exposure to bisphenol A is through products that directly contact food such as food and beverage containers with internal epoxy resin coatings and polycarbonate tableware and bottles, such as those used to feed infants (European-Union, 2003). Studies examining the extraction of bisphenol A from polycarbonate bottles or tableware into food simulants are summarized in Table 4. Studies measuring bisphenol A concentrations in canned infant foods are summarized in Table 5 and studies measuring bisphenol A concentrations in canned food are summarized in Table 6. Table 18 summarizes the general findings from all the food contact–material studies. Bisphenol A concentrations were measured in canned foods produced and purchased from various countries.

Table 19 summarizes BPA concentrations reported in human body fluids. Measurement of bisphenol A concentrations are affected by measurement technique,

Table 19
Maximum Reported Biological Measures of Bisphenol A Concentrations in Humans

Biological medium	Population	Concentration free BPA[a] (μg/L)	Total BPA[a] (μg/L)	Reference
Urine	Adult	≤2.36 (<0.6 in U.S. study)	≤3950 (<19.8 U.S. studies)	Table 8
	Children		<54 (2 U.S. studies)	
Blood	General	<LOD (0.5)	<LOD (0.5)	Table 7
	Infertile women	<0.87		Table 7
	Women	<1.6		Table 7
	Men	<1		Table 7
	Fetal	<9.2		Table 9
Breast milk	Women	<6.3 (U.S.)	<7.3	Table 3
Amniotic fluid	Fetus	<1.96 (U.S)		Table 9
Semen	Adult	<0.5		Inoue et al.(2002)
Saliva after dental sealant	Adult	<2800		Arenholt-Bindslev et al. (1999)

[a]Measurements by HPLC, GC/MS, and LC/MS only.

Table 20
Summary of Reported Human Dose Estimates

Exposure source	Population	BPA μg/kg bw/day	Notes	Source
Estimates based on intake				
Formula	Infant	1.6–8	8 assumes 700 ml formula with 50 μg/L	Table 14
Formula	Infant	1.0	Assumes 4.5 kg, 700 ml formula with 6.6 μg/L from U.S. canned formula	Expert Panel
Breast milk	Infant	1.0	Assumes 4.5 kg, 700 ml with 6.3 μg/L from breast milk	Expert Panel
Food	Infant	1.65–5	5 assumes 0.375 kg canned food at 100 μg/kg	Table 14
	Child	0.00475–1.2	1.2 assumes 1 kg canned food at 20 μg/kg	Table 14
	Adult	0.00195–1.4	1.4 assumes 1 kg canned food at 100 μg/kg	Table 14
Aggregate	Infant (formula)	0.055–0.18	Assumes 0–0.17 μg/L in formula	Table 14
	Infant (breast milk)	0.028–0.16	Assumes 0 exposure from breast milk	Table 14
	Child	0.042981–14.7	14.7 assumes 2 kg canned food at 100 μg/kg	Table 14
	Adult	0.36–0.43	Assumes 0–602 μg/kg in canned food	Table 14
Occupational	Adult	0.043–100		EPA and Expert Panel
Estimates based on urinary metabolites				
Aggregate	Child	0.07 (2.17)	Median (max) U.S. 6–8-year-old girls	Table 15
	Adult	0.026	Median NHANES	Table 15
	Adult	0.66	Assume max 19.8 μg/L from U.S., 2 L urine/day, 60 kg	Ye et al. (2005)

particularly at the very low concentrations that can now be measured. ELISA has poor correlation with the LC-ECD method and also the different ELISA kits correlate poorly with each other. ELISA methods may overestimate bisphenol A in biologic samples due to lack of specificity of the antibody and effects of the biologic matrix (Inoue et al., 2002; Fukata et al., 2006). In addition, contamination from labware and reagents or sample degradation during storage can impact the accuracy of measurements. **[The panel therefore finds the greatest utility in studies that use sensitive and specific analytical methods for biological samples (LC-MS or GC-MS) and report quality control measures for sample handling and analysis.]**

Table 20 summarizes food and/or aggregate exposure estimates calculated from bisphenol A concentrations in food, environmental and toy exposures along with estimates of consumption and body weights. It was noted that dietary sources account for 99% of exposure (Wilson et al., 2006). Metabolite-based estimates of bisphenol A used urinary concentrations along with estimates of urinary and/or creatinine excretion, and body weight.

Dental sealant exposure to bisphenol A occurs primarily with use of dental sealants bisphenol A dimethylacylate. This exposure is considered an acute and infrequent event with little relevance to estimating general population exposures.

Very limited information is available for bisphenol A exposure in the U.S. workplace. Data obtained from the U.S. and Europe indicate highest potential exposures during spraying of powdered bisphenol A-containing coatings and during tank filling, plant operation activities, and maintenance work in plants where bisphenol A is manufactured. (European-Union, 2003). One study measured total urinary bisphenol A in Japanese workers who sprayed an epoxy compound (Hanaoka et al., 2002).

2.0 GENERAL TOXICOLOGY AND BIOLOGICAL EFFECTS

As discussed in **Section 1.4**, the quantified amount of free bisphenol A present in biological samples may be affected by contamination with bisphenol A in plastic laboratory ware and in reagents (Tsukioka et al., 2004; Völkel et al., 2005). In addition, the accuracy may also be affected by measurement technique, particularly at the very low concentrations that can now be measured. ELISA have the potential to overestimate bisphenol A in biologic samples due to lack of specificity of the antibody and effects of the biologic matrix (Inoue et al., 2002; Fukata et al., 2006). High performance liquid chromatography (HPLC) with ultraviolet, fluorescence, or electrochemical detection is unable to make definitive identification of bisphenol A or bisphenol A glucuronides, because similar retention times may occur for the metabolites of other endogenous and exogenous compounds (Völkel et al., 2005). Use of LC-tandem mass spectrometry (MS/MS) with and without hydrolysis of bisphenol A glucuronide permits determination of free and total bisphenol A with a limit of quantification of 1 μg/L (Völkel et al., 2005). Gas chromatography (GC)/MS/MS has been used with solid phase extraction after treatment with glucuronidase and derivatization to measure total bisphenol A with a limit of detection of 0.1 μg/L (Calafat et al., 2005). Bisphenol A glucuronidate has been shown to be unstable and can be hydrolyzed to free bisphenol A at neutral pH and room temperature in diluted urine of rats and in rat placental and fetal tissue homogenates at room temperature. Bisphenol A glucuronide can also be hydrolyzed and in some cases degraded to unknown components either in acidic or basic pH solutions of diluted urine, adding another potential source of error in the measurement of sample levels of bisphenol A and its conjugates (Waechter et al., 2007). These considerations taken together, suggest that it is possible that free bisphenol A concentrations measured in biological samples may be overestimated.

2.1 Toxicokinetics and Metabolism

The studies presented in this section demonstrate that bisphenol A is absorbed in humans and experimental animals following oral exposure. In humans and experimental animals, most of the dose is present in blood as the main metabolite, bisphenol A glucuronide, and smaller percentages are present as the parent compound. Bisphenol A and its metabolites are widely distributed in humans and animals. More than 90% of unmetabolized bisphenol A is reportedly bound to plasma protein. Bisphenol A is distributed to fetal fluids in humans and experimental animals, and a limited number of studies in humans demonstrate fetal concentrations of bisphenol A within an order of magnitude of concentrations in maternal blood. None of the studies detected bisphenol A glucuronide in fetal fluids. Transfer of bisphenol A to milk was demonstrated in humans and experimental animals. One study in humans reported bisphenol A in milk at concentrations exceeding maternal blood concentrations. In humans and experimental animals, most of a bisphenol A dose is metabolized to bisphenol A glucuronide before absorption. Studies in humans and experimental animals demonstrated that glucuronidation of bisphenol A can occur in the liver, and one study in rats demonstrated that bisphenol A is glucuronidated upon passage through the intestine. Bisphenol A glucuronide is excreted in the bile of rats, and enterohepatic cycling is thought to occur in rats but not humans. In humans, most of a bisphenol A dose is eliminated through urine as bisphenol A glucuronide. In rats, bisphenol A is eliminated through feces as bisphenol A and in urine as bisphenol A glucuronide.

2.1.1 Humans. Human toxicokinetics studies that were judged potentially important to interpret developmental and reproductive toxicity were reviewed in full. These studies include reports of potential exposure of fetuses during pregnancy or of infants through human milk and reports of toxicokinetics or metabolism following low-dose exposure of humans. Information from secondary sources was included if the information was not considered to be critical to the interpretation of developmental and reproductive toxicity data.

2.1.1.1 Absorption: Two studies described here examined oral absorption of bisphenol A from dental sealants, and one study examined in vitro dermal absorption. Bisphenol A (as parent or the monoglucuronide) is absorbed in humans as indicated by the detection of bisphenol A (and metabolites) in blood from the general population (Section 1) and in maternal and fetal fluids (Table 9).

Fung et al. (2000) examined the toxicokinetics of bisphenol A leaching from dental sealant. Volunteers included 18 men and 22 non-pregnant women (20–55 years of age) who did not have dental disease, existing composite resin restorations or pit and fissure sealants, or a history of resin exposure. Volunteers were treated with a widely used commercial dental sealant (Delton Opaque Light-cure Pit and Fissure Sealant). Components of the sealant were analyzed by HPLC. The low-dose group ($n = 7$ men, 11 women) received 8 mg dental sealant on 1 tooth, and the high-dose group (11 men, 11 women) received 32 mg sealant on 4 teeth. Saliva and blood samples were collected before the procedure and at 1 and 3 hr and 1, 3, and 5 days after the procedure. Blood and saliva were analyzed by HPLC. Statistical analyses of data were conducted by nonparametric test, Wilcoxon signed rank test, and χ^2 test. Analysis of the dental sealant revealed that bisphenol A concentrations were below the detection limit of 5 ppb. At 1 hr following treatment, bisphenol A was detected in samples from 3 of 18 volunteers in the low-dose group and 13 of 22 samples from volunteers in the high-dose group. At 3-hr post-treatment, bisphenol A was detected in samples from 1 of 18 volunteers in the low-dose group and 7 of 22 volunteers in the high-dose group. Concentrations of bisphenol A in saliva at 1 and 3 hr following exposure were reported at 5.8–105.6 ppb **[μg/L]**. No bisphenol A was detected in saliva samples at 24 hr or in serum samples at any time point. Differences between the low-dose and high-dose groups in bisphenol A saliva concentrations and in the proportion of bisphenol A-positive saliva samples at 1 and 3 hr achieved statistical significance. In the high-dose group, a significant difference in "readings" was observed between 1 and 3 hr. **[The data as presented did not illustrate possible quantitative differences in saliva bisphenol A concentrations from the 2 dose groups or at different sampling times.]**

Joskow et al. (2006) examined bisphenol A in urine and saliva of 14 adults (19–42 years old) treated with dental

Table 21
Saliva and Urinary Concentrations of Total Bisphenol A in Adults Receiving Dental Sealants[a]

Collection time	Mean ± SD bisphenol A concentration (ng/mL)[b]		
	Both sealants	Delton LC	Helioseal F
Saliva			
Pretreatment	0.30 ± 0.17	0.34 ± 0.19	**0.22 ± 0.03**
Immediately after treatment	26.5 ± 30.7	42.8 ± 28.9	**0.54 ± 0.45**
1 hr post-treatment	5.12 ± 10.7	7.86 ± 12.73	**0.21 ± 0.03**
Urine (creatinine-adjusted)			
Pretreatment	2.41 ± 1.24	2.6 ± 1.4	**2.12 ± 0.93**
1 hr post-treatment	20.1 ± 33.1	27.3 ± 39.1	**7.26 ± 13.5**
24 hr post-treatment	5.14 ± 3.96	7.34 ± 3.81	**2.06 ± 1.04**

[a]Joskow et al. (2006).
[b]Samples were treated with β-glucuronidase.

sealants. Excluded from the study were individuals with resin-based materials on their teeth, smokers, users of antihistamines, and patients with Gilbert syndrome. The volunteers received either Helioseal F ($n = 5$) or Delton LC ($n = 9$) sealant. Sealant was weighed before and after application to determine the amount applied, and the number of treated teeth was recorded. The mean number of teeth treated was 6/person and the mean total weight of sealant applied was 40.35 mg/person. In a comparison of the 2 sealants, no differences were reported for number of teeth treated or amount of sealant applied. Saliva samples were collected before treatment, immediately after, and at 1 hr following sealant application. Urine samples were collected before treatment and at 1 and 24 hr following sealant placement. A total of 14–15 saliva samples and 12–14 urine samples were collected at each time point. Samples were treated with β-glucuronidase and analyzed for bisphenol A concentrations using selective and sensitive isotope-dilution-MS-based methods. Table 21 summarizes changes in saliva and bisphenol A concentrations. Immediately and at 1 hr after sealant application, salivary concentrations of bisphenol A compared to baseline were significantly higher in the patients who received the Delton LC sealant. Bisphenol A concentrations in saliva increased >84-fold following application of the Delton LC sealant. Urinary concentrations of bisphenol A were increased 1 hr following application of the Delton LC sealant. Concentrations of bisphenol A in saliva and urine following application of Helioseal F were reported to be similar to baseline.

The European Union (2003) reviewed unpublished preliminary data from a human dermal absorption study. Skin samples obtained from 3 human donors (6 samples/donor/dose) were exposed to 5 or 50 mg/cm^2 (3.18 or 31.8 mg/mL) ^{14}C-bisphenol A in ethanol vehicle. Following evaporation of the vehicle, bisphenol A was resuspended in artificial sweat. Radioactivity was measured in receptor fluid at various time intervals over a 24-hr period. Radioactivity was measured in the stratum corneum and ''lower'' skin layer at 24 hr. Authors of the European Union report noted that tritiated water was not used as a marker for skin integrity. However, based on the patterns of results, they concluded that skin integrity was likely lost after 4–8 hr. The European Union authors therefore concluded that the only reliable data from the study were those for the cumulative percentage of the dose in receptor fluid at 8 hr, which was reported at 0.57–1.22% at 5 mg/cm^2 and 0.491–0.835% at 50 mg/cm^2. Because radioactivity in skin was not measured at 8 hr, the percentage of the applied dose remaining on skin and available for future absorption could not be determined. Based on ratios of receptor fluid concentrations and lower skin levels (1:2 to 1:8) at 24 hr, and assuming that the higher ratio applies to skin at 8 hr, the authors of the European Union report predicted that 10% of the dose would be present in ''lower'' skin layers. Therefore, dermal absorption of bisphenol A was estimated at 10%.

2.1.1.2 Distribution: In humans, bisphenol A was measured in cord blood and amniotic fluid, demonstrating distribution to the embryo or fetus. Studies reporting bisphenol A concentrations in fetal and/or maternal compartments are summarized in Table 9. Detailed descriptions of those studies are also presented below.

Engel et al. (2006) reported concentrations of bisphenol A in human amniotic fluid. Twenty-one samples were obtained during amniocentesis conducted before 20 weeks gestation in women who were referred to a U.S. medical center for advanced maternal age. Bisphenol A concentrations in amniotic fluid were measured using LC with electrochemical detection. Bisphenol A was detected in 10% of samples at concentrations exceeding the LOD (0.5 μg/L). Bisphenol A concentration ranges of 0.5–1.96 μg/L were reported.

Schönfelder et al. (2002b) examined bisphenol A concentrations in maternal and fetal blood and compared bisphenol A concentrations in blood of male and female fetuses. In a study conducted at a German medical center, blood samples were obtained from 37 Caucasian women between 32–41 weeks gestation. At parturition, blood was collected from the umbilical vein after expulsion of the placenta. Bisphenol A concentrations in plasma were measured by GC/MS. Control experiments were conducted to verify that bisphenol A did not leach from collection, storage, or testing equipment. Bisphenol A was detected in all samples tested, and concentrations measured in maternal and fetal blood are summarized in Table 9. Mean bisphenol A concentrations were higher in maternal (4.4 ± 3.9 [SD] μg/L) than fetal blood (2.9 ± 2.5 μg/L). Study authors noted that in 14 cases fetal bisphenol A plasma concentrations exceeded those detected in maternal plasma. Among those 14 cases, 12 fetuses were male. Analysis by paired *t*-test showed significantly higher mean bisphenol A concentrations in the blood of male than female

fetuses (3.5 ± 2.7 vs. 1.7 ± 1.5 ng/mL, $P = 0.016$). Bisphenol A concentrations were measured in placenta samples at 1.0–104.9 μg/kg.

Ikezuki et al. (2002) measured concentrations of bisphenol A in serum from 30 healthy premenopausal women, 37 women in early pregnancy, 37 women in late pregnancy, and 32 umbilical cord blood samples. Concentrations of bisphenol A were also measured in 32 samples of amniotic fluid obtained during weeks 15–18 of gestation, 38 samples of amniotic fluid obtained at full-term cesarean section, and 36 samples of ovarian follicular fluid collected during in vitro fertilization procedures. **[It was not stated if different sample types were obtained from the same subjects.]** An ELISA method was used to measure bisphenol A concentrations and results were verified by HPLC. The mean ± SD concentration of bisphenol A in follicular fluid was reported at 2.4 ± 0.8 μg/L. As summarized in Table 7 for nonpregnant women and Table 9 for maternal and fetal samples, concentrations of bisphenol A in follicular fluid were similar to those detected in the serum of fetuses and pregnant and non-pregnant women and in amniotic fluid collected in late pregnancy (~1–2 μg/L). Bisphenol A concentrations in amniotic fluid samples collected in early pregnancy were ~5-fold higher than in other samples, and the difference achieved statistical significance ($P < 0.0001$). Study authors postulated that the higher concentrations of bisphenol A in amniotic fluid collected during gestation weeks 15–18 may have resulted from immature fetal liver function. They noted that according to unpublished data from their laboratory, the percentage of glucuronidated bisphenol A in mid-term amniotic fluid was ~34%, which is much lower than reported values for other human fluids (>90%).

Yamada et al. (2002) measured bisphenol A concentrations in maternal serum and amniotic fluid from Japanese women. Samples were collected between 1989–1998 in women undergoing amniocentesis around gestation week 16. One group of samples was obtained from 200 women carrying fetuses with normal karyotypes, and a second group of samples was obtained from 48 women carrying fetuses with abnormal karyotypes. An ELISA method was used to measure bisphenol A concentrations. **[As discussed in Section 1.1.5, ELISA may overestimate bisphenol A.]** Concentrations of bisphenol A measured in maternal plasma and amniotic fluid are summarized in Table 9. Median concentrations of bisphenol A in maternal serum (~2–3 μg/L) were significantly higher **[~10-fold]** than concentrations in amniotic fluid (~0–0.26 μg/L) in the groups carrying fetuses with normal and abnormal karyotypes. However, in 8 samples from women carrying fetuses with normal karyotypes, high concentrations (2.80–5.62 μg/L) of bisphenol A were measured in amniotic fluid. The study authors interpreted the data as indicating that bisphenol A does not accumulate in amniotic fluid in most cases but accumulation is possible in some individuals. Bisphenol A concentrations in maternal blood were significantly higher **[by ~33%]** in woman carrying fetuses with abnormal versus normal karyotypes. However, the study authors noted that the effect may not be related to bisphenol A exposure because there was no adjustment for maternal age, and concentrations in amniotic fluid did not differ between groups. In the group carrying fetuses with normal karyotypes, data obtained from 1989–1998 were summarized by year. Median bisphenol A concentrations in serum significantly decreased over that time from a concentration of 5.62 μg/L detected in 1989 to 0.99 μg/L in 1998.

Kuroda et al. (2003) used an HPLC method to measure bisphenol A concentrations in 9 sets of maternal and cord blood samples obtained from Japanese patients at the time of delivery. Bisphenol A concentrations were also measured in 21 sets of serum and ascitic fluid samples collected from sterile Japanese patients of unspecified sexes and ages. Results for pregnant women are summarized in Table 9. Mean ± SD concentrations of bisphenol A were lower in maternal (0.46 ± 0.20 ppb **[μg/L]**) than cord blood (0.62 ± 0.13 ppb **[μg/L]**). There was a weak positive correlation ($r = 0.626$) between bisphenol A concentrations in maternal and cord blood. Concentrations of bisphenol A in the blood of sterile patients are summarized Table 7. There were no differences between pregnant and non-pregnant blood levels (Kuroda et al., 2003). Mean ± SD concentrations of bisphenol A were higher in ascitic fluid (0.56 ± 0.19 ppb **[μg/L]**) than in serum (0.46 ± 0.20 ppb **[μg/L]**). The correlation between bisphenol A concentration in serum and ascitic fluid was relatively strong ($r = 0.785$).

Tan and Mohd (2003) used a GC/MS method to measure bisphenol A concentrations in cord blood at delivery in 180 patients at a Malaysian medical center. Bisphenol A was detected in 88% of samples. As noted in Table 9 concentrations ranged from <0.10–4.05 μg/L.

Calafat et al. (2006) reported a median bisphenol A concentration of ~1.4 μg/L **[as estimated from a graph]** in milk from 32 women. Bisphenol A was measured after enzymatic hydrolysis of conjugates. Ye et al. (2006) found measurable milk concentrations of bisphenol A in samples from 18 of 20 lactating women. Free bisphenol A was found in samples from 12 women. The median total bisphenol concentration in milk was 1.1 μg/L (range: undetectable to 7.3 μg/L). The median free bisphenol A concentration was 0.4 μg/L (range: undetectable to 6.3 μg/L).

Sun et al. (2004) used an HPLC method to measure bisphenol A concentrations in milk from 23 healthy lactating Japanese women. Bisphenol A concentrations ranged from 0.28–0.97 μg/L, and the mean ± SD concentration was reported at 0.61 ± 0.20 μg/L. No correlations were observed between bisphenol A and triglyceride concentrations in milk. Values from six milk samples were compared to maternal and umbilical blood samples previously reported in a study by Kuroda et al. (2003). Bisphenol A values were higher in milk, and the milk/serum ratio was reported at 1.3. Bisphenol A values in milk were comparable to those in umbilical cord serum. **[It was not clear whether milk and serum samples were obtained from the same volunteers in the two studies.]**

Schaefer et al. (2000) measured concentrations of bisphenol A and other compounds in uterine endometrium of women undergoing hysterectomy for uterine myoma at a German medical center. Endometrial and fat samples were obtained between 1995–1998 from 23 women (34–51 years old) with no occupational exposure. Samples were handled with plastic-free materials and stored in glass containers. Concentrations of environmental chemicals were measured in samples by GC/MS. None of 21 fat samples had detectable concentrations of

bisphenol A. Bisphenol A was detected in 1 of 23 endometrial samples; the median concentration was reported at <1 μg/kg wet weight, and the range was reported at 0–13 μg/kg. **[It is not known why a median value and range were reported when bisphenol A was only detected in 1 sample.]**

As part of a study to compare an ELISA and an LC/MS method for biological monitoring of bisphenol A, Inoue et al. (2002) measured concentrations of bisphenol A in semen samples obtained from 41 healthy Japanese volunteers (18–38 years old). Analysis by the ELISA method indicated bisphenol A concentrations ranging from concentrations below the detection limit (2.0 μg/L) to 12.0 μg/L. The LC/MS method indicated that the bisphenol A concentration in all samples was <0.5 μg/L, the LOQ. The study authors concluded that the LC/MS method was more accurate and sensitive and that the ELISA method overestimated bisphenol A concentrations, possibly due in part to nonspecific antibody interactions.

2.1.1.3 Metabolism: Völkel et al. (2005) measured bisphenol A and metabolite concentrations in human urine following exposure to a low bisphenol A dose. The human volunteers consisted of 3 healthy females (25–32 years old) and 3 healthy males (37–49 years old) who were asked to refrain from alcohol and medicine intake for 2 days before and during the study. Volunteers received 25 μg D_{16}-bisphenol A in drinking water **[0.00028–0.00063 mg/kg bw based on reported body weights]**, a dose reported to represent a worst-case human exposure. Urine samples were collected at 0, 1, 3, 5, and 7 hr following exposure. Analyses for D_{16}-bisphenol A and D_{16}-bisphenol A-glucuronide were conducted by LC/MS and HPLC. Recovery of D_{16}-bisphenol A-glucuronide in urine within 5 hr of dosing was 85% of dose in males and 75% of dose in females. Analysis following treatment of urine with glucuronidase resulted in recovery of 97% of the dose in males and 84% of the dose in females. The highest concentrations of bisphenol A glucuronide in urine were measured at 1 hr (221–611 pmol **[50–139 ng bisphenol A eq]**/mg creatinine) and 3 hr (117–345 pmol **[27–79 ng bisphenol A eq]**/mg creatinine) following exposure. Elimination half-life was estimated at 4 hr. Bisphenol A concentrations exceeding the detection limit were detected in only 2 urine samples at concentrations of ~10 pmol **[2 ng]**/mg creatinine.

Völkel et al. (2002) examined toxicokinetics and metabolism of bisphenol A in humans administered a low dose. Volunteers in this study consisted of 3 healthy females (24–31 years of age) and 6 healthy males (28–54 years of age) who were non- or occasional smokers; volunteers were asked to refrain from alcohol and medicine intake for 2 days before and during the study. In two different studies, D_{16}-bisphenol A was orally administered to volunteers via gelatin capsules at a dose of 5 mg (0.054–0.090 mg/kg bw). The dose was reported to be ~10-fold higher than the estimated human exposure level of 0.6 mg/day. In the first study, urine samples were collected at 6-hr intervals until 42 hr following exposure and blood samples were collected at 4-hr intervals until 32 hr following exposure in 3 males and 3 females. In a second, more detailed study conducted in 4 of the male volunteers, blood samples were collected at 30–60-min intervals until 381 min following exposure. Samples were analyzed by GC/MS and LC/MS. In the first study, a terminal half-life of 5.3 hr was reported for D_{16}-bisphenol A glucuronide clearance from blood. The half-life for urinary elimination was reported at 5.4 hr. D_{16}-Bisphenol A glucuronide concentrations in plasma and urine fell below LOD at 24–34 hr post-dosing. Complete urinary recovery (100%) was reported for the D_{16}-bisphenol A glucuronide. In the second study, maximum plasma concentration of D_{16}-bisphenol A glucuronide (~800 pmol **[183 ng bisphenol A eq]**/mL) was obtained 80 min after oral administration. The half-life for initial decline in plasma was reported at 89 min. Free D_{16}-bisphenol A was not detected in plasma. According to study authors, the study demonstrated rapid absorption of bisphenol A from the gastrointestinal tract, conjugation with glucuronic acid in the liver, and rapid elimination of the glucuronide in urine. Study authors noted that the rapid and complete excretion of bisphenol A glucuronide in urine suggested that in contrast to rats, enterohepatic circulation did not occur in humans.

Table 8 in Section 1 provides information on bisphenol A and metabolites detected in human urine. A study conducted in the U.S. used an HPLC method to examine 30 urine samples collected from a demographically diverse adult population in 2000–2004 (Ye et al., 2005). Mean urinary compound composition was 9.5% bisphenol A, 69.5% bisphenol A glucuronide, and 21% bisphenol A sulfate conjugate. A study conducted in Korea used an HPLC method to examine urine collected from 15 men (mean age = 42.6 years) and 15 women (mean age = 43.0 years) (Kim et al., 2003b). Sex-related differences were observed for urinary metabolic profiles. Mean urinary compound composition in men was reported at 29.1% bisphenol A, 66.2% bisphenol A glucuronide, and 4.78% bisphenol sulfate conjugate. The urinary metabolite profile in females was 33.4% bisphenol A, 33.1% bisphenol A glucuronide, and 33.5% bisphenol A sulfate conjugate. The study authors concluded that women had a greater ability for sulfation than men.

2.1.1.4 Excretion: As discussed in greater detail in Section 2.1.1.3, two studies in which human volunteers were administered low doses of D_{16}-bisphenol A (~0.00028–0.090 mg/kg bw) demonstrated that most of the dose (85–100%) was eliminated through urine (Völkel et al., 2002, 2005). In those studies, the half-lives for urinary elimination were reported at 4–5.4 hr. As discussed in more detail in Section 2.1.1.3, examination of human urine samples revealed that bisphenol A glucuronide and sulfate conjugates are present at higher concentrations than is the parent compound (Kim et al., 2003b; Ye et al., 2005).

2.1.2 Experimental animal. Original animal studies that were potentially important for the interpretation of developmental and reproductive toxicity were reviewed thoroughly. Examples included:

- Studies examining toxicokinetics or metabolism in pregnant or lactating animals;
- Studies examining toxicokinetic difference observed with different doses or exposure routes;
- Studies looking at age-related differences in toxicokinetics or metabolism; and
- Studies in non-rodent species such as primates.

Secondary sources were utilized for general information not considered critical to the interpretation of developmental and reproductive toxicity data.

2.1.2.1 Absorption: In rats orally exposed to bisphenol A at doses ≤100 mg/kg bw, maximum bisphenol A concentrations (C_{max}) were generally measured in plasma within 0.083–0.75 hr following exposure (Pottenger et al., 2000; Takahashi and Oishi, 2000; Yoo et al., 2001; Domoradzki et al., 2004; Negishi et al., 2004b). At doses of 1 or 10 mg/kg bw, time to maximum bisphenol A concentration (T_{max}) in plasma was longer in postnatal day (PND) 21 rats (1.5–3 hr) than in PND 4 and 7 rats (0.25–0.75 hr) (Domoradzki et al., 2004). In a limited number of studies in which rats were subcutaneously (s.c.) dosed with up to 100 mg/kg bw bisphenol A, time (0.5–4 hours) to reach C_{max} was longer than with oral dosing, although the findings were not always consistent (Pottenger et al., 2000; Negishi et al., 2004b). In one study, T_{max} was comparable in oral and intraperitoneal (i.p.) dosing of rats (Pottenger et al., 2000). Another study reported that C_{max} was attained at 0.7 hr in monkeys orally exposed to 10 or 100 mg/kg bw bisphenol A and at 0.5 hr in chimpanzees orally exposed to 10 mg/kg bw bisphenol A (Negishi et al., 2004b). In the same study, a longer T_{max} (2 hr) was observed following exposure of monkeys and chimpanzees to the same doses by s.c. injection compared to oral intake. Additional details for these studies are presented below.

As discussed in greater detail in Section 2.1.2.3, bisphenol A is glucuronidated in the liver and intestine, and most of the dose is absorbed as bisphenol A glucuronide following oral exposure of rats (Domoradzki et al., 2004). In ovariectomized rats gavaged with bisphenol A, bioavailability of bisphenol A was reported at 16.4% at a 10 mg/kg bw dose and 5.6% at a 100 mg/kg bw dose (Upmeier et al., 2000). The findings are fairly consistent with a second study in which maximum plasma values of free bisphenol A represented low percentages **[<2–8%]** of the total radioactive dose in rats orally administered bisphenol A at 10 or 100 mg/kg bw (Pottenger et al., 2000); maximum values of free bisphenol A represented higher percentages of the radioactive dose in rats given 10 or 100 mg/kg bw s.c. **[64–82% free bisphenol A]** or i.p. **[19–54%]** (Pottenger et al., 2000). Percentages of parent bisphenol A in blood were also higher in monkeys exposed intravenously (i.v.; 5–29%) than orally (0–1%) (Kurebayashi et al., 2002). Similarly, HPLC analysis of plasma conducted 1 hr following s.c. or gavage dosing of 4 female 21-day-old Sprague–Dawley rats/group with bisphenol A revealed higher bisphenol A plasma concentrations with s.c. than with gavage dosing (Table 22) (Yamasaki et al., 2000). One study in male and female rats gavaged with 10 mg/kg bw bisphenol A demonstrated higher plasma concentrations of bisphenol A in immature animals than in adults (10.2–48.3 μg/g **[mg/L]** plasma at 4 days of age; 1.1–1.4 μg/g **[mg/L]** plasma at 7 days of age; 0.2 μg/g **[mg/L]** plasma at 21 days of age; and 0.024–0.063 μg/g **[mg/L]** plasma in adulthood) (Domoradzki et al., 2004).

A review by the European Union (2003) noted that in the study by Pottenger et al. (2000), fecal excretion represented the highest proportion of the eliminated dose (74–83% in males and 52–72% in females) following oral or parenteral exposure of rats to 10 or 100 mg/kg bw bisphenol A. The authors of the European Union report therefore concluded that absorption **[assumed to be of the radioactive dose]** is likely extensive following oral intake. Adding to the proof of extensive oral absorption is the observation that >50% of fecal elimination occurred at 24 hr post-dosing, a time period beyond the average gastrointestinal transit time of 12–18 hr for rats. Possible explanations provided for the detection of parent compound in feces were cleavage of conjugates within intestines and enterohepatic circulation.

Table 22
Plasma Bisphenol A Concentrations in 21-Day-Old Rats at 1 Hr Following Oral Gavage or S.C. Dosing[a]

	Plasma concentration, μg/L	
Dose, mg/kg bw	Injection (s.c.)	Oral gavage
0 (sesame oil vehicle)	Not detected	Not detected
8	94.6±58.0	Not examined
40	886.3±56.4	Not detected
160	2948±768.8	198.8±88.2
800	Not examined	2879.0±2328.3

Values presented as mean±SD.
[a]Yamasaki et al. (2000).

2.1.2.2 Distribution

2.1.2.2.1 Pregnant or lactating animals: Information on distribution in pregnant or lactating rats is presented first followed by other species. Studies including oral exposures are summarized before those with parenteral exposures.

Takahashi and Oishi (2000) examined disposition and placental transfer of bisphenol A in F344 rats. Rats were orally administered 1000 mg/kg bw bisphenol A (>95% purity) in propylene glycol on gestation day (GD) 18 (GD 0 = day of vaginal plug). Rats were killed at various time points between 10 min and 48 hr after bisphenol A dosing. At each time point, 2–6 dams and 8–12 fetuses obtained from 2–3 dams were analyzed. Blood was collected from dams and kidneys, livers, and fetuses were removed for measurement of bisphenol A concentrations by HPLC. Results are summarized in Table 23. Study authors noted the rapid appearance of bisphenol A in maternal blood and organs and in fetuses. Concentrations of bisphenol A at 6 hr following dosing were 2% of peak concentrations in maternal blood and 5% of peak concentrations in fetuses. It was noted that in fetuses, area under the time-concentration curve (AUC) was higher and mean retention time, variance of retention time, and terminal half-life were longer than in maternal blood.

Dormoradzki et al. (2003) examined metabolism, toxicokinetics, and embryo-fetal distribution of bisphenol A in rats during 3 different gestation stages. Sprague–Dawley rats were gavaged with bisphenol A (99.7% purity)/radiolabeled ^{14}C-bisphenol A (98.8% radiochemical purity) at 10 mg/kg bw. Bisphenol A was administered to 1 group of non-pregnant rats and 3 different groups of pregnant rats on GD 6 (early gestation), 14 (mid gestation), or 17 (late gestation). GD 0 was defined as the day that sperm or a vaginal plug were detected. Blood, urine, and feces were collected at multiple time points between 0.25 and 96 hr post-dosing. It appears that most and possibly all samples were pooled. Four rats

in each group were killed at 96 hr post-dosing. Maternal organs, 6 embryos or fetuses/dam (when possible), and placentas were collected. Samples were analyzed for radioactivity and bisphenol A and/or bisphenol A glucuronide by HPLC/liquid scintillation spectrometry.

In all groups, 90–94% of radioactivity was recovered. Elimination of bisphenol A and its metabolites is discussed in Section 2.1.2.4. At 96 hr following dosing, low percentages of the dose were present in carcass (~1–6%) and tissues such as brain, fat, liver, kidney, ovary, uterus, and skin. The only quantifiable data in placentas and fetuses at 96 hr were obtained in the GD 17 group, and those samples contained 0.01–0.07% of the bisphenol A dose. Standard deviations for maternal and fetal tissues generally exceeded 50% of the mean. Study authors concluded that disposition of radioactivity was similar in pregnant and non-pregnant rats.

Toxicokinetic data obtained from plasma profiles are summarized in Table 24. The authors stated that there was high inter-animal variability. The presence of two C_{max} values was noted by the authors, and they stated that it was the result of enterohepatic circulation of radioactivity. Bisphenol A was not quantifiable in most plasma samples. Because bisphenol A glucuronide represented most (~95–99%) of the radioactivity, plasma profiles for that metabolite were nearly identical to profiles for radioactivity.

Table 23
Toxicokinetic Endpoints for Bisphenol A in Rats Dosed With 1000 mg/kg bw Bisphenol A on GD 18[a]

	Maternal tissue			
Endpoint	Blood	Liver	Kidney	Fetus
C_{max}, mg/L	14.7	171	36.2	9.22
T_{max}, min	20	20	20	20
AUC, mg hr/L	13.1	700	84.0	22.6
Mean retention time, hr	10.6	29.3	12.0	20.0
Variance in retention time, hr squared	203	657	227	419
Half-life, hr				
From 20–40 min	0.0952	0.178	0.245	0.55
From 40 min–6 hr	2.58	1.75	2.98	1.60
From 6–48 hr	4.65	No data	No data	173

[a]Takahashi and Oishi (2000).

A second study was conducted by Dormoradzki et al. (2003) to measure bisphenol A and bisphenol A glucuronide concentrations in maternal and fetal tissues. Rats were gavaged with radiolabeled bisphenol A at 10 mg/kg bw on GD 11, 13, or 16. Blood was collected over a 24-hr period. Five rats/group/time period were killed at 0.25, 12, and 96 hr post-dosing. Maternal blood and organs, yolk sacs/placentas, and embryos/fetuses were removed for measurement of bisphenol A and bisphenol A glucuronide. Yolk sacs/placentas and fetuses were pooled at most time periods. Results are summarized in Table 25.

At 0.25 hr following dosing, bisphenol A glucuronide concentrations in maternal plasma were similar in groups dosed on GD 11 and 13 but concentrations were 1.7–2 times higher in the group dosed on GD 16. At 12 hr post-dosing in all exposure groups, bisphenol A glucuronide concentrations in maternal plasma were reduced 7- to 11-fold from values observed at 0.25 hr. Levels of radioactivity in plasma were not sufficient for analysis at 96 hr post-dosing. Bisphenol A was detected in maternal plasma at 0.25 hr post-dosing in rats that were exposed to a higher radioactive concentration (0.5 mCi compared to 0.2 mCi) on GD 16; bisphenol A concentrations were 26.5-fold lower than bisphenol A glucuronide concentrations.

In animals dosed on GD 11, bisphenol A glucuronide was only detected in yolk sac/placenta at 0.25 hr post-dosing and the concentration was ~17 times lower than the concentration detected in maternal blood for the same time period. With dosing on GD 11, bisphenol A glucuronide was not detected in embryos and bisphenol A was not detected in yolk sac/placenta or embryos. In animals dosed on GD 13, bisphenol A glucuronide was detected in yolk sac/placenta at 0.25 and 12 hr post-dosing and concentrations were 9–24-fold lower than those detected in maternal plasma for the same time period. Bisphenol A was also detected in yolk sac/placenta at 0.25 and 12 hr after dosing on GD 13 and concentrations were similar to those detected in the blood of 2 dams. A lower concentration of bisphenol A was detected in embryos of dams at 0.25 hr following dosing on GD 13, and bisphenol A was the only moiety detected in embryos. Following dosing on GD 16, bisphenol A glucuronide and bisphenol A were detected in yolk sac/placenta at 0.25 and 12 hr post-dosing. Concentrations of bisphenol A glucuronide in yolk sac/

Table 24
Toxicokinetic Data for Radioactivity in Pregnant and Non-Pregnant Rats Gavaged With 10 mg/kg bw ^{14}C-bisphenol A[a]

Endpoint	Non-pregnant	GD 6–10	GD 14–18	GD 17–21
C_{max1}, mg eq/L	0.716	0.370	0.482	1.006
T_{max1}, hr	0.25	0.25	0.25	0.25
C_{max2}, mg eq/L	0.171	0.336	0.211	0.278
T_{max2}, hr	18	12	24	12
Time to non-quantifiable level, hr	72	Not determined	72	96
AUC				
^{14}C, mg-eq · hr/L	6.1	12.4	7.1	10.2
Bisphenol A glucuronide, mg-eq · hr/L	5.8	12.3	6.8	9.7
Percent as bisphenol A glucuronide	95.1	99.2	95.8	95.1

[a]Dormoradzki et al. (2003).

Table 25
Maternal and Fetal Concentrations of Bisphenol A Following Gavage Dosing of Dams With 10 mg/kg bw Bisphenol A[a]

	Bisphenol A concentration, mg/L or mg/kg					
		Maternal plasma		Yolk sac/placenta		Embryo/fetus
Exposure	Glucuronide	Parent	Glucuronide	Parent	Glucuronide	Parent
GD 11, 0.2 mCi						
0.25 hr	1.060 ± 0.258	0.041	0.062	<LOD[b]	<LOD	<LOD
12 hr	0.099 ± 0.036	<LOD	<LOD	<LOD	<LOD	<LOD
96 hr	NA	NA	<LOD	<LOD	<LOD	<LOD
GD 13, 0.2 mCi						
0.25 hr	0.868 ± 0.189	0.078	0.036	0.019	<LOD	0.013
12 hr	0.117 ± 0.033	0.008	0.013	0.009	<LOD	<LOD
96 hr		Not analyzed due to insufficient radioactivity				
GD 16, 0.2 mCi						
0.25 hr	1.768 ± 0.783	0.485, 0.129[c]	0.223 ± 0.104	0.166 ± 0.069	0.031, 0.009[c]	0.122, 0.020[c]
12 hr	0.174 ± 0.045	<LOD	0.025 ± 0.005	0.034 ± 0.002	NA	NA
96 hr		Not analyzed due to insufficient radioactivity			0.016	0.008
GD 16, 0.5 mCi						
0.25 hr	1.699 ± 0.501	0.064 ± 0.025	0.342 ± 0.104	0.095 ± 0.031	0.013 ± 0.008	0.018 ± 0.011

Data expressed as mean ± SD or single values for individual or pooled data.
[a]Dormoradzki et al. (2003).
[b]Limit of detection (LOD) for bisphenol A reported at 0.005–0.029.
[c]Detected only in two animals at the concentrations listed.

placenta were 7- to 8-fold lower than concentrations detected in maternal plasma. From 0.25 to 12 hr, concentrations of bisphenol A decreased 4.9-fold and concentrations of bisphenol A glucuronide decreased 9-fold. Mean concentrations of bisphenol A in yolk/sac placenta following exposure on GD 16 were similar to the blood concentration detected in 1 of 2 dams.

In yolk sac/placenta and fetuses of dams dosed with a higher level of radioactivity (0.5 mCi) on GD 16, bisphenol A glucuronide and bisphenol A were detected at 0.25 hr following dosing. Compared to concentrations detected in placenta, fetal concentrations of bisphenol A glucuronide were ~26-fold lower and bisphenol A concentrations were 5-fold lower. Bisphenol A concentrations were lower than bisphenol A glucuronide concentrations by 3.6-fold in yolk sac/placenta and by 0.7-fold in fetuses. Study authors concluded that there is no selective affinity for bisphenol A or bisphenol A glucuronide by the yolk sac/placenta or embryo/fetus.

Kurebayashi et al. (2005) examined distribution of radioactivity in pregnant and lactating rats dosed with ^{14}C-bisphenol A. Pregnant rats were orally dosed with 0.5 mg/kg bw ^{14}C-bisphenol A on GD 12, 15, or 18. The rats were killed at 30 min or 24 hr following dosing ($n = 1$/time period) and examined by whole-body radioluminography. Study authors noted that the distribution of label was nearly identical in dams at each gestation time point. At 30 min following dosing, the concentration of radioactivity in dam blood was ~31–43 μg bisphenol A eq/L. The highest concentration of radioactivity was detected in maternal liver (~219–317 μg bisphenol A eq/kg) and kidney (~138–270 μg bisphenol A eq/kg); concentrations in other tissues (lung, ovary, placenta, skin, and uterus) were ~10-fold lower. Fetuses, fetal membranes, and yolk sacs did not contain quantifiable levels of radioactivity at 30 min following maternal exposure at any gestation time point. At 24 hr following exposure of dams, radioactivity concentrations in blood (~4–11 μg bisphenol A eq/L) were nearly 3–10-fold lower than values obtained at 30 min following exposure. Levels of radioactivity remained highest in liver. At 24 hr following exposure, radioactivity was only detected in fetuses and fetal tissues from dams dosed on GD 18. Radioactivity levels in fetuses or fetal tissues compared to maternal blood were ~30% in fetuses, nearly equal in fetal membranes, and ~5-fold higher in yolk sacs. Study authors concluded that there was limited distribution of radiolabel to fetuses.

In another study by Kurebayashi et al. (2005), a lactating rat was orally dosed with 0.5 mg/kg bw ^{14}C-bisphenol A on PND 11 and caged with 5 neonatal rats for 24 hours. One male and one female neonatal rat were killed at the end of the 24-hr period and examined by whole-body radioluminography. The 3 remaining neonates were caged for 24 hr with a dam that was not exposed to bisphenol A. One male and one female neonate were then killed and examined by whole-body radioluminography. In pups killed immediately after being nursed by the lactating dam exposed to ^{14}C-bisphenol A, most of the radioactivity was detected in intestinal contents (~30–46 μg bisphenol A eq/kg) and lower levels were found in gastric contents and urinary bladder (<10 μg bisphenol A eq/kg). After being nursed for 24 hr by a dam that was not exposed to bisphenol A, radioactivity was only detected in intestinal contents and the level was ~20–40% of that measured in pups examined immediately after being nursed by dams receiving ^{14}C-bisphenol A.

An additional 3 lactating dams were dosed with 0.5 mg/kg bw ^{14}C-bisphenol A on PND 11 for examination of radioactivity in plasma and milk over a 48-hr period. Table 26 summarizes toxicokinetic endpoints for radioactivity in milk and plasma. Study authors concluded that there was significant secretion of ^{14}C-associated radioactivity into milk.

Table 26
Toxicokinetic Endpoints for Radioactivity in Lactating Rats Orally Administered 0.5 mg/kg bw ^{14}C-Bisphenol A on PND 11[a]

Endpoint	Milk	Maternal plasma
C_{max}, μg-eq/L	4.46	27.2
T_{max}, hr	8	4
Elimination half-life, hr	26	31
AUC (0–48 hr), μg-eq · hr/L)	156	689

[a]Kurebayashi et al. (2005).

Miyakoda et al. (1999) examined placental transfer of bisphenol A in rats. Wistar rats were administered an oral dose of bisphenol A (99% purity) at 10 mg/kg bw on GD 19. Blood was collected and fetuses were removed at 1, 3, and 24 hr following dosing. Bisphenol A concentrations were measured in plasma and fetuses by GC/MS. **[A statement in Figure 3 of the study indicated that values were the means of 5 or 7 experiments; it is possible the authors meant that 5 or 7 dams were dosed.]** Concentrations of bisphenol A peaked in maternal plasma and fetuses within 1 hr of dosing, with bisphenol A concentrations measured at ~34 ppb **[μg/L]** in maternal plasma and 11 ppb **[μg/kg]** in fetuses. At 3 hr after dosing, bisphenol A concentrations were ~10% of peak concentrations in maternal plasma and 40% of peak concentrations in fetuses. At 24 hr post-dosing, bisphenol A concentrations in fetuses were detected at 70% of peak value and concentrations in fetuses were more than twice the concentrations in maternal plasma. Study authors concluded that bisphenol A is rapidly transferred to the fetus and tends to remain longer in fetuses than in maternal blood.

Snyder et al. (2000) examined the toxicokinetics of bisphenol A in lactating rats. On PND 14, lactating CD rats were gavaged with 100 mg/kg bw ^{14}C-bisphenol A. Milk, blood, and organs were collected from 2–4 dams/group at 1, 8, 24, or 26 hr after dosing. **[While the text indicates collection of samples at 26 hr, Table 3 of the study indicates collection at 24 hr. The collection time reported in the study table was used when there were discrepancies between text and table.]** Animals were injected with oxytocin before milk collection. Radioactivity in pup carcasses was measured at 2, 4, 6, and 24 hr following exposure of dams; 8–16 pups/time period were examined **[pup data does not appear to be analyzed by litter]**. Samples were analyzed by scintillation counting, HPLC, and/or nuclear magnetic resonance. At 1 and 8 hr following exposure, the highest percentage of the radioactive dose was detected in intestine with contents (75–83%). Among the other organs examined, the highest percentage of the radioactive dose was detected in liver (0.38–0.74%) and much lower percentages were detected in kidney and lung (≤0.02%). Low percentages of the radioactive dose were also detected in milk (≤0.0020%), blood (~0.006%), plasma (~0.01%), and fat (≤0.004%). Compared to earlier time periods, radioactivity levels were lower at 24 hr post-dosing (26% of the dose detected in intestine and contents), but distribution was similar. At all 3 sampling time points, radioactivity levels were highest in plasma > blood > milk. The major radioactivity peak in plasma was represented by bisphenol A glucuronide at 1, 8, and 26 hr following exposure. Bisphenol A glucuronide also represented the major radioactive peak detected in milk. Radioactivity levels in pups amounted to <0.01% of the maternal dose. Radioactivity levels in pups tended to increase over time. From 2–24 hr following exposure, mean ± SD radioactivity levels rose from 44 ± 24 to 78 ± 11 μg bisphenol A eq/pup.

Yoshida et al. (2004) compared bisphenol A concentrations in rats and their offspring during the lactation period. The main focus of the study was developmental toxicity, which is discussed in Section 3.2.3.2. In the distribution study, Donryu rats (12–19/group) were gavaged with bisphenol A at 0 (carboxymethylcellulose solution), 0.006, or 6 mg/kg bw/day from GD 2 to the day before weaning (21 days post-delivery). Bisphenol A concentrations were measured in maternal and pup serum, milk, and pup liver by GC/MS on PND 10, 14, and/or 21. Milk samples were obtained from pup stomachs. Pup serum and liver samples were pooled. Two to six dams/litter were examined in each dose group and time period. Samples of tap water, drinking water from plastic containers, and feed were measured for bisphenol A content by HPLC. Bisphenol A was not detected in fresh tap water but was detected at ~3 μg/L following storage of that water in plastic containers. Bisphenol A concentration in feed was ~40 μg/kg. Results for maternal and fetal tissues are summarized in Table 27. Bisphenol A concentrations in the serum of high-dose-dams were significantly elevated compared to the control group on PND 21. No other significant differences were observed in bisphenol A concentrations in samples between treated and control groups.

Kim and Huang (2003) used an HPLC method to measure bisphenol A concentrations in rat dams and their offspring. Dams were gavaged with bisphenol A (>99.7% purity) at doses of 0 (corn oil vehicle), 0.002, 0.020, 0.200, 2, or 20 mg/kg bw/day on GD 7–17. Dams and offspring were killed at 21 days following parturition, and serum was collected for measurement of bisphenol A. Development effects observed in this study are summarized in Section 3.2.1.1. Bisphenol A was not detected in the serum of dams at the two lowest doses. Respective concentrations of bisphenol A in the serum of dams at the 3 highest doses were 0.900, 0.987, and 1.00 mg/L. In offspring, bisphenol A was not detected in serum at the 3 lowest doses. At the 2 highest doses, the respective concentrations of bisphenol A in offspring were 0.69 and 0.74 mg/L in males and 0.71 and 0.82 mg/L in females.

Shin et al. (2002) examined elimination of bisphenol A from maternal–fetal compartments of rats. On 1 day between GD 17 and 19, four Sprague–Dawley rats were i.v. injected with 2 mg/kg bw bisphenol A. Amniotic fluid, placenta, and fetuses were collected at multiple intervals between 5 min and 8 hr following injection. Bisphenol A concentrations in samples were measured by HPLC. Transfer rate constants and clearance rates were determined using a five-compartment model consisting of maternal central, maternal tissue, placental, fetal, and amniotic fluid compartments. Toxicokinetic findings are summarized in Moors et al. (2006) evaluated the kinetics of bisphenol A in pregnant rats on GD 18 after a single i.v. dose of 10 mg/kg bw. Unconjugated bisphenol A represented almost 80% of total bisphenol A

Table 27
Bisphenol A Concentrations in Maternal and Pup Samples During Lactation in Rats Gavaged With Bisphenol A[a]

			Dose group, mg/kg bw/day		
			0	0.006	6
Sample	Time of analysis	Sex	Bisphenol A concentration, ppb [μg/L or μg/kg]		
Dam[b]					
Serum	PND 21		3±0	4±0	11±4
Milk	PND 10		28±9	8±21	8±3
	PND 14		255±78	205±7	185±50
Pup[c]					
Serum	PND 10	Female	4	10	23
		Male	15	5	7
	PND 14	Female	5	4	3
		Male	4	5	4
	PND 21	Female	9	3	9
		Male	14	9	20
Liver	PND 10	Female	13	12	17
		Male	9	9	14
Pup[c]					
Liver	PND 14	Female	22	100	18
		Male	45	14	16
	PND 21	Female	60	70	37
		Male	69	9	60

[a]Yoshida et al. (2004).
[b]Values are presented as mean±SD.
[c]Pup samples were pooled.

Table 28
Toxicokinetic Endpoints for Bisphenol A in Pregnant Rats iv Dosed With 2 mg/kg bw Bisphenol A[a]

Endpoint	Compartment			
	Maternal serum	Placenta	Fetus	Amniotic fluid
AUC, μg·hr/L	905.5±275.8	4009±962.7	1964.7±678.5	180.4±102.0
Elimination half-life, hr	2.5±0.9	2.2±0.8	2.2±0.8	3.9±3.1
Mean residence time, hr	3.0±1.1	2.0±0.5	3.0±0.9	5.6±4.7
C_{max}, μg/L	927.3±194.3	1399.2±323.7	794±360.6	75.1±59.7
T_{max}, hr	No data	0.1±0.1	0.6±0.3	0.3±0.2

Values presented as mean±SD.
[a]Shin et al. (2002).

Table 29
Intercompartmental Transfer and Clearances in Pregnant Rats Following Intravenous Bisphenol A[a]

Compartment	Transfer rate constant, hr^{-1}	Clearance rate mL/min
Maternal central to maternal tissue	3.4±2.6	38.2±26.5
Maternal tissue to maternal central	1.7±1.3	50.2±36.7
Maternal central to placental	0.7±0.5	8.3±5.4
Placental to maternal central	23.6±14.7	2.2±1.3
Placental to fetal	46.4±29.2	4.1±2.1
Fetal to placental	22.8±28.0	7.6±6.0
Fetal to amniotic fluid	0.00001±0.00002	0.00001±0.00001
Fetal	0.0062±0.0044	0.0024±0.0015
Amniotic fluid to fetal	14.0±21.0	0.8±1.1
Amniotic fluid to placental	7.9±6.7	0.7±0.7
Placental to amniotic fluid	1.0±1.3	0.1±0.1
Maternal central	0.9±0.6	9.7±5.3

Values presented as mean±SD.
[a]Shin et al. (2002).

Table 30
Toxicokinetic Endpoints in Lactating Rats Infused With Bisphenol A[a]

Endpoint	Bisphenol A infusion rate, mg/hr		
	0.13	0.27	0.54
Systemic clearance, mL/min/kg	119.2 ± 23.8	142.4 ± 45.3	154.1 ± 44.6
Steady state serum bisphenol A concentration, ng/mL	66.1 ± 15.5	120.0 ± 34.7	217.1 ± 65.0
Steady state milk bisphenol A concentration, ng/mL	173.1 ± 43.3	317.4 ± 154.4	493.9 ± 142.2
Milk/serum ratio	2.7 ± 0.9	2.6 ± 1.2	2.4 ± 0.6

Data presented as mean ± SD.
[a]Yoo et al. (2001).

5 min after injection, 50% of total bisphenol A 20 min after injection, and ~10% of total bisphenol A 6 hr after the injection. The half-life of free bisphenol A in the dam's blood was 0.34 hr, and the half-life of total bisphenol A was 0.58 hr. Bisphenol A in fetal tissues peaked 20–30 min after maternal injection at 4.0 mg/kg in placenta, 3.4 mg/kg in fetal liver, and 2.4 mg/kg in remaining fetal tissues. Peak maternal blood bisphenol A had been 3.8 mg/L shortly after injection.

Rapid distribution of bisphenol A was observed in placenta, fetus, and amniotic fluid. Bisphenol A concentrations in placenta and fetus remained higher than those in maternal serum over most of the sampling period. Amniotic fluid contained the lowest concentration of bisphenol A. Decay curves in amniotic fluid, fetus, and placenta paralleled decay curves in maternal serum. Transfer rate constants and clearance rates are summarized in Table 29. Transfer rate constants were greater in the direction of amniotic fluid to fetus or placenta than in the opposite direction. The elimination rate constant and clearance rate from the fetal compartment were much lower than for the maternal central compartment. The clearance rate from placenta to fetus was higher than clearance rate from fetus to placenta. The authors calculated that 65.4% of the bisphenol A dose was delivered to the fetus, 33.2% to the maternal central compartment, and 1.4% to amniotic fluid. According to the study authors, the low transfer rate from the fetal to amniotic compartment suggested minimal fetal excretion of unchanged bisphenol A through urine and feces into the amniotic fluid. They also noted that the small fetal compartment transfer constant compared to the relative fetal–placental transfer constant indicated minimal metabolism by the fetus. Authors estimated that 100% of bisphenol A was eliminated from the fetus via the placental route and concluded that fetal elimination represents 0.05% of total elimination from the maternal–fetal unit.

Moors et al. (2006) evaluated the kinetics of bisphenol A in pregnant rats on GD 18 after a single i.v. dose of 10 mg/kg bw. Unconjugated bisphenol A represented almost 80% of total bisphenol A 5 min after injection, 50% of total bisphenol A 20 min after injection, and ~10% of total bisphenol A 6 hr after the injection. The half-life of free bisphenol A in the dam's blood was 0.34 hr, and the half-life of total bisphenol A was 0.58 hr. Bisphenol A in fetal tissues peaked 20–30 min after maternal injection at 4.0 mg/kg in placenta, 3.4 mg/kg in fetal liver, and 2.4 mg/kg in remaining fetal tissues. Peak maternal blood bisphenol A had been 3.8 mg/L shortly after injection.

Yoo et al. (2001) examined mammary excretion of bisphenol A in rats. At 4–6 days postpartum, 4–6 lactating female Sprague–Dawley rats/group were i.v. injected with bisphenol A at 0.47, 0.94, or 1.88 mg/kg bw and then infused with bisphenol A over a 4-hr period at rates of 0.13, 0.27, or 0.54 mg/hour. Blood samples were collected at 2, 3, and 4 hr, and milk was collected at 4 hr following initiation of infusion. Before collection of milk, rats were injected with oxytocin to increase milk production. HPLC was used to measure bisphenol A concentrations in serum. Differences in data for mean systemic clearance were analyzed by analysis of variance (ANOVA). Results are summarized in Table 30. The study authors noted extensive excretion of bisphenol A into milk, with milk concentrations exceeding serum concentrations. No significant differences were reported for systemic clearance rates between the 3 doses. Steady state concentrations of bisphenol A in maternal serum and milk increased linearly according to dose.

Kabuto et al. (2004) reported bisphenol A concentrations in mice indirectly exposed to bisphenol A during gestation and lactation. The focus of the study was oxidative stress; more details are presented in Section 3.2.7. Six ICR mouse dams were given drinking water containing 1% ethanol vehicle or bisphenol A at 5 or 10 μg/L. **[Based on the reported water intake of 5 mL/day and an assumed body weight of 0.02 kg (USEPA, 1988), it is estimated that bisphenol A intakes in mice at the start of pregnancy were 0.0013 and 0.0025 mg/kg bw/day.]** Mice gave birth about 3 weeks following mating and pups were housed with dams for 4 weeks. **[Based on an assumed body weight of 0.0085 kg and assumed water intake rate of 0.003 L/day (USEPA, 1988), it is estimated that intake of bisphenol A in weanling males was 0.0018 and 0.0035 mg/kg bw/day.]** At 4 weeks of age, male pups were killed and a GC/MS technique was used to measure bisphenol A concentrations in brain, kidney, liver, and testis in an unspecified number of control pups and in four pups from the 10 μg/L group. Study authors reported that they could not detect bisphenol A in control pups. In pups from the 10 μg/L group, the highest concentration of bisphenol A was detected in kidney (~24 μg/kg wet weight), followed by testis (~20 μg/kg wet weight), brain (~18 μg/kg wet weight), and liver (~11 μg/kg wet weight).

Zalko et al. (2003) examined metabolism and distribution of bisphenol A in pregnant CD-1 mice. A series of studies was conducted in which mice were treated with ^{3}H-bisphenol A (>99.9% purity)/unlabeled bisphenol A (>99% purity). Mice were exposed to different regimens; biological samples examined included blood, liver, fat,

Table 31
Qualitative Analysis of Maternal and Fetal Tissues Following Injection of Mice With 0.025 mg/kg bw Radiolabeled Bisphenol A on GD 17[a]

	Bisphenol A-associated compound detected										
	Hydroxylated glucuronide		Double glucuronide		Metabolite F[b]		Glucuronide		Parent		Others
Hr after dose	11.5 ng/g	%	17.5 ng/g	%	24.0 ng/g	%	25.0 ng/g	%	33.5 ng/g	%	%
Maternal plasma											
0.5	0.07±0.01	3	0.11±0.02	4	0.11±0.02	4	1.01±0.19	39	1.06±0.19	41	9
2	0.02±0.01	2	0.03±0.01	4	0.03±0.01	4	0.55±0.14	63	0.15±0.04	17	10
24	0.04±0.04	20		0		0	0.13±0.05	65		0	15
Placenta											
0.5		0		0	0.46±0.48	2	5.50±4.24	25	15.98±12.02	72	1
2	0.03±0.02	1	0.04±0.03	1	0.37±0.07	7	3.13±2.34	62	1.32±0.95	26	3
24	0.05±0.04	5	0.04±0.02	4	0.64±0.19	59	0.21±0.22	19	0.06±0.04	6	6
Fetus											
0.5	0.05±0.03	1	0.04±0.04	0	0.46±0.27	5	3.83±2.65	44	4.20±2.16	49	1
2	0.02±0.02	1	0.01±0.02	0	0.37±0.22	13	1.93±0.45	66	0.48±0.55	16	3
24	0.01±0.01	1		0	0.11±0.07	13	0.51±0.12	60	0.13±0.16	15	2
Amniotic fluid											
0.5	0.10±0.14	1	0.19±0.14	2	0.09±0.13	1	8.17±6.55	83	0.90±0.89	9	4
2	0.06±0.03	1	0.07±0.03	1	0.26±0.15	5	4.82±4.81	88	0.10±0.07	2	2
24	0.13±0.05	8	0.01±0.02	1	0.37±0.09	24	0.70±0.13	44	0.03±0.03	2	20
Maternal liver											
0.5	0.12±0.12	0	0.18±0.24	0	6.22±1.75	18	12.90±2.81	37	10.85±2.77	31	12
2	0.08±0.08	1	0.77±0.25	8	2.16±0.91	20	4.95±1.82	45	1.51±0.97	13	13
24	0.16±0.14	2	0.35±0.13	7	0.99±0.42	16	2.56±1.62	36	1.72±1.18	23	17

Data presented as mean±SD
[a]Zalko et al. (2003).
[b]Most likely bisphenol A glucuronide conjugated to acetylated galactosamine or glucosamine.

gall bladder, uterus, ovaries, digestive tract and contents, urine, and feces. In the first exposure scenario, mice were s.c. injected with 0.025 mg/kg bw labeled/unlabeled bisphenol A on GD 17; three animals/time period were examined at 0.5, 2, and 24 hr following dosing. In the second exposure scenario, 2 mice/group were s.c. injected with 50 mg/kg bw bisphenol A on GD 17 and killed 24 hr following dosing. In the third scenario, 3 non-pregnant female mice/group were "force-fed" a single oral dose of 0.025 mg/kg bw bisphenol A; urine and feces were collected over 24 hr, and animals were killed at 24 hr. Biological samples were analyzed by scintillation analysis, HPLC, MS, and/or nuclear magnetic resonance.

In pregnant mice injected with 0.025 mg/kg bw/day bisphenol A and examined 24 hr later, 85.68% of the radioactivity was recovered. The highest percentages of radioactivity were detected in the digestive tract and contents (~45%) and feces (~21%). Less radioactivity was detected in the litter (~4%), liver (~2%), bile (~2%), urine (~6%), and carcass (~3%). Blood, ovaries, uterus, placenta, amniotic fluid, fat, and cage washes each contained <1% of the radioactive dose. At 0.5 hr following dosing, levels of radioactivity were highest in uterus > liver > placenta > fetus > amniotic fluid > ovaries > carcass > blood. Radioactivity levels in tissues were lower by 24 hr following exposure. **[Compared to radioactive levels detected in tissues at 24 hr, levels detected at 0.5 hr were ~12-fold higher in uterus, 3-fold higher in liver, 8-fold higher in placenta, 3.5-fold higher in fetuses, 2-fold higher in amniotic fluid, and 3.5-fold higher in ovaries.]** The only information provided for mice s.c. dosed with 50 mg/kg bw bisphenol A and examined 24 hr later was for radioactivity levels in organs; the highest levels (pg/g) were detected in uterus > blood > ovary > carcass > liver. Study authors stated that distribution of radioactivity was comparable in mice treated with 50 and 0.025 mg/kg bw bisphenol A. In the mice orally dosed with 0.025 mg/kg bw bisphenol A and examined 24 hr later, levels of radioactivity in blood, ovaries, and uterus were reported to be significantly lower **[by ~1–2 orders of magnitude]** than levels in animals exposed by s.c. injection, but the level in the liver was not significantly different. There was significantly more residue in mouse carcass after oral than s.c. dosing (~2.5 fold) (A. Soto, personal communication, March 2, 2007). No qualitative differences in metabolites were observed following oral or s.c. exposure. **[Data were not shown by study authors.]** Distribution of parent compound and metabolites detected in maternal and fetal tissues is summarized in Table 31. Further discussion on metabolites is included in Section 2.1.2.3.

Uchida et al. (2002) examined distribution of bisphenol A in pregnant mice and monkeys. On GD 17 (GD 0 = day of vaginal plug), ICR mice were s.c. injected with bisphenol A 100 mg/kg bw in sesame oil vehicle. More than 3 mice/time point were killed at various points between 0.5–24 hr following injection. An untreated control group consisted of 6 mice. **[Data were not presented for controls.]** Maternal and fetal serum and organs were collected. Among organs collected were fetal uteri and testes, which were pooled. On GD 150, 2 Japanese monkeys (*Macaca fuscata*) were s.c. injected with 50 mg bisphenol A/kg bw and at 1 hr following injection,

Table 32
Toxicokinetic Values for Bisphenol A in Rats Following Gavage Dosing With 1 or 10 mg/kg bw[a]

	Age at exposure and sex							
	PND 4		PND 7		PND 21		Adult	
Endpoint	Male	Female	Male	Female	Male	Female	Male	Female
Bisphenol A dose: 1 mg/kg bw								
T_{max}, hr	0.25	0.25	0.25	0.25	3	3		
C_{max}, mg/L	0.03	0.06	0.04	0.08	0.005	0.006		
Half-life, hr	7.2	7.3	21.8	8.8				
AUC, mg · hr/L	0.2	0.1	0.1	0.1				
Bisphenol A dose: 10 mg/kg bw								
T_{max}, hr	0.25	0.25	0.25	0.25	1.5	1.5	0.25	0.75
C_{max}, mg/L	48.3	10.2	1.1	1.4	0.2	0.2	0.024	0.063
Half-life, hr	17	6.7	11.4	8.5	4.3	6.6	"0"	"0"
AUC, mg · hr/L	23.1	7.2	1.9	1.7	1.1	1	"0"	"0"
Ratio of value at 10 to 1 mg/kg bw/day								
C_{max}	1610	170	27.5	17.5				
AUC	115.2	72	19	17				

Data missing from table cells were not determined.
[a]Domoradzki et al. (2004).

fetuses were removed by cesarean section. Two untreated fetuses were used as controls. Maternal and fetal serum and organs, not including reproductive organs, were collected from monkeys. Bisphenol A concentrations were measured by GC/MS in mouse and monkey samples.

In mice, bisphenol A was detected within 0.5 hr of exposure in all tissues examined, including placenta, maternal and fetal serum, liver, and brain, and fetal uterus, and testis. Bisphenol A concentrations were higher in fetal than maternal serum and liver. **[Peak concentrations were observed within 0.5–1 hr in most tissues, with the exception of fetal brain (2 hr), and concentrations remained elevated for 1–6 hr, depending on tissue. More than one peak was observed in fetal serum, uterus, and testis.]** In exposed monkeys, bisphenol A was found at the highest concentrations (15.6–72.50 mg/kg) in fetal heart, intestine, liver, spleen, kidney, thymus, muscle, cerebrum, pons, and cerebellum; bisphenol A concentrations in the same organs from control monkeys were measured at 3.70–22.80 mg/kg. Lower concentrations of bisphenol A were detected in umbilical cord and maternal and fetal serum of the exposed group (1.70–6.10 mg/kg) and control group (0.02–0.25 mg/kg). The study authors stated that the most likely source of bisphenol A in control monkeys was the feed, which was found to contain bisphenol A. The study authors concluded that the placental barrier does not protect the fetus from bisphenol A exposure.

Halldin et al. (2001) examined distribution of bisphenol A in quail eggs or hens. After injection of fertilized quail egg yolk sacs with 67 μg/g ^{14}C-bisphenol A egg on incubation day 3, <1% of radioactivity was detected in embryos at incubation day 6 or 9. A similar finding was reported for diethylstilbestrol. At incubation day 6, no specific localization was observed in the embryo but in 10- and 15-day-old embryos a high amount of radioactivity was observed in liver and bile. **[Low transfer of labeled bisphenol A to the egg was reported after oral or i.v. dosing of quail hens (with apparently 105 μg bisphenol A), but concentrations in eggs were not quantified by study authors.]**

2.1.2.2.2 Non-pregnant and non-lactating animals: Domoradzki et al. (2004), examined the effects of dose and age on toxicokinetics and metabolism of bisphenol A in rats. Neonatal and adult male and female Sprague–Dawley rats were gavaged with ^{14}C-bisphenol A (~99% radiochemical purity)/non-radiolabeled bisphenol A (99.7% purity). Three neonatal rats/age/sex/time period were dosed on PND 4, 7, and 21 with 1 or 10 mg/kg bw bisphenol A. Adult rats (11 weeks old) **[number treated not specified]** were dosed with 10 mg/kg bw bisphenol A. Blood samples were collected at various time points from 0.25–24 hr post-dosing in neonatal rats and from 0.25–96 hr in adult rats. Plasma samples were pooled on PND 4. Immature rats were killed at 24 hr post-dosing, and adult rats were killed at 96 hr post-dosing. Brain, liver, kidneys, skin, and reproductive organs were collected from neonatal rats. Levels of radioactivity, bisphenol A, and/or metabolites were analyzed in blood and tissue samples using HPLC and liquid scintillation spectrometry.

In neonatal and adult rats, radioactivity levels in plasma generally peaked within 0.25–0.75 hr. With the exception of 0.25 hr post-dosing on PND 4, when plasma radioactivity levels were ~4-fold higher in males than females, plasma radioactivity levels were generally similar in male and female rats. At 24 hr post-dosing, plasma radioactivity levels were 4–100 times lower in all groups of neonatal rats. Trends were noted for decreasing radioactivity levels with increasing age. Information related to dose- and age-related effects on metabolism is presented in Section 2.1.2.3.

Toxicokinetic values for bisphenol A are listed in Table 32. C_{max} and AUC values for bisphenol A decreased with increasing age, especially following dosing with 10 mg/kg bw. Bisphenol A concentrations were lower in adults than neonates. No patterns were observed for half-lives, and the authors stated that values in neonates may not have been reliable because bisphenol A concentrations were near the LOD at the end of the 24-hr observation period. Ratios of C_{max} and AUC values for the 10 and 1 mg/kg bw doses were different at

Table 33
Toxicokinetic Values for Bisphenol A Glucuronide in Rats Following Gavage Dosing With 1 or 10 mg/kg bw Bisphenol A[a]

	Age at exposure and sex							
	PND 4		PND 7		PND 21		Adult	
Endpoint	Male	Female	Male	Female	Male	Female	Male	Female
Bisphenol A dose: 1 mg/kg bw								
T_{max}, hr	0.75	0.75	0.75	0.25	0.25	0.25		
C_{max}, mg/L	1.3	1.5	2	1.1	0.8	0.8		
Half-life, hr	26.1	24.2	6.6	6.4	4.2	4.1		
AUC, mg · hr/L	9	9.6	7.7	7.7	4.1	3.3		
$AUC_{BPA\text{-}glucuronide}/AUC_{BPA}$	45	96	77	77				
Bisphenol A dose: 10 mg/kg bw								
T_{max}, hr	1.5	1.5	1.5	0.75	0.75	0.75	0.25	0.25
C_{max}, mg/L	13.1	6.3	6.6	10.3	10.4	7.8	0.6	0.7
Half-life, hr	7.3	9.8	9.1	8.4	4.4	4.4	22.5	10.8
AUC, mg · hr/L	80	50.3	58.9	60.9	60.3	56.1	31.5	9.8
$AUC_{BPA\text{-}glucuronide}/AUC_{BPA}$	3.5	7	31	36	55	56		
Ratio of value at 10 to 1 mg/kg bw/day								
C_{max}	10.1	4.2	3.3	9.4	13	9.8		
AUC	8.9	5.2	7.6	7.9	14.7	17		

Data missing from table cells were not determined.
[a]Domoradzki et al. (2004).

each age and generally decreased with age. Plasma bisphenol A concentrations were very low in adults dosed with 10 mg/kg bw; therefore, few data were available.

Toxicokinetic values for bisphenol A glucuronide are listed in Table 33. Peak plasma concentrations of bisphenol A glucuronide were 9–22 times higher in neonates than adult rats dosed with 10 mg/kg bw bisphenol A. AUC values for bisphenol A glucuronide were also higher in neonates than adults **[~2–6 times higher]**. In neonates dosed with 1 mg/kg bw, AUC values and elimination half-lives for bisphenol A glucuronide decreased with age. Ratios of C_{max} and AUC values for the 10 and 1 mg/kg bw doses were nearly proportional. In adults dosed with 10 mg/kg bw, bisphenol A glucuronide concentrations peaked at 0.25 hr and secondary peaks were observed at 18 and 24 hr. In neonates dosed with 10 mg/kg bw, concentrations of bisphenol A glucuronide peaked at 0.75–1.5 hr and then bisphenol A glucuronide was eliminated in an apparently monophasic manner. Half-lives of elimination were shorter in neonates compared to adults. In neonatal rats, the bisphenol A glucuronide represented 94–100% of the 1 mg/kg bw dose and 71–97% of the 10 mg/kg bw/day dose. In adult rats, ~100% of the dose was represented by bisphenol A glucuronide.

Half-life and AUC data for bisphenol A-derived radioactivity in organs of neonatal rats are summarized in Table 34. Radioactivity was distributed to all organs and dose-related increases were observed. The study authors noted lower concentrations in brain than in other tissues. **[Levels of radioactivity in reproductive organs compared to those in plasma varied at each evaluation period but were usually within the same or one order of magnitude lower.]** With the exception of males dosed with 10 mg/kg bw bisphenol A, half-lives decreased with age. There were some disproportionate increases in ratios of AUC at 10 and 1 mg/kg bw.

The study authors concluded:

- Metabolism of bisphenol A to its glucuronide conjugate occurred as early as PND 4 in rats;
- Dose-dependent differences occurred in neonatal rats, as noted by a larger fraction of the lower dose being metabolized to the glucuronide; and
- There were no major sex differences in metabolism or toxicokinetics of bisphenol A.

Pottenger et al. (2000) examined the effects of dose and route on metabolism and toxicokinetics of bisphenol A in rats. Information focusing on toxicokinetics is summarized primarily in this section, while metabolic data are summarized primarily in Section 2.1.2.3. Adult male and female F344 rats were dosed with ^{14}C-bisphenol A (99.3% radiochemical purity)/non-radiolabeled bisphenol A (99.7% purity) at doses of 10 or 100 mg/kg bw by oral gavage or i.p. or s.c. injection. Blood was collected at multiple time points between 0.083 and 168 hr post-dosing, and excreta were collected for 7 days. Animals were killed 7 days post-dosing. Blood, brain, gonads, kidneys, liver, fat, skin, uterus, and carcass were analyzed by liquid scintillation counting and HPLC. Some samples were analyzed by HPLC/electrospray ionization/MS.

Toxicokinetic endpoints for bisphenol A in blood are summarized in Table 35. Study authors noted that concentration-time profiles of bisphenol were dependent on dose, exposure route, and sex. The longest T_{max} was observed with s.c. dosing. C_{max} and AUC values were lowest following oral administration. Time to non-quantifiable concentrations of bisphenol A was longest following s.c. exposure. The only sex-related difference was a higher C_{max} value in females than males following oral dosing. In most cases, bisphenol A toxicokinetics were linear across doses within the same administration

Table 34
Distribution of Radioactivity to Tissues at 24 Hr Following Dosing With Radiolabeled Bisphenol A[a]

	PND 4			PND 7			PND 21		
Tissue	Half-life hr	AUC mg ·← hr/kg	AUC ratio of doses	Half-life hr	AUC mg ·← hr/kg	AUC ratio of doses	Half-life hr	AUC mg ·← hr/kg	AUC ratio of doses
Females, 1 mg/kg bw									
Brain	11.7	0.4		6.7	0.2		3.6	0.1	
Liver	18	7.5		7.9	7.1		3.6	2.9	
Kidney	18.1	9.4		7.3	9.5		5.0	3.0	
Ovary	11.7	7.3		6.0	3.5		3.7	0.9	
Uterus	7.4	8.3		6.2	3.0		3.4	1.0	
Carcass	11.2	22.2		10.0	16.6		4.0	8.3	
Plasma	19.5	9.4		6.4	7.8		3.6	3.5	
Females, 10 mg/kg bw									
Brain	7.2	3.3	8.3	8.0	2.5	12.5	4.9	1.7	17.0
Liver	11.1	44.8	6.0	10.0	59.6	8.4	4.5	39.1	13.5
Kidney	15.2	43.9	4.7	8.6	66.6	7.0	5.3	36.5	12.2
Ovary	6.5	136.2	18.7	5.0	69.7	19.9	3.6	21.1	23.4
Uterus	15.2	127.0	15.3	4.8	108.5	36.2	3.4	30.6	30.6
Carcass	6.6	112.8	5.1	7.0	130.7	7.9	4.8	100.9	12.2
Plasma	9.2	61.0	6.5	8.1	67.0	8.6	3.7	59.0	16.9
Males, 1 mg/kg bw									
Brain	14.1	0.3		6.0	0.3		3.4	0.1	
Liver	19.7	6.1		6.6	7.3		3.7	3.2	
Kidney	19.3	8.5		7.0	8.6		4.6	3.4	
Testis	10.3	3.4		5.7	2.0		3.4	0.8	
Carcass	11.1	22.2		9.0	17.3		4.1	9.0	
Plasma	24.0	9.2		6.6	7.7		3.4	4.2	
Males, 10 mg/kg bw									
Brain	3.1	4.7	15.7	8.0	2.9	9.7	4.7	1.7	17.0
Liver	11.6	48.4	7.9	11.8	62.0	8.5	5.1	40.9	12.8
Kidney	5.4	68.9	8.1	9.8	59.6	6.9	6.9	30.4	8.9
Testes	5.8	36.8	10.8	7.6	22.1	11.1	5.2	8.1	10.1
Carcass	8.3	111.7	5.0	8.6	135.5	7.8	4.8	95.2	10.6
Plasma	6.9	113.0	12.3	9.9	69.0	9.0	4.0	62.0	14.8

[a]Domoradzki et al. (2004).

Table 35
Toxicokinetic Endpoints for Bisphenol A in Blood Following Dosing of Rats by Gavage or Injection[a]

	Exposure route and doses (mg/kg bw)					
Endpoint	10 oral	100 oral	10 i.p.	100 i.p.	10 s.c.	100 s.c.
Males						
T_{max}, hr	N/A	0.083	0.5	0.25	0.75	0.5
C_{max}, mg/L, hr[b]	[c]	0.22±0.09	0.69±0.08	9.7±1.27	0.39±0.16	5.19±0.98
Time to non-quantifiable concentration, hr	0.083	0.75	8	12	18	24
AUC, mg ·←hr/L		0.1	1.1	16.4	2.6	24.5
Females						
T_{max}, hr	0.25	0.25	0.25	0.25	4	0.75
C_{max}, mg/L, hr**b**	0.04±0.03	2.29±1.82	0.87±0.15	13.13±4.13	0.34±0.06	3.97±0.6
Time to non-quantifiable concentration, hr	1		24	72	48	72
AUC, mg · hr/L	0.42	4.4	1.4	26.2	3.1	31.5

Missing values were not determined.
[a]Pottenger et al. (2000).
[b]Mean±SD.
[c]Non-quantifiable (0.01 μg/g at 10 mg/kg bw and 0.1 μg/g at 100 mg/kg bw).

route, as noted by approximate proportionate increases in C_{max} and AUC values from the low to the high-dose. Toxicokinetics data for radioactivity in plasma are summarized in Table 36. Concentrations of radioactivity were dependent on exposure route and to a lesser extent, dose and sex. AUC values for radioactivity were lowest

Table 36
Toxicokinetics for Radioactivity Following Dosing of Rats with Bisphenol A Through Different Exposure Routes[a]

Endpoint	Exposure route and doses (mg/kg bw)					
	10 oral	100 oral	10 i.p.	100 i.p.	10 s.c.	100 s.c.
Males						
T_{max}, hr	0.25	0.25	0.5	0.25	1	0.75
C_{max}, mg eq/L, hr	0.73±0.22	3.92±1.93	1.26±0.09	29.3±11.7	0.61±0.24	6.33±0.43
Time to non-quantifiable concentration, hr	72	72	96	96	96	144
AUC, mg-eq ·hr/L	8.1	66.5	16.9	170	15.5	218
Females						
T_{max}, hr	0.083	0.25	0.25	0.5	0.75	0.75
C_{max}, mg eq/L, hr	1.82±0.66	28.33±8.64	2.27±0.19	67.81±7.33	0.52±0.06	5.66±0.95
Time to non-quantifiable concentration, hr	72	72	72	120	120	168
AUC, mg-eq · hr/L	9.54	94.9	15.3	247	21.6	297

[a]Pottenger et al. (2000).

Table 37
Toxicokinetic Values for Bisphenol A in Adult Rats Exposed to Bisphenol A Through the Intravenous or Oral Route[a]

Endpoint	Bisphenol A dosing	
	0.1 mg/kg bw, i.v.	10 mg/kg bw, gavage
Distribution half-life, min	6.1±1.3	
Terminal elimination half-life, hr	0.9±0.3	21.3±7.4
AUC, μg · hr/L	16.1±3.2	85.6±33.7
Systemic clearance, mL/min/kg	107.9±28.7	
Steady-state volume of distribution, L/kg	5.6±2.4	
C_{max}, μg/L		14.7±10.9
T_{max}, hr		0.2±0.2
Apparent volume of distribution, L/kg		4273±2007.3
Oral clearance, mL/min/kg		2352.1±944.7
Absolute oral bioavailability, %		5.3±2.1

Data presented as mean±SD.
[a]Yoo et al. (2001).

following oral exposure. Time to non-quantifiable concentration was longest following s.c. dosing. For most groups, C_{max} and AUC values were proportionate across doses within the same exposure route. A second part of the study examined metabolites and is summarized in Section 2.1.2.3.

Upmeier et al. (2000) examined toxicokinetics in rats exposed to bisphenol A through the oral or i.v. route. Ovariectomized DA/Han rats (130–150 g bw) were exposed to bisphenol A by i.v. injection with 10 mg/kg bw or oral gavage with 10 or 100 mg/kg bw. Blood was collected from treated rats at multiple time points until 2 hr following i.v. dosing and 3 hr following oral dosing. Three to five rats were sampled during each time period. To reduce stress, only some of the rats were sampled at each time point. In control animals, blood was collected 2 hr following dosing with vehicle. Bisphenol A concentrations in plasma were measured by GC/MS. Dosing with 10 mg/kg bw i.v. resulted in a maximum plasma concentration of 15,000 μg/L bisphenol A. Concentrations decreased to 700 μg/L within 1 hr, 100 μg/L within 2 hr, and non-detectable concentrations by 24 hr followingexposure. The apparent final elimination half-life was estimated at 38.5 hr. In rats gavaged with 10 mg/kg bw, an initial maximum blood concentration of 30 μg/L was obtained at 1.5 hr. A decrease in bisphenol A blood concentration at 2.5 hr was followed by a second peak of 40 μg/L at 6 hr, leading study authors to conclude that enterohepatic cycling was occurring. The same patterns of bisphenol A concentrations in blood were observed following gavage dosing with 100 mg/kg bw. Peak concentrations were observed at 30 min (150 μg/L) and 3 hr (134 μg/L) following exposure. According to the study authors, the differences in peak concentrations observed between the two doses suggested lower bioavailability at the high-dose than at the low dose. Oral bioavailability of bisphenol A was estimated at 16.4% at the low dose and 5.6% at the high-dose.

Yoo et al. (2001) examined toxicokinetics of a low i.v. dose and a higher gavage dose of bisphenol A in male rats. Five adult male Sprague–Dawley rats/group were administered bisphenol A by i.v. injection at a dose of 0.1 mg/kg bw or by gavage at a dose of 10 mg/kg bw. Multiple blood samples were collected until 3 hr following i.v. dosing and 24 hr following gavage dosing. HPLC was used to measure bisphenol A concentrations in serum. Route-specific differences in mean systemic clearance were analyzed by Student *t*-test. Results are summarized in Table 37. The study authors noted bi-exponential decay of serum bisphenol A concentrations following i.v. dosing, significantly longer elimination half-life with oral than i.v. exposure, and low oral bioavailability of bisphenol A.

Kurebayashi et al. (2003) conducted a series of studies to examine toxicokinetics and metabolism of bisphenol A in adult F344N rats exposed through the oral or i.v. route. In these studies, radioactivity levels were measured by scintillation counting. Bisphenol A or its metabolites were quantified by HPLC, electrospray ionization/MS, or nuclear magnetic resonance. As discussed in greater detail in Section 2.1.2.4, fecal excretion was the main

route of elimination for radioactivity following oral or i.v. dosing of rats with 0.1 mg/kg bw ^{14}C-bisphenol A. A study describing biliary excretion and metabolites in bile is summarized in Section 2.1.2.3. Toxicokinetic endpoints were determined in a study in which blood was drawn from 3 male rats/group at various time points between 0.25–48 hr following oral gavage or i.v. dosing with 0.1 mg/kg bw bisphenol A. Results of the study are summarized in Table 38. Rapid absorption of radioactivity was observed following oral dosing. AUC values were significantly lower for oral than i.v. dosing. In a another study, rats were administered ^{14}C-bisphenol A by i.v. injection and blood was collected 30 min later for determination of blood/plasma distribution and protein binding. At a blood radioactivity level of 80 nM **[18 μg bisphenol A eq/L], preferential distribution to plasma was observed, with the blood/plasma ratio reported at 0.67. At radioactivity levels of 6–31 μg-eq/L (27–135 nM), plasma protein binding was reported at 95.4%. Additional studies reviewed by Teeguarden et al. (2005) reported plasma protein binding of bisphenol A at ~90–95%. An additional study by Kurebayashi et al. (2003) compared metabolic patterns and excretion following exposure to a higher bisphenol A dose; that study is discussed in Section 2.1.2.3.**

Table 38
Toxicokinetic Endpoints for ^{14}C-Bisphenol A-Derived Radioactivity in Rats Exposed to 0.1 mg/kg bw ^{14}C-Bisphenol A Through the Oral or I.V. Route[a]

Endpoint	I.V. exposure	Oral exposure
T_{max}, hr		0.38±0.10
C_{max}, μg-eq/L		5.5±0.3
Half-life-α, hr	0.59±0.09	No data
Half-life-β, hr	39.5±2.1	44.5±4.1
Absorbance rate, hr^{-1}		3.6±1.0
Volume of distribution, L/kg	27.0±0.7	No data
Total body clearance. L/hr/kg	0.522±0.011	0.544±0.049
Mean residence time, hr	51.7±2.4	No data
AUC, μg-eq · hr/L		
0–6 hr	33.9±1.6	18.4±0.7[b]
0–24 hr	79.3±3.3	60.0±7.1[b]
0–48 hr	118±4	102±13[b]
0–∞	192±4	185±16
Oral bioavailability[c]		
0–6 hr		0.54
0–24 hr		0.76
0–48 hr		0.86
0–∞		0.97

Data presented as mean±SD. Missing values are not applicable or were not reported.
[a]Kurebayashi et al. (2003).
[b]$P < 0.05$ compared to i.v. exposure.
[c]Variances not reported.

Kurebayashi et al. (2005) administered ^{14}C-bisphenol A to adult male and female F344 rats (3/dose/sex) at doses of 0.020, 0.1, or 0.5 mg/kg bw orally or 0.1 or 0.5 mg/kg bw by i.v. injection. Plasma samples were analyzed for radioactivity over a 72-hr period to determine toxicokinetic endpoints. Results are summarized in Table 39. Study authors noted that the AUC was almost linearly correlated with dose. Several peaks were observed with oral or i.v. exposure, indicating enterohepatic cycling, according to the study authors. Study authors noted that substantially lower AUC values in females than in males following oral exposure could have resulted from lower absorption and/or a higher elimination rate. Distribution of radioactivity was evaluated 0.5, 24, and 72 hr following oral administration of 0.1 mg/kg bw bisphenol A to adult male and female Wistar rats (3/sex/time point). At 0.5 hr following exposure, most of the radioactivity (~12–51 μg bisphenol A eq/kg) was found in kidney and liver. **[A large amount of radioactivity was also reported for intestinal contents, but those data were not shown by the study authors.]**. Lower amounts of radioactivity (~2–7 μg bisphenol A eq/kg or L) were detected in adrenal gland, blood, lung, pituitary gland, skin, and thyroid gland of both sexes; uterus; and bone marrow, brown fat, and mandibular gland of males. In males, <μg bisphenol A eq/kg was detected in skeletal muscle and testis. Radioactivity was non-quantifiable in brain and eye of both sexes; epididymis, prostate gland, and heart of males; and bone marrow, brown fat, skeletal muscle, and mandibular gland of females. At ≥24 hr following exposure, radioactivity was detected primarily in only kidney, liver, and intestinal contents, with the exception of ~3 μg bisphenol A eq/L detected in blood of males at 24 hr following dosing. Study authors noted that elimination of radioactivity from some tissues

Table 39
Toxicokinetic Endpoints for Plasma Radioactivity in Rats Dosed With ^{14}C Bisphenol A[a]

	Route and dose (mg/kg bw)				
	Oral			I.V.	
Endpoints	20	100	500	100	500
Males					
Elimination half-life, hr	78±52	18±3	21±3	19±2	21±3
AUC, μg-eq · hr/L	36±6	178±44	663±164	266±46	865±97
Apparent absorption, %	82	81	60		
Females					
Elimination half-life, hr	20±7	22±13	18±8	13±3	16±2
AUC, μg-eq · hr/L	14±5	99±19	500±43	190±45	1029±81
Apparent absorption, %	35	50	50		

Data presented as mean±SD.
[a]Kurebayashi et al. (2005).

appeared to occur more rapidly in females than in males. Distribution in pregnant animals was also examined and is described in Section 2.1.2.2.1.

Kabuto et al. (2003) reported distribution of bisphenol A in mice. Male ICR mice were i.p. dosed with bisphenol A at 0, 25, or 50 mg/kg bw/day for 5 days and killed 6 hr following the last dose. Bisphenol A concentrations in tissues of animals from the high-dose group were determined by GC/MS. In mice of the high-dose group, the highest concentrations of bisphenol A were detected in kidney (~2.02 mg/kg wet weight) and body fat (~1.25 mg/kg wet weight). Lower concentrations of bisphenol A (≤0.42 mg/kg wet weight or mg/L) were detected in brain, lung, liver, testis, and plasma.

Kurebayashi et al. (2002) examined the toxicokinetics of a low bisphenol A dose in Cynomolgus monkeys following gavage or i.v. dosing. Three adult male and female monkeys were dosed with 0.1 mg/kg bw ^{14}C-bisphenol A (99% radiochemical purity)/non-radiolabeled bisphenol A **[purity not reported]**. Monkeys were dosed by i.v. injection on Day 1 of the study and by gavage on Day 15 of the study. Urine and feces were collected for 7 days post-dosing. Blood samples were collected at various time points from 0.083–72 hr following i.v. dosing and for 0.25–71 hr after oral dosing. Binding to plasma protein was determined at some time points over 0.25–4 hr. Samples were analyzed by liquid scintillation counting and HPLC. Following oral or i.v. exposure, the percentage of radioactivity recovered in excreta and cage washes was 81–88% over a 1-week period. As discussed in greater detail in Section 2.1.2.4, most of the radioactivity was excreted in urine and very little was excreted in feces. Toxicokinetic endpoints are summarized in Table 40. Based on the toxicokinetic values, study authors concluded that absorption of bisphenol A following oral exposure was rapid and high, and terminal elimination half-lives of bisphenol A/metabolites were longer following i.v. than oral exposure. As discussed in more detail in Section 2.1.2.3, glucuronide compounds were the major metabolites detected in urine, and higher percentages of the radioactive dose in plasma were represented by bisphenol A following i.v. than oral dosing.

Table 40
Toxicokinetic Endpoints for Radioactivity in Male and Female Cynomolgus Monkeys Exposed to ^{14}C-Bisphenol A Through IV Injection or by Gavage[a]

Endpoint	Male	Female
Intravenous exposure		
AUC, μg-eq · hr/L	377 ± 85	382 ± 96
Volume of distribution, L/kg	1.58 ± 0.11	1.82 ± 0.41
Half-life, hr	13.5 ± 2.6	14.7 ± 2.1
Total body clearance, L/hr/kg	0.27 ± 0.05	0.28 ± 0.08
Mean residence time, hr	5.93 ± 0.91	6.68 ± 0.72
Oral exposure		
AUC, μg-eq · hr/L	265 ± 74	244 ± 21
T_{max}, hr	1.00 ± 0.87	0.33 ± 0.14
C_{max}. μg-eq/L	104 ± 85	107 ± 37
Half-life. hr	9.63 ± 2.74	9.80 ± 2.15
Bioavailability	0.70 ± 0.16	0.66 ± 0.13

[Mean ± SD assumed based on data presentations elsewhere in this study.]
[a]Kurebayashi et al. (2002).

Negishi et al. (2004b) compared toxicokinetics of bisphenol A in female F344/N rats, Cynomolgus monkeys, and Western chimpanzees. Bisphenol A was administered by oral gavage and s.c. injection at doses of 10 or 100 mg/kg bw/day to rats and monkeys and 10 mg/kg bw to chimpanzees. Three rats/dose/time point were killed before and at various times between 0.5 and 24 hr following bisphenol A administration. Three monkeys/group and 2 chimpanzees were first exposed orally and 1 week later by s.c. injection. In monkeys, blood samples were drawn before and at various times from 0.5–24 hr after dosing. In chimpanzees, blood was drawn before and at multiple time points between 0.25–24 hr following dosing. Bisphenol A was measured in serum by ELISA, and toxicokinetics endpoints were determined. Results are summarized in Table 41. The study authors noted that the bioavailability of bisphenol

Table 41
Toxicokinetic Endpoints for Bisphenol A by ELISA in Rats, Monkeys, and Chimpanzees[a]

	10 mg/kg bw		100 mg/kg bw	
Endpoints	Oral	S.C.	Oral	S.C.
Rat (data presented as mean ± SD)				
C_{max}, μg/L		872 ± 164	580 ± 398	3439 ± 679
T_{max}, hr		1.0	0.5	1.0
$AUC_{0-4\,h}$, μg · hr/L		1912 ± 262	506 ± 313	9314 ± 2634
$AUC_{0-24\,h}$, μg · hr/L		3377 ± 334	1353 ± 462	23,001 ± 6387
Monkey (data presented as mean ± SD)				
C_{max}, μg/L	279 ± ±920	57,934 ± 1902	5732 ± 525	10,851 ± 3915
T_{max}, hr	0.7 ± 0.2	2.0 ± 0.0	0.7 ± 0.2	2.0 ± 0.0
$AUC_{0-4\,h}$, μg · hr/L	3209 ± 536	15,316 ± 5856	14,747 ± 2495	48,010 ± 11,641
$AUC_{0-24\,h}$, μg · hr/L	3247 ± 587	39,040 ± 10,738	52,595 ± 8951	189,627 ± 21,790
Chimpanzee (data presented for 2 animals)				
C_{max}, μg/L	325; 96	2058; 1026	Dose not administered	
T_{max}, hr	0.5; 0.5	2.0; 2.0		
$AUC_{0-4\,h}$, μg · hr/L	491; 235	5658; 3109		
$AUC_{0-24\,h}$, μg · hr/L	1167; 813	21,141; 12,492		

Data were not reported in cases where table cells are empty.
[a]Negishi et al. (2004b).

Table 42
Toxicokinetic Endpoints for Bisphenol A by LC-MS/MS in Rats, Monkeys, and Chimpanzees[a]

Endpoints	10 mg/kg bw		100 mg/kg bw	
	Oral	S.C.	Oral	S.C.
Rat (data presented as mean ± SD)				
C_{max}, μg/L	2.1 ± 1.6	746 ± 80	47.5 ± 10.6	2631 ± 439
T_{max}, hr	0.7 ± 0.3	0.8 ± 0.3	0.5 ± 0.0	1.2 ± 0.8
$t_{1/2}$, hr	not calculated	3.2 ± 0.7	not calculated	4.5 ± 0.7
AUC_{0-4h}, μg · hr/L	4.2[b]	1542 ± 200	43.2 ± 9.7	6926 ± 1071
AUC_{0-24h}, μg · hr/L	7.2[b]	1977 ± 182	350 ± 294	15,576 ± 2263
Monkey (data presented as mean ± SD)				
C_{max}, μg/L	11.5 ± 2.2	4213 ± 3319	28.6 ± 3.9	7010 ± 3045
T_{max}, hr	1.0 ± 0.9	1.7 ± 0.6	3.3 ± 1.2	2.7 ± 1.2
$t_{1/2}$, hr	8.9 ± 3.0	3.8 ± 0.8	4.5 ± 0.7	12.9 ± 3.6
AUC_{0-4h}, μg · hr/L	21.4 ± 6.1	8828 ± 4309	85.3 ± 18.6	19,981 ± 7567
AUC_{0-24h}, μg · hr/L	42.5 ± 7.3	18,855 ± 3870	350 ± 13	79,796 ± 21,750
Chimpanzee (data presented as mean for 2 animals)				
C_{max}, μg/L	5.5	703	Dose not administered	
T_{max}, hr	0.8	1.0		
$t_{1/2}$, hr	6.8	4.2		
AUC_{0-4h}, μg · hr/L	13.3	2148		
AUC_{0-24h}, μg · hr/L	33.1	6000		

[a]Tominaga et al. (2006).
[b]1 or 2 animals.

was lowest in rats < chimpanzees < monkeys following exposure through either route. In most cases, bisphenol A was not detected in rat serum following oral administration of the 10 mg/kg bw dose. In all species, higher bioavailability was observed with s.c. than oral dosing.

In a subsequent report (Tominaga et al., 2006), these authors noted that ELISA may overestimate bisphenol A concentrations due to non-specific binding. They reported measurements by LC-MS/MS in animals evaluated using the same study design **[possibly the same specimens reported previously]**. These results are summarized in Table 42. The authors proposed that primates, including humans, may completely glucuronidate orally-administered bisphenol A on its first pass through the liver and excrete it in the urine whereas bisphenol A remains in the rat for a more extended period due to enterohepatic recirculation. They suggested that the rat may not be a good model for human bisphenol A kinetics.

2.1.2.3 Metabolism: Information is arranged in this section according to species. In rats, study summaries are arranged in order of those providing general or route-specific information on metabolites, specifics on organs or enzyme isoforms involved in metabolism, and pregnancy-, sex-, or age-related effects on metabolism.

Pottenger et al. (2000) examined the effects of dose and route on toxicokinetics of bisphenol A in rats. Disposition of bisphenol A and its metabolites in urine and feces is described in this section, while results of the toxicokinetics study are described in Section 2.1.2.2. Five adult F344 rats/sex/group were dosed with ^{14}C-bisphenol A (99.3% radiochemical purity)/non-radiolabeled bisphenol A (99.7% purity) at doses of 10 or 100 mg/kg bw by oral gavage or i.p. or s.c. injection. Excreta were collected for 7 days. Samples were analyzed by HPLC or HPLC/electrospray ionization/MS. The percentage of radioactivity recovered from all groups was 84–98%. Fecal elimination represented the largest percentage of radioactivity in all exposure groups (52–83%). Eight peaks were identified in feces, and the largest peak (representing 86–93% of radioactivity) was for unchanged bisphenol A. Elimination of radioactivity through urine was ~2-fold higher in females (21–34%) than males (13–16%) in all dose groups. Fourteen different peaks were identified in urine. It was estimated that radioactivity in urine was represented by bisphenol A monoglucuronide (57–87%), bisphenol A (3–12%), and bisphenol A sulfate (2–7%). Some differences were noted for retention of radioactivity following dosing by gavage (0.03–0.26%), i.p. injection (0.65–0.85%), and s.c. injection (1.03–1.29%).

Metabolites associated with bisphenol A exposure were examined in a second study by Pottenger et al. (2000). Three rats/sex/dose/route/time point were dosed with ^{14}C-bisphenol A/non-radiolabeled bisphenol A at 10 or 100 mg/kg bw by oral gavage or i.p. or s.c. injection. Rats were killed at 2 different time points following dosing, T_{max}, and the time when bisphenol A concentrations were no longer quantifiable. Times at which rats were killed were determined by data obtained during the first study. Plasma samples were pooled at each time period and examined by HPLC or HPLC/electrospray ionization/MS. Qualitative and quantitative differences were observed for parent compound and metabolites in plasma following exposure through different routes. Following oral exposure, bisphenol A glucuronide was the most abundant compound detected in plasma at both time periods (C_{max} and time when parent compound was not quantifiable) and represented 68–100% of total radioactivity. Following i.p. or s.c. exposure, unmetabolized bisphenol A was the most abundant compound at T_{max}; levels of radioactivity represented by unmetabolized bisphenol A were 27–51% following i.p. exposure and 65–76% following s.c. exposure. Only 2–8% of radioactivity was represented by bisphenol A following oral exposure. Some compounds observed following i.p. or s.c. exposure were not

Table 43
Biliary Excretion in Male and Female Rats Exposed to 0.1 mg/kg bw ^{14}C-Bisphenol A Through the Oral or Intravenous Route[a]

	Male		Female	
Parameters	I.V.	Oral	I.V.	Oral
Biliary excretion, %				
0–2 hr	48	32	35	28
0–4 hr	61	44	50	39
0–6 hr	66	50	58	45
Radioactivity in bile represented by glucuronide, %	84	86	87	88
Dose excreted as glucuronide in bile, %	55	43	50	40

[a]Kurebayashi et al. (2003).

observed following oral exposure. A compound tentatively identified as a sulfate conjugate was observed following i.p. exposure and represented a small portion of radioactivity. An unresolved peak of 3 compounds was observed following i.p. or s.c. exposure, at the time when parent compound was not quantifiable and represented that major percent of radioactivity for that time point. Three additional unidentified, minor peaks were observed following i.p. or s.c. but not oral exposure. The major sex differences observed were higher C_{max} values for bisphenol A and bisphenol A glucuronide in females than males, especially following i.p. administration. A review by the European Union (2003) noted that the substantially higher concentrations of parent compound with i.p. and s.c. compared to oral exposure indicated the occurrence of first-pass metabolism following oral intake.

Elsby et al. (2001) examined bisphenol A metabolism by rat hepatocytes. In the hepatocyte metabolism study, hepatocytes were isolated from livers of adult female Wistar rats and incubated in dimethyl sulfoxide (DMSO) vehicle or bisphenol A 100 or 500 μM **[23 or 114 mg/L]** for 2 hr. Metabolites were identified by HPLC or LC/MS. Data were obtained from 4 experiments conducted in duplicate. At both concentrations, the major metabolite was identified as bisphenol A glucuronide, which was the only metabolite identified following incubation with 100 μM bisphenol A. Two additional minor metabolites identified at the 500 μM concentration included 5-hydroxy-bisphenol A-sulfate and bisphenol A sulfate. Another part of the study comparing metabolism of bisphenol A by rat and human metabolites is discussed in Section 2.1.1.3. Another study (Pritchett et al., 2002) comparing metabolism of bisphenol A in humans, rats, and mice is also summarized in Section 2.1.1.3.

In neonatal rats gavaged with 1 or 10 mg/kg bw ^{14}C-bisphenol A on PND 4, 7, and 21 and adult rats gavaged with 10 mg/kg bw bisphenol A, the major compounds detected in plasma were bisphenol A glucuronide and bisphenol A (Domoradzki et al., 2004). Up to 13 radioactive peaks were identified in neonatal rats dosed with 10 mg/kg bw and 2 were identified in neonates dosed with 1 mg/kg bw/day. At the 10 mg/kg bw dose, the concentration of bisphenol A glucuronide detected in plasma increased with age. Metabolic profiles were generally similar in males and females. The study authors noted that metabolism of bisphenol A to its glucuronide conjugate occurs as early as PND 4 in rats. However, age-dependent differences were observed in neonatal rats, as noted by a larger fraction of the lower dose being metabolized to the glucuronide. More details from this study are included in Section 2.1.2.2.

Kurebayashi et al. (2005) used a thin layer chromatography technique to examine metabolite profiles in blood, urine, and feces of 3 male rats orally dosed with 0.5 mg/kg bw ^{14}C-bisphenol A. **[The procedure did not identify metabolites.]** Parent bisphenol A represented ~2% of the dose in plasma at 0.25 and 6 hr post-dosing and ~0.3% of the dose at 24 hr after exposure. Unmetabolized bisphenol A represented 1.6% of compounds in urine and 77.2% of compounds in feces collected over a 24-hr period. Free bisphenol A represented 47.1% of compounds in urine following β-glucuronidase hydrolysis of urine, and there was an almost equivalent decrease in a metabolite the study authors identified as "M2." Therefore, the study authors stated that M2 was most likely bisphenol A glucuronide. M2 was the major metabolite identified in plasma (~74–77%) and urine (~40%).

The European Union (2003) reviewed studies by Atkinson and Roy ([1995a,b) that reported two major and several minor adducts in DNA obtained from the liver of CD-1 rats dosed orally or i.p. with 200 mg/kg bw bisphenol A. Chromatographic mobility of the two major adducts was the same as that observed when bisphenol A was incubated with purified DNA and a peroxidase or microsomal P450 activation system. The profile closely matched that of adducts formed with the interaction between bisphenol *O*-quinone and purified rat DNA deoxyguanosine 3′-monophosphate. Formation of the adduct appeared to be inhibited by known inhibiters of cytochrome P (CYP) 450. It was concluded that bisphenol A is possibly metabolized to bisphenol *O*-quinone by CYP450.

Biliary excretion of bisphenol A and its metabolites following oral or i.v. dosing with bisphenol A was examined by Kurebayashi et al. (2003). Bile ducts of 3 rats/sex/group were cannulated, and the rats were dosed with 0.1 mg/kg bw ^{14}C-bisphenol A (>99% radiochemical purity) in phosphate buffer vehicle by oral gavage or i.v. injection. Biliary fluid was collected every 2 hr over a 6-hr period to determine percent total biliary excretion and percent of dose represented by bisphenol A glucuronide. Results are summarized in Table 43. The study authors noted that the importance of biliary excretion following oral or i.v. dosing. ^{14}C-bisphenol A-glucuronide was the predominant metabolite in bile.

In another study by Kurebayashi et al. (2003), biliary, fecal, and urinary metabolites were examined in male rats gavaged with 100 mg/kg bw bisphenol A or D_{16}-bisphenol A in corn oil. Bile was collected over an 18-hr period, and urine and feces were collected over a 72-hr period. The primary metabolite detected in urine was bisphenol A glucuronide, which represented 6.5% of the dose. Lower percentages of the dose (≤1.1%) were present in urine as bisphenol A and bisphenol A sulfate. In feces, the primary compound detected was bisphenol A, which represented 61% of the dose. No glucuronide or sulfate conjugated metabolites of bisphenol A were detected in feces. Most of the dose in bile consisted of

bisphenol A glucuronide (41% of the dose). Bisphenol A represented 0.3% of the dose in bile. The study authors noted that as with oral or i.v. exposure to a smaller dose, feces was the main route of elimination for bisphenol A and bile was the main elimination route for bisphenol A glucuronide.

A study by Yokota et al. (1999) examined the hepatic isoform of uridine diphosphate glucuronosyltransferase (UDPGT) involved in the metabolism of bisphenol A and distribution of the enzyme in organs of Wistar rats. Using yeast cells genetically engineered to express single rat UDPGT enzymes, it was determined that UGT2B1 was the only isoform capable of glucuronidating bisphenol A. Microsomal UDPGT activity toward bisphenol A was demonstrated in liver, kidney, and testis, but activity was minimal in lung and brain. **[Minimal activity was also observed for intestine]**. Northern blot analyses revealed high expression of UGTB1 only in liver. It was demonstrated that 65% of glucuronidation activity was absorbed by binding with anti-UGTB1, indicating that additional isoforms are likely involved in glucuronidation of bisphenol A.

The intestine was determined to play a role in the metabolism of bisphenol A in rats. Nine-week-old male Sprague–Dawley rats were orally administered 0.1 mL of a solution containing 50 g/L bisphenol A **[5 mg total or ~17 mg/kg bw assuming a body weight of ~0.3 kg (USEPA, 1988)]** (Sakamoto et al., 2002). Rats were killed at multiple time intervals between 15 min and 12 hr following exposure. The small intestine was removed and separated into upper and lower portions. Intestinal contents were removed from each section. Bisphenol A and metabolite concentrations were measured by HPLC. Activities and expression of β-glucuronidase were determined. A large amount of bisphenol A glucuronide was detected in the upper and lower portions of the small intestine, and a large amount of free bisphenol A was detected in the cecum. Less bisphenol A was detected in colon and feces. The observations lead the study authors to conclude that free bisphenol A generated in the cecum as a result of deconjugation was reabsorbed in the colon. The presence of large amounts of bisphenol A glucuronide in the small intestine at 12 hr following exposure suggested that bisphenol A was reabsorbed in the colon and re-excreted as the glucuronide. As determined in an assay using *p*-nitrophenol-β-*d*-glucuronide as a substrate, ~70% of total β-glucuronidase activity was present in the cecum and 30% in the colon. Western blot analysis revealed a large amount of bacterial β-glucuronidase protein in cecum and colon contents.

Glucuronidation and absorption of bisphenol A in rat intestine were studied by Inoue et al. (2003a). Intestines were obtained from 8-week-old male Sprague–Dawley rats, and the small intestine was divided into 4 sections. Small intestine and colon were everted and exposed to 40 mL of a solution containing bisphenol A at 10, 50, or 100 μM **[2.3, 11, or 23 mg/L, resulting in delivery of 91, 456, or 913 μg bisphenol A to the everted intestine]**. Every 20 min during a 60-min time period, reaction products were collected from serosal and mucosal sides and analyzed by HPLC. Optimal glucuronidation was observed at 50 μM **[11 mg/L]**. At 60 min following exposure to 50 μM bisphenol A, ~37% of bisphenol A was absorbed by the small intestine and ~83% was glucuronidated. Approximately 74.7% of the glucuronide was excreted on the mucosal side and ~25.3% transported to the serosal side of small intestine. Slightly greater absorption of bisphenol A in the colon (48.6%) compared to the proximal jejunum (37.5%) was observed at 60 min following exposure to the 50 μM solution. Transport of both bisphenol A and bisphenol A glucuronide to the serosal side of intestine increased distally and was greatest in the colon. Minimal mucosal excretion was observed in the colon.

Inoue et al. (2004) compared glucuronidation of bisphenol A in pregnant, non-pregnant, and male rats. Livers of 4 male and non-pregnant Sprague–Dawley rats/group were perfused via the portal vein for 1 hr with solutions containing bisphenol A at 10 or 50 μM **[2.3 or 11 mg/L]**. The total amount of bisphenol A infused into livers was 1.5 or 7.5 μmol **[0.34 or 1.7 mg]**. On GD 20 or 21, livers of 4 pregnant Sprague–Dawley rats were perfused for 1 hr with 10 μM **[2.3 mg/L]** bisphenol A. At the start of perfusion, excreted bile and perfusate in the vein were collected every 5 min for 1 hr. Samples were analyzed by HPLC. Statistical analyses were conducted by Student *t*-test and ANOVA. Bisphenol A glucuronidation in the liver was 59% in male rats and 84% in non-pregnant female rats perfused with the 10 μM solution. The glucuronide was excreted primarily through bile in both males and females, but a significantly higher amount was excreted through bile in non-pregnant females than in males. The total amount of glucuronide excreted into bile and vein was ~1.4-fold higher in females than males following perfusion with the 10 μM **[2.3 mg/L]** solution. At the 50 μM **[11 mg/L]** concentration, bisphenol A glucuronidated within liver was 66% in males and 91% in females. In males the glucuronide was excreted mainly in bile, and in females, a higher amount of glucuronide was excreted in the vein. In livers of pregnant rats perfused with the 10 μM **[2.3 mg/L]** solution, 69% of bisphenol A was glucuronidated in the liver. Percentages of glucuronide excretion were 54.5% through bile and 45.5% through the vein in pregnant rats. In a comparison of pregnant rats and non-pregnant rats perfused with 10 μM **[2.3 mg/L]** bisphenol A, biliary excretion in pregnant rats was half that observed in non-pregnant rats, and venous excretion in pregnant rats was 3-fold higher than in non-pregnant rats. To determine the pathway of bisphenol A glucuronide excretion, livers of 4 male Eisai hyperbilirubinemic rats, a strain deficient in multidrug resistance-associated protein, were perfused with 50 μM **[11 mg/L]** bisphenol A. During and after perfusion, nearly all of the bisphenol A was excreted into the vein, thus indicating that multidrug resistance-associated protein mediates biliary excretion of bisphenol A glucuronide. The study authors concluded that bisphenol A is highly glucuronidated and excreted into bile using a multidrug resistance-associated protein-dependent mechanism, and that venous excretion increases and biliary excretion decreases during pregnancy.

Miyakoda et al. (2000) examined the production of bisphenol A glucuronide in fetal and adult rats. Bisphenol A was orally administered at 10 mg/kg bw to pregnant Wistar rats on GD 19 and to 10-week-old adult male Wistar rats. **[The number of animals exposed was not reported. In some legends for study figures, it was stated that the data were from 4 experiments,**

suggesting that 4 pregnant rats and adult males may have been exposed.] Fetuses were removed at 1 hr following dosing. Blood was drawn and testes were removed from adult males at 1, 3, and 8 hr following dosing. GC/MS was used to measure bisphenol A concentrations in 19 fetuses and in testis of adult rats before and following homogenization with β-glucuronidase. In fetal extracts, there were no differences in bisphenol A concentrations before or after treatment with β-glucuronidase, suggesting that bisphenol A glucuronide was not present at detectable concentrations. The study authors noted the possibility that bisphenol A glucuronide was not transferred from dams to fetuses and stated that glucuronidation by the rat fetus is unlikely. At 1 hr following dosing of adult male rats, 90% of bisphenol A was detected as glucuronide in plasma and testis. Bisphenol A glucuronide concentrations gradually decreased and bisphenol A concentrations increased slightly in testis over the 8-hr sampling period. In plasma, bisphenol A-glucuronide decreased to 55% of the maximum observed concentration at 3 hr following dosing and increased to 100% of maximum observed concentration at 8 hr following dosing. Based on concentrations of bisphenol A glucuronide in testis and blood (40 ppb **[μg/kg]** and 600 ppb **[μg/L]**) at 8 hr, the study authors concluded that bisphenol A glucuronide passage through the testicular barrier was unlikely. It was thought that bisphenol A passed through the testicular barrier, was converted to the glucuronide within the testis, and was then gradually released following digestion of the glucuronide by β-glucuronidase.

Matsumoto et al. (2002), studied developmental changes in expression and activity of the UDPGT isoform UGT2B toward bisphenol A in Wistar rats. Activity toward other compounds was also examined but this summary focuses on bisphenol A. Microsomes were prepared from livers of fetuses, neonates on PND 3, 7, 14, and 21, and pregnant rats on GD 10, 15, and 19. Activity toward the bisphenol A substrate was measured using an HPLC method. Expression of UGT2B1 protein was examined by Western blot and messenger ribonucleic acid (mRNA) expression was examined by Northern blot. Little to no UGT2B activity toward bisphenol A was detected in microsomes of fetuses. Activity increased linearly following birth and reached adult concentrations by PND 21. **[No data on UGT2B activity for non-pregnant adult rats were shown and it was not clear if activity in adults was examined in this study.]** The same developmental patterns were observed for expression of UGT2B1 protein and mRNA. Activity and protein expression of UGT2B1 were also found to be reduced in pregnant rats.

The European Union (2003) reviewed an unpublished study by Sipes that compared clearance of bisphenol A by hepatic microsome from fetal ($n = 8$/sex), immature ($n = 4$/sex), and adult ($n = 4$) rats. The clearance rate in microsomes from male and female GD 19 rat fetuses (0.7–09 mL/min/mg) was lower than clearance rates in microsomes from 4-day-old males and females (1.2–2.6 mL/min/mg), 21-day-old males and females (2.4–2.7 mL/min/mg), and their dams (2.6 mL/min/mg). The European Union concluded that clearance rate was lower in fetuses but reached adult concentrations by 4 days of age.

In a qualitative study of bisphenol A metabolites in pregnant mice injected with 0.025 mg/kg bw bisphenol A, 10 radioactive peaks were observed in urine by Zalko et al. (2003). The major metabolites detected in urine were bisphenol A glucuronide and a hydroxylated bisphenol A glucuronide. Unchanged bisphenol A was the major compound detected in feces (>95%). Bisphenol A glucuronide represented >90% of the compounds detected in bile. Additional compounds detected in urine, feces, digestive tract, or liver included a double glucuronide of bisphenol A and sulfate conjugates. Unchanged bisphenol A, bisphenol A glucuronide, and "metabolite F" (disaccharide conjugate of BPA) were the major compounds detected in all tissues. **[Authors state that formation of glucuronic acid conjugate of BPA, several double conjugates, and conjugated methoxylated compounds, demonstrate the formation of potentially reactive intermediates.]** The most abundant compound in all tissues was bisphenol A glucuronide, except in placenta where bisphenol A and metabolite F were the major compounds detected. Concentrations of bisphenol A decreased rapidly in all tissues. It was determined that metabolite F was most likely bisphenol A glucuronide conjugated to acetylated galactosamine or glucosamine. Distribution of bisphenol A and its metabolites in maternal and fetal tissues in summarized in Table 31. Additional details of this study are included in Section 2.1.2.2.

Jaeg et al. (2004) reported metabolites observed following incubation of CD-1 mouse liver microsomes or S9 fractions with bisphenol A at 20–500 μM **[4.6–114 mg/L]**. The metabolites included isopropyl-hydroxyphenol, bisphenol A glutathione conjugate, glutythionylphenol, glutathionyl 4-isopropylphenol, 2,2-bis-(4-hydroxyphenyl)1-propanol, 5-hydroxy bisphenol A, and bisphenol A dimers. It was postulated that bisphenol A-ortho-quinone, produced from 5-hydroxy bisphenol A (catechol), may be the reactive intermediate leading to the formation of these metabolites.

Kurebayashi et al., (2002) examined metabolism of bisphenol A in monkeys. Three adult male and female Cynomolgus monkeys were dosed with 0.1 mg/kg bw ^{14}C-bisphenol A/non-radiolabeled bisphenol A by i.v. injection on Study Day 1 and by gavage on Study Day 15 (Kurebayashi et al., 2002). Additional details of the study are included in Section 2.1.2.2. Up to five peaks were identified in urine. Analysis by radio-HPLC suggested that the major peaks in both sexes treated by either exposure route were mono- and diglucuronides. Five peaks were identified in plasma, and some differences were noted in comparisons of i.v. to oral exposure. In the 2 hr following dosing, most of the radioactivity in plasma was represented by bisphenol A glucuronide after i.v. dosing (57–82%) and oral dosing (89–100%). The percentage of radioactivity represented by unchanged bisphenol A was higher following i.v. (5–29%) than oral (0–1%) dosing.

Kang et al. (2006) reviewed studies that provided some information about metabolism of bisphenol A in fish and birds. One study reported bisphenol A sulfate and bisphenol A glucuronide as the major metabolites detected in zebra fish exposed to bisphenol A. A second study conducted in carp reported an increase in UDPGT activity for bisphenol A in microsomes and metabolism of bisphenol A to bisphenol A glucuronide in intestine. In

Table 44
Excretion of Radioactivity Following Oral or Intravenous Dosing of Rats With 0.1 mg/kg bw ^{14}C-Bisphenol A[a]

	Percent radioactive dose excreted		
Time post-dosing, hr	Urine	Feces	Total
Oral			
0–24	6.3±1.1	49.3±2.1	55.7±2.8
24–48	3.8±1.0	32.3±2.1	36.1±3.0
Total	10.1±1.6	81.6±3.7	91.8±5.0
Intravenous			
0–24	8.4±1.8	46.2±1.8	54.6±3.4
24–48	4.1±0.9	31.4±1.5	35.4±1.8
Total	12.5±0.9	77.6±1.8	90.1±2.7

Values presented as mean±SD.
[a]Kurebayashi et al. (2003).

quail embryos, metabolism and excretion of bisphenol A was reported, but specific metabolites were not indicated. Another study reported that ^{14}C-bisphenol A administered orally or i.v. to laying quail was rapidly removed via bile and excreted through feces.

2.1.2.4 Elimination: Elimination of bisphenol A and its metabolites was examined in Sprague–Dawley rats that were gavaged with bisphenol A and ^{14}C-bisphenol A at 10 mg/kg bw (Domoradzki et al., 2003). One group of rats was not pregnant, and three additional groups were treated on either GD 6 (early gestation), 14 (mid gestation), or 17 (late gestation). More details of this study are available in Section 2.1.2.2. Most of the radioactivity (65–78%) was eliminated in feces. Elimination in urine accounted for 14–22% of the dose, and considerable variability for urinary elimination among animals was evident by the large standard deviations, which were 50% of means. The authors stated that bisphenol A glucuronide represented 62–70% of radioactivity in urine and bisphenol A represented 19–23% of radioactivity in urine **[data were not shown by authors]**. Nine peaks were identified in urine. In feces, 83–89% of radioactivity was represented by bisphenol A and 2–3% was represented by bisphenol A glucuronide; 7 peaks were identified in feces. The study authors concluded that urinary elimination and fecal elimination of radioactivity were similar in pregnant and non-pregnant rats.

Difference in excretion following oral or i.v. exposure of rats to a low bisphenol A dose was examined by Kurebayashi et al. (2003). Three male rats/group were exposed to 0.1 mg/kg bw ^{14}C-bisphenol A (>99% radiochemical purity) in phosphate buffer vehicle by oral gavage or i.v. injection. Radioactivity levels were measured in urine and feces, which were collected over a 48-hr period. Additional details of the study are included in Section 2.1.2.2. Results of that study are summarized in Table 44. With both oral and i.v. dosing, fecal excretion was the main route of elimination.

Kurebayashi et al. (2005) examined elimination of radioactivity in 3 adult male and female F344 rats that were orally dosed with 0.1 mg/kg bw ^{14}C-bisphenol A. Urine and feces were collected over a 168-hr period and analyzed by liquid scintillation counting. Total radioactivity excreted in urine and feces over the 168-hr period was ~98% in males and females. In male rats, ~10% was excreted in urine and ~88% was excreted in feces. Female rats excreted ~34% of the radioactivity in urine and ~64% in feces. **[The majority of radioactivity, ~90%, was excreted over 48 hr by males and 72 hr by females.]**

Snyder et al. (2000) compared toxicokinetics of bisphenol A in CD and F344 rats. Four CD and F344 rats were gavaged with 100 mg/kg bw ^{14}C-bisphenol A in propylene glycol vehicle. Disposition of radioactivity in urine, feces, and carcass was examined over a 144-hr period. Samples were analyzed by scintillation counting, HPLC, or nuclear magnetic resonance. Data were analyzed by ArcSin transformation of the square root of the mean and using two-sample *t*-test. Recovery of radioactivity was 93% in both strains. The highest concentrations of radioactivity were detected in feces (70% of dose in CD rat and 50% of dose in F344 rats) followed by urine (21% of dose in CD rat and 42% of dose in F344 rats). The percentages of the dose excreted in urine and feces differed significantly by strain. Much lower percentages of radioactivity were detected in the carcass (~1%). Bisphenol A glucuronide, representing 81–89% of the dose, was the major urinary metabolite detected in both strains. A much lower percentage (2.2–10%) of the dose was represented by urinary bisphenol A.

Kim et al. (2002b) reported urinary excretion of bisphenol A in 4-week-old male F344 rats given bisphenol A in drinking water at 0 (ethanol vehicle), 0.1, 1, 10, or 100 ppm (equivalent to 0.011, 0.116, 1.094, or 11.846 mg/kg bw/day) for 13 weeks. Urine samples were collected for 24 hr following administration of the last dose and analyzed by HPLC before and after digestion with β-glucuronidase. The focus of the study was male reproductive toxicity; the study is described in detail in Section 4.2.2.1. Bisphenol A was not detected in the urine of rats from the control and 2 lowest dose groups. **[At the 2 highest doses, free bisphenol A represented 60 and 30% of the total urinary bisphenol A concentrations.]**

In rats exposed to 10 or 100 mg/kg bw/day ^{14}C-bisphenol A through the oral, i.p., or s.c. routes, fecal elimination represented the highest percentage of radioactivity in all exposure groups (52–83%) (Pottenger et al., 2000). Elimination of radioactivity through urine was ~2-fold higher in females (21–34%) than males (13–16%) in all dose groups. Additional details of this study are included in Section 2.1.2.3.

Elimination of bisphenol A and metabolites was examined in 3 adult male and female Cynomolgus monkeys dosed with 0.1 mg/kg bw ^{14}C-bisphenol A/non-radiolabeled bisphenol A by i.v. injection on Study Day 1 and by gavage on Study Day 15 (Kurebayashi et al., 2002). Additional details of the study are included in Section 2.1.2.2. Following oral or i.v. exposure, the percentage of radioactivity recovered in excreta and cage washes was 81–88% over a 1-week period. Most of the radioactivity was recovered in urine (combination of urine and cage washes), with most of the radioactivity excreted in urine within 12 hr and essentially all of the dose excreted within 24 hr following treatment. Percentages of radioactive doses recovered in urine within 1 week after dosing were ~79–86% following i.v. dosing and 82–85% following oral dosing. Much smaller amounts were recovered in feces during the week following i.v. or oral exposure (~2–3%). The study authors concluded that because fecal excretion was very

low following oral exposure, absorption was considered to be complete. The authors also noted that there were no obvious route or sex differences in excretion of radioactivity. The study authors concluded that terminal elimination half-lives were longer following i.v. than oral exposure. A limited amount of information was presented for the fast phase, defined as the 2 hr following i.v. injection. Fast-phase elimination half-life of bisphenol A following i.v. exposure was significantly lower in females (0.39 hr) than males (0.57 hr). There were no sex-related differences in fast-phase half-life for bisphenol A glucuronide (0.79–0.82 hr) or total radioactivity (0.61–0.67 hr).

2.1.3 Comparison of humans and experimental animals. Studies comparing toxicokinetics and metabolism of bisphenol A in humans and laboratory animals were reviewed and are summarized below. In most cases the data were from original sources, but information from secondary sources was included if the information was not new or critical to the evaluation of developmental or reproductive toxicity.

Elsby et al. (2001) compared bisphenol A metabolism by rat and human microsomes. Microsomes were obtained from 8 immature Wistar rats (21–25 days old) and histologically normal livers from 4 male (25–57 years old) and 4 female (35–65 years old) Caucasian donors who were killed in accidents. Human microsomes were pooled according to sex of the donor. Glucuronidation was examined following exposure of microsomes to bisphenol A concentrations of 0–1000 μM **[0–228 mg/L]** for 30 min with human microsomes and 10 min with rat microsomes. Metabolites were identified by HPLC or LC/MS. Data were obtained from 4 experiments conducted in duplicate. Data were analyzed by Mann–Whitney test. Maximum velocity (V_{max}) and the rate constant (K_m) values are summarized in Table 45. The study authors reported a significant difference between the V_{max} for glucuronidation in immature rats and humans. No sex-related difference was reported for glucuronidation by human microsomes. As a result of less extensive glucuronidation by human than rat microsomes, the study authors noted that estrogen target tissues in humans may receive higher exposure to bisphenol A than tissues of immature female rats used in estrogenicity studies. Lastly, oxidation of bisphenol A by female rat or human microsomes was examined following incubation with 200 μM **[46 mg/L]** bisphenol A and NADPH. The only metabolite identified was 5-hydroxybisphenol A.

Table 45
Glucuronidation Kinetics in Microsomes From Immature Rats and Adult Humans[a]

Sex/species	V_{max}, nmol/minute/mg protein	K_m, μM
Male/human	5.9 ± 0.4	77.5 ± 8.3
Female/human	5.2 ± 0.3	66.3 ± 7.5
Female/immature rat	31.6 ± 8.1	27.0 ± 1.2

Data presented as mean ± SEM.
[a]Elsby et al. (Elsby et al., 2001).

The European Union (2003) reviewed a series of studies by Sipes that compared metabolism of bisphenol A in microsomes from male and female humans (15 pooled samples/sex and 3–5 individual samples/sex), rats (4/sex), and mice (4/sex). It was concluded that the studies generally agreed with the findings of Elsby et al. (2001). Clearance rates (V_{max}/K_m) in human microsomes (0.4–0.9 mL/min/mg for pooled samples and 0.3–0.5 mL/min/mg in individual samples) were lower than those observed in rats (1.0–1.7 mL/min/mg) and mice (1.3–3.0 mL/min/mg).

Pritchett et al. (2002) compared metabolism of bisphenol A in hepatocyte cultures from humans, rats, and mice. Cell cultures were prepared from adult male and female F344 rats, Sprague–Dawley rats, and CF1 mice. Human hepatocyte cultures were obtained from 3 females and 2 males. **[No information was provided about the age of human donors.]** Cells were exposed to ^{14}C-bisphenol A (99.3% purity)/bisphenol A (>99% purity) in a DMSO vehicle. In a cytotoxicity assessment,

Table 46
Metabolites Obtained From Incubation of Human, Rat, and Mouse Hepatocyte Cultures With 20 μM [4.6 mg/L] Bisphenol A[a]

Sex and species	Percentage of parent compound or metabolites			
	Glucuronide/sulfate	Sulfate	Glucuronide	Bisphenol A
Human samples				
Female–1	4	0	93	0
Female–2	2	0	84	2
Female–3	43	2	55	0
Male–1	1	0	85	0
Male–2	0	7.5	75	0
Rodent samples				
Male F344 rat	70	0	30	0
Female F344 rat	10	0	86	0
Male Sprague–Dawley rat	30	2	58	0
Female Sprague–Dawley rat	0	0	100	0
Male CF1 Mouse	0	0	100	0
Female CF1 mouse	0	0	93	0

Human cells were incubated for 3 hr, and animal cells were incubated for 6 hr.
[a]Pritchett et al. (2002).

lactate dehydrogenase activity was measured in rat cells following incubation for 18 hr in 5–100 μM **[1.1–23 mg/L]** bisphenol A, and cytotoxicity was observed at ≥75 μM bisphenol A. Bisphenol A concentrations tested and times of exposure were 5–20 μM **[1.1–4.6 mg/L]** for up to 6 hr in time-dependent metabolism studies and 2.5–30 μM **[0.57–6.8 mg/L]** for 10 min in concentration-dependent metabolism studies. Metabolites in cell media were analyzed by HPLC and LC-MS/MS.

Analysis of media from human hepatocytes incubated with bisphenol A indicated that the major metabolite was bisphenol A glucuronide, and compounds found at lower concentrations were bisphenol A glucuronide/sulfate diconjugate, and bisphenol A sulfate conjugate. Table 46 summarizes percentages of each type of metabolite detected in media following incubation with 20 μM **[4.6 mg/L]** bisphenol A for 3 hr in human cells and 6 hr in rodent cells. In cells from all sexes and species except male F344 rats, bisphenol A glucuronide was the major metabolite detected. The glucuronide/sulfate diconjugate was the major metabolite detected in cells from male F344 rats. In concentration-dependent studies conducted in F344 rat hepatocytes, a biphasic curve was obtained following a 10-min incubation, with a V_{max} of 0.36 nmol/min at bisphenol A concentrations of 20–30 μM **[4.6–6.8 mg/L]** and a V_{max} of ~0.15 nmol/min at bisphenol A concentrations of 2.5–10 nM **[0.57–2.3 mg/L]**. Table 47 summarizes the higher V_{max} values obtained with cells from human, rat, and mouse livers. Total hepatic capacity was determined by multiplying V_{max} by total number of hepatocytes/liver in vivo. **[The only graphical data presented were for male F344 rats]**. The authors noted that V_{max} values were highest in mice > rats > humans. However, when adjusted for total hepatocyte number in vivo, the values were predicted to be highest in humans > rats > mice.

Table 47
Rates of Bisphenol A Glucuronide Formation Following Incubation of Human, Rat, and Mouse Hepatocytes With Bisphenol A[a]

Species and sex	V_{max}, nmol/min/ 0.5×10^6 hepatocytes	Hepatic capacity, μmol/hr[b]
Human female	0.27	8000
F344 rat female	0.46	46.5
F344 rat male	0.36	61.8
Sprague Dawley female	0.39	54.5
Sprague Dawley male	0.45	79.9
CF1 mouse female	0.50	13.8
CF1 mouse male	0.82	23.6

[a]Pritchett et al. (2002).
[b]Hepatic capacity was estimated by multiplying V_{max} by total numbers of hepatic cells in vivo.

Table 48
Toxicokinetic Endpoints for Bisphenol A in Mice, Rats, Rabbits, and Dogs Intravenously Dosed With 2 mg/kg bw Bisphenol A[a]

Endpoint	Mouse[b]	Rat	Rabbit	Dog
Systemic clearance, L/hr	0.3	1.9 ± 0.4	12.6 ± 4.9	27.1 ± 8.0
Volume of distribution, L	0.1	1.3 ± 0.4	7.1 ± 2.3	20.0 ± 5.4
Half-life, min	39.9	37.6 ± 12.8	40.8 ± 17.1	43.7 ± 21.9

Data are presented as mean ± SD.
[a]Cho et al. (2002).
[b]Variances not reported.

Data from Pritchett et al. (2002) appeared to be included in a series of unpublished studies by Sipes that were reviewed by the European Union (2003). In their review, the European Union noted that metabolic patterns appear to be similar in humans, rats, and mice. It was stated that the biphasic kinetic profile indicated involvement of a high-affinity glucuronidase enzyme at low concentrations and a high-capacity enzyme at high concentrations. In the interpretation of kinetic profiles in humans and experimental animals, the authors of the European Union report noted that the study calculations did not consider in vivo conditions such as varying metabolic capacity of hepatic cells, relationship of hepatic size to body size, and possibly important physiological endpoints such as blood flow. In addition, it was noted that calculations were based on limited data that did not address inter-individual variability in enzyme expression.

Cho et al. (2002) examined toxicokinetics of bisphenol A in mouse, rat, rabbit, and dog and used that information to predict toxicokinetic values in humans. Bisphenol A was administered by i.v. injection at 2 mg/kg bw to 5 male ICR mice and at 1 mg/kg bw to 7 male Sprague–Dawley rats, 7 male New Zealand White rabbits, and 5 male beagle dogs. Blood samples were drawn before dosing and at multiple time points between 2 min and 6 hr following injection. Serum bisphenol A concentrations were measured by HPLC. Toxicokinetic endpoints in animals are summarized in Table 48. The study authors noted that clearance and volume of distribution increased with increasing animal weight but that terminal half-life remained relatively constant across the different species. Simple allometric

Table 49
Predicted Bisphenol A Toxicokinetic Endpoints in Humans Based on Results From Experimental Animal Studies[a]

	Prediction method			
Endpoint	Allometric scaling	Kallynochrons	Apolysichrons	Dienetichrons
Systemic clearance, L/hr	127.1	123	120.7	46.0
Volume of distribution, L	125.3	229.7	138.0	149.3
Half-life, min	43.6	110.4	67.8	196.2

[a]Cho et al. (2002).

scaling and species-invariant time methods were used to predict values for a 70-kg human, and those values are summarized in Table 49. Regression analyses of estimates using the species-invariant time methods demonstrated that data from the 4 animal species were superimposable ($r = 0.94$–0.949).

Teeguarden et al. (2005) developed a physiologically based pharmacokinetic (PBPK) model for bisphenol A. Rat toxicokinetic data for the model were obtained from the studies by Pottenger et al. (2000) and Upmeier et al. (2000). Human toxicokinetic data were obtained from the study by Völkel et al. (2002). The model was developed to simulate blood and uterine concentrations of bisphenol A following exposure of humans through relevant routes. Correlations were determined for simulated bisphenol A binding to uterine receptors and increases in uterine wet weight, as determined by an unpublished study by Twomey. Although intestinal metabolism of bisphenol A to the glucuronide metabolite had been demonstrated recently, the model attributed bisphenol A metabolism entirely to the liver. Plasma protein binding was considered in both the rat and human model. The model accurately simulated plasma bisphenol A glucuronide concentrations in humans orally administered 5 mg bisphenol A, with the exception of underpredicting bisphenol A glucuronide concentrations at the 24–48-hr period following exposures. Cumulative urinary elimination of bisphenol A glucuronide in human males and females was simulated accurately. Less accurate simulations were observed for toxicokinetics in orally exposed rats, and the study authors indicated that a likely cause was oversimplification of the rat gastrointestinal compartment. Comparisons in metabolic clearance rates for i.v. and oral exposure suggested significant intestinal glucuronidation of bisphenol A. Enterohepatic recirculation strongly affected terminal elimination in rats but not humans. Consideration of bound versus unbound bisphenol A was found to be important in simulating occupancy of the estrogen receptor (ER) and uterine weight response. No increase in uterine weight was reported with simulated receptor occupancy of ~1–15%. An increase in uterine weight was reported with ~25% receptor occupancy, and doubling of uterine weight was reported with 63% receptor occupancy.

Shin et al. (2004) developed a PBPK model to predict the tissue distribution (lung, liver, spleen, kidneys, heart, testes, muscle, brain, adipose tissue, stomach, and small intestine) and blood pharmacokinetics of bisphenol A in rats and humans. The model was based on experimentally determined steady state blood-to-serum and tissue-to-blood partition ratios and does not include parameters to account for elimination via glucuronidation or differences in metabolism between rats and humans (e.g., enterohepatic circulation). Predicted concentration-time profiles were then compared to actual rat toxicokinetic data and to a profile for a simulated 70-kg human. Rat toxicokinetic information was obtained by administering multiple i.v. injections of bisphenol A (0.5 mg/kg) to adult male rats to achieve steady state. Bisphenol A concentrations were determined by a modified HPLC method with fluorescence detection. The authors noted good agreement between predicted and observed concentration-time profiles for blood and all tissues but did not present any statistical analysis or evaluate the performance of alternative models in order to establish goodness of fit. Based on the figures presented in the article, the PBPK model appeared to more accurately predict concentrations of bisphenol A in some tissues (e.g., blood, lung, and liver) better than others such as the small intestine and adipose tissue. The model was then applied to predict blood and tissue levels of bisphenol A in a 70 kg human after single i.v. injection (5-mg dose) and multiple oral administrations to steady state (100-mg doses every 24 hr). Tissue volumes and blood flow rates for a 70 kg human were taken from the literature. The authors concluded that simulated steady-state human blood levels (0.9–1.6 ng/ml) were comparable to blood levels of bisphenol A reported in the literature (1.49 ng/ml). In addition, the authors noted the similarity of predicted toxicokinetic endpoints obtained from their PBPK model to those predicted by Cho et al. (2002) based on simple allometric scaling on rat data.

Table 50
LD50 s for Bisphenol A

Species	Exposure route	LD_{50} (mg/kg bw)
Rat	Oral	3300–4100[a]
		5000[b]
		3250[c]
	Inhalation	>170 mg/m^3[b]
Mouse	Oral	4100–5200[a]
		2400[c]
	Intraperitoneally	150[c]
Guinea pig	Oral	4000[c]
Rabbit	Oral	2230[b,c]
	Dermal	>2000[b]
		3 mL/kg[c]

[a]National Toxicology Program (NTP, 1982).
[b]Reviewed by the European Union (2003).
[c]Reviewed in ChemIDplus (2006).

2.2 General Toxicity, Estrogenicity, and Androgenicity

This section includes information on general toxicity as well as information on estrogenicity and androgenicity; however, results of estrogenicity and androgenicity testing are not considered a priori evidence of toxicity.

2.2.1 General toxicity. The European Union (2003) reported there were no adequate studies for assessing acute toxicity of bisphenol A in humans.

In an acute toxicity study in rats orally dosed with bisphenol A at ≥2000 mg/kg bw, clinical signs included lethargy, prostration, hunched posture, and piloerection [reviewed by (European-Union, 2003)]. Gross signs in animals that died included pale livers and hemorrhage in the gastrointestinal tract. In a study in which male and female rats were subjected to whole body inhalation exposure to 170 mg/m^3 bisphenol A dust for 6 hr, there were no gross signs of toxicity [reviewed by (European-Union, 2003)]. Effects observed in the respiratory tract at 2 but not 14 days following exposure included slight inflammation of nasal epithelium and slight ulceration of the oronasal duct. LD_{50} reported in studies with oral, dermal, inhalation, or i.p. exposure are summarized in Table 50. The European Union (2003) concluded that bisphenol A is of low acute toxicity through all exposure routes relevant to humans.

The European Union (2003) noted limited anecdotal data reporting skin, eye, and respiratory tract irritation in workers exposed to bisphenol A, but concluded that the reports were of uncertain reliability. It was noted that a recent, well-conducted study in rabbits demonstrated that bisphenol A is not a skin irritant. Other studies conducted in rabbits demonstrated eye irritation and damage, and it was concluded the bisphenol A can potentially cause serious eye damage. Slight respiratory tract inflammation occurred in rats inhaling $\geq 50\,mg/m^3$ bisphenol A, and it was concluded that bisphenol A had limited potential for respiratory irritation. Based on the results of the studies described above, the European Union concluded that bisphenol A is not corrosive.

The European Union (2003) reviewed studies examining possible sensitization reactions in humans exposed to products containing bisphenol A, and those studies reported mixed results. In studies reporting positive findings, it was unclear if bisphenol A or epoxy resins were the cause of hypersensitivity. Cross-sensitization responses in individuals exposed to compounds similar to bisphenol A were also reported. Animal studies were determined unreliable for assessing sensitization. Based on the results of human studies, it was concluded that bisphenol A may have potential for sensitization in individuals exposed to resins. Human studies suggested that bisphenol A can induce dermal photosensitization responses. Photosensitization studies in mice resulted in reproducible positive results. Mechanistic studies in mice suggested that sensitization occurs through an immune-mediated process. The overall conclusion of the European Union was that it was somewhat unclear if bisphenol A induces orthodox skin sensitization, photosensitization, or responses in individuals previously sensitized to another substance, such as epoxy resins. No information was available on potential respiratory sensitization by bisphenol A.

The European Union (2003) summarized systemic toxicity reported in subchronic, chronic, and reproductive toxicity studies of rats, mice, and dogs. CERHR also reviewed the studies that examined reproductive organs, and those studies are summarized in detail in the appropriate section of this report. A relevant study by Yamasaki et al. (2002a) was published subsequent to the European Union review and was reviewed in detail by CERHR.

In studies reviewed by the European Union (2003) and in a study by Yamasaki et al. (2002a), rats were orally exposed to bisphenol A for periods of 28 days to 2 years. Cecal enlargement occurring at doses $\geq 25\,mg/kg$ bw/day was the effect observed most frequently in those studies but was not considered toxicologically significant by the European Union. Histological alteration in the cecum consisting of mucosal hyperplasia was only reported in one study at doses $\geq 200\,mg/kg$ bw/day. Histopathological changes in liver and kidney were reported at doses $\geq 500\,mg/kg$ bw/day. The changes in liver were characterized by prominent hepatocyte nuclei or inflammation. Histopathology in kidney was characterized by renal tubule degeneration or necrosis. Testicular toxicity (degeneration of seminiferous tubules and arrested spermatogenesis) was observed in 1 study at doses $\geq 235\,mg/kg$ bw/day.

The European Union (2003) found subchronic and chronic studies conducted by the NTP (NTP, 1982) to be the only reliable studies for assessing systemic toxicity in mice orally exposed to bisphenol A. The liver was found to be the target organ of toxicity, with multinucleated giant hepatocytes observed in male mice exposed to $\geq 120\,mg/kg$ bw/day and female mice exposed to 650 mg/kg bw/day.

In a 90-day dietary study in dogs reviewed by the European Union (2003), an increase in relative liver weight with no accompanying histopathological alterations was found to be the only effect at doses $\geq 270\,mg/kg$ bw/day. This finding was considered by the European Union to be of doubtful toxicological significance.

In a subchronic inhalation exposure study in rats reviewed by the European Union (2003), cecal enlargement as a result of distention by food was observed at $\geq 50\,mg/m^3$. Also observed at $\geq 50\,mg/m^3$ were slight hyperplasia and inflammation of epithelium in the anterior nasal cavity.

A limited number of repeat-dose systemic toxicity studies were summarized in detail by CERHR because they included examination of reproductive organs. Those studies are summarized in detail below.

NTP (1982), conducted acute, subacute, and subchronic bisphenol A toxicity studies in F344 rats and $B6C3F_1$ mice. Animals were randomly assigned to treatment groups. Purity of bisphenol A was <98.2%. Concentration and stability of bisphenol A in feed were verified. In acute studies, single doses of bisphenol A in a 1.5% acacia vehicle were administered by gavage to 5 rats/group/sex at doses of 2150, 3160, 4640, or 6810 mg/kg bw/day and 5 mice/group/sex at 1470, 2150, 3160, 4640, 6810, or 10,000 mg/kg bw. LD_{50} values for that study are summarized in Table 50.

In a 14-day repeat dose study, survival and body weight gain were evaluated in 5 rats and mice/sex/group that were fed diets containing bisphenol A at 0, 500, 1000, 2500, 5000, or 10,000 ppm. Survival was unaffected by treatment. Weight gain was reduced by 60% or more in male rats exposed to ≥ 2500 ppm and 40% or more in female rats exposed to ≥ 5000 ppm bisphenol A. Survival and weight gain in mice were not affected by bisphenol A exposure.

In subchronic studies, 10 rats and mice/sex/group were exposed to bisphenol A in diet for 13 weeks. Dietary doses were 0, 250, 500, 1000, 2000, or 4000 ppm for rats and 0, 5000, 10,000, 15,000, 20,000, or 25,000 ppm for mice. A review by the European Union (2003) estimated bisphenol A intake at 0, 25, 50, 100, 200, and 400 mg/kg bw/day for rats, 0, 600, 1200, 1800, 2400, and 3000 mg/kg bw in male mice, and 0, 650, 1300, 1950, 2600, and 3250 mg/kg bw/day in female mice. Animals were observed and weighed during the study and killed and necropsied on Day 91 of the study. **[Histopathological evaluations were conducted but it was not clear if all dose groups and all animals/dose group were examined. There was no mention of statistical analyses.]** In rats, the only deaths occurred in 2/10 males of the 1000 ppm group. Weight gain was reduced by 18% or more in male rats and 10% or more in female rats exposed to ≥ 1000 ppm. There were no effects on feed intake. Hyaline masses in the bladder lumen were not observed in control male rats but were observed in 5 of 10 males exposed to 250 ppm, 3 of 10 exposed to 500 ppm, 3 of 10 exposed to 1000 ppm, 6 of 10 exposed to 2000 ppm, and 4 of 10 exposed to 4000 ppm. Cecal enlargement, which was observed in rats at a rate of

60–100% in each dose group with the exception of females exposed to 250 ppm was considered to be treatment-related. No histological alterations were observed in the cecum. Death in mice was limited to 2 of 10 females in the 5000 ppm group. Weight gain was reduced by at least 14% in male mice exposed to ≥15,000 ppm. Non-dose-related decreases in weight gain of 17% or more occurred in female mice of all dose groups. A dose-related increase in multinucleated giant hepatocytes was observed in all dose groups of male mice; the only incidence data reported for multinucleated giant hepatocytes were 0 of 10 female controls and 9 of 10 male mice of the 25,000 ppm group. **[A complete set of data for histopathological findings was not presented for rats or mice.]**

Yamasaki et al. (2002a) examined the effects of bisphenol A exposure on male and female CD rats in a study conducted according to Good Laboratory Practices (GLP). **[Because this study included a number of reproductive organ and hormone endpoints, it is also discussed in Sections 4.2.1.1 and 4.2.2.1.]** Rats were fed a commercial diet (MF Oriental Yeast Co.) and housed in stainless steel wire-mesh cages. Rats were groups according to body weight and then randomly assigned to treatment groups. Ten 7-week-old rats/sex/group were gavaged with bisphenol A at 0 (olive oil vehicle), 40, 200, or 1000 mg/kg bw/day for 28 days. Due to the death of 1 animal exhibiting clinical signs in the 1000 mg/kg bw/day group, the high-dose was reduced to 600 mg/kg bw/day on Study Day 8. In an additional study, rats were exposed to ethinyl estradiol at 0, 10, 50, or 200 μg/kg bw/day for 28 days. Endpoints examined during the study were clinical signs, body weight gain, and food intake. Estrous cyclicity was examined in females for 2 weeks beginning on Study Day 15. Males were killed on Study Day 29 and females were killed in diestrus on Study Day 30, 31, or 32. Hematology and clinical chemistry endpoints were assessed, and blood hormone concentrations were measured by immunoassay systems. Sperm motility and viability were evaluated. Organs, including those of the reproductive system, were weighed and subjected to histopathological evaluation. With the exception of the testis and epididymis, which were fixed in Bouin solution, the organs were fixed in 10% neutral buffered formalin. Statistical analyses included Bartlett test for homogeneity of variance, ANOVA, Dunnett test, and/or Kruskall–Wallis test.

One female and 3 males from the high-dose group died; clinical signs observed in those animals included soft stools, decreased mobility, reduced respiration rate, and decreased body temperature. Soft stools were also observed in surviving males and females of the mid- and high-dose groups. Results of the study are summarized in Table 51. Terminal body weights were lower in females of the mid- and high-dose groups and males of the high-dose group. During the first week of study, food intake was decreased in both sexes of the mid- and high-dose group. **[Data were not shown by study authors.]** As noted in Table 51, some alterations in hematological and clinical chemistry endpoints were observed, mainly at the high-dose. **[Data were not shown by study authors.]** There were no treatment-related abnormalities in sperm or alterations in blood concentrations of thyroid hormones, follicle stimulating hormone (FSH), luteinizing hormone (LH), 17β-estradiol, prolactin, or testosterone. Number of females with diestrus lasting 4 or more days was increased in the high-dose group. Changes in relative organ weights **[assumed to be relative to body**

Table 51
Toxicological Effects in Rats Gavaged With Bisphenol A for 28 Days[a]

Endpoint	Bisphenol A dose (mg/kg bw/day)		
	40	200	600–1000[c]
Males			
Terminal body weight	↔	↔	↓ 17%
Relative testes weight	↔	↔	↑ 21%
Relative ventral prostate weight	↔	↔	↓ 28%
Relative adrenal weight	↔	↔	↑ 19%
Feed intake[b]	↔	↓	↓
Prothrombin time[b]	↔	↔	↑
Glutamic-oxaloacetic transaminase[b]	↔	↑	↑
Triglyceride[b]	↔	↔	↓
Alkaline phosphatase[b]	↔	↔	↑
γ-Glutamyl transpeptidase[b]	↔	↔	↑
Chloride[b]	↔	↔	↑
Renal tubular degeneration and necrosis	0/10	0/10	7/7
Forestomach squamous epithelial cell hyperplasia	0/10	0/10	6/7
Lacteal dilatation in duodenum	0/10	10/10	2/7
Lacteal dilation in jejunum	0/10	0/10	2/7
Mucosal hyperplasia in cecum	0/10	3/10	6/7
Mucosal hyperplasia in colon	0/10	2/10	7/7
Adrenal cortical vacuolization	0/10	0/10	3/7
Females			
Terminal body weight	↔	↓ 7%	↓ 5%
Relative thyroid weight	↔	↔	↑ 22%
Relative liver weight	↔	↔	↑ 10%
Relative heart weight	↔	↓ 9%	↓ 15%
Feed intake[b]	↔	↓	↓
Hemoglobin and hematocrit values[b]	↔	↔	↓
Cholinesterase[b]	↔	↓	↓
Glutamic-oxaloacetic transaminase[b]	↔	↔	↑
Albumin and albumin: globulin rats[b]	↔	↔	↓
Diestrus ≥4 days	0/10	0/10	3/9
Prominent hepatocyte nuclei	0/10	0/10	4/9
Renal tubular degeneration and necrosis	0/10	0/10	9/9
Forestomach squamous epithelial cell hyperplasia	0/10	0/10	5/9
Lacteal dilatation in duodenum	0/10	7/10	6/9
Mucosal hyperplasia in cecum	0/10	6/10	4/9
Adrenal cortical vacuolization	0/10	0/10	3/9

[a]Yamasaki et al. (2002a).
[b]Data were not shown by study authors.
[c]The dose was 1000 mg/kg bw/day at the beginning of the study, but was decreased to 600 mg/kg bw/day in the second week of the study due to excessive toxicity.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

weight] included decreased heart weight in females from the mid- and high-dose groups. At the high-dose, there were decreases in relative weight of ventral prostate and increases in relative weights of testis and adrenals in males and thyroid and liver in females. Gross signs observed in animals that died included enlarged kidney, elevated mucosa in the forestomach, and atrophied spleen and thymus. In surviving animals, the cecum was enlarged in the mid- and high-dose group and forestomach mucosa was elevated in the high-dose group. As described in more detail in Table 51, histopathological alterations were observed in the intestine, cecum, and colon of males and intestine and cecum of females in the mid- and high-dose groups. Additional histopathological alterations were observed in the high-dose group in the kidney, forestomach, and adrenals of males and females and livers of females.

Male rats from the mid- and high-dose ethinyl estradiol groups experienced decreased prostate, seminal vesicle, and pituitary weights, increased testis weight, and histopathological alterations in prostate, seminal vesicle, mammary gland, and testis. Females from the mid- and high-dose ethinyl estradiol group experienced alterations in estrous cyclicity. Females from the high-dose group experienced decreased ovary weight, increased uterine weight, and histopathological changes in ovary, uterus, and vagina.

General Electric (1984) conducted a subchronic toxicity study in Beagle dogs orally dosed with bisphenol A **[purity not reported]**. Dogs weighing 6.5–13.4 kg were housed in metal metabolism cages and fed Purina Dog Chow. During a 90-day period, 4 dogs/sex/group were given feed containing bisphenol A at 0, 1000, 3000, or 9000 ppm. The European Union (2003) estimated bisphenol A intake at 0, 28, 74, or 261 mg/kg bw/day in males and 0, 31, 87, or 286 mg/kg bw/day in females. Dogs were observed for body weight gain, food, intake, and clinical signs. Ophthalmoscopic examination was conducted before and following the treatment period. Hematology, clinical chemistry, and urinalysis evaluations were conducted before treatment and at 1, 2, and 3 months into the study. Dogs were killed at the end of the treatment period. Organs were weighed and fixed in 10% neutral buffered formalin. Histopathological evaluations were conducted in organs from the control and high-dose groups; prostate, uterus, testis, and ovary were among organs evaluated. **[Procedures for statistical analyses were not described.]** No treatment-related clinical signs (conducted monthly), ophthalmological changes, or death were observed during the study. Bisphenol A treatment did not affect body weight gain or food intake. There were no treatment-related effects on hematology, biochemistry, or urinalysis. Relative liver weight was significantly increased **[by 18% in males and 26% in females]** in the high-dose group, and the study authors considered the effect to be treatment-related. No treatment-related gross or histopathological lesions were observed in the high-dose group.

Nitschke et al. (1988) conducted a subchronic inhalation toxicity test with bisphenol A in F344 rats. Rats were fed Purina Certified Rodent Chow 5002 and housed in stainless steel wire cages. At 7 weeks of age, rats were stratified according to body weight and randomly assigned to treatment groups. Thirty rats/sex/group received whole-body exposures to polycarbonate grade bisphenol A dust (99.7% purity) at 0, 10, 50, or 150 mg/m^3 for 6 hr/day, 5 days/week, for 13 weeks. Mass median aerodynamic diameter of bisphenol A dust was measured at $\leq$5.2 microns. Stability and concentrations of bisphenol A were verified. Rats were observed for clinical signs, body weight gain, and food intake. Ten rats/sex/group in each time period were killed and necropsied on the day following and at 4 and 12 weeks following exposure. At each necropsy period, hematological and clinical chemistry endpoints were examined. The lungs, brain, kidneys, and testes were weighed. Numerous organs were preserved in 10% phosphate-buffered formalin. In most cases, histological examinations were conducted in organs from the control and high-dose groups. Respiratory organs and organs with lesions or signs of toxicity were histologically examined at all dose levels. Included among organs undergoing histopathological examination immediately after the exposure period were the epididymis, mammary gland, ovary, oviduct, prostate, seminal vesicles, testis, uterus, and vagina. No reproductive organs were examined following the recovery periods. Statistical analyses included Bartlett's test, ANOVA, Dunnett test, Wilcoxon Rank-Sum test, and Bonferroni correction for multiple comparisons. Gross pathology and histopathology data did not appear to have been statistically analyzed.

During the exposure period, a reddish material around the nose (most likely porphyrin) was observed in 2–10 of 10 animals/sex in the 50 and 150 mg/m^3 groups. Perineal soiling was observed in 2 of 10 females in the 10 mg/m^3 group and 9–10 of 10 animals/sex in the 50 and 150 mg/m^3 groups. Decreased body weight gain during treatment was observed in males from all dose groups and females in the 50 and 150 mg/m^3 groups. Immediately following the treatment period, terminal body weights were reduced by ~5% in males and ~11% in females from the 150 mg/m^3 group. **[Body weights were ~4% lower in males from the 50 mg/m^3 group.]** No differences in feed intake were observed at this or any other time period in the study. The only hematological effect observed was slightly increased hemoglobin in males exposed to 10 mg/m^3, but the study authors did not consider the effect to be biologically significant. Clinical chemistry observations in the 150 mg/m^3 group included decreased serum glutamic pyruvic transaminase activity, serum glutamic oxaloacetic transaminase activity, and glucose in males and decreased total protein and albumin and increased alkaline phosphatase activity in females. Alkaline phosphatase activity was also increased in females exposed to 50 mg/m^3. The study authors did not consider any of the clinical chemistry changes to be biologically **[toxicologically]** significant. Absolute liver weight was decreased in males exposed to $\geq$10 and 150 mg/m^3, and relative brain weight was increased in females exposed to $\geq$50 mg/m^3. Additional organ weight changes observed in females from the 150 mg/m^3 group included decreased absolute liver and kidney weights and increased relative lung weights. Because the organ weight changes were not associated with microscopic changes in organs, the study authors concluded that the effects reflected decreases in body weight and were not toxicologically significant. Cecal size was increased as a result of distention by food in all (10/dose/sex) males and females exposed to $\geq$50 mg/m^3, and the effect was considered to be treatment-

related. No histopathological alterations were observed for cecal wall morphology. Hemolyzed blood was observed in the stomachs of three to seven of 10 males/group exposed to 50 and 150 mg/m^3, but there were no signs of histopathological alterations in the gastrointestinal tract. Slight histopathological alterations, consisting of hyperplasia in stratified squamous and ciliated epithelium lining and inflammation of submucosal tissues was observed in the anterior nasal cavities of all (10/dose/sex) males and females exposed to $\geq$50 mg/m^3. Slight-to-moderate hyperplasia of goblet cells was also observed in the lateral nasal wall. No other treatment-related histopathological alterations were observed, including in reproductive organs.

During the 4-week recovery period, body weights remained lower in males and females of the 50 and 150 mg/m^3 groups At 4 weeks following exposure, terminal body weights of males and females in the 150 mg/m^3 group were $\sim$6% lower than control values. A decrease in white blood cell count in females from the 10 and 150 mg/m^3 groups was the only hematological effect observed. The clinical chemistry effects that were somewhat consistent with effects observed immediately following treatment were increased alkaline phosphatase activity in females exposed to 10 and 150 mg/m^3 and decreased serum glutamic pyruvic activity transaminase activity in females exposed to 150 mg/m^3; the study authors did not consider the clinical chemistry changes to be treatment-related. The study authors concluded that an increase in relative brain weight in males of the 150 mg/m^3 group was related to decreased body weights in those animals. Enlarged cecal size was observed in 5 of 10 males of the 150 mg/m^3 group, a decreased incidence compared to the period immediately following treatment. Nasal histopathology was observed in the 150 mg/m^3 but was reduced in magnitude and severity compared to rats observed immediately following exposure.

In rats examined following 12 weeks of recovery, body weights of males in the 150 mg/m^3 group remained lower than controls, and terminal body weight was decreased by $\sim$6%. An increase in white blood cell counts but not differential counts was observed in male rats of the 10 and 150 mg/m^3 group. The only clinical chemistry finding consistent with earlier observations was decreased total protein and globulin in females from the 150 mg/m^3 group, but the study authors did not consider the effect to be biologically significant. Organ weight changes in the 150 mg/m^3 group included decreased absolute kidney and lung weights in males and decreased absolute and relative kidney weights in females. No histopathological alterations were observed in kidney or lung. No other gross or histopathological alterations were observed, including cecal enlargement and nasal histopathology, which were observed at earlier time periods.

2.2.2 Estrogenicity. The first identification of bisphenol A as an estrogen has been attributed to Dodds and Lawson (1936), who reported that 100 mg injected by an unspecified route twice daily for 3 days resulted in maintenance of 5 of 5 rats in vaginal estrus for 40 days. The estrogenicity of bisphenol A has since been evaluated using several different kinds of assays. In vitro studies are summarized in Table 52, and in vivo studies are summarized in Table 53 using comparisons with 17β-estradiol, ethinyl estradiol, diethylstilbestrol, and, in one study, estrone. There is considerable variability in the results of these studies with the estrogenic potency of bisphenol A ranging over about 8 orders of magnitude, but similar means (Fig. 2).

The most common method of comparing potency is to test responses over a range of concentrations and to compare the concentrations producing the half-maximal (or other fractional) response of the comparator estrogen. An alternative is to compare the magnitude of the response at an equimolar concentration of the 2 estrogens. The difference in these two methods is illustrated in Figure 3. An example of the difference in potency estimations according to comparison method is the study of Vivacqua et al. (2003), in which the fold-increase in reporter activity for an estrogen-responsive gene was compared over a range of concentrations for bisphenol A and for 17β-estradiol. This study's Figure 3 presents curves analogous to Figure 3, but also presents a bar graph comparing response of the reporter at a 10^{-7} M concentration of each estrogen. Based on the half-maximal response to 17β-estradiol, bisphenol-A appeared 1000 times less potent than 17β-estradiol, but based on the fold-difference in reporter activity at 10^{-7} M, bisphenol A was about half as potent. Data for other estrogenicity comparisons in this study and in many other studies are presented only using bar graphs comparing responses at the same molar concentrations of the 2 estrogens, thereby overestimating the estrogenic potency of bisphenol A compared to studies in which comparisons are based on the half-maximal response.

Competitive binding assays, which evaluate the concentration at which bisphenol A displaces labeled 17β-estradiol from ER, are summarized in the top part of Table 52. The receptor binding of bisphenol A in these assays varies over 3 orders of magnitude. Bisphenol A competes for human ER binding at molar concentrations 20–10,000 times that of the native ligand. When bisphenol A binding to ERα and ERβ was compared in the same study, 3 reports found little difference by receptor subtype (Kuiper et al., 1998; Paris et al., 2002; Takayanagi et al., 2006), and 3 studies found binding to ERβ to be 4, 10, 47, and 254 times greater than binding to ERα (Routledge et al., 2000; Matthews et al., 2001; Seidlová-Wuttke et al., 2004, 2005; Takemura et al., 2005). Yeast reporter systems, which reflect activation of post-receptor pathways, show less variability; these studies show bisphenol A activity to be 10,000–26,000 times less than that of 17β-estradiol.

Some variability in estimating bisphenol A potency appears to be due to differences between laboratories. Andersen et al. (1999a) reported results from 3 laboratories that evaluated the proliferative response of MCF-7 breast cancer cells to bisphenol A. The laboratories, which were in the U.S., Spain, and Denmark, were sent samples of the same stock of bisphenol A, 17β-estradiol, and MCF-7 cells. Procedures were similar in the labs, although two different counting methods were used. The bisphenol A potencies relative to 17β-estradiol were 5×10^{-7}, 3×10^{-6}, and 1×10^{-5}. Laboratory variability may underlie some of the large differences in cell-based assays for ER activation; in those studies bisphenol A molar potency compared to 17β-estradiol were reported to vary by over 7 orders of magnitude (Table 52). Another explanation for this wide range of reported values is the

Table 52
In Vitro Estrogenicity Testing of Bisphenol A

Endpoint	Molar potency relative to 17β-estradiol	Reference
Binding assays		
Frog liver cytosol binding	[1.4×10^{-3}]	Lutz and Kloas (1999)
Carp liver cytosol binding	[1.3×10^{-3}]	Segner et al. (2003)
Rainbow trout ER binding	5.8×10^{-5}	Olsen et al. (2005)
Rainbow trout ER binding	2.1×10^{-3}	Matthews et al. (2000)
Anole ER binding	1.3×10^{-3}	Matthews et al. (2000)
Chicken ER binding	4.4×10^{-4}	Matthews et al. (2000)
Mouse ERα binding	8.6×10^{-5}	Matthews et al. (2000)
Mouse uterine cytosol binding	[1.2×10^{-4}]	Matthews et al. (2001)
Rabbit uterine ER binding	[1.3×10^{-5}]	Andersen et al. (1999a)
Rat uterine cytosol binding	$\sim 5 \times 10^{-4}$	Krishnan et al. (1993)
Rat uterine cytosol binding	8×10^{-5}	Blair et al. (2000)
Rat uterine cytosol binding	$1–2 \times 10^{-4}$	Kim et al. (2001a)
Rat ERα binding	[2.5×10^{-4}]	Strunck et al. (2000)
ER binding in rat lactotroph	$1–10 \times 10^{-5}$	Chun and Gorski (2000)
Rat ERα binding	5×10^{-4}	Kuiper et al. (1997)
Rat ERβ binding	3.3×10^{-4}	Kuiper et al. (1997)
Rat uterine ERα and β binding	6.2×10^{-5}	Washington et al. (2001)
Rat uterine Type II estrogen-binding site	4×10^{-3}	Washington et al. (2001)
ER binding in MCF-7 lysates	1×10^{-2}	Dodge et al. (1996)
Human ERα binding	4×10^{-4}	Bolger et al. (1998)
Human ERα binding	1×10^{-4}	Kuiper et al. (1998)
Human ERβ binding	1×10^{-4}	Kuiper et al. (1998)
Human ER binding	5.6×10^{-4}	Perez et al. (1998)
Human ER binding	[1.3×10^{-4}]	Andersen et al. (1999a)
ER binding in ECC-1 cells	3×10^{-3}	Bergeron et al. (1999)
Human ERα binding	8×10^{-5}	Matthews et al. (2000)
Human ERα binding	[2.5×10^{-3} diethylstilbestrol]	Nakagawa and Suzuki (2001)
Human ERα binding	7.3×10^{-4}	Routledge et al. (2000)
Human ERβ binding	7.5×10^{-3}	Routledge et al. (2000)
Human ER binding	[7.1×10^{-5}]	Sheeler et al. (2000)
Human ERα binding	[8×10^{-5}]	Matthews et al. (2001)
Human ERβ binding	[3.8×10^{-3}]	Matthews et al. (2001)
Human ERα binding	5×10^{-2}	Paris et al. (2002)
Human ERβ binding	4×10^{-2}	Paris et al. (2002)
Human ER binding	[3×10^{-4}]	Stroheker et al. (2004)
Human ERα binding	[2.4×10^{-4}]	Seidlová-Wuttke et al. (2005)
Human ERβ binding	[2.8×10^{-2}]	Seidlová-Wuttke et al. (2004)
Human ERα binding	[1.1×10^{-4}]	Takemura et al. (2005)
Human ERβ binding	[4.4×10^{-4}]	Takemura et al. (2005)
Human ER binding	3.15×10^{-3}	Olsen et al. (2005)
ERα binding	[9.4×10^{-4}]	Takayanagi et al. (2006)
ERβ binding	[9.6×10^{-4}]	Takayanagi et al. (2006)
Recombinant yeast reporter systems		
Human ER activation	5×10^{-5}	Coldham et al. (1997)
Human ER activation	6.7×10^{-5}	Gaido et al. (1997)
Human ER activation	[2.5×10^{-5}]	Harris et al. (1997)
Human ER activation	[$4–8 \times 10^{-5}$]	Andersen et al. (1999a)
Human ER activation	[3.9×10^{-5}]	Sheeler et al. (2000)
Human ER activation	$\sim 1 \times 10^{-4}$	Sohoni and Sumpter (1998)
Human ER activation	3.7×10^{-5}	Metcalfe et al. (2001)
ERα activation	6.2×10^{-5}	Silva et al. (2002)
ERα activation	[1×10^{-4}]	Nishihara et al. (2000)
ERα activation	[$\sim 1 \times 10^{-4}$]	Beresford et al. (2000)
Human ERα	[3.3×10^{-5}]	Rajapakse et al. (2001)
Human ERα, no microsomes	[5.5×10^{-5}]	Elsby et al. (2001)
Human ERα, human liver microsomes	[6.6×10^{-6}]	Elsby et al. (2001)
ER activation	$\sim 10^{-5}$	Chen et al. (2002)
Human ER activation	[8.1×10^{-5}]	Segner et al. (2003)
Human ER activation	9×10^{-5}	Li et al. (2004)
ERα activation	[4×10^{-5}]	Singleton et al. (2006)
Human ERα, with denatured rat S9	[2.4×10^{-6}]	Yoshihara et al. (2004)
Human ERα, with active rat S9	[9.2×10^{-6}]	Yoshihara et al. (2004)
Human ERα, with denatured mouse S9	[3.0×10^{-6}]	Yoshihara et al. (2004)
Human ERα, with active mouse S9	[7.8×10^{-6}]	Yoshihara et al. (2004)

Table 52
Continued

Endpoint	Molar potency relative to 17β-estradiol	Reference
Human ERα, with denatured monkey S9	[2.4×10^{-6}]	Yoshihara et al. (2004)
Human ERα, with active monkey S9	[6.0×10^{-6}]	Yoshihara et al. (2004)
Human ERα, with denatured human S9	[2.2×10^{-6}]	Yoshihara et al. (2004)
Human ERα, with active human S9	[4.6×10^{-6}]	Yoshihara et al. (2004)
Human ERα activity	[2.3×10^{-5}]	Terasaki et al. (2005)
Medaka ERα activity	[3.3×10^{-4}]	Terasaki et al. (2005)
"Estrogenic activity"	3.4×10^{-5}	Kawagoshi et al. (2003)
ERα activation	[2.3×10^{-4}]	Singleton et al. (2006)
Fish ERα activation	4.1×10^{-4}	Fu et al. (2007)
Fish ERβ2 activation	3.2×10^{-5}	Fu et al. (2007)
Other cell-based recombinant reporter systems		
ER activation in trout gonad cell line	5.4×10^{-3}	Ackerman et al. (2002)
Mouse ERα in HeLa cells	[$<1 \times 10^{-5}$]	Ranhotra and Teng (2005)
Mouse ERβ in HeLa cells	[$\sim 1 \times 10^{-2}$]	Ranhotra and Teng, 2005
HepG2 cells, human ERα	[3.0×10^{-3}]	Snyder et al. (2000)
HepG2 cells, human ERβ	[1.1×10^{-2}]	Snyder et al. (2000)
Rat ERα in HeLa cells	[1.6×10^{-7}]	Yamasaki et al. (2002b)
ER activation in HeLa cells	[8.8×10^{-4}]	Takahashi et al. (2004)
ERα activation in HeLa cells	[2.5×10^{-2}]	Hiroi et al. (1999)
ERβ activation in HeLa cells	[2.3×10^{-2}]	Hiroi et al. (1999)
ERα activation in HeLa cells	[6.1×10^{-1}]	Vivacqua et al. (2003)
ERβ activation in HeLa cells	[5.6×10^{-1}]	Vivacqua et al. (2003)
ERα activation in HeLa cells	[7.7×10^{-1}]	Recchia et al. (2004)
ERβ activation in HeLa cells	[1.2]	Recchia et al. (2004)
ERα activation in T47D cells	[$6.2–7.9 \times 10^{-1}$]	Recchia et al. (2004)
Proliferation in T47D cells	[6.6×10^{-1}]	Recchia et al. (2004)
Human ER in hepatoma cells	[3×10^{-2}]	Gould et al. (1998)
Human ERα, human embryonal kidney	[4.8×10^{-3}]	Kurosawa et al. (2002)
Human ERβ, human embryonal kidney	[4.6×10^{-3}]	Kurosawa et al. (2002)
Human ERα, endometrial carcinoma	[5.4×10^{-3}]	Kurosawa et al. (2002)
Human ERβ, endometrial carcinoma	[4.9×10^{-3}]	Kurosawa et al. (2002)
Human ERα, osteosarcoma	[7.3×10^{-3}]	Kurosawa et al. (2002)
Human ERβ, osteosarcoma	[7.7×10^{-3}]	Kurosawa et al. (2002)
Human ERα, human hepatoma cells	[2.7×10^{-1}]	Gaido et al. (2000)
Human ERβ, human hepatoma cells	[1.8×10^{-1}]	Gaido et al. (2000)
Human ERα, 239HEK cells	2×10^{-4} diethylstilbestrol	Lemmen et al. (2004)
Human ERβ, 239HEK cells	7×10^{-4} diethylstilbestrol	Lemmen et al. (2004)
Human ERα, endometrial carcinoma	[6.1×10^{-3}]	Singleton et al. (2006)
MCF-7 cells		
G6PD activity	[1×10^{-1}]	Kim et al. (2003a)
Expression of proteins	[1×10^{-3}]	Perez et al. (1998)
Progesterone receptor mRNA	Not increased at 10^{-6} M[a]	Diel et al. (2002)
Androgen receptor mRNA	Not decreased at 10^{-6} M[a]	Diel et al. (2002)
Progesterone receptor	$\sim 2 \times 10^{-4}$	Krishnan et al. (1993)
ER binding, serum-free	3.3×10^{-4}	Samuelsen et al. (2001)
ER binding, 100% human serum	1.7×10^{-4}	Samuelsen et al. (2001)
ER binding	3.2×10^{-3}	Olsen et al. (2003)
ER activation	[1.4×10^{-5}]	Kitamura et al. (2005)
*ER*α expression	[7.5×10^{-5}]	Matthews et al. (2001)
*ER*β expression	[1.8×10^{-4}]	Matthews et al. (2001)
ERα activation	[$4.7–6.9 \times 10^{-1}$]	Vivacqua et al. (2003)
ERα activation	[$5.5–6.7 \times 10^{-1}$]	Recchia et al. (2004)
pS2 induction	[1.8×10^{-6}]	Leffers et al. (2001)
ER production	[7×10^{-8}]	Olsen et al. (2003)
Progesterone receptor production	[6.8×10^{-8}]	Olsen et al. (2003)
pS2 production	[10^{-7}]	Olsen et al. (2003)
pS2 mRNA	[1.1]	Vivacqua et al. (2003)
pS2 mRNA	[8.9×10^{-1}]	Recchia et al. (2004)
Cathepsin D mRNA	[8.2×10^{-1}]	Recchia et al. (2004)
Transcription of human telomerase reverse transcriptase	[$\sim 10^{-2}$]	Takahashi et al. (2004)
Proliferation	[3.8×10^{-4}]	Krishnan et al. (1993)
Proliferation	1×10^{-3}	Brotons et al. (1995)
Proliferation	1×10^{-4}	Soto et al. (1997)
Proliferation	[$\sim 1 \times 10^{-3}$]	Dodge et al. (1996)
Proliferation	[1×10^{-4}]	Perez et al. (1998)

Table 52
Continued

Endpoint	Molar potency relative to 17β-estradiol	Reference
Proliferation	$[9.8 \times 10^{-4}]$	Schafer et al. (1999)
Proliferation (3 different laboratories)	$5–100 \times 10^{-7}$	Andersen et al. (1999a)
Proliferation	6×10^{-5}	Körner et al. (2000)
Proliferation	3×10^{-5}	Kim et al. (2001a)
Proliferation	$[2.5 \times 10^{-6}]$	Suzuki et al. (2001)
Proliferation	2×10^{-5}	Samuelsen et al. (2001)
Proliferation	$[9.2 \times 10^{-4}]$	Nakagawa and Suzuki (2001)
Proliferation	$[\sim 1 \times 10^{-3}]$	Shimizu et al. (2002)
Proliferation	$[7 \times 10^{-9}]$	Diel et al. (2002)
Proliferation	1.6×10^{-5}	Olsen et al. (2003)
Proliferation	$[4.5–5 \times 10^{-1}]$	Vivacqua et al. (2003)
Proliferation	$[1.1 \times 10^{-4}]$	Stroheker et al. (2004)
Proliferation	$[6 \times 10^{-1}]$	Recchia et al. (2004)
Proliferation	2×10^{-5}	Olsen et al. (2005)
Proliferation, with denatured rat S9	$[6.5 \times 10^{-5}]$	Yoshihara et al (2001)
Proliferation, with active rat S9	$[3.4 \times 10^{-4}]$	Yoshihara et al (2001)
Rat pituitary cells		
Proliferation	$1–10 \times 10^{-6}$	Chun and Gorski (2000)
Proliferation	$[\sim 8.4 \times 10^{-3}]$	Steinmetz et al. (1997)
Prolactin release	1×10^{-5}	Chun and Gorski (2000)
Prolactin release (GH_3 cell)	$[6 \times 10^{-3}]$	Steinmetz et al. (1997)
Prolactin release (F344 pituitary)	$2–10 \times 10^{-4}$	Steinmetz et al. (1997)
Prolactin gene expression	$[\sim 1 \times 10^{-3}]$	Steinmetz et al. (1997)
Rat uterine adenocarcinoma cells		
Induction of complement C3 mRNA	$[8 \times 10^{-3}]$	Strunck et al. (2000)
Human uterine adenocarcinoma cells		
Progesterone receptor mRNA/protein	$[\sim 1 \times 10^{-2}]$	Bergeron et al. (1999)
Proliferation	No effect at 10^{-5} M	Bergeron et al. (1999)
Vitellogenin production, fish hepatocytes		
Carp	1×10^{-4}	Smeets et al. (1999)
Carp	$[3.1 \times 10^{-3}]$	Segner et al. (2003)
Carp	$[1 \times 10^{-5}]$	Letcher et al. (2005)
Carp	$[3 \times 10^{-4}]$	Rankouhi et al. (2002)
Trout	2×10^{-5}	Shilling and Williams (2000)
Trout	$[8 \times 10^{-4}]$	Segner et al. (2003)
Trout	2.9×10^{-5}	Olsen et al. (2005)
Frog hepatocytes		
Vitellogenin mRNA expression	$[\sim 1 \times 10^{-3}]$	Kloas et al. (1999)
Vitellogenin production	No effect at 100 μM	Rankouhi et al. (2004)
ER mRNA expression	$\sim 10^{-2}$	Lutz et al. (2005)

[a]Progesterone receptor was increased and androgen receptor was decreased by 17β-estradiol 10^{-10} M.

difference in defining relative potency in some assays, as discussed above. [According to a study author, the wide variability in relative bisphenol A potency was due to a wide fluctuation in the 17β-estradiol dose at which half-maximal proliferation was achieved (0.1–70 pM) (A. Soto, personal communication, March 2, 2007).]

A study using ERα- and ERβ-reporting systems in 3 human cell lines found that bisphenol A had a small antagonistic effect on ERα activation in the presence of 17β-estradiol in human embryonal kidney and endometrial carcinoma cells (Kurosawa et al., 2002). There were no significant interactions between bisphenol A and 17β-estradiol on ERα activation in human osteosarcoma cells or on ERβ activation in any tested cell type. By contrast, a study using a recombinant yeast assay for ERα activation found 17β-estradiol and bisphenol A to have additive effects (Rajapakse et al., 2001), and a study using MCF-7 cell proliferation found 17β-estradiol and bisphenol A to have synergistic effects (Suzuki et al., 2001).

The data in Table 52 are applicable only to unconjugated bisphenol A. Estrogenic activity has not been identified for bisphenol A glucuronide (Matthews et al., 2001) or sulfate (Shimizu et al., 2002).

In vivo tests (Table 53) have been conducted principally in rats and mice. Most endpoints in these studies involved the uterus, and effects on uterine weight in immature or ovariectomized animals are the uterine endpoints reported most commonly. The potency of bisphenol A in increasing uterine weight varies over ~4 orders of magnitude. Some of this variation may be related to the short half-life of bisphenol A. Uterotrophic evaluations are typically performed 24 hr after the last dose of the test agent is administered. Laws et al. (2000) showed no significant effect of bisphenol A at doses ≤400 mg/kg bw/day given orally on uterine wet weight assessed 24 hr after administering the last dose. When assessed 6 hr after the last oral dose, bisphenol A 200 mg/kg bw/day increased uterine wet weight to ~2.5 times the control **[estimated from a graph]**, which was about the same as the increase produced by administering 17β-estradiol 0.005 mg/kg bw/day sc. Increase in uterine weight in the first 6 hr after treatment

Table 53
In Vivo Estrogenicity Tests of Bisphenol A

Model and exposure	Husbandry[a]	Endpoint	Molar potency/comparator[b]	Reference
Rat uterus				
Adult ovariectomized Sprague–Dawley, gavage × 4 days	TD89222 diet, metal cage	Uterine wet weight	**[3.9 × 10^{-3}]**/ethinyl estradiol	Dodge et al. (1996)
Immature Sprague–Dawley, bisphenol A given "orally" × 3 days; 17β-estradiol i.p. × 3 days	Not indicated	Uterine weight	Not affected by bisphenol A at up to 150 mg/kg bw/day; 17β-estradiol was positive at 0.005 mg/day **[∼0.089 mg/kg bw/day]**	Gould et al. (1998)
		Progesterone receptor	**[5.9 × 10^{-3}]**/17β-estradiol	
		Peroxidase activity	**[7.6 × 10^{-3}]**/17β-estradiol	
Adult ovariectomized Crl:CD BR, gavage × 4 days	Purina 5002 diet, steel cage	Uterine weight	**[3.5 × 10^{-5}]**/17β-estradiol	Cook et al. (1997)
		Stromal cell proliferation	**[4.1 × 10^{-5}]**/17β-estradiol	
Adult ovariectomized F344, i.p. × 1	Not indicated	*cfos* expression	**[2.1 × 10^{-4}]**/17β-estradiol	Steinmetz et al. (1998)
Adult ovariectomized F344 or Sprague–Dawley, silastic implant × 3 days	Not indicated	Uterine wet weight:		Steinmetz et al. (1998)
		F344	**[8.2 × 10^{-3}]**/17β-estradiol	
		Sprague–Dawley	**[6.0 × 10^{-3}]**/17β-estradiol	
		Uterine cell height:		
		F344	**[1.1 × 10^{-2}]**/17β-estradiol	
		Sprague–Dawley	**[9.2 × 10^{-3}]**/17β-estradiol	
Juvenile ovariectomized DA/Han, Wistar, or Sprague–Dawley, gavage × 3 days	Not indicated	Uterine wet weight:		Diel et al. (2004)
		DA/Han	**[1.8 × 10^{-5}]**/ethinyl estradiol	
		Wistar	No response to 200 mg/kg/d	
		Sprague–Dawley	**[1.7 × 10^{-5}]**/ethinyl estradiol	
		Uterine epithelium	No response to 200 mg/kg/day	
		Vaginal epithelium	No response to 200 mg/kg/day	
		Clusterin mRNA	No response to 200 mg/kg/day	
Immature Alpk:AP, s.c. × 3 days	RM3 diet, wire cage	Uterine wet weight	**[2.6–2.7 × 10^{-5}]**/diethylstilbestrol	Ashby and Tinwell (1998)
		Uterine dry weight	**[2.5–3.0 × 10^{-5}]**/diethylstilbestrol	
Immature Alpk:AP, gavage × 3 days	RM3 diet, wire cage	Uterine wet weight	**[2.3–3.1 × 10^{-5}]**/diethylstilbestrol	
		Uterine dry weight	**[2.7–3.6 × 10^{-5}]**/diethylstilbestrol	
Immature Long–Evans, gavage × 3 days	Purina 5001 diet	Uterine wet weight 6 hr after dosing	**[1.4 × 10^{-5}]**/17β-estradiol	Laws et al. (2000)
		Uterine wet weight 24 hr after dosing	No effect at bisphenol A at ≤400 mg/kg bw/day	
Adult ovariectomized Long–Evans	Purina 5001 diet	Uterine wet weight	No effect of bisphenol A at ≤100 mg/kg bw/day	Laws et al. (2000)
Juvenile ovariectomized DA/Han, gavage × 3 days	Ssniff R-10 diet	Uterine wet weight relative to bw	**[1.2 × 10^{-5}]**/ethinyl estradiol	Diel et al. (2000)
		Expression of:		
		Androgen receptor	**[3.9 × 10^{-4}]**/ethinyl estradiol	
		ER	**[1.9 × 10^{-4}]**/ethinyl estradiol	
		Progesterone receptor	bisphenol A and ethinyl estradiol produced opposite effects	
		Complement C3	**[2.2 × 10^{-5}]**/ethinyl estradiol	
		Clusterine	No bisphenol A effect at 200 mg/kg bw/day; ethinyl estradiol showed an effect at 0.1 mg/kg bw/day	
		Glyceraldehyde phosphate dehydrogenase		

Table 53
Continued

Model and exposure	Husbandry[a]	Endpoint	Molar potency/comparator[b]	Reference
Adult ovariectomized Alpk:ApfSD, s.c. × 3 days	Not indicated	Uterine wet weight	**[1.7 × 10^{-4}]**/17β-estradiol	Ashby et al. (2000)
		Uterine dry weight	**[1.8 × 10^{-4}]**/17β-estradiol	
Immature Crj:CD (SD), s.c. × 3 days	MF diet, steel cage	Wet and blotted uterine weight	Effect noted at ≥8 mg/kg bw/day bisphenol A/no comparator	Yamasaki et al. (2000)
Immature Crj:CD (SD), gavage × 3 days	MF diet, steel cage	Wet and blotted uterine weight	Effect noted at ≥160 mg/kg bw/day bisphenol A/no comparator	
Adult ovariectomized Wistar, s.c. × 7 days	Not indicated	Blotted uterine weight	Increased relative weight compared to placebo at ≥11 mg/kg bw/day; uterus reached 83% of weight of sham-ovariectomized control at bisphenol A dose of 250 mg/kg bw/day.	Goloubkova et al. (2000)
Adult ovariectomized Sprague–Dawley, exposed in drinking water × 3 days	Glass water bottles, plastic cage (negative E-Screen of ethanol cage washes)	Uterine wet weight	No effect of bisphenol A at up to 16.9 mg/kg bw/day; estrone positive at 0.12 mg/kg bw/day	Rubin et al. (2001)
Adult ovariectomized Sprague–Dawley, s.c. × 3 days	PMI Certified Rodent Diet, polycarbonate cage, elm bedding	Uterine wet weight	**[1.7 × 10^{-6}]**/17β-estradiol	Kim et al. (2001a)
		Uterine dry weight	**[2.3 × 10^{-6}]**/17β-estradiol	
Immature Alpk:ApfSD, s.c. × 3 days	RM1 diet	Uterine wet weight	**[2.9 × 10^{-4}]**/17β-estradiol	Matthews et al. (2001)
		Uterine dry weight	No effect of bisphenol A at 800 mg/kg bw/day; 17β-estradiol positive at 0.4 mg/kg bw/day	
Immature Alpk:ApfSD, gavage × 3 days	RM1 diet	Uterine wet weight	**[2.3–5.5 × 10^{-4}]**/17β-estradiol	
		Uterine dry weight	**[2.4–7.1 × 10^{-4}]**/17β-estradiol	
Immature Sprague–Dawley, s.c. × 3 days	Soy-free diet, polycarbonate cage	Uterine wet weight	No effect of bisphenol A at ≤1000 mg/kg bw/day; 17β-estradiol was positive at 0.04 mg/kg bw/day	An et al. (2002)
		Calbindin D_{9k} expression	**[8.4 × 10^{-6}]**/17β-estradiol	
		ERα expression	**[3.4 × 10^{-5}]**/17β-estradiol	
Immature Crj:CD (SD), s.c. × 3 days	MF diet, steel cage	Uterine wet weight	**[5.1 × 10^{-5}]**/ethinyl estradiol	Yamasaki et al. (2002b)
Immature Sprague–Dawley, s.c. × 3 days	Soy-free diet, polycarbonate cage, corncob bedding	Blotted uterine weight	**[8 × 10^{-7}]**/ethinyl estradiol	Wade et al. (2003)
		Epithelial cell height	**[1.2 × 10^{-6}]**/ethinyl estradiol	
Pubertal Sprague–Dawley, gavage PND 22–42/43	Purina 5002 diet, polycarbonate cage, chip bedding	Blotted uterine weight	Absolute organ weight decreased with increase dose (400 and 600 mg/kg bw/day); no effect on relative organ weight	George et al. (2003)
		Vaginal opening	No effect at 400 and 600 mg/kg bw/day	
Pregnant Sprague–Dawley, s.c. bisphenol A on GD 17–19 (17β-estradiol s.c. × 1)	Soy-free diet, polycarbonate cage	Maternal uterine weight	**[1.8 × 10^{-5}]**/17β-estradiol	Hong et al. (2003)
Pregnant Sprague–Dawley, s.c. bisphenol A on GD 17–19 (17β-estradiol s.c. × 1)	Soy-free diet, polycarbonate cage	Maternal uterine calbindin D_{9k} protein	**[1.7 × 10^{-5}]**/17β-estradiol	Hong et al. (2003)
Lactating Sprague–Dawley, s.c. bisphenol A × 5 days (17β-estradiol s.c. × 1)	Soy-free diet	Maternal uterine calbindin D_{9k} mRNA calbindin D_{9k} protein	**[2.2 × 10^{-5}]**/17β-estradiol **[6.9 × 10^{-5}]**/17β-estradiol	Hong et al. (2004)

Table 53
Continued

Model and exposure	Husbandry[a]	Endpoint	Molar potency/comparator[b]	Reference
Immature and adult ovariectomized Wistar, gavage × 4 days	AO4C diet, wire cage	Uterine wet and dry weight	No effect in either model of bisphenol A at ≤200 mg/kg bw/day/17β-estradiol positive at 0.025–0.035 mg/kg bw/day	Stroheker et al. (2003)
Immature Sprague–Dawley, s.c. × 3 days	Soy-free feed, polycarbonate cage	Calbindin D_{9k} protein	**[5.1 × 10^{-5}]**/17β-estradiol	An et al. (2003)
Immature Sprague–Dawley, s.c. × 3 days	Shinchon diet	Uterine wet weight	**[1.5 × 10^{-6}]**/17β-estradiol	Kim et al. (2003a)
		Uterine wet weight relative to bw	**[1.3 × 10^{-6}]**/17β-estradiol	
		Glutathione peroxidase activity	**[4.2 × 10^{-3}]**/17β-estradiol	
Immature Alpk:ApfSD, gavage × 3 days	RM1 diet, polycarbonate cage	Blotted uterine weight	**[2.5 × 10^{-4}]**/17β-estradiol	Ashby and Odum (2004)
		Expression of:		
		Progesterone receptor A	**[3.8 × 10^{-4}]**/17β-estradiol	
		Progesterone receptor B	**[4.2 × 10^{-4}]**/17β-estradiol	
		Complement C3	**[1.8 × 10^{-4}]**/17β-estradiol	
		Lipocalcin	**[2.3 × 10^{-4}]**/17β-estradiol	
Immature AP, s.c. × 3 days	RM1 diet, polypropylene cages, sawdust and shredded paper bedding	Uterine wet weight	**[1.0 × 10^{-6}]**/ethinyl estradiol	Tinwell and Ashby (2004)
		Uterine dry weight	**[1.2 × 10^{-6}]**/ethinyl estradiol	
Adult ovariectomized Sprague–Dawley, diet × 3 months	Phytoestrogen-free diet	Uterine weight, endometrial thickness, *ERα*, *ERβ* expression	No bisphenol A effect at 0.37 mg/kg bw/day; estradiol benzoate positive control	Seidlová-Wuttke et al. (2004)
		Complement C3 expression	Bisphenol A and estradiol benzoate produced opposite effects	
Immature Sprague–Dawley, s.c. × 3 days	PMI Certified Rodent Diet	Uterine wet weight	**[4.5 × 10^{-7}]**/ethinyl estradiol	Kim et al. (2005)
		Uterine dry weight	**[4.9 × 10^{-7}]**/ethinyl estradiol	
Adult ovariectomized Crj:CD (SD), s.c. × 3 days	Estrogen-free NIH-07PLD diet, aluminum cage, paper bedding	Uterine wet weight, relative to bw	**[2.1 × 10^{-5}]**/17β-estradiol	Koda et al. (2005)
		Blotted uterine weight, relative to bw	**[1.7 × 10^{-6}]**/17β-estradiol	
Adult Holzman, progesterone-treated to delay implantation, given test agent s.c. on GD 7	Unspecified Purina rodent chow, plastic cage, pine shavings	Implantation	**[4–34 × 10^{-6}]**/estrone	Cummings and Laws (2000)
Rat vagina				
Adult ovariectomized F344, i.p. × 1	Not indicated	BrdU labeling	Increased at bisphenol A dose of 37.5 but not 18.5 mg/kg bw/no comparator	Steinmetz et al. (1998)
		cfos expression	**[1.3 × 10^{-4}]**/17β-estradiol	
Adult ovariectomized Long Evans, bisphenol A by gavage × 11 days; 17β-estradiol by s.c.	Purina 5001 diet	Vaginal cytology	No effect at bisphenol A dose of 100 mg/kg bw/day; 17β-estradiol 0.005 mg/kg bw/day resulted in persistent estrus	Laws et al. (2000)
Long–Evans treated PND 21–35 by gavage	Purina 5001 diet	Vaginal opening	No effect at bisphenol A dose ≤400 mg/kg bw/day; ethinyl estradiol was active at 0.01 mg/kg bw/day	Laws et al. (2000)
Adult ovariectomized F344 and Sprague–Dawley, i.p. × 1	Not indicated	BrdU labeling	F344: **[4.5 × 10^{-6}]**/17β-estradiol Sprague–Dawley: **[1.4 × 10^{-6}]**/17β-estradiol	Long et al. (2000)

Table 53
Continued

Model and exposure	Husbandry[a]	Endpoint	Molar potency/comparator[b]	Reference
Immature Wistar, gavage × 4 days	AO4C diet, wire cage	Vaginal cornification	**[3.8 × 10^{-4}]**/17β-estradiol	
Adult ovariectomized Wistar, gavage × 4 days	AO4C diet, wire cage	Vaginal cornification	No effect at bisphenol A dose of 100 mg/kg bw/day; 17β-estradiol was positive at 0.1 mg/kg bw/day	Stroheker et al. (2003)
Immature Sprague–Dawley, s.c. × 3 days	PMI Certified Rodent Diet	Vaginal weight	**[5.3 × 10^{-7}]**/ethinyl estradiol	Kim et al. (2005)
Other rat organs				
Ovariectomized Sprague–Dawley, daily gavage for 5 weeks	TD89222 diet, metal cage	Prevention of bone mineral density decline	No effect at bisphenol A dose up to 10 mg/kg bw/day; no standard estrogen comparator	Dodge et al. (1996)
Adult ovariectomized Sprague–Dawley, treated in feed	Phytoestrogen-free diet	Prevention of bone mineral density decline	No effect at bisphenol A dose ≤ 370 μg/kg bw/day; estradiol benzoate was effective at 1.18 mg/kg bw/day	Seidlová-Wuttke et al. (2004)
Adult ovariectomized Sprague–Dawley and F344, by s.c. implant × 3 days	Not indicated	Serum prolactin	F344: **[1.7 × 10^{-2}]**/17β-estradiol Sprague Dawley: no effect of bisphenol A at 40–45 μg/day or 17β-estradiol at 1.2–1.5 μg/day	Steinmetz et al. (1997)
Adult ovariectomized Wistar, s.c. × 7 days	Not indicated	Pituitary weight	Increased compared to vehicle control at 128 but not 78 mg/kg bw/day	Goloubkova et al. (2000)
		Serum prolactin	Increased compared to vehicle control at 128 mg/kg bw/day	
Mouse uterus				
Immature CFLP, s.c. × 3 days	Not indicated	Relative uterine weight	No response at up to 0.5 mg **[∼50 mg/kg bw/day]**	Coldham et al. (1997)
Adult ovariectomized CD-1, s.c. × 1	Not indicated	*IGF1* expression	**[8.4 × 10^{-4}]**/17β-estradiol	Klotz et al. (2000)
Juvenile-adult ovariectomized B6C3F$_1$, s.c. × 4 days	Purina 5001, polypropylene cage, chip bedding	Uterine wet weight	**[2.3 × 10^{-5}]**/17β-estradiol	
Papaconstantinou et al. (2000)				
		Endothelial proliferation	**[6.9 × 10^{-6}]**/17β-estradiol	
Juvenile-adult ovariectomized B6C3F$_1$, s.c. × 4 days	Purina 5001, polypropylene cage, cellulose fiber bedding	Induction of grp94	**[2.4 × 10^{-5}]**/17β-estradiol	
Papaconstantinou et al. (2001)				
		Induction of hsp72	**[3.5 × 10^{-6}]**/17β-estradiol	
		Induction of hsp90	**[5.3 × 10^{-6}]**/17β-estradiol	
Juvenile-adult ovariectomized B6C3F$_1$, s.c. × 4 days	Purina 5001, polypropylene cage, cellulose fiber bedding	Uterine weight	**[5.3 × 10^{-6}]**/17β-estradiol	
Papaconstantinou et al. (2002)				
		Induction of hsp90α	**[1.2 × 10^{-5}]**/17β-estradiol	
		Induction of grp24	**[8.4 × 10^{-6}]**/17β-estradiol	
Juvenile-adult ovariectomized B6C3F$_1$, s.c. × 1	Purina 5001, polypropylene cage, cellulose fiber bedding	Blotted uterine weight, 6 hr after dose	**[8.4 × 10^{-6}]**/17β-estradiol	
Papaconstantinou et al. (2003)				
		Blotted uterine weight, 12 hr after dose	**[4.2 × 10^{-6}]**/17β-estradiol	
Adult ovariectomized transgenic ER-reporter, s.c. × 1	Purina 5001, polystyrene cage	Uterine wet weight	**[2.9 × 10^{-5}]**/diethylstilbestrol	Nagel et al. (2001)
		ER activation	**[1.0 × 10^{-4}]**/diethylstilbestrol	Nagel et al. (2001)

Table 53
Continued

Model and exposure	Husbandry[a]	Endpoint	Molar potency/comparator[b]	Reference
Immature AP, s.c. × 3 days	RM1 diet, plastic cage, sawdust and shredded paper bedding	Blotted uterine weight	**[2.3 × 10^{-5}]**/diethylstilbestrol in 4 of 8 trials; other trials showed no effect at bisphenol doses up to 300 mg/kg bw/day	Tinwell and Joiner (2000)
Immature AP, gavage × 3 days	RM1 diet, plastic cage, sawdust and shredded paper bedding	Blotted uterine weight	No effect at bisphenol A doses up to 300 mg/kg bw/day; diethylstilbestrol produced response at 10 μg/kg bw/day	Tinwell and Joiner (2000)
Immature CD-1, s.c. × 3 days	RM1 diet	Lactoferrin expression	No effect at bisphenol A doses up to 1000 mg/kg bw/day; diethylstilbestrol showed effect at 0.1 μg/kg bw/day	Mehmood et al. (2000)
		Uterine weight, BrdU incorporation, peroxidase production	No effect at bisphenol A doses up to 100 mg/kg bw/day; diethylstilbestrol showed effect at 1–5 μg/kg bw/day	
Immature CD-1, s.c. minipump × 3 days	RMH 3000 diet, cage, and bedding estrogen-negative by E-Screen	Uterine wet weight	**[1.6 × 10^{-5}]**/17β-estradiol	Markey et al. (2001b)
		Epithelial cell height	**[3.8 × 10^{-5}]**/17β-estradiol	
		Lactoferrin expression	**[3.9 × 10^{-5}]**/17β-estradiol	
Ovariectomized adult B6C3F$_1$, i.p. × 3 days	Not indicated	Relative uterine to body weight	**[3.6–74 × 10^{-5}]**/17β-estradiol	Kitamura et al. (2005)
Ovariectomized adult Swiss, s.c. × 1	Economy Rodent Maintenance diet	Increased uterine vascular permeability	~1 × 10^{-4}/17β-estradiol	Milligan et al. (1998)
Other mouse organs				
Juvenile-adult aromatase knock-out, diet × 4 months	NMF diet	Uterine and ovarian histology, bone mineral density	Dietary bisphenol A (0.1%) exerted estrogenic effects. Mean ± SD serum bisphenol A 84.3 ± 8.7 μg/L. No comparator estrogen was used for these endpoints	Toda et al. (2002)
Fish				
Immature rainbow trout, injected		Plasma vitellogenin	**[3 × 10^{-4}]**/17β-estradiol	Christiansen et al. (1997)
Juvenile rainbow trout, injected		Plasma vitellogenin	**[5.6 × 10^{-3}]**/17β-estradiol	Andersen et al. (1999a)
Juvenile rainbow trout, exposed in water		Plasma vitellogenin	**[~8.4 × 10^{-5}]**/17β-estradiol	Lindholst et al. (2000)
Male medaka, exposed in feed		Plasma vitellogenin	**[1.4 × 10^{-4}]**/ethinyl estradiol	Chikae et al. (2003)
Male medaka, exposed in water		Hepatic vitellogenin and ERα mRNA	**[8.4 × 10^{-6}]**/17β-estradiol	Yamaguchi et al. (2005)
Male killfish, injected		Plasma vitellogenin	**[2.7 × 10^{-4}]**/17β-estradiol	Pait and Nelson (2003)
Male zebrafish, juvenile rainbow trout, exposed in water		Plasma vitellogenin	**[~0.2]**/ethinyl estradiol	Van den Belt et al.(2003)
Invertebrates				
Mud snail, exposed in water		New embryo production	**[1.5 × 10^{-4}]**/ethinyl estradiol	Jobling et al. (2004)
Ramshorn snail, exposed in water		Egg production	Increased (EC$_{10}$ 13.9 ng/L); blocked by Faslodex and tamoxifen. No comparison to reference estrogen	Oehlmann et al. (2006)

[a]Husbandry information for rodent studies includes caging and bedding materials and diet when indicated by the authors.
[b]Estimates include comparison of administered dose, magnitude of effect, and molecular weight.

represents fluid inbibition and not true tissue growth. A dose-related decrease in blotted uterine weight and body weight, with no effect on weight-adjusted uterine weight, was shown in pubertal rats treated on PND 22–42/43 with bisphenol A by gavage at 400 or 600 mg/kg bw/day (George et al., 2003).

For studies showing an increase in uterine weight after bisphenol A treatment, dose route affects response;

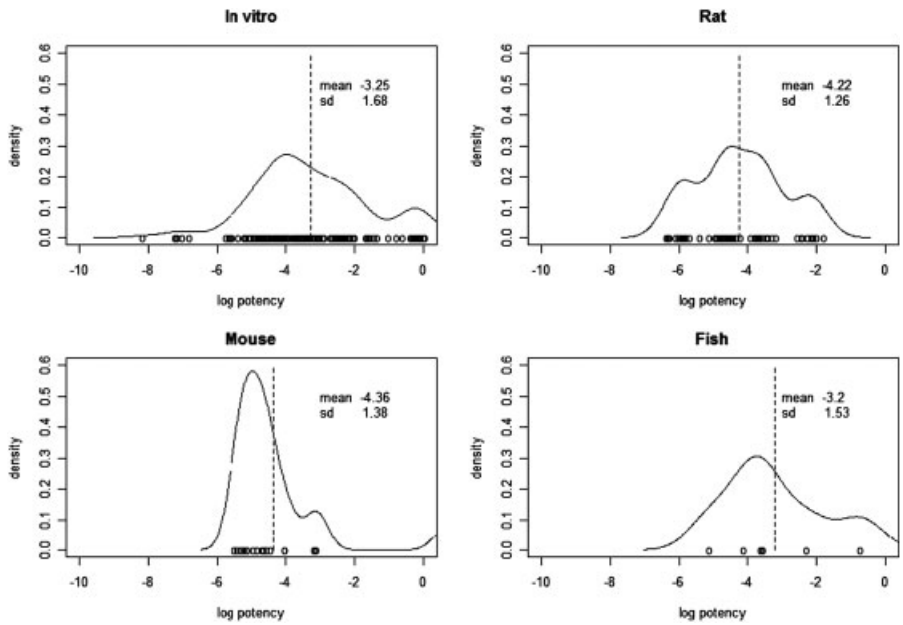

Fig. 2. In vitro estrogenic potency ($_{log10}$) in ER α and β binding and transcriptional assays and estrogen-dependent cell proliferation assays) distributions of bisphenol A and estrogen responses in vivo in rats, mice and fish. Each data point represents one bisphenol A study in which bisphenol A was compared to a reference estrogen in rats, mice, fish, or in vitro. Data summarized from Table 52 and Table 53, midrange values used when a range is given in the table.

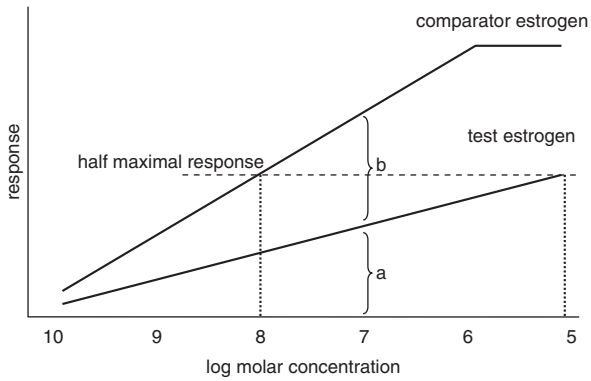

Fig. 3. Alternative approaches to comparing estrogenic potency. In this example, the half-maximal response to the comparator estrogen occurs at 10^{-8} M. A similar response occurs with the test estrogen at 10^{-5} M, suggesting a 1000-fold difference in potency. If the magnitudes of response at equimolar concentrations are compared, the apparent potency may be much different. The response to the test estrogen at 10^{-7} M (a) is about half the response to the comparator estrogen at 10^{-7} M (a+b).

bisphenol A given by gavage increased uterine weight by approximately 25% while the same dose given s.c. increased uterine weight by approximately 170% (Laws et al., 2000). A greater response by the s.c. than oral route was also shown by Yamasaki et al. (2000) and Kanno et al. (2003b) in the OECD multilaboratory study who showed a lowest effective bisphenol A dose of 8 mg/kg bw/day by the s.c. route and 160 mg/kg bw/day by the oral route. The greater activity per unit dose of s.c. than oral bisphenol A is due presumably to glucuronidation of the orally administered compound with consequent loss of estrogenicity (Matthews et al., 2001). A few studies could not confirm the greater effect of s.c. compared to oral bisphenol A on uterine weight. Ashby and Tinwell (1998) concluded that the magnitude of uterine weight response was similar for s.c. and oral routes. **[The Expert Panel notes a greater numerical magnitude of response after s.c. than oral exposure in most of the experiments reviewed in this report, and that statistical comparison of the dose routes was not reported.]** Matthews et al. (2001) found a similar increase in uterine weight in rats given s.c. or oral bisphenol A at 800 mg/kg bw/day.

Nagel et al. ([1997, 1999) noted that 17β-estradiol is extensively protein-bound in vivo and bisphenol A is minimally protein-bound. A recent study indicated more extensive binding of bisphenol A to plasma binding proteins (Teeguarden et al., 2005). Nagel suggested that estrogenicity of BPA (as well as other steroid hormones) can be predicted more accurately in rats by considering the free fraction of a chemical in human serum. **[The Expert Panel notes that** Figure 2 **does not suggest that bisphenol A is more potent than 17β-estradiol in vivo than in vitro. The developmental effects of bisphenol A in the prostate are discussed in Section 3.2.]**

Inter-strain variability in rats has been evaluated as a source of variability in estrogenicity assays. Inspection of Table 53 does not suggest large sensitivity differences between Sprague–Dawley, Wistar, and Long-Evans rats. Greater sensitivity of F344 than Sprague–Dawley rats has been shown with respect to uterine weight and epithelial cell height (Steinmetz et al., 1998), where 17β-estradiol-adjusted potencies differed by 20–37% between the strains. BrDu labeling of vaginal epithelium was 3 times greater in F344 than Sprague–Dawley rats in another study (Long et al., 2000), and a third study (Steinmetz et al., 1997) showed that both bisphenol A and 17β-estradiol increase serum prolactin in ovariectomized F344 but not ovariectomized Sprague–Dawley rats. Diel et al. (2004) evaluated estrogenic response to bisphenol A

Table 54
Differences Between Laboratories in Rat Uterotrophic Assay With Bisphenol A[a]

Laboratory	Rat strain	Lowest effective dose level (mg/kg bw/day)				
Immature, gavage × 3 days		200	375	600	1000	
2	CD(SD)IGS		×←			
7	CD(SD)IGS			×←		
12	CD(SD)IGS BR		×←			
13	Wistar				×←	
Immature, s.c. × 3 days		10	100	300	600	1000
2	CD(SD)IGS		×←			
6	CD(SD)IGS BR			×←		
7	CD(SD)IGS		×←			
8	Alpk:ApfSD		×←			
12	CD(SD)IGS BR				×←	
13	Wistar			×←		
15	Wistar			×←		
18	Sprague–Dawley	×←				
20	Sprague–Dawley	×←				
21	CD(SD) BR	×←				
Adult, s.c. × 3 days		10	100	300	600	1000
2	CD(SD)IGS		×←			
6	CD(SD)IGS BR		×←			
7	CD(SD)IGS		×←			
8	Alpk:ApfSD		×←			
12	CD(SD)IGS BR		×←			
Adult, s.c. × 7 days		10	100	300	600	1000
2	CD(SD)IGS		×←			
7	CD(SD)IGS		×←			

[a]Kanno et al. (2003b).

in juvenile ovariectomized DA/Han, Sprague–Dawley, and Wistar rats. After 3 days of treatment with bisphenol A 200 mg/kg bw/day, there were small statistically significant increases in uterine weight in DA/Han and Sprague–Dawley rats but not in Wistar rats. There were no alterations in uterine or vaginal epithelium or in uterine clusterin mRNA expression in any of the strains after bisphenol A treatment.

Inter-laboratory variation in the uterotrophic assay was evaluated by the Organization for Economic Cooperation and Development (OECD) (Kanno et al., 2003b). Coded chemicals, including bisphenol A, were sent to up to 212 different laboratories. Four assay protocols were evaluated including oral treatment of intact immature rats for 3 days, s.c. treatment of intact immature rats for 3 days, s.c. treatment of ovariectomized 6–8-week-old rats for 3 days, and s.c. treatment of ovariectomized 6–8-week-old rats for 7 days. Not all laboratories used all protocols or tested all compounds. Rat strains and suppliers were not standardized across laboratories. Comparisons were made between labs based on the lowest dose level at which body weight-adjusted blotted uterine weight was significantly different from the control. Results are summarized in Table 54. The lowest effective dose of bisphenol A was uniformly identified for the assays performed in ovariectomized adults. Assays performed in immature animals varied in identification of the lowest effective bisphenol A dose

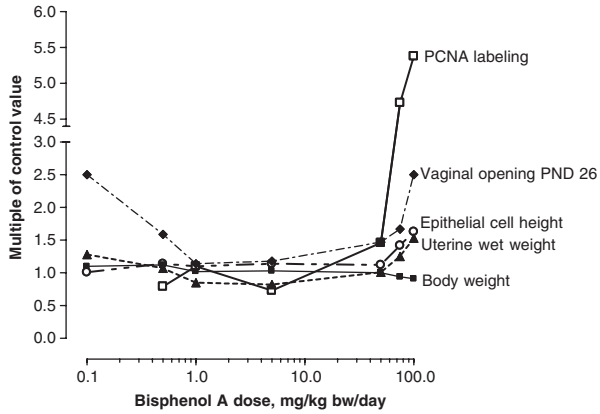

Fig. 4. Dose–response curves for endpoints of estrogenic activity in s.c.-dosed mice. On pair-wise testing, body weight was increased at 0.5 mg/kg bw/day and decreased at 100 mg/kg bw/day; vaginal opening was advanced at 0.1 and 100 mg/kg bw/day; epithelial cell height was increased at 5, 75, and 100 mg/kg bw/day; PCNA labeling was increased at 75 and 100 mg/kg bw/day; and uterine wet weight was increased at 100 mg/kg bw/day. Data from Markey et al. (2001b).

level. There was no apparent effect of strain on sensitivity of the uterotrophic response in immature (intact or castrate) or adult female rats.

Intra-laboratory variability has been noted for the bisphenol A uterotrophic assay in immature mice (Tinwell and Joiner, 2000). Of 8 studies performed over a 2-year period at s.c. bisphenol A dose levels up to 200 or 300 mg/kg bw/day, 4 showed a significant increase in uterine weight at 200 mg/kg bw/day. The other 4 studies, including the 2studies that went to 300 mg/kg bw/day, showed no effect of bisphenol A treatment on uterine weight despite the expected response to diethylstilbestrol. Study authors noted that reducing the permissible body weight of the mice selected for study resulted in lower and less variable control uterine weights and greater likelihood of bisphenol A effect (Tinwell and Joiner, 2000; Ashby et al., 2004). **[The Expert Panel notes that these studies all used high s.c. doses of bisphenol A.]**

Markey et al. (2001b) proposed that the rodent uterotrophic assay is relatively insensitive to the estrogenic effects of bisphenol A. These authors treated immature CD-1 mice with bisphenol A in s.c. minipumps and evaluated uterine weight, relative area of uterine compartments, epithelial height, expression of lactoferrin and proliferating cell nuclear antigen (PCNA), and induction of vaginal opening. Dose–response curves for the endpoints that showed significant changes from control are illustrated in Figure 4. The study authors also noted that significant alterations in some endpoints were observed at much lower doses (0.1 mg/kg bw/day for vaginal opening and 5 mg/kg bw/day for epithelial cell height), giving rise to a U-shaped dose–response curve. **[The assertions of some investigators notwithstanding, the Expert Panel notes that oral bisphenol A does not consistently produce robust estrogenic responses and, when seen, estrogenic effects after oral treatment occur at high-dose levels.]**

Transgenic reporter mice have permitted in vivo identification of activation of the estrogen response

element. Eight hr after i.p. injection on GD 13.5 of wild-type dams carrying transgenic fetuses, luciferase reporter activity was increased for bisphenol A 1 and 10 mg/kg bw (Lemmen et al., 2004). The luciferase response after bisphenol A was about 50% of that after a similar dose of estradiol dipropionate and ~25% of that after a 10-fold higher dose of diethylstilbestrol **[estimated from a graph]**. Use of an in vitro reporter system showed bisphenol A potency to be 3–4 orders of magnitude less than that of diethylstilbestrol (Table 52). The authors concluded that the in vivo estrogenic potency of bisphenol A may be greater than predicted by in vitro assays.

Nagel et al. (2001) developed a transgenic mouse with a thymidine kinase-*lacZ* reporter linked to 3 copies of the vitellogenin estrogen response element. This model showed an increase in ER activity after a single s.c. bisphenol A dose of 25 μg/kg bw ($P = 0.052$), with further increases in activity after 0.8 and 25 mg/kg bw. Uterine weight was only increased at the 25 mg/kg bw dose level. Normalized to the diethylstilbestrol response, uterine weight response to bisphenol A 25 mg/kg bw was less than one-third the response in ER activity **[estimated from a graph]**.

Gene expression profiles have been performed to compare the presumably ER-mediated response to bisphenol A with the response to reference ER agonists. Naciff et al. (2002) evaluated expression in the uteri and ovaries of Sprague–Dawley fetuses after s.c. dosing of dams on GD 11–20 with ethinyl estradiol 0, 0.5, 1, or 10 μg/kg bw/day or bisphenol A 0, 5, 50, or 400 mg/kg bw/day. The high-dose of both compounds induced nipples and areolae in male and female fetuses. There were 366 genes in which expression was altered by ethinyl estradiol and 397 genes in which expression was altered by bisphenol A. Expression of 66 genes was changed in the same direction with high-doses of ethinyl estradiol, bisphenol A, and genistein (which was also tested in this model). Of the 40 genes with at least a 1.8-fold change in expression, 17 responded similarly to ethinyl estradiol and bisphenol A. The authors identified 50 mg/kg bw/day as the lowest dose level at which estrogen-like gene expression activity could be identified, which is lower than the 400–800 mg/kg bw/day dose range at which uterotrophic activity is typically reported in rats (Ashby and Tinwell, 1998).

Terasaka et al. (2006) used expression of 120 estrogen-responsive genes (based on previous work) in MCF-7 cells to compare the profiles of bisphenol A and 17β-estradiol. Response was highly correlated ($R = 0.92$) between the 2 compounds. Another gene array study (Singleton et al., 2004) used MCF-7 cells that had lost ER and were re-engineered to express ERα. Among 40 estrogen-responsive genes, 12 responded to both bisphenol A and 17β-estradiol, 9 responded only to bisphenol A, and 19 responded only to 17β-estradiol. In the ER-deficient MCF-7 cell line from which these cells had been engineered, 1 gene responded to both bisphenol A and 17β-estradiol and 14 responded to bisphenol A alone, suggesting ER-independent activity. The same group reported the response of an additional 31 genes, associated with growth and development, from the same chip (Singleton et al., 2006). In the ERα-containing cells, 5 of these genes showed regulation with both 17β-estradiol and bisphenol A, 13 were regulated only by bisphenol A, and 13 were regulated by only 17β-estradiol.

Table 55
Bisphenol A Receptor Binding and Recruitment of Co-Activator Proteins[a]

	Activity relative to 17β-estradiol	
Assay	ERα	ERβ
Receptor binding	7.3×10^{-4}	7.5×10^{-3}
TIF2 recruitment	$< 1 \times 10^{-6}$	5×10^{-4}
SRC-1a recruitment	3×10^{-4}	2×10^{-4}

[a]Routledge et al. (2000).

Differences in the estrogenic activity of bisphenol A and reference estrogens may be due to differences in recruiting by the liganded receptor of co-regulatory proteins. Singleton et al. (2006) used a co-regulator-independent yeast reporter system to evaluate the estrogenicity of bisphenol A and 17β-estradiol. Bisphenol A activity was more than 3 orders of magnitude less than 17β-estradiol in the yeast system, compared to about a 2-order-of-magnitude difference in an MCF-7 cell assay, leading the authors to postulate that mammalian co-activators may be involved in enhancing bisphenol A activity. In a comparison of ER binding and co-activator recruitment, Routledge et al. (2000) showed bisphenol A to bind the receptor more avidly than the liganded receptor recruited 2 co-activator proteins, normalized to 17β-estradiol (Table 55).

The classical ERs are receptors that, when bound, produce their activity through alterations in genomic transcription. In contrast, a membrane-bound ER has been described in murine pancreatic islet cells (Nadal et al., 2000, 2004; Quesada et al., 2002; Alonso-Magdalena et al., 2005). This membrane-bound receptor regulates calcium channels and modulates insulin and glucagon release. Bisphenol A has been shown to activate this receptor in vitro at a concentration of 1 nM, which is similar to the active concentration of diethylstilbestrol (Nadal et al., 2000; Alonso-Magdalena et al., 2005). Treatment of mice with bisphenol A or 17β-estradiol s.c. at 10 μg/kg bw acutely or daily for 4 days resulted in decreased plasma glucose and increased insulin (Alonso-Magdalena et al., 2006). By contrast, Adachi et al. (2005) reported that exposure of rat pancreatic islets to 0.1–1 μg/L **[0.4–4.4 nM]** bisphenol A did not alter insulin secretion over a 1-hr period. Exposure of islets to bisphenol A 10 μg/L **[44 nM]** for 24 hr increased insulin release. This response was prevented by actinomycin D and by ICI 182,780, supporting the conclusion that bisphenol A insulin release occurs through interaction with the cytoplasmic ER rather than the membrane-bound receptor.

A membrane-bound ERα in the pituitary could be related to regulation of the release of stored prolactin in response to estrogens, a non-genomic response mediated by calcium influx. Using a rat prolactinoma cell line, bisphenol A was shown to promote calcium influx and release prolactin over a concentration range similar to that for 17β-estradiol (Wozniak et al., 2005; Watson et al., 2007). The response to bisphenol was bimodal, with maximal responses at concentrations of 10^{-12} and 10^{-8} M and little-to-no response at intermediate concentrations. Calcium influx in MCF-7 cells has been shown to occur

Table 56
Anti-Androgenicity Studies of Bisphenol A in Cells Transfected With Androgen Receptor Reporter

Cell type	Reference androgen concentration (nM)	Bisphenol A median inhibitory concentration (IC_{50}) μM **[mg/L]**	Reference
Human prostate adenocarcinoma	R1881 0.1	7 **[1.6]**	Paris et al. (2002)
Chinese hamster ovary	R1881 0.1	19.6 **[4.5]**	Roy et al. (2005)
Yeast	Testosterone 10	1.8 **[0.4]**	Lee et al. (2003a)
Yeast	Dihydrotestosterone 1.25	2[a] **[0.5]**	Sohoni and Sumpter (1998)
Monkey kidney	Dihydrotestosterone 1	0.746 **[0.2]**	Xu et al. (2005)
Monkey kidney	Dihydrotestosterone 1	2.14 **[0.5]**	Sun et al. (2006)
Mouse fibroblast	Dihydrotestosterone 0.01	4.3 **[1.0]**	Kitamura et al. (2005)
Human hepatoma	Dihydrotestosterone 100	No anti-androgenic activity	Gaido et al. (2000)

[a]Estimated from a graph.

rapidly after exposure to bisphenol and 17β-estradiol concentrations of 10^{-10} M through a non-ER-mediated mechanism (Walsh et al., 2005).

Recently, bisphenol A was identified as competitor to 17β-estradiol for binding to the GPR30 receptor; a novel seven-transmembrane receptor that mediates nongenomic estrogen actions to upregulate adenylyl cyclase and MAPK activities (Thomas and Dong, 2006). Similar to findings reported previously with nuclear estrogen receptors and membrane estrogen receptors, bisphenol A was identified as a relatively effective competitor of 17β-estradiol binding, with relative binding affinities of 2.8% that of the natural estradiol ligand and an IC_{50} of $630 \times 10-9$ M. Bisphenol A, at a concentration of 200 nM significantly increased cAMP levels in transfected cells 30 min after compound addition.

Bisphenol A has been found to bind estrogen-related receptor γ, a nuclear receptor with no known natural ligand that shows little affinity for 17β-estradiol (Takayanagi et al., 2006). Estrogen-receptor γ demonstrates high constitutive activity that is maintained by bisphenol A in the presence of 4-hydroxytamoxifen, which otherwise blocks nuclear ER activity. This observation led to the suggestion that bisphenol A may maintain estrogen-related receptor γ activity in the presence of a yet-to-be-identified natural antagonist and that cross talk between the estrogen-related receptor and ER systems could be responsible for the estrogenic activity of bisphenol A in spite of low binding affinity for ERα and β (Takayanagi et al., 2006).

In addition to the studies reviewed for this section, there are studies in which the putative estrogenicity of environmental samples or synthetic products were evaluated using one or another assay. For example, Olea et al. (1996) evaluated resin-based dental composites in an MCF-7 culture system. The response of the system was attributed to the bisphenol and its methacrylate detected in the composites, but bisphenol A was not specifically tested. These articles were not reviewed for this section.

2.2.3 Androgen activity. Transfected cell-based assays have not identified bisphenol A as having androgenic activity (Sohoni and Sumpter, 1998; Gaido et al., 2000; Kitamura et al., 2005; Xu et al., 2005). However, bisphenol A is mitogenic in cultured human prostate carcinoma cells at a concentration of 1 nM (Wetherill, 2002). Based on stimulated cell growth in this system, the potency of bisphenol A is about 5% that of dihydrotestosterone **[estimated from a graph]**. This bisphenol A activity was shown to be mediated by interaction with a mutant tumor-derived androgen receptor called AR-T877A. Anti-androgenic activity has been demonstrated using cells transfected with androgen receptor reporting systems (Table 56). The anti-androgenic activity of bisphenol A is expressed as the concentration needed to halve the androgen reporter response to a reference androgen. Studies in transfected cells have shown that bisphenol A interferes with the binding of dihydrotestosterone to the androgen receptor, interferes with translocation of the liganded receptor to the nucleus, and prevents transactivation at the androgen-response element (Lee et al., 2003a).

Kim et al. (2002a) conducted a Hershberger assay to determine the effects of bisphenol A exposure on reproductive organs of rats. Sprague–Dawley rats were fed PMI Certified Rodent LabDiet and housed in polycarbonate cages. No information was provided about bedding materials. One experiment was conducted to determine the optimum dose and age for observing testosterone exposure effects. In a second experiment, 10 rats/group rats were castrated at 5 weeks of age and 7 days later gavaged with bisphenol A (99% purity) at doses of 0 (ethanol/corn oil vehicle) 10, 100, or 1000 mg/kg bw/day for 7 days. A second group of castrated 6-week-old males rats was gavaged with bisphenol A at 0, 50, 100, 250, or 500 mg/kg bw/day for 7 days. In a third experiment, 10 castrated 6-week-old rats/group were treated with 0.4 mg/kg bw/day testosterone by s.c. injection in addition to gavaged bisphenol A at 50, 100, 250, or 500 mg/kg bw/day or flutamide at 1, 5, 10, or 25 mg/kg bw/day for 7 days. A positive control group was given 0.4 mg/kg bw/day testosterone for 7 days. **[There is some confusion in the article regarding ages at castration and start of treatment. For the first group of bisphenol A-treated rats, it is reported that rats were castrated at 5 weeks of age and treated at 6 weeks of age. For the other groups of bisphenol A-treated rats, the Methods section reported that treatment began at 6 weeks of age, but tables in the Results section indicated that rats were castrated at 6 weeks of age.]** During the study, clinical signs were observed and body weights were measured. Blood was collected and rats were killed ~24 hr after administration of the last dose. Accessory reproductive organs were removed and weighed. Serum luteinizing hormone (LH) and testosterone concentrations were measured by

radioimmunoassay (RIA). Statistical analyses included Bartlett test, analysis of covariance (ANCOVA), Dunnett test, and Bonferroni test. Exposure to bisphenol A did not affect weights of the ventral prostate, seminal vesicles, glans penis, or levator ani plus bulbocavernosus muscle; or serum concentrations of LH or testosterone. Testosterone increased the weights of accessory reproductive organs. Flutamide increased serum LH concentrations and inhibited testosterone-induced increases in accessory reproductive organ weights. Study authors concluded that bisphenol A did not exhibit androgenic or antiandrogenic effects in rats.

Yamasaki et al. (2003) conducted a Hershberger assay in rats exposed to bisphenol A or 1 of 29 other chemicals. In this study, which was conducted according to GLP, animals were housed in stainless steel wire-mesh cages. Assuming these males were fed the same diets as rats used in an uterotrophic assay also described in this study, they received MF Oriental Yeast feed. Rats were randomly assigned to treatment groups. Beginning at 56 days of age and continuing for 10 days, 6 castrated male Brl Han: WIST Jcl (GALAS) rats/group were administered bisphenol A by stomach tube at doses of 0 (olive oil vehicle), 50, 200, or 600 mg/kg bw/day. An additional group of rats was administered the same vehicle and doses of bisphenol A in addition to 0.2 mg/kg bw/day testosterone propionate by s.c. injection. Dose selection was based on results of preliminary studies. A positive control group was given 10 mg/kg bw/day flutamide in addition to 0.2 mg/kg bw/day testosterone propionate. Rats were killed 24 hr after receiving the final dose. Ventral prostate with fluid, seminal vesicles with fluid, bulbocavernosus/levator ani muscle, glans penis, and Cowper gland were collected and weighed. Data were analyzed by Student *t*-test. Bisphenol A did not affect body weight and there were no clinical signs of toxicity. The only statistically significant effect on relative organ weight was a **[24%]** increase in glans penis weight in rats given 600 mg/kg bw/day bisphenol A without coadministration of testosterone. In contrast, rats treated with flutamide plus testosterone propionate experienced increases in weights of ventral prostate, seminal vesicle, bulbocavernosus/levator ani muscle, glans penis, and Cowper gland. **[Absolute organ weights were not reported. It is assumed but was not stated that relative weights were based on body weight.]** Study authors noted that because glans penis weights were variable in control rats and weights of other accessory reproductive organs were not affected, bisphenol A could not be clearly determined to have androgen agonistic properties.

Nishino et al. (2006) performed a Hershberger assay in Wistar rats. At 2 weeks of age, rats were given ssniffR 10 diet and housed in Makrolon cages with ssniff bedding. Seven days after orchiectomy, rats were placed in groups of 13 **[randomization not discussed]** and treated orally **[gavage assumed]** with bisphenol A **[purity not indicated]** in propylene glycol at 0, 3, 50, 200, or 500 mg/kg bw/day for 7 days or s.c. with testosterone propionate 1 mg/kg bw. Another group was given oral bisphenol A 500 mg/kg bw/day and flutamide 3 mg/kg bw/day. Rats were killed by decapitation after treatment. Seminal vesicles and prostates were weighed and fixed in 4% neutral buffered paraformaldehyde. Immunohistochemical evaluation of androgen receptor, PCNA, and MIB-5 was performed. Epithelial cell height and duct luminal area were determined morphometrically. Review by the Expert Panel indicated that this study was inadequate due to methodological issues.

2.3 Genetic Toxicity

Assessment of mutagenicity associated with bisphenol A was based primarily on reviews by the European Union (2003) and Haighton et al. (2002). CERHR summarized a limited number of studies that were not included in reviews. Results of in vitro genetic toxicity testing are summarized in Table 57, and results of in vivo genetic toxicity tests are summarized in Table 58.

The European Union (2003) noted that bisphenol A demonstrated aneugenic potential and micronuclei formation in in vitro tests without metabolic activation. However, there was no evidence of micronuclei formation in an in vivo mouse study. Other studies demonstrated disruption of microtubule formation and the presence of DNA adducts. In the studies reviewed by the European Union, there was no evidence of gene mutations or structural chromosomal aberrations in in vitro tests and negative results were obtained in a dominant lethal test in rats; however, the European Union noted several limitations for those studies. Based on their review of genotoxicity data and the lack of significant tumors reported in animal studies, the European Union (2003) concluded that bisphenol A does not appear to have significant mutagenicity potential in vivo. Because aneugenic potential was apparently observed only in in vitro tests, it was judged to be of no concern. The relevance of DNA adduct formation was unclear, but based on weight of evidence, i.e., negative findings for gene mutation and clastogenicity in cultured mammalian cells, DNA adduct formation was thought unlikely to be of concern for humans.

Haighton et al. (2002) concluded that results of standardized and validated genetic toxicity tests demonstrated the lack of mutagenic and genotoxic activity of bisphenol A in vivo. Studies demonstrating disrupted microtubule formation or DNA adduct formation were noted, but because the studies used high-doses, they were judged to be of limited relevance. The lack of activity in an in vivo micronucleus assay in mice was said to confirm negative results observed in in vivo tests. Lastly, it was concluded that bisphenol A (parent) had no structural features that suggested mutagenic activity.

Subsequent to the release of the European Union (2003) and Haighton et al. (2002) reviews, Hunt et al. (2003), published a study examining meiotic aneuploidy potential of bisphenol A in female mice. In 1998, a large increase in background rate of congression failure (from 1–2 to 40%) and in aneuploidy (from 0.7 to 5.8%) was observed in the study authors' laboratory. The increase was found to coincide with damage to polycarbonate caging material. Removal of the most damaged cages and change to polysulfone cages resulted in decreased background rates of congression failure. Intentionally damaging polycarbonate cages and water bottles resulted in increased rates of congression failure. As noted in Table 58, congression failure rates were increased in juvenile female mice orally exposed to $\geq 20\,\mu g/kg$ bw/day bisphenol A for 6–8 days or $20\,\mu g/kg$ bw/day for 7

Table 57
In Vitro Genetic Toxicity Studies of Bisphenol A

Concentration	Cell	Endpoint	Results	Reference
3.3–333.3 μg/plate, with and without metabolic activation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537	Mutagenicity	Negative	Haworth et al. (1983)[a,b]
50–500 μg/plate, with and without metabolic activation	*Salmonella typhimurium* strains TA97a, TA98, TA100, TA102	Mutagenicity	Negative	Schweikl et al. (1998)[a,b]
≤5000 μg/plate with and without metabolic activation	*Salmonella typhimurium* strains TA97, TA98, TA100, TA102	Mutagenicity	Negative	Takahata et al. (1990)[a,b]
≤1000 μg/mL, with and without metabolic activation	*Salmonella typhimurium* strain TA1538 and *Escherichia coli* strains WP2 and WP2uvrA	Mutagenicity	Negative	Dean and Brooks (1978)[a,b]
5–1250 μg/plate, with and without metabolic activation	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and *Escherichia coli* strain WP2uvrA	Mutagenicity	Negative	JETOC (1996)[a,b]
1 mM **[228 mg/L]**, with and without metabolic activation	*Salmonella typhimurium* strains TA98 and TA100	Mutagenicity	Negative	Masuda et al. (2005)
0.1–0.2 mM **[23–46 mg/L]**, without metabolic activation	Chinese hamster V79 cells, hprt locus	Mutagenicity	Negative	Schweikl et al. (1998)[a,b]
5–60 mg/L without metabolic activation, 25–200 mg/L with metabolic activation, or 5–60 mg/L with and without metabolic activation[d]	Mouse lymphoma L5178Y cells, $tk^{+/-}$ locus	Mutagenicity	Negative (results questioned due to possible inability to count small colonies)	Myhr and Caspary (1991)[a,b]
Concentrations not specified, with and without metabolic activation	Mouse lymphoma L5178Y cells, $tk^{+/-}$ locus	Mutagenicity	Inconclusive without and negative with metabolic activation	Honma et al. (1999)[a,b]; Moore et al. (1999)[a,b]
25–200 μM **[5.7–46 mg/L]**, without metabolic activation	Syrian hamster embryo cells (Na+/K+ ATPase and hprt loci)	Mutagenicity	Negative	Tsutsui et al. (1998)[a,b]
10^{-8}–10^{-5} M **[0.002–2 mg/L]**, without metabolic activation	Human RSa cells	Mutagenicity	↑ at all doses	Takahashi et al. (2001)
≤500 mg/L, with and without metabolic activation	*Saccharomyces cerevisiae* strain JDI	Mutagenicity	Negative	Dean and Brooks (1978)[a,b]
10^{-8}–10^{-4} M **[0.002–23 mg/L]**, without metabolic activation	MCF-7 cells	DNA damage (assessed by comet assay)	↑ at ≥10^{-6} M **[0.2 mg/L]**	Iso et al. (2006)
10^{-4} M **[23 mg/L]**, without metabolic activation	MDA-MB-231 cells	DNA damage (assessed by comet assay)	↑	
20–40 mg/L, without metabolic activation and 30–50 mg/L with metabolic activation	Chinese hamster ovary (CHO) cells	Chromosomal aberration	Negative (inconsistent ↑ at high dose with metabolic activation)	Ivett et al. (1989)[a,b]; Tennant et al. (1986, 1987)[b]
350–450 μM **[80–103 mg/L]**, without metabolic activation and ≤250 μM **[57 mg/L]** with metabolic activation	CHO cells, clone WBL	Chromosomal aberration	Positive at ≥400 μM **[91.3 mg/L]** without metabolic activation[c]; negative with metabolic activation	Hilliard et al. (1998)[a]
400 and 450 μM **[91 and 103 mg/L]**, without metabolic activation	CHO cells, clone WBL	Chromosomal aberration	Positive[c]	Galloway et al. (1998)[a]
25–200 μM **[5.7–46 mg/L]**, without metabolic activation	Syrian hamster embryo cells	Chromosomal aberration	Negative	Tsutsui et al. (1998)[a,b]
10–30 mg/L	Epithelial-type rat liver cell line (RL1)	Chromosomal aberration	Negative	Dean and Brooks (1978)[b]
25–200 μM **[5.7–46 mg/L]**, without metabolic activation	Syrian hamster embryo cells	Aneuploidy/polyploidy		Tsutsui et al. (1998)[a,b]

			Inconclusive (non-dose-related ↑ in aneuploidy at ≥50 μM **[11 mg/L]**)[e]; apparently positive[f]	
0.8–25 mg/L, without metabolic activation and 30–50 μg/mL, with metabolic activation	CHO cells	Sister chromatid exchange	Negative (one small ↑ was not reproducible)	Ivett et al. (1989)[a,b] Tennant et al. (1986)[b]
0.2–0.5 mM or nM[d] **[46–114 mg/L or μg/L]**	Rat hepatocytes	DNA strand breaks	Negative (↑ noted but scored as negative by study authors due to excessive cytotoxicity)	Storer et al. (1996)[a,b]
10^{-9}–10^{-5} M **[0.0002–2 mg/L]**, without metabolic activation	Human RSa cells	Unscheduled DNA synthesis	↑ at 10^{-6} M **[0.2 mg/L]** and ↓ at 10^{-7} **[0.02 mg/L]** and 10^{-5} M **[2 mg/L]**	Takahashi et al. (2001)
Not specified, but stated to cover range of cytotoxicity	A31-1-13 clone of BALB/c-3T3 cells	Transformation	Negative	Matthews et al. (1993)[a]
25–200 μM **[5.7–46 mg/L]**, without metabolic activation	Syrian hamster embryo cells	Transformation	Positive at ≥50 μM **[11.4 mg/L]** (non-dose-related ↑)[e]; equivocal[f]	Tsutsui et al. (1998, 2000)[a,b]
≤50 mg/L for 24 hr; ≤30 mg/L for 7 days, without metabolic activation	Syrian hamster embryo cells	Transformation	Negative	LeBoeuf et al. (1996)[a]
2–60 mg/L	Syrian hamster embryo cells	Transformation	Negative	Jones et al. (1988)[b]
50–200 μM **[11.5–46 mg/L]**, without metabolic activation	Syrian hamster embryo cells	DNA adduct formation	Positive at ≥50 μM **[11 mg/L]** (dose-related ↑)	Tsutsui et al. (1998)[a,b]
1000 μg presence of peroxidase and hydrogen peroxide	Purified rat DNA	DNA adduct formation	Positive	Atkinson and Roy (1995a)
10–100 μM **[2.3–23 mg/L]**, metabolic activation unknown	Bovine brain microtubule protein	Inhibited microtubule polymerization	Positive	Metzler and Pfeiffer (1995)[a]
50–200 μM **[11.5–46 mg/L]**, no metabolic activation	Bovine brain microtubule protein	Inhibited microtubule polymerization	Positive (dose-related)	Pfeiffer et al. (1996)[b]
20–200 μM **[4.6–46 mg/L]**, metabolic activation unknown	Bovine brain microtubule protein	Inhibited microtubule polymerization	Positive (EC_{50} = 150 μM **[34 mg/L]**)	Pfeiffer et al. (1997)[a,b]
200 μM **[46 mg/L]**, without metabolic activation; 100 μM **[23 mg/L]** for metaphase arrest assay	Chinese hamster V79 cells	Aneuploidogenic potential as assessed by micronuclei formation, microtubule assay, and metaphase arrest	Positive	Pfeiffer et al. (1997)[a,b]
100–200 μM **[23–46 mg/L]**, without metabolic activation	Chinese hamster V79 cells	Aneuploidogenic potential as assessed by micronuclei formation	Positive	Ochi (1999)[a,b]
10 or 30 μM **[2.3 or 6.9 mg/L]**	Oocytes from Balb/c mice	Meiotic spindle formation	Centrosomes and spindles disorganized	Can et al. (2005)
0.05–0.4 mg/L	Oocytes from MF_1 mice	Aneuploidy	No hyper haploidy but ↑ diploid metaphase II oocytes at 0.2 mg/L	Pacchierotti et al. (2007)

[a]Reviewed by Haighton et al. (2002).
[b]Reviewed by European-Union (2003).
[c]According to the Haighton et al. (2002) review, positive results occurred at cytotoxic concentrations.
[d]Discrepancies noted between information presented by Haighton et al. (2002) and European-Union (2003).
[e]Conclusion by Haighton et al. (2002).
[f]Conclusion by European-Union (2003).
↑,↓ increase, decrease.

Table 58
In Vivo Genetic Toxicity Studies of Bisphenol A

Species and sex	Dose (route)	Cells	Endpoint	Results	Reference
Male rat	85 mg/kg bw/day for 5 days (i.p.)	Germ	Dominant lethality	Negative	Bond et al. (1980)[a,b] (abstract only)
Male rat	200 mg/kg bw (i.p.) and 200 mg/kg bw for 4, 8, 12, or 16 days (oral)	DNA	Adduct formation	Positive	Atkinson and Roy (1995b)
Male and female mouse	500–2000 mg/kg bw (oral)	Bone marrow	Micronuclei	Negative	Gudi and Krsmanovic (1999)[a]; Shell Oil Co. (1999)[b]
Male mouse	1 mmol/kg bw **[228 mg/kg bw]** (oral)	Peripheral blood reticulocyte	Micronuclei	Negative	Masuda et al. (2005)
20–22-day-old female mouse	0.02–0.100 mg/kg bw/day (oral) for 6–8 days or 0.02 mg/kg bw for 3, 5, or 7 days	Oocyte	Congression failure	Positive at all doses; statistically significant with 7-day exposure	Hunt et al. (2003)
Pregnant mouse GD 11.5–18.5	0.4 μg/day s.c. pellet **[∼20 μg/kg bw/day]**	Oocyte	Evaluation of pachytene fetal oocyte and of ploidy in oocytes and 2-cell embryos from adults that were exposed in utero	Incomplete synapsis, end-to-end association of sister chromatids, ↑hyperploidy	Susiarjo et al. (2007)
Female mouse	0.2 or 20 mg/kg bw acutely or daily for 7 days or 0.4 mg/L in drinking water for 7 weeks	Oocyte	Aneuploidy	Negative	Pacchierotti et al. (2007)
Male (102/ElxC3H/El) F_1 mouse	0.002–0.2 mg/kg bw for 6 days (oral)	Spermatocyte	Meiotic delay and aneuploidy	Negative	Pacchierotti et al. (2007)
Drosophila melanogaster	10,000 ppm (oral)	Offspring	Sex-linked recessive lethal test	Negative	Foureman et al. (1994)[a,b]
Turbot	50 ppb in aquarium water for 2 weeks	Erythrocyte	Micronuclei	Positive	Bolognesi et al. (2006)

[a]Reviewed by Haighton et al. (2002).
[b]Reviewed by European-Union (2003).

days. The study authors concluded that bisphenol A was a potential meiotic aneugen.

In a follow-up study (Susiarjo et al., 2007), pregnant C57Bl/6 mice on GD 11.5 were implanted with s.c. pellets designed to release bisphenol A 0 or 0.4 μg/day. **[The authors assume a 20 g bw, giving an estimated dose level of 20 μg/kg bw/day.]** Oocytes from GD 18.5 female fetuses showed an increase in pachytene synaptic abnormalities including incomplete synapsis and end-to-end associations of sister chromatids. There was also paradoxically an increase in recombinant foci in pachytene oocytes of bisphenol A-exposed females. Some female offspring of bisphenol A-treated dams were fostered to untreated dams. Eggs or 2-cell embryos from these female offspring at 4–5 weeks of age showed an increase in hyperploidy. Pachytene oocyte abnormalities similar to those identified in fetuses exposed to bisphenol A were seen in oocytes obtained from ERβ knock-out mice, suggesting to the authors that bisphenol A may exert adverse effects on meiosis by blocking ERβ.

In response to the study of Hunt et al. (2003), Pacchierotti et al. (2007) investigated the aneugenic effects of bisphenol A in mouse somatic and germ cells. C57Bl/6 female mice were superovulated using pregnant mare serum and hCG after which they were gavaged with bisphenol A 0.2 or 20 mg/kg bw. Metaphase II oocytes were collected after 17 hr and evaluated using C-banding. Additional female mice were gavaged with bisphenol A 0.04 mg/kg bw/day for 7 days or were given bisphenol A in drinking water at a concentration of 0.4 mg/L for 7 weeks. These mice were superovulated at the end of the 7-day or 7-week treatment period and housed overnight with untreated males. Females without vaginal plugs were killed for evaluation of oocytes by C-banding. Females with vaginal plugs were treated with colchicine to prevent the first embryonic cleavage, and zygotes were collected the next morning for evaluation by C-banding. There were no bisphenol A effects on induction of aneuploidy. There was a statistically significant increase in premature centromere separation in the group treated for 7 weeks, but there was no effect of bisphenol A treatment on the proportion of zygotes with structural or numeric chromosome changes. Male mice were treated with bromodeoxyuridine 8 days before being treated with bisphenol A 0.2 mg/kg bw/day for 6 days. Evaluation of sperm after 21–25 days did not show a significant mitotic delay in spermatocytes. Additional male mice were given bisphenol A orally at doses of 0,

0.002, 0.02, and 2 mg/kg bw/day for 6 days. Epididymal sperm were collected 22 days after the end of bisphenol A treatment and multicolor fluorescent in situ hybridization was used to evaluate decondensed sperm for aneuploidy. Sperm count was decreased by bisphenol A 0.002 mg/kg bw/day, but there was no increase in the frequency of hyperhaploidy or diploidy. Bisphenol A was negative in a bone marrow micronucleus test at dose levels up to 2 mg/kg/day for 2 days.

2.4. Carcinogenicity

No human data examining the carcinogenicity of bisphenol A were identified.

NTP (1982) and Huff (2001) examined carcinogenicity of bisphenol A in F344 rats and B6C3F$_1$ mice. Animals were randomly assigned to treatment groups. Bisphenol A (<98.2% purity) was administered through feed for 103 weeks to 50 rats/sex/dose at 0, 1000, or 2000 ppm, 50 male mice/group at 0, 1000, or 5000 ppm, and 50 female mice/group at 0, 5000, or 10,000 ppm. NTP estimated mean bisphenol A intakes of 74 and 148 mg/kg bw/day for male rats and 74 and 135 mg/kg bw/day for female rats. **[Data on bisphenol A intake, food intake, and body weights were not provided for mice.]** Using default values, the European Union (2003) estimated bisphenol A intakes of 120 and 600 mg/kg bw/day in male mice and 650 and 1300 mg/kg bw/day in female mice. Concentration and stability of bisphenol A in feed were verified. Body weights and clinical signs were observed during the study. Following the exposure period, animals were killed and necropsied. Organs, including seminal vesicle, prostate, testis, ovary, and uterus, were preserved in 10% neutral buffered formalin and examined histologically. Statistical analyses included Cox and Tarone methods, 1-tailed Fisher exact test, Bonferroni inequality criterion, Cochran-Armitage test, and life table methods for linear trend.

In rats, body weights of males and females from both dose groups were lower than controls throughout the study. Feed intake was decreased in females of both dose groups beginning at Week 12. No adverse effects on survival were observed. There were no non-neoplastic lesions **[including in male and female reproductive organs]** that appeared to be treatment-related. The incidence of leukemia was increased in males (13 of 50, 12 of 50, and 23 of 50 in control and each respective dose group) and females (7 of 50, 13 of 50, and 12 of 50). In males the trend for leukemia was significant by Cochran-Armitage test, but statistical significance was not shown by life table analysis for trend or incidence in the high-dose group, according to the unpublished version of the study. The published version of the study indicated statistical significance at the high-dose. Statistical significance was not attained for leukemia incidence in female rats. An increased incidence of testicular interstitial cell tumors (35 of 49, 48 of 50, 46 of 49) was statistically significant in both dose groups. An increased incidence of mammary fibroadenomas in males of the high-dose group (0 of 50, 0 of 50, and 4 of 50) achieved statistical significance for trend by Cochran-Armitage test but not by Fisher exact test. In bisphenol A groups, there were decreased incidences of adrenal pheochromocytomas in males, adrenal cortical adenomas in females, and uterine endometrial stromal polyps. The NTP concluded that none of the increases in tumor incidence in rats was clearly associated with bisphenol A exposure.

In mice, body weights were lower in high-dose males and in females of both dose groups. Feed intake could not be accurately determined because of spillage. Bisphenol A did not affect the survival of mice. Incidence of multinucleated hepatocellular giant cells was increased in treated males (1 of 49, 41 of 49, and 41 of 50). **[A review of the data indicated no increases in incidence of non-neoplastic lesions in the reproductive organs of male or female mice.]** The incidence of leukemia or lymphoma in male mice by dose group (2 of 49, 9 of 50, and 5 of 50) was not statistically significant. The published version of the report indicated an increasing trend for lymphoma. The linear trend for increased pituitary chromophobe carcinomas in male mice (0 of 37, 0 of 36, 3 of 42) was reported to be statistically significant by Cochran-Armitage test but statistical significance was not shown by Fisher exact test. The study authors concluded that none of the increases in tumor incidence in mice could be unequivocally associated with bisphenol A exposure.

NTP concluded that under the conditions of this study, there was no convincing evidence the bisphenol A was carcinogenic in F344 rats or B6C3F$_1$ mice. However, study authors stated that there was suggestive evidence of increased cancer in the hematopoietic system based on marginally significant increases in leukemia in male rats, non-statistically significant increases in leukemia in female rats, and a marginally significant increase in combined incidence of lymphoma and leukemia in male mice. A statistically significant increase in testicular interstitial cell tumors in aging F344 rats was also considered suggestive evidence of carcinogenesis. The effect was not considered conclusive evidence because of the high incidence of the testicular neoplasm in aging F344 rats (88% incidence in historical controls).

The NTP study was reviewed by the European Union (2003) and Haighton et al. (2002). For increases in leukemia, mammary gland fibroadenoma, and Leydig cell tumors in male rats, both groups noted the lack of statistical significance using the appropriate analyses and the common occurrence of these tumor types in F344 rats. The European Union (2003) concluded, "Overall, all of these [tumor] findings in rats and mice are not considered toxicologically significant. Consequently, it is concluded that bisphenol A was not carcinogenic in this study in both species." Haighton et al. (2002) concluded, "Overall, the results of this bioassay did not provide any compelling evidence to indicate that [bisphenol A] was carcinogenic in F344 rats or in B6C3F$_1$ mice." Based on the experimental animal data, the European Union concluded that "…the evidence suggests that bisphenol A does not have carcinogenic potential." Using a weight of evidence approach, Haighton et al. (2002) concluded that bisphenol A was not likely to be carcinogenic to humans. This conclusion was based on NTP study results; lack of activity at noncytotoxic concentrations in both in vitro genetic toxicity tests and in an in vivo mouse micronucleus test; and data from metabolism studies that show rapid glucuronidation and no formation of possibly reactive intermediates, with the possible exception of reactive intermediates potentially generated as a result of saturated detoxification pathways at high-doses.

Table 59
Development of UDPGT Activity in Humans[a]

Age	UDPGT activity, nmol/min/mg protein		
	Bilirubin	Testosterone	1-Napthol
30 weeks gestation	0.05	0	0.56
30 weeks gestation with 10 weeks survival	0.4; 1	0.14; 0.85	3.0; 1.8
Full-term infants surviving 1–10 days (n = 7)	0.07 ± 0.04	0.10 ± 0.06	0.75 ± 0.68
Full-term infants surviving 8–15 weeks (n = 6)	0.64 ± 0.32	0.12 ± 0.05	2.4 ± 1.1
Full-term infants surviving 22–55 weeks (n = 5)	0.99 ± 1.1	0.09 ± 0.06	3.6 ± 2.1
Adult males (n = 3)	0.76 ± 0.43	0.46 ± 0.61	7.2 ± 2.2

[a]Coughtrie et al. (1988).
Data presented as individual values or mean ± SD.

2.5 Potentially Susceptible Subpopulations

As noted in Section 2.1.1.3, one pathway of bisphenol A metabolism in humans and experimental animals is glucuronidation. Studies in experimental animals demonstrated that both the intestine and liver can glucuronidate bisphenol A. UGT2B1 was identified as the isoform involved in bisphenol A glucuronidation in rat liver (Yokota et al., 1999). The UDPGT isoform involved in human intestinal glucuronidation of bisphenol A is not known to have been identified. Despite uncertain isoform identification, studies in humans and experimental animals demonstrate developmental changes in expression of activities of several UDPGT isoforms that potentially affect bisphenol A metabolism.

Coughtrie et al. (1988) examined the ontogeny of UDPGT activity in human liver microsome samples obtained postmortem from adults and premature or full-term infants. Results of this analysis are listed in Table 59. Activities for isoenzymes catalyzing glucuronidation of bilirubin, testosterone, and 1-napthol were very low at birth in premature and full-term infants. Activities increased with age for the isoenzymes catalyzing glucuronidation of bilirubin (~80% of adult levels by 8–15 weeks of age) and 1-naphthol (~30% of adult levels at 8–15 weeks of age). During the first 55 weeks of life, no consistent increase in activity was noted for the isoenzyme catalyzing glucuronidation of testosterone. Using an immunoblot technique with antibodies developed toward liver testosterone/4-nitrophenol and kidney naphthol/bilirubin, 1 immunoreactive protein was observed in microsomes of 18- and 27-week-old fetuses and 3 immunoreactive proteins were observed in microsomes of full-term infants. Most isoenzymes present in adults were observed in infants within 3 months of age at levels ~25% those of adults.

Strassburg et al. (2002) used a reverse transcript (RT)-polymerized chain reaction (PCR) technique to examine developmental changes in expression for 13 *UDPGT* genes in liver samples obtained from 16 pediatric patients undergoing liver transplant for extrahepatic biliary atresia (6–24 months old) and 12 adults undergoing liver transplant for carcinoma (25–75 years). Changes in gene expression were also assessed in hepatic RNA samples for two 20-week-old fetuses. No transcripts for UDPGT were detected in samples from 20-week-old fetuses. In infant and adult livers, transcripts were detected for *UGT1A1*, *UGT1A3*, *UGT1A4*, *UGT1A6*, *UGT1A9*, *UGT2B4*, *UGT2B7*, *UGT2B10*, and *UGT2B15*; there were no age-related differences in expression. Expression of *UGT1A9* and *UGT2B4* mRNA was lower in the pediatric samples. Western blot analyses of protein expression for UGT1A1, UGT1A6, and UGT2B7 were consistent with findings for mRNA expression. Activities toward 18 specific substrates were assessed in microsomes. In 13–24-month-old children compared to adults, glucuronidation activity was lower for ibuprofen (24-fold), amitriptyline (16-fold), 4-*tert*-butylphenol (40-fold), estrone (15-fold), and buprenorphine (12-fold).

Cappiello et al. (2000) compared uridine 5′-diphosphoglucuronic acid concentrations in livers and kidneys of human fetuses and adults and in placenta. In adults undergoing surgery, liver samples were obtained from 1 man and 4 women (23–72 years of age) and kidney samples were obtained from 1 woman and 4 men (55–63 years of age). Fetal livers and kidneys were obtained from 5 fetuses legally aborted between 16 and 25 weeks gestation. Five placenta samples were obtained on delivery at 17–25 weeks gestation. Compared to adults, fetal uridine 5′-diphosphoglucuronic acid concentrations were 5-fold lower in liver and 1.5-fold lower in kidney. Concentrations of uridine 5′-diphosphoglucuronic acid in placenta were 3–4-fold lower than in fetal liver. Based on these findings, study authors concluded that glucuronidation is potentially limited in the human fetus.

As noted in Sections 2.1.2.2 and 2.1.2.3, rat fetuses appear to have no or low ability to glucuronidate bisphenol A (Miyakoda et al., 2000; Matsumoto et al., 2002; Domoradzki et al., 2003). Although rats glucuronidate bisphenol A at birth, glucuronidation capacity appears to increase with age (Matsumoto et al., 2002; European-Union, 2003; Domoradzki et al., 2004).

Some possible interindividual or sex-related differences in the ability to produce the bisphenol A sulfate conjugate were identified in a limited number of human studies. As discussed in more detail in Section 2.1.1.3 and shown in Table 8, higher amounts of urinary bisphenol A sulfate were detected in 15 adult women than in 15 adult males (Kim et al., 2003b). In a study examining bisphenol A metabolism by human hepatocytes, an ~10-fold higher concentration of a bisphenol A glucuronide/sulfate conjugate was observed in the sample from 1 female than in samples from 2 other females and 2 males (Pritchett et al., 2002).

Yang et al. (2003) examined the effects of polymorphisms in sulfotransferase enzymes on urinary excretion of total bisphenol A (conjugated and free) in Korean volunteers. Urinary bisphenol A concentrations were measured by HPLC and a PCR method was used to determine sulfotransferase genotype. The SULT1A1*1 allele was reported to have greater enzyme activity than the SULT1A1*2 enzyme and it was expected that individuals with the SULT1A1*1 allele would be able to rapidly eliminate bisphenol A. However, no significant differences in urinary bisphenol A concentrations were observed between 57 individuals with the SULT1A1*1 allele (geometric mean ± SD = 10.10 ± 8.71 μg/L) and 15

individuals with the SULT1A1*2 enzyme (6.31 ± 8.91 μg/L). Adjustment for possible bisphenol A exposure through vinyl wrap use also did not result in significant differences between the 2 groups. The study authors concluded that additional enzymes involved in bisphenol A metabolism should be studied to determine possible sensitivity differences.

One animal study demonstrated sex-related differences in sulfation. Male versus female Sprague–Dawley and F344 rats were found to produce higher amounts of a bisphenol A glucuronide/sulfate conjugate (Pritchett et al., 2002).

As noted in Table 7, one human study reported ~2-fold higher blood bisphenol A concentrations in Japanese men than women (Takeuchi and Tsutsumi, 2002). Based on positive correlation between serum bisphenol A and testosterone concentrations, authors speculated that sex-related differences in bisphenol A concentrations might be due to androgen-related metabolism (Takeuchi and Tsutsumi, 2002). There are no known human studies showing inter-individual or sex-related variations in metabolism that could lead to higher bisphenol A concentrations in blood. Experimental animal studies have not consistently demonstrated higher concentrations of bisphenol A or radioactive dose in one sex (Pottenger et al., 2000; Kurebayashi et al., 2005). In Wistar rats orally administered 1 mg bisphenol A every 2 days for 2 or 4 weeks, liver microsomal UDPGT activity toward 17β-estradiol and testosterone and expression of UGT2B1 protein and mRNA were reduced in males but not females (Shibata et al., 2002). One study reported an ~3-fold higher concentration of blood bisphenol A in male than in female Wistar–Imamichi rats that were apparently not treated, but there were was no sex-related difference in percent glucuronidated bisphenol A in serum (Takeuchi et al., 2004b). However, in an in vitro study conducted with hepatic microsomes, glucuronidation of bisphenol A and expression of *UGT2B1* mRNA were higher in microsomes from female than male rats. As described in more detail in Section 2.1.2.3, one study showed reduced capacity to glucuronidate bisphenol A in livers from pregnant than in non-pregnant rats (Inoue et al., 2004).

Table 60
Human Toxicokinetic Values for Total Bisphenol A Dose

Endpoint	Value	Reference
Oral absorption, %	≥84%	Völkel et al.(2002, 2005)
Dermal absorption, in vitro, %	~10%	European-Union (2003)
T_{max}, min	80	Völkel et al. (2002)
Elimination half-life, hr	4–5.4	Völkel et al. (2002, 2005)

2.6 Summary of General Toxicology and Biologic Effects

Analytical considerations. Free concentrations of BPA measured in various matrices can be affected by analytic techniques and methodology. Free bisphenol A contamination from reagents and plastic ware may contribute to the measured free concentration of bisphenol A (Tsukioka et al., 2004; Völkel et al., 2005). Analytical techniques employed may incorrectly overestimate the free concentration of measured bisphenol A. HPLC with ultraviolet, fluorescence, or electrochemical detection is unable to make definitive identification of bisphenol A or bisphenol A glucuronides, because similar retention times may occur for the metabolites of other endogenous and exogenous compounds (Völkel et al., 2005). Bisphenol A glucuronide can also be hydrolyzed and in some cases degraded to unknown components either in acidic or basic pH solutions of diluted urine, adding another potential source of error in the measurement of sample levels of bisphenol A and its

Table 61
Concentrations of Bisphenol A in Maternal and Fetal Samples[a]

	Bisphenol A concentrations, μg/L, median (range) or mean ± SD			
	Serum or plasma			
Study description; analytical method	Maternal	Fetal	Other fetal compartments	Reference
21 samples collected in women in the U.S. before 20 weeks gestation; LC with electrochemical detection			0.5 (Non-detectable <0.5–1.96) 10% of amniotic fluid samples detectable	Engel et al. (2006)
37 German women, 32–41 weeks gestation; GC/MS	3.1 (0.3–18.9); 4.4 ± 3.9	2.3 (0.2–9.2); 2.9 ± 2.5	12.7 (ng/g) (1.0–104.9) 11.2 ± 9.1) Placental tissue	Schönfelder et al. (2002b)
9 sets of maternal and umbilical cord blood samples obtained at birth in Japanese patients; HPLC	0.43 (0.21–0.79) 0.46 ± 0.2	0.64 (0.45–0.76) 0.62 ± 0.13		Kuroda et al. (2003)
180 Malaysian newborns; GC/MS		Non-detectable (<0.05) to 4.05 88% of samples detectable		Tan and Mohd (2003)

[a]As discussed in Section 1.1.5, ELISA may overestimate bisphenol A concentrations so only results from studies based on HPLC, GC/MS, and LC/MS are presented.

Table 62
Toxicokinetic Values for Bisphenol A in Non-Pregnant Animals

Model	Endpoint	Value	Reference
Rats orally exposed to ≤100 mg/kg bw	T_{max}, hr	0.083–0.75	Domoradzk et al. (2004); Pottenger et al. (2000); Negishi et al. (2004b); Takahashi and Oishi (2000); Yoo et al. (2001)
Ovariectomized, adult rats gavaged with bisphenol A at 10 and 100 mg/kg bw	T_{max1} /T_{max2}, hr	0.5–1.5 / 3–6	Upmeier et al. (2000)
Immature rats orally dosed with ≤10 mg/kg bw	T_{max} hr	0.25–3	Domoradzk et al. (2004)
Monkeys orally dosed with ≤100 mg/kg bw	$T_{max,}$ hr	0.7	Negeshi et al. (2004b)
Chimpanzees orally dosed with 10 mg/kg bw	T_{max}, hr	0.5	Negeshi et al. (2004b)
Rats s.c. dosed with ≤100 mg/kg bw	T_{max}, hr	1	Negeshi et al. (2004b)
Monkeys s.c. dosed with ≤100 mg/kg bw	T_{max}, hr	2	Negeshi et al. (2004b)
Chimpanzees s.c. dosed with 10 mg/kg bw	T_{max}, hr	2	Negeshi et al. (2004b)
Ovariectomized, adult rats orally dosed with bisphenol A at 10 and 100 mg/kg bw	Bioavailability, %	16.4 and 5.6[a]	Upmeier et al. (2000)
Rats orally dosed with 10 mg/kg bw	Bioavailability, %	5.3	Yoo et al. (2001)
Rat	Plasma protein binding, %	90–95%.	Kurebayashi et al. (2003); Teeguarden et al. (2005)
Rats orally dosed with 10 mg/kg bw	C_{max}, μg/L	14.7–63	Domoradzk et al., (2004); Yoo et al. (2001)
Rats orally dosed with 100 mg/kg bw	C_{max}, μg/L	580	Negeshi et al. (2004b).
Ovariectomized, adult rats orally dosed with (mg/kg bw):	C_{max1}/C_{max2}, μg/L		Upmeier et al. (2000)
10			30/40
100			150/134
Oral doses (mg/kg bw) in immature rats at each age:	C_{max} (μg/L)	Range of values for males and females:	Domoradzki et al. (2004)
1 (PND 4)		30–60	
10 (PND 4)		10,200–48,300	
1 (PND 7)		40–80	
10 (PND 7)		1100–1400	
1 (PND 21)		5–6	
10 (PND 21)		200	
Monkeys orally dosed with 10 and 100 mg/kg bw	C_{max}, μg/L	2793 and 5732[a]	Negeshi et al. (2004b)
Monkeys orally dosed with 10 mg/kg bw	C_{max}, μg/L	96–325	Negeshi et al. (2004b)
Rats s.c. dosed with 10 and 100 mg/kg bw	C_{max}, μg/L	872 and 3439[a]	Negeshi et al. (2004b)
Monkeys s.c. dosed with 10 and 100 mg/kg bw	C_{max}, μg/L	57,934 and 10,851[a]	Negeshi et al. (2004b)
Chimpanzees s.c. dosed with 10 mg/kg bw	C_{max}, μg/L	1026–2058	Negeshi et al. (2004b)
Oral doses (mg/kg bw) in immature rats at each age:	AUC, μg-hr/L	Range of values for males and females:	Domoradzki et al. (2004)
1 (PND 4)			100–200
10 (PND 4)			7200–23,100
1 (PND 7)			100
10 (PND 7)			1700–1900
10 (PND 21)			1000–1100
Rats orally dosed with 10 mg/kg bw	AUC, μg-hr/L	85.6	Yoo et al. (2001)
Rats orally dosed with 100 mg/kg bw	$AUC_{0-24\,h}$, μg-hr/L	1353	Negeshi et al. (2004b).
Monkeys orally dosed with 10 and 100 mg/kg bw	$AUC_{0-24\,h}$, μg-hr/L	3247 and 52,595[a]	Negeshi et al. (2004b).
Chimpanzees orally dosed with 10 mg/kg bw	$AUC_{0-24\,h}$, μg-hr/L	813–1167	Negeshi et al. (2004b).
Rats s.c. dosed with 10 and 100 mg/kg bw	$AUC_{0-24\,h}$, μg-hr/L	3377 and 23,001[a]	Negeshi et al. (2004b).

Table 62
Continued

Model	Endpoint	Value	Reference
Monkeys s.c. dosed with 10 and 100 mg/kg bw	$AUC_{0-24\,h}$, μg-hr/L	3247 and 39,040[a]	Negeshi et al. (2004b).
Chimpanzees s.c. dosed with 10 mg/kg bw	$AUC_{0-24\,h}$, μg-hr/L	12,492–21,141	Negeshi et al. (2004b).
Rats orally dosed with 10 mg/kg bw	Apparent volume of distribution, L/kg	4273	Yoo et al. (2001)
Immature rats orally dosed with ≤10 mg/kg bw	Half-life, hr	4.3–21.8	Domoradzki et al. (2004)
Rats orally dosed with 10 mg/kg bw	Terminal elimination half-life, hr	21.3	Yoo et al. (2001)
Rats orally dosed with 10 mg/kg bw	Oral clearance, mL/min/kg	2352.1	Yoo et al. (2001)

[a]Results presented for low and high dose.

Table 63
Age and Route Factors Affecting Free Bisphenol A Concentrations in Blood

Model and regimen	Findings for free bisphenol A in blood	Reference
Age effects of rat oral dosing at 1 or 10 mg/kg:		Domoradzki et al. (2004)
4 days of age	1.5–56.8 mg/L	
7 days of age	1.1–12.2 mg/L	
21 days of age	0.8–8 mg/L	
Adulthood	0.07–0.6 mg/L	
S.C. or gavage dosing of 18–21-day-old rats with 160 mg/kg bw/day	**[93% lower]** with oral than s.c. dosing 2.9 mg/L s.c. (plasma) 0.2 mg/L oral (plasma)	Yamasaki et al. (2000)
Route effects in rats administered 10 or 100 mg/kg bw:		Pottenger et al. (2000)
Oral	[<2–8%] BLQ (males); 0.04 mg/L (females) (at 10 mg/kg)	
s.c.	[65–76%] 0.69 (males); 0.87 mg/L (females) (at 10 mg/kg)	
i.p.	[27–51%] 0.39 (males); 0.34 mg/L (females) (at 10 mg/kg)	
Route effects in monkeys:	Percent of dose:	Kurebayashi et al. (2002)
i.v.	5–29	
Oral	0–1	

conjugates (Waechter et al., 2007). These considerations taken together, suggest that it is possible that free bisphenol A concentrations reported in biological samples may be overestimated.

2.6.1 Toxicokinetics and metabolism. Human toxicokinetic data for bisphenol A are summarized in Table 60. In humans ingested bisphenol A is rapidly glucuronidated and circulated as bisphenol A glucuronide (Völkel et al., 2002). There is no evidence of enterohepatic circulation (Völkel et al., 2002). Most of the bisphenol A dose is excreted by humans through urine; bisphenol A recoveries in urine were reported at ≥84% within 5 hr of dosing (Völkel et al., 2005) and 100% within 42 hr of dosing (Völkel et al., 2002). Human urinary profiles were reported at ∼33–70% bisphenol A glucuronide, ∼10–33% parent compound, and ∼5–34% bisphenol A sulfate conjugate (Kim et al., 2003b; Ye et al., 2005). The presence of bisphenol A in human fetal tissues or fluids demonstrates that bisphenol A is distributed to the human conceptus (Ikezuki et al., 2002; Schönfelder et al., 2002b; Yamada et al., 2002; Kuroda et al., 2003; Tan and Mohd, 2003; Engel et al., 2006) (Table 61). Results from a limited number of studies indicated that fetal bisphenol A concentrations are within the same order of magnitude as maternal blood concentrations (Schönfelder et al., 2002b; Kuroda et al., 2003) and amniotic fluid bisphenol A concentrations are ∼1 order of magnitude lower than maternal blood concentrations (Yamada et al., 2002). Significantly higher mean bisphenol A concentrations were reported in the blood of male than female fetuses (3.5±2.7 vs. 1.7±1.5 ng/mL, $P = 0.016$). Bisphenol A concentrations were measured in placenta samples at 1.0–104.9, median 12.7 μg/kg (Schönfelder et al., 2002b). There were no differences between pregnant and non-pregnant blood levels (median in μg/L 0.44, range = 0.22–0.87; mean+SD = 0.46+0.20) (Kuroda et al., 2003). Median bisphenol A concentrations in human milk were reported to be ≤1.4 μg/L (Calafat et al., 2006; Ye et al., 2006). One of the studies reported a milk/serum ratio of 1.3 (Sun et al., 2004).

Animal toxicokinetic data for bisphenol A are summarized in Table 62. Following oral intake of bisphenol A by rats, most of the dose (≥77%) is glucuronidated and circulated as bisphenol A glucuronide (Miyakoda et al., 2000; Domoradzki et al., 2003; Kurebayashi et al., 2005). Most bisphenol A (90–95%) circulates bound to plasma proteins (Kurebayashi et al., 2003) [reviewed in (Teeguarden et al., 2005)]. In a single study in mice injected

Table 64
Tissue Radioactivity in Pregnant and Fetal Rats After Oral Administration of 500 μg/kg ^{14}C-Bisphenol A to Dams

	Radioactivity concentration (ng bisphenol A eq. g^{-1} or mL^{-1})					
	2 days of gestation		15 days of gestation		18 days of gestation	
Dam and fetal tissues	30 min[a]	124 hr	30 min[a]	24 hr	30 min[a]	24 hr
Dams						
Amniotic fluid	ND	ND	NQ	NQ	NQ	NQ
Blood	43.32	4.33	37.51	3.83	30.99	10.79
Ovary	21.94	3.96	13.91	NQ	15.67	3.49
Placenta	15.43	NQ	18.12	NQ	9.91	3.86
Uterus	22.68	ND	NQ	NQ	15.31	NQ
Fetus	NQ	NQ	NQ	NQ	NQ	3.28
Fetal membrane	NQ	NQ	NQ	NQ	NQ	10.87
Yolk sac	NQ	ND	ND	ND	NQ	54.14

[a]Each time shows the sacrifice time after oral administration of ^{14}C-bisphenol A to each pregnant rat.
ND, not determined (indistinguishable); NQ, nonquantifiable (below LOQ).

Table 65
Summary of Elimination Information for Bisphenol A

Model	Elimination route	Dose eliminated (%)	Compound and metabolite profile	Reference
Pregnant or non-pregnant rats orally, i.p., or s.c. dosed with <100 mg/kg bw	Feces	50–83	Bisphenol A (83–93%); Bisphenol A glucuronide (2–3%)	Domoradzki et al. (2003); Snyder et al. (2000); Pottenger et al. (2000)
	Urine	13–42	Bisphenol A (3–23%); Bisphenol A glucuronide (57–87%); Bisphenol A sulfate (2–7%)	
Rats orally or i.v. dosed with 0.1 mg/kg bw	Feces	64–88	Not reported	Kurebayashi et al. (2003)
	Urine	10–34		
Rats orally or i.v. dosed with 0.1 mg/kg bw	Bile	45–66 within 5 hr	Bisphenol A glucuronide (84–88%)	Kurebayashi et al. (2003)
Rats orally dosed with 100 mg/kg bw/day	Feces	Not reported	Bisphenol A (61% of dose)	Kurebayashi et al. (2003)
	Urine		Bisphenol A and bisphenol A sulfate (≤1.1% of dose); Bisphenol A glucuronide (6.5% of the dose)	
	Bile		Bisphenol A glucuronide (41% of dose)	
Pregnant mice injected with 0.025 mg/kg bw bisphenol A	Feces	Not reported	Bisphenol A (>95%)	Zalko et al. (2003)
	Urine		Major metabolites: bisphenol A glucuronide and hydroxylated bisphenol A glucuronide	
	Bile		Bisphenol A glucuronide (>90%)	
Monkeys orally or i.v. dosed with 0.1 mg/kg bw	Feces	2–3	Not reported	Kurebayashi et al. (2002)
	Urine	79–86		

with a low dose (0.025 mg/kg bw), the most abundant compound in most tissues was bisphenol A glucuronide (Zalko et al., 2003). Most of a bisphenol A dose is circulated as the glucuronide in monkeys (Kurebayashi et al., 2002). As noted in Table 63, free bisphenol A in blood represents ≤8% of the dose in rats and ≤1% of the dose in monkeys following oral dosing; higher concentrations of free bisphenol A in blood were observed following parenteral dosing. The presence of 2 or more C_{max} values for radioactivity or bisphenol A, an indication of enterohepatic circulation, was noted in some rat studies (Upmeier et al., 2000; Domoradzki et al., 2003; Kurebayashi et al., 2005). In rats, glucuronidation of bisphenol A was shown to occur in intestine (Sakamoto et al., 2002; Inoue et al., 2003a) and liver (Inoue, 2004). UGT2B1 was identified as a liver enzyme capable of

glucuronidating bisphenol A, and possible involvement of other liver isoforms was noted (Yokota et al., 1999). There are some data indicating that glucuronidation capacity is very limited in fetuses and lower in immature than adult animals. Little-to-no UGT2B activity toward bisphenol A was detected in microsomes of rat fetuses; activity of the enzyme increased linearly following birth (Matsumoto et al., 2002). In an in vitro study comparing clearance of bisphenol A by hepatic microsomes from rats of different ages, activity was lower in microsomes from fetuses than in those from immature animals and adults [reviewed in (European-Union, 2003)]. As noted in Table 63, immature rats are capable of glucuronidating bisphenol A, but activity appears to increase with age. One study demonstrated that neonatal rats were able to glucuronidate a larger fraction of a lower (1 mg/kg bw) than higher (10 mg/kg bw) bisphenol A dose (Domoradzki et al., 2004).

Kurbayashi et al. (2005) evaluated fetal and maternal rat bisphenol A during different stages of pregnancy. Bisphenol A labeled with carbon-14 was administered p.o. to male and female Fischer (F344) rats at relatively low doses (20, 100, and 500 μg/kg), and i.v. injected at 100 and 500 μg/kg). ^{14}C-bisphenol A (500 μg/kg) was also administered orally to pregnant and lactating rats to examine the transfer of radioactivity to fetuses, neonatal rats, and milk (Table 64). Radioluminographic determination using phosphor imaging plates was employed to achieve highly sensitive determination of radioactivity. Absorption ratios of radioactivity after three oral doses were high (35–82%); parent ^{14}C- bisphenol A in the circulating blood was quite low, however, suggesting considerable first-pass effect. After an oral dose of 100 μg/kg ^{14}C- bisphenol A, the radioactivity was distributed and eliminated rapidly, but remained in the intestinal contents, liver, and kidney for 72 hr. The major metabolite in the plasma and urine was bisphenol A glucuronide, whereas most of the bisphenol A was excreted with the feces as free bisphenol A. A second peak in the time-course of plasma radioactivity suggested enterohepatic recirculation of bisphenol A glucuronide. There was limited distribution of ^{14}C- bisphenol A to the fetus and neonate after oral administration to the dam. Significant radioactivity was not detected in fetuses on GD 12 and 15. On GD 18, however, radioactivity was detected in the fetal intestine and urinary bladder 24 hr after oral dosing of ^{14}C- bisphenol A to the dam. The distribution pattern of radioactivity in pregnant rats was essentially the same as that in non-pregnant female rats. The distribution levels were dose-dependent in most of the tissues. There was limited distribution of ^{14}C-bisphenol A to the fetus. Radioactivity in fetal tissues was undetectable except on GD 18 in the fetal urinary bladder and intestine. On GD 18, the amount of radioactivity in fetal tissues at 24 hr was about 30% that in maternal blood, and the yolk sac contained a much higher level of radioactivity than the maternal blood. The Expert Panel thought these differences were a consequence of the routes of administration, i.v. or p.o., because only trace amounts of parent bisphenol A dosed orally appeared in the plasma.

The major metabolite of bisphenol A is the glucuronide conjugate. Another metabolite that has been commonly detected in urine is bisphenol A sulfate. Excretion patterns for bisphenol A are summarized in Table 65. As noted in Table 65, the major elimination routes for bisphenol A in rodents are feces and bile; a lower percentage of the dose is eliminated through urine. The major compound detected in feces is bisphenol A and the major compound detected in bile and urine is bisphenol A glucuronide. Excretion patterns and metabolic profiles observed in rodents dosed orally or parenterally with low (<1 mg/kg bw/

Table 67
Toxicokinetic Values for Radioactive Dose in Pregnant Rats (Total Bisphenol A)[a]

Endpoint	Value
C_{max1}/ C_{max2}, μg eq/L	370–1006/211–336
T_{max1}/ T_{max2}, hr	0.25/12–24
Time to non-quantifiable concentration, hr	72–96
AUC ^{14}C, μg –eq ·←hr/L	7100–12,400
AUC Bisphenol A glucuronide, μg –eq ·←hr/L	6800–12,300

[a]Dormoradzki et al. (2003).
Dams were gavaged with 10 mg/kg bw/day on GD 6–10, 14–18, or 17–21.
GD, gestation day.

Table 66
Toxicokinetic Values for Free Bisphenol A in Pregnant Rats and Fetuses

Dose	Endpoint	Maternal	Fetal	Reference
1000 mg/kg bw orally on GD 18	C_{max}, μg/L	14,700	9220	Takahashi and Oishi (2000)
10 mg/kg bw orally on GD 19	Concentration 1-hr post-dosing, μg/L	34	11	Miyakoda et al. (1999)
2 mg/kg bw i.v. on 1 day between GD 17–19	C_{max}, μg/L	927.3	794	Shin et al. (2002)
1000 mg/kg bw orally on GD 18	T_{max}, min	20	20	Takahashi and Oishi (2000)
2 mg/kg bw i.v. on 1 day between GD 17–19	T_{max}, hr	No data	0.6 ± 0.3	Shin et al. (2002)
1000 mg/kg bw orally on GD 18	AUC, μg ·←hr/L	13,100	22,600	Takahashi and Oishi (2000)
2 mg/kg bw i.v. on 1 day between GD 17–19	AUC, μg ·←hr/L	905.5	1964.7	Shin et al. (2002)
1000 mg/kg bw orally on GD 18	Mean retention time, hr	10.6	20.0	Takahashi and Oishi (2000)
1000 mg/kg bw orally on GD 18	Variance in retention time, hr^2	203	419	Takahashi and Oishi (2000)
2 mg/kg bw i.v. on 1 day between GD 17–19	Mean residence time, hr	3.0	3.0	Shin et al. (2002)
1000 mg/kg bw orally on GD 18	Half-life, hr:			Takahashi and Oishi (2000)
	From 20–40 min	0.0952	0.55	
	From 40 min–6 hr	2.58	1.60	
	From 6–48 hr	4.65	173	
2 mg/kg bw i.v. on 1 day between GD 17–19	Elimination half-life, hr	2.5	2.2	Shin et al. (2002)

Table 68
Toxicokinetic Values for Free Bisphenol A in Lactating Rats[a]

Endpoint	Blood value	Milk value
Systemic clearance, mL/min/kg	119.2/142.4/154.1[b]	
Steady state bisphenol A concentration, ng/mL	66.1/120.0/217.1[b]	173.1/317.4/493.9[b]
Milk/serum ratio	2.7/2.6/2.4[b]	

Rats were i.v. injected 0.47, 0.94, or 1.88 mg/kg bw and then infused over a 4-hr time period with 0.13, 0.27, 0.54 mg/hr.
[a]Yoo et al. (2001).
[b]Effect at each dose, from low to high dose.

Table 69
Toxicokinetic Values for Radioactive Dose in Lactating Rats (Total Bisphenol A)[a]

Endpoint	Blood value	Milk value
C_{max}, μg – eq/L	27.2	4.46
T_{max}, hr	4	8
Elimination half-life, hr	31	26
AUC (0–48 hr), μg – eq ·hr/L)	689	156

[a]Kurebayashi et al. (2005).
Rats were orally dosed with 0.5 mg/kg bw on PND 11.

day) or high-doses (10–100 mg/kg bw/day) were similar. In contrast to rodents and similar to humans, most of the dose in orally- or i.v.-exposed monkeys was eliminated through urine.

Toxicokinetics of bisphenol A were examined in pregnant rats and are summarized in Table 66 for free bisphenol A and Table 67 for total dose. One study demonstrated similar disposition, metabolism, and elimination of bisphenol A in pregnant and non-pregnant rats (Domoradzki et al., 2003). A number of rodent studies demonstrated distribution of bisphenol A or radioactive dose to fetuses following oral dosing of the dam (Miyakoda et al., 1999; Takahashi and Oishi, 2000; Domoradzki et al., 2003; Kim and Hwang, 2003; Kabuto et al., 2004; Kurebayashi et al., 2005). Bisphenol A distribution to fetus was also demonstrated with i.v. dosing of rats (Shin et al., 2002) and s.c. dosing of mice or monkeys (Uchida et al., 2002; Zalko et al., 2003). In a study in which bisphenol A was orally administered to rats on GD 19, bisphenol A glucuronide was not detected in fetuses (Miyakoda et al., 2000); study authors noted the possibilities that bisphenol A glucuronide was not likely transferred from dams to fetuses and that fetuses do not likely possess glucuronidation ability. Some of the studies demonstrated slower elimination of bisphenol A from fetuses than maternal blood following oral dosing (Miyakoda et al., 1999; Takahashi and Oishi, 2000).

Toxicokinetics data in lactating rats are summarized in Table 68 for free bisphenol A and Table 69 for total dose. Distribution of bisphenol A to milk and/or nursing pups was demonstrated in rodent studies with oral or i.v. exposures (Snyder et al., 2000; Yoo et al., 2001; Kurebayashi et al., 2005). One study reported that most of the bisphenol A dose is present as bisphenol A glucuronide in milk of lactating rats (Snyder et al., 2000). In a study that compared bisphenol A concentrations in maternal serum, milk, and offspring after rat dams were administered low oral doses (0.006 or 6 mg/kg bw/day), a significant increase in bisphenol A concentration was only observed in the serum of dams from the high-dose group on PND 21; no increase was observed in milk or pups (Yoshida et al., 2004). Another study demonstrated higher concentrations of bisphenol A in milk compared to maternal serum after i.v. dosing of rat dams (Yoo et al., 2001).

A number of in vitro studies compared bisphenol A metabolic velocity rates in microsomes or hepatocytes from rodents and humans. Generally, faster rates were demonstrated by rodent than human hepatocytes and microsomes (Elsby et al., 2001; Pritchett et al., 2002) [reviewed in (European-Union, 2003)]. One of the studies noted that adjustment for total hepatocyte number in vivo resulted in higher predicted rates for humans than rodents (Pritchett et al., 2002). The European Union (2003) noted that the interpretation of such studies should included knowledge about in vivo conditions such as varying metabolic capacity of hepatic cells, relationship of hepatic size to body size, and possibly important physiological endpoints such as blood flow.

2.6.2 General toxicity. Gross signs of toxicity observed in rats acutely exposed to bisphenol A included pale livers and gastrointestinal hemorrhage [reviewed by the (European-Union, 2003)]. Acute effects of inhalation exposure in rats included transient and slight inflammation of nasal epithelium and ulceration of the oronasal duct. Based on LD_{50} observed in animals, the European Union (2003) concluded that bisphenol A is of low acute toxicity through all exposure routes relevant to humans. According to the European Union (2003), there is evidence that bisphenol A is irritating and damaging to the eye and is irritating to the respiratory tract and possibly the skin. Findings regarding sensitization potential were not clear.

Possible target organs or systems of toxicity identified in repeat-dose animal studies with oral dosing included intestine, liver, kidney, and male, and female reproductive systems [reviewed in (NTP, 1982; Yamasaki et al., 2002a; European-Union, 2003)]. Intestinal findings (effect levels) in rats included cecal enlargement ($\geq$25 mg/kg bw/day) and cecal mucosal hyperplasia ($\geq$200 mg/kg bw/day). Hepatic effects included prominent hepatocyte nuclei or inflammation in rats ($\geq$500 mg/kg bw/day), multinucleated giant hepatocytes in mice ($\geq$120 mg/kg bw/day), and increased weight with no evidence of histopathology in dogs ($\geq$270 mg/kg bw/day). Renal tubule degeneration or necrosis was observed in rats dosed with $\geq$500 mg/kg bw/day. Reproductive findings are discussed in Section 4.0. Effects in subchronic inhalation studies in rats included cecal enlargement resulting from distention by food and transient, slight hyperplasia and inflammation of epithelium in the anterior nasal cavity; both effects occurred at ($\geq$50 mg/m^3).

2.6.3 Estrogenicity. Estrogenicity of bisphenol A has been evaluated using in vitro (Table 52) and in vivo (Table 53) assays. In those studies estrogenic potency was compared to 17β-estradiol, ethinyl estradiol, diethylstilbestrol, and, in one study, estrone. There is considerable variability in the results of these studies with the estrogenic potency of bisphenol A ranging over about 8 orders of magnitude (Fig. 2). On the other hand, the average potency only differs by 1 order of magnitude and there is very little difference between rat and mouse means.

Most in vivo estrogenicity studies examined effects on uterine weights of intact weanling or ovariectomized adult rats or mice. The potency of bisphenol A in increasing uterine weight varied over ~4 orders of magnitude. Uterine weight findings can be affected by the time period between dosing and measurement. Most, but not all studies, showed a greater effect on uterine weight with s.c. than with oral dosing. The greater activity of s.c. than oral bisphenol A is presumably due to glucuronidation of the orally administered compound with consequent loss of estrogenicity (Matthews et al., 2001). Inter-strain variability in rats has been evaluated as a source of variability in estrogenicity assays. (see Section 4.0 for additional discussion) Inter-laboratory variability has been noted for uterotropic effects in intact weanling mice exposed to bisphenol A (Tinwell and Joiner, 2000); one factor that can result in variability is body weight of the animal. Use of mice with lower body weights results in lower and less variable control uterine weights and greater likelihood of bisphenol A effect (Tinwell and Joiner, 2000; Ashby et al., 2004). In in vivo studies examining gene expression profiles, some but not all gene expression changes were consistent between bisphenol A and reference estrogens (Tinwell and Joiner, 2000; Naciff et al., 2002; Singleton et al., 2004; Terasaka et al., 2006); ER-independent activity was suggested by 1investigator (Singleton et al., 2004). **[Based on one comprehensive study of the effects of bisphenol A orally delivered from 60–1000 mg/kg for 3–7 days, the Expert Panel concludes that the uterotrophic responses were only found at higher does (Ashby, 2002; Kanno et al., 2003a) whereas s.c. dosing produced consistent uterine weight increases at lower doses.]**

2.6.4 Androgenic activity. In the majority of in vitro tests conducted, bisphenol A was not demonstrated to have androgenic activity (Sohoni and Sumpter, 1998; Gaido et al., 2000; Kitamura et al., 2005; Xu et al., 2005). Anti-androgenic activity was demonstrated in systems using cells transfected with three different androgen receptor reporting systems (ARE-luc, MMTV-lacZ, and C3-luc) (Table 56). No consistent effects were observed on male accessory reproductive organ weights in 3 in vivo studies in which rats were dosed with bisphenol A at $\leq$600 mg/kg bw/day; the study authors concluded that bisphenol A does not have anti-androgenic or androgenic activity (Kim et al., 2002a; Yamasaki et al., 2003; Nishino et al., 2006).

2.6.5 Genetic toxicity. In in vitro genetic toxicity studies reviewed by the European Union (2003) and/or Haighton et al. (2002), evidence of aneugenic potential, chromosomal aberration, micronuclei formation, and DNA adducts was observed (Table 57). Because of the lack of chromosomal effects in in vivo studies (Table 58) and unknown relevance of DNA adduct formation, which only occurred at high-doses, both groups concluded that bisphenol A is not likely to have genotoxic activity in vivo.

2.6.6 Carcinogenicity. Carcinogenic potential of bisphenol A was evaluated in rats and mice by the NTP (1982) and Huff (2001). NTP concluded that under the conditions of the study, there was no convincing evidence that bisphenol A was carcinogenic in F344 rats or B6C3F$_1$ mice. However, NTP stated that there was suggestive evidence of increased cancer in the hematopoietic system based on marginally significant increases in leukemia in male rats, non-statistically significant increases in leukemia in female rats, and a marginally significant increase in combined incidence of lymphoma and leukemia in male mice. A statistically significant increase in testicular interstitial cell tumors in aging F344 rats was also considered suggestive evidence of carcinogenesis. The effect was not considered conclusive evidence because of the high incidence of the testicular neoplasm in aging F344 rats (88% incidence in historical controls). Both the European Union (2003) and Haighton et al. (2002) stated that the evidence does not suggest carcinogenic activity of bisphenol A in rats or mice. Conclusions by the European Union (2003) and Haighton et al. (2002) were based on factors such as lack of statistical significance for leukemia, mammary gland fibroadenoma, and Leydig cell tumors, lack of activity at noncytotoxic concentrations in both in vitro genetic toxicity tests and an in vivo mouse micronucleus test, and unlikely formation of reactive intermediates at doses that do not saturate detoxification pathways.

2.6.7 Potentially sensitive subpopulations. Studies in humans and laboratory animals demonstrated developmental changes in UDPGT gene expression or enzyme activity that could potentially affect the concentration of free bisphenol A reaching target organs because of a differential capacity for bisphenol A glucuronidation. In humans, activities for some UDPGT isozymes were reported to be very low at birth but increased with age (Coughtrie et al., 1988). No transcripts for UDPGT were detected in samples from 20-week-old human fetuses and activity for some UDPGT enzymes was lower in children than adults (Strassburg et al., 2002). Compared to adults, human fetal uridine 5′-diphosphoglucuronic acid concentrations were 5-fold lower in liver and 1.5-fold lower in kidney (Cappiello et al., 2000). It is not clear if any of the isozymes examined are involved in bisphenol A glucuronidation by humans. Human findings were consistent with rodent studies that demonstrated no or limited glucuronidation capacity by fetuses (Miyakoda et al., 2000; Matsumoto et al., 2002; Domoradzki et al., 2003) and lower glucuronidation capacity in immature than adult rats (Matsumoto et al., 2002; European-Union, 2003; Matsumoto et al., 2004).

Some studies suggested possible gender-related differences in sulfation capacity in humans (Pritchett et al., 2002; Kim et al., 2003b) and laboratory animals (Pritchett et al., 2002). One study in humans demonstrated no differences in urinary bisphenol A concentrations in individuals carrying a sulfotransferase genotype associated with greater activity (Yang et al., 2003).

3.0 DEVELOPMENTAL TOXICITY DATA

The Panel attended to multiple design and analysis characteristics in judging the acceptability of individual studies. It was our consensus that for a study to be acceptable for this review process, several conditions had to be met. First, effects related to litter of origin needed to be accounted for in design and statistical procedures. Second, animals needed to be dosed via the dam or directly under individual housing conditions. Concern that multiple exposures within a cage to different animals could cause cross-animal contamination across cage-mates led to the determination that this design was not acceptable. Third, a minimum of 6 animals per

treatment condition needed to be used to provide minimal confidence in results. Fourth, if similar tests were conducted at multiple ages, the statistical analyses needed to account for repeated measurement in order not to inflate degrees of freedom. The Panel carefullyconsidered the merits of each study according to these primary criteria, and the related design characteristics represent the most common reasons for judging a study to be unacceptable for our review process. Our intent was to have our review depend most heavily on studies that would have reduced risks for false negative or false positive findings.

In addition, the Panel carefully considered the value of studies where bisphenol A was administered anywhere other than to the mouth or stomach of the experimental animal. Human exposure is overwhelmingly oral, and oral exposure produces an internal metabolite profile which is overwhelmingly dominated by the (inactive) glucuronide in both rats and humans. Subcutaneous or parenteral injections result in blood levels of active parent compound which are much higher than those seen after oral exposure. In light of these pharmacokinetic differences, the Panel concluded that injection studies, unless they proved otherwise, would produce irrelevantly high internal doses of the active parent compound, and would tend to produce "false positive" effects from the point of view of the human oral situation. Thus, the Panel viewed those otherwise adequate studies that injected bisphenol A as providing "supplemental" information (i.e., of limited utility), unless they also analyzed the levels of parent compound and metabolites after the injection. The intent of this approach is limit the impact of those studies which produced an unrealistic and irrelevant internal metabolite profile (i.e., one which is significantly different from that experienced by humans). Thus, the closer any given study came to replicating the human situation, the more weight it had in the final analysis.

The report below mentions "dosing procedures" as reasons for limiting the adequacy or utility of various studies. This has been used to mean non-gastric administration (s.c. injection, intramuscular [i.m.] injection, i.p. injection, or intracerebroventricular injection).

The Panel also had extensive discussion about dosing vehicles. Dimethyl sulfoxide (DMSO) has significant biological activities of its own (Santos et al., 2003), and the experience of the Panel is that DMSO can help move solutes into cells. Increasing the DMSO concentration can produce a greater solute effect, even when holding that solute concentration stable. The real impact of this for in vivo injections is uncertain, and this effect is likely to be small at the dosing volumes administered in the studies considered here. The use of 100% DMSO as a vehicle for ALZET mini-pump studies is a clear contravention of the directions for mini-pump use[3], as it accelerates the breakdown of the mini-pumps and produces blood levels that are not predictable and therefore not useful for the Evaluative Process. Various oils each can bring their own potential issues, such as oxidative damage, but these were considered and discussed by a sub-team of the Panel and not considered to be consequential for this analysis

The Panel also examined the issue of data that would be expected to result when positive controls were employed. While we did not feel that positive controls were required for studies, when they were used, expected effects needed to be demonstrated to validate that the experimental model was capable of responding to a certain stimulus. This is of even more value when there is no response to the main exposure under study. When looking for estrogenic responses, investigators often use 17β estradiol or diethylstilbestrol. These must be used at adequate doses to produce the desired response. Inadequate challenge by the positive control, resulting in no response, leaves the reader uncertain whether the lack of response is due to the selection of too low a dose, or whether the experimental model is incapable of responding to a sufficient challenge. Even though the Panel, based on its own scientific experience, might conclude that inappropriately low doses had been selected and thus a lack of response is not surprising, the Panel was left with little choice in such situations but to give much less weight to studies where non-effective doses of a positive control compound were used.

The Panel is confident in our assessment of those studies judged adequate and useful, and are focusing our limited time on the consistency and utilization of these data.

3.1 Human

No studies were located on possible human developmental effects of bisphenol A.

3.2 Experimental Animal

Studies are presented by species (rat, mouse, other), route (oral, parenteral), and by whether exposure was during pregnancy or the postnatal period. Studies in which exposures were started during pregnancy and continued after pregnancy are discussed with studies in which exposures occurred postnatally.

3.2.1 Rat—oral exposure only during pregnancy.

3.2.1.1 Evaluation of pre- or perinatal growth and development: Morrissey et al. (1987), supported by NTP/NCTR, examined the effects of prenatal bisphenol A exposure in rats and mice in a study conducted according to GLP. Studies are also available as NTP publications for rats (NTP, 1985c) and mice (NTP, 1985b). The study was conducted in two sets of rats and mice, and data were pooled for each species. **[The data for mice are discussed in Section 3.2.5.1.]** Pregnant CD rats were randomly assigned to groups of ≥ 10 animals in each set of the study, for a total of ≥ 20 animals/dose. On GD 6–15 (GD 0 = sperm or plug), rats were gavaged with bisphenol A at 0 (corn oil vehicle), 160, 320, 640, or 1280 mg/kg bw/day. Doses were based on results of preliminary studies and were expected to result in 10% maternal mortality at the high-dose and no toxicity at the low dose. Purity of bisphenol A was >95% and 2,4′-bisphenol A was reported as an impurity. Dosing

[3]Manufacturer instructions specify use of up to 50% DMSO (http://www.alzet.com/products/checklist.php). One hundred percent DMSO is completely incompatible with the pump reservoir material and will dissolve reservoirs within 24–36 hr. Eighty percent DMSO causes pinholes to appear in the reservoirs within 4–7 days. Thus, if a high concentration of DMSO is used, one most likely will infuse both degraded reservoir material as well as the salt compound which makes up the osmotic layer. These two things, combined with DMSO itself (a strong tissue irritant) will most likely cause tissue inflammation and edema (Kurt Kemling ALZET Associate Product Manager, personal communication, September 14, 2007).

Table 70
Maternal and Developmental Effects in Rats Exposed to Bisphenol A[a]

Endpoint	Dose, mg/kg bw/day						
	100	300	1000	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Dams							
No. pregnant	↔	↔	↓30%				
Body weight gain	↔	↓35%	↓52%	178	152	379	304
Corrected body weight	↔	↓14%	↓15%	631	490	566	424
Food intake on GD 4	↔	↓24%	↓57%	168	147	313	257
No. fetal deaths	↔	↔	↑6.5-fold	827	13	978	585
No. early resorptions	↔	↔	↑6-fold	821	14	980	584
Post-implantation losses	↔	↔	↑11-fold	1278	394		
Fetuses							
No. live /litter	↔	↔	↓36%	929	348	982	713
Male body weight	↔	↓14%	↓20%	456	339	694	497
Female body weight	↔	↔	↓21%	439	328	682	490
Ossification	↔	↔	↓				

[a]Kim et al. (2001b).
↑,↓ Statistically significant increase, decrease compared to controls; ↔ No statistically significant effect compared to controls.

solution concentrations were verified. Pregnant animals were weighed during the study. Rats were killed on GD 20. Liver and uterus were weighed, and corpora lutea and implantation sites were examined. Fetuses were sexed, weighed, and examined for viability and external, visceral, and skeletal malformations. Data were analyzed by Bartlett test for homogeneity of variance, ANOVA and/or William multiple comparison, Dunnett, or Fisher exact probability tests. **[Data were presented and analyzed on a per litter basis.]**

An unexpectedly high number of dams (7 of 27) died in the 1280 mg/kg bw/day group, with most deaths occurring in the second set of animals. Because of the high death rate, the study authors decided not to evaluate data in the 1280 mg/kg bw/day group. Clinical signs that occurred most frequently in dams from the 640 mg/kg bw/day group included lethargy, piloerection, pica, rough coat, wet urogenital area, weight loss, and alopecia. Significant and dose-related decreases in maternal body weights were observed during the entire gestation period and thus were not confined to the GD 6–15 treatment period in rats from the 160, 320, and 640 mg/kg bw/day groups. Body weight corrected for gravid uterine weight was also decreased in all three dose groups. Effects on maternal body weight were most pronounced during the treatment period. **[During the treatment period, dam body weights were 35, 53, and 54% lower in the 160, 320, and 640 mg/kg bw/day groups than in control groups; estimated benchmark doses[4] in mg/kg bw/day were BMD_{10} 113, $BMDL_{10}$ 94, BMD_{1SD} 416, $BMDL_{1SD}$ 321.]** Despite this large effect on maternal body weight, there were no effects on numbers of implantation sites or resorptions, gravid uterine weight, or liver weight. The numbers of litters available for evaluation in the control and 160, 320, and 640 mg/kg bw/day dose group were 23, 26, 24, or 29. There were no significant effects on fetal body weight or viability, percentage males/litter, or malformed fetuses/litter. Study authors concluded that bisphenol A was not teratogenic in rats at doses that cause maternal toxicity.

Strengths/Weaknesses: This study used adequate sample sizes to evaluate the effects of GD 6–15 exposure on maternal body weight during gestation and on implantation and resorption sites/dam, fetal body weight, and fetal viability to GD 20. Strengths are the verification of dosing solutions, use of GLP, adequate n, sensitive evaluation of soft and hard-tissue structures. Weaknesses include no postnatal examination, as well as the absence of data from the 1280 mg/kg bw/day group, the absence of a no-effect dose. The absence of effects on fetal endpoints despite marked reductions in maternal body weight corrected for gravid uterine weight warrants the appropriate conclusion that bisphenol was not teratogenic when based on GD 20 data. Further, a gross visceral exam is likely insensitive to certain abnormalities of the reproductive tract and brain, as noted above.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Kim et al. (2001b), support not indicated, examined the effects of prenatal bisphenol A exposure on developmental toxicity in rats. Sprague–Dawley rats were fed commercial rodent chow (Jeil Feed Co., Daejon, Korea) and housed in polycarbonate cages; no information was provided about bedding. Twenty dams/group were gavaged with 0 (corn oil vehicle), 100, 300, or 1000 mg/kg bw/day bisphenol A [purity not provided] on GD 1–20 (GD 0 = first 24 hr after detection of vaginal sperm or plug). Dose selection was based on the results of a preliminary study that demonstrated maternal and developmental toxicity at doses ≥400 mg/kg bw/day and a lack of effect at doses ≤200 mg/kg bw/day. Endpoints examined in dams during the study were

[4]Benchmark doses are used commonly in a regulatory setting; however, they are used in this study when the underlying data permit their calculation, and are only supplied to provide one kind of description of the dose–response relationship in the underlying study. Calculation of a benchmark dose in this report does not mean that regulation based on the underlying data is recommended, or even that the underlying data are suitable for regulatory decision-making. The BMD10 is the benchmark dose associated with a 10% effect, estimated from a curve fit to the experimental data. The BMDL10 represents the dose associated with the lower 95% confidence interval around this estimate. Unless otherwise indicated, BMD values in this report were calculated using a power model for continuous data and a probit model for dichotomous data using Environmental Protection Agency (EPA) Benchmark Dose Software version 1.3.2.

clinical signs, body weight gain, and food intake. Dams were killed on GD 21 and examined for corpora lutea and implantation sites. Fetuses were sexed, weighed, and examined for viability and external abnormalities. Anogenital distance was measured and alternate fetuses were examined for visceral and skeletal malformations. The dam or litter was considered the statistical unit. Data were analyzed by ANOVA, Scheffé multiple comparison test, Kruskal–Wallis nonparametric ANOVA, Mann–Whitney *U*-test, and Fisher exact probability test.

Statistically significant effects are summarized in Table 70. Dose-dependent clinical signs observed in dams at the 2 highest doses included piloerection, dull fur, reduced locomotor activity, emaciation, sedation, red-colored tears, soft stool, diarrhea, urination, and perineal soiling. Pregnancy failure, as observed by lack of implantation sites, was increased in females from the high-dose group. Maternal body weight, body weight gain, and body weight corrected for gravid uterus weight were reduced at the mid- and high-dose. GD 4 was the only time period when food intake was significantly reduced at the mid- and high-dose. Expansion and congestion of stomach and/or intestines were observed in dams from the high-dose group. Body weights of male fetuses were decreased at the mid- and high-dose, and body weights of female fetuses were reduced at the high-dose. Increases in fetal death, early resorption, and post-implantation loss, accompanied by reduced number of live fetuses, were observed at the high-dose. Anogenital distance was significantly reduced in males from the mid- and high-dose groups, but there were no differences in anogenital distance of males or females when the values were normalized by the cube root of body weight. Significantly reduced ossification was observed in the high-dose group. There were no treatment-related differences in fetal sex ratio or external, visceral, or skeletal malformations. Study authors concluded that exposure of rats to a maternally toxic dose of bisphenol A during the entire gestation period resulted in pregnancy failure, post-implantation loss, reduced fetal body weight, and retarded fetal ossification but not dysmorphogenesis.

Strengths/Weaknesses: This report presents a fairly standard embryo–fetal developmental toxicity study. One strength is that the doses utilized incorporated both a no-effect dose and a high maternally toxic dose, revealing fetal effects only at the high-dose that showed marked maternal toxicity. Measurement of anogenital distance is another strength. Weaknesses include the absence in all groups of information about postnatal viability, and postnatal function. Further, a gross visceral exam is likely insensitive to certain abnormalities of the reproductive tract and brain. However, this type of study does report on the ability of the exposure to cause structural malformations, which are notably absent.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Kim et al. (2003), support not indicated, examined the effects of prenatal bisphenol A exposure on postnatal body and organ weights of Sprague–Dawley rats. Rats were housed in polycarbonate cages. **[No information was provided on feed or bedding material.]** Rats were grouped according to body weight and randomly assigned to dose groups. On GD 7–17 (GD 0 = day of vaginal sperm or plug), at least 10 rats/dose group were gavaged with bisphenol A (>99.7% purity) at doses of 0 (corn oil vehicle), 0.002, 0.020, 0.200, 2, or 20 mg/kg bw/day. Dosing solution concentrations were verified. Dams were weighed and observed for clinical signs of toxicity during the study. Dams were killed on Day 21 of the postpartum period. Corpora lutea, implantation sites, resorptions, and fetal viability were assessed. Maternal liver, kidney, spleen, ovary, and gravid uterus were weighed. Live fetuses were weighed and examined for external and visceral abnormalities. Fetal liver, kidneys, spleen, and reproductive organs were weighed in half the fetuses. **[These methods are produced here as written in the original; although dams were clearly stated to have been killed on PND 21, the "fetal" examinations described appear more consistent with killing of the dams on GD 21.]** Data were analyzed by ANOVA and Student *t*-test. **[It was not clear if the litter or fetus was considered the statistical unit in the evaluation of developmental toxicity data.]**

A significant but non-dose-related increase in dam body weight occurred in the 0.2 mg/kg bw/day group on GD 0–15. Dam body weight was significantly increased on GD 21 in the 2 (by 53%) and 20 (by 43%) mg/kg bw/day groups. No significant differences in dam body weight were noted during the lactation period. Significant changes in dam relative organ weights (dose at which effects were observed) were: increased liver (0.002, 0.020, and 20 mg/kg bw/day); decreased right kidney (0.2 mg/kg bw/day); increased right kidney (2 mg/kg bw/day), and increased uterine (0.2 mg/kg bw/day). There was no effect on ovary weight of dams. The majority of dams were in diestrus when killed. One of 7 dams in the 0.2 mg/kg bw/day group was in proestrus. One of 7 dams in the 0.2 mg/kg bw/day, 1 of 6 dams in the 2 mg/kg bw/day group, and 2 of 8 dams in the 20 mg/kg bw/day group were in diestrus. Body weight effects in male and female offspring were reported in most treatment groups when evaluated at various time points between birth and PND 22. In general, when body weights effects were detected it was an increase in weight of ~12–65%. **[Changes occurred at most dose levels but were not consistent over time and there was little evidence of dose–response relationships. In general, effects appeared to be most pronounced in the lowest dose group.]** Relative weights for several tissues attained statistical significance at 1 or more doses in offspring of both sexes: liver, spleen and right kidney. In addition, relative organ weights for were altered in males for the left kidney, both testes, right epididymis, left seminal vesicle, and prostate gland. There were no effects on ovary or uterus weights. **[In most cases, there was little evidence of a dose–response relationship for organ weights, including male reproductive organs, in offspring.]** Study authors concluded that bisphenol A had estrogenic effects on rat dams and offspring exposed during the gestation period.

Strengths/Weaknesses: While the verification of the dosing solution is a strength, this study is of unclear quality, to the point that there is real confusion about what was actually done. It is indicated that 10 dams were assigned to each dose group but numbers at sacrifice were 7, 7, 6, and 8 across the 4 doses. It is unclear whether fetal data were appropriately analyzed with

litter as the unit. It is unclear when the dams were killed and analyzed. The absence of understandable dose-related effects complicates interpretation at these low doses; although the possibility of unusual low dose effects cannot be discounted.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for inclusion into the evaluation process, due to small sample size and poor documentation and communication about what was done.

3.2.1.2 Evaluation of reproductive organ development: Talsness et al. (2000), supported by the German Federal Ministry for Environmental Protection and Radiation Security, examined the effect of prenatal bisphenol A exposure on the reproductive systems of male and female rats. **[No information was provided about feed, caging, and bedding materials used.]** On GD 6–21, Sprague–Dawley rats ($n = 18–20$/group) were gavaged with 2% corn starch vehicle or bisphenol A [purity not indicated] at 0.1 or 50 mg/kg bw/day. A group of 11 dams was gavaged with 0.2 mg/kg bw/day ethinyl estradiol. Litters were weighed during the lactation period. Pups were weaned on PND 22 (according to Table 1 of the study, PND 1 was apparently the day of birth) and males and females were separated around PND 30. Vaginal opening was examined in 42–91 female offspring/group, and estrous cyclicity was monitored over a 3-week period in 42–53 females/group. At 4 months of age, 5–10 females/group were killed during diestrus and 20 females/group were killed while in estrus. A histopathological evaluation of vaginal tissue was conducted in 5 animals **[assumed 5/group]**. In 44–112 male offspring/group, anogenital distance was measured on PND 3, 15, and 21 and days of testicular descent and preputial separation were recorded. Males were killed on PND 70 ($n = 20$/group) or 170 ($n = 17–20$/group). Blood LH and testosterone concentrations were measured in 14–20 animals/group/time period. Sperm and spermatid numbers and sperm production and transit rates were determined in all offspring. Histopathological evaluation of the testis was conducted in 2 animals **[assumed/group]**. Body, reproductive organ, and liver weights were measured in all male and female offspring killed. Data from female rats were analyzed by ANOVA with post-hoc Dunnett test or Fisher test. Data from male rats were analyzed by ANOVA and Dunnett test. **[It appears that offspring were considered the statistical unit.]**

Pup body weights at birth were unaffected in the bisphenol A group, but on PND 22, pup body weights were lower **[by 28%]** in the low-dose group than in the control group. Study authors noted that the mean litter size in the low-dose group was larger by 2.6 pups than in the control group. Vaginal opening was delayed in the low-dose group and accelerated in the high-dose group. When estrous cyclicity data were evaluated according to total number of cycles, there was an increase in estrous phases lasting more than 1 day and prolongation of the cycle length in the high-dose group. Evaluation of estrous cycles by individual rat indicated a decrease in the percentage of low-dose females with 3 consecutive 1-day estrus phases. The only terminal body and organ weight effects occurred in the low-dose group and included decreased absolute liver weight in females killed in estrus and decreased body and uterus weights in females killed in diestrus or in estrus. There were no effects on relative organ weights. Histological observations in vaginal tissue of bisphenol A-exposed rats included less pronounced cornification during estrus and more pronounced mucification during diestrus, with magnitude of effect greater in the low- than the high-dose group. Observations in the animals exposed to ethinyl estradiol included decreased pup birth weight, delayed vaginal opening, near-persistent estrus, decreased absolute and relative uterus weights, and changes in vaginal histology similar to those described for the low-dose bisphenol A group.

Decreased anogenital distances was observed in the bisphenol A groups during all three time periods for male offspring, but the effect remained statistically significant only in the high-dose group when normalized for body weight. Testicular descent and preputial separation were delayed in the low-dose group. Organ weight effects that remained significant following adjustment for body weight included increased prostate weight in the high-dose group on PND 70 and increased testicular and epididymal weights in the low-dose group on PND 170. There was no effect on sperm morphology. Blood testosterone concentration was decreased in the high-dose group on PND 70, and blood LH concentration was increased in the high-dose group on PND 170. Testicular histopathology observations in the low-dose group on PND 70 included cellular debris in lumens, pyknotic nuclei in spermatids, and apoptotic debris in the region of the spermatogonia and primary spermatocyte. In testes of 70-day-old animals of the high-dose group, there were central necrotic masses, low numbers of meiotic figures in spermatocytes, and low spermatozoa numbers. On PND 170, observations in testes from the low-dose group included low spermatozoa numbers, a thin layer of spermatocyte meiotic figures, and apoptotic debris in region of spermatids. Low spermatocyte meiotic figures were the only testicular observation in the high-group on PND 170. Effects observed in the ethinyl estradiol group included increased anogenital distance, delayed testicular descent, accelerated preputial separation, decreased testis and prostate weights, decreased sperm counts and production, increased LH concentrations, increased testosterone concentrations on PND 170, apoptotic debris, and/or low sperm numbers in testes.

Study authors concluded that prenatal exposure to bisphenol A disrupts the reproductive systems of both male and female rats and that the effects do not occur according to a classic dose–response curve, which is generally observed in toxicology studies.

Strengths/Weaknesses: Strengths are the postnatal evaluation of various endpoints to "pup" adulthood and that the concentration of the dosing solutions was verified. Based on the description of numbers of pups contributing to various endpoints, however, the authors do not appear to have used the litter as the unit of analysis. These inflated numbers subjected to analysis complicate the interpretation of findings, especially for PND 1–21 measures. A weakness also is that only 2 dose levels were examined. The vaginal opening data for the controls were outside the normal range for Sprague–Dawley rats. It is unclear how the estrous cycle data were analyzed. The F_1 data were not analyzed correctly. Data may be suggestive of developmental disruptions at both doses, but the magnitudes are likely unreliable, and the

Table 71
Benchmark Doses for Rat Reproductive Organ Endpoints Affected by Prenatal Bisphenol A[a]

Endpoint	Benchmark dose, mg/kg bw/day			
	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Delayed vaginal opening	68	51	35	16
Sperm count/testis	55	30	57	31
Sperm count/g testis	81	41	68	34
Daily sperm count/testis	56	31	59	31
Daily sperm count/g testis	83	42	70	34

[a]Calculated from data in Tinwell et al. (2002).

authors' statements about dose–response peculiarities must be viewed with caution until more complete dose–response assessments are published.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Tinwell et al. (2002), support not indicated, examined the effects of in utero exposure to bisphenol A on sexual development of male rats. The study attempted to duplicate findings that were reported in several abstracts and as a full report (Talsness et al., 2000). Sprague–Dawley and Wistar-derived Alderley Park rats were housed in plastic-bottomed cages containing sawdust and shredded paper bedding. Rats were assigned to groups based on body weights and 6–7/group/strain were gavaged on GD 6–21 with bisphenol A (99% purity) at 0 (arachis oil vehicle), 0.020, 0.100, or 50 mg/kg bw/day. A positive control group initially received 200 μg/kg bw/day ethinyl estradiol, but the dose was reduced to 100 μg/kg bw/day between GD 11 and 14 due to maternal toxicity. Dosing solution concentrations and stability were verified. Dams were fed RM3 breeding diet (18.5% soybean protein; Special Diet Services, Ltd.) during gestation and lactation. At birth, pups were counted, sexed, and weighed. Anogenital distance was measured 24 hr following birth (PND 1). On PND 5, pups were culled to 8/litter, with equal numbers of males and females when possible. On PND 23, rats were weighed and housed according to sex. Following weaning, pups were fed RM1 feed (6.5% soybean protein). Pups were weighed throughout the post-lactation period. Ages at preputial separation, vaginal opening, and first estrus were assessed. Males were killed on PND 90–91 and females on PND 98. Liver and reproductive organs were weighed. Daily sperm production was determined. Data were analyzed using the litter and grouped individuals as the statistical unit. **[Litter values are discussed below.]** Data were analyzed by ANOVA, ANCOVA, and Dunnett test.

The only significant effect observed in female rats exposed to bisphenol A was a 1.6-day delay in vaginal opening in Alderley-Park rats of the high-dose group. The study authors stated that effect on vaginal opening was correlated with body weight. **[Data were not shown by study authors.]** In Alderley Park males of the high-dose group, significant reductions were observed for total sperm count/testis **[12% lower than controls]**, sperm count/g testis **[10% reduction]**, daily sperm count/testis **[12% reduction]**, and daily sperm count/g testis **[10% reduction]**. Benchmark doses for the endpoints with statistically significant changes are shown in Table 71. In both strains, bisphenol A treatment had no effect on litter size, sex ratio, birth weight, anogenital distance, first day of estrus, or age of preputial separation. There were no significant effects on weights of liver, ovary, cervix, uterus, vagina, testis, epididymis, seminal vesicle, or prostate. Rats treated with ethinyl estradiol also experienced decreased sperm counts, in addition to decreased weights of male reproductive organs and advanced age of vaginal opening. Several findings (Talsness et al., 2000) were not duplicated in this study including: reduced anogenital distance; altered age of sexual maturation in males and females; variable changes in male reproductive organ weight, including prostate weight; and reduced sperm production at low doses. Study authors concluded that this study failed to confirm low-dose endocrine effects.

Strengths/Weaknesses: Strengths of this study are the range and appropriateness of selected measures, the use of 2 strains of rat, the verification of dosing solutions, and the use of ethinyl estradiol, which produced expected responses. An unfortunate weakness is the small sample size of 6–7 dams/strain/group. Nevertheless, data were analyzed appropriately with the litter as the experimental unit, and significance judgments were apparently based on 7/group. Modest effects were noted in male and female offspring in the 50 mg/kg exposure group. While effects on the lowest doses in this study were not seen, it is important to recognize the effects seen at 50 mg/kg bw/day (the high-dose in this study) dosing on GD 6–21.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Schönfelder et al. (2002a), supported by the German Federal Ministry for Education and Research, examined the effects of prenatal bisphenol A exposure on the rat vagina. Sprague–Dawley rats were gavaged on GD 6–21 with bisphenol A at 0 [2% corn starch vehicle (Mondamin)], 0.1, or 50 mg/kg bw/day. A positive control group was treated with 0.2 mg/kg bw/day 17α-ethinyl estradiol in a peanut oil vehicle. **[No information was provided on the number of dams treated, the day of vaginal plug, purity of bisphenol A, or the type of chow, bedding, and caging materials used.] [According to the author the number of litters treated were: Mondamin = 20, 0.1 mg/kg bw/day bisphenol A = 20, 50 mg/kg bw/day bisphenol A = 18, and 0.2 mg/kg bw/day 17α ethinyl estradiol = 11; day of sperm positive smear was considered to be GD 0 and was used instead of day of vaginal plug; purity of bisphenol A was ≥98%; Altromin 1324 rodent chow was used (obtained from Altromin GmbH); bedding was wood shavings obtained from Altromin GmbH; caging was Type III macrolon cages (G. Schönfelder, personal communication, July 20, 2007).]** At 3 months of age, estrous cyclicity was evaluated for 3 weeks in 42 female offspring of the control group, 21 offspring of the 0.1 mg/kg bw/day group, 18 offspring of the 50 mg/kg bw/day group, and 24 offspring of the 17β-estradiol group. **[The number of litters represented was not stated.]** At 4 months of age, female offspring were killed in either estrus or diestrus. **[Authored clarified that each estrus group contained 22 offspring from 20 dams in the cornstarch group, 13 offspring from 13 dams in the 0.1 mg/kg/d and 12 offspring from 12 dams in the 50 mg/kg/d bisphenol A**

group, as well as 19 offspring from 11 dams in the 0.2 mg/kg/d 17α-ethinyl estradiol group (G. Schönfelder, personal communication, July 20, 2007)]. [Exact litter representation for animals collected during diestrus was not provided.]Vaginas were fixed in Bouin solution and a histopathological evaluation was conducted. Western blot analyses were conducted to measure expression of ERα and ERβ. **[It does not appear that statistical evaluations were conducted.]**

Qualitative descriptions of vaginal histopathology changes and ER expression were provided by the study authors. Low-dose animals killed during the estrous stage lacked keratinization of the surface epithelium and demonstrated reduced thickness of the total epithelium. Similar but less pronounced effects were observed in rats of the high-dose bisphenol A group. Vaginal findings were similar in the positive control group, and slight desquamation of the superficial layers was also observed. There were no differences in vaginal histopathology findings in rats killed during the diestrous stage. No ERβ was observed in vaginas of rats from any treatment group. Full-length ERα expression was not observed in either bisphenol A group during estrus, but ERα in the bisphenol A-exposed groups did not differ from the control group during the diestrous stage. ERα in vaginas obtained from the positive control group was either reduced or was not detected. The study authors concluded that altered vaginal morphology following bisphenol A treatment appears to be due to down-regulation of ERα.

Strengths/Weaknesses: Vaginal histopathology of female offspring is of interest but the quality of the study cannot be judged due to unclear methodology. Uncertainty about the numbers of animals, the number of offspring examined and the lack of statistical accounting for litter effects are significant weaknesses.

Utility (Adequacy) of the CERHR Evaluation Process: This study is inadequate for the evaluation process for the reasons detailed above.

Schönfelder et al. (2004), supported by the German Federal Ministry for Environmental Protection and Radiation Security, examined the effects of prenatal bisphenol A exposure on the rat uterus. **[No information was provided about composition of feed, caging, or bedding.]** Sprague–Dawley rats **[number treated not specified]** were gavaged with bisphenol A **[purity not reported]** at 0 (2% corn starch vehicle), 0.1, or 50 mg/kg bw/day on GD 6–21. **[Author clarified that the purity of bisphenol A was ≥98%; Altromin 1324 rodent chow was used (obtained from Altromin GmbH); bedding was wood shavings obtained from Altromin GmbH; caging was Type III macrolon cages (G. Schönfelder, personal communication, July 20, 2007).]** The high bisphenol A dose was selected because it was reported to be the no observed effect level (NOEL) recommended by the Society of the Plastics Industry. A positive control group was gavaged with 0.2 mg/kg bw/day ethinyl estradiol on GD 6–21. Estrous cyclicity was examined for 3 weeks in 6 female offspring/group beginning at 3 months of age. Six female offspring/group were killed at 4 months of age on the day of estrus. Body and reproductive organ weights were measured. Uteri were fixed in methacarn solution and sectioned. Examinations of uterine morphology were conducted. Immunohistochemistry techniques were used to detect ERα and ERβ in the uterus, and results were verified by Western blot. Data were analyzed by Mann–Whitney test. **[It was not clear if data were analyzed on a per litter or per offspring basis.] [Author states that each female came from a different litter so the data were analyzed on a per litter basis (G. Schönfelder, personal communication, July 20, 2007).]** Statistically significant findings are summarized in Table 72. Effects observed at both dose levels were increased epithelial cell nuclei, epithelial nuclei with condensed chromatin, and epithelial cells with cavities and reduced ERβ-positive cells in uterine tissue. Additional effects observed only at the high-dose included decreased thickness of luminal epithelium and increased ERα-positive cells in the epithelium. Similar findings were observed following treatment with ethinyl estradiol. The study authors concluded that prenatal bisphenol A exposure causes uterine effects in rat offspring.

Table 72
Uterine Effects in Rats Exposed to Bisphenol A During Prenatal Development

	Dose, mg/kg bw/day	
Endpoint	0.1	50
Thickness of luminal epithelium	↔	↓38%
Epithelial nuclei[a]	↑69%	↑89%
Epithelial nuclei with condensed chromatin	↑2.7-fold	↑3.1-fold
Epithelial cells with cavities	↑2.1-fold	↑ 1.9-fold
ERα positive cells in epithelium	↔	↑67%
ERβ-positive cells in uterine tissue	↓88%	↓88%

[a]It is unclear if authors were referring to numbers of nuclei.

Strengths/Weaknesses: A strength is the examination of effects on uterine indices in female offspring. A slight weakness is the use of only 6 females per group; however, the panel noted that the results appeared to be consistent across animals and across endpoints, especially in the 50 mg/kg bw/day treatment group.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Wistuba et al. (2003), supported by the German Federal Ministry of Education and Science, examined the effects of prenatal exposure on testicular histology and sperm endpoints in rats. **[No information was provided about chow, bedding, or caging.]** Sprague–Dawley rats were gavaged with 0 (2% corn starch suspension vehicle), 0.1, or 50 mg/kg bw/day bisphenol A **[purity not reported]** on GD 6–21 (GD 0 = day of sperm detection). A third group was treated with 0.02 mg/kg bw/day ethinyl estradiol. The high-dose was said to correspond to the current accepted no observed adverse effect level (NOAEL) and the lower dose was selected to determine if effects occurred at lower doses. It appears that the number of dams treated was 2 in the control group, 4 in the low-dose group, 1 in the high-dose group, and 4 in the ethinyl estradiol group. Litters were weighed during the lactation period. Pups were weaned on PND 22 **[day of birth not defined]**. Male offspring were killed between the ages of ~9–12 months. The number of males killed was 5 from 2 litters in the control group, 15 from 4 litters in the low-dose

group, 5 from 1 litter in the high-dose group, and 10 from 4litters in the ethinyl estradiol group. Testes were fixed in Bouin solution, and Sertoli cells were counted. Spermatogenesis was evaluated by examining germinal epithelia for cell death and distribution of various cell populations. Data were analyzed by ANOVA. **[It appears that at least some data were analyzed on a per litter basis. In addition, analyses were done to determine intralitter variability and thus the numbers of animals per group that needed to be analyzed.]**

Examination of tubule cross sections revealed qualitatively normal spermatogenesis in the bisphenol A groups. A comparison of Sertoli cell numbers in littermates revealed high variability (20–27%) in the 0.1 mg/kg bw/day group. A comparison of Sertoli cell numbers in the 4 litters from the 0.1 mg/kg bw/day group revealed almost identical results between litters. Sertoli cell numbers/organ were significantly increased by 19.4% in the low-dose group and 19% in the high-dose group. Bisphenol A had no significant effect on Sertoli cell numbers/g testis weight. The opposite situation occurred in the ethinyl estradiol group, with no significant effects on Sertoli cell numbers/organ but a significant increase in Sertoli cell numbers/g testis weight. Testis weight was not affected by bisphenol A treatment but was decreased in the ethinyl estradiol group. The study authors concluded that the study does not support the hypothesis of disruption of the male reproductive system by bisphenol A exposure.

Strengths/Weaknesses: The conceptual strength is the focus on the male reproductive tract/function. However, a weakness is that there were too few animals to provide reliable data.

Utility (adequacy) for the CERHR Evaluation Process: This study is inadequate based on insufficient sample size ($n = 2$–4).

Thuillier et al. (2003), supported by National Institute of Environmental Health Sciences (NIEHS), examined a possible role for the platelet-derived growth factor system in estrogenic effects induced by bisphenol A in rats exposed during gestation. The effects of other compounds such as genistein and coumestrol were also examined but will not be discussed here. Pregnant Sprague–Dawley rats were gavaged with bisphenol A at 0 (corn oil vehicle) or 0.1, 1, 10, or 200 mg/kg bw/day from GD 14 through birth (PND 0). Additional rats were s.c. injected with diethylstilbestrol at 0.01–2 μg/kg bw/day during the same period. **[No information was provided about number of rats treated, purity of bisphenol A, feed, or materials used in bedding and caging.]** Male offspring were killed on GD 21 or PND 3 and testes were collected. Expression of mRNA or protein for platelet-derived growth factor receptor-α and platelet-derived growth factor receptor-β were determined in testes using RT-PCR, in situ hybridization, or immunohistochemistry. Statistical analyses included unpaired *t*-test with Welch correction. **[It was not clear if the litter or offspring were considered the statistical unit.]**

Expression of mRNA for platelet-derived growth factor receptor-α and -β was significantly increased at bisphenol A doses ≥1 mg/kg bw/day in testes from 3-day-old rats. All other experiments with bisphenol A were conducted with a single dose of 200 mg/kg bw/day. In situ hybridization examination of testes from 3-day-old rats from the bisphenol A group revealed an increase in expression of platelet-derived growth factor receptor-α mRNA in testicular interstitium and platelet-derived growth factor receptor-β mRNA in interstitium and seminiferous cords. Exposure to bisphenol A resulted in slightly increased platelet-derived growth factor receptor-α protein expression and strong expression of platelet-derived growth factor receptor-β in gonocytes from 3-day old rat testes. Immunolocalization studies in testes from 21-day-old fetuses revealed that exposure to 200 mg/kg bw/day bisphenol A did not affect expression of platelet-derived growth factor receptor-α protein in gonocytes, but platelet-derived growth factor receptor-β protein appeared to be increased in gonocytes and Sertoli cells. Diethylstilbestrol tended to have a biphasic effect with increased expression of platelet-derived growth factor receptor-α and -β mRNA in 3-day-old rat testis at low doses and decreased expression at the high-dose. Treatment with 1 μg/kg bw/day diethylstilbestrol decreased mRNA expression of platelet-derived growth factor receptor-α in interstitium and increased platelet-derived growth factor receptor-β mRNA expression in seminiferous cords. Immunoreactivity for platelet-derived growth factor receptor-α protein was maintained but there was a minimal level of platelet-derived growth factor receptor-β protein expression in 3-day-old rat testes following exposure to 1 μg/kg bw/day diethylstilbestrol. In testes obtained from 21-day-old fetuses, expression of platelet-derived growth factor receptor-α protein was decreased in Sertoli and interstitial cells and expression of platelet-derived growth factor receptor-β protein was apparently increased following exposure to diethylstilbestrol. The study authors concluded that the platelet-derived growth factor receptor pathway may be a target for estrogens in the testis, but the findings do not exclude the possibility that effects may have occurred through an ER-independent mechanism.

Strengths/Weaknesses: Endpoints are a strength, but inadequate methodological detail (i.e., sample size or adequate control for litter effects) precludes any informed judgment of study quality.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process based on insufficient methodological details.

Wang et al. (2004), supported by NIEHS, examined the effects of prenatal bisphenol A exposure on expression of ER-associated proteins in rat testis. The effects of genistein and coumestrol were also examined but will not be discussed here. Pregnant Sprague–Dawley rats **[apparently 3/group]** were gavaged with corn oil vehicle or bisphenol A at 0.1–200 mg/kg bw/day from GD 14 (14 days post-coitum) through birth. Additional rats were s.c. injected with 0.01–2 μg/kg bw/day diethylstilbestrol during the same time period. **[No information was provided about feed, caging and bedding material, or compound purity.]** Male offspring from three independent litters were killed on GD 21, PND 3, or PND 21. Western blot, RT-PCR, and immunohistochemistry techniques were used to measure expression of protein or mRNA for *Hsp90*, *Hsp90α*, *p23*, *CYP40*, *Hsp70*, and/or *ERβ*. Spermatogonia were quantified in PND 21 rat testis. Data were analyzed by unpaired *t*-test. The dam was considered the statistical unit.

In testes from 3-day-old rats, RT-PCR revealed significant increases in mRNA for *hsp90* at bisphenol A dose levels of 10 and 200 mg/kg bw/day, and significant decreases in expression of *CYP40* at 200 mg/kg bw/day and *p23* at 1 mg/kg bw/day. In situ hybridization analyses in 3-day-old rat testes revealed that bisphenol A tended to increase expression of *hsp90* throughout the testis, with patterns indicating increased expression in gonocytes and interstitial Leydig cells. Examination of protein in testes from 3-day old rats exposed to 200 mg/kg bw/day bisphenol A revealed significantly increased levels of hsp90 and hsp70, but no effect on levels of CYP40, p23, or ERβ. Immunohistochemistry revealed that hsp90 protein in testes from 3-day-old rats was most increased in gonocytes and less so in interstitium following exposure to 200 mg/kg bw/day bisphenol A. Use of a probe specific for hsp90α protein revealed that increased protein expression of hsp90 was due in a large part to the hsp90α isoform. Examination of testes from GD 21 fetuses and PND 21 pups revealed that the amount of hsp90 protein in the bisphenol A treatment group was similar to that observed on PND 3 but that the amount of protein did not differ from controls on PND 21. In 21-day-old rats from the bisphenol A group, the number of spermatogonia/tubule was significantly higher by ~2-fold compared to the control group. **[It is not clear which bisphenol A dose induced an increase in spermatogonia, but it was most likely 200 mg/kg bw/day, because that dose appeared to be used in all studies not examining dose–response relationships.]** Effects following diethylstilbestrol exposure included increased expression of *hsp90* mRNA at 1.0 μg/kg bw/day and decreased *CYP40* mRNA expression at 0.01 and 1 μg/kg bw/day, but no effect on protein levels of those compounds was reported in testes from 3-day-old rats. The number of spermatogonia/tubule was also increased after prenatal exposure to diethylstilbestrol. The study authors concluded that prenatal exposure to bisphenol A affects *hsp90* expression in gonocytes of rats, and because hsp90 interacts with several signaling molecules, changes in its expression could affect gonocyte development.

Strengths/Weaknesses: This study was generally well conceived, but the small sample size suggests it presents pilot data only. A full study is needed to provide reliable data.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate based on insufficient sample size ($n = 3$).

3.2.1.3 Neurodevelopmental endpoints: Funabashi et al. (2004a), supported in part by Yokohama City University, examined the effects of bisphenol A on the numbers of corticotropin-releasing hormone neurons in the preoptic area and bed nucleus of the stria terminalis of rats exposed during development. **[No information was provided about chow or composition of bedding and caging.]** Pregnant Wistar rats ($n = 8$–11/treatment group) were given drinking water containing the 0.1% ethanol vehicle or 10 mg/L bisphenol A **[purity not reported]** until their offspring were weaned at 3 weeks of age. **[It is implied but not stated that exposure occurred during the entire gestation period.]** Bisphenol A intake was estimated by study authors at 2.5 mg/kg bw/day. Male and female offspring ($n = 8$–11/group) were killed at 4–7 months of age, and immunocytochemistry techniques were used to determine the number of corticotropin-releasing hormone neurons in brain. Female rats were killed during proestrus. **[Although the number of litters represented in each group was not specified, the number of rats examined suggests that 1 rat/sex/litter was examined.]** Histological slides of brain were evaluated by an investigator blinded to treatment conditions. Two series of experiments were conducted, and data from both experiments were combined. Data were analyzed by ANOVA followed by Fisher protected least significant difference post-hoc test. **[It was not stated if data were analyzed on a per litter or per offspring basis, but as stated earlier, it appears that 1 rat/sex/litter was examined.]** In the control group, females had more corticotropin-releasing hormone neurons in the preoptic area and anterior and posterior bed nucleus of the stria terminalis than males. Bisphenol A treatment did not change the number of corticotropin-releasing hormone neurons in the preoptic areas of males. A loss in sex difference occurred in the anterior and posterior bed nuclei of the stria terminalis following bisphenol A treatment because differences in numbers of corticotropin-releasing hormone neurons between males and females were no longer evident. It appears that bisphenol A treatment increased the number of corticotropin-releasing hormone neurons in males and decreased the number in females. The study authors concluded that exposure to bisphenol A during gestation and lactation results in a loss of sex difference in corticotropin-releasing hormone neurons in the bed nucleus of the stria terminalis but not in the preoptic area.

Strengths/Weaknesses: This study was appropriately designed to examine effects on the development of brain areas known to be influenced by hormonal levels. Strengths include the relevance and subtleties of the endpoints measured; weaknesses include uncertainties about the numbers of animals examined and the duration of the dosing period. The results suggest a disruption of the normal pattern of sexually dimorphic neurons, a result of critical importance to concerns about disruptions relevant to reproductive function and sexually dimorphic behaviors. While the sample size was 8–11/group, the design and statistics appear to be appropriate. It is a weakness that the control for litter effects was not clear.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion in the evaluation process, although of limited utility due to uncertainties about the sample size, duration of dosing, and control for litter effects.

Fujimoto et al. (2006), supported by the Japanese Ministry of Education, Culture, Sports, Science, and Technology, examined the effect of prenatal bisphenol A exposure on sexual differentiation of neurobehavioral development in rats. Wistar rats were fed CE-2 feed (CLEA, Japan). **[Caging and bedding materials were not described.]** From GD 13 (day of vaginal sperm not defined) to the day of birth (PND 0), 6 rats/group were given tap water containing bisphenol A **[purity not reported]** at 0 or 0.1 ppm. The study authors estimated the bisphenol A dose at 0.015 mg/kg bw/day. On PND 1, pups were weighed and litters were culled to 4 pups/sex. Pups were weaned on PND 21. Neurobehavioral evaluations conducted in 20–24 offspring/sex/group at 6–9 weeks of age included open-field, elevated plus maze, passive avoidance, and forced swimming tests. Statistical analyses included ANOVA, Fisher protected

least significant difference test, and Mann–Whitney *U*-test. **[It appears that offspring were considered the statistical unit.]**

In the control group, rearing frequency and duration were significantly higher in females than males, but there were no sex-related differences in rearing frequency or duration in the bisphenol A group. Bisphenol A exposure caused an increase in rearing duration in males when compared to males from the control group. In the forced swim test, females in the control group struggled more than males but no sex-related differences in struggling were observed in the bisphenol A group. The duration of immobility in the swimming test was longer in males from the bisphenol A compared to males from the control group. Immobility was described as non-significantly increased in females exposed to bisphenol A compared to control females. Bisphenol A exposure had no effect on performance in passive avoidance and elevated plus maze test. The study authors concluded that exposure of male offspring to bisphenol A during the final week of gestation resulted in impaired sexual differentiation in rearing and struggling behaviors and facilitated depression-like behavior.

Strengths/Weaknesses: This study used a good choice of methods to examine functional disruptions in sexually dimorphic behaviors. Weaknesses include a lack of clarity about the nature of disruption of sexually dimorphic behavior patterns that was indicated in the authors' conclusions, the somewhat small sample size, the use of a single dose level, which was not confirmed, and the lack of clarity of the statistical methods regarding litter.

Utility (Adequacy) for CERHR Process: This study is inadequate for the evaluation process due to statistical methodology.

3.2.2 Rat—parenteral exposure only during pregnancy. **Ramos et al. (2001)**, supported by the Argentine National Council for Science and Technology, the Argentine National Agency for the Promotion of Science and Technology, and the Ministry of Health, examined the effects of bisphenol A exposure on the rat prostate. Wistar rats were housed in stainless steel cages. **[No information was provided about chow or bedding material.]** Four dams/group were exposed to bisphenol A **[purity not reported]** at 0 (DMSO vehicle), 0.025, or 0.250 mg/kg bw/day by s.c. pump on GD 8–23 (GD 1 = day of vaginal sperm). Pups were weighed and sexed at birth. Litters were culled to 8 pups, with 4/sex when possible. Pups were weaned on PND 22 **[day of birth not defined]**. On PND 30, pups were injected with bromodeoxyuridine and killed 2 hr later. Ventral prostates were dissected and fixed in 10% neutral buffered formalin. Immunohistochemical techniques were used to measure proteins associated with cell proliferation and cell phenotypes. Morphometric measurements were taken. **[It was not clear how many rats/treatment group were examined for each endpoint. Although a statement was made that males from a single dam were evaluated, it was later stated that siblings were excluded from the same experimental group. Therefore it appears that different litters were represented.]** Data were analyzed by Kruskal–Wallis ANOVA and Mann–Whitney *U*-test. **[It was not clear if the dam or offspring were considered the statistical unit.]**

In the periductal stroma, the fibroblastic layer was increased, the smooth muscle layer was reduced, and androgen receptor-positive cells were decreased. Prostatic acid phosphatase-positive cells were reduced in epithelial cells. There were no effects on cell proliferation and ERα was not detected. No changes were observed in interductal stromal cells.

Strengths/Weaknesses: This study has an interesting design with respect to choice of endpoints. Certain design aspects are unclear and statistical approaches are inadequate. The sample size was small (4 dams/group) and there was considerable uncertainty about numbers of offspring examined and accounting for litter effects. The use of DMSO (% not specified) is of concern, as this can modify the effects of the solute. Of additional concern is the route of administration (s.c. pump).

Utility (Adequacy) for CERHR Evaluation Process: This study is considered inadequate.

Ramos et al. (2003), supported by the Argentine Ministry of Health, Argentine National Agency for the Promotion of Science and Technology, and the National University of Litoral, examined the effects of bisphenol A exposure on the prostate and the hypothalamic-pituitary-gonadal axis in Wistar rats. Rats were housed in stainless steel cages and 7–9/group were administered DMSO vehicle or bisphenol A at 0.025 or 0.250 mg/kg bw/day by s.c. pump on GD 8–23 (GD 1 = day of vaginal sperm). **[No information was provided on purity of bisphenol A, the type of feed used, or composition of bedding.]** After birth, pups were weighed and sexed. Litters were culled to eight pups with equal numbers of male and female pups when possible. Pups were weaned on PND 22 **[day of birth not defined]**. During prepuberty (PND 15), peripuberty (PND 30), and adulthood (PND 120), 6–8 males/group were injected with bromodeoxyuridine and killed 2 hr later. Serum was collected for measurement of LH and prolactin by RIA. Immunohistochemistry techniques were used to evaluate markers of cell proliferation, estrogen/androgen receptors, and prostatic cells. Expression of mRNA for *ER*α and *ER*β in the preoptic area and medial basal hypothalamus was determined by RT-PCR. Data were analyzed by Kruskal–Wallis 1-way ANOVA using Dunn post-test.

No significant effects were observed for ventral prostate weight. Numerous transient effects were observed in both bisphenol A dose groups. On PND 15, cellular proliferation was increased in the periductal stroma of the prostate, and serum testosterone levels were elevated. On PND 30, the fibroblasts (vimentin-positive cells) in the prostatic periductal stroma was increased and the area of smooth muscle cells α-smooth muscle actin) was decreased. Also observed on PND 30 was a reduction in androgen-receptor positive stromal cells, a decrease in epithelial cells positive for prostatic acid phosphatase, and an increase in serum prolactin levels. Expression of *ER*β mRNA was increased in the preoptic areas on PND 30 and 120, and the study authors considered the effect to be permanent because it occurred on both days. The study authors concluded that prenatal exposure to environmental concentrations of bisphenol A during gestation results in transient and permanent changes in the male reproductive axis.

Strengths/Weaknesses: The design appears reasonable as a means to address the study questions. Like many of these studies, altered values are given without addressing the normal range of variation or the likely functional

significance of the changes. Weaknesses include use of the s.c. pump as a route of administration and use of DMSO as a vehicle.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for inclusion due to the use of 99.9% DMSO as a vehicle to administer BPA via s.c. pump. As discussed in earlier, the use of >50% DMSO as a vehicle for ALZET mini-pump studies is a clear contravention of the directions for mini-pump use, as it accelerates the breakdown of the mini-pumps

Naciff et al. (2002), from the Procter and Gamble Company, examined the effects of prenatal bisphenol A exposure on gene expression and, to a limited extent, development in female rat reproductive organs. Pregnant Sprague–Dawley rats were fed Purina 5K96, a casein-based soy- and alfalfa-free diet. **[Composition of caging and bedding materials was not reported.]** The rats were assigned to groups (≥7 rats/group) s.c. injected with bisphenol A (~99% purity) in DMSO vehicle at 0, 5, 50, or 400 mg/kg bw/day on GD 11–20 (day of sperm detection = GD 0). Dams were killed on GD 20, and ovaries and uteri were removed from fetuses. In 4 litters/group, 1 female fetus/litter was examined for ovarian and uterine histopathology. In 5 litters/group, ovaries and uteri from at least 5 littermates were pooled for a microarray analysis of gene expression. Changes in gene expression were further quantified using RT-PCR. Data were analyzed by *t*-test, ANOVA, and Jonkheere-Terpstra test. Comparisons of gene expression among estrogenic compounds were made by Wilcoxon-Mann–Whitney and Jonkheere-Terpstra tests. Results of gene expression assays are discussed in Section 2. Vaginal bleeding and early parturition occurred in 1 of 8 dams in the high-dose group. Bisphenol A treatment had no effect on maternal body weight or number of live fetuses/litter, and there were no gross or histopathological effects on ovary or uterus. Prominent nipples and areolas were observed in males and females in the high-dose bisphenol A group **[number of fetuses and litters affected were not reported]**.

Strengths/Weaknesses: Strengths are that these endpoints appear appropriate; weaknesses are the limited nature of the endpoints and the use of neat DMSO as vehicle. The sample sizes are 4–5/endpoint/group and judged to be inadequate. Of additional concern is the route of administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Naciff et al. (2005), from The Procter and Gamble Company, examined the effect of prenatal bisphenol A exposure on male rat reproductive organ histology and gene expression. Pregnant Sprague–Dawley rats were fed Purina 5K96, a casein-based soy- and alfalfa-free diet. Rats were housed in stainless steel cages before mating. Rats were randomly assigned to groups (≥8 rats/group) and s.c. injected with bisphenol A (~99% purity) in DMSO at 0, 0.002, 0.02, 0.5, 50, or 400 mg/kg bw/day on GD 11–20 (day of sperm detection = GD 0). Dams were killed on GD 20, and testes and epididymides were removed from fetuses. In 4 litters/dose group, 1 male fetus/litter was examined for testicular histopathology. In 5 litters/group, testes and epididymides from 5 littermates were pooled for a microarray analysis of gene expression. Changes in gene expression were further quantified using RT-PCR. Data were analyzed by *t*-test, ANOVA, and Jonkheere-Terpstra test. Comparisons of gene expression among estrogenic compounds were analyzed by Wilcoxon-Mann–Whitney and Jonkheere-Terpstra tests.

Bisphenol A treatment had no effect on maternal body weight or number of live fetuses/litter, and there were no gross or histopathological effects on the testis or epididymis. Prominent nipples/areolas were observed in male and female fetuses from the high-dose group **[numbers of fetuses and litters affected were not reported]**. In pooled testis and epididymis samples from the high-dose bisphenol A group, expression of 15 genes was significantly altered in a dose-related manner. When bisphenol A data were pooled with data obtained from ethinyl estradiol and genistein and globally analyzed, there were 50 genes that were significantly altered in the same direction by all three compounds. The study authors concluded that transplacental exposure to high-doses of bisphenol A alters the expression of certain genes in the testis and epididymis of fetal rats without causing malformations in those organs. The study authors noted that the dose response to bisphenol A was monotonic with no evidence of robust quantifiable responses at low doses.

Strengths/Weaknesses: Strengths are that these endpoints appear appropriate; weaknesses are the limited nature of the endpoints and the use of neat DMSO as vehicle. The sample sizes are 4–5/endpoint/group and judged to be inadequate. Of additional concern is the route of administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Saito et al. (2003b), support not indicated, examined the effect of prenatal bisphenol A exposure on testosterone production during adulthood in rats. On GD 12–19 (day of vaginal plug not reported), 2 Wistar rats were s.c. injected with the corn oil vehicle, 4 rats were s.c. injected with 0.005 mg/day bisphenol A **[purity not indicated]**, and 2 rats were injected with 5 μg/day 17β-estradiol. **[Assuming a pregnant Wistar rat weights ~0.33 kg, 0.005 mg/day would be equivalent to 0.015 mg/kg bw/day bisphenol A.]** Other materials found in dental composites were also evaluated but will not be discussed. During the lactation period, rats were housed in polypropylene cages with synthetic bedding. **[No information was provided on feed.]** Offspring were housed separately at 3 weeks of age and killed at 13 weeks of age. Body and testis weights were measured in all male offspring (22 in the bisphenol A group, 11 in the vehicle control group, and 5 in the 17β-estradiol group). Plasma testosterone level was measured by RIA, and plasma cholesterol level was measured using a kit. Activities of testicular enzymes involved in the production of testosterone from progesterone were also examined in an in vitro assay in which testicular microsomes were incubated with ^{14}C-progesterone and ^{14}C-δ^4-androstenedione for 20 min. Data were analyzed using unspecified post-hoc tests. **[Although not clear, it appears that offspring were considered the statistical unit for some analyses.]**

Bisphenol A exposure had no effect on pup sex ratio. No effects on body weight or absolute testicular weight were observed in the bisphenol A group at 13 weeks of age. However, relative (to body weight) testicular weight was lower **[by 6%]** in rats of the bisphenol A compared to the control group. Also observed in the bisphenol A

group was a reduction in plasma testosterone level **[by ~28%]**. No effect was observed on cholesterol level. In the ex vivo study, prenatal bisphenol A exposure increased activities of 17α -hydroxysteroid dehydrogenase **[by ~140%]** and 17β-hydroxysteroid dehydrogenase **[by ~70%]**. Observations in the 17β-estradiol compared to the control group included decreased numbers of offspring delivered, higher body weight of male offspring at 13 weeks of age, reduced plasma testosterone level, and increased testicular 17α-hydroxysteroid dehydrogenase activity. The study authors concluded that bisphenol A had an estrogenic effect on the testis but did not decrease activities of enzymes involved in testosterone production.

Strengths/Weaknesses: A strength of this study is the examination of testosterone levels at 13 weeks of age. This strength is negated by the sample size ($n = 2$–4), which is too small to draw any firm conclusions.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate based on insufficient sample size.

Murray et al. (2007), supported by NIH, examined the effect of prenatal bisphenol A exposure on in situ induction of mammary tumors. Wistar-Furth rats were fed Harlan Teklad 2018, which was reported to contain 20 fmol/g estrogen equivalents. Water was supplied in glass bottles. Caging and bedding materials were not reported, but they were stated that to test negative in the E-SCREEN. From GD 9 (GD 1 = day of vaginal sperm) through PND 1 **[The day of birth was PND 0 (A. Soto, personal communication, March 2, 2007)]**, rats received the 50% DMSO vehicle or bisphenol A **[purity not reported]** at 0.0025, 0.025, 0.250, or 1 mg/kg bw/day. Dosing solutions were delivered by implanted **[assumed s.c.]** osmotic pumps. **[Number of dams treated was not reported. Based on a limited amount of information provided on the number of offspring examined, it appears that ≤6 dams/group were treated.]** Pup viability was assessed on PND 1. On PND 2 pups were sexed and litters were culled to 8 pups. Anogenital distance was measured on PND 4. Litters were weighed during the lactation period. Female offspring were monitored for body weight and vaginal opening in the post weaning period. Female offspring were killed on PND 50 or 95. Mammary glands were collected and whole-mounted or sectioned for histopathological examination. Morphometric analyses were conducted to examine possible presence of preneoplastic lesions. Mammary glands were examined for ERα and Ki-67 protein by an immunohistochemistry technique. Maximal numbers of "maternal units" were represented in each dose group. One female/litter was included in histological examinations. **[Apparently ≤6 offspring/group were examined in histopathological examinations. Number of offspring examined for other endpoints was not reported in the manuscript. According to an author, $n = 7$–21 for the other endpoints (A. Soto, personal communication, March 2, 2007).]** Statistical analyses included ANOVA followed by post-hoc tests (Bonferroni or *t*-test) when significant effects were observed by ANOVA. **[It was not clear if dams or offspring were considered the statistical unit.]**

Bisphenol A exposure did not affect offspring viability, sex ratio, age at vaginal opening, or female anogenital distance. Anogenital distance was reduced on PND 4 in males from the 0.250 mg/kg bw/day group. Percent hyperplastic ducts was increased in all dose groups on PND 50 and in the 0.0025 mg/kg bw/day group on PND 95; the study authors noted that the effect on PND 50 was quantitatively similar in all dose groups (i.e. 3–4-fold increase). Cribriform structures were observed in the 0.25 and 1 mg/kg bw/day groups. **[Incidence was not reported for the control and lower dose groups.]** The structures were classified as carcinomas-in-situ and were characterized by increased ductal size resulting from luminal epithelium proliferation, enlarged luminal epithelial cells, presence of a nucleolus, variable chromatin pattern, and rounded luminal spaces consisting of trabecular rods of cells perpendicularly aligned to the longer duct axis. Numbers of Ki-67- and ER-α positive cells were increased in aberrant compared to normal tissues, regardless of dose. **[Results in treated compared to control groups were not reported.]** The study authors concluded that fetal bisphenol A exposure is rats is sufficient to induce development of preneoplastic and neoplastic mammary lesions.

Strengths/Weaknesses: Relevance of endpoints is a strength, as is the use of multiple dose levels. Weaknesses include an unstated number of dams (and by inference, a small number of these, and thus, because of dam-related effects, a small overall n), the uncertainty of the response rate of histopathology in the controls, and the use of 50% DMSO as vehicle.

Utility/Adequacy for CERHR Evaluation: This study was inadequate due to small sample size, route of administration, and lack of clarity on statistical analysis.

Durando et al. (2007), supported by Universidad National del Litoral, Argentine National Agency for the Promotion of Science and technology, and NIH, examined the effects of prenatal bisphenol A exposure on susceptibility to mammary tumors in rats. Wistar rats were fed Cooperación (Buenos Aires, Argentina) and housed in stainless steel cages. **[It was not clear if bedding was used.]** On GD 8–23 (GD 1 = day of vaginal sperm), 11–14 dams/group were s.c. dosed by osmotic pump with the DMSO vehicle or 0.025 mg/kg bw/day bisphenol A **[purity not indicated]**. Pups were delivered on GD 23 and weaned on PND 21. It was not indicated if day of birth was considered PND 0 or 1. During the study, body weights and day of vaginal opening were monitored. Offspring were killed before puberty (PND 30), after puberty (PND 50), or in adulthood (PND 110 and 180). In mammary gland stroma and epithelium, proliferation was assessed by BrdU incorporation and apoptotic cells were identified by TUNEL method. Morphometric analyses were conducted in sectioned mammary glands. Mast cells were identified by immunostaining for proteinase. At least 6 offspring/group/time point were evaluated. **[No littermates were used in the evaluation at any given time point (A. Soto, personal communication, March 2, 2007).]** Additional offspring were examined for responsiveness to chemically-induced mammary preneoplastic or neoplastic lesions. On PND 50, *N*-nitroso-*N*-methylurea was administered to 10–16 offspring from the vehicle control group at 25 or 50 mg/kg bw and 21 offspring from the bisphenol A group at 25 mg/kg bw. Based on findings from a pilot study, 25 mg/kg bw was considered a subcarcinogenic dose and 50 mg/kg bw was considered a positive control. During the study, rats were palpated for tumors. Rats that received 50 mg/kg bw *N*-nitroso-

N-methylurea were killed on PND 180 and rats that received 25 mg/kg bw *N*-nitroso-*N*-methylurea were killed on PND 110 or 180. Whole-mounted mammary glands were examined for tumors. Immunostaining was conducted to identify cytokeratin 8 (an epithelial marker) and p63 (a myoepithelial marker). Data were statistically analyzed using the Mann–Whitney *U*-test.

Bisphenol A exposure did not affect successful pregnancies, dam weight gain, pregnancy duration, number of pups/litter, or percent females/litter. Anogenital distance on PND 1 or 5 and postnatal body weights were unaffected in pups exposed to bisphenol A. Vaginal opening was accelerated in pups from the bisphenol A group (mean 34 days of age compared to 39 days of age in controls). On PND 50, the BrdU/apoptosis ratio was significantly increased and apoptosis was significantly decreased in mammary parenchyma and stroma of bisphenol A-exposed animals; the effects were not observed on PND 30 or 110. Significantly increased percentages of hyperplastic ducts, density of stromal nuclei, and numbers of mast cells were observed in the bisphenol A group on PND 110 and 180. Exposure to bisphenol A resulted in formation of a dense stroma layer around mammary epithelial structures and replacement of normal adipose tissue with a fibroblastic stroma. In rats exposed to 25 mg/kg bw *N*-nitroso-*N*-methylurea on PND 50, incidence of hyperplastic lesions on PND 180 was significantly higher in the group with prenatal bisphenol A compared to DMSO exposure (mean incidence of 35.5% compared to 15.7% in controls). Although statistical significance was not achieved, exposure to 25 mg/kg bw *N*-nitroso-*N*-methylurea resulted in tumors in 2 of 15 rats in the prenatal bisphenol A group and 0 of 10 rats in the prenatal vehicle control group on PND 180. Cytokeratin 8 immunostaining revealed no invasion by stromal epithelial cells. The study authors concluded that rats prenatally exposed to environmentally relevant doses of bisphenol A may have an increased risk of developing mammary tumors.

Strengths/Weaknesses: Weaknesses include route of administration and the high single dose is a weakness as is the use of pure DMSO.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for inclusion due to the use of 99.9% DMSO as a vehicle to administer bisphenol A via s.c. osmotic pump.

Hong et al. (2005), sponsored by the Korea Research Foundation, investigated the effects of acute exposures to bisphenol A during late pregnancy on expression and protein level of calbindin-D_{9k}, a putative biomarker of estrogen activity, in the uteri of offspring and lactating rats on PND 5. Pregnant Sprague–Dawley rats were given free access to water and a diet of soy-free pellets in polycarbonate caging. **[Housing conditions (individual or group) and bedding material were not indicated.]** On GD 17–19, pregnant rats were s.c. injected daily with 200, 400, or 600 mg/kg bw/day bisphenol A **[purity not provided]** in corn oil ($n = 5$/group). Negative and positive control groups ($n = 10$/group) were administered corn oil or 17β-estradiol 40 μg/kg bw/day. On PND 5, lactating dams and female pups were killed and their uteri harvested. Dose response changes in calbindin-D_{9k}, expression levels in uteri of lactating dams and female offspring (3/group) were analyzed by Northern blot and RT-PCR, with appropriate housekeeping gene controls. Protein levels and localization of calbindin-D_{9k} were performed by Western blot and immunohistochemistry for lactating dams only. Statistical analyses were performed using the Kruskall–Wallis and Dunnett tests. **[It was not clear if dams or offspring were considered the statistical unit.]**

Northern blot analysis revealed a significant increase **[~6.4-fold]** in the level of calbindin-D_{9k} expression in the uteri of lactating dams exposed to 600 mg/kg bw/day bisphenol A compared to oil controls. 17β-Estradiol treatment produced a significant **[~3.9-fold]** increase in calbindin-D_{9k} mRNA expression in the dam uterus that was not statistically distinct from the effect of the high bisphenol A dose. Uteri of offspring exposed to the highest dose level of bisphenol A also showed a significant upregulation **[~4.4-fold]** in calbindin-D_{9k} expression. Expression levels of *ER*α were unaffected in maternal uteri exposed to bisphenol A. However, *ER*α expression was increased significantly in uteri of pups exposed to 400 and 600 mg/kg bw bisphenol A **[↑33% and 66%, estimated from a graph]**. Protein levels of calbindin-D_{9k} in lactating dam uteri were elevated significantly at all dose points **[50, 40, and 50%, for 200, 400, and 600 mg/kg bw/day, respectively]**. 17β-Estradiol-treatment was not associated with a significant increase in calbindin-D_{9k} protein. The density of calbindin-D_{9k}-immunopositive cells was increased in uterine sections from lactating dams exposed to all doses of bisphenol A relative to oil controls, correlating with Western blot results. Authors note insufficient material or low detectability of calbindin-D_{9k} protein in offspring tissue, and protein analyses were not performed.

The authors suggest that calbindin-D_{9k} can serve as a reliable biomarker of acute estrogenic exposure, particularly for insight into maternal-fetal metabolic exchange, given that calbindin-D_{9k} is tightly regulated and rapidly induced by 17β-estradiol, diethylstilbestrol, alkylphenols, and now, bisphenol A. They further point out that calbindin-D_{9k} expression is absent in immature rat and ovariectomized rat uteri.

Strengths/Weaknesses: This study supports the use of calbindin-D_{9k} as a uterine biomarker of estrogenic effect in the perinatal period in the rat, and provides some dose–response information for bisphenol A induction of an estrogenic response. Limitations are the subcutaneous route of exposure, small sample size, high-doses and uncertain statistical analyses of the F1 data.

Utility (Adequacy) for CERHR Evaluation Process: While providing some dose–response information regarding bisphenol A-induced estrogenic effects following exposure of rats in the perinatal period, the lack of clarity regarding whether the dam or offspring was considered the statistical unit, route of exposure, and use of high doses render this study inadequate for consideration in the evaluation process.

3.2.3 Rat—oral exposure postnatally with or without prenatal exposure.

3.2.3.1 Reproductive studies: The International Research and Development Corporation (General Electric, 1976), sponsored by General Electric, examined the effects of bisphenol A exposure on CD rats and their offspring. Male and female F_0 rats were housed in wire mesh cages and fed Purina Laboratory Chow. Ten rats/sex/group (body weights of 110–170 g for males and

100–151 g for females) were given feed containing bisphenol A **[purity not specified]** at 0, 1000, 3000, or 9000 ppm for 17 weeks. **[It was not clear how long before mating that the dosing was started or if dosing was continued through the gestation and lactation periods.]** The European Union (2003) estimated bisphenol A intake at 0, 70, 200, or 650 mg/kg bw/day in males and 0, 100, 300, or 950 mg/kg bw/day in females. F0 rats were mated at ~100 days of age and assessed for fertility. F_1 pups were counted and weighed at birth and on PND 21 (day of birth not defined). Fifteen male and female F_1 rats/group/sex that were exposed in utero were selected for a 13-week feeding study and were fed diets containing the same concentration of bisphenol A as their parents. F_1 rats were weighed and observed for clinical signs. Hematological, clinical chemistry, and urinalysis parameters were examined in 5 rats/sex/group in the control and 2 highest dose groups at 1, 2, and 3 months of F_1 exposure. Ophthalmoscopic examinations were conducted at 3 months of F_1 exposure. After 13 weeks of dosing, the F_1 rats were killed and necropsied. Organs were weighed and fixed in 10% neutral buffered formalin. Included among organs weighed were testis and ovary. Histopathological examinations were conducted in tissues from 10 rats/sex/group in the control and high-dose group. Included among organs histologically examined were prostate, uterus, testis, and ovary. Statistical analyses included χ^2 test with Yates correction, Fisher exact probability test, Mann–Whitney *U*-test, ANOVA, *t*-test, and Dunnett multiple comparison test.

Fertility was unaffected in F_0 rats. Body weight gain was lower in F_0 rats from the 3000 and 9000 ppm groups. Body weight at Week 17 followed the same patterns as body weight gain **[6–7% decrease in the 3000** ppm **group and 12–18% decrease in the 9000** ppm **group compared to controls]**. There were no differences in food intake. **[Statistical significance for body weight effects was not reported. It was not clear if statistical analyses were not conducted or if the effects did not attain statistical significance.]**

There were no effects on number of F_1 pups/litter or survival of pups. Pup birth weights in the 9000 ppm group were slighter decreased but were said to be within normal range. Body weight gains on PND 21 were slightly decreased in pups from the 3000 and 9000 ppm dose groups. Body weights on PND 21 were significantly lower in pups from the 3000 and 9000 ppm groups **[7 and 12% lower compared to controls; benchmark dose analysis not conducted because variances not reported]**. One male F_1 rat in the control group and 2 female F_1 rats in each of the 3000 and 9000 ppm group died during the study. Post-weaning body weight gain was lower in females from all dose group and in males from the 3000 and 9000 ppm dose groups. Body weight at week 13 followed the same patterns as body weight gain **[13% decrease in the 1000** ppm **group, 11–17% in the 3000** ppm **group, and 22–24% decrease in the 9000** ppm **group compared to controls]**. Food intake was decreased in females from all dose groups and in males from the 9000 ppm group. Examination by ophthalmoscopy revealed no treatment-related effects. No treatment-related effects were observed for hematology, biochemistry, or urinalysis. No changes in organ weights or gross or histopathological lesions were considered treatment-related. The study authors noted increases in mean weights of spleen, brain, thyroid, and adrenals in the treated groups but concluded that the effects resulted from decreased body weight. **[With the exception of PND 21 pup weights, there was no discussion of statistical significance for effects observed in F_1 rats. It was not clear if statistical analyses were not conducted or if statistical significance was not attained.]**

Strengths/Weaknesses: This study is a conventional, state-of-the-art-at-the-time two-generation toxicity study. The inclusion of a breeding period and a second generation are strengths. Weaknesses are magnified in hindsight: these include the limited number of animals examined, the lack of close examination of the reproductive processes in the F_1 animals, and uncertainty about the statistical significances. The study has not been peer-reviewed.

Utility (Adequacy) for CERHR Evaluation Process: Although this study was not designed to find non-linear dose–responses, it represents a conventional-for-the-time 2-generation toxicity study, and is adequate for the evaluation process but of limited utility because the high doses preclude evaluation of low dose effects and limit its utility in showing a lack of marked organ toxicity or gross reproductive toxicity in a limited number of animals at very high-doses.

The International Research and Development Corporation (General Electric, 1978), sponsored by General Electric, examined the effects of bisphenol A exposure on male and female CD rats and their offspring. In the first part of the experiment, male and female rats were housed in wire mesh cages and were fed Purina Laboratory Chow containing bisphenol A **[purity not specified]** for 18 weeks. Ten rats/group (body weights of 135–179 g for males and 114–158 g for females) were assigned to each treatment group based on even distribution of body weight and litter mates. **[Based on information provided in study tables, it appears that the rats were ~30 days old at the start of dosing.]** Bisphenol A was added to feed at concentrations of 0, 100, 250, 500, 750, or 1000 ppm. The European Union (2003) estimated bisphenol A intake at 0, 5, 15, 30, 50, and 60 mg/kg bw/day in males and 0, 10, 25, 50, 75, and 100 mg/kg bw/day in females. Rats were examined for clinical signs, body weight gain, and food intake throughout the study. Estrous cyclicity was examined in females for 3 weeks before breeding and during breeding. At 100 days of age (Week 10 of the study), rats were moved to plastic cages with corncob bedding and mated for 3 weeks. GD 0 was defined as the day that vaginal sperm or plug was observed. Rats were assessed for fertility and gestation length. Day of delivery was designated lactation day 0 (PND 0). Pups were counted, sexed, and weighed, assessed for viability at birth and through the lactation period. After weaning, 15 male and female F_1 rats/group that were exposed in utero were selected for a 90-day feeding study. Parental rats and unselected F_1 rats were killed and discarded.

During a 90-day period, F_1 rats were fed diets containing the same concentration of bisphenol A as their parents. **[Ages at the start of dosing were not reported, but based on body weight ranges reported (64–138 g for males and 57–118 g for females) it appears that rats were different ages at the start of dosing.]** F_1 rats were weighed and observed for clinical signs. Hematological, clinical chemistry, and urinalysis

parameters were examined at Day 30, 60, and 90 of the study. Ophthalmoscopic examinations were conducted before initiation of and following 90 days of dosing. The rats were killed and organs weighed. Adrenals, pituitary, ovaries, and thyroid were weighed following fixation in 10% neutral buffered formalin. Histopathological examinations were conducted in tissues from 10 rats/sex/group in the control and high-dose groups. Organs histologically examined included prostate, uterus, testis, and ovary. Statistical analyses included χ^2 test with Yates correction, Fisher exact probability test, Mann–Whitney *U*-test, ANOVA, *t*-test, and Dunnett multiple comparison test.

In parental rats, bisphenol A exposure did not affect general behavior, appearance, or survival. Mean body weight of males in the 1000 ppm group was 6% lower than control males. Food intake was increased **[by ~7–11%, no dose–response]** in females of all dose groups. Bisphenol A exposure had no effect on estrous cyclicity or gestation length **[data were not shown]**, male or female fertility, number of pups/litter, or pup survival Body weights of pups in the 750 ppm group were significantly higher **[by ~10%]** compared to controls on PND 21, but the study authors did not consider the effect to be treatment-related.

In the F_1 offspring, a slight decrease in body weight gain was observed for males in the 750 ppm group. **[At the end of the study, body weights of males in the 750 ppm group were ~7% less than controls]**. Food intake was similar in treated and control groups. Ophthalmoscope examinations did not reveal any treatment-related effects. Although mean blood urea nitrogen levels were slightly lower and mean serum glutamic-oxaloacetic transaminase values were sporadically increased in treated rats, the study authors noted that the values were within physiological ranges. There were no effects on hematological or urinalysis parameters. Some significant organ weight changes were noted by the study authors, but they stated that the biological significance of the effects was not known. **[There did not appear to be dose–response relationships for any organ weight change.]** The study authors stated that no compound-related lesions were observed in organs, including reproductive organs.

Strengths/Weaknesses: The use of multiple dose levels (going down to fairly low exposure levels) is a plus, as is a breeding phase. Weaknesses include the limited number of animals per group, discarding of the parental animals without examination, the fact that not all F_1 animals were examined at least for structural effects, the lack of close examination of F_1 animals for reproductive effects (cyclicity and sperm measures), and the use of the conventional "top-down" pathology evaluation, wherein the lower dose groups were examined only if effects were noted in the high-dose. The study has not been peer-reviewed.

Utility (Adequacy) for CERHR Evaluation Process: For what it is, this study is adequate and of limited utility for the evaluative process, showing no gross changes in the structure of a limited number of tissues in a limited number of F_1 animals, exposed from preconception. This study was not designed to find unusual effects or non-linear dose–response relationships or to address the issue of low-dose functional responses or non-linear responses.

Ema et al. (2001), supported by the Japanese Ministry of Health and Welfare, examined developmental toxicity endpoints, in a 2 generation rats study described in detail in Section 4.2.3.1. Two generations of rats were gavaged with 0, 0.0002, 0.002, 0.020, or 0.200 mg/kg bw/day bisphenol A (99.9% purity) before and during mating and throughout the gestation and lactation period. These doses were based on previous studies that found effects at 0.002 and 0.020 mg/kg bw/day. There were some non-dose-related and sporadic effects, but the study authors concluded that none of the effects were related to bisphenol A treatment. Bisphenol A exposure did not adversely affect prenatal or postnatal growth or survival, developmental landmarks, anogenital distance, or age of puberty. In adult animals exposed to bisphenol A during development, there was no evidence of adverse effects on reproductive endpoints such as fertility, estrous cyclicity, or sperm counts. Prostate and other male reproductive organ weights were unaffected.

Strengths/Weaknesses: Strengths of this study were the thoroughness of the evaluation, the size of the dose range, the large number of animals, the litter-based analysis, and the verification of the dosing solution. A minor weakness is the lack of a positive control group, which leaves a question about the ability of this group of rats to respond.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Tyl et al. (2002b), supported by The Society of the Plastics Industry, Inc., reported some developmental toxicity effects in a multigeneration bisphenol A study in Sprague–Dawley rats that is reported in detail in Section 4.2.3.1. In that study, F_1, F_2, and F_3 rats were exposed to bisphenol A [99.70%-99.76% pure] indirectly during gestation and lactation and directly through feed after weaning. Dietary doses were 0, 0.015, 0.3, 4.5, 75, 750, or 7500 ppm, and target intakes were ~0.001, 0.02, 0.30, 5, 50, and 500 mg/kg bw/day. At the 7500 ppm dose there were fewer pups and live pups/litter and body weight gain of pups was lower during the lactation period. Delayed puberty in both males and females of the 7500 ppm group was most likely related to reduced body weights according to the study authors. Bisphenol A exposure during development did not increase the weight of the prostate in adult rats. Although some decreases in epididymal sperm concentration and daily sperm endpoints were each observed in 1 generation of males from the high-dose group, the study authors concluded there were no treatment-related effects on sperm endpoints or reproductive function. The study authors identified an offspring and reproductive NOAEL of 750 ppm (~50 mg/kg bw/day). A systemic NOAEL for adult rats was identified at 75 ppm (~5 mg/kg bw/day) by the study authors; therefore, bisphenol A was not considered a selective developmental toxicant.

Strengths/Weaknesses: This study has numerous strengths, including the quality and number of the endpoints evaluated, the number of dose groups and generations examined, and the confirmation of dosing solutions. This study incorporated screening-level endpoints within the context of a multigeneration study. As such, it addresses gross issues but does provide helpful data regarding the NOAEL.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

3.2.3.2 Development of the reproductive or endocrine systems: Cagen et al. (1999b), support not indicated (but all authors affiliated with industry), conducted a study to examine the effects of prenatal and lactational bisphenol A exposure on reproductive development of rats. The study attempted to replicate findings by Sharpe et al. that appeared in an unpublished meeting abstract. The protocol used by Cagen et al. (1999b) was the same as that used by Sharpe et al. with the exception that more dose levels were included, group sizes were larger, and a greater number of reproductive endpoints were examined. Animals were fed Certified Rodent Chow 5002. Music was played at a low volume to provide background noise. Female Han-Wistar rats were randomly assigned to groups. For 2 weeks before mating, during a 2-week mating period, and during the gestation and lactation periods, 28 rats/group were given drinking water containing bisphenol A (>99% purity) at 0.01, 0.1, 1.0, or 10 ppm (0.001–0.004, 0.008–0.038, 0.100–0.391, or 0.775–4.022 mg/kg bw/day). Two negative control groups of 28 rats each were given undosed drinking water. Because the two control groups were determined to be statistically equivalent, data from the two groups were pooled. A positive control group of 28 rats was given drinking water with diethylstilbestrol at 0.1 ppm (0.006–0.036 mg/kg bw/day). Dosing solutions were prepared weekly, and concentrations were verified. Dams were evaluated for food and water intake, weight gain, and fertility endpoints. Pups were sexed, weighed, and counted at birth. During the postnatal period, pups were evaluated for growth and survival. On PND 4, litters were culled to 8 pups with as many male pups retained as possible. At weaning on PND 22, up to 4 males/litter (86–109 pups/group) were randomly selected to continue in the study until 90 days of age and were individually housed. At necropsy, brain, liver, kidneys, and reproductive organs were weighed, daily sperm production was determined, and testes were examined histologically. Technicians were blinded to treatment group. The litter was considered the experimental unit in statistical analyses. Data were analyzed by Levene test, ANOVA, Dunnett test, rank transformation, and Wilcoxon rank sum test with Bonferroni correction.

In the bisphenol A groups, there were no significant effects on dam body weight gain or food or water intake. **[Food and water intake data were not shown.]** There were also no effects on dam fertility, mating, gestation index and duration, live litter size, or pup survival and body weight gain during the postnatal period. Male sex ratio was increased in the 0.1 ppm bisphenol A group (56.7% males vs. 48.4% in control), but the study authors did not consider the effect to be treatment-related due to the lack of a dose response relationship. Dams in the diethylstilbestrol group experienced decreased body weight gain and food intake, increased duration of gestation, smaller litter size at birth, and decreased pup survival in the postnatal period.

In adult offspring from the bisphenol A groups, there were no significant effects on terminal body weight or organ weights including prostate, epididymis, preputial gland, seminal vesicle, or testis. There were also no significant effects on epididymal sperm concentration, efficiency of sperm production, or daily sperm production. No histopathological alterations were observed in the testis. Reproductive development in male offspring was also unaffected by prenatal exposure to diethylstilbestrol. The study authors noted that the reduced testis weight and sperm production reported by Sharpe et al. was not confirmed in this study and that bisphenol A should not be considered a selective reproductive or developmental toxicant.

[The NTP Statistics Subpanel (NTP, 2001) concluded that the statistical methods used by Cagen et al. (1999b) were appropriate. Although the Subpanel agreed with the study author conclusions, 2 matters were noted. The first was that a significant ANOVA is not a requirement for Dunnett test. The second was that a Bonferroni correction of Wilcoxon-rank sum test was not needed because the authors already required significance by ANOVA, which was sufficient.]

Strengths/Weaknesses: Significant strengths of this study include the large number of dose levels and animals per dose level and the technical care with which the study was performed, as well as the inclusion of a positive control group and two negative controls. The lack of much effect with diethylstilbestrol treatment is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: Although only weak effects were observed for the diethylstilbestrol positive control the panel considered this study adequate and of high utility.

Elswick et al. (2000), from the Chemical Industry Institute of Toxicology [CIIT], examined the effects of sampling design on conclusions made about bisphenol A effects on prostate weight. Two of the 3 studies discussed in the study relate to bisphenol A research in Sprague–Dawley rats performed at CIIT between 1997–1999. One study is Kwon et al. (2000) which is discussed in detail in Section 3.2.3.3. The other study was unreferenced at the time and remains so. This section discussed the analysis of the unpublished study. In that study, the litter was considered the experimental unit in statistical analyses. Organ weights were analyzed using a nested ANOVA with litter within dose as the random effect. Post-hoc tests were conducted when appropriate.

Dams were given drinking water containing 0, 0.005, 0.05, 0.5, 5, or 50 mg/L bisphenol A **[purity not indicated]** from GD 2 to PND 21. The study authors estimated bisphenol A intakes at ~0.001–10 mg/kg bw/day. The lowest doses were reported to be similar to human exposure levels. The study was conducted in 2 blocks separated by 4 months. A total of 16 dams/group were exposed, and the overall sample size was ultimately 13–16/group. In the first block, 2 males/litter were most often retained and in the second block, 1 male/litter was retained until 6 months of age. Fresh ventral prostate weights were recorded. Analysis of data from the first study block revealed no treatment-related effects on ventral prostate weight. Within litters, ventral prostate weights were observed to be very variable, with weights sometimes differing by values of 2-fold or more. In the second study block, mean weights in the 0.05, 5, and 50 mg/kg bw/day groups were significantly higher than those of the control group. It was noted that mean prostate weight in the control group from Block 2 (0.387 g) was much lower than the mean weight observed in Block 1 (0.517 g) and that the standard error in Block 2 (0.174 g) was almost two times higher than the standard error in Block 1 (0.092 g). When data from the 2 blocks were combined, statistical significance remained. The

study authors noted that no historical control database was available at CIIT at the time of the analysis.

[The NTP Statistics Subpanel (NTP, 2001) reanalyzed these data agreed with its results and conclusions showed a consistent increase in ventral prostate weight in the 2 replicates. Note that the NTP Statistics Subpanel rejected the conclusions in Elswick et al. (2000) that use of multiple pups per litter can decrease false positive rates in these studies.]

Strengths/Weaknesses: This study demonstrated an increase in ventral prostate weight. These data argue for multiple pup/litter sampling, a characteristics that has been uncommon in this literature. The fact that significant effects were noted in only in 1 block raise the question of a lack of experience or training among the technicians. The study referred to in Elswick et al. (2000) is unpublished and not peer-reviewed.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate because it is primarily a discussion of results published previously and the new data presented have inconsistencies in block replicates.

Rubin et al. (2001), supported by the Tufts Institute of the Environment and NIH, examined the effects of perinatal bisphenol A exposure on estrous cyclicity and LH levels in rats. Uterotropic responses were examined in a second group of rats, and those results are listed in Table 53. Sprague–Dawley rats were fed Purina Rodent Chow and provided drinking water in glass bottles. The rats were housed in plastic cages; estrogenicity testing of ethanol extracts indicated that estrogenic compounds did not leach from cages at detectable levels. **[No information was provided about bedding.]** Dams were weighed and randomly assigned to treatment groups of 6 animals given drinking water containing bisphenol A **[purity not reported]** at 0 (1% ethanol vehicle), 1, or 10 mg/L from GD 6 (plug day not indicated) through the lactation period. Mean bisphenol A doses were estimated by study authors at 0.1 and 1.2 mg/kg bw/day. At weaning, pups were given untreated water. Dams were examined and weighed during the studies. Offspring were sexed on PND 2 and weighed beginning in the postnatal period and continuing through adulthood ($n = 40$–53/group during the neonatal period and 19–27/sex/group during adulthood). Anogenital distance was examined during the neonatal period. **[It was not clear how many time points and animals were examined. According to 1 study author, anogenital distance was measured on PND 2 (A. Soto, personal communication, March 2, 2007).]** Genital tracts were examined for gross abnormalities in males killed during the neonatal period, at 3 months, and at 5 months of age and in females killed during the neonatal period, at 8 months, and at 12–16 months of age. **[The total number of animals examined at each time period was reported as 12–34, but it is not known how many/dose group were examined.]** Animals were selected from as many different litters as possible at each time point. Day of vaginal opening was monitored. Estrous cyclicity was evaluated daily for 18 days at 4 and 6 months of age in 18–28 rats/group. Eight female offspring/group were killed 3 months later following ovariectomy to measure serum LH levels using an LH assay kit; a total of 6–8 values/group were obtained. Body and uterine weights and LH levels were analyzed by ANOVA followed by *t*-test, Tukey test, or least significant difference test. Mammary tumors were analyzed by χ^2 test, and estrous cyclicity data were analyzed by Kruskall–Wallis test and Mann–Whitney *U*-test. **[It appears that offspring were considered the statistical unit.]**

On PND 4, 7, and 11, body weights were significantly higher in pups from the bisphenol A groups than in the control group; body weights were higher in animals of the low compared to the high-dose group. Body weights of low-dose females were higher than body weights of control and high-dose females at PND 28 and beyond. Although the percentage of control females with regular estrous cycles was 83% at 4 months of age and 60% at 6 months of age, the values were reduced significantly in the high-dose group to 21% at 4 months of age and 23% at 6 months of age. There were no clear patterns of estrous cycle changes. Periods of diestrus were extended in some animals and other animals had extended periods of proestrus and/or estrus. The mean number of 4–5-day estrous cycles was reduced significantly in rats of the high-dose group at 6 months of age. Serum LH levels in the high-dose group were reduced significantly by ~19% compared to the control group **[$BMD_{10} = 0.94$, $BMDL_{10} = 0.48$, $BMD_{1\ SD} = 1.6$, and $BMDL_{1\ SD} = 0.78$ mg/kg bw/day]**. The treatment group incidences of females with mammary tumors (10% in controls, 20% in the low-dose group, and 28% in the high-dose group) were not statistically different. The study authors noted that the study was not designed to detect mammary tumors and that the tumors were detected during routine handling. No effects were reported for mean number of pups/litter, sex ratio, day of vaginal opening, or anogenital distance in the neonatal period. **[Data were not shown for anogenital distance.]** In comparing the effects on estrous cycles and LH levels in animals exposed in the perinatal period to the lack of uterotropic effects in animals exposed in the post-pubertal period, the study authors concluded that there was evidence of increased sensitivity to bisphenol A during the perinatal period.

Strengths/Weaknesses: This study incorporates a range of basic developmental and gross functional reproductive endpoints, but the sample sizes are small (6 dams/group) and the statistical approach does not appear to use litter as the unit. Actual exposures are poorly defined, particularly postnatally. The plausibility of the estrous cycle changes is a strength.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process, based on a lack of adequate control for litter effects

Takashima et al. (2001), supported by a Grant-in-Aid for Health Sciences Research **[sponsor not indicated]**, examined the effect of bisphenol A exposure during development on carcinogenicity induced by *N*-nitrosobis (2-hydroxypropyl)amine. **[No information was provided about caging and bedding materials used in this study.]** Female Wistar rats were fed either MF diet or soybean-devoid powder diet (Oriental Yeast Co.). In each dietary group, 10–11 rats/group received bisphenol A **[purity not indicated]** at 0 or 1.0% diet. Bisphenol A exposure commenced 10 weeks before mating and was continued through the mating, gestation, and lactation periods. Total intakes of bisphenol A were reported at 21–22 g/rat. **[Assuming an exposure period of ~16 weeks, mean bisphenol A intake over the course of the study was estimated at ~200 mg/day. Based on reported body**

weights, bisphenol A intake was ~1600 mg/kg bw/day during the prebreeding stage and 1000 mg/kg bw/day during gestation and at weaning.] The rats were mated to males fed CE-2 basal pellet diet (Clea, Inc.), and GD 0 was defined as the day of the vaginal plug. Endpoints associated with pregnancy, delivery, and nursing were evaluated. Dam body weight and food intake were measured. Offspring were not culled and were weaned at 3 weeks of age. Dams were killed following weaning of offspring. Serum levels of thyroid hormones were measured in 2–4 dams/group. Implantation sites were evaluated. Weights of several organs, including ovary, were measured. The organs were fixed in 10% buffered formalin and processed for histopathological evaluation. Offspring ($n = 32$–50/group) were evaluated for body weight gain, preputial separation, and vaginal opening. Beginning at 5 weeks of age and continuing for 12 weeks, offspring in each group were subdivided into 2 groups ($n = 17$–21/group/sex) that received either undosed tap water or tap water containing 2000 ppm *N*-nitrosobis (2-hydroxypropyl)amine. Offspring were killed at 25 weeks of age. Serum thyroid hormone levels were measured. Organs, including testis, ovary, and uterus were weighed. In 5–19 offspring/sex/group, histopathological examinations were conducted in organs targeted by *N*-nitrosobis (2-hydroxypropyl)amine (lungs, thyroid, esophagus, liver, and thymus). Data were analyzed by Dunnett and χ^2 tests. **[Data for pre-and postnatal survival were presented and apparently analyzed on a litter basis. The offspring were apparently used as the statistical unit in body weight analyses. It was not clear if the dam or offspring were considered the statistical unit in other analyses.]**

Dam body weight was lower in the 1.0% bisphenol A group fed MF diet compared to the MF diet control during the gestation period and at weaning. Food intake and maternal serum levels of triiodothyronine, thyroxine, and thyroid-stimulating hormone were unaffected by bisphenol A exposure. Changes in weights or histopathological alterations of maternal organs, including uterus and ovary, were not observed in the bisphenol A groups. **[Data were not shown by the study authors.]** Bisphenol A had no significant effect on mating, fertility, duration of gestation, live-born pups, implantation loss, or offspring viability through PND 21. In pups from dams exposed to 1.0% bisphenol A fed MF diet compared to pups from MF controls, body weights were higher **[by 11%]** in females at 3 days of age and lower in males and females at 10 days and 2 weeks of age **[16–22% decreases in males and 12–19% decreases in females]**. In pups from dams exposed to 1.0% bisphenol A and fed soybean-free diet compared to pups from the soybean-free controls, body weights of pups were increased in males at 3 weeks of age **[13% increase]** and in females at 10 days and 3 weeks of age **[13–19% increase]**. Prenatal exposure to bisphenol A did not affect preputial separation or vaginal opening. In 25-week-old rats that were not exposed to N-nitrosobis (2-hydroxypropyl)amine, prenatal bisphenol A exposure was associated with some thyroid-stimulating hormone elevations in males and females from the MF and soybean-free diet groups. According to a statement in the study abstract, the study authors did not consider the effect on thyroid-stimulating hormone to be related to bisphenol A exposure. There were no effects of *N*-nitrosobis (2-hydroxypropyl)amine exposure on serum thyroid-stimulating hormone, triiodothyronine, or thyroxin levels or on thyroid histopathology. No effects were observed on offspring organ weights. **[With the exception of uterus and ovary, no organ weight data were shown.]** Prenatal bisphenol A exposure was not associated with significant differences in the development of *N*-nitrosobis (2-hydroxypropyl)amine-induced neoplasms in the offspring. The study authors concluded that bisphenol A exposure did not induce tissue injury in rat dams or their offspring or affect the development of tumors in offspring exposed to *N*-nitrosobis (2-hydroxypropyl)amine.

Strengths/Weaknesses: Weaknesses include high-doses and inadequate sample sizes. This study appears to discount the importance of certain effects on body weight and thyroid-stimulating hormone levels that might have received more attention in a study with a non-tumor focus. Sample size is inadequate to address neoplasm endpoints. Information is insufficient to judge the appropriateness of the statistical analyses and hence the reliability of findings.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to small sample size, high-dose levels, and inappropriate statistics.

Kobayashi et al. (2002), supported by the Japanese Ministry of the Environment, examined the effect of prenatal and lactational bisphenol A exposure on somatic growth and anogenital distance in Sprague–Dawley rats. The same rats were used to measure plasma hormone levels and testicular testosterone content in a study by Watanabe et al. (2003) and apparently thyroid function in a study by Koybayashi et al. (2005). Rats were fed standard laboratory feed (CE-2, CLEA Japan, Inc.). **[No information was provided about caging or bedding materials.]** Rats were randomly assigned to groups and 6 rats/group were gavaged with bisphenol A (99.8% purity) at 0 (corn oil vehicle), 4, 40, or 400 mg/kg bw/day from GD 6 through PND 20. GD 0 was defined as the day a vaginal plug was observed, but the day of birth was not defined. Doses were based on the study by Kwon et al. (2000) **[discussed in Section 3.2.3.3]**. On PND 7, litters were culled to 10 pups, with equal numbers of males and females when possible. Offspring were weaned on PND 21. Dams were weighed during the study. Body weight and anogenital distance were measured in offspring at 1, 3, and 9 weeks of age. Plasma and testicular testosterone levels were measured at 9 and 36 weeks of age, and plasma LH and FSH concentration were measured at 9 weeks of age Weights of liver, kidney, and testis were examined in offspring at 3 and 9 weeks of age. One to 10 (most often 6–10) offspring/group/sex were examined for body weight and anogenital distance at 1 week of age and 4–6/sex/group at 3 and 9 weeks of age. A pair of male and female offspring/litter **[assuming authors meant 1/sex/litter]** was examined for organ weights, and 4–6 males/group were used in hormone analyses at 3 and 9 weeks of age. Statistical analyses included ANOVA followed by Dunnett test. **[It was not clear if the dam or litter was considered the statistical unit.]**

In the 40 mg/kg bw/day group, all pups from 1 dam were found dead on PND 2. Four of 6 dams of the 400 mg/kg bw/day group died on GD 21, and all pups

born to 1 dam in that group died by PND 2. Maternal body weight gain during pregnancy was reduced in the 400 mg/kg bw/day group. A transient decrease in body weight gain was observed early in the lactation period in dams of the 40 mg/kg bw/day group. In offspring from the 4 and 40 mg/kg bw/day group, no statistically significant effects were observed for body or organ weights, anogenital distance, anogenital distance/g body weight, or anogenital distance/body weight cubed at any time point in the study. At 9 weeks of age, plasma testosterone levels were significantly increased by 88% in the 4 mg/kg bw/day group and by 123% in the 40 mg/kg bw/day group. **[Benchmark dose was not calculated because the SD was provided only graphically.]** The study authors stated that there was a tendency for plasma testosterone to increase with dose at 36 weeks, but neither of the values were significantly increased compared to control. Testis testosterone was not statistically different from control at either dose at 9 or 36 weeks of age. There were no significant effects on plasma LH and FSH levels at 9 weeks of age. Plasma levels of 17β-estradiol were also unaffected by bisphenol A exposure. **[Data were not shown.]** The study authors concluded that gestational and lactational exposure to bisphenol A did not affect somatic growth or anogenital distance but did have a significant effect on testosterone homeostasis in rat offspring.

Strengths/Weaknesses: The study appears better able to address maternal toxicity than offspring outcomes, for which it appears to be best considered a screening study. Sample sizes are too small to reliably judge postnatal endpoints.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation of bisphenol A effects on postnatal outcome.

Yoshino et al. (2002), supported by the Japanese Ministry of Health, Labor, and Welfare, examined the effects of prenatal and lactational bisphenol A exposure in the prostate and testis of rats. In this study, pregnant and lactating dams were fed nMF feed and offspring were fed MF feed (Oriental Yeast Co., Tokyo). The animals were housed in an unspecified type of cage containing wood chip bedding. F344 rat dams ($n = 19$–22/group) were gavaged with bisphenol A (99.9% purity) at 0 (0.5% sodium carboxymethylcellulose vehicle), 7.5, or 120 mg/kg bw/day during mating, gestation, and lactation periods. Doses were based on the result of an NTP study (NTP, 1982). Clinical signs, food intake, and body weight were monitored in dams during the study. After birth, pups were counted and weighed. Pups were randomly culled to 8/litter on PND 4 (day of birth not defined). On PND 21, weaning occurred and female pups were killed and discarded. Dams were killed at weaning for examination of implantation sites. Male pups were weighed during the post-weaning period. On PND 23, 28, and 91, five male offspring/group were killed. Ventral prostate weights were measured during each evaluation period, and anterior and dorsolateral prostate, testis, and epididymis weight were also measured on PND 91. Reproductive organs were preserved in 10% buffered formalin and examined histologically. Sperm count, motility, and morphology were examined on PND 91. The study was repeated with evaluation of 10 male offspring/group. **[The number of dams treated/group in the repeat study was not reported. Based on body weights reported for rats in Experiment 2, it appears they were evaluated at adulthood, but it was not specified if they were evaluated on PND 91.]** Data were analyzed by Student *t*-test. **[It appears that offspring were considered the statistical unit.]**

In the first experiment, bisphenol A exposure had no effect on dam body weights during gestation or lactation, duration of the gestation period, or number of implantation sites. There were no effects on litter size, pup viability, or sex ratio. On PND 21, relative dorsolateral prostate weight was significantly higher **[by 23%]** in the low-dose group than in controls. **[It was not stated if organ weights were relative to body weight.]** There were no effects on final body weight or weights of anterior and ventral prostate, testis, or epididymis. There were no increases in malformations of reproductive organs. **[Data were not shown by study authors.]** Testicular sperm counts were significantly lower **[by 22%]** in males of the high-dose group, but there were no effects on epididymal sperm counts. There were also no effects on sperm motility or abnormalities. **[Data were not shown by authors.]** In the second experiment examining 10 males/group, exposure to bisphenol A had no effects on final body weights or relative weights of testis, epididymis, or ventral, anterior, or dorsolateral prostate. There were no adverse effects on testicular or epididymal sperm count, motility, or morphology. Morphologically abnormal sperm were reduced in rats of the low-dose group. Study authors concluded that under the conditions of their study, exposure of dams to bisphenol A during the gestation and lactation periods did not result in adverse effect on the reproductive system of male offspring.

Strengths/Weaknesses: The number of dams used in Experiment 1 appears adequate and 10 males/group were used to examine various organ endpoints at multiple time points. It is unfortunate that these data were then analyzed by many *t*-tests rather than multivariate analyses.

Utility (Adequacy) for CERHR Evaluation Process: This study is considered inadequate due to statistical insufficiencies.

Ichihara et al. (2003), supported by the Japanese Ministry of Health, Labor, and Welfare, examined the effects of prenatal and lactational exposure to bisphenol A on the development of prostate cancer in rats. F344 rat dams were fed nMF feed during pregnancy and lactation and their offspring were fed MF (Oriental Yeast Co.) following weaning. Rats were housed in cages containing wood chip bedding. **[No information was provided about caging materials.]** During pregnancy and lactation, ~8–15 dams/group were gavaged with bisphenol A (99.9% purity) at 0 (0.5% carboxymethyl cellulose sodium salt vehicle), 0.05, 7.5, 30, or 120 mg/kg bw/day. Doses were based on findings from an NTP study **[citation not provided]**. Dam body weight and food intake were monitored during the study. Gestation period duration and implantation sites were evaluated. Pups were counted and sexed at birth. Litters were culled randomly to 8 pups on PND 4, and pups were weaned on PND 21 **[day of birth not defined]**. At 5 weeks of age, 21 male rats/group were injected s.c. with 50 mg/kg bw 3,2-dimethyl-4-aminobiphenyl 10 times at 2-week intervals. An additional 12 rats/group in the 0, 0.05, 7.5, and

120 mg/kg bw/day bisphenol A groups were injected with corn oil during the same time period. Surviving male offspring were killed and necropsied at 65 weeks of age. Blood was collected for analysis of serum testosterone levels in 5 rats/group. Reproductive organs were examined for gross abnormalities, weighed, and fixed in 10% buffered formalin. A histopathological examination of the prostate was conducted. Body and organ weight data were analyzed by Student *t*-test. The incidence of histopathological lesions was evaluated by Fisher exact probability test. **[It appears that the litter was considered the statistical unit in analyses for numbers and survival of pups at birth. Offspring were apparently considered the statistical unit for other analyses.]**

Body weights of dams in the 120 mg/kg bw/day group were significantly lower than control values from GD 14–20. There were no consistent or dose-related effects on dam body weights during lactation, although a significant increase in body weight was observed in dams of the 0.05 mg/kg bw/day group on PND 14. Exposure to bisphenol A had no effect on gestation period duration or number of implantation sites. In pups exposed to bisphenol A, there were no differences in number of live births, sex ratio, external anomalies, or body weights during the lactation period. **[Data for pup body weights were not shown by study authors.]** Terminal body weight of pups exposed to 0.05 mg/kg bw/day bisphenol A before treatment with 3,2-dimethyl-4-aminobiphenyl were significantly higher than controls **[by 12%]**. Exposure to bisphenol A had no effect on weights of prostate, testis, or epididymis. Incidences of prostatic intraepithelial neoplasia, carcinoma, and atypical hyperplasia were not increased by bisphenol A treatment, and there were no increases in tumors found in non-reproductive organs. No effect was observed on serum testosterone levels. The study authors concluded that exposure of rat dams to bisphenol A during the gestation and lactation periods does not predispose their offspring to prostate cancer development.

Strengths/Weaknesses: Strengths are the range of low dose levels, the use of an additional strain (Fischer 344 rat), and the endpoints evaluated. The design is reasonable for some of the endpoints measured, but sample sizes are inadequate for the prostate cancer endpoint and hormonal endpoints in particular. Statistical accounting for litter effects is unclear for neonatal measures, body weight, and fertility endpoints.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate based on insufficient sample size given the endpoints (i.e., tumors response) and lack of consistently accounting for litter effects.

Yoshida et al. (2004), supported by the Japanese Ministry of Health, Labor, and Welfare, examined the effects of bisphenol A exposure on development of the rat female reproductive tract. Donryu rats (12–19/group) were gavaged with bisphenol A **[purity not reported]** at 0 (carboxymethylcellulose solution), 0.006, or 6 mg/kg bw/day from GD 2 to the day before weaning of pups at 21 days post delivery. The low dose was said to represent average daily intake from canned foods and the high-dose was reported to represent the maximum dose level detected in plastic plates for children. **[It is assumed the authors meant estimated exposure levels for children eating off plastic plates.]** Bisphenol A levels were measured in maternal and pup tissues, and those values are reported in Section 2.1.2.2.1. After delivery, dams and litters were housed in plastic cages with wood chip bedding. Tap water was stored in plastic containers. The only information provided about feed was that it was a commercial pellet diet. Samples of tap water, drinking water from plastic containers, and feed were measured for bisphenol A content by HPLC. Offspring were sexed, weighed, and examined for external abnormalities on PND 1 and then weighed weekly through PND 21. Litters were adjusted to 8–10 pups at PND 4 or 6 (day of birth = PND 0). Dams were weighed, and observed during the study and killed following weaning of litters on PND 21. Implantation sites were examined and organs including uterus, vagina, and ovaries were fixed in 10% neutral buffered formalin and examined histologically. **[It does not appear that results of histopathological testing in dams were reported.]** All female offspring were examined for vaginal opening, and following vaginal opening, vaginal smears were taken for the remainder of the study. Three to 5 offspring/group from different litters were killed on PND 10, 14, 21, or 28 and at 8 weeks of age. At most time periods, uteri were weighed, preserved in 10% neutral buffered formalin, and examined histopathologically to determine development of uterine glands. Ovaries and vagina were also examined histologically. ERα was determined using an immunohistochemical method. Serum was collected for measurement of FSH and LH by RIA. Four offspring/group from different litters were killed at 8 weeks of age on the morning of estrus to examine ovulation by counting ova in oviducts. Initiation of carcinogenesis following injection of the uterine horn with *N*-ethyl-*N*′-nitro-nitrosoguanidine was examined at 11 weeks of age in 35 or 36 animals/group. At ~24 weeks following cancer initiation, the 24–30 surviving animals/group were killed and uteri were examined histologically to determine the presence of tumors and other lesions. Statistical analyses included ANOVA and Dunnett test. **[Most of the data for endpoints evaluated at birth appeared to be presented and apparently analyzed on a litter basis. For other data, it appears that offspring were considered the statistical unit.]**

Bisphenol A was not detected in fresh tap water but was detected at ~3 ng/mL following storage in plastic containers. The bisphenol A concentration in feed was ~40 ng/g. In dams exposed to bisphenol A, there were no clinical signs of toxicity or effects on body weight, implantation sites, or gestation length. Bisphenol A exposure had no effect on litter size, pup body weight at birth and through PND 21, external abnormalities in pups or age of vaginal opening. In uteri of bisphenol A-exposed offspring, there were no effects on weight, gland development, ERα, or cell proliferation. No increase in lesions was reported in organs of the alimentary, urinary, respiratory, or nervous system. **[Data were not shown by study authors.]** Bisphenol A exposure had no effect on ovulation, estrous cyclicity, or serum FSH or LH levels. There were no effects on uterine preneoplastic or neoplastic lesions or ovarian histopathology following bisphenol A treatment. The study authors concluded that perinatal exposure to bisphenol A at levels comparable to human exposure did not affect the reproductive system of female rats.

Strengths/Weaknesses: Strengths of this study were the bisphenol A determinations that were made and the anchoring of animal exposure levels to human exposures. The design appears sound with a good range of endpoints measured. Small numbers of animals were sacrificed at several time points and cellular analyses were performed; these numbers were too small for a definitive cancer evaluation and were, in fact, too small for definitive conclusions to be reached for most of the adult reproductive endpoints. Statistics are not described in enough detail to determine how data from multiple sampling points were evaluated. This experiment represents a thorough screening study.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate based on insufficient sample size (3–5/group).

Takagi et al. (2004), supported by the Japanese Ministry of Health, Labor, and Welfare, examined the effect of perinatal bisphenol A exposure on the reproductive and endocrine systems of rats. Nonylphenol was also examined but will not be discussed. Sprague–Dawley rat dams were fed a soy-free diet (Oriental Yeast Co.) prepared according to the formula for NIH-07. At weaning, the offspring were fed CRF-1 diet (Oriental Yeast Co.), which contains soybean and alfalfa-derived proteins. Rats were housed in polycarbonate cages containing wood chip bedding. Dams were randomly assigned to groups, and 5–6 dams/group were fed diets containing bisphenol A (96.5% purity) at 0, 60, 600, or 3000 ppm from GD 15 (GD 0 = day of vaginal plug) to PND10 (PND 1 = day of birth). The study authors estimated bisphenol A intake at ~5, 49, and 232 mg/kg bw/day during the gestation period and ~9, 80, and 384 mg/kg bw/day during the lactation period. Dose levels were based on results of preliminary studies, and selected with a goal of achieving weak to moderate toxicity in dams at the highest dose. In a separate study, rats were fed diets containing ethinyl estradiol at 0 or 0.5 ppm from GD 15 to PND 10. On PND 2, offspring were counted, sexed, and weighed and anogenital distance was measured. Litters were culled to 6 pups on PND 10, and pups were weaned on PND 21. Five pups/sex/group (1/sex/litter) were selected for necropsy on PND 21 and brain, adrenals, testis, ovary, and uterus were weighed. Eight offspring/sex/group (at least 1/sex/litter) were selected for evaluation in adulthood, and these rats were observed for age and body weight at puberty. Estrous cyclicity was observed from 8–11 weeks of age. Offspring were killed at 11 weeks of age, on the day of diestrus for cycling female rats. Brain, pituitary, thyroid, adrenal mammary gland, epididymis, prostate, seminal vesicles, ovary, uterus, and vagina were weighed and examined histologically. The testis was fixed in Bouin solution, and other organs were fixed in 10% neutral buffered formalin. The volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) was measured. It appears that endpoints were assessed in 8 adult rats/sex/group, with the exception of histopathological evaluations, which were conducted in 5 rats/sex/group. The litter was considered the experimental unit in statistical analyses of data from PND 21 offspring, and the individual animal was considered the statistical unit for data obtained from adult offspring. Homogenous numerical data were analyzed by ANOVA and Dunnett test, and heterogeneous numerical data were analyzed by Kruskall–Wallis *H*-test and Dunnett-type rank sum test. Data for histopathological lesions and vaginal cyclicity were analyzed by Fisher exact probability test or Mann–Whitney *U*-test.

Maternal body weight gain was significantly decreased the high-dose bisphenol A group during gestation, but there were no effects on body weight gain during lactation or food intake. In offspring evaluated on PND 2, there were significant decreases in body weight in low- and high-dose males **[13 and 22%]** and in high-dose females **[20%]**, but there were no effects on number of live offspring or anogenital distance. Body weight gain was lower in high-dose males **[21%]** and females **[29%]** from PND 2–10. Increased relative brain weight as a result of growth retardation was reported in high-dose offspring evaluated on PND 21. **[Data were not shown by study authors.]** Exposure to bisphenol A did not affect onset of vaginal opening, preputial separation, or estrous cyclicity. Body weight of males was significantly lower **[by 9.3%]** at adult necropsy. Weights and histopathology of brain, pituitary, thyroid, adrenal mammary gland, epididymis, prostate, seminal vesicles, ovary uterus, and vagina in adulthood were unaffected in rats from the bisphenol A group. **[Organ weight data were not shown by study authors.]** Bisphenol A did not affect SDN-POA volume. Effects observed in offspring from the ethinyl estradiol study included reduced numbers of live offspring, increased male:female ratio, decreased body weight and body weight gain, accelerated vaginal opening, delayed preputial separation, increased estrous cycle irregularities, and histopathological alterations in pituitary, ovary, uterus, vagina, and mammary gland. The study authors concluded that bisphenol A did not affect endocrine or reproductive system development of rats at doses that induced maternal toxicity.

Strengths/Weaknesses: Strengths include the range of doses and endpoints measured and the use of the ethinyl estradiol comparator group. The study used small sample sizes of dams (n = 5–6/group) and inadequate statistical procedures to control for litter effects.

Utility (Adequacy) for CERHR Evaluation Process: This study is considered inadequate for the evaluative process, based on sample size and statistical procedures.

Akingbemi et al. (2004), supported by NIEHS, USEPA, NICHHD, and NIH, conducted a series of studies in Long–Evans rats to determine the effects of postweaning and perinatal exposure to bisphenol A on testicular steroidogenesis. In vitro studies were also conducted and are described in Section 4 because cells used in the studies were obtained from adult animals. Rats were fed Purina chow, which contains soybean meal, and given drinking water in polycarbonate bottles. Pregnant and nursing dams were housed in polycarbonate cages lined with wood bedding, but no information was provided on caging used at the other life stages. To reduce leaching of bisphenol A, the cages were washed, rinsed, and dried at least twice/week and were discarded once they began getting cloudy; water bottles were cleaned daily. Corn oil vehicle was used for bisphenol A and was administered to control animals. Rats were stratified according to body weight and randomly assigned to treatment groups. RIA methods were used to measure steroid hormone concentrations in serum or testicular fluid. RT/PCR methods were used to examine changes in mRNA expression. Statistical analyses included ANOVA with multiple

comparisons conducted by the Duncan multiple range test. **[In the part of the study in which dams were dosed, it appears that offspring were considered the statistical unit.]**

In the first study, rats were gavaged with bisphenol A **[purity not given]** at 0, 0.0024, 0.010, 100, or 200 mg/kg bw/day from PND 21–35. The two lowest doses were selected to represent environmental exposures, and the higher doses were selected to compare the effects between low and high-doses. Rats were killed at the end of treatment and blood was collected for measurement of serum LH, testosterone, and 17β-estradiol levels. Leydig cell cultures were prepared for measurement of ex vivo testosterone production with and without the addition of LH, testosterone precursors, or metabolizing enzymes. Additional weanling rats were exposed to bisphenol A at 0 or 0.0024 mg/kg bw/day on PND 21–35. At the end of treatment, mRNA for *LH*β, *ER*β, and *ER*α was measured in pituitary using an RT-PCR technique. All endpoints were reported for 7–12 rats/group. Compared to rats in the control group, rats exposed to bisphenol A at 0.0024 mg/kg bw/day had significantly lower levels of serum LH **[by 62%]** and testosterone **[by 40%]**. Serum 17β-estradiol levels were decreased in rats exposed to 0.0024, 0.010, and 100 mg/kg bw/day bisphenol A **[by ~30, 40, and 25% in each respective dose group]**. There were no effects on basal ex vivo testosterone production by Leydig cells. In Leydig cells obtained from rats exposed to 0.0024 mg/kg bw/day bisphenol A, testosterone production was significantly reduced when cells were exposed to LH or CYP450 17α-hydroxylase/17–20 lyase. In Leydig cells obtained from rats exposed to 0.0024 or 0.010 mg/kg bw/day bisphenol A, testosterone production was significantly reduced following exposure of the cells to pregnenolone or progesterone. No effects on blood hormone levels or ex vivo testosterone production were observed at higher doses. Significant effects observed in pituitaries obtained from rats exposed to 0.0024 mg/kg bw/day bisphenol A were decreased *LH*β mRNA and increased *ER*β mRNA. The study authors concluded that the decreased serum LH level resulted from bisphenol A effects on the pituitary and that decreased LH stimulation of Leydig cells was the cause of reduced serum testosterone levels.

In the second experiment, 7 dams/group were gavaged with bisphenol A at 0 or 0.0024 mg/kg bw/day from GD 12–PND 21. Male offspring received no further treatment following weaning. Males were randomly selected from each dam and killed on PND 90. Endpoints evaluated in 10–12 male offspring/group included serum LH and testosterone levels, ex vivo testosterone production by Leydig cells, testosterone levels in testicular interstitial fluid, and seminal vesicle and prostate weight. Significant ($P<0.01$ or 0.05) effects observed in 90-day-old males that had been perinatally exposed to bisphenol A compared to the control group included increased body weight **[10%]**, decreased relative weight (to body weight) of paired testes **[17%]** and seminal vesicles **[17%]**, reduced testicular testosterone level **[~39%]**, and reduced basal and LH-induced ex vivo testosterone production.

In the third experiment, 10–12 rats/group were gavaged with bisphenol A at 0 or 0.0024 mg/kg bw/day from PND 21–90. Within 24 hr following treatment, rats were killed and examined for the same endpoints described for the second experiment. Significant ($P<0.01$ or 0.05) effects compared to the control group included increased serum LH level **[117%]**, decreased seminal vesicles weight **[absolute: 15%, relative: 16%]**, reduced testicular testosterone level **[~24%]**, and decreased basal and LH-induced ex vivo testosterone production. For the second and third experiments, the study authors concluded that bisphenol A exposure inhibits androgen production by Leydig cells.

Strengths/Weaknesses: Significant strengths of this report were the sequential nature of the work, in which later studies built on the previous data, and the clear expertise that the authors brought to this endeavor. Experiment 1 provided a helpful examination of postnatal effects following adolescent exposure and examined hormonal levels under stimulated and unstimulated conditions, thus separating pituitary from target organ contributions to serum levels. In Experiment 2, the sample size of 7 dams/prenatal treatment group and the examination of 10–12 offspring/group raise questions about the adequacy of the sample size with respect to the number of litters represented and the number of offspring used to represent each litter. In Experiment 3, 10–12 rats/group were treated from PND 21–90 (through adolescence and into early adulthood) and then examined according to endpoints common to Experiments 1 and 2. Weaknesses include an inadequate number of animals to obtain confidence about the hormonal changes (indeed, LH was decreased in the first experiment and increased in the third), the lack of histopathology evaluation, and lack of an estrogenic positive control.

Utility (Adequacy) for CERHR Evaluation Process: Experiments 1 and 3 are adequate but of limited utility because of the mechanistic nature of the endpoints examined. Experiment 2 is inadequate for consideration due to inappropriate statistics that failed to account for litter effects.

Masutomi et al. (2004), supported by the Japanese Ministry of Health, Labour, and Welfare, examined the potential effects in rats of neonatal bisphenol A exposure through maternal dietary intake on the number of offspring pituitary cells positive for LH, FSH, and prolactin. The authors exposed 5–8 pregnant CD(SG)IGS dams from GD 15–PND 10 to soy-free diet containing: (1) genistein 20, 200, or 1000 ppm; (2) diisononyl phthalate 400, 4000, or 20,000 ppm; (3) methoxyclor 24, 240, or 1200 ppm; (4) 4-nonylphenol 60, 600, or 3000 ppm; or (5) bisphenol A **[96.5% purity]** 60, 600, or 3000 ppm. Ethinyl estradiol at 0.5 ppm was also administered to a positive control group and the regular soy-free diet to a control group. **[Only the bisphenol A-treated group will be considered here. Feed consumption and dam body weight were not reported, but would be expected to have changed dramatically over the treatment period, making it difficult to estimate the bisphenol A doses received by the rats.]** After weaning, offspring were placed on CRF-1 rodent chow. Animals were housed in polycarbonate cages with wood-chip bedding.

During postnatal week 3 or 11, offspring were killed and anterior pituitary glands from 5 male and 5 female offspring/group were harvested. Immunohistochemistry using paraffin-embedded sections for LH, FSH, and prolactin was conducted and the percentage of cells positive for LH, FSH, and prolactin was determined in 2 sections/gland. Statistical analyses were performed by

Student or Welch's *t*-test using values from the highest bisphenol A dose group and the control. There was no effect of bisphenol A treatment on relative pituitary weight or on cell counts for LH, FSH, or prolactin. There was an increase in cells staining for prolactin in female offspring from the ethinyl estradiol-treated dams at 3 weeks.

Strengths/Weaknesses: This hypothesis-driven study was carefully designed with respect to exposure during established periods relevant to the sexual differentiation of the brain and with respect to assessment of appropriate parameters related to reproductive function. A large number of dose levels were examined across 5 compounds, one being bisphenol A with evaluation of 4 dose levels, including controls. Five to eight animals/sex/dose were used in evaluations and animals were selected as 1 male and 1 female/litter. Findings were judged against an incorporated positive control that resulted in predicted findings.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility for the evaluation because of the secondary nature of the endpoints for a human health evaluation.

Tan et al. (2003), supported by the University of Malaya and the Ministry of Science, Technology, and Environment, examined the effects of bisphenol A exposure on pubertal development of male rats. Sprague–Dawley rats were fed soy-free feed (Gold Coin Feedmills) and housed in aluminum cages containing shredded recycled paper as bedding. On PND 23–53, 12 rats/group were gavaged with 100 mg/kg bw/day bisphenol A **[purity not reported]** in a Tween-80/water solution (1:9 v/v), 100 mg/kg bw/day nonylphenol in corn oil, or a mixture of 100 mg/kg bw/day bisphenol A and nonylphenol. A control group of 12 rats was gavaged with Tween 80 in corn oil. Dosage selection was based on published studies reporting NOAELs of 50 mg/kg bw/day for both compounds. Rats were examined for preputial separation during the study. Six rats/group were killed on PND 52, and the other 6/group were killed on PND 53. Testes, epididymides, liver, kidney, adrenal, seminal vesicles plus coagulation gland, and thyroid were weighed. **[The Expert Panel assumes that by coagulation gland, the authors mean the anterior prostate or coagulating gland.]** Thyroid, testis, kidney, and liver were fixed in 10% formalin and examined histologically. Statistical analyses included ANOVA and Fisher protected least significant difference test.

There was no significant effect on weight gain in rats treated with bisphenol A. In the bisphenol A group, preputial separation occurred by PND 53 in 66.7% of rats compared to 100% of rats in the control group. In the bisphenol A group, significant increases were observed in absolute and relative (to body weight) kidney and thyroid weights and significant decreases were observed for absolute and relative liver weight. Cortical thickness of the kidney was significantly decreased **[by ~13% compared to controls according to CERHR calculations and ~30% according to study authors]**. There was no effect on testicular weight or tubule diameter. Normal patterns of spermatogenesis were observed in rats from the control group. Multinucleated giant cells were observed in seminiferous tubules and there was no indication of spermatogenesis in 4 of 12 rats of the bisphenol A group. Giant cells were observed and spermatogenesis was found to occur in only some seminiferous tubules of the remaining rats treated with bisphenol A. Moderate-to-severe hydronephrosis was observed in 50% of rats and mild hydronephrosis was observed in the other 50% of rats from the bisphenol A group.

Preputial separation occurred by PND 53 in 33.3% of rats in the nonylphenol group and 58.3% of rats exposed to the bisphenol A/nonylphenol mixture. In animals treated with nonylphenol, relative liver weight was increased, absolute and relative seminal vesicle weights were decreased, and the diameter of testicular tubules was reduced. A decrease in relative seminal vesicle weight was the only significant organ weight effect observed in the group treated with both bisphenol A and nonylphenol. Moderate hydronephrosis was observed in 25% of rats exposed to the bisphenol A/nonylphenol mixture and mild hydronephrosis was observed in the other rats from that exposure group. No spermatogenesis was observed in 3–5 of 12 rats/group treated with nonylphenol or the mixture of bisphenol A/nonylphenol. The study authors concluded that both bisphenol A and nonylphenol affected the reproductive system of rats, while only bisphenol A affected the kidneys. They also noted a less-than-additive effect with administration of the bisphenol A/nonylphenol mixture.

Strengths/Weaknesses: This study was apparently well performed and documents the endpoints tested. A weakness is the use of a single high-dose level of bisphenol A.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Kobayashi et al. (2005), supported by the Japanese Ministry of Health, Labor, and Welfare, examined the effects of developmental exposure to bisphenol A on thyroid status in rats. Rats used in this study were fed standard laboratory chow (CE-2, Clea Japan). **[No information was provided about caging or bedding materials.]** From GD 6 (day of copulatory plug = GD 0) through PND 20 (day of birth not defined), 6 maternal CD rats/group were gavaged with bisphenol A (>99.8% purity) at 0 (corn oil vehicle), 4, 40, or 400 mg/kg bw/day. The 400 mg/kg bw/day maternal group was excluded from analysis because of excessive maternal toxicity. Details about maternal toxicity and additional aspects of this study are available in the summary for the study by Kobayashi et al. (2002). On PND 7, litters were culled to 5 pups/sex when possible. It appears that culled pups may have been used in analyses conducted at 1 week of age. Pups were weaned on PND 21. Approximately 1 male and 1 female pup/litter were killed at 3 and 9 weeks of age. Plasma thyroxin levels were measured by chemiluminescence immunoassay in 1–9 offspring/group/sex at 1 week of age and 3–6 offspring/sex/group at 3 and 9 weeks of age. At 9 weeks of age, thyroid stimulating hormone test was conducted in 2–7 rats/sex/group by measuring thyroxin levels after injection with bovine thyroid stimulating hormone. Statistical analyses included ANOVA followed by Dunnet test or Student or Welch's *t*-test. **[It was not clear if the litter or offspring were considered the statistical unit.]**

In the 4 and 40 mg/kg bw/day groups, there were no significant differences in thyroxine levels at 1, 3, or 9 weeks of age or in thyroid stimulating hormone-induced increases in thyroxine levels at 9 weeks of age. Based on

the findings of this study, the study authors concluded that prenatal and lactational exposure of rats to bisphenol A does not appear to affect thyroid function.

Strengths/Weaknesses: Strengths of this study include the use of a range of dose levels of bisphenol A. Weaknesses include the limited endpoints addressed (thyroid function), concern that the number of animals used (6 dams per treatment group) may not provide adequate statistical power to assess changes in hormone levels and response given the variability inherent in these measures, and failure to account for litter in the analyses.

Utility (Adequacy) for CERHR Evaluation Process: As presented, this study is inadequate because it is unclear whether there were adequate controls for litter effects.

Zoeller et al. (2005), supported in part by NIH, examined the effect of bisphenol A exposure on the thyroid of developing rats. Sprague–Dawley rats were housed in plastic cages. **[No information was provided about composition of feed or bedding materials.]** Before initiation of dosing, rats were trained to eat an untreated wafer each day. On GD 6 (day of vaginal plug not defined) through the remainder of the experiment (the remainder of the gestation and lactation periods), 9 rats/group were given a wafer dosed with bisphenol A **[purity not reported]** at levels resulting in exposure to 0 (methanol vehicle), 1, 10, or 50 mg/kg bw/day. Doses were based on those used in the study by Tyl et al. (2002b). Pups ($n = 7$–9/group/sex/time period) were weighed and killed on PND 4, 8, 15, or 35 (day of birth not defined). During each of those time periods, serum thyroxin was measured by RIA. On PND 15, brains of male pups were sectioned and examined for presence of RC3/neurogranin mRNA, a thyroid hormone-responsive gene, using an in situ hybridization and autoradiography technique. Serum thyroid-stimulating hormone was measured using an unspecified method in 6–8 male pups/group (1/litter) on PND 15. Statistical analyses included ANOVA and Bonferroni *t*-test.

The text of the study indicated a significant reduction in maternal body weight gain during pregnancy in the high-dose group, while Figure 1 of the study indicated a significant reduction in maternal body weight gain during pregnancy at all dose levels. Maternal body weight gain during the lactation period was unaffected by bisphenol A treatment. Bisphenol A exposure had no effect on litter size at birth. **[Data were not shown by study authors.]** Bisphenol A had no effect on pup body weights on PND 4, 8, or 15. On PND 15, but at no other time period, there was a significant increase in serum thyroxin levels in all dose groups of male and female pups **[percent increases compared to controls were ~11, 35, and 37% in each respective dose group.]** Significant increases in expression of RC3/neurogranin mRNA were observed in the upper and lower dentate gyrus in males from each treatment group **[with no apparent dose–response relationship]**. Expression of RC3/neurogranin mRNA in cortex was unaffected by bisphenol A treatment. No significant effects were observed for thyroid-stimulating hormone levels in males on PND 15. The study authors concluded that bisphenol A acts as a thyroid antagonist at these concentrations.

Strengths/Weaknesses: Strengths of the study include use of a range of doses and examination of a role of bisphenol A as a thyroid hormone antagonist. Weaknesses include the lack of litter-based analysis.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate based on inappropriate statistics (i.e., not accounting for repeated measures over time or the use of more than one pup per litter for a given endpoint).

3.2.3.3 Studies with neurobehavioral endpoints: **Kwon et al. (2000)**, from CIIT, examined the effects of bisphenol A exposure during pre- and postnatal development on reproductive endpoints and the SDN-POA of rats. Sprague–Dawley rats were fed NIH-07 feed and housed in polycarbonate cages with cellulose fiber-chip bedding. Water was provided in glass bottles with Teflon-lined caps. Pregnant rats were randomly assigned to groups according to body weight. Rats ($n = 8$/group) were gavaged with bisphenol A (~99% purity) at 0 (corn oil vehicle), 3.2, 32, or 320 mg/kg bw/day from GD 11 (GD 0 = day of sperm detection) through PND 20, excluding the day of parturition. It was not stated if the day of parturition was considered PND 0 or 1. A positive control group was treated with 15 μg/kg bw/day diethylstilbestrol. Rats were examined for clinical signs of toxicity and weighed during the study. Pups were weighed on PND 1 and 7. Pups were weaned on PND 21. After pups were weaned, dams were killed for assessment of body and organ weights. On PND 10, brains were collected from 1–3 female offspring/litter from 6–8 litters/group for measurement of SDN-POA volume. All remaining female pups were examined for age of vaginal opening and day of first estrus, and estrous cyclicity was monitored for 22 days, beginning at ~4 months of age. Lordosis behavior was examined at 6 months of age in 1–2 female offspring/litter from 7–9 litters/group that had been ovariectomized 2 weeks before reproductive behavior testing and primed with estradiol benzoate and progesterone. Male offspring were killed on PND 180 for measurement of body and reproductive organ weights and histopathological evaluation of ventral prostates fixed in 10% neutral buffered formalin. **[Based on information presented in the Results section, it also appears that ovaries and uteri were examined in an unspecified number of female offspring at 6 months of age.]** Statistical analyses included ANOVA, Dunnett test, ANCOVA, and pair-wise comparison of least square means. The litter was considered the experimental unit.

Bisphenol A treatment had no significant effect on maternal body weight during pregnancy or lactation or on maternal liver, kidney, adrenal, ovary, or uterus weights. There was no effect on number of live pups/litter at birth or pup weight on PND 1 or 7. In female offspring, bisphenol A exposure had no significant effect on volume of SDN-POA, age or weight at vaginal opening or first estrus, estrous cyclicity, or mean lordosis intensity. In male offspring, there were no significant effects on body weight or weights of testis, epididymis, seminal vesicle, or prostate. The study authors noted that a 23% increase in ventral prostate weight in the high-dose group did not reach statistical significance. No treatment-related histopathological alterations were reported for ventral prostate, ovary, or uterus. Effects observed in the diethylstilbestrol group included increased maternal liver weight, increased SDN-POA volume in female offspring, and disrupted estrous

cycles. The study authors concluded that indirect exposure of offspring to bisphenol A at these levels during gestation and lactation did not affect estrogen-mediated reproductive endpoints. A similar study with comparable findings in females was reported in abstract form (Kwon et al, 1999).

Strengths/Weaknesses: This study was well performed and presented. The wide coverage of the dose range (across a three-log range) is a major strength. The use of diethylstilbestrol as a positive control is a strength, as is the number of reproductive organs and endpoints evaluated. A weakness was the limited analysis of those reproductive organs (wet weight only; histopathology was only performed on the prostate) and a lack of determination of pup exposure during lactation.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Kubo et al. (2001), supported by the Japanese Ministry of Education, Science, and Culture, examined the effect of prenatal bisphenol A exposure on sexually dimorphic behavior and brain development in rats. **[No information was provided about the type of chow, bedding, or caging materials used.]** Throughout the gestation (from the day that sperm were detected in the vagina) and lactation periods, 5 Wistar rats/group were administered bisphenol A through drinking water at 0 or 5 mg/L. **[No information was provided on bisphenol A purity or use of a vehicle.]** The study authors estimated the bisphenol A dose at 1.5 mg/kg bw/day. **[It is not clear whether this is an estimate based on water consumption or dosing by a separate, unspecified route. If based on drinking, this estimate is suspect because it implies a daily consumption of approximately 70 mL water (because the weight of the rats is not supplied this must of necessity be a guess), which is well in excess of published intakes for post-partum rats (generally noted as around 20 mL/day). It is also noted that water consumption varies widely in non-lactating rats and throughout the period of lactation in rats, reflecting milk production, so any such estimate would of necessity be suspect, and doses will vary with time post-partum.]** Litters were adjusted to 5 pups/sex on the day following birth. Pups were weaned on PND 21 **[day of birth not defined]** and housed according to sex and litter. Behavior was tested for 10 min in an open-field apparatus at 6 weeks of age ($n = 11–14$) **[It was not clear if the number of animals examined included total animals, total/group, or total/sex/group. Litter distribution was not indicated.]** A passive avoidance test was conducted at 7 weeks of age ($n = 11–14$); the test included a habituation period and testing of retention 24 hr later. An unspecified number of rats were killed and necropsied at 12 weeks of age, with females killed in diestrus. Reproductive organs were weighed ($n = 12–14$) and sperm endpoints were evaluated in an unspecified number of rats. Serum hormone levels were measured by RIA ($n = 5–10$/group). At 20 weeks of age, 6 rats/sex from the control group and 7 rats/sex from the treated group were killed to measure the volume of the SDN-POA and the locus ceruleus. Behavioral data were analyzed by Student *t*-test or Mann–Whitney *U*-test, and brain morphology data were analyzed by Student *t*-test. **[It was not clear if the litter or offspring was considered the statistical unit.] No information was provided on data analyses for reproductive organ weight, serum hormone levels, or sperm endpoints.]**

In open-field testing of controls, females moved significantly greater distances, reared more times, and stayed in the center of the apparatus for a longer period of time than males. In passive avoidance testing of controls, latency to enter the dark chamber following electric shock was significantly longer in male than female rats. In rats exposed to bisphenol A, there were no significant differences in the behaviors of males compared to females. Study authors attributed the loss of sexually dimorphic behaviors to demasculinization of male behavior and defeminization of female behavior. Bisphenol A treatment did not affect brain weight, which was higher in male than female controls. The larger size of SDN-POA in male compared to female controls was retained following bisphenol A treatment. The volume of the locus ceruleus was significantly larger in females than males of the control group. In the bisphenol A group, the volume of the locus ceruleus was described as larger in males than females, but the stated increase was not statistically significant ($P = 0.12$). **[Graphically, there is an estimated 14% difference between male and female locus ceruleus volume in controls and in bisphenol A-exposed animals, with the direction of the difference apparently reversed by treatment.]** Bisphenol A treatment had no effect on absolute weight of the testis or epididymis or relative weights of the ventral prostate, ovaries, or uterus. There were no significant effects on serum levels of LH, FSH, testosterone, or 17β-estradiol. Sperm count and motility were also unaffected by bisphenol A exposure. The study authors concluded that current methods for establishing NOAELs may not be sufficient to detect disrupted sexual dimorphism in the brain.

Strengths/Weaknesses: A strength is the variety of biological and behavioral endpoints assessed. The major weakness of the study is the lack of experimental detail, which makes it difficult to determine whether litter effects were adequately controlled for and how much bisphenol A was received by the animals.

Utility (Adequacy) for CERHR Evaluation Process: Given the lack of methodological data provided in the study, this communication is inadequate for the evaluation process.

Kubo et al. (2003) examined the effect of prenatal bisphenol A exposure on sexually dimorphic behavior and brain structure of rats. **[No information was provided on the type of feed or materials used in bedding or caging.]** Wistar rats were dosed with the 0.1% ethanol in distilled water vehicle ($n = 5$ dams/group) or bisphenol A **[purity not reported]** at 0.1 or 1 mg/L ($n = 6$ dams/group). The study authors estimated bisphenol A intake at 0.030 and 0.3 mg/kg bw/day and noted that the levels were below the tolerated daily intake. **[Although not clearly stated, it appears that as in the previous study by Kubo et al. (2001), exposures occurred through drinking water during the entire gestation and lactation period.]** Five dams/group were exposed to *trans*-resveratrol, an estrogenic compound found in grapes, at 5 mg/L or diethylstilbestrol at 50 μg/L. Body weight and anogenital distance were measured in pups on PND 1 (the day following birth). **[All litters were examined and although the number of pups examined in each litter was not clearly stated, it was implied that all pups were**

analyzed.] Anogenital distance was adjusted by the cube root of body weight. Following the evaluations on PND 1, litters were standardized to 5 pups/sex. Pups were weaned on PND 21 and housed according to sex and litter. Day of testicular descent or vaginal opening was monitored in all remaining offspring ($n = 25$/sex in the control group and 30–31/sex in the treated group). Open-field testing was conducted in 20–24 animals/group at 6 weeks of age. **[It is not clear if the authors meant 20–24 animals/group or animals/group/sex]**. Sexual behavior of 7–13 male and female rats/sex/group was tested at 11–12 weeks of age. Males and females ($n = 11$–15/sex/group) were killed at 12 weeks of age, females during proestrus. Reproductive organs were weighed. Serum hormone levels were measured by RIA. Sperm from one testis and cauda epididymis were counted. Histological examinations were conducted on testis fixed in Bouin solution and ovary fixed in 10% neutral buffered formalin. Rats were killed at 14 weeks of age for measurement of SDN-POA and locus ceruleus volume in 7–8 males and females/group.

Because of the large number of animals used, the experiment was conducted in 3 blocks representing identical experiments. All data were collected and analyzed following completion of the third block of the study. The litter was considered the statistical unit in analyses of data collected before weaning of animals. Individual animals were considered the statistical unit in analyses of data collected subsequent to weaning.

Behavior and brain structure data were analyzed by ANOVA and differences between sexes were analyzed by Student *t*-test. Reproductive data were analyzed by ANOVA followed by Fisher protected least significant difference test for each sex.

Bisphenol A exposure had no significant effect on body weight on PND 1, anogenital distance in males and females, day of testicular descent, or day of vaginal opening. Body weight at vaginal opening was significantly higher **[by 7%]** in the high-dose bisphenol A group. In sexual behavior testing of males, a non-dose-related decrease in the intromission rate observed in the low-dose group was the only significant effect reported following bisphenol A exposure. There were no effects on mounting or ejaculation. Bisphenol A exposure had no significant effects on female sexual behavior as measured by ear wiggle, lordosis quotient, and rejection of males. The study authors concluded that bisphenol A exposure had no remarkable effects on male or female sexual behavior. The only significant effect on organ weights was an **[9%]** increase in testis weight in the high-dose bisphenol group. There were no significant effects on absolute weight or relative (to body weight) weights of ventral prostate, seminal vesicle, uterus, or ovary. Bisphenol A treatment did not affect sperm count or motility or estrous cycles. Serum levels of LH, FSH, prolactin, testosterone, and 17β-estradiol were also unaffected by treatment. No histopathological findings were observed in testis or ovary. **[Data were not shown.]**

In open-field testing of control rats, females moved greater distances, reared more often, and spent more time in the center of the testing apparatus. Following treatment with the low or high-dose of bisphenol A, there were no longer significant differences between males and females in frequency of rearing and or duration of time spent in the center of the apparatus. Differences in distances moved by males versus females were no longer significant following exposure to the high bisphenol A dose. Males in the low bisphenol A group reared significantly more times than males in the control group. Bisphenol A treatment had no significant effect on the sex-related difference in size of the SDN-POA, which was significantly larger in males than females in the control and treatment groups. Although the volume of the locus ceruleus was significantly greater in females than males of the control group, locus ceruleus volume was significantly larger in males than females of both bisphenol A groups. The change was due to a significant increase in volume in males at the low dose and significant decrease in volume in females at both dose levels of bisphenol A. **[Magnitude of locus ceruleus volume changes in males and females was ~12–17% compared to controls, as estimated from a graph.]** The numbers of neurons in the locus ceruleus was affected in the same manner as volume by bisphenol A treatment, except that increases in neuron numbers following bisphenol A treatment were also significant in males of the high-dose group.

Diethylstilbestrol mainly affected open-field behavior, locus ceruleus volume, and the reproductive system. *Trans*-resveratrol mainly affected locus ceruleus volume and the reproductive system. The study authors concluded that the brain is highly sensitive to bisphenol A at levels below the tolerable daily intake and disruptions in sexual differentiation may differ from effects observed with diethylstilbestrol and *trans*-resveratrol.

Strengths/Weaknesses: As with the previous study by this group (Kubo et al., 2001) the main weakness of the study lies in the failure to accurately describe the methods to allow a reader to determine how much bisphenol A the dams received during the experiment. Despite well-selected endpoints, the sample size of 5 dams/group and lack of clarity on the number of pups analyzed per litter are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to insufficient sample size and lack of experimental detail.

Facciolo et al. (2002), supported in part by the Italian Ministry of University Education and Research, examined the effects of developmental exposure to bisphenol A on the somatostatin receptor subtype sst_2 in the limbic circuit of rats. Sprague–Dawley dams were exposed orally to bisphenol A at 0 (arachis oil vehicle), 0.040, or 0.400 mg/kg bw/day. **[No information was provided on the specific method of oral dosing, the purity of bisphenol A, or the number of dams treated/group. There was no information on the type of chow used or composition of cage and bedding materials.] [Author states that 32 dams were subdivided into three treatment subgroups: controls ($n = 8$;), low bisphenol A and high bisphenol A ($n = 12$/group) (R. Facciolo, personal communication, July 17, 2007).]** The authors stated that the doses selected were relevant to human exposures from can linings and dental sealants and had been reported to induce morphometric changes in offspring. The rats were mated for 5 days during the treatment period, and treatment was continued through gestation and lactation. Litters (minimum 8/group) were culled to 8 pups at birth and 1 pup/litter was randomly assigned to a dam in the same treatment group for

postnatal rearing. Pups were weaned on PND 23 (day of birth not defined). On PND 10 and 23, 4–7 rats/group **[10–11/group according to figures in the study]** were killed and their brains were removed to examine effects on sst_2 receptors in the limbic region. Receptor binding was assessed using ^{125}I-Tyr^0-somatostatin-14 as a ligand. At the same ages, interactions of sst_2 with α-containing γ-aminobutyric acid (GABA) receptors, using the agonists zolpidem and Ro 15-4513, were examined in 12–13 rats/group. Results were reported for only the high-dose of bisphenol A (0.400 mg/kg bw/day) because higher affinity was obtained for receptor ligand binding. Statistical analyses included Student *t*-test, ANOVA, and Newman–Keuls multiple range test. Analyses did not account for litter of origin.

Strengths/Weaknesses: Strengths of this study are the fact that it appears to have been carefully performed and used biologically-relevant concentrations delivered orally. A weakness is the purposeful confounding of litter of origin through cross-fostering

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate due to experimental design that did not sufficiently account for litter effects.

Facciollo et al. (2005), supported by the Italian Ministry of University Education and Research, examined the effects of bisphenol A on expression of somatostatin subtype 3 (sst_3) receptor mRNA in brains of female rats exposed during development and investigated whether the $\alpha GABA_A$ receptor is also involved in this effect. Sprague–Dawley rats were housed in stainless steel cages. **[No information was provided about the type of feed or bedding used.]** Beginning 8 days before mating and continuing through the mating period (5 or 8 days) and during pregnancy and lactation (42 days), 8 rats received the arachis oil vehicle and 12 rats/group received bisphenol A **[purity not reported]** at 0.040 or 0.400 mg/kg bw/day. Vehicle or bisphenol A were orally administered by pipette. To minimize litter effects, 1 female pup from each litter was fostered to a dam from the same treatment group (8 pups/dam). Pups were weaned on PND 23. On PND 7 and at 55 days of age, 4 rats/group/time period were killed. Brains were sectioned and a ^{32}S-labeled probe was used in an in situ hybridization method to measure sst_3 mRNA expression. The effects of $\alpha GABA_A$ receptor subunits on expression of sst_3 mRNA was examined by incubating the brain sections in 1 nM–100 μM of $\alpha GABA_A$ receptor agonists (zolpidem, flunitrazepam, RY 080, and RO 15-4513). Additional brain sections from high-dose rats were used to determine interactions between sst_3 with α_1 and α_5 subunits with or without addition of 5–500 nM zolpidem or RY 080. Statistical analyses included ANOVA followed by Dunnett *t*-test or Neuman–Keuls multiple range post-hoc test, when analysis by ANOVA indicated statistical significance.

Changes in sst_3 expression varied with dose and age. Expression patterns were changed in the presence of $\alpha GABA_A$ receptor agonists. Based on their findings, the study authors concluded that bisphenol A exposure can affect cross-talking mechanisms involved in the plasticity of neural circuits with resulting influences on neuroendocrine/sociosexual behaviors.

Strengths/Weaknesses: Strengths of this study are the fact that it appears to have been carefully performed and used biologically-relevant concentrations. A weakness is the purposeful confounding of litter of origin through cross-fostering.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate due to experimental design.

Aloisi et al. (2002), supported in part by the Italian Ministry for Universities and Scientific and Technological Research (MURST), examined the effects of prenatal or postnatal bisphenol A exposure on the pain response of rats. **[No information was provided in the manuscript on chow or composition of caging and bedding. The Expert Panel has been informed that Harlan Teklad 2018 feed, Lignocel bedding, and polysulfone cages were used (F. Farabollini et al., personal communication, March 1, 2007).]** Sprague–Dawley rats were fed peanut oil vehicle ($n = 13$) or 0.040 mg/kg bw/day bisphenol A **[purity not given in the manuscript; ≥95% according to the authors (F. Farabollini et al., personal communication, March 1, 2007)]** ($n = 7$/group) by pipette during pregnancy and lactation. Within 48 hr after birth, the offspring were sexed and cross-fostered to form the following groups:

- Prenatal exposure group—born to dams receiving bisphenol A and nursed by dams receiving the peanut oil vehicle ($n = 11$ males; 9 females);
- Postnatal exposure group—born to vehicle control dams but fostered to bisphenol A treated dams ($n = 11$ males; 9 females); and
- Vehicle control group—born to and nursed by dams exposed to the vehicle control ($n = 16$ males and 11 females).

At 22 weeks of age, the rats were randomly assigned to sham or formalin treatment groups, but the sham group was not analyzed. The formalin group was s.c. injected with 10% formalin on the dorsal surface of the right hind paw. Pain behaviors, such as licking, flexing, and jerking of the paw were recorded for 60 min. Following testing, the phase of the estrous cycle was determined and blood was drawn to measure plasma levels of testosterone in males and corticosterone and 17β-estradiol in both sexes by RIA. Data were analyzed by ANOVA followed by post-hoc least significant difference test.

The frequency of paw jerking was decreased at 30–60 min following formalin injection in postnatally exposed rats. **[The study abstract and results section indicate that the effect occurred in males and females, but according to data presented in figures of the study, the effect only appeared to have occurred in males.]** Duration of flexion was increased 0–30 min following formalin injection in both sexes exposed prenatally to bisphenol A. Although statistical significance was not attained, the study authors noted an increase in licking duration at 0–30 min following formalin injection in females exposed to bisphenol A during prenatal development. No effects were observed on open-field behaviors or plasma levels of testosterone, 17β-estradiol, or corticosterone. The study authors concluded that their findings indicated sex- and exposure-related modifications of neural pathway activity or nociception centers following exposure to bisphenol A.

Strengths/Weaknesses: A strength of this study is the added dimension being investigated (pain response). A weaknesses, however, are use of a single dose and the

purposeful confounding of litter of origin during the cross-fostering process. In addition, the sample size of 7 dams in the 0.040 mg/kg bw/day bisphenol A group and the examination of $n = 11$ male and $n = 9$ female offspring in the prenatal exposure group raise questions about experimental or statistical accounting for litter effects.

Utility (Adequacy) for CERHR Evaluation Process: The data presented are inadequate due to the methodological design and lack of clarity on accounting for litter effects.

Negishi et al. (2003), support not indicated, examined the effect of perinatal bisphenol A exposure on behavior of rats. F344/N rats ($n = 8$–9/group) were orally exposed to bisphenol A at 0 (olive oil vehicle), 4, 40, or 400 mg/kg bw/day from GD 10–PND 20. GD 0 was defined as the day that vaginal sperm were detected and PND 0 was defined as the day of parturition. **[No information was provided on purity of bisphenol A, the specific method of oral dosing, type of chow used, or composition of bedding or caging materials.]** Dams were observed and weighed throughout the study. On PND 0, pups were counted, weighed, and culled to 8/litter with equal numbers/sex when possible. Pups were weighed periodically from PND 7–84. Pups were housed as same-sex littermates following weaning on PND 21. On weaning of pups, dams were killed and body and organ weights were recorded. Behavioral testing of offspring consisted of spontaneous motor activity measured at 28–34 days of age ($n = 12$–27/group), active avoidance testing conducted at 28–34 and 56–62 days of age ($n = 8$–9/group), and open-field behavior evaluations at 56–62 days of age ($n = 9$–18/group). Litter was not accounted for in the analyses. On PND 62, offspring were randomly selected (8/sex/group) and killed for evaluation of body and organ weights. Statistical analyses included ANOVA, nested ANCOVA, and post-hoc Fisher protected least significant difference test. **[Data analyzed at birth were presented and analyzed on a per litter basis. Postnatal data were apparently analyzed on a pup basis.]**

Maternal body weight gain was reduced during the gestation and lactation period in dams exposed to the mid- or high-dose. The only organ weight effects in dams were reduced absolute and relative (to body weight) thymus weight. There were no effects on weights of liver, kidney, or spleen in dams. Bisphenol A treatment did not affect the number of pups/litter or sex ratio. In male offspring, body weights were lower than control values on PND 7 and 28 at the mid-dose, and PND 7, 21, 28, and 56 at the high-dose. Body weights of female offspring were lower than controls at PND 7 and 28 at the low- and mid-dose and PND 7, 21, and 28 at the high-dose. On PND 62, there were no effects on body weight or liver, kidney, spleen, thymus, brain, or testis weights. There were no effects on spontaneous activity, but total immobile time was increased in females of the mid-dose group. Performance of males in avoidance testing improved in the mid- and high-dose group at 4 weeks of age but decreased in the low-dose group at 8 weeks of age. Increased grooming by males of the low-dose group was observed in open-field testing. The study authors concluded that perinatal bisphenol A exposure caused behavioral alterations that differed by sex.

Strengths/Weaknesses: Doses were sufficiently high to produce gross body weight changes, and 3 different measures of behavior were collected, as well as organ weights at necropsy from the same animals. Weaknesses include a lack of statistical accounting for possible litter effects in the postnatal analysis, the lack of an evaluation of hormone-dependent behaviors, and the lack of assessment of more hormone-dependent tissues (prostate, levator ani muscle, etc.) or processes (age at balanopreputial separation, postnatal anogenital distance).

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to a failure to account for litter effects.

Negishi et al. (2004a), support not indicated, examined the effect of perinatal bisphenol A **[purity not indicated]** exposure on the behavior of rats. The effects of nonylphenol were also examined but will not be discussed. F344/N rats (10 or 11/group) were gavaged with bisphenol A at 0 (corn oil vehicle) or 0.1 mg/kg bw/day from GD 3–PND 20. GD 0 was defined as the day that vaginal sperm was detected, and PND 0 was the day of parturition. At birth, pups were counted and weighed. Litters were culled to 6 pups, with equal numbers of each sex when possible. Pups were weighed throughout the postnatal period. At weaning, dams were killed and organ weights were measured. One male pup/litter ($n = 8$–10/group) was subjected to a series of behavioral tests. The remaining male pups were killed for measurement of organ weights at 21 days or 8 weeks of age. Neurobehavioral endpoints evaluated included open-field behavior at 8 weeks of age, spontaneous motor activity at 12 weeks of age, passive avoidance at 13 weeks of age, performance in the elevated-plus maze at 14 weeks of age, and active avoidance at 15 weeks of age. At 22–24 weeks of age, a monoamine reduction test was performed: rats were injected with the monoamine oxidase inhibitor trans-2-phenylcyclopropylamine hydrochloride or with saline, and behavior was then evaluated. Data were analyzed by ANOVA, and if statistical significance was obtained, Fisher protected least significant difference test was conducted. Behavioral endpoints were measured on 1 male pup/litter, thus accounting for litter issues.

Bisphenol A exposure did not affect dam body weights during gestation or lactation, gestation duration, litter size, number of male and female pups, or final dam body and organ weights. **[Data were not shown.]** Body and organ weights of male offspring at 21 days and 8 weeks of age, behavior in open-field testing, spontaneous motor activity, and performance in the elevated-plus maze were also unaffected by bisphenol A exposure. **[Data were not shown by study authors.]** Bisphenol A had no significant effect on performance in the passive avoidance test, although tendencies for increased latency were observed. In active avoidance testing, rats from the bisphenol A group had significantly ($P < 0.01$) fewer correct avoidance responses during the first, second, and third of five sessions, and failure of avoidance was significantly increased **[$\sim$2.5% in the bisphenol A group compared to 0.2% in controls]**. In contrast to control rats, bisphenol A-treated rats did not show an increase in locomotion following a challenge with trans-2-phenylcyclopropylamine hydrochloride. The number of rearings following 2-phenylcyclopropylamine hydrochloride exposure did not differ significantly between rats from the bisphenol A and control groups. The study authors concluded that perinatal exposure of rat dams to bisphenol A at concentrations

slightly higher than environmental exposures irreversibly affected perception of fear-provoking stimuli and monoaminergic neural pathways in male offspring.

Strengths/Weaknesses: The use of a single dose level is a weakness. Strengths include the variety of endpoints used to provide data, which point to effects that are not gross structural changes but relatively subtle behavioral effects.

Utility (Adequacy) for CERHR Evaluation Process: These data are adequate and of high utility for the evaluation process.

Farabollini et al. (1999), supported by the University of Siena, University of Firenze, MURST, and the Italian National Research Council, examined the effects of perinatal bisphenol A exposure on behavior in male and female rats. **[No information was provided in the manuscript about chow or composition of cage and bedding materials. The Expert Panel has been informed that Morini MIL chow, lignocel bedding, and polysulfone cages were used (F. Farabolli et al., personal communication, March 1, 2007).]** Three groups of Sprague–Dawley rats were orally dosed with the arachis oil vehicle or bisphenol A **[purity not reported in the manuscript; ≥95% according to the authors (F. Farabolli et al., personal communication, March 1, 2007)]** by micropipette. One group of 11 rats was administered 0.040 mg/kg bw/day bisphenol A from 10 days before conception until weaning of pups at 21 days of age. A second group of 11 rats was given arachis oil from 10 days before conception through GD 13, 0.400 mg/kg bw/day bisphenol A from GD 14 **[day of vaginal plug not stated]** through 6 days following delivery of pups, and arachis oil until weaning of pups. A control group of 9 rats was given arachis oil from 10 days before conception until weaning of pups. Beginning at 85 days of age and continuing for 3 days, behavioral testing was conducted using a hole board and elevated-plus maze in 15 offspring/sex from the low-dose group, 11–12 offspring/sex from the high-dose group, and 14 pups/sex from the control group. **[Litter distribution was not reported.]** Separate sessions were conducted for each sex and treatment group. Data were analyzed by ANOVA and Fisher least significant difference test. A factor analysis was conducted using the principal components method with an orthogonal rotation of the factor matrix. **[It appears that offspring were considered the statistical unit.]**

In general, head dipping (extending head over edge of apparatus) and arm entries were reduced and self-grooming was increased in exposed females. Head dipping and stretched-attend posture (moving body forward without moving paws and then returning to original position) were inhibited and arm entries were increased in exposed males. A factor analysis indicated reduced anxiety and motivation to explore in treated males and reduced activity and motivation to explore in treated females. The study authors concluded that although sex-related differences in behavior were noted following bisphenol A treatment, there was no clear masculinization of behavior in females. The authors also noted the lack of substantial differences in results between the two exposure protocols.

Strengths/Weaknesses: The unusual exposure scenario in this study is both a strength and a weakness; however, the use of 11–15 pups from 9–11 litters raises concern for possible litter effects that were unaccounted for in the statistical analysis.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate due to insufficient control for possible litter effects.

Farabollini et al., (2002), supported by the University of Siena, University of Firenze, and MURST, examined the effects of perinatal bisphenol A exposure on sociosexual behavior in rats. Sprague–Dawley rats were housed in polysulfone cages. **[No information was provided in the manuscript on type of feed or composition of bedding materials. The Expert Panel has been informed that Harlan Teklad 2018 chow and Lignocel bedding were used (F. Farabolli et al., personal communication, March 1, 2007).]** Dams received arachis oil vehicle ($n = 13$) or 0.040 mg/kg bw/day bisphenol A **[purity not indicated]** ($n = 7$) through a micropipette from mating through weaning of pups. On Day 2 following delivery, litters were culled to 4 pups/sex and cross-fostered to obtain the following exposure groups of 12 animals/sex:

- Prenatal exposure group: born to bisphenol A-treated dams and nursed by vehicle-treated dams;
- Postnatal group: born to vehicle-treated dams and nursed by bisphenol A-treated dams; and
- Control group: born to and nursed by vehicle-treated dams.

Litters were weaned on PND 21 (day of birth not defined). On day 45 **[assumed to be PND 45]**, animals of the same sex were randomly chosen and housed 4/cage, with no siblings in any cage. At 100 days of age, behavior in the presence of an intruder rat was observed. In female rats, vaginal smears were taken at the end of intruder testing and only females in diestrus were considered ($n = 8$–9/group). One week later, sexual orientation was tested in 12 rats/sex/group by placing a rat between cages containing a sexually receptive female and sexually mature male and recording the number of visits to each rat. Sexual performance was tested next in males; evaluation was restricted to only males that ejaculated ($n = 10$–12 group). One week later, sexual behavior was tested in females during the diestrous or proestrous phase. **[It is not clear how many females were evaluated for sexual behavior.]** Behavior testing sessions were video recorded and later evaluated by a blinded observer. Data were analyzed by ANOVA followed by post-hoc Fisher least significant difference test. Litter effects were purposely confounded through cross-fostering.

In intruder testing, statistically significant effects observed in males exposed prenatally to bisphenol A included an increased number showing defensive behavior (9 of 10 vs. 4 of 10 in the control group), a decreased number showing ambivalent behavior (3/10 vs. 8/10 in the control group), and increased ratio of defensive/agonistic behaviors **[by 280% compared to controls]**. No significant effects were observed in intruder testing of female rats. There was no effect on sexual preference of males or females. For sexual behavior testing of females, data from the pre- and postnatal exposure groups were pooled because there were no significant differences between groups. Bisphenol A exposure significantly decreased exit latency in females in diestrus **[by ~66%]** and proestrus **[by ~83%]** and significantly ($P < 0.05$)

increased lordosis frequency in females in proestrus **[∼11.75 vs. 3.75 times in controls]**. Statistically significant effects on sexual performance of treated males included an increased number of intromissions **[∼15 compared to 11 in controls]** in the postnatal exposure group and increased duration of intromission latency **[∼115 vs. 40 sec in controls]** and genital sniffing **[∼40 vs. 16 sec in controls]** in the prenatal exposure group. The study authors stated that the results suggested a slight intensification of sexual behavior in females, slightly reduced performance in a limited number of endpoints in males, but no effect on other important sexual endpoints in males (e.g., latency of ejaculation and refractory period). It was concluded that pre- or postnatal exposure to bisphenol A potentiated female behavior and depotentiated male behavior.

Strengths/Weaknesses: The work was carefully performed. The use of a single dose level of bisphenol A is a weakness; however, this dosing paradigm is consistent with many other studies by this group making comparisons between the studies relevant. Addressing aggressive/defensive behavior as well as sexual performance and interest in both male and female offspring is a strength. The failure to address underlying biological mechanisms is a weakness. Further weaknesses include the inability to account for litter effects as the use of multiple pups from some litters without appropriate statistical control raises concern for possible litter effects due to unequal litter representation

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for evaluation purposes due to the inability to fully account for possible litter effects.

Dessi-Fulgheri et al. (2002), supported by the University of Firenze, University of Siena, and MURST, examined the effect of perinatal bisphenol A exposure on play behavior in rats. Sprague–Dawley rats were housed in polysulfone cages. **[No information was provided in the manuscript on chow or bedding material. The Expert Panel has been informed that Morini MIL chow and Lignocel bedding were used (F. Farabolli et al., personal communication, March 1, 2007).]** Using a pipette, rats were fed solutions containing the arachis oil vehicle and/or bisphenol A according to 1 of 3 exposure scenarios. A control group of 9 rats was given arachis oil from 10 days before mating until weaning of pups on PND 21 **[day of birth not defined]**. Eleven rats in the low-dose group were given 0.040 mg/kg bw/day bisphenol A **[purity not provided]** from 10 days before mating until weaning of pups. Eleven rats in the high-dose group received arachis oil vehicle from 10 days before mating until GD 13 **[day of vaginal plug not defined]**, 0.400 mg/kg bw/day bisphenol A from GD 14–PND 6, and arachis oil from PND 7 until weaning. Both doses were considered to be within the range of human exposure. The low dose was said to represent exposures through food occurring over a long period of time. The high-dose was said to represent exposures occurring through dental procedures occurring over a short period of time. Litters were culled to 8 pups at birth. **[No information was provided in the study on the sex distribution of the retained pups; the Expert Panel was advised that there were 4 males and 4 females/litter (F. Farabolli et al., personal communication, March 1, 2007).]** After pups were weaned, 3 male and 3 female pups were randomly caged together, with no siblings co-housed in any cage. Behavioral testing was conducted on PND 35, 45, and 55. For the behavioral testing, rats from the same cage were individually identified by marking them with dye. On each day of testing, the 6 cage mates were transferred to a neutral arena that was covered in clean sawdust and video recorded for 6 min. Behaviors recorded during the second and third minute of each testing session were evaluated. There were 12–15rats/sex/group. **[The methods section indicates that 15 rats/sex were tested at the high-dose, 12 rats/sex at the low dose, and 15 rats/sex in the control group. According to Table 4 of the study, which gives the pooled number of rats tested for 3 age periods, it appears that 12/sex were tested in the high-dose group, 15/sex in the low-dose group, and 15/sex in the control group. The Expert Panel has been informed that Table 4 is correct (F. Farabolli et al., personal communication, March 1, 2007).] For statistical analyses, individual factor scores were used as independent variables in a 3-way ANOVA that considered treatment, sex, and age. Fisher least significant difference test was used when appropriate. At weaning, housing conditions confounded litter of origin that was not then accounted for in statistical analyses.**

Behavioral elements were categorized under 8 general factors. The authors first presented results that were pooled for the 3 different age groups. In females of the low-dose group, bisphenol A treatment was found to significantly increase factors addressing play directed toward females. Factors affecting low-intensity mating elements (e.g., crawling-under behavior) were reduced significantly in high-dose males and females. Factors of sociosexual exploration (e.g., genital and body sniffing) were reduced significantly in high-dose females and in males from both dose groups. Factors of social interest (e.g., approaching) were reduced significantly in both sexes at the high-dose but increased in low-dose males. The authors next discussed results for PND 35, because it is the approximate time period of vaginal opening in females. Factors that were affected significantly at PND 35 included increased social interest by males and females of the low-dose group, decreased low-intensity mating elements by females of both dose groups, and decreased sociosexual exploration by males of both dose groups. The study authors concluded that 2 factors of female behavior were masculinized by treatment: play with females and sociosexual exploration.

Strengths/Weaknesses: A strength of this work is that it evaluated the socio-sexual consequences of exposure, and specifically at a young age. Weaknesses include absence of accounting for litter influences and inadequate statistical procedures (i.e., failure to consider the repeated measures design). In addition, the hypothesis is not biologically plausible (i.e., consistent with expected effects of a chemical with an estrogenic mode of action) and the factor analysis does not necessarily cluster the play behaviors that are known to be sexually dimorphic.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for evaluation process due to faulty statistical procedures.

Porrini et al. (2005), supported by MURST, the University of Firenze, and the University of Siena, examined the effects of perinatal bisphenol A exposure on play behavior of female rats. **[No information was provided in the manuscript about the type of feed or**

bedding and caging materials. The Expert Panel has been informed that Harlan Teklad 2018 chow, polysulfone cages, and Lignocel bedding were used (F. Farabolli et al., personal communication, March 1, 2007).] Female Sprague–Dawley rats were co-housed with males for 36 hr and then fed the peanut oil vehicle (n = 10) or 0.040 mg/kg bw/day bisphenol A **[purity not stated]** ($n = 12$) by micropipette during the gestation and lactation period. Two days following delivery, litters were adjusted to 4 pups/sex and pups were fostered by a dam from the same treatment group. Pups were weaned on Day 21 **[assumed to be PND 21; day of birth not defined]**. Offspring were housed in cages containing 3 pairs of male–female siblings, with no siblings of the same sex in the same cage. Each group contained 18 female pups. Before examination of behavior in rats from the same cages at 35, 45, and 55 days of age, animals were individually identified with dye. Behavior was observed in a neutral arena in which the floor was covered with clean sawdust. Animals were allowed to familiarize themselves to the new environment for 1 min and then behavior was video recorded for 6 min. Video recordings were analyzed by an investigator blinded to treatment conditions. Only behavior of female rats was considered. Data were analyzed by ANOVA for repeated measures. The cross fostering design precluded the ability to examine litter effects.

Factors were defined by study authors based on groups of behaviors. Significant effects were reported for 3 factors. Social and non-social exploration was increased **[by ~34%]** at 35 days of age and **[by ~25%]** at 45 days of age. Frequency of play behavior with males was decreased **[by ~100%]** at 45 days of age. Grooming behavior was also decreased **[by ~63%]** at 45 days of age. The study authors concluded that bisphenol A does not clearly induce masculinization of female behavior, but some aspects of female behavior were defeminized.

Strengths/Weakness: This study reports a well-performed study with a poorly researched endpoint (juvenile play behavior) that has implications for reproductive behavior later in life. The use of only a single dose level of bisphenol A is a weakness. The fostering of pups within treatment group prevents the evaluation of litter effects and the use of multiple pups from some litters without appropriate statistical correction raises concern for possible litter effects.

Utility (Adequacy) for CERHR Evaluation Process: This work is inadequate for the evaluation process due to insufficient control for possible litter effects.

Adriani et al. (2003), supported by the Nervous and Mental Disorders Research Area, Istituto Superiore di Sanità, Italy, and by MURST, examined the effects of perinatal exposure to bisphenol A on behavior in rats. Sprague–Dawley rats were housed in Plexiglas cages with sawdust bedding. **[No information was provided in the manuscript about feed. The Expert Panel has been informed that Morini MIL feed was used (F. Farabolli et al., personal communication, March 1, 2007).]** Nine dams/group were dosed with bisphenol A **[purity not reported]** orally by micropipette at doses of 0 (arachis oil vehicle) or 0.040 mg/kg bw/day from the day of mating to the day pups were weaned. Pups were weaned on PND 25 (PND 0 = day of birth) and housed in groups of 3 according to sex. One male and 1 female/litter were observed in testing that included novelty-seeking behavior during adolescence (PND 30–45), impulsivity during adulthood (PND 70), and open-field behavior following injection with 1 mg/kg bw *d*-amphetamine during adulthood. It appears that the same animals were tested at each time period. Data were analyzed by Tukey HSD test and ANOVA.

In novelty testing, the time spent in a new area of the testing apparatus was lower in females exposed to bisphenol A **[~45–55% compared to vehicle control, $P < 0.05$]**. Males and females of the bisphenol A group exhibited increased activity in the novel area **[increases of ~75% in males and 35–55% in females, $P < 0.05$]**. The study authors interpreted the effects of novelty testing as suggesting a less pronounced habituation profile and increased stress in a novel situation. In the impulsivity testing, food-restricted animals were placed in an apparatus that involved nose poking in a small hole to immediately deliver 1 pellet of feed or a larger hole to deliver 5 pellets of feed following a delay that was increased over the time of the study. Lights were turned on during the delay periods following nose poking and for 25 sec after delivery of feed, time periods in which no feed could be delivered. Both groups of rats preferred the larger hole with delayed delivery, but treatment with bisphenol A resulted in a more marked preference for the larger hole ($P < 0.05$), thus indicating reduced impulsivity. When the length of the delay was increased for the large hole, the frequency of inadequate responding (i.e., nose poking during the delay) was decreased in males from the bisphenol A group; the study authors interpreted the effect as indicating a demasculinization of the restlessness profile. **[The study report originally mislabeled the control and bisphenol A-treated groups in Figure 3a. A corrected version of the figure was included in an erratum statement released by the study authors (Adriani et al., 2005).] In open-field testing, vehicle control males displayed significantly more rearing and crossing behaviors following injection with *d*-amphetamine, but an increase in rearing and crossing behavior following *d*-amphetamine injection did not occur in males exposed perinatally to bisphenol A. The study authors concluded that perinatal exposure of rats to bisphenol A resulted in altered behavior in rats.**

Strengths/Weakness: This study used protocols that are well established by this group. The use of only a single exposure level of bisphenol A is a weakness, with the proviso that the dose used is directly comparable to other studies. The degrees of freedom reported for behavioral measures suggest inflation of sample size due to failure to account for multiple time sampling.

Utility (Adequacy) for CERHR Evaluation Process: The study is inadequate for evaluation due to inappropriate statistical procedures.

Carr et al. (2003), supported by the National Science Foundation, the Mississippi Agricultural and Forestry Experiment Station, and the College of Veterinary Medicine at Mississippi State University, examined the effects of bisphenol A exposure on performance of rats in the Morris water maze. In this study, F344 rat dams and pups were fed Purina Test Diet 8117, a casein-based rodent chow. **[No information was provided about caging or bedding materials.]** Treatment groups were assembled by including pups from different litters such

that there was a member of each treatment group from each sex from each litter: a control animal was always present in each litter. Ten pups/sex/group were gavage dosed from PND 1 (day of birth = PND 14 with bisphenol A (>99% purity) at 0 (safflower oil vehicle), 0.1, and 0.25 mg/kg bw/day. An additional group of rats was gavaged with 17β-estradiol 72 μg/kg bw/day during the same time period. Straight channel swimming was tested on PND 33. Spatial learning and memory were tested by Morris water maze for 4 days beginning on PND 34. In the test, acquisition of maze solution occurred when the rat found a platform. A probe trial measuring the amount of time spent in an escape quadrant from which the platform had been removed was conducted on PND 40. Data were analyzed by ANOVA followed by means separation by least squared means or Greenhouse-Geisser adjusted *F* ratios.

There were no significant effects of bisphenol A treatment on straight channel swimming or time to acquisition of maze solution in the Morris maze test. Time spent in the escape quadrant was significantly lower in females of the high-dose group **[by ~38%]** than in controls. The study authors noted that acquisition of maze performance was significantly better in control males than control females. However, no sex-related difference was observed following treatment with the low bisphenol A dose. Increased time to acquisition in males on the third day of testing, and no sex-related differences in performance were reported for the 17β-estradiol group. The study authors concluded ''These data indicate that [17β-estradiol] and low dosages of [bisphenol A] can alter the normal sex-dependent pattern of acquisition, while higher dosages of [bisphenol A] alter the retention of spatial information without significantly affecting acquisition.''

Strengths/Weaknesses: Strengths are the additional behavioral dimensions captured by this paper and the use of a positive control. The analyses appeared appropriate. The within litter dosing design raises concerns about cross-contamination that would decrease differences between groups and challenge interpretation of results of non-standard dose–response curves. Analyses did not account for the repeated measures design, thus inflating degrees of freedom. A weakness is the limited number of endpoints investigated.

Utility (Adequacy) for CERHR Evaluation Process: This study is considered inadequate because of the limitations noted.

Della Seta et al. (2006), supported by MURST and the University of Siena, examined the effects of pubertal bisphenol A exposure on behavior of male rats. **[No information was provided in the manuscript about feed, caging, or bedding. The Expert Panel has been informed that Harlan Teklad 2018 chow, Lignocel bedding, and polysulfone cages were used (F. Farabolli et al., personal communication, March 1, 2007).]** Seventy-eight Sprague–Dawley males were obtained from 16 dams and housed in groups of 4 with each from a different litter. On PND 23–30 (day of birth not defined), the rats were fed (by micropipette) peanut oil vehicle, 0.040 mg/kg bw/day bisphenol A **[purity not reported in the manuscript; ≥95% according to the authors (F. Farabollini et al., personal communication, March 1, 2007)]**, or 0.4 μg/kg bw/day ethinyl estradiol. **[The number of rats treated in each group was not specifically indicated, but can be inferred to be 24–26/group.]** On PND 45, 12 males/group were tested for social and non-social behavior in response to a black PVC tube introduced into the cage. Behaviors were examined according to factor clusters of play and social interaction, environmental exploration and social investigation, and elements directed to the object. Twelve adults/group (>90 days of age) were tested for sexual behavior with a sexually receptive female. Males that were not used in behavioral testing were killed on PND 37 ($n = 7$ or 8/group) and 105 ($n = 5$ or 6/group) to measure plasma 17β-estradiol and testosterone levels by RIA. Data were assessed by ANOVA and Fisher least significant difference test.

Around the time of treatment, bisphenol A effects on juvenile behavior were not found on factors associated with environmental exploration and social investigation or with play and social interaction. However, juvenile behaviors directed to the object (biting, sniffing, climbing) occurred at a significantly lower frequency in the bisphenol A than control group. Compared to the vehicle controls, the ethinyl estradiol group exhibited lower frequencies of behaviors associated with environmental exploration or social investigation and with behaviors directed to the object. With respect to adult sexual behavior, data from the 9 or 10 of 12 animals/group that were sexually active were analyzed. Decreased intromission latency was significantly affected in males from the bisphenol A group. Significant effects in the ethinyl estradiol compared to the control group included decreased intromission latency as well as decreased latency to mount, increased frequency of intromission, increased ratio of intromissions/mount, and decreased duration of genital sniffing. On PND 37, the plasma testosterone level was significantly lower in the bisphenol A and ethinyl estradiol group than in controls. The plasma testosterone level was also significantly lower in the bisphenol A than control group on PND 105. No effects were observed on plasma 17β-estradiol levels. The study authors concluded that the behavioral effects observed in the bisphenol A-exposed rats occurred in the same direction as those observed in the ethinyl estradiol group and could be interpreted as consistent with estrogenic mediation.

Strengths/Weaknesses: This study was well-conceived and executed. Appropriate dosing periods, design, and testing methods and timeframes were used to capture developmental effects of pubertal bisphenol A exposure of a short-term (juvenile period) and long-term (into adulthood) nature. Sample sizes were adequate.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for use in the evaluation process.

Ceccarelli et al. (2007), supported by the University of Siena and MIUR, investigated the effects of orally administered bisphenol A and ethinyl estradiol during puberty in Sprague–Dawley rats. Sixteen pregnant Sprague–Dawley rats gave birth to offspring that were cross-fostered on PND 1, weaned on PND 21, and housed in groups of 4 males and 4 females. **[No details of housing conditions during gestation were provided, including individual or group residency, bedding or cage material, or diet.]** On PND 31, male and female offspring were separately housed in groups of 4 in Plexiglas cages with free access to water and food and

Table 73
Effects of Pubertal Exposure to Bisphenol A on ERα Levels in Sexually Dimorphic Hypothalamic Regions in the Rat[a]

		Comparison, % change						
		To oil control				Males to females		
		Bisphenol A		Ethinyl estradiol				
Region	PND	Males	Females	Males	Females	Control	Bisphenol A	Ethinyl estradiol
Arcuate nucleus	37	↔	↔	↔	↔	↔	↔	↔
	90	↔	↔	↔	↔	↔	↔	↔
Ventromedial nucleus	37	↔	**[↑50]**	**[↑112]**	↔	↔	↔	**[↑70 in males]**
	90	↔	↔	↔	↔	↔	↔	↔
Medial preoptic area	37	↔	↔	↔	↔	↔	↔	↔
	90	↔	↔	↔	**[↑85]**	↔	**[↑50 in females]**	**[↑118 in females]**

[a]Ceccarelli et al. (2007).
Comparisons estimated from a graph.
↑,↓,↔ Statistically significant increase, decrease, or no change compared to vehicle-treated, orchiectomized control.

maintained under a reversed light cycle. On PND 23–30, rats ($n = 14$/group) were given bisphenol A 40 μg/kg bw/day, ethinyl estradiol 0.4 μg/kg bw/day, or peanut oil vehicle. Half the offspring ($n = 7$/group) were killed on PND 37 and half on PND 90. Females killed on PND 90 were killed in estrus. Blood samples were taken and animals were formalin perfused. Brains were harvested, post-fixed, and cryopreserved. Immuno-histochemistry was performed on frozen sections for comparative ERα level analysis, with a focus on sexually dimorphic regions of the hypothalamus: the arcuate nucleus, ventromedial nucleus, and medial preoptic area. Two or three sections/rat were stained, equivalent field areas outlined, and ERα-positively stained nuclei counted under light microscopy by an evaluator blinded to all experimental parameters. Serum testosterone and 17β-estradiol were determined by RIA. Statistical analyses were performed using ANOVA and post-hoc least significant difference test.

The results for ERα are shown in Table 73. There were few statistically significant difference between controls and treated rats. Effects identified for ethinyl estradiol were not seen with bisphenol A with the exception of an increase in bisphenol A-treated females compared to males in ERα at 90 days in the medial preoptic area**.** On PND 37, testosterone was significantly reduced **[∼40%]** in bisphenol A treated males compared to control males. There were no significant effects of bisphenol A treatment on 17β-estradiol or on testosterone/17β-estradiol ratio.

The authors conclude that exposures to bisphenol A at 40 μg/kg bw/day during early puberty can induce both short-term and long-term changes in sexually dimorphic regions of the brain and circulating testosterone/17β-estradiol ratio.

Strengths/Weaknesses: This interesting and novel manuscript examined the potential for the ethinyl estradiol positive control and bisphenol A administered before puberty, but after the most sensitive period (i.e., PND 3–10), to modulate ER and steroid hormones during puberty and sexual maturity. It appears that the authors tried to remove the potential for bias by blinded quantification of ER-positive neurons. The oral route of exposure was relevant. These data must be linked functionally to the results of Della-Seta et al. (2006). A weakness is that hormonal measurements were taken at single time points.

Utility (Adequacy) for CERHR Evaluation Process: These data are adequate and of high utility for the evaluation process.

3.2.4 Rat—parenteral exposure postnatally.

3.2.4.1 Reproductive endpoints: Fisher et al. (1999), supported by the European Centre for Ecotoxicology of Chemicals and Zeneca, examined the effect of neonatal bisphenol A exposure on excurrent ducts of the rat testis. On PND 2–12 (PND 1 = day of birth), Wistar rat pups were s.c. injected with the corn oil vehicle or 37 mg/kg bw/day bisphenol A **[purity not given]**. The dose was based on the solubility limit in oil. **[The number of rats treated was not indicated nor was relationship to litter, but based on the number of rats examined in each time period (∼3–7 in treated group and 5–20 in control group), it appears that there were ∼25/group in the bisphenol A group and ∼48 in the vehicle control group. No information was provided about caging or bedding materials.]** Seven other compounds were also examined but will not be discussed, with the exception of a brief explanation of results obtained with 0.0037–0.37 mg/kg bw/day diethylstilbestrol. Rats were killed at 10, 18, 25, 35, and 75 days of age. Testes and epididymides were removed and fixed in Bouin solution. Immunohistochemistry techniques were used to examine water channel aquaporin-1 levels. Morphology of rete testis and efferent duct were examined. Data were analyzed by ANOVA.

In the bisphenol A group, the only effect on testis weight was a significant decrease **[∼40%]** at 35 days of age. Epithelial cell height in the efferent ducts was significantly reduced **[by ∼15%]** at 18 and 25 days of age, but not at later time periods. There was no effect on expression of water channel aquaporin-1 protein or morphology of the rete testis. Treatment with most diethylstilbestrol doses resulted in reduced testicular weights at all ages, decreased expression of water channel aquaporin-1 protein, and decreased epithelial cell height in efferent ducts at 25 days of age and younger, and fluid retention and enlargement of rete testis, which was most severe at PND 18 and 25. The study authors concluded that the magnitude and

duration of adverse effects induced by estrogenic compounds were broadly similar to the estrogenic potencies of the compounds.

Strengths/Weaknesses: This is a carefully performed study, although the inclusion of many methodological details (*vide supra*) would have improved it. Strengths include the use of a wide range of estrogenic compounds to alter testicular development. A limitation for the present purpose is that only a single dose level of bisphenol A was administered subcutaneously. A weakness is that tissues other than the testis were not examined. Other weaknesses include sample sizes ranging from 3–20 examined pups across groups and s.c. administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for evaluation due to lack of clarity about experimental or statistical control for litter effects.

Nagao et al. (1999), supported by the Japanese Ministry of Health and Welfare, examined the effects of neonatal bisphenol A exposure on reproductive function of male and female Sprague–Dawley given CE-2 feed (Clea Japan). **[No information was provided about caging or bedding materials.]** From PND 1–5 (birth by 16:00 considered PND 0), 28–31 pups/sex/group were s.c. injected with corn oil vehicle, 300 mg/kg bw/day bisphenol A **[purity not reported]**, or 2 mg/kg bw/day estradiol benzoate. Pups within litters were treated with the same dose. Doses were based on results of preliminary studies that demonstrated no effect on growth or viability at bisphenol A doses up to 300 mg/kg bw/day administered by s.c. injection in the neonatal period. Pups were examined for viability from PND 6–21. On PND 21, 5 pups/sex/group were randomly selected and killed. Pups were transcardially perfused, and reproductive organs were collected for histopathological evaluation. At 12 weeks of age, 22–25 rats/sex were mated with untreated rats. Females were killed on GD 13 for an evaluation of implant number and viability of embryos. After fertility evaluation, sexual behavior with a sexually receptive female was assessed in 10 males/group. Following evaluation of sexual behavior, 15 male rats/group were killed for measurement of reproductive organ and brain weight. Histopathology of reproductive organs and SDN-POA volume were measured in 5 males/group. Copulation and fertility indices were analyzed by χ^2 and Fisher exact one-tailed test. Data for other endpoints were analyzed by Student *t*-test.

In rats treated with bisphenol A, there were no clinical signs of toxicity or effects on pup viability or body weight gain during or following the lactation period **[data for pup viability not shown by study authors]**. There were no effects on age of vaginal opening or preputial separation. Copulation and fertility indices and numbers of live embryos/litter were not affected in male or female rats treated with bisphenol A. Bisphenol A treatment did not affect sexual behaviors of males, as determined by number of mounts, intromissions, and ejaculations. No histopathological alterations were observed in the ovaries of treated females at 21 days of age or in the epididymis, prostate, or seminal vesicles of treated male rats at 21 days or 14 weeks of age. **[The prostatic lobe not specified; based on the figure provided, the lobe appears to have been ventral prostate. The Expert Panel notes that the number of apically located nuclei may be elevated by 14 weeks of age over what would normally be expected; however, this observation cannot be determined definitively based on a single high power field and in the absence of a matched control.]** No effect of treatment was observed on the SDN-POA of males. In contrast to the bisphenol A groups, rats treated with estradiol benzoate experienced decreased body weight gain, compromised male sexual behavior, infertility, lesions in reproductive organs, and reduced volume of the SDN-POA. The study authors concluded that neonatal exposure to a relatively high-dose of bisphenol A had no effect on morphological development or function of the reproductive system.

Strengths/Weaknesses: Strengths include a well performed and documented study that compared effects of bisphenol A and estradiol benzoate. Additional strengths include documentation of both behavioral (mating behavior) and biological (genital tract development) endpoints in both male and female rats. Weaknesses include the use of only a single dose level of bisphenol A via s.c. injection, and no accounting for litter effects within the context of individual animal treatments within litters.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for evaluation, however, utility is limited by subcutaneous administration.

Stoker et al. (1999), support not indicated, examined the effects of prepubertal bisphenol A exposure on prolactin secretion and prostate size in rats. **[No information was provided about feed, bedding, or caging materials.]** On PND 22–32 (day of birth = PND 0), 15–17 male Wistar rats from different litters/group were s.c. injected with bisphenol A **[purity not reported]** at 0 (sesame oil vehicle) or 50 mg/kg bw **[assumed to be 50 mg/kg bw/day]**. Another group of rats was administered 17β-estradiol through a s.c. Silastic tube implant **[dose administered not clear]**. On PND 29, 6 animals/dose were killed and blood was collected for measurement of serum prolactin concentration. The remaining rats ($n = 9$–11/group) were killed at 120 days of age. Prolactin levels were measured in serum and anterior pituitary by RIA. Inflammation was visually examined in the ventral and lateral prostate. Left lateral and ventral prostates were weighed and lateral prostate was analyzed for myeloperoxidase (an indicator of neutrophil numbers) and DNA. The right lateral prostate was subjected to histological examination. Statistical analyses included ANOVA, Dunnett *t*-test for multiple comparison, and Fisher exact probability test.

On PND 29, serum prolactin levels were significantly increased by ~210% in rats of the bisphenol A group compared to the control group. On PND 120, there was no effect on prolactin levels in serum or pituitary in the bisphenol A group. Ventral prostate weight was unaffected but lateral prostate weight was increased **[by ~25%]** in the bisphenol A group. Exposure to bisphenol A had no effect on body or testis weight. **[Data were not shown by study authors.]** The myeloperoxidase assay was reported to show a "trend" for lateral prostate inflammation in the bisphenol A group. **[Trend was not defined; there was no statistical difference between the bisphenol A group and the control in the myeloperoxidase assay.]** No histological evidence of inflammation was observed in prostates from the control group. In the bisphenol A group, histopathological analyses revealed

that 44.4% of prostates contained increased a focal luminal polymorphonuclear cellular infiltrate that was milder in severity compared to prostates from the 17β-estradiol group. The study authors noted the discrepancy between the results obtained by myeloperoxidase assay and histological observation in the bisphenol A group and stated that the discrepancy may have been due to evaluation of the whole tissue by myeloperoxidase assay versus only one section of the tissue by histological evaluation. Bisphenol A had no effect on prostate DNA content. In addition to prostate inflammation, effects observed in the 17β-estradiol group were increased serum prolactin levels on PND 29 and elevated myeloperoxidase and DNA content in lateral prostate on PND 120. Based on these findings, the study authors concluded that chemically induced, transient increases in prolactin secretion in the prepubertal period can lead to increased incidence of lateral prostate inflammation in 120-day-old rats.

Strengths/Weaknesses: Comparison with other agents is a strength. Weaknesses include low to moderate sample sizes and the use of a single high-dose level of bisphenol A through subcutaneous administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process but has limited utility due to concerns about sample sizes and route of administration of treatment.

Atanassova et al. (2000), supported by the European Center for the Ecotoxicology of Chemicals and AstraZeneca, examined the effects of neonatal bisphenol A exposure on the reproductive system of male rats. Wistar rats were fed rat and mouse breeding diet No. 3, which contains 15.5% soy meal flour. **[No information was provided about caging and bedding materials.]** Litters of 8–12 male rats from randomized litter origin were assembled by cross-fostering pups on PND 1 (day of birth). On PND 2–12, rats were s.c. injected with corn oil vehicle or bisphenol A **[purity not given]** 0.5 mg/day. **[Assuming a 5–25 g body weight during this interval, this dose would be ~100 mg/kg bw/day at the beginning of the interval and ~20 mg/kg bw/day at the end of the interval.]** Other groups of rats were s.c. injected with 0.01–10 μg diethylstilbestrol every other day between PND 2–12 or 2 mg 4-*tert*-octylphenol/day during PND 2–12. Rats were killed on PND 18, 25, and 90–100. At PND 18 and 25, testes were weighed and fixed in Bouin solution. Testicular cell numbers and seminiferous tubule lumen formation were determined by standard point counting of cell nuclei. Apoptosis was assessed by DNA fragmentation detected by in situ DNA 3′-end labeling. Spermatocyte nuclear volume as a fraction of Sertoli cell nuclear volume was calculated as ''an index of spermatogenic efficiency.'' Plasma FSH and inhibin B were measured by RIA and ELISA methods, respectively. Fertility was assessed at 80–90 days of age; rats were mated for 7 days and number of pups was counted at birth. The number of rats/group examined was 7–14 at 18 days of age, 4–12 at 25 days of age, and 6 in fertility testing. Data were analyzed by ANOVA.

Significant effects observed on PND 18 were advanced testicular lumen formation and increases in testis weight, Sertoli cell volume/testis, and spermatocyte nuclear volume/unit Sertoli cell. A decrease in germ cell apoptosis was also described on PND 18 but was not statistically significant. Plasma FSH levels were increased significantly on PND 18, but there was no effect on plasma inhibin B concentration. The only significant effect observed on PND 25 was increased plasma FSH levels. Testis weight was increased in adulthood, but there were no effects on fertility or litter size. Effects observed with octylphenol were similar to those observed with bisphenol A. In contrast, exposure to one or more doses of diethylstilbestrol resulted in increased apoptosis, decreased plasma inhibin levels, decreased Sertoli cell nuclear volume, and changes in spermatocyte/Sertoli cell ratios. The study authors concluded that the effect of bisphenol A on spermatogenic processes is benign.

Strengths/Weaknesses: Comparison with other agents is a strength. Weaknesses include low to moderate sample sizes, the use of a single high-dose level of bisphenol A through subcutaneous administration, and no accounting for litter effects within the context of individual animal treatments within litters.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process but has limited utility due to concerns about sample sizes and route of administration of treatment.

Williams et al. (2001), supported by the European Centre for Ecotoxicology, examined the effect of neonatal bisphenol A exposure on seminal vesicle structure and expression of sex steroid receptors in rats. On PND 2 (day of birth = PND 1), litters consisting of 8–14 male Wistar rat pups were derived through cross-fostering. Rats were s.c. injected with corn oil vehicle or 0.5 mg/day bisphenol A on PND 2–12. **[Assuming a 5–25 g body weight during this interval, the dose would be ~100 mg/kg/day at the beginning of the interval and ~20 mg/kg bw/day at the end of the interval.]** The dose was based on the highest amount that could remain in solution. A positive control group was injected with diethylstilbestrol at 0.1, 1, or 10 μg/day on PND 2, 4, 6, 8, 10, and 12. Ethinyl estradiol was administered at 10 μg/day, according to the protocol for diethylstilbestrol. Control animals for each compound were dosed with vehicle on the appropriate days, and because no differences were noted for controls, data were pooled. The effects of 4-*tert*-octylphenol, genistein, Antarelix, flutamide, and tamoxifen were also examined but will not be discussed. **[No information was provided about feed, caging or bedding materials, or purity of compounds.]** Animals were killed on PND 18, and seminal vesicles from 11–15 animals/group were collected and stored in Bouin solution. Seminal vesicles were examined for gross abnormalities in stroma and epithelium. Immunolocalization studies were conducted to assess ERβ, ERα androgen receptor, and progesterone receptor proteins in the seminal vesicle. Studies were replicated 3–5 times using samples from at least 6 animals/group. Results were scored subjectively.

The gross structure of the seminal vesicles from bisphenol A-treated rats appeared normal, and there were no changes in ERβ, ERα, androgen receptor, or progesterone receptor proteins in the seminal vesicle. In contrast, diethylstilbestrol induced changes in seminal vesicle morphology, increased ERα and progesterone receptor, and decreased androgen receptor. Effects of ethinyl estradiol were similar to those observed with diethylstilbestrol. The study authors concluded that the lack of bisphenol A effects suggested that only high-

doses of potent estrogens induce gross abnormalities in the male reproductive system; and that only agents that suppress androgen receptor while increasing ERα and progesterone receptor are likely to cause gross developmental abnormalities in the male reproductive system.

Strengths/Weaknesses: Strengths include expertise of the group coupled to well-performed experiments, data recording, and interpretation. Bisphenol A was not a primary target in this study but was one of a series of estrogenic compounds, allowing comparison with other similar compounds. However, a significant weakness are the s.c. route of administration, only a single varying dose level of bisphenol A was used, and there was no accounting for litter effects within the context of individual animal treatments within litters.

Utility (Adequacy) for CERHR Evaluation Process: This work is inadequate for the evaluation process, based on lack of clarity for experimental or statistical control for litter effects.

Rivas et al. (2002), supported by the European Union and the Spanish Ministry of Education, examined the effects of bisphenol A exposure on reproductive tract development of male rats. The main focus of the study was determining the effects of decreased androgen production in combination with a low dose of diethylstilbestrol. Effects of flutamide were also examined but will not be discussed. Wistar rats were fed a soy-free diet (rat and mouse soya-free breeding diet; SDS, Dundee, Scotland). **[No information was provided about caging and bedding materials.]** Litters of 8–12 male pups were assembled by cross-fostering on PND 1 (day of birth). Male rats were s.c. injected with the corn oil vehicle or 0.1 mg bisphenol A **[purity not indicated]** on PND 2, 4, 6, 8, 10, and 12 with and without co-administration of 10 mg/kg GnRH antagonist (a suppressor of androgen production). **[Assuming a 5–25 g body weight during this interval, the bisphenol A dose would be ~20 mg/kg bw/day at the beginning of the interval and ~4 mg/kg bw/day at the end of the interval.]** Additional rats were s.c. injected with diethylstilbestrol at doses of 0.1 or 10 μg on PND 2, 4, 6, 8, 10, and 12 with and without administration of GnRH antagonist. Rats were killed on PND 15. The testis was fixed in Bouin solution and testicular structures were measured. Plasma testosterone levels were measured using an ELISA technique. From 3–10 animals/group were examined for each endpoint. Data were analyzed by ANOVA.

Treatment with bisphenol A alone did not affect plasma testosterone levels but treatment with GnRH antagonist alone and in combination with bisphenol A significantly lowered plasma testosterone levels. Treatment of rats with bisphenol A alone or in combination with GnRH antagonist had no significant effect on rete testis luminal area, efferent duct luminal area, efferent duct epithelial cell height, or vas deferens epithelial cell height. Exposure to the high diethylstilbestrol dose increased rete area, and both doses of diethylstilbestrol decreased plasma testosterone levels, increased efferent duct luminal area, and decreased epithelial cell height in efferent duct and vas deferens. The study authors concluded that the estrogenicity of bisphenol A when injected at a moderately high-dose was insufficient for disrupting the estrogen-androgen balance in rats.

Strengths/Weaknesses: This study was performed carefully and well-documented. Weaknesses include: the dose of bisphenol A was high and only a single dose level administered subcutaneously was examined; and litter effects were not addressed in the context within litter dosing of cross-fostered litters.

Utility (Adequacy) for CERHR Evaluation Process: This work is inadequate for the evaluation process, based on lack of clarity on control for litter effects.

Sharpe et al. (2003), supported in part by the European Union and the Spanish Ministry of Education, examined the effects of neonatal exposure of rats to bisphenol A on Leydig cell development and function. Wistar rat dams were fed a standard soy-containing feed (rat and mouse breeding diet; SDS). **[No information was provided on feed given to male offspring following weaning or bedding and caging materials.]** Litters of 9–12 male pups were created by cross fostering pups on PND 1 (day of birth). Male pups were s.c. injected with the corn oil vehicle or 0.5 mg/day bisphenol A **[purity not reported]** on PND 2–12. **[Assuming 5–25 g body weight during this interval, the dose would be ~100 mg/kg bw/day at the beginning of the interval and ~20 mg/kg bw/day at the end of the interval.]** Other groups of rats received diethylstilbestrol at 0.1–10 μg/day on PND 2, 4, 6, 8, 10, and 12. Additional rats were treated with GnRH antagonist Antarelix or 4-*tert*-octylphenol, but those results will not be discussed. Rats were killed on PND 18, 25, 35, or 90. Testes were weighed and fixed in Bouin solution. Sections of testes were immunostained with the Leydig cell marker 3β-hydroxysteroid dehydrogenase to evaluate Leydig cell development in 5–7 animals/group. Plasma testosterone levels were measured by ELISA. Group sizes for evaluation of testes weight and plasma testosterone were 2–23, with most groups containing at least 8 animals. Data were analyzed by ANOVA.

The only significant effect on plasma testosterone level following exposure to bisphenol A was an increase on PND 18 ($n = 4$). In rats of the bisphenol A group examined at each time period, there were no significant effects on testis weight, percent Leydig cell nuclear volume/testis, Leydig cell nuclear volume/testis, or total Leydig cell volume (nuclear+cytoplasmic volume/testis). Significant results in rats exposed to diethylstilbestrol included decreased Leydig nuclear cell volume at the mid- or high-dose on or before PND 35 and reduced plasma testosterone level and testis weight at all doses and most time points of evaluation. The study authors concluded that there were no consistent changes in Leydig cell development following exposure to bisphenol A.

Strengths/Weaknesses: A strength is that bisphenol A was one of a number of compounds examined enabling internal comparison with other similar molecules. Limitations include use of a single high but variable dose of bisphenol A and small sample sizes for critical endpoints.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate due to small or uncertain sample sizes for key endpoints.

Khurana et al. (2000), supported by NIH, March of Dimes, and Pardee Foundation, examined the effects of neonatal bisphenol A exposure on prolactin levels in rats. **[The type of chow used and composition of bedding and caging materials were not reported.]** On PND 1–5 (day of birth = PND 0), 8–10 Fischer 344 rat pups/sex/group (litter relationships are unclear) were s.c.

injected with the tocopherol-stripped corn oil vehicle, bisphenol A **[purity not indicated]** at 0.1 or 0.5 mg/day, diethylstilbestrol at 5 μg/day, or octylphenol at 0.1 or 0.5 mg/day. **[Assuming a pup body weight of 5 g, bisphenol A intakes were estimated at 20 and 100 mg/kg bw/day.]** On PND 15, 20, and 25, blood was collected for measurement of serum prolactin level by RIA. A final sample for prolactin analysis was obtained when animals were killed on PND 30. Medial basal hypothalamus, anterior pituitary, uterus, and prostate were collected for measurement of *ER*α and *ER*β mRNA expression by RT-PCR in animals of the low-dose group. Statistical analyses included ANOVA followed by Student-Newman–Keuls test.

In male and female rats, hyperprolactemia was observed on PND 25 and 30. **[On PND 30, prolactin levels in the low- and high-dose groups compared to the control group were ~150 and 95% higher in females and 120 and 80% higher in males]**. In females exposed to the low dose, *ER*α mRNA in the medial basal hypothalamus was higher **[by 25%]** than control levels. In anterior pituitary of low-dose males, *ER*α mRNA was higher **[by ~80%]** and *ER*β mRNA was higher by 35–40% compared to control levels. There were no effects on *ER*β mRNA in female tissues. Most effects observed with octylphenol exposure were similar to those observed with bisphenol A exposure. Diethylstilbestrol induced transient increases in prolactin levels, decreased expression of *ER*α in medial basal hypothalamus of males, upregulated *ER*α and *ER*β expression in the pituitary of males, decreased expression of *ER*α in the uterus, and upregulated *ER*β expression in prostate. The study authors concluded that exposure of neonatal rats to bisphenol A resulted in delayed and sustained hyperprolactemia and changes in *ER* mRNA expression.

Strengths/Weaknesses: A strength is that both male and female animals were assessed following administration of two dose levels. Weaknesses include small treatment groups consisting of unclear numbers of litters and composition and limited experimental details regarding design.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to lack of design clarity.

Fukumori et al. (2003), support not indicated, examined the effect of postnatal bisphenol A exposure on ultrastructure of the prostate in rats. **[The study was published in Japanese; a translation was provided by the American Plastics Council.]** On Day 1–21 following birth, F344 rats were s.c. injected with bisphenol A 5 days/week at doses of 0 (DMSO vehicle), 0.0008, 0.004, 0.020, and 0.500 mg/kg bw/day. A positive control group received 100 μg/kg bw 17β-estradiol by s.c. injection during the same time period. Rats were killed at 22 days of age. Ventral prostates were fixed in glutaraldehyde, sectioned, and examined by electron microscopy. **[The number of rats treated and examined/group and the number of litters represented were not reported. No information was provided on purity of bisphenol A, type of feed, or composition of bedding and caging. The translated version of the report did not include figures from the original report.]**

In ventral prostates obtained from rats exposed to 17β-estradiol, there was an increase in secretory granules accompanied by reductions in microvilli on the surface of the glandular epithelium. Proliferation of fibroblasts was observed in the fibromuscular layer of the stroma in rats from the 17β-estradiol group. In the 0.020 and 0.500 mg/kg bw/day bisphenol A groups, a slight increase in secretory granules and slight decrease in microvilli was observed in glandular epithelium. Effects in stroma were described as unremarkable for the bisphenol A groups. The study authors concluded that bisphenol A may have ultrastructural effects on the ventral prostates of suckling rats.

Strengths/Weaknesses: This is a translation of an apparently carefully performed study to assess the effects of low doses of perinatal bisphenol A on prostatic structure. A major weakness is that the original figures were not provided in the translated version of the report, and the route is s.c. injection in DMSO. The young age at which the animals were sacrificed is also a concern because prostatic development is not complete at 22 days of age making comparisons with the bulk of established data problematic. The lack of data specifics raise the level of uncertainty about this study.

Utility (Adequacy) for CERHR Evaluation Process: This study is considered inadequate for inclusion in the evaluative process because of the lack of detail on study design (i.e., litter representation, number of animals per group).

Kato et al. (2003), supported by the Japanese Ministry of Education, Culture, Sports, Science, and Technology and the Ministry of Health, Labor, and Welfare, examined the effects of neonatal bisphenol A exposure on the reproductive organs of rats. Sprague–Dawley rats were fed CRF-1 diet. **[No information was provided on caging or bedding materials.]** Female offspring from 8 dams were grouped to achieve equal distribution of body weight. At least 8 female offspring/group were s.c. injected with 0 (ethanol/corn oil vehicle), 0.25, 1, or 4 mg/day bisphenol A **[purity not reported]** from PND 0 to 9 (day of delivery = PND 0). **[Based on body weights reported on PND 0 and 9, CERHR calculated mean bisphenol A intakes of ~26, 105, and 427 mg/kg bw/day.]** A positive control group was given 10 μg/day 17β-estradiol **[~3 mg/kg bw/day]** during the same time period. Rats were weighed during and following the lactation period and examined for day of vaginal opening. External reproductive organs were examined on PND 60, and estrous cycles were assessed from PND 61–94. One group of rats was ovariectomized on PND 80; ovaries were weighed, and fixed in 10% neutral buffered formalin for evaluation of corpora lutea and polyovular follicles. Another group of bisphenol A-exposed and the vehicle-treated control females were given 1 μg/kg 17β-estradiol from PND 94–96 and killed the day following final injection; uterus and vagina were weighed, and fixed in 10% formalin. For all endpoints, 5–8 rats/group were examined. Statistical analyses included Student *t*-test and Fisher exact probability test.

Treatment–related results are summarized in Table 74. Two rats of the high-dose group died. Body weights of rats in the high-dose group were lower than controls on PND 9–30 but higher than controls on PND 61–97. Effects observed at the mid- and high-dose included accelerated vaginal opening, increased incidence of polycystic ovaries, decreased area of corpora lutea, and decreased uterine fluid weight. All rats of the mid-dose group had partial clefts in the clitoris, and all rats of the high-dose

Table 74
Effects in Female Rats Exposed to Bisphenol A During the Neonatal Period[a]

Endpoint	Dose, mg/kg bw/day **[CERHR estimate]**						
	26	105	427	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Body weight gain							
PND 9	↔	↔	↓16%	286	200	233	156
PND 97	↔	↔	↑10%	432	261	430	253
Day of vaginal opening	↔	↓2.9 days	↓4.1 days	345	267	159	116
No. with normal estrous cycles[b]	↔ (8/8)	↔ (2/8)	↓ (0/6)	81	28		
No. with cleft clitoris[c]	↔ (0/8)	↑ (0/8)	↑ (6/6)	299	failed		
Relative organ weight							
Ovary	↔	↔	↓59%	85	59	140	93
Uterus, wet	↔	↔	↓60%	66	55	128	96
Uterus, blotted	↔	↔	↓21%	273	128	318	168
Uterine fluid weight	↔	↓42%	↓97%	42	34	139	104
No. with polycystic ovaries[c]	No data	↑ (4/8)	↑ (5/5)	81	24		
No. with corpora lutea[b]	No data	↔ (8/8)	↓ (0/5)	238	90		
No. of corpora lutea	No data	↔	↓ (none)	65	38	137	83
Corpora lutea area	No data	↓ 30%	↓ (none)	42	37	84	66

[a]Kato et al. (2003).
[b]Control rate 8/8.
[c]Control rate 0/8.
↑,↓ Statistically significant increase or decrease compared to controls; ↔ no statistically significant effect.

group had deep clefts in the clitoris. Additional effects observed in rats of the high-dose group included disrupted estrous cycles (e.g., irregular cycles or persistent estrous) and decreased relative (to body weight) ovary and wet or blotted uterus weights. Absolute weights of wet uterus and ovary were also reduced in the high-dose group. No corpora lutea were observed in rats of the high-dose group. Qualitatively similar effects were observed in the group treated with 17β-estradiol. The study authors concluded that exposure of rats to bisphenol A during the neonatal period resulted in changes in female reproductive organs.

Strengths/Weaknesses: The strengths are the carefully performed and documented experiments. The major limitation is that the s.c. route of administration and the doses of bisphenol A were relatively high. The changes in the female reproductive organs seen are well documented, but given the extremely high-dose of agent used, broadly unsurprising.

Utility (Adequacy) for CERHR Evaluation Process: The results of this study reflect a careful documentation of the experiments performed. The study is adequate for the evaluation process but has limited utility due to concerns about the route of administration.

Toyama and Yuasa (2004), supported in part by the Japanese Ministry of Environment and Ministry of Education, Science, Sports and Culture, examined the effects of neonatal bisphenol A **[purity not reported]** exposure on spermatogenesis during puberty and adulthood in rats and mice. **[No information was provided about chow or bedding and caging materials. The mouse data are reported in Section 3.2.8.]** Wistar rats were s.c. injected on a μg/pup basis with bisphenol A in a DMSO and olive oil vehicle on PND 1, 3, 5, 7, 9, and 11 (PND 0 = day of birth). Bisphenol A doses were 1.0, 10.0, 100.0, and 600.0 μg/pup. Additional animals were treated with 17β-estradiol and estradiol benzoate. Animals were killed weekly at 2–10 weeks of age, and other pups were killed at 24 and 31 days of age. There were 5 animals/dose/time point in bisphenol A groups and apparently 5 vehicle control rats/time period. Testes were examined by light and electron microscopy. Males from each experimental group (a total of 11 rats) were mated with 2 females **[number tested in each dose group not reported]**. A total of 11 rat dams were allowed to complete pregnancy. **[It does not appear that statistical analyses were conducted.]**

All rats given 0.600 μg/pup bisphenol A died before 20 days of age and were excluded from analysis. In mature spermatids of 8-week-old rats in the vehicle control group, the incidences of deformed acrosomes, deformed nuclei, and abnormal ectoplasmic specialization were <0.3%. In 8-week-old rats treated with ≥0.010 μg/pup bisphenol A, the incidence of deformed acrosomes was >50–60%, the incidence of deformed nuclei was >40%, and the incidence of abnormal ectoplasmic specialization was >60–70%. **[Data were not shown for individual dose levels.]** Similar effects were observed in the groups treated with 17β-estradiol and estradiol benzoate. No effects were reported at other ages. **[Data were not shown by study authors.]** The blood–testis barrier remained intact based on histologic observations. All tested males from the bisphenol A group were fertile, and sex ratio, litter sizes, and pup weights were reported to be normal. **[No results were shown for individual dose levels. Fertility data presented in Table 4 and 5 of the study, were not clearly identified by dose level.] The study authors concluded that bisphenol A acts as an estrogen and induces transient changes in the male reproductive system of rodents that resolve in adulthood.**

Strengths/Weaknesses: The strengths include the use of multiple doses of bisphenol A and the use of both rats and mice, allowing interspecies comparisons. Weaknesses include selective and unclear data presentation, absence of statistical analyses, subcutaneous injection on a per pup basis, and failure to examine sperm morphology in the fertile 15-week-old animals to

determine whether the changes in sperm maturation seen at earlier time points had resolved or whether the animals were fertile in the face of such abnormalities.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful for the evaluation process due to lack of clarity of design and analyses, route of administration, and dosing procedures.

Kato et al. (2006), supported by the Japanese Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labor and Welfare, examined the effects of neonatal exposure to bisphenol A on reproductive function of male rats. Sprague–Dawley rats were fed CRF-1 diet, which was described as having relatively low estrogenic activity compared to other Japanese rodent feeds. **[No information was provided on caging or bedding materials.]** Male rats used in this study were born to 12 dams, assigned to 8 foster dams in groups of seven based on body weights, and distributed to dose groups. From PND 0–9 (PND 0 = day of birth), 24 male pups/group were s.c. injected with bisphenol A **[purity not indicated]** at 0 (ethanol/corn oil vehicle), 0.000024, 0.000120, 0.000600, 0.003, or 1 mg/pup/day bisphenol A. Study authors calculated average exposures of 0.002, 0.011, 0.056, 0.277, or 97 mg/kg bw/day. An additional group was treated with 10 μg/day 17β-estradiol (0.9 mg/kg bw/day) during the same time period. Eight rats/group were killed and necropsied at PND 10, 35, and 150. At the PND 10 necropsy, serum testosterone levels were measured by RIA, the testis was weighed and examined histologically, and expression changes in genes for hormone receptors and steroidogenic enzymes were determined by RT-PCR. The same endpoints were examined at the PND 35 necropsy in addition to measuring seminal vesicle, ventral prostate, and epididymis weights. The remaining rats were assessed for day of preputial separation. From PND 105–130, they were mated for 1 day a maximum of 4 times with an untreated female in proestrus. Females were killed on GD 13 (day of sperm = GD 0) and examined for corpora lutea, embryonic mortality, and implantation sites. Male rats were killed on PND 150. In addition to endpoints examined at earlier time periods, sperm endpoints and histopathology of ventral prostate were assessed. Statistical analyses included Bartlett method for homogeneity of variance followed by Dunnett method for homogeneous variances or Dunnett-type method with rank order for heterogeneous variances. Reproductive data were analyzed by Fisher exact probability test. Data obtained from the 17β-estradiol group were analyzed by Student *t*-test.

There were no deaths or decreases in body weight in animals of the bisphenol A group. There were no effects on age of preputial separation, copulation rate, or fertility. In dams impregnated by bisphenol A-treated males, there were no effects on numbers of implantation sites, implantation losses, or live fetuses. Bisphenol A treatment had no adverse effects on sperm count, motility, or morphology. There were no effects on serum testosterone levels, histopathology of testis or prostate, or weights of testis, epididymis, seminal vesicle, ventral prostate, or penis. No significant changes were observed in mRNA for estrogen, androgen, or progesterone receptor or steroidogenic enzymes. In contrast to the bisphenol A groups, rats treated with 17β-estradiol experienced decreases in reproductive organ weights, altered gene expression, delayed and incomplete preputial separation, decreased copulatory rate, and decreased sperm numbers. The study authors concluded that neonatal bisphenol A exposure caused no adverse effects on reproductive function or gene expression of steroidogenic enzymes in the rat testis.

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Strengths/Weaknesses: This study has a number of major strengths, notably the wide range of doses, appropriate use of statistics, inclusion of a positive control, and use of relevant endpoints. Weaknesses include route of administration and dosing on a per pup basis, thus not adjusting for body weight.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility due to route of administration and dosing on a per pup basis.

Noda et al. (2005), support not indicated, examined the effect of neonatal bisphenol A exposure on reproductive organs of Sprague–Dawley rats. For 5 days beginning on PND 1 (day of birth = PND 0), 6–10 pups/sex/group (drawn from 2 litters) were s.c. injected with olive oil vehicle or bisphenol A **[purity not reported]** at 0.0001, 0.001, or 0.010 mg/rat/day. According to the study authors, the doses were equivalent to ~0.010, 0.100, or 1 μg/kg bw/day. A positive control group received diethylstilbestrol at the same doses as bisphenol A. Nonylphenol and genistein were also examined but will not be discussed here. Dose selection was based on diethylstilbestrol doses reported to have an effect. Stability, homogeneity, and concentration of dosing solutions were verified. Pups in each group were obtained from 2 dams. On PND 7, litters were adjusted to 4 males and 4 females/dam when possible. Dams and pups were housed in polycarbonate cages until weaning at PND 21. At that time, pups were housed in wire mesh cages. Animals were fed MF feed (Oriental Yeast Co.). **[No information was provided on bedding used in polycarbonate cages.]** During the study, animals were examined for clinical signs, body weight, anogenital distance on PND 7, and day of vaginal opening or preputial separation. Estrous cycles were assessed from the time of vaginal opening until animals were killed on PND 47–50 (females in diestrus). Rats in persistent estrus were killed on PND 70. Reproductive organs were weighed. Testis was fixed in Bouin solution and all other

reproductive organs were fixed in 10% neutral buffered formalin for histopathological examination. **[It was not indicated, but it is assumed that all pups were examined in each analysis.]** Data were analyzed by Bartlett test for homogeneity of variance, ANOVA, Dunnett test, or Kruskal–Wallis test.

In the bisphenol A groups, there were no abnormal clinical signs or effects on body weight. Absolute anogenital distance was not affected, but anogenital distance adjusted by the square root of body weight cubed was decreased in females treated with the mid- and high-bisphenol A dose. There were no effects on day of vaginal opening or preputial separation or on estrous cycles. **[Data were not shown.]** No gross or histopathological abnormalities were reported in male or female reproductive organs. The study authors only reported organ weight effects relative to body weight, because the rats were killed at different ages. The only dose-related effect on reproductive organ weight was increased relative ventral prostate weight in the high-dose group. Relative pituitary weight was increased in males of the low-dose group and females of the high-dose group. There were no effects on weights of testis, epididymis, seminal vesicle, uterus, or ovary in bisphenol A-treated animals. Effects observed in animals treated with 1 or more dose of diethylstilbestrol included delayed or incomplete preputial separation, estrous cycle disruption, underdeveloped reproductive organs (including ventral prostate), malformations in male and female reproductive organs, ovarian cysts, and uterine squamous metaplasia in glandular epithelium. The study authors noted that the shortened anogenital distance in females appeared to be biologically significant. However, it was stated that the effect is of unknown relevance in female rats and was not observed in the rats treated with diethylstilbestrol. The study authors concluded that findings observed with bisphenol A were not toxicologically relevant.

Strengths/Weaknesses: Strengths of this report include the use of 3 dose levels, the use of a positive control (diethylstilbestrol), and the use of multiple endpoints to evaluate estrogenic effects. Weaknesses include the use of only 2 litters to constitute exposure groups, exposure by the subcutaneous route to bisphenol A (not the anticipated route of exposure in humans), and dosing on a per pup basis.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate due to the combination of small sample size (i.e., 2 litters) and s.c. route of administration.

Ho et al. (2006b), supported by NIH and Department of Defense, examined the effect of developmental exposure to bisphenol A on susceptibility of Sprague–Dawley rats to prostate cancer. The dams and offspring used in this study were fed a soybean-free phytoestrogen-reduced diet (Zeigler Reduced Rodent Diet 2, Ziegler Brothers, Inc.), housed in polysulfone cages **[with unspecified bedding]**, and provided drinking water in glass bottles. On PND 1, 3, and 5 (day of birth = PND 0), 20–30 male pups/group were s.c. injected with tocopherol-stripped corn oil vehicle, bisphenol A **[purity not indicated]** at 0.1 μg/pup (0.010 mg/kg bw), or estradiol benzoate at 0.001 μg/pup (0.1 μg/kg bw) or 25 μg/pup (2500 μg/kg bw). Male rats from each litter were randomly assigned to treatment groups, but the total number of litters from which the pups were selected was not reported. Likewise, it is unclear, but assumed, that all doses were represented within litter rearing units. Pups were weaned on PND 21. At PND 90, half the rats from each treatment group were implanted with Silastic capsules containing 17β- estradiol and testosterone and the other half were implanted with empty capsules; the capsules were left in place for 16 weeks. The treatment was designed to result in a serum 17β-estradiol level of ~75 ng/L and testosterone level of ~3 μg/L, levels reported to induce prostatic intraepithelial neoplasia in 33% of Sprague–Dawley rats. Rats were killed at 28 weeks of age. Prostates were removed, and histopathological evaluations were conducted on each lobe. Immunohistochemistry techniques were used to measure proliferation. Apoptosis was measured using the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique. PCR techniques were used to study methylation pattern and expression changes in prostate cell signaling proteins on PND 10, 90, and 200. Statistical analyses included χ^2 test, ANOVA, Fisher exact test, and Bonferroni test. The study authors stated that similar responses were observed in each of the 3 prostate lobes; and thus results were presented only for dorsal prostate. In bisphenol A-exposed compared to vehicle controls rats that did not receive 17β-estradiol/testosterone exposure in adulthood, there were no effects on dorsal prostate weight, histopathology alterations, proliferation index, or apoptotic index. In bisphenol A-treated compared to vehicle control rats that received 17β-estradiol/testosterone exposure in adulthood, there was increased incidence and severity of prostatic intraepithelial neoplasia (100 vs. 40% incidence). In the bisphenol A/17β-estradiol/testosterone group, proliferation and apoptosis indices were increased in regions where prostatic intraepithelial neoplasia (PIN) was observed. In humans PIN is an accepted precursor lesion to prostate cancer. In rodents the significance of PIN is less clear. Some transgenic mouse models will form PIN lesions that progress to adenocarcinoma in a manner broadly similar to that seen in humans. However, there are many examples in which mice form PIN lesions that do not progress to invasive disease. In rats, testosterone plus estradiol classically induces PIN lesions that progress to adenocarcinoma. The increase in incidence of PIN lesions seen following testosterone and estradiol treatment in BPA exposed rats in this study are certainly a cause for concern. The data presented do not address whether these lesions progress to cancer in a manner similar to PIN lesions seen in the classic testosterone plus estradiol model, or whether such progression occurs at a higher or lower rate. Changes observed in rats exposed to the high estradiol benzoate dose in the neonatal period but not 17β-estradiol/testosterone during adulthood included increased incidence and severity of prostatic intraepithelial neoplasia and elevated apoptosis and proliferation indices. The same effects, in addition to decreased prostate weight, were observed in rats receiving neonatal exposure to the high estradiol benzoate dose and adult exposure to 17β-estradiol/testosterone.

In the investigation of a molecular basis for increased susceptibility to PIN, exposure to estrogenic compounds altered methylation pattern in several cell signaling genes. Phosphodiesterase type 4 variant, an enzyme

involved in cyclic AMP breakdown, was selected for further study. Neonatal bisphenol A exposure resulted in hypomethylation of the phosphodiesterase type 4 variant gene and increased expression of that gene at 90 and 200 days of age, with or without 17β-estradiol/testosterone exposure in adulthood. Similar responses in phosphodiesterase type 4 variant gene methylation and expression were observed with exposure to the low and high 17 estradiol benzoate doses. The study authors concluded that developmental exposures of rats to bisphenol A increased susceptibility to precancerous prostate lesions resulting from prostate epigenomic alteration.

Strengths/Weaknesses: This is a carefully performed study by a group with significant expertise in this area of work. The study has many strengths, from the use of a relatively low dose level of bisphenol A to the search to identify molecular mechanisms, possibly including site-specific promoter methylation, underlying the observations made. Weaknesses include the use of a single dose level with subcutaneous dosing. It could be suggested that carrying the study further in terms of animal age might have produced more dramatic phenotypes and clarified the relevance of PIN resulting from BPA exposure to prostate cancer (potentially enhancing cancer incidence) in this model. Failure to do this could be considered a weakness of the work.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of limited utility for the evaluation process due to use of subcutaneous route of administration.

3.2.4.2 Neurobehavioral endpoints: Ishido et al. (2004), supported by the National Institute for Environmental Studies and the Ministry of Economy, Trade, and Industry, examined the effects of postnatal intracisternal bisphenol A exposure on behavior of rats. Dams in this study were fed standard laboratory chow (MF Diet; Oriental Yeast Corp.). **[No information was provided about caging or bedding materials.]** At 5 days of age, 5–7 male Wistar rat pups/group were injected intracisternally with a bisphenol A dose **[purity not indicated]** of 0 (ethanol/olive oil vehicle), 0.00002, 0.0002, 0.002, or 0.020 mg. Pups were weaned at 3 weeks of age. Spontaneous motor activity was measured over a 12–24-hr period at 4–5 weeks of age. Rats were killed at 4 and 8 weeks of age, and brains were removed. RNA was isolated from midbrain and striatum for DNA microarray analysis. Expression of the gene for dopamine transporter in midbrain was studied by RT-PCR. Tyrosine hydroxylase expression in brain was measured at 8 weeks of age using an immunostaining method. Statistical analyses included ANOVA and Student *t*-test.

In 4–5-week-old rats from the 0.020 mg bisphenol A group, motor activity was significantly increased and was 1.6 times higher than in control rats during the nocturnal period. In a dose response experiment, it was noted that hyperactivity was increased significantly at doses $\geq$0.0002 mg. Microarray analysis revealed that bisphenol A **[at an unspecified dose]** downregulated expression of dopamine D4 receptor gene 2-fold at 4 weeks of age and dopamine transporter gene 2.8-fold at 8 weeks of age. Numerous other gene expression changes were observed but not discussed in detail by study authors. Analysis by RT-PCR confirmed that expression of the dopamine transporter gene was downregulated 3-fold in the midbrain of 8-week-old rats treated with bisphenol A in the neonatal period. In rats from the 0.020 mg bisphenol A group, tyrosine hydroxylase immunoreactivity was reduced in the substantia nigra at 8 weeks of age. The study authors interpreted the decrease in tyrosine hydroxylase immunoreactivity as degeneration of dopaminergic neurons. They concluded that bisphenol A affected the central dopaminergic system, resulting in hyperactivity that most likely occurred as a result of decreased tyrosine hydroxylase activity in midbrain.

Strengths/Weaknesses: A significant weakness is the inability to correlate the internal exposure to bisphenol A provided by the intracisternal route with that seen by the oral route. Strengths of this study include the use of a range of concentrations of bisphenol A. The correlation of changes in behavior patterns induced by bisphenol A with expression of specific dopamine receptor sets is also a strength. A significant weakness is the inability to correlate the doses of bisphenol A provided by this dosing mechanism with those seen by more common s.c. or oral routes, as well as uncertainty about the disposition of the bisphenol A that is injected into the cerebrospinal fluid.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to uncertainties around the intracisternal route of administration.

Masuo et al. (2004a), of the Japanese National Institute of Advanced Industrial Science and Technology and National Institute for Environmental Studies, investigated the effects in rats of an acute neonatal exposure to 6-hydroxydopamine, bisphenol A, nonylphenol, *p*-octylphenol, or diethylhexyl phthalate on spontaneous motor activity, as well as catecholamine levels, dopaminergic neuron integrity by immunohistochemistry, and gene expression profiles. In the 6-hydroxydopamine group, 5-day-old male Wistar pups weighing about 10 g were first pretreated with 25 mg/kg desipramine i.p. on PND 5 in order to protect noradrenergic neurons from the effects of 6-hydroxydopamine. These pups were then injected intracisternally 30 min later with 6-hydroxydopamine **[not discussed here]**. Other groups of pups were treated intracisternally with 0 (olive oil vehicle) or 87 nmol bisphenol A **[purity not provided]**, nonylphenol, *p*-octylphenol, or diethylhexyl phthalate in olive oil ($n = 6$ or 7/group). In additional experiments, intracisternal bisphenol A treatments were used over a 0.087–87 nmol **[19.8 ng to 19.8 μg]** dose range. Following treatment, pups were randomly assigned to lactating dams and weaned at 3 weeks of age. Animals were housed in acrylic cages at 22°C under 12-hr light/12-hr dark conditions and given free access to water and chow from Oriental Yeast Co.

Spontaneous motor activity was assessed at 4–5 weeks of age using an automated activity-monitoring system over a 12-hr light/12-hr dark cycle, apparently for a single 24-hr period. **[Total number of cycles not indicated.]** Brain sections from 8–10-week-old rats were snap frozen in liquid nitrogen. The striatum and whole mid-brains were used for cDNA microarray analyses. The frontal cortex, striatum, limbic regions including nucleus accumbens, septum, and olfactory tubercles were used to measure catecholamine levels by HPLC. Immunohistochemistry from whole brain sections was used to evaluate dopamine neuron integrity using

tyrosine-hydroxlyase monoclonal antibody reactivity **[number of rats not indicated]**. Most statistical analyses were performed using ANOVA techniques. Activity data were analyzed using repeated measures ANOVA to examine activity in 2-hr intervals, as well as across the dark, light, or full 24-hr period. Student *t*-tests were used to compare catecholamine levels.

Spontaneous motor activity in rats treated with bisphenol A increased in a dose-dependent manner over the 0.087–87 nmol range, with significance on pair-wise comparison with controls at dose levels ≥ 0.87 nmol **[198 ng]**. Activity was increased in both the dark and light periods. Tyrosine hydroxylase activity was reduced in bisphenol A-treated rats, compared to controls. **[Quantification of immunohistochemical sections was not provided.]** Gene expression patterns in the midbrain differed in bisphenol A and 6-hydroxydopamine-treated animals.

The authors concluded that neonatal exposure to bisphenol A was associated with an increase in spontaneous motor activity and reduced tyrosine hydroxylase activity. They hypothesized that bisphenol A may cause a deficit in the development of mesostriatal dopaminergic neurons, and that this increase either is greater than that produced by 6-hydroxydopamine lesions or involves additional neurochemical systems. A follow-up study (Masuo et al., 2004b) addressed these issues. The authors also proposed that bisphenol A-exposed rats can serve as animal models of attention deficit-hyperactivity disorder.

Strengths/Weaknesses: A significant weakness is the inability to correlate the internal exposure to bisphenol A provided by the intracisternal route with that seen by the oral route.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to uncertainties around the intracisternal route of administration.

Masuo et al. (2004b), funded by the New Energy and Industrial Technology Development Organization, the Ministry of the Environment, and the Ministry of Economy, Trade, and Industry, Japan, followed up their previous study (Masuo et al., 2004a) with additional gene expression microarrays to elucidate potential molecular pathways associated with the effects of an acute, neonatal exposure to 6-hydroxydopamine, bisphenol A, nonylphenol, diethylhexyl phthalate, or dibutyl phthalate on spontaneous motor activity levels at 4–5 weeks of age. Pregnant Wistar rats were housed in acrylic cages with free access to tap water and laboratory chow (Oriental Yeast) and maintained on a 12-hr light/12-hr dark cycle. In the 6-hydroxydopamine group, 5-day-old male pups, each about 10 g, were first pretreated with 25 mg/kg desipramine by i.p. injection (to protect noradrenergic neurons from the effects of 6-hydroxydopamine) and then given 6-hydroxydopamine intracisternally 30 min later. PND 5 male pups in other groups were intracisternally injected with olive oil vehicle, 87 nM bisphenol A **[19.8 μg] [purity not provided]**, nonylphenol, diethylhexyl phthalate, or dibutyl phthalate. **[Only the bisphenol A experiments will be discussed here.]** Following treatments, pups were randomly fostered to lactating dams (5–7 pups/per dam) and weaned at 3 weeks of age. At 4–5 weeks of age, the spontaneous motor activity of bisphenol A treated rats was compared to vehicle treated rats ($n = 6$ or 7/group) using an automated activity-monitoring system over a 12-hr light/12-hr dark cycle. Bisphenol A and vehicle-treated rats were killed at 8–10 weeks of age and the striatum and midbrain were harvested. RNA was extracted from 2 pooled striata/rat ($n = 3$/group) or 1 midbrain/rat ($n = 3$/group) for cDNA microarray analyses. Gene expression values were evaluated relative to those of control-treated rats. Repeated measures ANOVA was used for statistical analyses of spontaneous motor activity during 2-hr time intervals. Statistics were not described for microarray results.

Neonatal exposure to bisphenol A in male rats increased spontaneous motor activity significantly at 4–5 weeks during both the dark and light periods of the cycle when compared to controls. Gene expression profiles examined at 8–10 weeks of age for select genes potentially impinging on dopamine function and/or other pathways were altered in the adult striatum and midbrain of bisphenol A treated mice. The authors concluded that neonatal exposure to bisphenol A resulted in elevated spontaneous motor activity during both the light and dark phases. 6-Hydroxydopamine lesions increased motor activity only during the dark period. Comparisons of genetic expression in 6-hydroxydopamine and bisphenol A-treated rats suggested that the effects of bisphenol A may be mediated by alterations in dopamine as well as other systems. This profile of adverse effects was suggested to potentially serve as a model for human hyperactivity disorders.

Strengths/Weaknesses: A significant weakness is the inability to correlate the internal exposure to bisphenol A provided by the intracisternal route with that seen by the oral route.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to uncertainties around the intracisternal route of administration.

Ishido et al. (2005), support not indicated, examined the effects of neonatal bisphenol A exposure of rats on motor activity and gene expression in brain. Wistar rat dams were fed MF diet (Oriental Yeast). Pups were born from 10 pregnant dams and 5–7 male pups were assigned to each dam. At 5 days of age, male pups were injected intracisternally with vehicle (50% ethanol in olive oil) or 87 nmol **[19.8 μg]** bisphenol A. **[No information was provided on number of pups treated, purity of bisphenol A, or caging and bedding materials.]** Pups were also treated with 2 nonylphenol compounds and 3 phthalate compounds, but results for those compounds will not be discussed. Pups were weaned at 3 weeks of age. Spontaneous motor activity was measured in pups at 4–5 weeks of age. Rats were killed at 8 weeks of age, and RNA was isolated from midbrain for microarray analyses of gene expression. **[The number of rats examined was not reported for any endpoint.]** Data for spontaneous motor activity were analyzed by ANOVA or Student *t*-test. **[There were no statistical analyses for gene expression data.]**

Rats exposed to bisphenol A were significantly more active during the nocturnal phase than control rats (by ~1.4–1.6-fold). In midbrains of 8-week-old rats, expression levels were altered for 46 G protein-coupled receptor genes, which are involved in dopaminergic neurotransduction and many peptidergic neurotransduction

processes. The study authors noted altered dopamine transporter gene expression, which was impaired by all chemicals tested. Bisphenol A also lowered galanin receptor 2 expression. The study authors concluded that intracisternal exposure to bisphenol A induced hyperactivity in rats, possibly by regulating gene or protein expression of G protein-coupled receptor and dopaminergic neurotransduction systems.

Strengths/Weaknesses: Despite certain strengths, a significant weakness is the inability to correlate the internal exposure to bisphenol A provided by the intracisternal route with that seen by the oral route.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Patisaul et al. (2006), supported by the American Chemistry Council, evaluated the effect of neonatal bisphenol A on the anteroventral periventricular nucleus of the Sprague–Dawley rat. Pregnant rats ($n = 5$) were fed a phytoestrogen-free diet (Purina 5K96) during the last week of gestation. **[No information was provided about caging or bedding.]** Dams were permitted to litter. Pups were cross-fostered among all dams so that 4 dams reared 6 females and 6 males and 1 dam reared 5 males. Pups ($n = 5$–8/group) were randomly assigned to receive s.c. injections of 17β-estradiol 50 μg/pup, genistein 250 μg/pup, bisphenol A **[purity not indicated]** 250 μg/pup, or sesame oil vehicle every 12 hr for 48 hr. The authors estimated that the twice daily dosing with 250 μg/pup was approximately equivalent to 100 mg/kg bw/day. Injections began the morning of PND 1 (delivery = PND 0). On PND 19, the pups were transcardially perfused with ice-cold saline followed by paraformaldehyde. Brains were post-fixed in 20% sucrose in paraformaldehyde, sectioned coronally, and processed for immunohistochemistry for ERα and tyrosine hydroxylase. Sections were counterstained with Nissl stain. Cells of the anteroventral periventricular nucleus positive for ERα, tyrosine hydroxylase, or both were counted. Statistical analysis used 2-way ANOVA with sex and treatment as factors, followed by 1-way ANOVA and post-hoc Fisher least significant difference test.

There was a significant, sex-related effect on tyrosine hydroxylase-positive cells in the anteroventral periventricular nucleus with the number in males about 29% that of females **[estimated from a graph]**. The authors concluded that neonatal treatment with bisphenol A interfered with the normal testosterone-associated masculinization of the anteroventral periventricular nucleus. Because 17β-estradiol is aromatized to testosterone in the brain, the authors interpreted this effect of bisphenol A as anti-estrogenic. Cells staining for both ERα and tyrosine hydroxylase are not present in rodents after puberty, and the authors stated that these cells may play a role in the organization of the LH-surge. They postulated that the decrease in these cells with neonatal exposure to bisphenol A may result in cycle disruption in adulthood.

Strengths/Weaknesses: Strengths of this study are the use of 17β-estradiol as a positive control and the measurement of ERα receptors. Weaknesses are the relatively high-dose level of bisphenol A and the use of the subcutaneous route of exposure on a per pup basis without adjustment for body weight. Critical weakness include small sample size (5 treated dams) and lack of adequate experimental and statistical control for litter effects.

Utility (Adequacy) for CERHR Evaluation Process: Despite certain strengths, this study is inadequate for the evaluation process for the reasons cited above.

Patisaul et al. (2006), supported by the American Chemistry Council, investigated the effects of an acute neonatal exposure to bisphenol A or genistein (**not discussed here**) on the SDN-POA and the anteroventral periventricular nucleus in the adult male rat. Five pregnant Sprague–Dawley rats were obtained and maintained on a 12-hr light/12-hr dark cycle, with free access to water and a soy-free, phytoestrogen-free diet that was maintained throughout the duration of the experiment. **[Details on housing (individual or group), type of caging, and bedding material were not provided.]** Most of the dams were cross-fostered with 6 male and 6 female pups. Starting on PND 1, all male pups were given s.c. injections every 12 hr over 48 hr with 250 μg bisphenol A **[purity not provided]** or oil vehicle. **[Assuming a Sprague–Dawley pup weighs ~7.5 g, this dose would be equivalent to ~66 mg/kg bw/day.]** On PND 85, males were gonadectomized. Six ovariectomized female rats served as controls. After a recovery period, the rats were given s.c. injections of 10 μg estradiol benzoate, and 48 hr later, a s.c. injection of 500 μg progesterone. The authors note that this protocol has consistently induced *fos* expression in GnRH neurons, leading to LH release in females. About 8 hr later, the animals were killed, formalin-perfused, and brains were harvested. Regions containing the SDN-POA and anteroventral periventricular nucleus were cryopreserved. SDN-POA sections were serially stained with Nissl or labeled for calbindin-d28 K. The vascular organ of the lamina terminalis was double-immunostained for Fos and GnRH. An automated stereomicroscope was used to gauge the volume areas of the anteroventral periventricular nucleus, the SDN-POA, the calbindin-immunoreactive regions of the SDN-POA, and number of calbindin-positive nuclei. Calbindin-positive nuclei were also counted by independent evaluators blinded to the treatments. Quantification analyses of GnRH and Fos staining were evaluated visually. Statistical analysis was performed using ANOVA, and Fisher least significant difference test.

Acute neonatal treatment of bisphenol A did not affect the volume of the SDN-POA. Similarly, the volumes of the calbindin-immunoreactive regions of the SDN-POA were roughly equivalent to SDN volumes **[estimated from a graph]** with no apparent bisphenol A treatment effect. Bisphenol A treatment induced a significant increase **[~50–60% estimated from a graph]** in calbindin-positive nuclei. Bisphenol A had no effect on the volume of the anteroventral periventricular nucleus or the total number of GNRH-positive nuclei, and no induction of Fos protein was identified.

The authors noted that the long-term effect of neonatal exposure to bisphenol A on male brain development and reproductive behavior cannot be predicted solely on anatomical changes in sexually dimorphic brain regions. They concluded that the development of more precise and predictive biomarkers is needed.

Strengths/Weaknesses: Strengths of this study are the use of 17β-estradiol as a positive control. Weaknesses are the relatively high-dose level of bisphenol A and the use of the subcutaneous route of exposure on a per pup basis without adjustment for body weight. Critical weakness

include small sample size (5 treated dams) and lack of adequate experimental and statistical control for litter effects.

Utility (Adequacy) for CERHR Evaluation Process: Despite certain strengths, this study is inadequate for the evaluation process for the reasons cited above.

Shikimi et al. (2004), supported by the Japan Society for the Promotion of Science for Young Scientists, examined the effects of bisphenol A exposure on Purkinje cell development in rats. **[No information was provided about feed or composition of caging and bedding materials.]** At 6–9 days of age, 4 male or female Fisher rats/group received bisphenol A **[purity not provided]** at 0 (sesame oil vehicle), 0.050, or 0.500 mg/day by injection into the cerebrospinal fluid near the region of the cerebellum. During the same time period, additional groups of 4 rats received 0.500 mg/day tamoxifen, 0.500 mg/day bisphenol A + 0.500 mg/day tamoxifen, or 5 μg/day estradiol benzoate through the same exposure route. **[Both male and female rats were treated, but it was not indicated if there were equal numbers in each group; both sexes were apparently evaluated together.]** At 10 days of age, pups were killed and vermal cerebella were removed and sectioned. Purkinje cells were examined morphologically following identification by calbindin-D28 K immunostaining. Data were analyzed by ANOVA, followed by Duncan multiple range test.

Treatment with the high-dose of bisphenol A increased Purkinje fiber length. There was no effect on cross-sectional soma area or Purkinje cell number as a result of bisphenol A treatment. Co-treatment with tamoxifen inhibited the increase in dendritic length that was observed following treatment with bisphenol A alone. Estradiol benzoate also induced an increase in dendritic length of Purkinje fibers that was blocked by tamoxifen. Treatment with tamoxifen alone also reduced dendritic fiber length. The effects of octylphenol were also examined and an increase in dendrite length was observed. The study authors concluded that bisphenol A induced Purkinje dendritic growth, possibly through the ER.

Strengths/Weaknesses: The use of estradiol benzoate as a positive control is a strength of this study. Weaknesses are the injection into cerebrospinal fluid.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to uncertainties surrounding the route of administration (i.e., difficulty of relating a cerebrospinal injection to human exposures).

Zsarnovszky et al. (2005), supported by NIH, NIEHS, and the American Heart Association, evaluated the effect of intracerebellar injection of bisphenol A on the development of activated extracellular signal-regulated kinase (ERK)-positive cells in cerebellar sections in Sprague–Dawley rats. Neonatal rats on PND 4–19 underwent a single direct injection under anesthesia of bisphenol A or 17β-estradiol under stereotactic guidance into cerebellar folia 6 and 7. **[For bisphenol A, only PND 10 results were given. The number of animals at each age was not specified, but a figure legend indicated at least 6/dose group. The purity of the chemicals was not specified. The day of birth was not defined.]** Concentrations of the chemicals were 10^{-12}–10^{-6} M **[bisphenol A concentrations of 0.23 ng/L to 0.23 mg/L]**. Uninjected, mock-injected, and vehicle-injected controls were used. Brains were removed and fixed 6 min after the onset of the injection. Sections were processed for immunohistochemistry using an antibody that recognized activated ERK. Quantitative analysis was performed on images of folium 9. Statistical analysis was performed using ANOVA with post-hoc Tukey–Kramer multiple comparison test. Response to different chemicals and different concentrations on PND 10 were compared using 2-factor ANOVA with post-hoc Bonferroni test. Adult rats were also treated but were not included in the quantitative analysis.

The qualitative appearance of the immunostained sections was similar after bisphenol A and 17β-estradiol. In the 10^{-12}–10^{-9} M dose range, the quantitative responses to the two chemicals were similar. Activated ERK-positive cells increased with a median effect concentration of 7.46 pM for 17β-estradiol and 3.25 pM **[0.74 ng/L]** for bisphenol A. Both chemicals were described as having an inhibitory effect at higher doses. **[The data graph shows drop-offs to control densities at 10^{-9} and 10^{-10} M, with a second increase in density at 10^{-7} and 10^{-5} M.]** Co-administration of 10^{-10} M 17β-estradiol with bisphenol A 10^{-12}–10^{-10} M **[0.23–23 ng/L]** resulted in a concentration-dependent decrease in activated ERK-positive cells compared to the administration of 17β-estradiol alone. The authors concluded that 17β-estradiol regulates ERK signaling in the developing cerebellum and that bisphenol A can mimic and also inhibit this estrogenic effect, with potentially adverse affects on brain development and function.

Strengths/Weaknesses: The use of 17β-estradiol as a positive control is a strength of this study. Weaknesses are the intracerebellar injection and the administration on a per pup basis.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due uncertainties surrounding the route of administration (i.e., difficulty of relating a cerebrospinal injection to human exposures).

3.2.5 Mouse—oral exposure only during pregnancy.

3.2.5.1 Studies without neurobehavioral endpoints: Morrissey et al. (1987), supported by NTP/NCTR, examined the effects of prenatal bisphenol A exposure in rats and mice in studies conducted according to GLP. The studies are also available as NTP publications for rats (NTP, 1985c) and mice (NTP, 1985b). The study was conducted in two sets of rats and mice and data were pooled for each species. **[The data for rats were discussed in Section 3.2.1.]** Animals were fed Purina 5002 diet, housed in polypropylene or polycarbonate cages with stainless steel wire lids with Ab-Sorb-Dri cage bedding. Pregnant CD-1 mice were randomly assigned to groups of ≥10 animals in each set of the study, for a total of ≥20 animals/dose. On GD 6–15 (GD 0 = sperm or plug), mice were gavaged with bisphenol A at 0 (food-grade corn oil), 500, 750, 1000, or 1250 mg/kg bw/day. Doses were based on results of preliminary studies and were expected to result in 10% maternal mortality at the high-dose and no toxicity at the low dose. The purity of bisphenol A was >95%, and 2,4′-bisphenol A was reported as an impurity. Concentrations of dosing solutions were verified. Pregnant animals were

Table 75
Maternal and Developmental Toxicity in Mice Gavaged With Bisphenol A[a]

	Dose in mg/kg bw/day							
Endpoint	500	750	1000	1250	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Dam weight in treatment period	↔	↔	↔	↓43%	881	661	1159	1039
Gravid uterine weight	↔	↔	↔	↓32%	983	690	1243	1123
Relative dam liver weight	↑9%	↑13%	↑17%	↑26%	618	411	755	541
Resorptions/litter	↔	↔	↔	↑2.8-fold	817	377	1245	1162
Fetal body weight/litter	↔	↔	↔	↓15%	1079	785	1249	1024

[a]Morrissey et al. (1987).
↑,↓ Statistically significant increase, decrease; ↔ no statistically significant change.

Table 76
Sperm Production and Male Reproductive Organ Weights in Mice Exposed to Bisphenol A During Gestation[a]

	Dose in mg/kg bw/day[b]					
Endpoint	0.002	0.020	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Sperm production efficiency	↔	↓ 19%	0.011	0.007	0.010	0.007
Body weight	↓ 9%	↔				
Preputial weight	↑ 36%	↔				
Seminal vesicle weight	↓ 12%	↔				
Epididymal weight	↓ 12%	↓ 8%				

[a]vom Saal et al. (1998).
[b]Benchmark doses were not estimated for values obtained from graphs and non-dose-related effects; errors were assumed to be SEM, as reported earlier in the study.

weighed during the study. Mice were killed on GD 17. Liver and uteri were weighed, and corpora lutea and implantation sites were examined. Fetuses were sexed, weighed, and examined for viability and external, visceral, and skeletal malformations. Data were analyzed by Bartlett test for homogeneity of variance, ANOVA, and/or William multiple comparison, Dunnett, and/or Fisher exact probability tests. **[Data were presented and analyzed on a per litter basis.]**

Clinical signs reported in mice treated with bisphenol A included arched back, lethargy, piloerection, rough coat, vaginal bleeding, vocalization, alopecia, weight loss, and wheezing. One or 2 of 29–34 dams died in each of the 3 lowest dose groups and 6 of 33 dams died in the 1250 mg/kg bw/day group. Statistically significant effects are summarized in Table 75. Absolute liver weight was increased in the 500, 750, and 1000 mg/kg bw/day dose groups, and relative liver weights were increased in all bisphenol A dose groups. Decreased gravid uterine weight and dam body weight gain during the gestation and treatment periods attained statistical significance at the 1250 mg/kg bw/day dose. The number of litters available for evaluation in the control and each dose group was 26, 23, 21, 23, and 21. Increased resorptions/litter and decreased fetal body weights/litter attained statistical significance in the high-dose group. There was no effect on the number of live fetuses/litter at birth or on fetal malformations/litter. The study authors concluded that bisphenol A is not teratogenic in mice at doses that result in maternal toxicity.

Strengths/Weaknesses: Strengths include the oral route of exposure as well as the design and sample sizes used. The use of very high-doses is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility in the evaluation in providing information on conventional teratogenic endpoints.

vom Saal et al. (1998), supported by NIH, examined the effects of bisphenol A exposure on male reproductive organs and sperm production in mice. The CF-1 mice used in this study were purchased in 1979 and maintained as an outbred stock in a closed colony. Dams were fed Purina breeder chow (5008) during pregnancy and lactation, and male offspring were fed Purina 5001 standard lab chow after weaning. Housing consisted of polypropylene cages with corn cob bedding. Bisphenol A **[purity not reported]** in tocopherol-stripped corn oil vehicle was fed to 7 mice/group by electronic micropipette at 0.002 or 0.020 mg/kg bw/day on GD 11–17 (day of vaginal plug = GD 0). One group of 6 mice was given the vehicle control, and a group of 5 mice was not handled. Based on results of in vitro assays conducted by the study authors, the 0.02 mg/kg bw/day bisphenol A dose was predicted to be bioactive in mice. Additional mice were treated with the same doses of octylphenol. Females delivered pups naturally on GD 19, and pups were weaned on PND 23 (day of birth not defined). Male siblings were housed 3/cage until 5 months of age. Randomly selected males were housed individually at 5 months of age and killed 1 month later. Body, testes, epididymides, preputial glands, and seminal vesicles were weighed in 11 control mice and 7 treated mice/group. Data from the two control groups did not differ significantly and were combined for analyses of organ and body weight. Data for prostate weight were reported by Nagel et al. (1997). Daily sperm production was determined in 8 control males/group and 5 treated

males/group. **[It was not stated how data from the 2 control groups were handled for sperm analyses.]** Sperm data were analyzed by ANOVA. Organ weight data were analyzed by ANCOVA, Pearson's correlation analysis, ANOVA, and least significant means test. **[It was not clear if the offspring or litter were considered the statistical unit; only one randomly selected male per litter was used per F. vom Saal, personal communication, June 20, 2007.]**

Statistically significant findings are summarized in Table 76. Exposure to bisphenol A resulted in dose-related reductions in daily sperm production efficiency (i.e., per g testis) that attained statistical significance at the highest dose level. Some significant but non-dose-related effects were observed for body and organ weights. Epididymal weights were reduced at both doses. At the low dose, body and seminal vesicle weights were reduced and preputial weight was increased. In mice treated with octylphenol, daily sperm production was reduced at the low dose but there was no effect on reproductive organ weights. The study authors concluded that exposure of the fetus to low doses of endocrine-disrupting chemicals can affect the size and function of reproductive organs.

[The NTP Statistics Subpanel (NTP, 2001) noted that vom Saal et al. (1998) did not apparently require overall differences by ANOVA to be significant before applying the least significant difference test, which is prone to false positive findings without the overall protection of ANOVA. The NTP Subpanel was not able to confirm any of the significant findings reported for bisphenol A. The NTP Subpanel noted that in theory, their reanalysis of organ weights was not necessarily in conflict with the findings of the study authors because of the use of different statistical methods (Dunnett test vs. Fisher least significant difference test).]

Strengths/Weaknesses: Strengths are the use of oral delivery and low dose levels. Weakness are the inability to assume the genetic comparability and responsiveness of CF-1 mice maintained in a closed colony for almost 20 years is comparable to other sources of CF-1 mice), failure to weight-adjust the maternal dose daily, the lack of information on testis weight (that is needed for consideration of daily sperm production), small sample size for sperm production measurement, and the questions about the statistical analysis. An additional weakness is the unusual/unexplained findings of low dose only effect on weights.

Utility (Adequacy) for CERHR Evaluation Process: The body weight data contained in this study are adequate for the evaluation process, however overall utility is limited because of sample size and statistical concerns. Data on reproductive organ weights and sperm production are considered inadequate for the evaluation.

Nagel et al. (1997), supported by NIH and the University of Missouri-Columbia, examined the effect of prenatal bisphenol A exposure on mouse prostate weight. The mice used in this study were the same ones used in the study by vom Saal et al. (1998), and experimental details are provided in the above summary of that study. CF-1 mice were fed Purina Laboratory Chow 5001 and housed in polypropylene cages with corn cob bedding. The mice (7/group) were dosed with bisphenol A **[purity not reported]** at 0.002 and 0.020 mg/kg bw/day on GD 11–17. A control group of 6 mice was given the tocopherol-stripped corn oil vehicle during the same time period. Vehicle and dosing solutions were fed to the mice using a micropipette. A second control group of 5 dams was unhandled. Because there were no significant differences between the 2 control groups, data from the 2 groups were pooled. Females were allowed to litter. Pups were weaned at 23 days of age and housed 3/cage. One male/litter was selected and housed individually for 1 month. Body weights of males were measured throughout the study. Selected males were killed at 6 months of age for measurement of prostate weight. Data for prostate weight were analyzed by ANCOVA using body weight as the covariate. If it was determined that body weight did not account for differences in prostate weight, data were reanalyzed by ANOVA without adjustment for body weight. Selection of 1 male/litter controlled for litter effects. Body weights were lower in males from the 0.002 mg/kg bw/day group than in controls. Statistical analyses revealed that prostate weight was not related to body weight. Compared to control values, prostate weights were 30% higher in the 0.002 mg/kg bw/day group and 35% higher in the 0.020 mg/kg bw/day group. The study authors concluded that bisphenol A alters the reproductive system of mice at doses near reported ranges of human exposure.

[The NTP Statistics Subpanel (NTP, 2001) concluded that Nagel et al. (1997) used appropriate statistical methods, and the Subpanel reached essentially the same conclusions as the study authors regarding elevated prostate weight.]

Strengths/Weaknesses: Strengths are the use of the same methods as vom Saal et al. (1998) and the use of dose levels in the range of human exposure. The independent confirmation of the data analysis by the NTP Statistics Subpanel is another strength. The use of a small sample size, closed mouse colony, and the failure to present any histopathological analyses are weaknesses. The Purina 5001 chow has high and variable levels of soy phytoestrogens, and the corn cob bedding is known to be problematic due to antiestrogenic constituents. This study did not use a positive control, although there are earlier reports from this laboratory using diethylstilbestrol.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and useful for the evaluation process.

Cagen et al. (1999a), support not indicated **[authors noted to work in industry]**, examined the effects of prenatal bisphenol A exposure on the developing reproductive system of male mice. The study attempted to duplicate the findings by vom Saal et al. (1998) and Nagel et al. (1997) by repeating their procedures. Exceptions were: (1) use of larger group sizes to increase statistical power; (2) use of 4 dose levels instead of 2; (3) use of 2 methods to determine sperm counts; (4) killing of male offspring at 90 instead of 180 days; (5) conducting the study according to GLP; (6) obtaining mice from a commercial source instead of an in-house bred colony; and (7) housing males individually after weaning. In the study by Cagen et al. (1999a), CF-1 mice gaining more than 4.5 g weight from GD 0 to 10 were randomly assigned to groups of 28 animals and administered bisphenol A (>99% pure) 0.0002, 0.002, 0.020, or 0.2 mg/kg bw/day on GD 11–17. Two negative control groups

with 28 dams each were given the tocopherol-stripped corn oil vehicle. Because results from the two vehicle control groups were statistically equivalent, data from the two groups were pooled. A positive control group of 28 mice was given 0.2 μg/kg bw/day diethylstilbestrol. Dosing solutions were dripped into the animals' mouths using a micropipette. Concentrations of dosing solutions were verified before dosing. Animals were fed certified rodent chow 5002. Water was provided in glass bottles with Teflon seals. Cages were made of polypropylene with steel lids. Corn cob bedding was used. Music was played at low volume to provide background noise. Dams were monitored for clinical signs, food intake, body weight gain, and fertility endpoints. Pups were counted and sexed at birth (PND 0) and monitored for survival and weight gain until weaning on PND 22. Litters were culled to 8 pups on PND 4, leaving as many males as possible. At weaning, no more than 4 males/litter (65–95 males/group) were randomly selected to continue in the study and housed individually. The males were monitored for body weight gain and feed intake until they were killed on PND 90. Brain, liver, kidneys, and reproductive organs were weighed. Daily sperm production and epididymal sperm counts were determined and a histopathological examination of testes was conducted. The litter was considered the experimental unit in statistical analyses. Data were analyzed by Levene test, ANOVA, Dunnett test, rank transformation, Wilcoxon rank sum test with Bonferroni correction, Fisher exact probability test, and binomial distribution test.

There were no clinical signs or significant differences in body weight gain or feed intake in dams. The numbers of dams that died of unknown causes during the study were: 2 receiving vehicle controls; 1 dosed with diethylstilbestrol; 3 dosed with 0.0002 mg/kg bw/day bisphenol A; and 1 each in the 0.002 and 0.020 mg/kg bw/day bisphenol A groups. The number of total pups/litter was significantly lower than controls in the 0.2 mg/kg bw/day bisphenol group (mean ± SD = 9.60 ± 3.85 compared to 12.37 ± 3.02 in the control group). In communications with the animal vendor, it was determined that litter size in the control group exceeded typical litter sizes (9–10 pups), and the study authors therefore concluded that the effect was not treatment-related. Bisphenol A had no significant effects on gestation index or duration, percentage of male pups at birth, or pup survival and body weight during the lactation period. The same endpoints were unaffected in the diethylstilbestrol group.

Terminal body weights were increased **[by 7%]** in the 0.020 mg/kg bw/day group and **[by 5%]** in the 2 mg/kg bw/day group. Bisphenol A did not affect absolute or relative (to body or brain) weights of reproductive organs including prostate, preputial gland, seminal vesicle, or epididymis. Non-dose-related effects were observed for brain and kidney weights, and the study authors concluded that the effects were not treatment-related. There were no significant effects on cauda epididymal sperm concentration, daily sperm production, or efficiency of sperm production. Testicular histopathology was not affected by bisphenol A treatment. **[Data were not shown by authors.]** Reproductive development of male offspring was also unaffected by diethylstilbestrol. The study authors noted that the diethylstilbestrol dose was considered the "maximum effect" oral dose by vom Saal but was lower than doses affecting male offspring in other studies. The study authors also noted that the effects of bisphenol A on prostate weight and sperm production reported by vom Saal et al. (1998) and Nagel et al. (1997) were not repeated in this study. They concluded that bisphenol A should not be considered a selective reproductive or developmental toxicant.

[The NTP Statistics Subpanel (NTP, 2001) concluded that the statistical methods used by Cagen et al. (1999a) were appropriate. Although the Subpanel agreed with the study author conclusions, they noted that (1) a significant ANOVA is not a requirement for Dunnett test; and (2) a Bonferroni correction of the Wilcoxon-rank sum test was not needed because the study authors already required significance by ANOVA, which was sufficient.]

Strengths/Weaknesses: The attempt to replicate the studies of vom Saal et al. (1998) and Nagel et al. (1997), the use of litter analysis, the large sample sizes, and the agreement of the NTP Subpanel with the author conclusions are strengths. With respect to this study as a replication, weaknesses include design differences relating to strain, dietary differences, age at evaluation, and the use of solo housing rather than small group housing. The lack of response of the positive control DES group is problematic.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to absence of response of the positive control group.

Ashby et al. (1999), support not indicated [2 authors from industry], examined the effects of prenatal bisphenol A exposure on the mouse reproductive system. The study attempted to duplicate the findings reported by vom Saal et al. (1998) and Nagel et al. (1997). Both generations of CF-1 mice were fed RM1 diet containing 6.5% soy during periods when they were not pregnant or lactating, and dams were fed RM3 diet containing 18.5% soy during pregnancy and lactation. On post-conception days 11–17, 8 dams/group were dosed with bisphenol A (99% pure) at 0, 0.002, or 0.020 mg/kg bw/day. The negative control group was administered the tocopherol stripped corn oil vehicle. A positive control group of 7 dams received diethylstilbestrol at 0.2 μg/kg bw/day. A naïve group of 7 dams was not weighed or dosed. The dosing solution was slowly expelled from a pipette placed in the animals' mouths. Day of vaginal plug detection was designated post-conception day 1, however. females that had no vaginal plugs but gained >3.5 g were arbitrarily considered to be 10 days pregnant. Females with vaginal plugs and those that gained >3.5 g were distributed evenly among treatment and control groups. Females that gained >1 but <3.5 g were considered to be pregnant, but because the day of pregnancy could not be determined, they were assigned to the naïve control group. Dams were allowed to litter. All female offspring were weighed and monitored for vaginal opening. Females were killed at ~44 weeks of age, and liver, kidney, and reproductive organs were weighed. Male pups were housed as littermates until PND 112 (day of birth designated as PND 1). To determine the effects of housing, ~3 males from 4–7 litters/group (11–21 males/group) were randomly selected and housed separately from PND 112 until study

termination, which occurred ~71 days later. The remaining male pups from 4–5 litters/group from each litter (11–17/group) were housed together. Singly housed males were weighed and killed on PND 183–185, and group-housed males were weighed and killed on PND 186–187. Equal numbers of males from each group were killed each day. Liver, kidney, and reproductive organs were weighed, and testicular sperm count and efficiency were determined. Technicians were blinded to experimental conditions. Measures taken to reduce stress to animals included administering test agents by drip feeding, minimal handling of pups, and minimal environmental noise. Selection of 3 males from each litter increased statistical power compared to previous studies (Nagel et al., 1997; vom Saal et al., 1997). Statistical analyses were dually conducted using the individual offspring and the litter as the statistical unit. Data were evaluated by ANOVA and Dunnett test. Results from vehicle-treated and naïve controls were pooled when there was no evidence of a vehicle effect. Data from individually housed and group housed-males were pooled when they did not differ significantly.

There were no significant differences in litter sizes or percentage of males/litter. In female offspring from the bisphenol A groups, there were no significant effects on body weight or organ weights, including cervix, uterus, vagina, and ovary. Age and weight at vaginal opening were also unaffected in groups exposed to bisphenol A. Vaginal opening was delayed in the diethylstilbestrol-treated group and in the naïve control group.

Significant effects included increased terminal body weights in the low-dose group, increased testis weight in both dose groups, and increased epididymis weight in the high-dose group. Because testis and epididymis weights relative to body weights were nearly identical to controls **[data not shown by study authors]**, the authors considered the finding equivocal. Although prostate weights were slightly higher in the bisphenol A groups, there were no statistically significant effects on prostate weight when adjusted for body weight and litter effects. Daily sperm production was increased in both dose groups, but the study authors considered the finding equivocal due to low biological significance. The study authors noted that the study failed to confirm the increase in prostate weight and decrease in sperm production reported in the studies by vom Saal et al. (1997) and Nagel et al. (1997), but results were consistent with those reported by Cagen et al. (1999a). Possible reasons for variability between studies were stated as differences in background sound level, diet, and animal body weights. The study authors also mentioned the possibility of genetic drift occurring in mice bred in-house in the vom Saal laboratory.

[The NTP Statistics Subpanel (NTP, 2001) essentially reproduced the findings reported by Ashby et al. (1999).]

Strengths/Weaknesses: Strengths are the rather close replication of the designs of the studies by vom Saal et al. (1998) and Nagel et al. (1997) with diet as the only major difference, the use of both solo and group housed mice, and the support of the conclusions by the NTP Statistics Subpanel. The use of small samples is an understandable weakness given that this study was designed to be a replicate study. The lack of response of the positive control DES group is problematic.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to absence of response of the positive control group and small sample sizes.

Howdeshell et al. (1999), support not indicated, examined the effect of prenatal bisphenol A exposure on age of puberty in female mice. **[No information was provided about chow or composition of bedding and cage materials.]** CF-1 mice ($n = 21$/group) were fed oil vehicle **[type of oil not specified]** or bisphenol A **[purity not reported]** at 0.0024 mg/kg bw/day on GD 11–17 **[day of vaginal plug not defined]**. On GD 19, pups were obtained by cesarean section. Intrauterine position of pups (i.e., located next to male or female pups) was noted at that time. Pups were fostered by untreated mothers and weaned on PND 22. Body weights were measured, and pups were monitored for vaginal opening and time to estrus. Results were analyzed according to all pups from each dose group or in relation to intrauterine position. The study authors stated that fetuses positioned between 2 male mice were exposed to the lowest levels of 17β-estradiol, while exposures to 17β-estradiol were highest in fetuses positioned next to female fetuses. Data were analyzed on a litter basis to control for maternal effects. Age of vaginal opening was covaried with weight at weaning. Numbers of female offspring evaluated were 75–111/group for body weight and 51–58/group for vaginal opening. The study authors attempted to evaluate females from each intrauterine position in each litter. **[No additional information was provided for statistical analysis in this brief communication.]**

Body weight at weaning was significantly increased in females in the bisphenol A group. When analyzed according to intrauterine position, body weights were 22% higher than controls in females who were not positioned next to a male fetus and 9% higher in females who had been positioned next to 1 male in utero. There were no significant effects on age of vaginal opening. **[It was not clear if the data presented were covaried with body weight.]** Bisphenol A treatment significantly reduced the period between vaginal opening and first estrus by ~2.5 days. When evaluated according to intrauterine position, a significant decrease in time to first estrous was observed in females who were not positioned next to a male pup (accelerated by ~5 days) and in females positioned next to 1 male **[~2 days]**. No statistically significant findings were observed in females who had been positioned next to 2 males in utero. The study authors concluded that prenatal exposure to bisphenol A at environmentally relevant levels altered postnatal growth and reproductive function in female mice but that natural variations in individual endogenous 17β-estradiol levels influenced the response to bisphenol A.

The results of this study were also discussed in a publication by Howdeshell and vom Saal (2000), which indicated that the work was supported by NIH and reported additional findings. There was a bisphenol A-associated reduction in pup survival between birth and weaning. Complete litter death occurred in 6 of 21 litters in the bisphenol A group compared to 1 of 21 litters in the control group. Significantly increased body weight of male pups at weaning was also reported for the bisphenol A group. Body weights were highest in males who were positioned next to 2 female pups in

utero and were 10% higher than body weights of control males positioned next to 2 female fetuses in utero. No increase in body weight occurred in males that were positioned between two male fetuses in utero. Although the authors identified a litter-based analysis, it was not always clear that this applied to all analyses (in Study Figure 1, the n-values exceed the number of dams, suggesting that some of the data were analyzed on a per pup basis.

[The NTP Statistics Subpanel (NTP, 2001) requested the Howdeshell et al. (1999) data set for reanalysis, but it was not provided by study authors.]

Strengths/Weaknesses: Strengths are the oral route of exposure and the use of a low dose level of bisphenol A. The omission of a description of husbandry conditions and lack of clarity of statistical procedures are weaknesses. Use of only a single dose is a weakness. Further, the use of time from vaginal opening to first estrus is not a standard endpoint for assessing puberty in mice and is of questionable biological significance.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process but utility is limited due to uncertainties in data analyses.

Gupta (2000), supported by NIH, examined the effects of bisphenol A exposure on the reproductive system of male mice. CD-1 mice were received on GD 12 (GD 0 = day of breeding). The mice were fed Purina Chow-5 L9 at the Charles Rivers Laboratory and Purina Chow 5012 at the study author's laboratory. **[No information was provided on bedding or caging materials.]** On GD 16–18, 15 mice/group were fed the corn oil/12% ethanol vehicle or 0.050 mg/kg bw/day bisphenol A **[purity not reported]**. Additional groups of mice were administered diethylstilbestrol at 0.1 and 200 μg/kg bw/day and Aroclor at 0.050 mg/kg bw/day during the same time period. The bisphenol A dose level was based on a level reportedly considered safe by the FDA. Following delivery, litters were culled to 8 pups, with at least 3 males. Body weight and anogenital distance were examined in 3 pups/litter (45 pups) on PND 3, 2 pups/litter (30 pups) on PND 21, and 1 offspring/litter (15 offspring) on PND 60. **[Although Table 1 of the study lists the n-value as 15–45/group, a statement in the methods section indicated that an equal number of pups (n = 1–3) were pooled from each litter.] Prostate and epididymis were weighed in 15 offspring/group on PND 3, 21, and 60. Whole-tissue mounts of prostate were examined for growth in 15-day-old offspring (n = 4/group). Androgen binding was measured in prostates isolated at 3, 21, and 60 days of age, with 2–6 prostates pooled, depending on age; an n of 5 was reported in Figure 2 of the study. Data were analyzed by ANOVA. [It was not clear if the offspring or litter was considered the statistical unit.]**

Body weights of male offspring were not affected by bisphenol A treatment. In male pups of the bisphenol A group compared to the control group, anogenital distance adjusted for body weight was significantly increased **[by 22%]** on PND 3, **[by 25%]** on PND 21, and **[by 33%]** on PND 60. Prostate weights in males of the bisphenol A group were significantly increased **[by 56%]** on Day 3, **[by 39%]** on Day 21, and **[by 101%]** on Day 60. Relative (to body weight) epididymis weight in the bisphenol A group was significantly reduced **[by 35%]** on PND 60. Prostate growth was reported to be qualitatively increased by bisphenol A exposure. Androgen receptor binding was increased on PND 21 and 60 **[by ~344% on PND 21 and 358% on PND 60, estimated from a graph]**. Similar effects were reported following treatment with the low dose of diethylstilbestrol and Aroclor. In contrast, the high-dose of diethylstilbestrol reduced body weights, anogenital distance, prostate weight, and androgen receptor binding. Presentation of pathology data are superficial, thus questioning interpretation.

The report also included an in vitro study to examine the effects of bisphenol A on prostate growth. The urogenital sinus was dissected from GD 17 fetuses and cultured for 7 days in media containing 0, 5, or 50 ng/L bisphenol A with and without the addition of testosterone. The urogenital sinus was also incubated in 0.1 or 0.5 ng/L diethylstilbestrol and 5 or 30 ng/L Aroclor. Prostates obtained from cultures were then fixed in Bouin solution and examined histologically. A similar protocol was used to examine androgen binding in cultured prostates, except that only the high-doses of each compound were examined, and cells were cultured for 6 days. Bisphenol A at 50 ng/L increased prostate size **[by 140%]** in the absence of testosterone and **[by 150%]** in the presence of testosterone. Androgen binding in prostate was increased **[by 200%]** following treatment with bisphenol A. Similar effects were reported with diethylstilbestrol and the high Aroclor dose. The study authors concluded that the effects of in vivo studies were reproduced in in vitro studies, which suggests a direct effect on reproductive organs of fetal mice.

In a subsequent commentary, Elswick et al. (2000) noted several concerns and requested clarification of the data analysis performed by Gupta. It was noted that statistical analyses were insufficiently described to determine if analyses in addition to ANOVA were conducted. It was not indicated if post-hoc tests were used or if corrections were made for multiple comparisons. Table 1 of the study was noted to contain a footnote indicating $P < 0.05$ (larger) or $P < 0.05$ (smaller). It was stated that determining a mean and conducting a one-tailed post-hoc test based on whether the mean is larger or smaller is a source of potential bias in the statistical analyses. Analyses conducted by Elswick et al. (2000) indicated that the assumption of homogeneity of variance, a requirement for ANOVA, was not met for some data such as anogenital distance on PND 3 (Table 1 of the study) and prostate size (Table 3 of the study). Therefore, questions were raised about whether homogeneity testing was done or if data were transformed to account for lack of homogenous variances before ANOVA. Failure to consider the litter as the experimental unit was noted in cases where the sample size was listed as 30 and 45, while only 15 dams/group were treated. It was noted that if anogenital distance was measured in the same animal at different time points, a repeated-measures ANOVA would have been the appropriate statistical test. It was stated that correction of anogenital distance by the cube root of body weight instead of body weight would have been preferred to avoid overcorrection; ANCOVA with body weight as a covariate would have been a better method for correcting anogenital distance, and the best method would have been a nested ANCOVA (dam within treatment). Questions were raised about whether sampling 1 pup/litter on PND 60

provided a reliable estimate, especially for highly variable endpoints such as anogenital distance, which can be affected by sex of the adjacent fetuses in the uterus. Organ weights were also stated to be variable, and it was questioned whether sampling 1 offspring/litter on PND 60 resulted in a reliable estimate.

Gupta (2001) responded to the questions raised by Elswick et al. (2000). Regarding the question of post-hoc tests for data analyzed by ANOVA, Gupta stated that comparisons using the least significant difference test support the effect reported in the original study. Gupta stated that the use of 1-tailed tests was never mentioned and that the criticism was unfounded. The numbers of offspring examined at each age was reiterated **[with no mention of considering the litter the statistical unit]**. It was stated that individual animals were not identified because it would have required using a toe clip or tattoo, which is stressful to the animals. Therefore, it was not known if the same animals were examined for anogenital distance at the different time points and use of the repeated-measures ANOVA would not have been appropriate. Regarding use of 1 animal/litter, it was stated that it is the standard procedure accepted by NIEHS to control for litter effects. Correction of anogenital distance by body weight was stated to be appropriate because of a significant correlation between body weight and anogenital distance ($r = 0.47$, $P < 0.001$). Adjustment for litter effects was stated to occur because litter was nested within treatment in the ANOVA. Gupta noted a typographical error in Table 3 of the original study. Standard deviations for the 50 ng/L bisphenol A and Aroclor groups were mistakenly indicated to be 10-fold higher than the actual values (i.e., the actual values were 0.024 for bisphenol A and 0.032 for Aroclor). The errors made it appear that there were differences in variances between groups, when actually there were not. Gupta stood by his original conclusion that low levels of bisphenol A alter the development of the male reproductive tract.

Strengths/Weaknesses: Strengths are the oral route of administration, the use of a low dose level of bisphenol A, the use of diethylstilbestrol as a positive control, the prostate measurements at 3 postnatal time points, and the use of an in vitro study to support the in vivo results. The use of a single dose level, and questionable histopathological presentation and evaluation are weaknesses. An additional weakness is that more than one male per litter was used for some endpoints without adequate statistical control for litter effects.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for evaluation of prostate weight, biochemical endpoints, and body weight and AGD at PND 60 but not other endpoints where litter effects were not adequately controlled for (i.e., those where 30 or 45 pups were examined from 15 litters).

Iida et al. (2002), supported by the Japan Society for Promotion of Science, examined the effect of prenatal bisphenol A exposure on spermatogenesis in adult mice. **[No information was provided about composition of feed, caging, or bedding.]** On GD 10–17 **[day of vaginal plug not defined]**, ≥ 3 ddY mice/group were orally administered bisphenol A **[purity not reported]** at 0 (corn oil vehicle), 1, 10, or 100 mg/kg bw/day. **[The specific method of oral dosing was not stated.]** At 60 days of age, 4–5 male mice/dose group (obtained from 3 litters/dose group) were weighed and killed. Testes were removed and fixed in paraformaldehyde for histopathological evaluation by light microscopy. At 120 days of age, testicular histopathology was examined by light and electron microscopy in 3 mice/group from the control and 10 mg/kg bw/day groups. Data were analyzed by ANOVA. **[It was not clear if the litter or offspring were considered the statistical unit.]**

No effects on body weight were observed in 60-day-old mice. Significant and dose-related increases in the incidence of abnormal seminiferous tubules were observed in mice exposed to bisphenol A. The incidence of abnormal seminiferous tubules in the control and each respective treatment group was 3.7, 15.2, 17.7, and 31.5%. **[Benchmark dose analysis using a probit model and $n = 3$ litters gave a $BMD_{10} = 44$ and a $BMDL_{10} = 17$ mg/kg bw/day.]** Examples of seminiferous tubule lesions included luminal space loss in tubules, reduced numbers of maturing elongate spermatids, decreased tubular diameter, aberrant distribution of spermatogenic cells in epithelium, and accumulation of material within tubules. In the 120-day-old mice exposed to 10 mg/kg bw/day, the same types of lesions were observed at a higher incidence than controls (28.3 compared to 5.14%). Electron microscopic examinations of 2 abnormal seminiferous tubules from exposed 120-day-old mice revealed the presence of round but not elongated spermatids, leading study authors to suggest disrupted spermatogenesis. Disorganized arrangement of Sertoli cells was also observed in the 120-day-old mice of the 10 mg/kg bw/day group. The study authors noted that degeneration of Sertoli cells may be the cause of aberrant distribution of spermatogenic cells.

Strengths/Weaknesses: The oral route of delivery is a strength of this study. The lack of information on details of husbandry, the small sample size (4–5 male mice from 3 litters per dose group) and the lack of adjustment for litter effects, inadequate methods for histopathological preservation and evaluation (i.e., use of paraformaldehyde for paraffin embedding) are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process based on methodology.

Timms et al. (2005), supported by NIEHS and U.S. EPA, examined the effects of bisphenol A exposure on development of the prostate in mice. CD-1 mice were fed soy-based Purina 5008 chow, provided drinking water in glass bottles, and housed in polypropylene cages. **[The type of bedding material was not indicated.]** On GD 14–18 (day of mating = GD 0), pregnant mice were fed by micropipette with 0.010 mg/kg bw/day bisphenol A **[purity not indicated]** ($n = 6$), the tocopherol-stripped corn oil vehicle ($n = 5$), 0.1 μg/kg bw/day ethinyl estradiol ($n = 5$), or 0.1 μg/kg bw/day diethylstilbestrol ($n = 5$), the positive control. The dose of bisphenol A was based on previous findings that suggested bisphenol A was 100-fold less potent than diethylstilbestrol in permanently increasing prostate size in mice. On GD 19, fetuses were removed by cesarean section, and during the removal process, intrauterine position of male fetus relative to sex of adjacent fetuses was recorded. To reduce effects associated with sex hormone exposure from the adjacent fetus, 1 male/litter that developed

Table 77
Effects on Prostate Development in Mice After Prenatal Exposure to 0.010 mg/kg bw/day Bisphenol A[a]

	Prostate region		
Endpoint[b]	Dorsolateral	Ventral	Dorsolateral and ventral
No. of prostate ducts	↑ 41%	↔	↑ 40%
Prostate duct volume	↑ 99%	↑ 78%	↑ 91%
Proliferating cell nuclear antigen staining	↑ 44%	↔	No data

[a]Timms et al. (2005).
[b]Percent changes calculated by CERHR differed slightly from values presented by authors; it was not clear which part of the prostate the authors' values represented.
↑,↓ Statistically significant increase, decrease; ↔ no statistically significant effect.

Table 78
Maternal Behavior Effects in Mice Exposed to Bisphenol A During Gestation or Adulthood[a]

	Bisphenol A exposure during gestation/adulthood		
Percent time[b]	Bisphenol A/vehicle	Vehicle/ bisphenol A	Bisphenol A/bisphenol A
Nursing	↓ 15%	↓ 14%	↔
Nest building	↑ 73%	↑ 146%	↔
Resting alone	↑ 67%	↑ 29%	↑ 46%
Grooming	↑ 25%	↑ 18%	↔
Active	↔	↑ 18%	↔
In nest	↓ 12%	↓ 10%	↔
Out of nest	↑ 17%	↑ 12%	↔

[a]Palanza et al. (2002).
[b]Data were presented graphically. Values were provided by the study author (personal communication, P. Palanza, February 26, 2007).
↑,↓ Statistically significant increase/decrease compared to vehicle-vehicle group, ↔ no statistically significant effect.

between a male and female fetus was examined. Prostate morphology was determined by a 3D computer reconstruction technique. Immunohistochemistry techniques were used to measure levels of proliferating cell nuclear antigen and mouse keratin 5. Statistical analyses included ANOVA, followed by Fisher least-squares mean test when statistical significance was obtained. In a separate study, prostate morphology was examined in 4 pregnant mice/group that were dosed with vehicle or 200 μg/kg bw/day diethylstilbestrol according to the procedures described above.

Bisphenol A increased numbers of ducts, volume, and proliferation in one or more prostate regions, as outlined in Table 77. The pattern of proliferating cell nuclear antigen staining was similar to that observed with mouse keratin 5, a basal epithelial cell maker. The study authors also reported a 56% increase in the volume of the coagulating glands. **[Data were not shown by study authors.]** An abnormal narrowing was observed in the portion of the urethra near the neck of the bladder. **[The volume of the cranial urethra was reduced by 35% compared to controls. Malformation of prostatic sulci was reported, but no information was provided on incidence or severity.]** Similar effects on the prostate were reported in mice exposed to ethinyl estradiol and the low dose of diethylstilbestrol. Narrowing of the cranial urethra was observed in mice exposed to ethinyl estradiol. In contrast, exposure to the high diethylstilbestrol dose resulted in inhibited morphogenesis of the prostate. The study authors concluded that the differentiating urogenital system of male mice is very sensitive to a low dose of bisphenol A.

Strengths/Weaknesses: Strengths are the oral route of administration, the low dose level of bisphenol A, the use of diethylstilbestrol and ethinyl estradiol as positive controls, and the sophisticated measures applied to the prostate. Weaknesses are the use of a single dose level and small sample size, although the Panel judged it to be adequate for the methodology.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation.

Palanza et al. (2002), supported by NIEHS, NIH, MURST, the University of Parma, and the National Council for Research, examined the effects of bisphenol A treatment on maternal behavior following exposure of mice during prenatal development and/or adulthood. The CD-1 mice used in this study were maintained as an outbred colony. Mice were housed in polypropylene cages with corn cob bedding. During pregnancy and lactation, mice were fed Purina 5008 (soy-based) chow. After weaning, mice were fed Purina 5001 (soy-based) chow. Water was provided in glass bottles. On GD 14–18 (GD 0 = day of vaginal plug), 14 mice were fed the tocopherol-stripped corn oil vehicle and 9 mice were fed 0.010 mg/kg bw/day bisphenol A **[purity not reported]** using an electronic micropipette. Dams were housed 3/cage after mating and individually housed on GD 17. Body weights of dams were measured during gestation. The day of birth was considered PND 1, and offspring were weaned on PND 20. At 2–2.5 months of age, F_1 female offspring from vehicle- and bisphenol A-treated dams were mated and exposed to vehicle or 0.010 mg/kg bw/day bisphenol A on GD 14–18. There were 4 groups of F_1 females that were exposed during gestation–adulthood to vehicle–vehicle ($n = 20$), vehicle–bisphenol A ($n = 15$), bisphenol A–vehicle ($n = 15$), and bisphenol A–bisphenol A ($n = 15$). Maternal behavior was observed in F_1 dams every 4 min during a 120-min period on PND 2–15. On PND 1, F_2 pups were weighed, sexed, and counted. Litters were then culled to 10 pups, with equal numbers of male and female pups when possible. Pups were weighed during the lactation period and cliff-drop aversion and righting reflex were evaluated in all pups of a subset of 8 litters/group on PND 3, 5, 7, and 9. For statistical analyses, all pup data were adjusted for litter. Data were analyzed by ANOVA, Holms *t*-test, and/or Fisher protected least-squared difference test.

Bisphenol A treatment did not affect gestational body weight gain in F_0 or F_1 dams. Statistically significant effects for F_1 maternal behavior collapsed across 14 observation days are presented in Table 78. Exposure to bisphenol A either in gestation or in adulthood resulted in decreases in the percentage of time the dams spent nursing and in the nest and increases in the percentage of time the dams spent nest building, resting alone,

grooming, and out of the nest. Increased activity was also observed in the group exposed to bisphenol A in adulthood. The only significant effect observed in mice exposed to bisphenol A during gestation and adulthood was increased time resting. When data were presented for individual evaluation days, time resting was significantly increased on PND 9, 10, 11, 12, and 14 in the group exposed to bisphenol A during gestation. Time spent resting was significantly increased on PND 9 and 14 in the group exposed to bisphenol A during gestation and adulthood. No other significant effects were observed on specific evaluation days. There were no significant differences in the number of live F_2 pups/litter, sex ratio, or body weight at birth or in weight gain during the lactation period. **[Data were not shown]**. No significant effects were observed for cliff aversion or righting reflexes. The study authors concluded that reduced levels of nursing behavior were observed in mice exposed to bisphenol A only as fetuses or only as adults. **[Because this study involves effects of adult exposure on maternal behaviors, it is also discussed in Section 4.2.]**

Strengths/Weaknesses: Strengths are the oral route of administration, the low dose level of bisphenol A, and the exploration of effects on complex maternal behaviors. It is unusual that pre- and postnatal exposure had effects but not the combination of pre- and postnatal exposure, and failure to explain this finding is a weakness. The use of a diet high in soy isoflavones is an additional weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Nishizawa et al. (2003), supported by the Japanese Ministry of Education, Culture, Sports, Science, and Technology, examined the effects of prenatal bisphenol A exposure on expression of retinoic acid receptor α and retinoid X receptor α in mouse embryos. ICR mice were fed standard feed (CM; Oriental Yeast). **[No information was provided about caging and bedding materials.]** Mice were orally dosed with bisphenol A **[purity not indicated]** at 0 (olive oil vehicle) or 0.002 mg/kg bw/day on 6.5–11.5, 6.5–13.5, 6.5–15.5, and 6.5–17.5 days post-coitum. Day of vaginal plug was considered 0.5 days post-coitum. **[No information was provided about the specific method of oral dosing.]** Twelve dams/group were killed at 12.5, 14.5, 16.5, and 18.5 days post-coitum, 24 hr after receiving the last dose. Expression of mRNA for retinoic acid receptor α and retinoid X receptor α was measured by RT-PCR in fetal cerebrum, cerebellum, and gonads. Data were analyzed by ANOVA. **[It was not clear if the litter or offspring was considered the measurement unit.]** Numerous changes in mRNA expression were observed following in utero exposure to bisphenol A, and they varied according to sex, tissue, and dosing period. The study authors concluded that these findings suggest a novel mechanism of bisphenol A toxicity mediation by disruption of the expression of retinoic acid receptor α and retinoid X receptor α.

Strengths/Weaknesses: Strengths are the oral route of delivery, the use of a low dose level of bisphenol A, and the exposure at different time periods. The study has value for understanding mechanisms of action although these changes were not tied to any adverse findings that might be related to these changes. Weaknesses include the use of a single dose level and lack of clarity on number of embryos per litter sampled. This is not considered a critical weakness because it is known that standard procedures for these methods require pooling of embryos within litter.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility for the evaluation because of the mechanistic nature of the endpoints.

Nishizawa et al. (2005b), supported by the Japanese Ministry of Education, Culture, Sports, Science, and Technology and by the Japan Society for the Promotion of Science, examined the effects of bisphenol A exposure on expression of mRNA for arylhydrocarbon and retinoid receptors in mouse embryos. ICR mice were fed standard diet (CM; Oriental Yeast). **[No information was provided about caging or bedding materials.]** Pregnant mice were orally dosed with bisphenol A **[purity not indicated]** at 0 (olive oil vehicle), 0.00002, 0.002, 0.20, or 20 mg/kg bw/day from 6.5 to 13.5 days post-coitum or 6.5 to 17.5 days post-coitum. Day of vaginal plug detection was considered 0.5 days post-coitum. **[No information was provided about the specific method of oral dosing.]** Twelve pregnant mice/group were killed on 14 and 18.5 days post-coitum, 24 hr after the last bisphenol A dose was administered. RT-PCR analyses were conducted to determine expression of mRNA for retinoic acid, retinoid X, and arylhydrocarbon receptors in fetal cerebrum, cerebellum, ovary, and testis. Data were analyzed by ANOVA. **[It was not clear if the litter or offspring was considered the measurement unit.]** Numerous changes in mRNA expression were observed following bisphenol A exposure and they varied according to dose, sex, tissue, and exposure period. The study authors concluded the this study shows a novel mechanism by which bisphenol can induce endocrine disruption through upregulation of arylhydrocarbon receptor (a key factor in the metabolism of some xenobiotics compounds) and retinoid receptors (key factors in nuclear receptor signal transduction).

Strengths/Weaknesses: The wide dose range from 0.00002 to 20 mg/kg bw/day and the oral route are strengths. The study has value for understanding mechanisms of action although these changes were not tied to any adverse findings that might be related to these changes. Weaknesses include the lack of specification of the method of oral dosing and lack of clarity on sample origins and sizes for each assay. Again, this is not considered a critical weakness because it is known that standard procedures for these methods require pooling of embryos within litter.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility for the evaluation because of the mechanistic nature of the endpoints.

Nishizawa et al. (2005a), supported by the Japan Society for the Promotion of Science, examined the effects of bisphenol A exposure on expression of aryl hydrocarbon receptors, related factors, and metabolizing enzymes in mouse embryos. ICR mice were fed standard diet (CM; Oriental Yeast). **[No information was provided about caging and bedding materials.]** Mice were orally dosed with bisphenol A **[purity not indicated]** at 0 (olive oil vehicle), 0.00002, 0.002, 0.2, or 20 mg/kg bw/day from

6.5–13.5 days post-coitum and 6.5 to 17.5 days post-coitum. Day of vaginal plug was considered 0.5 days post-partum. **[No information was provided about the method of oral dosing.]** Another group of mice was dosed with 5 μg/kg bw/day 17β-estradiol during the same time periods. Twelve mice/group were killed at 14.5 and 18.5 days post-coitum, 24 hr after receiving the final dose. Embryos were dissected to obtain cerebrum, cerebellum, ovary, testis, and liver. RT-PCR analysis was used to measure mRNA levels of genes. Western immunoblotting was used to measure protein levels of CYP1A1 and glutathione-*S*-transferase in liver. Data were analyzed by ANOVA. **[It was not clear if the litter or offspring was considered the measurement or statistical unit.]**

Numerous changes in mRNA expression were observed following bisphenol A exposure, and they varied according to dose, sex, tissue, and exposure period. In at least one sex and time period, exposure to 17β-estradiol increased expression of mRNA arylhydrocarbon receptor in all tissues, arylhydrocarbon receptor repressor in testes and ovaries, arylhydrocarbon receptor nuclear translocator in brain or testes, *CYP1A1* in brain, and glutathione-*S*-transferase in brain. Changes in protein levels of CYP1A1 and glutathione-*S*-transferase in liver were also examined in embryos at 18.5 days post-coitum and levels of both proteins were increased with exposure to bisphenol A at doses ≥0.2 mg/kg bw/day and with exposure to 17β-estradiol. The study authors proposed a novel mechanism of toxicity involving upregulation of mRNA for arylhydrocarbon receptor and other factors by bisphenol A.

Strengths/Weaknesses: The wide dose range and the oral route are strengths. The study has value for understanding mechanisms of action although these changes were not tied to any adverse findings that might be related to these changes. Weaknesses include the lack of specification of the method of oral dosing and lack of clarity on sample origins and sizes for each assay. This is not considered a critical weakness because it is known that standard procedures for these methods require pooling of embryos within litter.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility for the evaluation because of the mechanistic nature of the endpoints.

Imanishi et al. (2003), supported by the Ministry of Education, Culture, Sports, Science, and Technology of Japan, used DNA microarrays to investigate potential mode of action of bisphenol A on alterations in expression of 20 nuclear hormone receptors and a few other genes in the mouse placenta. ICR male and female mice were housed in polycarbonate cages, given ad lib access to tap water and CM rodent feed (Oriental Yeast), and maintained under standard 12-hr/12-hr light/dark cycle. Between 6.5 and 17 days post-coitum, pregnant dams were orally administered 0 or 0.002 mg/kg bw/day bisphenol A **[purity not provided]** in olive oil **[method of oral administration not given]**. The dams were killed 18.5 days post-coitum, and placentas and fetuses were frozen at 80°C. Placental RNA from male and female embryos was separately extracted, reverse transcribed, and hybridized to a microarray chip for 18 hr at 42°C. Images were analyzed using Atlas navigator software, and statistical analyses were performed using the Pearson correlation coefficient, normalized to the Fisher *z* transformation. Differentially expressed genes were identified using paired *t*-test, and significant changes were noted in percent values increased or decreased relative to control mRNA expression values. **[The number of dams used and arrays run was not given. It was not clear if the litter or offspring were considered the statistical unit.]**

Nuclear receptor genes that showed differential expression in male and/or female fetuses were: neuron-derived orphan receptor 1, retinoic acid-related orphan receptor γ, estrogen receptor β, liver X receptor α, progesterone receptor, chicken ovalbumin upstream promoter transcription factor α, germ cell nuclear factor, steroidogenic factor 1, and photoreceptor-specific nuclear receptor. Nuclear receptor genes that did not show differential expression included thyroid hormone receptor β, peroxisome proliferators activated receptor α and γ, constitutive androstane receptor, farnesoid X receptor, chicken ovalbumin upstream promoter transcription factor β, testis receptor β, estrogen-related receptor γ, aryl hydrocarbon receptor, small heterodimer partner, and dosage-sensitive sex reversal receptor. Other genes the expression of which was both significantly altered in pair-wise comparison with control treatment and exhibited opposing up- or downregulation in a sex-dependent manner included fast skeletal troponin C, probasin, RNA-specific adenosine deaminase, and ADAM25/testase 2, α-fetoprotein, and kinesin light chain 1. These genes were downregulated in placentas of male fetuses and upregulated in placentas of female fetuses. Placentas of male and female fetuses exhibited downregulation if α-fetoprotein (↓60%, male and ↓24%, female) and kinesin light chain 1 (↓70%, male and ↓10%, female).

The authors conclude that fetal sex-based differences in placental physiology resulting from bisphenol A exposure may lead to subsequent sex-specific developmental perturbance. They also indicated that important but largely unknown effects of bisphenol A may occur with respect to a cluster of orphan nuclear receptors, which exhibited significant changes in gene expression.

Strengths/Weaknesses: Strengths: The evaluation of several molecular endpoints including gene activity for several receptors that are not commonly examined, oral dosing, and use of a low dose represent strengths. Weaknesses are the use of only one dose level of BPA and absence of many critical experimental details such as the number of litters used.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for inclusion due to lack of reporting key experimental details.

Yoshino et al. (2004), supported by the Japanese Ministry of Education, Science, Sports, and Culture and the Japan Private School Promotion Foundation, examined the effect of prenatal bisphenol A exposure on immune response in mice. **[No information was provided about feed or caging and bedding materials.]** DBA/1 J mice were fed bisphenol A **[purity not indicated]** at doses of 0 (ethanol/corn oil vehicle), 0.003, 0.030, 0.300, or 3 mg/kg bw/day for 18 days **[stated to be 17 days in the Methods section but 18 days in other parts of the report]**, beginning on the day of a 24-hr mating period (Day 0). Twelve mice/group were treated and 7–9/group became pregnant. **[The specific**

method of oral dosing was not described.] At 8 weeks of age (Day 77) 5 mice/group/sex were randomly selected and immunized by i.p. injection with hen egg lysozyme. Representation of litter was not specified. Blood was collected and spleens were removed 3 weeks following immunization (Day 98). Serum levels of hen egg lysozyme-specific immunoglobulin G (IgG), IgG1, and IgG2A were measured by ELISA. Spleen cell suspensions were prepared, and proliferation was assessed by incorporation of ^{3}H-thymidine following a 72-hr incubation with hen egg lysozyme. Spleen cell suspensions were also prepared for measurement of interferon-γ and interleukin-4 secretion by ELISA. An additional 6 mice/group/sex were killed at 8 weeks of age (Day 77). Spleens were removed and expression of $CD3^+CD8^+$ and $CD3^+CD4^+$ molecules on splenic lymphocytes was examined using monoclonal antibodies and flow cytometry. Thymus and spleen were fixed in 4% formaldehyde and examined histologically. Data were analyzed by Mann–Whitney *U*-test. **It was not clear if the litter of origin was accounted for in statistical analyses.**

Bisphenol A treatment had no significant effect on pregnancy rate, sex ratio, or body weight of offspring. There were several significant immune responses for male mice. **[Results in female mice were said to be similar to those observed in male mice but the data were not show by study authors.]** At bisphenol A doses ≥0.03 mg/kg bw/day, production of anti-hen egg lysozyme IgG2a following immunization was increased. Effects observed at ≥0.3 mg/kg bw/day included increases in production of anti-hen egg lysozyme IgG and secretion of interferon-γ and interleukin-4. Additional findings at the high-dose (3 mg/kg bw/day) were increases in spleen cell proliferation and production of anti-hen egg lysozyme IgG1 following immunization. Augmentation of interferon-γ and interleukin-4 secretion following incubation of spleen cells with hen egg lysozyme was examined in the high-dose group only and found to be increased. **[Increases in $CD3^+CD8^+$ and $CD3^+CD4^+$ expression on lymphocytes were reported in males and females exposed to bisphenol A, but the doses at which the effects occurred were not specified.]** No histopathological alterations were reported for the spleen or thymus. The study authors explained that effects on IgG2a and interferon-γ were indicators of T helper 1 immune responses and effects on IgG1 and interleukin-4 were indicators of T helper 2 responses. They concluded that the findings suggest that prenatal exposure to bisphenol A may upregulate immune responses in mice.

Strengths/Weaknesses: The oral route of administration and the wide range of doses are strengths. Weaknesses include small sample size ($n = 5$), lack of clarity regarding statistical handling of factors such as litter and sex effects.

Utility (Adequacy) of CERHR Evaluation Process: This study is inadequate for the evaluation process due to the reasons stated above.

Berger et al. (2007), supported by The Natural Sciences and Engineering Research Council of Canada, examined the effect of bisphenol A exposure on ovum implantation and pup survival in mice. CF-1 mice were housed in polypropylene cages and were fed Harlan Teklad 22/5 rodent feed, which was stated to contain soy. **[No information was provided about bedding materials.]** On GD 1–4 or 5 **[described as GD 1–5 in Methods section and GD 1–4 in study figures and tables]** (GD 0 = day of vaginal plug), 31 mice in the control group were s.c. injected with peanut oil vehicle and 5–15 mice/group were s.c. injected with bisphenol A (97% purity) at 0.0005, 0.0015, 0.0046, 0.0143, 0.0416, 0.125, 0.375, 1.125, 3.375, or 10.125 mg/animal/day. In a second experimental group, BPA was administered through a diet containing 3% or 6% BPA added to peanut butter and chow. In a third experimental group maintained on chow, BPA was administered at 0.11, 1.0, 3.0, or 9.0% in separate offerings of peanut butter alone. Pregnancy disruptions in orally exposed mice are discussed in Section 3.2.5.1. **[In the first experimental group, if it is assumed that the mice weighed 0.02 kg at the start of gestation (USEPA, 1988), CERHR estimated bisphenol A intakes of 0.025, 0.075, 0.23, 0.72, 2.1, 6.3, 19, 56, 170, and 500 mg/kg bw/day.]** Mice were allowed to litter. Pups were counted on the day of parturition and observed for survival for 5 days. Pups were weaned at 28 days after birth and at that time, body weight and sex ratio were determined. Data were analyzed by ANOVA, χ^2 test, and Newman–Keuls multiple comparisons. **[It was not clear if all offspring data were analyzed on a pup or litter basis.]** A study examining implantations in s.c.-treated females is discussed in Section 4.2.1.1 Percent of females giving birth was significantly decreased in the 10.125 mg/day group (~28% vs. 97% in control group). Numbers of pups born were significantly decreased in the 3.375 and 10.125 mg/day group (~8 and 2 pups in each of the dose groups and 13 pups in the control group). There were no treatment-related effects on pup weight or sex ratio at weaning. **[As discussed in Section 3.2.5.1, it appears that with oral exposure, pregnancy disruption occurred at higher bisphenol A levels (68.8 mg/day, 3440 mg/kg bw/day) than with s.c. exposure (10.125 mg/day, ~500 mg/kg bw/day)]**. The study authors concluded that the amount of bisphenol A required for pregnancy disruption was higher than typical environmental levels but that it is not known if bisphenol A could have additive or synergistic effects with other environmental estrogens.

Strengths/Weaknesses: A strength of the subcutaneous study is that it examined a wide range of bisphenol A dose levels. The comparison of the differential effects of s.c. and oral routes of bisphenol A administration is also a strength. Weaknesses include the limited/unequal number of mated mice in each dose group, absence of maternal data to ascertain the potential impact of maternal toxicity on pregnancy, methodological deficiencies regarding fertility assessment, and the use of a diet that contains phytoestrogens.

Utility (Adequacy) for CERHR Evaluation Process: Due to the limited number of mated mice per dose level ($n = 5$–15), methodological concerns, absence of key statistical information as well as maternal information, this study is inadequate for the CERHR evaluation process.

3.2.5.2 Studies with neurobehavioral endpoints:

Narita et al. (2006), supported by the Japanese Ministry of Health, Labor, and Welfare, and Ministry of Education, Culture, Sports, Science, and Technology, conducted a series of studies to examine the effects of bisphenol A on the dopaminergic system of mice exposed during development. Only brief details were provided about

the studies. In each study, ddY mice received feed containing bisphenol A from mating to weaning of their offspring. [**No information was provided on purity of bisphenol A, type of feed, caging and bedding materials, the number of dams treated, or the ages or sexes of offspring that were tested.**] Statistical analyses included ANOVA with Bonferroni/Dunnett test. [**It was not clear if the litter or offspring was considered the statistical unit.**] In a place conditioning-study, testing was conducted in 6–14 mice/group born to dams exposed to bisphenol A at 0, 0.03, 0.3, 3, 500, or 2000 mg/kg food. [**Assuming a female mouse eats ~0.2 kg feed/kg bw/day (USEPA, 1988), bisphenol A intake would have been 0.006, 0.06, 0.6, 100, or 400 mg/kg bw/day.**] During the preconditioning period, mice were placed in one section of a cage following injection with saline [**specific route not reported**] and in another section of the cage following s.c. injection with 1 mg/kg bw morphine. On the day of testing, the amount of time spent in each section of the cage was recorded. Mice from the lowest dose group (0.03 mg/kg food) and 2 highest dose groups (500 and 2000 mg/kg) food spent more time in the section of the cage associated with morphine injection. [**Compared to controls, the time spent in the morphine-associated section of the cage was ~9.5-, 7-, and 9-fold longer in each of the respective dose groups.**] Total locomotor activity was measured for 3 hr in 5–15 mice/group born to dams exposed to 0, 0.03, 3, or 2000 mg/kg food. Following s.c. injection with 10 mg/kg bw morphine, activity was increased in mice from the low- (0.03 mg/kg food) and high- (2000 mg/kg food) dose groups compared to the control group [**increased by ~9-fold in the low dose group and 12-fold in the high-dose group**]. Binding of ^{35}S-guanosine-5′[γ-thio]-triphosphate in the limbic system was measured in 3 samples/group obtained from offspring of dams exposed to 0.03, 3, or 2000 mg/kg food. Dopamine-induced binding of ^{35}S-guanosine-5′[γ-thio]-triphosphate in the limbic system was increased at each dose level compared to controls **[by ~32, 18, and 56%]**. Based on their findings, the study authors concluded that prenatal and neonatal exposures to low bisphenol A doses can potentiate central dopamine receptor-dependent neurotransmission in the mouse.

Strengths/Weaknesses: This study is so poorly written that it is extremely difficult to understand many sentences (let alone paragraphs) and to determine precisely what was done, why, and what happened. The main weakness of the study is therefore its inability to pass its message to the reader. Given this limitation, it is difficult to determine whether the study has any strengths, and if so what they might be.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process because of the lack of methodological details and the poor communication of the study results.

Kawai et al. (2003), supported by Core Research for Evolutional Science and Technology and Japan Science and Technology, examined the effects of prenatal bisphenol A exposure on aggressive behavior in male mice. **[No information was provided about feed, bedding, or caging materials.]** Pregnant CD-1 mice were randomly assigned to groups of 7 and orally dosed by micropipette with 0.002 or 0.020 mg/kg bw/day bisphenol A **[purity not reported]** on GD 11–17. A control group of 9 mice received the corn oil vehicle by micropipette during the same time period. Doses were said to be within the range of human exposures. Pups were weaned on PND 21 (day of birth = PND 0), and males randomly selected males from the same litter were housed in groups of 4 or 5. Aggression testing was conducted at 8, 12, and 16 weeks of age. For the testing, 15 control male mice from the 9 litters were randomly selected to be opponents and housed 5/cage. Opponents were used only once/day for testing. During testing of mice from the control and treated groups, the subject was housed alone for 5 min before placing the opponent mouse into the cage. Behavior with the opponent mouse was observed for 7 min. The numbers of mice evaluated were 26–32/group at 8 weeks of age, 18–24/group at 12 weeks of age, and 10–16/group at 16 weeks of age. MiceRandomly selected were killed at 9, 13, and 17 weeks of age, 1 week following behavior testing, for measurement of testis weight and serum testosterone level. **[The results section states that testis weights and serum testosterone levels were obtained at 8, 12, and 16 weeks of age.]** Eight mice/group were killed after the first 2 test periods and 10–16 mice/group were killed after the last test period. Mice that were not killed were tested at the next evaluation period, so that mice killed after 16 weeks of age were tested a total of 3 times. Statistical analyses included ANOVA and Spearman rank correlation test. **[It does not appear that the litter was considered the statistical unit.]**

Aggression scores, as determined by contact time, were increased significantly compared to the control group at 8 weeks of age in both the low- (124% increase) and high- (146% increase) dose bisphenol A groups. No treatment-related effects on aggression score were observed at 12 and 16 weeks of age. In the low-dose group, relative (to body weight) testis weight was 10% lower than controls at 8 weeks of age and 18% lower than controls at 12 weeks of age. Relative testis weight was 11% lower than control values in the high-dose group at 12 weeks of age. No significant effects were observed for serum testosterone levels. There were no correlations between serum testosterone levels and contact time in aggression testing. The study authors concluded that prenatal bisphenol A exposure of mice resulted in behavioral changes and decreased relative testis weight that was more pronounced at the lower dose.

Strengths/Weaknesses: Strengths are the use of 2 low dose levels and the oral route of administration. The lack of husbandry information, inappropriate presentation of testis weight data, variable degrees of repeated behavioral testing, and the apparent lack of consideration of possible litter effects are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to the reasons stated above.

Kawai et al. (2007), supported by Japan Sciences Technology and Core Research for Evolutional Science and Technology, evaluated the brain expression of *ER*α and *ER*β in male mice exposed in utero to bisphenol A. Pregnant ICR mice were fed bisphenol A in corn oil by micropipette on GD 11–17 at 0 or 0.002 mg/kg bw/Day 9, $n = 18$/group). Mice were housed singly in polypropylene cages. **[The first day of gestation was likely designated as GD 0, according to a figure. Type of feed and bedding material were not given.]** Litters were

reared by their dams until weaning on PND 21 **[birth = PND 0]**. Males from the same litters were housed 4 or 5/cage. Randomly selected males **[8–12/group, without mention of litter of origin]** were killed at 4–5, 8–9, or 12–13 weeks of age. Testosterone was measured by RIA in trunk blood serum. Brains were perfusion fixed and processed for immunostaining with antibody to ERα, ERβ, serotonin, and serotonin transporter. Fields were selected within the dorsal raphe nucleus and ERα- or ERβ-positive neurons were counted in every fourth section ($n = 8$ or 9 animals/group). Staining for serotonin and serotonin transporter involved overlapping dendrites, making it difficult to count positive neurons, and densitometric methods were used to quantify staining for serotonin and serotonin transporter ($n = 8$–12 animals/group). Data were analyzed using 2-way ANOVA and post-hoc Student *t*-test.

The number of neurons in the dorsal raphe nucleus expressing *ER*α and *ER*β was increased by bisphenol A at 5 and 13 weeks but not at 9 weeks. There were no significant differences at any time point in serum testosterone concentrations. The authors identified a ''tendency'' for serotonin and serotonin transporter immunoreactivity to be increased by bisphenol A in the dorsal raphe nucleus, but there were no statistical differences between bisphenol A-treated and control brains at any time point. The authors concluded that it is possible that alterations in ER in the brain may be responsible for emotional and behavioral alterations in mice.

Strengths/Weaknesses: This was a reasonable attempt to detect effects and explore a connection between bisphenol A, brain receptors, and aggressive behavior. This study is weakened by the use of only one dose, lack of experimental details, and uncertain accounting for litter and repeated measures/sections effects in analyses.

Utility (Adequacy) for CERHR Evaluation Process: This study is deemed inadequate for inclusion due to unclear statistical procedures regarding litter and nested factors associated with repeated measurements.

Laviola et al. (2005), supported by Italian Ministry of Health, Ministry of Universities and Research, and the University of Parma, examined the effect of prenatal bisphenol A exposure on *d*-amphetamine-reinforcing effects in mice. **[No information was provided about feed, housing, or bedding composition.]** CD-1 mice were trained to drink the tocopherol-purified corn oil vehicle through a syringe. The mice were randomly assigned to groups, and 10–12/group were exposed to bisphenol A **[purity not reported]** at 0 (vehicle) or 0.010 mg/kg bw by feeding from a syringe on GD 11–18 **[day of vaginal plug not defined]**. Another group of mice was exposed to methoxychlor; those findings will not be discussed. Litters were culled to 10 pups (5 ± 1 of each sex) within 12 hr of parturition. Offspring were weaned and group housed with littermates of the same sex on PND 25. At 60 days of age, 3 offspring/sex/litter (1 sex/litter at each *d*-amphetamine dose) were subjected to conditioned place-preference testing. For the test, animals were acclimated to the apparatus on the first day of testing. On alternate days over a 4-day period, animals were i.p. injected with 0, 1, or 2 mg/kg bw *d*-amphetamine and confined to one compartment of the apparatus for 20 min. On the other days of the 4-day period, animals were injected with saline and confined in another section of the apparatus for 20 min. On the fifth day of testing, animals were not treated and were given free access to the entire apparatus for 10 min. The amount of time spent in the compartment associated with *d*-amphetamine treatment was measured. Data were analyzed by a split-plot ANOVA, in which the litter was considered the block variable, and Tukey HSD test. Prenatal treatment was described as a between litters factor and all other variables were described as within litter factors.

No differences were reported for birth weight and sex ratio at birth. **[Data were not shown by authors.]** There were no significant effects of bisphenol A treatment on locomotor activity. Conditioned place-preference occurred in control females following injection with either *d*-amphetamine dose, but was not observed in females treated with bisphenol A. In males, both the vehicle control and the bisphenol A group displayed a preference for the *d*-amphetamine-associated compartment following treatment with the high *d*-amphetamine dose. Therefore, there was no change in preference following bisphenol A treatment of males. The study authors concluded that prenatal bisphenol A exposure affected organization of the brain dopaminergic system in female mice leading to long-term alterations in neurobehavioral function.

Strengths/Weaknesses: Strengths of this study include robust and appropriate design and analysis, adequate sample size, and oral dosing. The use of only one dose level is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility in the evaluation.

3.2.6 Mouse—parenteral exposure only during pregnancy. **Markey et al. (2001a)**, supported by NIH, the Massachusetts Department of Health, the International Union Against Cancer, and the World Bank, examined the effect of prenatal bisphenol A exposure on mammary gland development in mice. CD-1 mice were fed RMH 3000 rodent diet, which showed negligible activity in estrogenicity testing. Caging and bedding were also reported to test negative in estrogenicity assays. Dams (6–10/group) were estimated to have received the DMSO vehicle or bisphenol A **[purity not reported in the manuscript; 97 ± 2% per A. Soto, personal communication, March 2, 2007]** at 0.000025 or 0.000250 mg/kg bw/day through a s.c. pump from GD 9–20 (GD 1 = day of vaginal plug). **[The original publication stated that bisphenol A doses were 25 and 250 μg/kg bw/day, but units were corrected to ng/kg bw/day in an addendum released for the study].** Doses were not adjusted for increasing body weight as dams gained weight during pregnancy. Dams were allowed to litter and offspring were weaned at 19 days of age. At 10 days, 1 month, and 6 months of age, 6–10 female offspring/group were killed during each time period. **[Number of litters represented was not stated but there may have been 1 offspring/litter based on the numbers examined.]** Vaginal smears were assessed in mice following puberty, and post-pubertal mice were killed during proestrus. Before being killed, females were injected with bromodeoxyuridine, and incorporation of bromodeoxyuridine in mammary glands was determined by an immunohistochemistry method. Histological and morphometric analyses of mammary

glands were also conducted. Data were analyzed by ANOVA, least significant difference test, and *t*-test. [The statistical analyses considered litter differences, method unstated.]

At 1 month of age, the rate of ductal migration into the stroma was increased in the low-dose group and decreased in the high-dose group; values in the 2 treatment groups were significantly different from one another but neither dose group was significantly different from the control group. Bisphenol A treatment increased percentages of ducts and buds at 6 months of age. Bromodeoxyuridine incorporation was decreased in epithelial cells at both doses at 10 days of age, decreased in stromal cells at the high-dose at 1 month of age, and increased in stromal cells at both dose levels at 6 months of age. At 1 month of age, the ratio of bromodeoxyuridine-positive epithelial to stromal cells was 4:1 in the control group, 2:1 in the 0.000025 mg/kg bw/day group, and 6:1 in the 0.000250 mg/kg/bw/day group. The percentage of alveoli containing secretory products was increased at the low dose at 6 months of age. The study authors concluded gestational exposure to low doses of bisphenol A alters timing of DNA synthesis in mammary epithelium and stroma, resulting in a histoarchitecture that is not typical for a virgin mouse.

Strengths/Weaknesses: The examination of the mammary gland, a system not often studied, is a strength. A critical weakness is the uncertainty of the DMSO concentration as a vehicle and therefore pump performance. An additional weakness is that the proliferative changes reported in mammary tissues in virgin mice have not been satisfactorily established as precursors of breast cancer.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process given exposure uncertainties.

Markey et al. (2003), supported by NIH and the Massachusetts Department of Public Health, examined the effects of prenatal bisphenol A exposure on development of the female reproductive system and mammary gland in mice. CD-1 mice were fed Purina Rodent Chow that tested as having negligible estrogenicity. Cages and bedding tested negative for estrogenicity in the E-SCREEN assay. Water was provided in glass bottles. Mice ($n = 6$–10/group) were administered bisphenol A **[purity not indicated in the manuscript; 97 ± 2% per A. Soto, personal communication, March 2, 2007]** at 0 (DMSO vehicle), 0.000025, or 0.000250 mg/kg bw/day by s.c. pump from GD 9 through the remainder of pregnancy (GD 1 = day of vaginal plug). **[The dose levels were incorrect in the original and were corrected by an erratum (Markey et al., 2004).]** Number of offspring, sex ratio, body weight, and age at vaginal opening were assessed. Beginning at 3 months of age and continuing for 2 weeks, estrous cyclicity was assessed by visual examination of the external vagina and confirmation by vaginal smears. Female offspring (6–10/group) were killed at 1, 3, 4, 6, 9, and 12 months of age on the afternoon of proestrus. Reproductive organs were grossly assessed, and morphometric measurements were obtained for ovary and mammary gland. **[Although the methods section suggests that morphometric measurements were obtained at each time period of sacrifice, it does not appear that the measurements were taken at 12 months of age. The 1-month data were reported in a previous publication (Markey et al., 2001a).]** A histopathological evaluation of the ovary was conducted at 3 months of age. Reproductive organ weights were obtained at 1, 3, and 6 months of age. **[As in other studies reported from this laboratory, different litters were represented at each time period (A. Soto, personal communication March 2, 2007).]** Statistical analyses included ANOVA, Kruskal–Wallis, and Mann–Whitney tests. **[It was not clear if the litter or offspring was considered the statistical unit.]**

Bisphenol A exposure had no significant effect on litter size or sex ratio. A significant interaction between age for body weight and treatment was reported from 2–12 months of age but the effect on body weight was not explained. No significant effects were observed for vaginal opening in treated mice. Significant increases were observed in percentages of 3-month-old mice with estrus/metestrus for ≥4 or 8 days. At 6 months of age, the incidence of fluid-filled ovarian bursae was increased in both treatment groups. Reproductive organ weights were not affected at 1 or 6 months of age, but at 3 months of age, absolute and relative (to body weight) weights of vagina were decreased in the high-dose group. The percentage of ovary tissue consisting of antral follicles was increased in the high-dose group at 3 months of age. No significant differences were observed for mammary structures at 4 months of age. At 6 months of age, the percentage of alveolar buds/lobulo-alveoli was increased in both dose groups compared to the control group. The percentage of alveolar buds/lobulo-alveoli was decreased in the low-dose group compared to control group at 9 months of age. The study authors concluded that exposure of mice to environmentally relevant doses of bisphenol A during the development of estrogen-sensitive tissues results in effects that are manifested in adulthood.

Strengths/Weaknesses: The examination of the mammary gland, a system not often studied, is a strength. A critical weakness is the uncertainty of the DMSO concentration as a vehicle and therefore pump performance.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process given exposure uncertainties.

Vandenberg et al. (2007), supported by NIEHS and Tufts, examined the effects of prenatal bisphenol A exposure on mouse mammary gland development. CD-1 mice were fed Harlan Teklad 2008, which was reported to contain 20 fmol/g estrogen equivalents. The type of caging and bedding used was not reported but they were stated to test negative for estrogenicity in the E-SCREEN. Water was supplied in glass bottles. On GD 8 (GD 1 = day of vaginal plug) mice were implanted **[subcutaneous (A. Soto, personal communication, March 2, 2007)]** with osmotic pumps that delivered the 50% DMSO vehicle or bisphenol A **[purity not reported in manuscript; 97 ± 2% per A. Soto, personal communication, March 2, 2007]** at 0.000250 mg kg bw/day. The bisphenol A dose was selected because it was predicted (or estimated) to be environmentally relevant and shown to alter mammary endpoints (Markey et al., 2001a; Muñoz-de-Toro et al., 2005). Pumps were left in place until dams were killed on GD 18. **[The number of dams treated was not reported in the paper. The Expert Panel**

has been informed that there were 20–30/group (A. Soto, personal communication, March 2, 2007).] Fetal mammary glands were mounted whole or sectioned to examine mammary gland development in 36–40 offspring/group. Immunohistochemistry techniques were used to measure expression of *Ki67* and *Bax* in mammary structures from 4–8 offspring/group. Mammary collagen localization was assessed using Masson Trichrome stain in 6–17 mice/group. Expression of mRNA for *ER*α, *ER*β, adipocyte lipid binding protein, *Col-I*, and *PPAR*γ were measured by RT-PCR in mammary glands from 4–6 offspring/group. Litter was accounted for in design and analyses by assigning 1 individual/litter to each group or endpoint. Statistical analyses included *t*-tests, ANOVA, Mann–Whitney *U* non-parametric tests, and χ^2 tests.

Morphometric analysis revealed significantly higher ductal area and extension in the bisphenol A group than in controls. In the control group, females positioned next to two females in utero had significantly fewer branching points than females positioned next to 1 or 2 males; this difference was not observed in the bisphenol A group. In fetuses that were not positioned next to a male, significantly more branching points were observed in the bisphenol A than in the control group. Control females positioned next to 2 males had significantly larger epithelial duct area than control females not positioned next to a male; this difference was not observed in the bisphenol A group. In bisphenol A-treated females positioned next to 1 male, ductal extension was significantly greater than in control females positioned next to 1 male.

In the bisphenol A group, epithelial cells were less rounded, more evenly spaced, and more dense than in controls. Bisphenol A did not significantly affect Ki67 (a proliferation marker) expression in mammary epithelium. Lumen formation was observed in 6 of 16 control mice and 0 of 10 bisphenol A-exposed mice. Significantly decreased numbers of Bax-positive (apoptotic) cells were observed in the inner epithelial cord (not in contact with basement membrane) of bisphenol A-exposed than control mice. Optical density of histological staining was significantly lower in the fat pad of the bisphenol A-exposed than control group. Fat pads of the bisphenol A group compared to control group were found to be significantly less cellular, contain more Bax-positive cells, and have more vacuoles at a distance <1 mm from the epithelial compartment. Study authors interpreted the effect as increased epithelial penetration and advanced maturation of fat pads. No significant differences were observed for PPARγ or adipocyte lipid binding protein mRNA expression. Density of collagen deposits was lower in the entire mammary gland but higher in the periductal stroma (within 10 μM of the epithelium) of the bisphenol A than the control group. Bisphenol A exposure did not affect collagen type I, *ER*α, or *ER*β mRNA expression. ERα protein expression in the stroma was also unaffected by bisphenol A exposure. Study authors concluded that advanced maturation of fat pad and changes in extracellular matrix may be the cause of altered growth, cell size, and lumen formation in mammary epithelium of mouse fetuses exposed to bisphenol A.

Strengths/Weaknesses: Strengths of this study are the rigor with which the measurements were made, and the fact that the authors were trying to quantify endpoints that are difficult to measure (e.g., the relationship of the stroma to the epithelium). The relevance of the endpoints is a strength as is the low dose used. The single dose and subcutaneous route of administration are weaknesses. A critical weakness is inappropriate statistical analysis of a complex study design that may have produced too many positive findings and a lack of statistical accounting for litter effects (i.e.,. 36–40 pups presented in Table 1 of study and only 20–30 litters treated).

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process because of insufficient control for litter effects.

Honma et al. (2002), supported by the Japanese Ministry of Education, Culture, Sports, Sciences, and

Table 79
Effects in Mice Exposed to Bisphenol A During Prenatal Development[a]

	Dose (mg/kg bw/day)					
Endpoint	0.002	0.020	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Female body weight						
Weaning	↓ 10%	↓ 7%	0.065	0.017	0.088	0.021
PND 60	↔	↓ 4%	0.054	0.021	0.11	0.021
Male body weight						
Birth	↔	↓ 5%	0.054	0.020	0.031	0.015
PND 60	↔	↓ 6%	0.048	0.020	0.044	0.020
Anogenital distance						
Females at weaning	↑ 6%	↔				
Males on PND 60	↑ 6%	↑ 8%	0.035	0.020	0.035	0.020
Age at vaginal opening[b]	↔	↓ 1.3 days				
Body weight at vaginal opening[b]	↓ 10%	↓ 11%				
Age at 1st estrus[b]	↔	↓ 1 day				
Estrous cycle length	↑ 1.3 day	↑ 1 day	0.021	0.007	0.12	0.021
Cornified cells in vaginal smear	↑ 3.1 days	↔	0.17	0.020	0.44	0.021
Lymphocytes in vaginal smear	↓ 2.2 days	↔	0.26	0.020	0.26	0.020

[a]Honma et al. (2002).
[b]Value estimated from a graph by CERHR; data from graphs were not modeled.
↑,↓ Statistically significant increase, decrease; ↔ no significant effect.

Technology, examined the effect of prenatal bisphenol A exposure on the reproductive system of female mice. Mice were fed commercial diet (CE-2, CLEA, Tokyo, Japan). **[No information was provided about bedding or caging materials.]** Ten ICR/Jcl mice/group were s.c. injected with bisphenol A **[purity not reported]** in sesame oil at 0, 0.002, or 0.020 mg/kg bw/day on GD 11–17 (GD 0 = vaginal plug). Additional mice were injected with diethylstilbestrol at 0.02–2 μg/kg bw/day. Pups were sexed, counted, and weighed at birth. At 22 days of age, offspring were weaned and litter sizes were adjusted to 8 pups. Male and female offspring were weighed during the postnatal period. Anogenital distance was measured in males and females at 22 and 60 days of age. Females were monitored for vaginal opening. Vaginal smears were obtained for 30 days following vaginal opening. Female offspring were mated with untreated males from 90 to 120 days of age. F_2 pups were counted and sexed at birth. The litter was considered the experimental until in statistical analyses. Data were analyzed by ANOVA and Student or Welch *t*-test.

Statistically significant findings are summarized in Table 79. There were no effects on gestation duration, number of pups/litter, or sex ratio. Body weights were slightly lower in high-dose males at birth, both dose groups of females at weaning, and high-dose males and females at 60 days of age. Anogenital distance was increased in low-dose females at weaning and both dose groups of males at 60 days of age. Age of vaginal opening and first estrus was accelerated in the high-dose group, and body weight at vaginal opening was lower in both dose groups. Estrous cycle length was increased in both dose groups. Total days that cornified cells were present in vaginal smears was increased and total days that lymphocytes were detected was decreased in the low-dose group. In F_1 offspring there were no significant effects on mating, number of F_2 pups/litter, or sex ratio of F_2 pups. Results in mice dosed with diethylstilbestrol were similar to those observed in mice dosed with bisphenol A. The study authors concluded that prenatal exposure to low doses of bisphenol A results in early vaginal opening in mice but did not affect female reproductive function.

Strengths/Weaknesses: Strengths are that this study represents one of the few studies that appropriately examines the onset of puberty in the mouse as an endpoint, it uses low dose levels of bisphenol A, relatively large sample sizes, and effectively uses a positive control at 3 dose levels. The lack of AGD measurement at birth and difficulty of measurement at PND 60 are weaknesses. he Expert Panel was unable to confirm the statistical significance of the effects shown in Table 2 of the manuscript.

Utility (Adequacy) for CERHR Evaluation Process: The study is adequate for inclusion but of limited utility due to statistical questions about body weight and AGD and subcutaneous route of exposure.

Iwasaki and Totsukawa (2003), support not indicated, examined the effect of prenatal bisphenol A exposure on reproductive development of female mice. ICR mice were fed F1 diet (Funabashi, Chiba, Japan) and housed in polycarbonate cages containing an unspecified chip bedding. On GD 7–18 (GD 0 = day of copulatory plug), 6 dams/group received bisphenol A **[purity not reported]** at 0 (DMSO vehicle) 0.00025, 0.025, or 2.5 mg/kg bw/day by s.c. injection. A positive control group of mice received 100 μg/kg bw/day 17β-estradiol **[route not specified]**. Dams were weighed during the study. Pups were counted and sexed on PND 0, and pup viability was determined on PND 4. Pups were weaned on PND 21, and male pups were killed and discarded. Female pups (24–41/group) were observed for vaginal opening. On PND 21, 1 pup/litter(4/group) from the low- and mid-dose group was injected with 3 μg/kg bw/day 17β-estradiol for 2 days and then killed. Uterine weights were assessed and expression of the *ERα* gene in uterus was determined using a colorimetric method. Statistical analyses included ANOVA, ANOVA on ranks (Kruskall–Wallis test), and Dunnett test. **[It was not clear if the litter or offspring was considered the statistical unit.]**

Weight gain was described as increased in all treated dams compared to control dams, but there was no evidence of a dose–response relationship and statistical significance was not achieved. Pup birth weight was significantly lower **[6%]** in the low-dose group compared to the control group. There were no differences in litter size at birth. Pup viability on PND 4 was significantly reduced **[by 26%]** in the low-dose group. Age of vaginal opening was significantly delayed by 3 days in the low-dose group, but significantly accelerated by 2.2 days in the high-dose group. Following 17β-estradiol exposure, uterine weight was significantly decreased **[by ~85%]** in the low-dose bisphenol A group and significantly increased **[by ~29%]** in the mid-dose bisphenol A group. Although expression of *ERα* mRNA was observed at 132% of control levels in the mid-dose bisphenol A group following exposure to 17β-estradiol, the effect did not attain statistical significance. Expression of *ERα* gene was not detectable in the low-dose bisphenol A group following 17β-estradiol exposure. No significant effects were reported in mice treated with 17β-estradiol. The study authors concluded that "The levels tested in this study appear to be dangerous."

Strengths/Weaknesses: The use of 3 dose levels, including low doses, and the use of 17β-estradiol as a positive control are strengths of this study. Weaknesses include the use of DMSO as a vehicle, the subcutaneous route of administration, the small sample size, lack of significant effects detected in the 17β-estradiol positive control group, and the failure to account for litter in statistical analyses.

Utility (Adequacy) for CERHR Evaluation Process: The study is inadequate for the evaluation process.

Nakamura et al. (2006), supported by grants from the Japanese government, examined the effects of prenatal exposure to bisphenol A on the morphology and expression of certain genes related to brain development in the mouse neocortex. In the first experiment ICR/Jc1 mouse dams were injected subcutaneous with either 0 (sesame oil vehicle) or 20 μ/kg bw/day bisphenol A **[purity not indicated]** daily from GD 0 (defined as the day that a vaginal plug was detected) until GD 10.5, GD 12.5, GD 14.5 or GD 16.5. **[No information was provided on feed, caging materials, bedding.]** Dams were then given a single i.p. injection of 5-bromo-2′-deoxyuridine (BrdU). Fetuses were collected either 1 hr following BrdU treatment (to assess precursor cell proliferation) or 2 or 3 days following BrdU treatment (to assess neuronal migration and differentiation). Brains were fixed in 4%

buffered paraformaldehyde for morphometry and immunohistochemical evaluation. The sections of the neocortex were sectioned into three zones: ventricular zone, intermediate zone, and cortical plate (the neocortex at GD 12.5 was divided into the ventricular zone and the primordial plexiform layer). Ten fetuses from two or more dams were collected at each time point. In the second study, ICR/Jc1 dams were treated as described above and fetal telencephalons were collected on GD 12.5, GD 14.5, or GD 16.5 and frozen in liquid nitrogen and stored at −80°C for mRNA expression analyses ($n = 10$–15 fetuses in each group).

There were no significant differences in the pattern of immunoreactivity for K1-67 (a marker for cell proliferation), nestin (a marker for neural progenitors), Musashi (another marker for neural progenitors), and histone H_3. However, a marker for young neurons, Tuj1, was more prominent in the intermediate zone at GD 14.5 and GD 16.5 in the bisphenol A group. The authors also looked at the immunoreactivity pattern for PDI, a microsomal enzyme that contains binding sites for T3 and estradiol. PDI is believed to act as a buffer for these hormones in cells. PDI is of interest because bisphenol A has been reported to bind to the T_3 binding sites of PDI with 10 to 100-fold lower affinity than T_3 (Hiroi, 2006) and inhibit the binding of T_3 to PDI when bound. PDI immunoreactivity was increased in the neocortex of bisphenol A treated fetuses from GD 12.5–GD 16.5 and in subplate cells at GD 14.5.

There were no differences in BrdU labeled cells in any neocortical zone from brains collected 1 hr following BrdU treatment. However, the BrdU-labeled cells analyzed 2 days following BrdU injection were decreased in the ventricular zone of BPA-treated mice at GD 14.5 (labeled at GD 12.5) and GD 16.5 (labeled at 14.5) and increased in the cortical plate at GD 14.5 (labeled at GD 12.5). The authors used quantitative RT-PCR to examine the expression of several genes involved in brain development including those that help regulate the maintenance of neural stem cells and promote gliogenesis (*Hes1* and *Hes5*), promote neurogenesis (*Mash1*, *Math3*, and *Ngn2*), and relate to thyroid hormone action (*L1CAM*, *THR*-α, and *THR*-β). The gene expression of *Math3*, *Ngn2*, *Hes1*, *LICAM*, and *THR*-α were upregulated significantly in the bisphenol A-treated group at GD 14.5 (*Hes1* and *Hes5* were downregulated significantly at GD 12.5). Overall, the authors interpreted these findings as suggesting that bisphenol A might disrupt normal neocortical development by accelerating neuronal differentiation and migration.

Strengths/Weaknesses: The strengths of this study are that a reasonable sample size (10) for this type of study was used although the presumed dam effect was only partly controlled for by choosing 10 pups from two different dams. The study used a low dose (20 ug/kg) delivered s.c. to a pregnant mouse. The results revealed an effect on neocortical development in developing fetuses. Neurogenesis and gene expression were affected by BPA.

Utility (Adequacy) for CERHR Evaluation Process: This is an adequate study for evaluation purposes but of limited utility because dam effects were only partly controlled for and because of the subcutaneous route of administration.

Nikaido et al. (2004), supported by the Japanese Ministry of Health, Labor, and Welfare examined the effects of bisphenol A exposure on mammary glands and reproductive systems of mice. Outbred CD-1 (ICR) mice were fed NIH-07 (a low-phytoestrogen diet) and provided with water supplied in polycarbonate bottles with rubber stoppers. The mice were housed in polyisopentene cages with white pine chip bedding. Beginning on GD 15 (plug day not specified), mice were s.c. injected with 0 (DMSO vehicle), 0.5, or 10 mg/kg bw/day bisphenol A (≥99% purity) or 0.5 or10 μg/kg bw/day diethylstilbestrol for 4 days. **[The control group contained 6 dams/group, but the number of dams in treated groups was not clear.]** Additional groups of mice were treated with the same doses of genistein, resveratrol, or zearalenone. Female pups were weaned at 21 days of age. Onset of vaginal opening was monitored. Estrous cyclicity was monitored in 12 mice/group at 9–11 weeks of age. At 4, 8, 12, and 16 weeks of age, 6 randomly selected mice/group were weighed and killed. Ovaries, uterus, vagina, and mammary glands were preserved in 10% formalin for histopathological evaluation. Differentiation of mammary structures was evaluated in whole mounts. Statistical analyses included homogeneity of variance tests followed by ANOVA or Kruskal–Wallis test. When P-values were <0.05, Fisher protected least significant difference test was conducted. **[It appears that offspring were considered the statistical unit.]**

Body weight gain of offspring was increased by bisphenol A treatment, and at 16 weeks of age, body weight compared to controls was higher **[by ∼50%]** in the low-dose group and **[by ∼23%]** in the high-dose group. Vaginal opening was accelerated by 1.2 days at the high-dose group. Estrous cycle length was increased by 2.8 days in the low-dose group and 3 days in the high-dose group as a result of increased time spent in diestrus. Corpora lutea were observed in all control mice at each age. No corpora lutea were observed in 2 of 6 mice of the low-dose group and 3 of 6 mice of the high-dose group at 4 weeks of age, but all mice had corpora lutea at 4, 8, 12, and 16 weeks of age. With the exception of vaginal cornification observed in mice lacking corpora lutea, no histopathological abnormalities were observed in the uterus or vagina. Two of three mice with corpora lutea in the high-dose bisphenol group had greater mammary alveolar differentiation compared to control mice at 4 weeks of age. No differences in mammary differentiation were observed at later ages. The study authors concluded that both the high and low dose of bisphenol A produced transient changes in the mammary gland and reproductive tracts of mice. Transient effects on the reproductive tract and mammary gland were also observed with genistein and diethylstilbestrol, whereas prolonged effects were induced by zearalenone.

Strengths/Weaknesses: The lack of clarity regarding sample size and the weak description of the histopathology findings are weaknesses, as are the use of DMSO as a vehicle, the subcutaneous route of administration, and statistical concerns.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Park et al. (2005b), support not indicated, treated ICR mice during pregnancy. Bisphenol A **[purity not indicated]** in corn oil was given i.p. at dose levels of 0, 0.05, 0.5, or 5 mg/kg bw on the day of mating and every 3 days for a total of six doses ($n = 12$/group). Dams were

killed on GD 18 (plug = GD 0) for determination of litter size, fetal weight, and sex ratio. The uterus and right ovary were removed from each dam, fixed in Bouin fluid, and sections were stained with hematoxylin and eosin for light microscopy. Results were analyzed with least significant difference test **[apparently on a per fetus basis]**.

Maternal weight was not altered by treatment. Fetal body weight was decreased in the high-dose group by 14% for males and 12% for females. There was no effect on litter size or sex ratio. There was no treatment effect on dam uterine or ovarian weight. Histopathology of the dam ovary was reportedly not affected by treatment. Histopathology of the dam uterus showed thickening of the endometrium in the 0.05 and 0.5 mg/kg bw groups and uterine muscle damage in the 5 mg/kg bw group. **[The damage is not otherwise described. The photomicrographs available in the report were not interpretable due to poor reproduction quality.]** The authors concluded that bisphenol A at low doses does not produce reproductive toxicity in mice. **[This study was written in Korean with an English abstract and tables. A translation was provided to CERHR by the American Plastics Council.]**

Strengths/Weaknesses: The use of 3 dose levels is a strength. The lack of information on husbandry conditions, the i.p. dose route, failure to account for litter effects in statistical analyses, and the poor presentation of histopathology results are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Park et al. (2005a), support not indicated, treated ICR mice during pregnancy. Bisphenol A **[purity not indicated]** in corn oil was given i.p. at dose levels of 0, 0.05, 0.5, or 5 mg/kg bw on the day of mating, and every 3 days for a total of 6 doses ($n = 3$–6/group). Offspring were evaluated on PND 45 for body weight, reproductive organ weight and histopathology, semen analysis, complete blood count, and serum chemistry. **[There were 24 female and male offspring evaluated per dose group (not indicated whether 12 of each sex). Litter of origin appears not to have been considered. No information was provided on standardization of litters, diet, or cage/bedding materials.]** Statistical analysis was performed using the least significant difference test. **[It was not clear if the litter or offspring was considered the statistical unit.]**

There was a statistically significant 6% decrease in male body weight in the high-dose group; a comparable body weight decrement in female offspring was not statistically significant. There were no statistically significant treatment effects on the weights of the testis, epididymis, seminal vesicles, coagulating glands, uterus, or ovary. Sperm concentration, viability, motility, and morphology were not affected by treatment. Blood endpoints were not affected by treatment except for a statistically significant 6% increase in erythrocyte count in male offspring and a 2% decrease in serum albumin in female offspring. An 11% increase in blood urea nitrogen in mid-dose female offspring was not dose-related. Histopathology of the testis and ovaries was described as unaffected by treatment. Uterine intimal proliferation was described in the mid- and high-dose female offspring. **[The histological methods were not described. The photomicrographs available in the report were not interpretable due to poor reproduction quality.]** The authors concluded that bisphenol A at low doses does not produce reproductive toxicity in mice. **[This study was written in Korean with an English abstract and tables. A translation was provided to CERHR by the American Plastics Council.]**

Strengths/Weaknesses: The inadequate description of methods, unacceptable small sample size, the i.p. dosing, inappropriate statistical analyses, and the poor presentation of histology results are weaknesses of this study.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to the reasons stated above.

Sato et al. (2001), support not indicated, investigated the effects in mice of in utero exposure to bisphenol A on fetal growth, offspring reproductive and brain development, and behavior. Pregnant Jcl-ICR mice ($n = 20$) were given s.c. injections of bisphenol A **[purity not indicated]** 100 mg/kg bw/day, ethinyl estradiol 0.2 or 0.02 mg/kg bw/day, or olive oil vehicle on GD 11–19 **[Plug day was not defined. Information regarding caging material, animals per cage, feed, culling, and weaning was not provided.]** Pups were evaluated for onset of pivoting, righting, straight line walking, and grasp reflex. Open field testing was conducted at 40 days of age. Offspring were killed at 40 or 60 days of age and organs were weighed and processed for histology using hematoxylin and eosin **[fixation method not given]**. Brain myelin was evaluated using Klüver-Barrera staining. Statistical analyses were performed using the Student *t*-test. **[The pup appears to have been used as the statistical unit.]**

There were 11/93 stillborn fetuses after in utero exposure to bisphenol A, but no data were provided for the control group. There were no significant effects of bisphenol A treatment on litter size or offspring body weight at birth, 20, or 60 days of age. There were no significant effects of bisphenol A treatment on days at acquisition of pivoting, righting, straight-line walking, or grasp reflexes. In open field testing, mice in the bisphenol A-treated group showed significantly less defecation than controls **[39% less]**. There was no statistically significant difference between groups in grooming, rearing, line-crossing of inner and outer fields, or latency to first line crossing. At 60 days of age, seminiferous tubules from bisphenol A-exposed male offspring had a significant reduction in mean diameter **[↓16.6%]** and cell layer thickness **[↓25%]** compared to controls. There was no significant bisphenol A effect on brain myelination at 60 days of age or in mean diameter at 40 and 60 days of age of the tractus mamillothalamics. The authors suggest that in utero exposure to 100 mg/kg bw/day bisphenol A induces alterations in behavior similar to that seen at reduced plasma corticosterone levels and that bisphenol A exposure induces gross and cellular changes in seminiferous tubules, suggesting potential perturbation in hormone pathways involved in development.

Strengths/Weaknesses: The use of multiple doses of estrogen as a positive control is a strength. Weaknesses include the evaluation of a single dose of BPA, subcutaneous dosing, and lack of details regarding husbandry. Behavioral methods were chosen from less sophisticated screening approaches and data were not analyzed appropriately using the litter as the statistical

unit. Further, there is no description of sex ratios in groups given behavioral testing, despite established sex differences in endpoints measured in the open field evaluation. As a result, behavioral findings are unreliable.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for inclusion in the evaluation process due to the reasons stated above.

Rubin et al. (2006), supported by NIEHS, examined sexual differentiation in mice perinatally exposed to bisphenol A. Animals were fed rodent diet 2018 (Harlan Teklad, St. Louis, MO), which was reported to have negligible for estrogenicity (20 fmol 17β-estradiol equivalents/g). Caging and bedding materials were not indicated but were reported to have negligible estrogenic activity in the E-SCREEN assay. Water was supplied in glass bottles. On GD 8 (GD 1 = day of vaginal plug) through Day 16 of lactation, CD-1 mice were s.c. dosed by osmotic pump with the 50% DMSO vehicle or bisphenol A **[purity not reported]** at 0.000025 or 0.000250 mg/kg bw/day. **[The numbers of dams exposed was not indicated.]** Litters were culled to 8 pups (4/sex) on the day following birth. Litters were weaned on PND 22–24 (day of birth not defined). Anatomical examination and assessment of tyrosine hydroxylase neurons in the anteroventral periventricular preoptic area by an immunohistochemistry technique were conducted before puberty (PND 22–24) in 7 or 8 offspring/sex/group (2/sex/litter). Open-field testing was conducted in 14–17 offspring/group (1 offspring/sex/litter) at 6–9 weeks of age. The study authors expressed concern about possible hormonal effects because their historical records indicated that regular estrous cycles are not observed in group-housed females at 6–9 weeks of age. Therefore, open-field testing was repeated in 27–29-day-old offspring ($n = 10$–12/sex/group) exposed to 0 or 0.000250 mg/kg bw/day bisphenol A. Statistical analyses included 2-way ANOVA, *t*-test, and ANOVA with Bonferroni post-hoc test.

In control offspring, the total number of tissue sections through the anteroventral periventricular preoptic area was greater in females than males, but the sexually dimorphic difference was not observed in either treatment group. The number of sections through the anteroventral periventricular preoptic area was significantly lower in females from the high-dose bisphenol A than control group. In the control offspring, the number of tyrosine hydroxylase-positive neurons in the anteroventral periventricular preoptic area was higher in females and in males but this sexually dimorphic difference was not observed in the high-dose group. The number of tyrosine hydroxylase-positive neurons in the anteroventral periventricular preoptic area was lower in females in the high-dose bisphenol A than control group. The results for tyrosine hydroxylase-positive neurons were based on counting of all sections. When counting was limited to 7 sections or 4mid-sections, the sexually-dimorphic difference observed for tyrosine hydroxylase-positive neurons in the control group was not observed in either treatment group. When limited to 3 caudal sections, the sexually dimorphic difference observed for tyrosine hydroxylase-positive neurons was maintained in the low-dose group and was borderline significant ($P = 0.06$) in the high-dose group. Bisphenol A exposure had no significant effect on the number of tyrosine hydroxylase-positive neurons in the arcuate nucleus. In open-field testing of 6–9-week-old animals, significant effects in control females compared to control males included more rearing and time spent in the center and less time stopped. Sexually dimorphic differences in rearing and time spent in center were not observed in either bisphenol A treatment group and the sexually dimorphic difference in time stopped was not observed in the low-dose group. In open-field testing conducted at 4 weeks of age, control females compared to males reared more times and spent less time stopped. The sexually dimorphic differences were not observed in animals exposed to 0.000250 mg/kg bw/day (the only dose tested in 4-week-old animals). The number of rearings was significantly lower in 4-week-old females in the 0.000250 mg/kg bw/day group than in controls. The study authors concluded that bisphenol A may alter important events during critical periods of brain development.

Strengths/Weaknesses: The strengths of this study are the care taken to control for extraneous estrogenic exposure, the delivery of BPA at 2 doses, both low, delivery from GD 1–PND 16, the reasonable sample sizes, and the inclusion as outcome measurements of behavior, anatomy, and an index of neurochemical effects in the brain. Significant weaknesses include the use of s.c. osmotic pumps, uncertainty about sample size, and whether litter effects were adequately controlled.

Utility (Adequacy) for CERHR Evaluation Process: This is inadequate for the evaluation process due to the combination of route of administration and statistical concerns.

Toyama (2005), supported in part by the Japanese Ministry of Education, Culture, Sports Science, and Technology, examined the effects of prenatal Bisphenol A exposure in CL/P mice, a strain with a high background rate of cleft lip/palate. The study was published in Japanese and a translation was provided by the American Plastics Council. Mice were fed CA-1 (Japan CLEA, Inc.). **[No information was provided about caging or bedding materials.]** On GD 9.5 (GD 0 = day of vaginal plug), 25 dams/group were s.c. dosed with olive oil vehicle or bisphenol A **[purity not reported]** at 0.001, 0.01, 0.1, 1, or 10 mg/kg bw. Dams were killed on GD 18 and fetuses (169–184/group) were examined for cleft lip/palate or thymic anomaly (i.e., hypoplasia). Data were analyzed by Student *t*-test and χ^2 test. **[It appears that offspring were considered the statistical unit.]**

There were no significant differences for numbers of implantations or fetal survival. The incidence of cleft lip/palate in fetuses from the control and each respective treatment group was 8.3, 8.0, 6.1, 1.8, 4.9, and 6.2%. There were no differences in the types of cleft palate observed in each group. Incidence of thymic anomaly in the control and each respective dose group was 11.8, 10.8, 6.1, 1.8, 4.9, and 6.2%. Incidence of cleft/lip palate or thymus anomalies was lower in bisphenol A-treated than control groups and was lowest in the 0.1 mg/kg bw bisphenol A group. **[Results of statistical analyses for cleft lip/palate and thymic anomaly were difficult to interpret.]** A higher tendency for complication of cleft lip/palate and thymus hypoplasia **[possibly fetuses with both types of defects]** was observed in the bisphenol A groups; respective incidences in the control

and each treatment group was 36, 57.1, 61.8, 100, 77.8, and 72.7%. The study authors concluded that U-shaped dose response curves were observed for cleft lip/palate and thymus hypoplasia and that complication of cleft lip/palate and thymus hypoplasia tended to be lower in the bisphenol A groups.

Strengths/Weaknesses: Strengths of this study include that the authors explored a wide range of BPA doses. Time of dosing was appropriate with respect to palate development. The hypothesis that BPA administration is protective is interesting. Weaknesses include the route of administration, absence of exposure assessment, confusion on statistical analyses, absence of historical control perspective, and strain of mouse used. This strain of mouse has a high incidence of cleft palate making interpretation of these data challenging.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the CERHR evaluation process because of the combination of strain selection, confusion on statistical analyses, use of s.c. route of exposure, and use of offspring as the unit of analysis.

Berger et al. (2007), supported by The Natural Sciences and Engineering Research Council of Canada, examined the effect of bisphenol A exposure on blastocyst implantation and pup survival in mice. CF-1 mice were housed in polypropylene cages mice and fed Harlan Teklad 22/5 rodent chow, a soy-containing feed. **[No information was provided about bedding materials.]** On GD 1–4 or 5 **[inconsistently described in report]**, 6–15 mice/group were administered bisphenol A through a peanut butter supplement, or a mixture of feed and peanut butter. Mice were allowed to litter. Pups were counted on the day of parturition and observed for survival for 5 days. Pups were weaned at 28 days after birth and at that time, body weight and sex ratio were determined. Data were analyzed by χ^2 test. **[It was not clear if offspring data were analyzed on a pup or litter basis.]**

In the study in which the diet was supplemented with peanut butter, bisphenol A was added to the peanut butter at 0, 0.11, 1, 3, or 9%. Based on weights of unconsumed peanut butter, the study authors estimated mean bisphenol A intake at 0, 1.08, 8.33, 16.50, or 13.59 mg/day. **[Assuming that the mice weighed 0.02 kg at the start of gestation (USEPA, 1988), CERHR estimated bisphenol A intakes of 54, 417, 825, and 680 mg/kg bw/day.]** Peanut butter consumption was significantly decreased in the 9% group. There were no treatment effects on number of females delivering litters. Survival of pups from birth to weaning was lower in the 9% group (76.1%) than in the control group (98.2%) and 2 complete litters were lost in the 9% group. There was no significant difference in sex ratio of pups at weaning. There also did not appear to be an effect on pup weight at weaning.

In the study in which feed was dosed, mice were fed one part feed to two parts peanut butter. The feed/peanut butter mixture contained bisphenol A (97% purity) at 0, 3, or 6%. The study authors estimated bisphenol A intake at 0, 66.7, or 68.8 mg/day. **[Assuming that the mice weighed 0.02 kg at the start of gestation (USEPA, 1988), CERHR estimated bisphenol A intakes of 0, 3335, or 3440 mg/kg bw/day.]** Feed intake was significantly decreased in the 6% group. Controls were fed with the same quantity of food consumed by treated mice on the previous day. Delivery of litters in the 3% group was not affected but there were no litters delivered in the 6% group. Pup weight and sex ratio at weaning were not affected in the 3% group. Pregnancy disruption in the s.c. dosed mice is discussed in Section 3.2.6. **[It appears that with s.c. exposure, pregnancy disruption occurred at lower bisphenol A levels (10.125 mg/day, ~500 mg/kg bw/day) than with oral exposure (68.8 mg/day, 3440 mg/kg bw/day).]** The study authors concluded that the amount of bisphenol A required for pregnancy disruption was higher than typical environmental levels but that it is not known if bisphenol A could have additive or synergistic effects with other environmental estrogens.

Strengths/Weaknesses: Major weaknesses include absence of key statistical information on the appropriate control for possible litter effects, absence of similar effects at the same estimated dose level, inability to discriminate between potential maternal toxicity and the findings in the offspring, and the absence of exposure data (i.e., does the matrix affect exposure?).

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the CERHR evaluation process.

3.2.7 Mouse—oral exposure postnatally with or without prenatal exposure. **Nagao et al. (2002)**, support not indicated, examined the effects of bisphenol A in mice following exposure during different life stages. An initial study compared the sensitivity of male juvenile C57BL/6N and ICR mice to 17β-estradiol. Following s.c. dosing of 10 mice/strain/group with 10 μg/kg bw/day 17β-estradiol on PND 27–48, there were no weight changes or histopathological alterations in reproductive organs of ICR mice. In contrast, C57BL/6N mice exposed to 17β-estradiol experienced significant decreases in absolute and relative weights of testes, epididymides, and seminal vesicles. In addition, epididymal sperm was reduced and there was increased severity of seminal vesicle and Leydig cell atrophy. The study authors concluded that C57BL/6N mice are sensitive to estrogen and this strain of mice was used in the remaining experiments.

Life stages examined in experiments with bisphenol A included prenatal development, adolescence, and adulthood. The studies conducted during prenatal development and adolescence are described here, and the study conducted during adulthood is described in Section 4.2. C57BL/6N mice were fed PLD (phytoestrogen-low diet, Oriental Japan). They were housed in polycarbonate cages with wood bedding. Daidzein and genistein levels were analyzed in the diet, tap water, and bedding and found to be <0.5 mg/100 g. Bisphenol A (stated to be 99% pure in the study with adult mice) was administered to juvenile or pregnant mice by gavage at doses of 0.002, 0.020, or 0.200 mg/kg bw/day. Control animals were gavaged with 0.5% carboxymethyl cellulose **[assumed to be the vehicle]**. Juvenile males (30/group, obtained from 10 litters) were treated on PND 21–43 (day of birth not defined). At 6 weeks of age, 25 mice/group were necropsied. Ten pregnant C57BL/6N mice/group were treated on GD 11–17 (GD 0 = day of vaginal plug). Fetuses were removed by cesarean section on GD 18 and that day was considered PND 0. Litters were fostered to untreated dams. On PND 4, females were disposed and litters were culled to 3 males. Males were weaned on

PND 21 and housed individually in polycarbonate cages. At 12 weeks of age, males were weighed and 25 males/group were killed and necropsied. During necropsy of males that had been exposed during prenatal development or during adolescence, testes, epididymis, and seminal vesicles with coagulating glands were weighed. In the study conducted in adult mice, it was noted that ventral prostates were not weighed due to difficulties in obtaining only prostate and determining the precise weight of the organ. Epididymal sperm counts were obtained. Histopathological examinations were conducted for reproductive organs fixed in Bouin solution. For males exposed during gestation, the litter was considered a single sample. Data were analyzed by Bartlett's test to determine homogeneity of variance, followed by ANOVA when homogeneity of variance was obtained or Wallace–Wallace analysis of ranks when variance was not homogenous. Dunnett test was used for multiple comparisons.

There were no significant effects on embryo mortality after birth, body weight gain, or terminal body weight. **[Data were not shown.]** The only reproductive organ weight effect was a significant, but non-dose-related **[6%]** decrease in absolute seminal vesicle weight in the low-dose bisphenol A group. Organ weights were not affected in males exposed during adolescence. Sperm density was unaffected by bisphenol A exposure. No treatment-related lesions were observed in testes or other reproductive organs including ventral prostate. **[Data were not shown.]** The study authors concluded that low-dose bisphenol A exposure of mice did not reduce sperm density or disrupt male reproductive system development.

Strengths/Weaknesses: Strengths are the use of three low dose levels, the oral route of administration, the careful description of methods, the use of a low-phytoestrogen diet, and the confirmation that the strain of mice used was estrogen sensitive.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Kabuto et al. (2004), supported by the Kagawa Prefectural College of Health Sciences, examined the role of oxidative stress in bisphenol A-induced toxicity in mice. ICR mice were fed standard laboratory chow containing 24% protein (MF; Oriental Yeast Co.). **[No information was provided about bedding or caging materials.]** From 1 week before mating through gestation and lactation, 6 mice/group were given drinking water containing the 1% ethanol vehicle or bisphenol A **[purity not reported]** at 5 or 10 μg/L. **[Based on the reported water intake of 5 mL/day and an assumed body weight of 0.02 kg (USEPA, 1988), it is estimated that bisphenol A intake in mice at the start of pregnancy was 0.0013 or 0.0025 mg/kg bw/day.]** Mice gave birth about 3 weeks following mating and pups were housed with dams for 4 weeks. **[Based on an assumed body weight of 0.0085 kg and assumed water intake rate of 0.003 L/day (USEPA, 1988), it is estimated that intake of bisphenol A in weanling males was 0.0018 or 0.0035 mg/kg bw/day]**. At 4 weeks of age, male pups were killed and brain, kidney, liver, and testis were weighed in 8–13 mice/group. Tissues were homogenized to determine activities of superoxide dismutase, catalase, and glutathione peroxidase and concentrations of glutathione and L-ascorbic acid in 6–8 mice/group. Tissue level of thiobarbituric acid-reactive substance, a biogenic macromolecular peroxidation indicator, was measured in 6 mice/group. Data were analyzed by ANOVA followed by Scheffe *F* test. **[It appears that offspring were considered the statistical unit in some analyses.]**

Organ weight effects included decreased brain weight at the low dose, decreased kidney weight at the high-dose, and decreased testis weight at both doses. **[Relative organ weights were not determined.]** In the high-dose group, thiobarbituric acid-reactive substance levels were increased in brain, kidney, and testis. Changes in antioxidant enzyme levels included decreased catalase activity in testis and increased glutathione oxidase activity in kidney. No significant effects were observed for superoxide dismutase activity or glutathione or ascorbic acid levels in any of the tissues examined. The study authors concluded that bisphenol A exposure during gestation and lactation results in oxidative stress and peroxidation in offspring that ultimately lead to underdevelopment of brain, kidney, and testis.

Strengths/Weaknesses: The delivery of bisphenol A in drinking water at low dose levels is a strength. Weaknesses include small sample size of exposed dams ($n = 6$), inappropriate use of the pup as the experimental unit in statistics, and mechanistic data without functional correlates.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to inappropriate statistical procedures and small sample size.

Takao et al. (2003), support not indicated, examined the effects of bisphenol A exposure on expression of *ER*α and *ER*β in the testis of young mice. **[No information was provided about feed, caging, or bedding materials.]** Three-week-old male C57BL/6 mice ($n = 7$/group) were administered bisphenol A **[purity not indicated]** through drinking water at 0 (ethanol vehicle), 0.5, or 50 mg/L for 8 weeks. **[Assuming a weanling mouse drinks ~0.35 L/kg bw/day (USEPA, 1988), bisphenol A intake would have been ~0, 0.175, or 17.5 mg/kg bw/day.]** The stability of bisphenol A was not determined, but water bottles were changed two times a week to maintain a stable concentration of bisphenol A in drinking water. Mice were killed at an unspecified period following exposure, and the testis and spleen were weighed. The testis was examined for ERα- and ERβ-positive cells using an immunohistochemistry method and *ER*α and *ER*β mRNA using a semi-quantitative RT-PCR technique. Data were analyzed by ANOVA followed by Fisher protected least significant difference test.

Exposure to 50 mg/L bisphenol A resulted in a decreased number of ERβ-positive cells and increased number of ERα-positive cells. Expression of *ER*β mRNA was decreased and expression of *ER*α mRNA was increased following exposure to 50 mg/L bisphenol A. There were no differences in body weight or absolute or relative weights of testis or spleen following bisphenol A treatment. The study authors concluded that differential modulation of ERα and ERβ could be involved in effects observed following bisphenol A exposure.

Strengths/Weaknesses: The delivery of bisphenol A in drinking water and the measurement of ER in the testis are strengths. The lack of clarity on age at sacrifice, limited number of endpoints assessed, and marginal sample size ($n = 7$) are significant weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process based on the limitations noted above.

Matsumoto et al. (2004), support not indicated, examined the effect of maternal bisphenol A exposure on growth of offspring in mice. Mice were fed standard rodent chow (CE-2, Japan Clea). **[No information was provided on caging and bedding materials.]** Mice of the ddY strain were exposed to bisphenol A ($\geq$97% purity) through feed at 0 or 1% from GD 14–PND 7. The study authors stated that the bisphenol A dose was equivalent to 1000 mg/kg bw/day. **[The number of dams treated was not indicated. Day of vaginal plug and day of birth were not defined]**. Mice delivered pups on PND 21. During the postnatal period, body weight was monitored in 31 pups from the control group and 61–89 pups from the bisphenol A group. Serum prolactin levels were measured by RIA in 3 dams/group 4 days following delivery. Pups were killed on PND 7, and stomach weight was measured. Data were analyzed by Student *t*-test. **[It was not clear if the litter or offspring was considered the statistical unit.]**

No differences were reported for live pups at birth. During the postnatal period, body weights of pups in the bisphenol A group were significantly lower **[by ~40%]** than control group pups. No deaths were reported for pups in the control group, but 30% of pups in the bisphenol A group died before PND 7. On PND 1, milk could be seen in stomachs of pups from the control group, but not the bisphenol A group. **[The number of pups evaluated for milk in stomach was not reported.]** On PND 7, stomach weight was significantly lower **[by 40%]** in pups from the bisphenol A than control group. Serum prolactin level was significantly reduced **[by 46%]** in dams from the bisphenol A group. The authors concluded that administration of a high bisphenol A dose to mice resulted in suppressed postnatal growth of offspring that probably resulted from an insufficient supply of milk, which might have been due to decreased prolactin secretion. **[Because of the implications of this study for lactation competence, this study will be discussed again in Section 4.2.]**

Strengths/Weaknesses: Weaknesses of the study are the difficulty in calculating bisphenol A intake, the likely high exposure level, the lack of information on dam number and husbandry, and the high level of pup body weight decrement and mortality.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to the reasons stated above.

Suzuki et al. (2003), supported by the Japanese Ministry of Health, Labor, and Welfare and the Ministry of Education, Culture, Sports, Science, and Technology conducted a study to determine the effect of prenatal bisphenol A exposure on dopamine-receptor mediated actions in mice. Female ddY mice were fed chow containing bisphenol A at 0.002, 0.5, or 2 mg/g feed from mating through weaning of offspring. **[No information was provided on the number of dams treated, purity of bisphenol A, or the type of chow, bedding, or caging materials. Assuming a female mouse eats ~0.2 kg feed/kg bw/day (USEPA, 1988), bisphenol A intake would have been 0.4, 100, or 400 mg/kg bw/day.]** Male offspring were subjected to a series of tests **[age at testing not stated]**. In a conditioned place-preference test, groups of 6–10 mice were injected with 0.5 mg/kg bw methamphetamine and placed in either the dark or light area of the test apparatus for 3 days. On the other 3 days, males were injected with saline and placed in the other compartment of the testing apparatus. On Day 7, the divider in the apparatus was raised and the time spent in each compartment was measured. Activity was measured in groups of 9–10 mice for 3 hr following injection with saline or 2 mg/kg bw methamphetamine. Dopamine-induced binding of ^{35}S-guanosine-5′[γ-thio]-triphosphate in the limbic system was measured ($n = 3$ samples/group). Protein levels of dopamine and vesicle monoamine transporters in brain were determined by Western blot ($n = 6$ samples), and mRNA levels of dopamine receptor in brain were determined by RT-PCR. Data were analyzed by ANOVA with Bonferroni/Dunnett test. **[It was not clear if the litter or offspring was considered the statistical unit.]**

In conditioned-preference testing, exposure to all 3 bisphenol A doses resulted in a significant and dose-related increase in preference for compartments associated with methamphetamine exposure. **[Control mice showed no compartment preferences while the times spent in the methamphetamine-associated compartment were ~150, 200, and 275 sec by animals in each respective dose group.]** Preference for the methamphetamine compartment was eliminated by injecting the animals with SCH23390, A dopamine D_1 receptor antagonist. In mice exposed to the high-dose of bisphenol A, activity was significantly increased **[by ~80% at peak]** compared to the control group following methamphetamine challenge, and sensitization to methamphetamine-induced activity was also enhanced. Dopamine-induced binding of ^{35}S-guanosine-5′[γ-thio]-triphosphate in the limbic system was potentiated **[increased by ~15%; not clear if statistically significant]** and G-protein activation was increased **[by ~75%]** in mice exposed to the high bisphenol A dose. The effects on G-protein activation were eliminated following injection with SCH23390 or sulpiride, a dopamine D_2 receptor antagonist. No changes were observed for expression of dopamine and vesicle monoamine transporter proteins. Expression of dopamine D_1 receptor mRNA was upregulated significantly to 130% of control levels in the high-dose bisphenol A group. **[For all endpoints except for conditioned preference, only the data from the high-dose bisphenol A group was shown. It was not clear if that was the only dose tested for those endpoints or if the high-dose data were shown because it was the only dose that resulted in a statistically significant effect.]** The study authors concluded that "prenatal and neonatal exposure to bisphenol A can potentiate central dopamine D_1 receptor-dependent neurotransmission, resulting in supersensitivity of methamphetamine-induced pharmacological actions related to psychological dependence on psychostimulants."

Strengths/Weaknesses: Strengths include a wide range of doses administered orally. Weaknesses include absence of adequate experimental details, inappropriate statistical procedures that did not account for litter or repeated measurement, inadequate presentation of body weight data, and use of high-doses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process due to the reasons stated above.

Tando et al. (2007), supported by the Japanese Ministries, investigated the effects of bisphenol A exposure in the maternal diet during the prenatal and lactational period on the long-term development of the cortex and substantia nigra. ddY mice were maintained under a 12-hr/12-hr light/dark cycle before mating. From GD 0 through weaning on PND 21, dams had free access to a diet containing bisphenol A (purity >99%) at 0, 3, or 8000 mg/kg feed. Pups were weaned on PND 21 to a diet without bisphenol A. **[The basal feed, cage, and bedding were not specified. Daily feed consumption was not reported. Assuming a pregnant mouse eats ~0.15 kg feed/kg bw/day and a lactating mouse eats ~0.45 kg feed/kg bw/day, bisphenol A intake would have been ~0, 4.5, or 1200 mg/kg bw/day during gestation and ~0, 1.35, or 3600 mg/kg bw/day during lactation.]** At 8–11 weeks of age, male and female offspring ($n = 4$ and 5/sex/treatment group) were killed and formalin-perfused. Brains were harvested and embedded in paraffin. Immunohistochemical detection for tyrosine hydroxylase, calbindin D-28 K, calretinin, and parvalbumin proteins were performed. In situ TUNEL was also performed. Statistical analyses use ANOVA and post-hoc test using the Bonferroni/Dunn multiple comparison test. **[It was not clear if the litter or offspring was considered the statistical unit.]**

No cytoarchitectural anomalies were seen in brain sections of either sex across treatment groups, based on hematoxylin-eosin and Kluver-Barrera stains. **[Data were not shown.]** The distribution and density of immunopositive staining for calbindin D-28 K, calretinin, and parvalbumin showed no statistically significant differences in low or high-dose bisphenol A exposed groups. Female offspring exposed to the lower dose level of bisphenol A exhibited a significant decrease in the volume of the substantia nigra. The number of tyrosine hydroxlyase-positive nuclei and fibers in this region was significantly reduced in low-bisphenol exposed female mice compared to control females and high-dose bisphenol A-exposed females **[↓18%, and 16%, respectively, estimated from a graph]**. No significant differences in number of tyrosine hydroxylase positive cells were identified in bisphenol A-exposed males. Decreased values in immunopositive staining could not be attributed to apoptosis, based on TUNEL staining **[data not shown]**.

The authors concluded that there were sex- and dose-specific sensitivities of the developing substantia nigra, in the DDY mice with females exposed to a low but not a high-dose of bisphenol A showing a significant reduction in the number of tyrosine hydroxylase-positive nuclei. They indicated that the functional significance of this reduction was unknown. The authors suggested a putative mechanism involving interaction of bisphenol A with ERβ, which is abundantly present in the developing substantia nigra.

Strengths/Weaknesses: Strengths of this study are that BPA was delivered orally to the dams during the gestational and lactational period and the use of appropriate methods for assay of the anatomical and some molecular aspects of brain development. Weaknesses include the lack of specification of the feed, broad range of the two doses used, small sample size given high variability of endpoints (4 and 5/sex/treatment group), and absence of expected sexually dimorphisms in measures in the controls.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful for the evaluation process for the reasons stated above.

Mizuo et al. (2004), supported by the Japanese Ministry of Health, Labor, and Welfare and the Ministry of Education, Culture, Sports, Science, and Technology, examined the effect of perinatal bisphenol A exposure on morphine-induced rewarding effects and hyperlocomotion in mice. Testing was conducted in offspring of ddY mice that received chow containing 0, 0.002, 0.5, or 2 mg bisphenol A/g feed [**0, 2, 500, or 2000 ppm**] during gestation and the neonatal period of pup development. **[No information was provided on the number of dams treated/group, purity of bisphenol A, or feed, caging, or bedding materials.]** In place-conditioning testing, 6–10 offspring/group were placed in one compartment of a testing apparatus following saline injection and in a second compartment of the apparatus following morphine injection; on the second day, mice were given free access to both compartments and the time spent in each compartment was measured. Locomotor activity was measured after injecting 9–10 mice/bisphenol A group with saline or 10 mg/kg bw morphine. Guanosine-5′-diphosphate binding and expression of μ-opioid receptor mRNA were measured in 3 independent samples/group. Statistical analyses included 2-way ANOVA with Bonferroni/Dunnett test. **[No information was given on the ages that testing was conducted and the sex of mice tested. It was not clear if the litter or offspring was considered the statistical unit.]**

In place-preference conditioning testing, a dose-dependent increase was observed for the time spent in the compartment associated with morphine exposure and statistical significance was attained at the two highest dose levels. **[The time spent in the morphine-associated compartment was ~15 sec for controls, 150 sec for the mid-dose group, and 175 sec for the high-dose group.]** Locomotion in the high-dose bisphenol A group was significantly increased following morphine injection **[~130 compared to 10 activity counts in high-dose bisphenol A group compared to the control]**. Bisphenol A treatment had no effect on guanosine-5′-diphosphate binding (i.e., μ-opioid receptor mediated G-protein activation) or expression of μ-opioid receptor mRNA. The study authors concluded that chronic exposure to bisphenol A induces morphine-induced rewarding effect and hyperlocomotion that does not occur through activation of the μ-opioid receptor.

Strengths/Weaknesses: Strengths of this study are that BPA was delivered orally to the dams during the gestational and lactational period. Weaknesses include the lack of specification of the feed, broad range of the two doses used, small sample size ($n = 6$–10) and inappropriate statistics that do not account for litter or repeated measures.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful for the evaluation process.

Miyatake et al. (2006), supported by the Japanese Ministry of Health, Labor, and Welfare and the Ministry of Education, examined the effects of developmental bisphenol A exposure on morphine-induced rewarding effects in male ddY mice. Maternal mice were orally exposed to olive oil vehicle, bisphenol A **[purity not indicated]** at 0.003 or 200 mg/kg bw/day, or 17β-

Table 80
Behavior of Female Mice After Gestational and Lactational Exposures[a]

Endpoint[b]	Bisphenol A, μg/kg bw/day		Ethinyl estradiol
	2	200	
Puberty onset	↔	↓ 4.5 days	↓ 6.25 days
Time in open arms of plus maze	↔	↓ 41% ($P = 0.06$)	↓ 73%
Time in light part of light/dark preference box	↔	↓ 52%	↓ 69%
Errors in radial arm and Barnes mazes	↔	↔	↓

[a]Ryan and Vandenbergh (2006).
[b]The size of the difference from control was estimated from graphs.
↓ Statistically significant decrease from control value; ↔ no statistical difference from control value, ↓decrease identified by authors although statistical difference from control not shown.

estradiol at 3 μg/kg bw/day by gavage. The compounds were administered 3 times a day from the mating period through weaning of offspring. Seven male offspring/group were examined in a place-conditioning test at 7 weeks of age. During the preconditioning period, mice were placed in one compartment of a cage following injection with saline and in another compartment of the cage following s.c. injection with morphine. During testing, the amount of time spent in each compartment of the cage was measured. Statistical analyses included ANOVA followed by Bonferroni/Dunnett test. **[It was not clear if the litter or offspring was considered the statistical unit.]**

Developmental exposures to either bisphenol A dose resulted in a preference for the cage compartment associated with morphine exposure. Developmental exposure to 17β-estradiol at 3 μg/kg did not affect place preference. Based on the findings of this study and in vitro studies described in Section 3.2.1.1, the study authors concluded that bisphenol A alters dopamine responsiveness in mouse neurons and astrocytes, which could potentially contribute to development of psychological dependence on drugs of abuse.

Strengths/Weaknesses: Strengths include the use of a positive control and corresponding measurement of in vitro and behavioral endpoints. Weaknesses include the use of only 2 doses, 1 very low and 1 high (both had similar effects), inadequate experimental details regarding exposure and numbers of dams, small sample size for behavioral endpoints, inappropriate statistical procedures that did not account for litter of origin or repeated behavioral measurements.

Utility (Adequacy) for CERHR Evaluation Process: This report is inadequate and not useful for the evaluation process.

Ryan and Vandenbergh (2006), supported by North Carolina State University and EPA, evaluated the effects in mice of prenatal and postnatal exposure to bisphenol A on sexually dimorphic behaviors. C57BL/6 mice were maintained in polycarbonate cages (checked frequently for condition) with chip bedding and were given Purina 5001 chow. Females were mated and the day a vaginal plug was identified was considered GD 1. Beginning on GD 3, dams were treated with bisphenol A **[purity not indicated]** 2 or 200 μg/kg bw/day, ethinyl estradiol 5 μg/kg bw/day, or the tocopherol-stripped corn oil vehicle. The dose was placed in the back of the throat with a gavage needle. Daily dosing was continued to PND 21, when pups were weaned. One female per litter was randomly selected for behavioral testing and was ovariectomized. Pup anogenital distance was measured at weaning. Non-ovariectomized mice were checked for vaginal opening and vaginal smears taken daily thereafter. Puberty was defined as the first day on which cornified cells were detected in 4–7 females/group. Fourteen mice/treatment group were tested in an elevated plus maze and a light/dark preference chamber. Sixteen mice/treatment group were tested in a radial arm maze and a modified Barnes maze. Testing occurred 2 weeks after ovariectomy. Statistical analysis used ANOVA with post-hoc Student *t*-test. The radial arm and Barnes mazes were run for 5 consecutive days and a repeated measures design was added to the ANOVA.

There was no effect of treatment on anogenital distance or anogenital distance divided by body weight. Other results are summarized in Table 80. Puberty was advanced by exposure to ethinyl estradiol or the high-dose of bisphenol A. The results of the elevated plus and light/dark preference tests led the authors to conclude that bisphenol A and ethinyl estradiol increased anxiety. The improved performance in the radial arm and Barnes mazes led the authors to conclude that ethinyl estradiol masculinized spatial ability. **[The results from the elevated plus maze also suggest masculinization of behavior, because males show more "anxiety" in this paradigm.]** Bisphenol A 200 μg/kg bw/day resulted in a decrease in errors on earlier trials than the control in the radial arm maze, but this effect was not characterized by the authors as providing strong evidence of an alteration in spatial memory.

Strengths/Weaknesses: Selection of established measurements of sexually dimorphic behaviors and replication of previous work by Howdeshell et al. (1999), the use of positive controls, the appropriate evaluation of pubertal onset, adequate sample sizes for behavioral methods, weight, and AGD measures are all strengths of this work. A weakness is the small sample size for evaluating pubertal onset.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process with the exception of the pubertal data.

Tyl et al. (2006), sponsored by the American Plastics Council, conducted a two-generation GLP study of bisphenol A in CD-1 mice. **[This study is discussed in detail in Section 4.2.3.2. Results relevant to developmental toxicity are presented here.]** Mice were fed

Purina Certified Ground Rodent Diet No. 5002 containing 177–213 ppm genistein, 173–181 ppm daidzein, and 39–55 ppm glycitein. Mice were housed in polypropylene cages with Sani-Chip bedding. F_0 and F_1 mice (28 sex/group/generation) were fed diets containing bisphenol A (99.70–99.76% purity) at 0.018, 0.18, 1.8, 30, 300, or 3500 ppm. Target intakes were 0.003, 0.03, 0.3, 5, 50, or 600 mg/kg bw/day. The study authors estimated bisphenol A intake in males at 0.0024–0.0038, 0.024–0.037, 0.24–0.37, 3.98–6.13, 39.1–60.8, or 529–782 mg/kg bw/day. Bisphenol A intakes (in mg/kg bw/day) by females were estimated at 0.0030–0.0041, 0.030–0.042, 0.32–0.43, 5.12–7.12, 54.2–67.8, 653–910 during the pre-mating period; 0.0027–0.0029, 0.027–0.028, 0.28–0.29, 4.65–4.80, 47.0–48.6, 552–598 during the gestation period; and 0.0087–0.0063, 0.062–0.091, 0.61–0.89, 10.4–15.1, 103.2–146.4, 1264–1667 during the lactation period. In each generation, there were 2 vehicle control groups with 28 mice/sex/group. A positive control group was given feed containing 17β-estradiol at 0.5 ppm (target intake of 0.08 mg/kg bw/day). **[The Expert Panel notes that a separate 2-generation study was used to characterize the dose–response relationship for 17β-estradiol.]** Homogeneity, stability, and concentration of bisphenol A in feed were verified. Exposure of F_0 mice began at ~6 weeks of age. Exposure of F_1 animals began at weaning, although it was noted that pups began eating the dosed feed in the late lactation period. F_0 and F_1 mice were fed the bisphenol A-containing diets for a minimum of 8 weeks before mating and during a 2-week mating period. Exposures of females continued through the gestation and lactation period.

Live F_1 and F_2 pups and litters at birth, sex ratio, and survival during the lactation period were not affected and there were no clinical or gross signs of toxicity in F_1 or F_2 offspring. A non-dose-related decrease in PND 21 survival index and lactational index (pups surviving on PND 21/PND 4) was described in F_2 pups of the 300 ppm group. **[The biological significance of the effect was not discussed by the study authors, but because the effect was not dose-related it is unlikely to be of biological significance.]** In F_1 pups from the 3500 ppm group, body weights were reduced during PND 7, 14, and 21 in F_1 females and both sexes combined and on PND 7 and 21 in F_1 males. An increase in male pup body weight observed on PND 7 in the 1.8 ppm group was not considered to be treatment-related by the study authors because no dose–response relationship was observed. There was no effect on anogenital distance in F_1 or F_2 males or females on PND 0. Anogenital distance was also unaffected in F_2 males and F_1 and F_2 females on PND 21. Anogenital distance adjusted for body weight was reduced in F_1 males from the 300 and 3500 ppm groups on PND 21. Based on the lack of effect on anogenital distance at birth and inconsistencies between generations, the study authors did not consider the decreases in anogenital distance in F_1 males to be treatment-related. An increase in anogenital distance in F_2 females from the 0.018 ppm group on PND 0 was not considered to be treatment-related by the study authors. Preputial separation (absolute age and adjusted for body weight on day of acquisition) was delayed in parental and retained F_1 males of the 3500 ppm group. When adjusted for PND 30 body weight, preputial separation was delayed in retained but not parental F_1 males from the 3500 ppm group. Body weights on day of vaginal opening were lower in F_1 females from the 3500 ppm group. Day of vaginal opening was accelerated in the 3500 ppm group if adjusted for PND 21 body weight, but not body weight on the day of acquisition. Due to the lack of effect when adjusted for body weight on day of acquisition, the study authors did not consider effects on vaginal opening to be treatment-related.

Dose-related organ weight changes in F_1 weanlings that were considered to be treatment-related by study authors included decreased absolute and relative (to body or brain weight) spleen and paired testes weights at 3500 ppm. Treatment-related absolute organ weight changes in F_2 weanlings included decreased weights of spleen, paired testes, and seminal vesicles with coagulating glands in the 3500 ppm group. Changes in organ weights relative to body weight in F_2 weanlings included decreased spleen weight in males and females and increased relative left kidney weight in 3500 ppm males. Treatment-related changes in organ weight relative to brain weight in F_2 weanlings were decreased spleen weight in both sexes and decreased paired testes weight at 3500 ppm and seminal vesicles with coagulating glands at 300 and 3500 ppm. Other organ weight effects (e.g., affecting epididymides, thymus, brain, ovaries, and/or uterus with cervix and vagina weights) were not considered to be dose-related due to lack of dose–response relationships or no consistent effects across generations. The study authors reported no gross findings in F_1 or F_2 weanlings. **[Although not clear because the number of animals examined for gross testicular effects was not reported in Tables 23 and 49 of the study, it appeared that the incidence of undescended bilateral testes may have been increased in F_1 and F_2 weanling males of the 3500 ppm group.]** The incidence of hepatic cytoplasm alteration (clear hepatocellular cytoplasm, slightly more basophilic cytoplasm, and/or minute vacuoles) was apparently increased in F_1 males from the 300 and 3500 ppm groups and F_1 females and F_2 males from the 3500 ppm group. The incidence of seminiferous tubule hypoplasia was increased in F_1 and F_2 weanlings from the 3500 ppm group. **[Another histopathological finding that appeared to be possibly increased in weanlings from the 3500 ppm group was unilateral hydronephrosis in F_1 males. It did not appear that histopathological data were statistically analyzed.]**

The study authors identified bisphenol A NOELs of 30 ppm (~5 mg/kg bw/day) for systemic effects and 300 ppm (~50 mg/kg bw/day) for developmental toxicity. **[The lowest benchmark doses were obtained from F_1 body weight data on PND 21: BMD_{10} 548 mg/kg bw/day, $BMDL_{10}$ 267 mg/kg bw/day, $BMD_{1\ SD}$ 580 mg/kg bw/day, $BMDL_{1\ SD}$ 370 mg/kg bw/day.]**

Strengths/Weaknesses: Strengths include the large number and range of doses examined, the rigor with which the study was performed (including evaluation of phytoestrogen content of feed), the large sample size in each group, the number of additional animals per litter that were retained and examined, the use of a concurrent estrogenic positive control group, and the thoroughness of the histological evaluation.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

3.2.8 Mouse—parenteral exposure postnatally with or without prenatal exposure.

3.2.8.1 Female reproductive endpoints: Suzuki et al. (2002), supported by Japanese Ministry of Education, Culture, Sports, Sciences, and Technology, the Special Coordination Funds of Science and Technology Agency of the Japanese Government, and the Japanese Ministry of Health, Labor, and Welfare, conducted a study to examine the effects of bisphenol A exposure on the reproductive system of the female mouse. Two sets of studies were conducted, one with prenatal exposure, and one with postnatal exposure. In both studies, ICR/Jcl strain mice were fed a commercial diet (CE-2, CLEA, Tokyo, Japan). **[No information was provided about bedding or caging materials.]** Bisphenol A **[purity not reported]** was administered by s.c. injection in sesame oil vehicle. For histological examinations, organs were fixed in Bouin solution. Parametric data were analyzed by ANOVA, with post-hoc Student *t*-test or Welch *t*-test. Data expressed as proportions were analyzed by Fisher exact probability test. For exposures occurring in the prenatal period, the litter was maintained as the statistical unit.

In the prenatal exposure study, mice were administered bisphenol A by s.c. injection at 0 (vehicle), 10, or 100 mg/kg bw/day on GD 10–18 (day of vaginal plug = GD 0). Other groups of mice were treated with diethylstilbestrol at 0.0067–67 μg/kg bw/day during the same period. **[Numbers of dams treated were not specified.]** On GD 19, fetuses were removed by cesarean section, weighed, adjusted to 7 pups/litter **[numbers for each sex not indicated]**, and fostered to untreated mothers. Pups were weaned at 22 days of age. Some pups were ovariectomized at 30 days of age, and some were killed at 30 or 40 days of age for histological examination of reproductive organs, polyovular follicle numbers, corpora lutea numbers, and mitotic index in uterine and vaginal cells. In the remaining pups, vaginal smears were examined from 41–70 days of age. Fertility was then assessed by mating the mice with untreated males (2 or 3 females/male). Offspring were counted and sexed. The authors stated that 2 or 3 pups/litter were used in each analysis. Data tables list the sample size as 8–11/group/time period for the bisphenol A and control groups.

Bisphenol A treatment did not affect the histology of the uterus or vagina in ovariectomized mice. The study authors stated there was no evidence of increased mitogenicity compared to controls in uterine cells of intact or ovariectomized mice exposed to bisphenol A. **[**Figure 3 **of the study indicated a higher mitotic index in epithelial cells of ovariectomized mice of the high-dose group.]** Mitotic indices were significantly lower in stromal cells of intact mice of both dose groups and in glandular cells of the low-dose group. There was no increase in mitogenicity of vaginal cells compared to the control group; in intact mice, the mitotic index was lower than control values in vaginal epithelial cells of the high-dose group and stromal cells of the low-dose group. Number of vaginal epithelial layers was increased in both bisphenol A dose groups of intact mice compared to control mice. No effect was reported for uterine or vaginal epithelial stratification. There were no effects on numbers of polyovular follicles. **[Data were not shown by study authors.]** The number of mice with corpora lutea at 30 days of age was significantly reduced in the low-dose group (4 of 9 mice in low dose group compared to 7 of 9 mice in control group). Estrous cyclicity was not affected by bisphenol A treatment. In mating studies, bisphenol A exposure did not affect the number of mice giving birth, number of fetuses/litter, or sex ratio. Several effects were observed in mice prenatally exposed to diethylstilbestrol, and most of the effects occurred at the high-dose of 67 μg/kg bw/day. In the high-dose diethylstilbestrol group, there were changes in vaginal and uterine histology, increases in mitotic indices in vaginal and uterine cells of ovariectomized animals, vaginal stratification and increased layers of epithelial cells in ovariectomized animals, disrupted estrous cycles, and complete infertility. The number of mice with corpora lutea at 30 days was decreased at the two highest diethylstilbestrol doses ($\geq$6.7 at μg/kg bw/day).

In the postnatal exposure experiment, female mice (1.5 g bw) were s.c. injected with bisphenol A at 0.015 or 0.150 mg/pup/day or diethylstilbestrol at 0.3 or 3 μg/pup/day for 5 days, beginning on the day of birth. **[The number of animals treated was not stated. Based on body weights provided by authors, bisphenol A doses were estimated at 10 and 100 mg/kg bw/day; diethylstilbestrol doses were estimated at 200 and 2000 μg/kg bw/day.]** Two-thirds of mice were ovariectomized at 30 days of age and then killed at 30, 40, or 90 days of age for histological examination of reproductive organs. Numbers of polyovular follicles were determined at 30 days of age, and number of corpora lutea were counted at 30 and 90 days of age. Estrous cyclicity was monitored in the remaining mice at 61–90 days of age. The 90-day-old mice were s.c. injected with 5 mg/kg bw colchicine and killed 5 hr later. Mitotic rates of uterine and vaginal cells were determined, and histological examinations of reproductive organs were conducted. Sample sizes were 6–17/group/time period in analyses conducted in mice exposed postnatally.

Vaginal stratification was observed at 40 days of age in 4 of 7 ovariectomized mice of the high-dose bisphenol A group, which was higher than in the control. The incidence of vaginal stratification in 90-day-old ovariectomized mice of the high-dose group (4 of 10) did not attain statistical significance compared to control. In ovariectomized mice, significant increases in the mitotic rate compared to controls were observed in uterine stromal cells and vaginal epithelial cells at the high-dose. The number of vaginal epithelial layers was also increased in the high-dose bisphenol A group ($\sim$4 layers in treated group compared to 3.5 layers in control group). There were no significant changes in estrous cycles or number of mice with corpora lutea. In 30-day-old mice of the high-dose group, significant increases were observed in the number of mice with polyovular follicles (15 of 17 in exposed group compared to 6 of 15 in control group) and the numbers of polyovular follicles/mouse (mean $\pm$ SE: 0.8 $\pm$ 0.2 in the exposed group and 0.2 $\pm$ 0.1 in control group); polyovular follicles contained 2 oocytes in the control and bisphenol A groups. Effects observed in mice treated with both doses of diethylstilbestrol included increased stratification of vaginal cells in ovariectomized mice at 40 and 90 days of age, increased mitotic rates of vaginal and uterine cells in ovariectomized mice, disrupted estrous cycles, and increased polyovular follicles. The study authors concluded that

high-doses of bisphenol A induce ovary-independent vaginal stratification and polyovular follicles when administered during postnatal but not prenatal development.

Strengths/Weaknesses: The use of diethylstilbestrol as a positive control is a strength as are an experimental design that appropriately examined litter effects. The use of relatively high-doses by s.c. injection and small sample sizes for ovarian histopathology are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility due to the route and dose level.

Nikaido et al. (2005), supported by the Japanese Ministry of Health, Labor, and Welfare, examined the effects of bisphenol A exposure on the development of the reproductive system in female mice. Mice used in this study were housed in polyisopentene cages with white pine chip bedding. The mice were fed a low-phytoestrogen diet (NIH-07 PLD; Oriental Yeast Co.) and provided water in polycarbonate bottles with rubber stoppers. At 15 days of age, 17–24 female CD-1 mice/group were s.c. injected with DMSO vehicle, 10 mg/kg bw/day bisphenol A (≥99% purity), or 10 μg/kg bw/day diethylstilbestrol for 4 days. Additional groups were dosed with other compounds, but those results will not be discussed. **[No information was provided on the numbers of litters represented.]** Mice were weaned at 21 days of age. Body weights were measured weekly. Day of vaginal opening was determined and estrous cyclicity was assessed over 21-day periods beginning at 5, 9, and 21 weeks of age. Six mice/group/time period were killed and necropsied at 4, 8, 12, and 24 weeks of age. **[In contrast to the Materials and Methods section, there was no mention of animals killed at 12 weeks of age in the Abstract or Results section of the study.]** Ovaries, uteri, vaginas, and inguinal mammary glands were fixed in 10% neutral buffered formalin. Histopathological analyses were conducted of the ovary, uterus, and vagina. Mammary glands were examined as whole-mount preparations. It appears that all endpoints were assessed in every mouse. Statistical analyses included homogeneity of variance analysis and ANOVA or Kruskal–Wallis test. If statistical significance was obtained, data were further analyzed by Fisher protected least significant difference test.

Exposure to bisphenol A resulted in no effects on body weight gain, age of vaginal opening, estrous cyclicity, histopathological changes in the uterus or vagina, or growth or development of the mammary gland. At 4 weeks of age, 33% of mice in the control group, 83% of mice in the bisphenol A group, and 100% of mice in the diethylstilbestrol group lacked corpora lutea. **[It appears that the study authors considered the lack of corpora lutea to be normal based on the age of mice.]** No effects on corpora lutea numbers or numbers of polyovular follicles were observed at later ages. Mice treated with diethylstilbestrol experienced accelerated vaginal opening and increased time in estrus. In their conclusion, the study authors reiterated the lack of effects in the bisphenol A group.

Strengths/Weaknesses: The use of diethylstilbestrol as a positive control is a strength, but the lack of information on sample size of dams, small sample size for postnatal endpoints, subcutaneous route, and high-dose level are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful in the evaluation.

Markey et al. (2005), supported by NIH, examined the effects of perinatal bisphenol A exposure on reproductive development in mice. CD-1 mice were fed Purina rodent chow that tested "negligible for estrogenicity in the E-SCREEN assay." Cages and bedding tested negative for estrogenicity in the E-SCREEN assay. Tap water was supplied in glass bottles. From GD 9 (GD 1 = day of vaginal plug) through PND 4, 6–10 mice/group were exposed to bisphenol A **[purity not reported in the manuscript; 97 ± 2% per A. Soto, personal communication, March 2, 2007]** at 0 (DMSO vehicle), 0.000025, or 0.000250 mg/kg bw/day through a s.c. pump. Offspring were culled to 10/litter on PND 7 and weaned on PND 20. One pup/litter from 6–10 litters/treatment group was killed on the day of proestrus at 3 months of age. The uterus and vagina were weighed and subjected to morphometric analysis. The uterus was also assessed for cell proliferation by bromodeoxyuridine (BrdU) incorporation, apoptosis by TUNEL method, and expression of ERα and progesterone receptor by an immunostaining procedure. Data that were normally distributed and showed homogeneity of variance were analyzed by ANOVA and least significant difference test. Other data were analyzed by Kruskall–Wallis and Mann–Whitney *U* test.

Significant effects observed in 3-month-old offspring exposed to the high-dose included decreased absolute and relative (to body weight) vaginal weight, decreased volume of uterine lamina propria, and increased percentage of proliferating uterine glandular epithelial cells. In mice of both dose groups, there were significant increases in expression of ERα and progesterone receptor in uterine luminal epithelial cells; levels of both receptors were also increased in the subepithelial stroma. No treatment effects were observed for apoptosis in uterine luminal and glandular epithelial cells. No treatment effects were observed for vaginal morphometry or cell proliferation. The study authors concluded that environmentally relevant doses of bisphenol A affect the development of the genital tract at the gross and cellular level in the female offspring of mice exposed during pregnancy.

Strengths/Weaknesses: The administration of very low doses is a strength. A critical weakness is the use of DMSO as a vehicle which is known to degrade the pump apparatus, and is inappropriate as a vehicle for in vivo studies. A critical weakness is the uncertainty of the DMSO concentration as a vehicle and therefore pump performance.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process given exposure uncertainties.

Muñoz-de-Toro et al. (2005), supported by NIH and National University of Litoral (Argentina), examined the effect of perinatal bisphenol exposure on mammary gland development in mice. Food, caging, and bedding material were reported to test negligible for estrogenicity in the E-SCREEN. Water was provided in glass bottles. CD-1 mice (n = 6–10/group) were implanted with osmotic pumps designed to deliver bisphenol A **[purity not indicated]** at 0 (DMSO vehicle), 0.000025, or 0.000250 mg/kg bw/day from GD 9 (GD 1 = day of vaginal plug) through PND 3 (not defined). Offspring

were culled to 10 pups/litter on PND 7. One female offspring/litter, from 6–10 litters/group, was killed on PND 20 and 30 and at 4 months of age. The 4-month-old mice were killed on proestrus. Another group of mice **[number not specified]** was killed on the first proestrus. Mammary glands were collected for evaluation of mammary structures at 20 and 30 days and 4 months of age and day of first proestrus. Mammary glands were also collected from 30-day-old mice for analysis of DNA synthesis by BrdU incorporation, expression of ERα and progesterone receptor using immunohistochemistry techniques, apoptosis by TUNEL method, and *Wnt4* mRNA by RT-PCR. Plasma 17β-estradiol levels were measured in mice killed at first proestrus. In an experiment to monitor response to 17β-estradiol, 1 pup/litter ($n = 10$/group) was ovariectomized at 25 days of age and implanted with a s.c. pump supplying vehicle or 0.5 μg 17β-estradiol/kg bw/day on PND 25–35. Mice were killed following 17β-estradiol treatment for examination of mammary structures. Statistical analyses included ANOVA and Dunn post-hoc test. If the data were not normally distributed, statistical analyses were done by Kruskall–Wallis and Mann–Whitney test.

In 30-day-old mice, bisphenol A exposure increased numbers of terminal end buds at both doses and area of terminal end buds at the high-dose. Percentages of apoptotic cells were decreased on PND 30 in mice from both bisphenol A dose groups. The percentage of stromal cells undergoing proliferation on PND 30 was reduced in the high-dose bisphenol A group. The number of epithelial cells expressing progesterone receptors was increased in both dose groups on PND 30, but there were no treatment-related changes in ERα receptor expression. Clusters of progesterone receptors were often observed in the ductal epithelium of bisphenol A-treated mice. Slopes of the correlation between age of first proestrus and mammary length were significantly reduced in the high-dose group, suggesting slower ductal invasion of stroma. There were no significant differences in plasma 17β-estradiol levels in mice killed at first proestrus. Trends for increasing expression of mRNA for *Wnt4*, a mediator of lateral branching downstream from progesterone receptors, did not attain statistical significance. The number of lateral branches in mammary gland at 4 months of age was significantly increased at the low but not the high-dose. In mice exposed to the high-dose of bisphenol A during perinatal development and 17β-estradiol during postnatal development compared to mice who were exposed to 17β-estradiol but not bisphenol A, there were increases in numbers, area, and size of terminal end buds, terminal end bud numbers/ductal area, and terminal end bud area/ductal area. The study authors concluded that "…perinatal exposure to environmentally relevant [bisphenol A] doses results in persistent alterations in mammary gland morphogenesis."

Strengths/Weaknesses: This study was a follow-up on the study of Markey et al. (2005) and tested the same doses using a similar schedule for effects on mammary tissue. The administration of very low doses is a strength. The statistics appear to be inappropriate in not accounting for the significant number of comparisons made. A critical weakness is the uncertainty of the DMSO concentration as a vehicle and therefore pump performance.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process given exposure uncertainties.

3.2.8.2 Male reproductive endpoints:: Nakahashi et al. (2001), supported by the Japanese Ministry of Education, Science, Sports, and Culture, examined the effect of neonatal bisphenol A exposure on adult sperm count in mice. On the first 5 days of life, 10–15 neonatal SHN mice/group were injected **[route not indicated]** with sesame oil/DMSO vehicle or with bisphenol A **[purity not reported]** in sesame oil at 0.0005 or 0.050 mg/day. **[Assuming a neonatal mouse weights 2 g, the mice received doses of 0.25 and 25 mg/kg bw/day.]** A group of 12 mice received 0.050 mg/day bisphenol A in sesame oil in combination with 100 IU retinol acetate in DMSO vehicle. In a second exposure protocol, pregnant mice were fed a vitamin A-deficient diet (Low Vitamin A diet; Clea Japan) from 3 days before gestation to PND 5. After PND 5, the dams were fed commercial diet (CE-7, Clea Japan). On the first 5 days of life, their pups ($n = 7$–9/group) were injected with bisphenol A at 0 (sesame oil) or 0.0005 mg/day. Male offspring from both studies were weaned at 20 days of age and fed the CE-7 diet. Mice were killed at 14 weeks of age and epididymal sperm counts were obtained. **[No information was provided about caging and bedding materials. Numbers of litter represented were not indicated. Procedures for statistical analyses were not discussed.]**

A 35% reduction in sperm counts was observed in mice from the 0.050 mg/day group compared to the control group. A significant reduction in sperm counts was not observed in the group co-treated with 0.050 mg/day bisphenol A and retinol acetate. Administration of a vitamin A-deficient diet to dams had no effect on sperm counts in their offspring, but sperm counts were reduced in mice born to mothers fed a vitamin A-deficient diet and injected with 0.0005 mg/day bisphenol A in the neonatal period. The study authors concluded that vitamin protects infants from the effects of environmental xenoestrogens.

Strengths/Weaknesses: The subcutaneous route of administration, lack of clarity on exposure issues, lack of husbandry and statistical information are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for inclusion and not useful.

Aikawa et al. (2004), supported by the Japanese Ministry of Education, Science, Sports, and Culture, examined the effects of neonatal bisphenol A exposure on sperm endpoints in adult mice. Unless otherwise specified, dams were fed CE-7 and CA-1 (Clea Japan Inc.). **[No information was provided about caging or bedding materials.]** In the first experiment, SHN mice were s.c. injected with bisphenol A, bisphenol A plus retinol acetate, or vehicle for 5 days beginning on the day of birth. Doses of each compound were 0.5 or 50 μg/day bisphenol A **[purity not reported]** ($n = 10$–14/group), 50 μg bisphenol A plus 100 IU retinol acetate/day ($n = 5$), and vehicle control (sesame oil for bisphenol A and or DMSO for retinol acetate; $n = 11$). **[Assuming a neonatal mouse weighs 2 g, these bisphenol A doses would be 0.25 and 25 mg/kg bw/day.]** In another group, pregnant mice were fed a low vitamin A diet from 3 days before gestation to PND 5 and were fed a normal vitamin A-containing diet (CE-7 and CA-1) beginning on Day 6 following parturition **[number/group not stated]**. Pups

born to those dams ($n = 7$–8/group) were s.c. injected with 0.5 μg/day bisphenol A or vehicle for 5 days, beginning on the day of birth. In all groups, mice were weaned at 3 weeks of age, individually housed at 8 weeks of age, and killed at 10 weeks of age. Sperm were collected for analysis of motility and abnormalities. In pups not born to vitamin A-deprived dams, testes were fixed in formalin for histopathological evaluation. Data were analyzed by ANOVA and Fisher least significant difference test.

Sperm motility was significantly reduced in mice injected with 50 μg/day bisphenol A (~25 vs. 50% in controls) but was not affected in mice exposed to 50 μg/day bisphenol A plus retinol acetate. Sperm motility was not affected in mice born to mothers fed a normal diet and exposed to 0.5 μg/day bisphenol. Compared to the vehicle control group born to mothers fed a normal diet, the mice born to mothers fed a vitamin A-deficient diet and injected with 0.5 μg/day bisphenol A had significant reductions in sperm motility **[~19 compared to 50% in vehicle controls]**. Sperm motility was also reduced in the mice born to mothers fed a vitamin A-deficient diet but not exposed to bisphenol A. In groups born to mothers fed a vitamin A-deficient diet, there were no differences in sperm motility following exposure to vehicle or bisphenol A. Percentage abnormal sperm was 6.8% in the vehicle control group and was significantly increased in mice exposed to 0.5 μg/day bisphenol A **[~45%]**, 50 μg/day bisphenol A (78.2%), 50 μg/day bisphenol A plus retinol acetate (27.8%), vehicle following birth to vitamin A-deficient mothers **[~45%]**, or bisphenol A following birth to vitamin A-deficient mother **[~70%]**. No histopathological alterations were reported in testes of mice exposed to 0.5 or 50 μg/day bisphenol A or 50 μg/day bisphenol A plus retinol acetate. The study authors concluded that neonatal exposure to a relatively large dose of bisphenol A damages sperm motility and morphology, effects that are inhibited by vitamin A and enhanced by vitamin A-deficient diets.

In a second experiment, 3 pups/group were s.c. injected with 20 μg 17β-estradiol/day, 20 μg 17β-estradiol plus 100 IU acetate retinol acetate/day, 50 μg bisphenol A/day, or vehicle (sesame oil for bisphenol A and 17β-estradiol or DMSO for retinol acetate) for 5 days beginning on the day of birth. Mice were killed at 18 days of age. Testis, efferent duct, epididymis, and vas deferens were fixed in formalin and analyzed for ERα using an immunohistochemical method. Data were analyzed by ANOVA and Fisher least significant difference test.

In a second experiment, 3 pups/group were s.c. injected with 20 μg 17β-estradiol/day, 20 μg 17β-estradiol plus 100 IU acetate retinol acetate/day, 50 μg bisphenol A/day, or vehicle (sesame oil for bisphenol A and 17β-estradiol or DMSO for retinol acetate) for 5 days beginning on the day of birth. Mice were killed at 18 days of age. Testis, efferent duct, epididymis, and vas deferens were fixed in formalin and analyzed for ERα using an immunohistochemical method. Data were analyzed by ANOVA and Fisher least significant difference test. Bisphenol A exposure had no effect on ERα expression in male reproductive organs. Exposure to 17β-estradiol increased the numbers of ER-positive cells in vas deferens epithelium, but there was no increase when mice were treated with acetate retinol in addition to 17β-estradiol. The study authors concluded that the lack of effect of bisphenol A may be due to its weak estrogenic activity.

Strengths/Weaknesses: This study provided follow-up information to that of Nakahashi et al. (2001). The use of 17β-estradiol as a positive control in the testis histology study is a strength. Weaknesses include subcutaneous route of administration, lack of clarity on exposure issues, small sample sizes, lack of husbandry and statistical information.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful based on small sample sizes and inadequate presentation of statistical methods of analysis.

Toyama and Yuasa (2004), supported in part by the Japanese Ministry of Environment and Ministry of Education, Science, Sports, and Culture, examined the effects of neonatal bisphenol A **[purity not reported]** exposure on spermatogenesis during puberty and adulthood in rats and mice. **[No information was provided about chow or bedding and caging materials. The rat data are reported in Section 3.2.4.]** ICR mice were s.c. injected with bisphenol A in a DMSO and olive oil vehicle on PND 1, 3, 5, 7, 9, and 11 (PND 0 = day of birth). Bisphenol A doses were 0.0001, 0.001, 0.005, and 0.010 mg/kg bw in mice. Additional animals were treated with 17β-estradiol and estradiol benzoate. Animals were killed weekly at 2–10 weeks of age and some pups were also killed at 24 and 31 days of age. There were 5 animals/dose/time point in bisphenol groups A groups and apparently 3–4 vehicle control mice. Testes were examined by light and electron microscopy. Males from each experimental group (a total of 12 mice) were mated with 2 females **[numbers tested in each dose group not reported]**. A total of 12 mouse dams were allowed to complete pregnancy. **[It does not appear that any statistical analyses were conducted.]**

In mature spermatids of 7-week-old mice in the vehicle control group, incidences of deformed acrosome, deformed nucleus, and abnormal ectoplasmic specialization were $<0.3\%$. In 7-week-old mice treated with ≥ 0.001 mg/kg bw bisphenol A, the incidence of deformed acrosome was >50–60%, the incidence of deformed nucleus was $>40\%$, and the incidence of abnormal ectoplasmic specialization was >60–70%. **[Data were not shown for individual dose levels.]** Similar effects were observed in the groups treated with 17β-estradiol and estradiol benzoate. No effects were reported at other ages. **[Data were not shown by study authors.]** The blood–testis barrier remained intact based on histologic observations. All tested males from the bisphenol A group were fertile, and sex ratio, litter sizes, and pup weights were reported to be normal. **[No results were shown for individual dose levels. Fertility data were presented in Table 4 and 5 of the study, but it is not clear which dose level(s) were represented.]** The study authors concluded that bisphenol A acts as an estrogen and induces transient changes in the male reproductive system of rodents that resolve in adulthood.

Strengths/Weaknesses: The strengths include the use of multiple doses of bisphenol A and the use of both rats and mice, allowing interspecies comparisons. Weaknesses include small sample size, unclear data analyses, and s.c. route of administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful due to critically small sample size, route of administration, lack of clarity of design, and inappropriate statistical procedures.

3.2.9 Sheep. **Evans et al. (2004)**, supported by the British Council, Irish Health Research Board, and the Royal Society, examined the effects of bisphenol A exposure on gonadotropin secretion on prepubertal female lambs. **[No information was provided about feed or composition of bedding or caging materials.]** Starting at 3 weeks of age, female Poll Dorset lambs were weighed weekly, and blood samples were collected 2 times/week for measurement of LH and FSH levels. At 4 weeks of age, lambs were assigned to treatment groups according to body weight. From 4–11 weeks of age, 6 lambs/group received biweekly i.m. injections with the 10:1 corn oil/alcohol vehicle, 3.5 mg/kg bw bisphenol A **[purity not reported]**, 0.175 mg/kg bw diethylstilbestrol **[listed as 0.0175 in the legend for Figure 1 of the study]**, or 3.5 mg/kg bw octylphenol. Lambs were ovariectomized at 9 weeks of age. **[The text of the methods sections reported ovariectomy at the beginning of treatment, but that statement appears to be an error because it is not indicated elsewhere in the study.]** On the last day of treatment, blood was collected every 15 min for 6 hr to assess pulsatile LH secretion. All lambs were then killed. Adrenal glands, kidneys, and ovaries were weighed. Uteri were examined as discussed in Morrison et al. (2003). Data were analyzed by ANOVA, Dunnett multiple comparison post-hoc test, regression analysis, Munro algorithm, and paired *t*-tests.

Compared to the control group, the bisphenol A group did not experience significant changes in body, kidney, adrenal, or ovarian weights. **[No data were shown for body, kidney, and ovarian weights in the control vs. bisphenol A group.]** Uteri from the bisphenol A group were reported to be visually larger, but no uterine weights were provided. Over the 7-week treatment period, bisphenol A did not significantly affect blood LH or FSH levels compared to controls. Compared to controls, the bisphenol A group experienced significant decreases **[% change compared to controls]** in concentration **[48%]**, amplitude **[77%]**, and frequency **[66%]** of pulsatile LH secretion. Octylphenol did not have any effect on the endpoints examined. Diethylstilbestrol treatment resulted in decreased blood levels of LH and FSH over the treatment period, including the period following ovariectomy. Concentration, amplitude, and frequency of pulsatile LH secretion were also lower in the diethylstilbestrol group, with a greater magnitude of effect compared to bisphenol A. The study authors concluded that the bisphenol A dose tested can inhibit LH secretion in lambs.

Strengths/Weaknesses: The unique animal model and the use of LH pulsatile response are uncommon but interesting. The high-dose level via i.m. injection is a weakness as are small sample sizes ($n = 6$). The statistical tests for LH trends did not appear to take into account the repeated nature of the sampling leading to over stating the significance of trend effects.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion but of limited utility for the evaluation process.

Morrison et al. (2003), supported by the Wellcome Trust, Dr. Ferranti, and the Irish Health Research Board, examined the effects of bisphenol A exposure on the lamb uterus. **[No information was provided on feed or composition of bedding or caging materials.]** At 4 weeks of age, female Poll Dorsett lambs were randomly assigned to treatment groups according to body weight. Beginning at 4 weeks of age and continuing for 7 weeks, 6 lambs/group received biweekly i.m. injections with the 10:1 corn oil:alcohol vehicle, 3.5 mg/kg bw bisphenol A **[purity not reported]**, 0.175 mg/kg bw diethylstilbestrol, or 3.5 mg/kg bw octylphenol. Lambs were ovariectomized during the fifth week of exposure. Throughout the study, blood was collected for measurement of gonadotropin levels and the results of those analyses were reported in the study by Evans et al. (2004). Lambs were killed following 7 weeks of exposure. Uteri and cervices were fixed in Bouin solution for histopathological examination, morphometric measurement, and immunohistochemical detection of ERα and ERβ. Statistical analyses included ANOVA with Fisher protected least significant difference.

Significant effects observed with bisphenol A treatment **[% change compared to controls]** were increased uterine/cervical tract weight **[87%]**, endometrial area **[154%]**, and endometrial/myometrial ratio **[65%]**. Qualitative histopathological observations in uteri from bisphenol A-treated lambs included endometrial edema, decreased endometrial gland density compared to controls, and crowding of cells in the uterine epithelium, which contained substantial amounts of eosinophilic, non-vacuolated cytoplasm. In contrast to uteri from control lambs, mononuclear cell exocytosis was not a common observation in uteri from the bisphenol A group. The cervical epithelium was keratinized in the bisphenol A group. Qualitative analyses revealed that diffuse intracellular staining for ERα and ERβ in the uterine subepithelium was most pronounced in the bisphenol A and diethylstilbestrol groups. Similar to animals treated with bisphenol A, the diethylstilbestrol group had increased uterine weight, keratinized cervical epithelium, changes in uterine histology, and keratinized cervical epithelium, but there was no change in endometrial/myometrial ratios. No changes were observed following exposure to octylphenol. The study authors concluded that bisphenol A exposure altered the uterocervical environment of lambs.

Strengths/Weaknesses: This is a companion to the study of Evans et al. (2004) with similar strengths. The single high-dose level via i.m. injection is a weakness as is the exclusion of data from 2 lambs based on responses for E/M ratio endpoints, thus reducing the n to 5 and potentially biasing the data. The statistical analyses do not appropriately account for the number of multiple comparison made which can increase the probability of detecting an effect by chance.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion but of limited utility in the evaluation process.

Savabieasfahani et al. (2006), supported by the U.S. Public Health Service, NIH, and the University of Michigan, used Suffolk ewes to investigate the effects of maternal exposure to bisphenol A or methoxyclor **[not discussed here]** during gestation. Pregnant Suffolk ewes used in this experiment were exposed to a natural photoperiod in the same pasture and fed a diet of 1.25 kg alfalfa/grass hay. Pregnant ewes ($n = 10$) of similar

average weight were injected s.c. on GD 30–90 with 5 mg/kg bw day bisphenol A (99+% purity) dissolved in cottonseed oil. Control pregnant ewes ($n = 16$) were administered vehicle injections. Lambs were born over about a 1-month interval in early spring. Birth outcome measurements included number and gender of offspring, weight, height, chest circumference, genital development, and measurement of blood insulin and insulin-like growth factor-1. Lambs were cross-fostered and group housed on PND 3. Lactating ewes were fed a diet of corn and alfalfa hay. Lambs had free access to standardized Shur Gain feed pellets. **[The authors note the presence of phytoestrogens in the feed but did not provide quantification.]** At weaning, female were separated from male offspring, and the females were housed in open air pens under natural photoperiod with free access to feed pellets, as described above.

Maternal blood samples were taken on GD 50, 70, and 90 for measurement of bisphenol A using HPLC. The number and sex of offspring in each treatment group, weight, height, chest circumference, and genital development were noted. Blood levels of insulin and insulin-like growth factor 1 were assayed by RIA on PND 1. In female offspring **[n not indicated]**, blood was drawn biweekly during the first 2 postnatal months for determination of LH by RIA. Timing of puberty onset was estimated through twice weekly blood draws for progesterone ($n = 11$/group). Estrus cycling patterns were determined by frequent measurement of FSH, LH, and progesterone by RIA in 3 female offspring/group after synchronization with prostaglandin F2α at 40 weeks of age. Statistical analyses were performed using ANOVA, repeated measures ANOVA, or a linear mixed model. A cluster algorithm was used to identify LH pulses, with Student *t*-test to determine LH nadirs.

Blood levels of bisphenol A were significantly higher in exposed pregnant ewes than controls at all sampling times. The levels reached (37.4 ± 3.3 μg/L) were compared to exposure levels reported in pregnant women [0.3–18.9 μg/L (Schönfelder et al., 2002b)]. No statistical difference was reported in gestation length, number of offspring, or sex. There were no significant differences in female lambs in anogenital distance, insulin, or insulin-like growth factor levels on PND 1. In female offspring, prenatal bisphenol treatment significantly decreased birth weight **[by ~11%]**, height **[by ~5%]**, and chest circumferences **[by ~7%, all comparisons estimated from a graph]**. In male offspring exposed to bisphenol A, there were no significant differences from control in birth weight, height, chest circumference, or anogenital distance, but anoscrotal:anonavel ratio was increased significantly **[by 21%]**. Bisphenol A treatment increased significantly levels of circulating LH **[by ~89%, estimated from a graph]** during the first 2 months of life in female offspring. Onset of puberty was not affected by treatment in bisphenol A-exposed female offspring, but these females had a significantly longer first breeding season **[by ~2 weeks]** and larger number of cycles during the first breeding season). Estrous cycle length and progesterone levels were not different from controls. The bisphenol A group had significantly lower peak and total LH, and the amplitude of LH pulses was increased significantly, whereas frequency showed no difference from control group. No differences in FSH were seen between groups. Progesterone secretion pattern showed no difference between groups, despite perturbations in LH patterns.

The authors concluded that prenatal exposure to bisphenol A impairs growth in female fetuses and is associated with dampening of the LH surge. Although there was no apparent effect on progesterone production, the authors suggested that the changes induced by prenatal exposure of females could interfere with fertility.

Strengths/Weaknesses: This study appears to have been well-conducted with the utilization of multiple endpoints in sheep. Weaknesses are the use of a single dose level and the relatively small sample size. The single time point for bisphenol A plasma determination at an unknown time relative to s.c. injection is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate though of limited utility.

3.2.10 Non-mammalian species. Although these studies in non-mammalian species can be quite useful for understanding mechanisms and environmental impacts, the studies are not considered useful for the evaluation process, because of the uncertain relationship between human biology and that of the model species.

3.2.10.1 Invertebrates: Hill et al. (2002) supported by the Council on Undergraduate Research and the Association for Biological Laboratory Education, examined the effects of bisphenol A on the development of 2 freshwater sponge species. (*Heteromyenia* sp. and *Eunapius fragilis*). Sponge gemmules were incubated in tissue culture wells containing bisphenol A **[purity not indicated]** at 0, 0.16, 16, 80, or 160 ppm **[mg/L]**. The control group was incubated in the spring water vehicle. There were 5 replicates/treatment. Nonylphenol and ethylbenzene were also examined. Growth was measured on Days 3, 6, and 9. Because growth patterns were similar at all 3 evaluation periods, statistical analyses were conducted only for Day 6 data. Data were analyzed by ANOVA and Tukey multiple comparison test. In both species, abnormal development or malformation of the water vascular system was observed at a bisphenol A dose of 16 ppm and germination was completely inhibited at 80 and 160 ppm. Significantly reduced growth rates were observed in *Heteromyenia* sp. at 160 ppm. Similar effects were observed with nonylphenol and ethylbenzene. The study authors stated that sponges may prove useful for examining endocrine-disrupting compounds.

Strengths/Weaknesses: This study used a unique model with a focus on the aquatic system.

Utility (Adequacy) for CERHR Evaluation Process: This study may have utility for environmental assessment, but is not useful for human risk assessment.

Roepke et al. (2005), supported by the National Oceanic and Atmospheric Administration, examined the effects of bisphenol A exposure on development of two species of sea urchin, *Strongylocentrotus purpuratus* and *Lytechinus anamesus*. In dose–response studies, sea urchin embryos were incubated from 1–96 hr post-fertilization in media containing bisphenol A **[purity not indicated]** at 0, 250, 500, 750, or 1000 μg/L **[culture ware not discussed]**. Development toxicity was assessed at 96 hr by examining larvae at the pluteus stage. The larvae were categorized as normal, delayed, abnormal, elongated, or hatched. Data were obtained in 3 replicates.

Results were reported to be similar for the 2 species, and unless otherwise indicated, data were shown for *S. purpuratus*. In additional studies, sea urchin embryos were incubated in bisphenol A at 0–500 μg/L with and without addition of tamoxifen or bisphenol A at 0–750 μg/L with and without the addition of ICI 182,780. Data were analyzed by ANOVA followed by Tukey–Kramer test or Tukey or Student-Newman–Keuls tests for pair-wise multiple comparison. An EC_{50} of 226.6 μg/L (lower limit: 121.6, upper limit: 323.5 μg/L) was estimated for developmental toxicity associated with bisphenol A exposure. Based on EC_{50} values, 17β-estradiol was ~15 times more potent than bisphenol A. Tamoxifen inhibited developmental toxicity, and ICI 182,780 enhanced the developmental toxicity induced by bisphenol A; similar results were obtained for 17β-estradiol. The study authors concluded that bisphenol A induced developmental toxicity in sea urchins through a tamoxifen-sensitive mechanism at levels exceeding environmentally relevant concentrations.

Strengths/Weaknesses: The use of 2 species and multiple concentrations are strengths.

Utility (Adequacy) for CERHR Evaluation Process: This study may have utility for environmental assessment, but is not useful for human risk assessment.

Andersen et al. (1999b), supported by the Danish Strategic Environmental Research Program, evaluated the effects of bisphenol A on female sexual maturation in the zoo planktonic crustacean *Acartia tonsa*. Eggs were grown in the presence of the algal food source for the organism after exposure of the algae to bisphenol A (>99% purity) for 3 hr to promote sorption by the algae of the test chemical **[culture ware not discussed]**. The treated algae were added to *Acartia tonsa* eggs to give nominal bisphenol A concentrations of 0.2, 2, and 20 μg/L. **[Actual concentrations were not reported. An untreated or vehicle-treated control appears to have been used.]** 17β-Estradiol 23 μg/L was used as a positive control, and 2,3-dichlorophenol 13.6 μg/L was used as a negative control. On Day 8 of incubation, 10–25 juvenile *Acartia tonsa*/group were transformed to an egg-collection apparatus, in which exposure to treated algae continued. Eggs were collected daily and counted until Day 12, at which time a stable adult level of egg production was established. Egg production by group was compared using Student *t*-test. **[A repeated-measures test appears not to have been used.]** A significant increase in egg production was shown on Day 10 in animals treated with bisphenol A 20 μg/L and 17β-estradiol 23 μg/L compared to control. The authors concluded that bisphenol A accelerated female reproductive maturation in *Acartia tonsa* and that the effect appeared to be estrogenic.

Strengths/Weaknesses: Strengths are the use of multiple exposure levels, the inventive method of feeding bisphenol A to the test organisms, and the use of 17β-estradiol as a positive control.

Utility (Adequacy) for CERHR Evaluation Process: This study may have utility for environmental assessment, but is not useful for human risk assessment.

Watts et al. (2001), supported by the European Union, examined development and reproduction in 2 generations of non-biting midges (*Chironomus riparius*) exposed to bisphenol A. The study began with incubation of 4 egg ropes/group in media containing vehicle, bisphenol A, or ethinyl estradiol **[apparently at the same concentrations described below]**. Twenty first-instar larvae from the appropriate media were added to each exposure glass jar containing dechlorinated water and sediment spiked with bisphenol A **[purity not indicated]** at concentrations of 0 (ethanol vehicle control and dechlorinated tap water control), <0.010, 0.078, 0.55, 77, 750, or 10,400 μg/L. Four replicate jars were prepared for each dose level. Concentrations in sediment were verified. Numbers and sexes of adults emerging from each replicate jar were determined. Egg ropes produced by the first generation were counted and placed in media containing test solutions or vehicle controls. Four egg ropes/group were selected and used to reseed the sediments with the second generation of larvae. Adults emerging from the second generation were counted. Statistical significance was determined by ANOVA. In the first generation, adult emergence was delayed in females from the <0.010, 0.55, and 77 μg/L bisphenol A groups but was not affected in males. Males were reported to emerge significantly earlier than females. In the second generation, emergence of males and female adults was significantly delayed at ≥0.078 μg/L bisphenol A. At concentrations of 0.010–750 μg/L, there were no significant differences in the percentage of adults emerging in either generation. No second-generation adults emerged in the group exposed to 10,400 μg/L. There were no effects on sex ratio. Exposure to bisphenol A did not significantly affect the number of eggs produced by the first generation. In contrast to bisphenol A, exposure to ethinyl estradiol accelerated adult emergence. The study authors concluded that the endpoints evaluated indicated general sediment toxicity but were not useful for detecting estrogenic effects.

Strengths/Weaknesses: The wide range of exposure levels and the use of ethinyl estradiol as a positive control are strengths.

Utility (Adequacy) for CERHR Evaluation Process: This study may have utility for environmental assessment, but is not useful for human risk assessment.

Watts et al. (2003), supported by the European Union, examined the effects of bisphenol A exposure on moulting and mouthpart deformities in non-biting midge (*Chironomus riparius*) larvae. Four egg-ropes/group were incubated in glass jars in media containing bisphenol A **[purity not indicated]** at 0 (ethanol vehicle or dechlorinated water group), 0.010, 0.1, 1, 10, 100, or 1000 μg/L. Concentrations of bisphenol A were verified in the 1000 μg/L group. On hatching, exposures were continued in 10 larvae/group. Endpoints examined included survival, time of moulting to successive instars, wet weight 2 days after moulting to fourth instar, and mouthpart morphology in fourth-instar head capsules. Statistical analyses included ANOVA, Tukey–Kramer multiple comparison test, and Kruskal–Wallis test. **[Effects were similar in ethanol and water controls.]** Moulting was delayed and larval weights were significantly decreased in the 1000 μg/L bisphenol A group. Deformities of the mentum were significantly increased in the range of 0.010–1 μg/L bisphenol A. The effects of ethinyl estradiol were also examined, and the study authors noted similar patterns of malformations, with greater incidence following exposure to ethinyl estradiol than bisphenol A. The

study authors concluded that exposure to bisphenol A delayed moulting and increased mouth part deformities at concentrations that were at opposite ends of the exposure range.

Strengths/Weaknesses: This study is similar in its strengths to that of Watts et al. (2001).

Utility (Adequacy) for CERHR Evaluation Process: This study may have utility for environmental assessment, but is not useful for human risk assessment.

3.2.10.2 Frog: Iwamuro et al. (2003), support not indicated, conducted a series of studies to examine the effects of bisphenol A exposure on development of the frog *Xenopus laevis*. In a study to assess survival and morphological abnormalities, 60–100 Stage 7 embryos/group were exposed to bisphenol A **[purity not indicated]** at 0 (ethanol vehicle), 10, 20, 25, 30, 50, or 100 μM **[0, 2.3, 4.6, 5.7, 6.8, 11, or 23 mg/L; culture ware not discussed]**. Siblings were randomly distributed among different treatment groups. Survival was assessed at 48, 96, and 120 hr. At least 3 embryos/group were examined for malformations at 5–7 days following fertilization. Data were analyzed by χ^2 test. Survival of embryos was significantly reduced following exposure to ≥25 μM **[5.7 mg/L]** bisphenol A for 96 or 120 hr. Complete mortality was observed at concentrations ≥50 μM **[11 mg/L]**. The study authors calculated a median LD_{50} for survival of 21 μM **[4.8 mg/L]**. The malformation rate was reported for the 10 and 25 μM **[2.3 and 4.6 mg/L]** group, and significant increases in malformations occurred in the 25 μM **[4.6 mg/L]** group. The types of malformations were reported as scoliosis, swollen head, and shortened distance between eyes. The effects of 17β-estradiol were also examined. An increase in malformations was observed with exposure to 10 μM 17β-estradiol, but there was no effect on survival.

In a second study, metamorphosis was observed in 10–12 tadpoles (Stage 52) placed in solutions containing 10 or 25 μM **[2.3 or 5.7 mg/L]** bisphenol A **[purity not indicated]** with and without the addition of 0.1 μM thyroxin for 21 days. Expression of thyroid hormone receptor-β gene was measured by RT-PCR in three regions (head, trunk, and tail) of tadpoles that were exposed to 10 or 100 μM **[2.3 or 23 mg/L]** bisphenol A with and without the addition of 0.1 μM triiodothyronine or thyroxin. Negative controls were exposed to ethanol/DMSO vehicle. Metamorphosis data were analyzed by Duncan new multiple range test. Bisphenol A inhibited significantly both spontaneous and thyroxine-induced metamorphosis. All concentrations of bisphenol A reduced expression of thyroid hormone receptor-β hormone and inhibited increases in thyroxine- and triiodothyronine-induced expression.

In a third study, tails were removed from 4 tadpoles/group and cultured for 4 days in media containing 10 or 100 μM **[2.3 or 23 mg/L]** bisphenol A with and without the addition of 0.1 μM triiodothyronine. Negative controls were exposed to ethanol/DMSO vehicles. Data were analyzed by Duncan new multiple range test. Growth of the tails was measured over a 4-day period. Neither bisphenol A dose significantly affected tail growth. Both bisphenol A doses blocked tail shortening that was induced by triiodothyronine. The study authors concluded that high-doses of bisphenol A adversely affect development of *Xenopus laevis* embryos and larvae.

Strengths/Weaknesses: The wide range of exposure levels is a strength.

Utility (Adequacy) for CERHR Evaluation Process: This study may have utility for environmental assessment, but is not useful for human risk assessment.

Oka et al. (2003), support not indicated, examined the effects of bisphenol A exposure on development of the frog *Xenopus laevis*. Embryos were exposed to the ethanol vehicle or 10–100 μM **[2.3–23 mg/L]** bisphenol A from developmental stage 6 until the early tadpole stage (late stage 10) **[purity not indicated, and culture ware not discussed]**. Embryos were harvested at Stages 19, 23, 33/34, and 40 and prepared for histological examination to determine the presence of apoptotic cells. Apoptosis was also assessed using a TUNEL staining method. Ten embryos were killed at the tail bud stage (Stage 35/36, 37/38, and 40), and genomic DNA was isolated and examined by electrophoreses to determine if 180 base pair ladders indicative of apoptosis were present. **[No information was provided on the number of individual doses examined or the number of embryos exposed/dose. No quantitative data were presented by authors, and it does not appear that data were statistically analyzed.]** Embryos exposed to 40–100 μM **[9.1–23 mg/L]** bisphenol A died during the gastrula stage. Developmental abnormalities were observed in embryos exposed to 20 μM **[4.6 mg/L]** bisphenol A. The abnormalities included open neural tubes at Stage 19, morphological defects at Stages 23 and 33/34, and crooked vertebrate, swollen abdomen, and malformed head at Stage 40. Malformations persisted following Stage 40, and death occurred during the tadpole stage. In Stage 33/34 and 40 embryos of the 20 μM **[4.6 mg/L]** group, apoptotic cells were observed in the prosencephalon, mesencephalon, rhombencephalon, and spinal cord. Apoptosis was confirmed using the TUNEL staining method. Using the DNA ladder method, it was found that apoptosis also occurred at Stages 35/36, 37/38, and 40. The authors briefly stated that they tested Stage 10, 19, or 23 embryos and found normal development following bisphenol A exposure. **[No additional details were provided.]** The effects of 17β-estradiol were also examined. Malformations were observed in embryos exposed to 10 μM 17β-estradiol, but apoptotic cells were not observed in the nervous system. A very brief description was provided of a study in which embryos were exposed simultaneously to 20 μM **[4.6 mg/L]** bisphenol A and 1–10 μM 17β-estradiol. Co-exposure with 17β-estradiol did not inhibit bisphenol A-induced apoptosis. The study authors concluded that bisphenol A induced malformations and apoptosis in *Xenopus laevis* at concentrations exceeding environmental levels and that the effects did not appear to occur through an estrogenic mechanism.

Strengths/Weaknesses: The use of 17β-estradiol exposure to suggest a non-estrogenic mechanism of bisphenol A toxicity is a strength. The omission of some important details and the high concentrations are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study may have utility for environmental assessment, but is not useful for human risk assessment.

Sone et al. (2004), supported by the Japanese Ministry of Environment and Ministry of Education, Culture, Sports, Science, and Technology, examined the effects of

bisphenol A exposure on the development of *Xenopus laevis* embryos. Three different sets of experiments were conducted. Data were analyzed by ANOVA followed by Fisher protected least significant difference test. From 3–96 hr following fertilization, embryos were exposed to bisphenol A **[purity not indicated]** at 1, 2.5, 5, 10, 15, 20, 25, or 30 μM (0.3, 0.6, 1.1, 2.3, 3.4, 4.6, 5.7, or 6.8 mg/L). Each exposure was replicated 3 times. Negative control groups consisted of the ethanol vehicle, medium alone, or dilution medium. Rates of normal embryo development were equivalent in the 3 different negative control groups. In groups exposed to ≥20 μM bisphenol A, there was a significant decrease in normal embryos and a non-significant increase in mortality rate. Teratogenicity was characterized by short body length, microcephaly, flexure, edema, and abnormal gut coiling. Increases in embryo abnormalities were also observed following exposure to ≥10 μM 17β-estradiol or nonylphenol.

To determine sensitive stages, embryos were exposed to control media or 20 μM **[4.6 mg/L]** bisphenol A for 45–48-hour periods ranging from 3–48 hr post-fertilization, 12–60 hr post-fertilization, 24–72 hr post-fertilization, 36–84 hr post-fertilization, or 48–96 hr post-fertilization. Body length, gross malformations, and distance between eyes were measured at 96 hr following exposure. **[The Methods section indicated that 59–71 embryos were examined in the bisphenol A group for each time period of exposure. However, a figure in the study reported the sample size as 3/time period.]** During the period of 3–48 hr following fertilization, statistically significant effects in the bisphenol A group included decreased body length and increased incidences of microcephaly, flexure, edema, and abnormal gut coiling. No increases in abnormal effects were observed following exposure at later time periods. Abnormalities were observed following exposure to 17β-estradiol or nonylphenol at early or late stages.

In the third part of the study, embryos were exposed to 20 μM **[4.6 mg/L]** bisphenol A from 3–96 hr following fertilization. RNA was isolated from whole embryos and subjected to analysis by cDNA microarray. Results obtained in microarray analyses were confirmed by PCR analysis. The sample size was reported as 2. The microarray analysis revealed 179 upregulated and 103 downregulated genes following exposure of embryos to bisphenol A. The study authors identified 27 genes in which expression was changed following exposure to bisphenol A, nonylphenol, or 17β-estradiol. The identified genes included: *KNP-Ia*, *CmaB*, *XIRG*, α-skeletal tropomyosin, apelin, cyclin G1, *Ube213*, *HGF*, toponin C2, ribosomal protein L9, and *Rattus norvegicus* similar to *CG10042-PA*. The other genes were not identified. The study authors concluded that these findings might provide clues to deciphering mechanisms of teratogenic effects associated with bisphenol A and the other compounds examined in this study.

Strengths/Weaknesses: The inclusion of 17β-estradiol as a comparator was a strength and the high bisphenol A concentration is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation process.

Pickford et al. (2003), supported by the Bisphenol A Global Industry group, the Society of the Plastics Industry, the Bisphenol A Sector Group of the European Chemical Industry Council, and the Japan Chemical Industry Association, examined the effects of bisphenol A exposure on development of frog gonads. Beginning at Stage 43/45 (~2 days post-hatching, 4 days post-fertilization, exposure day 0) and continuing through Stage 66, *Xenopus laevis* larvae were exposed to bisphenol A **[purity not indicated]** at nominal concentrations of 0 (water control), 1.0, 2.3, 10, 23, 100, or 500 μg/L in a flow-through test system **[culture ware not discussed]**. Actual concentrations were verified as 0.83, 2.1, 9.5, 23.8, 100, and 497 μg/L. A positive control group was exposed to 2.7 μg/L 17β-estradiol. There were 4 replicate test vessels/dose, with each containing 40 larvae (i.e., 160 larvae/test condition). Larvae were observed daily for mortality, behavior, and appearance. Growth and development were assessed on all larvae of a replicate tank on exposure days 32 and 62 (36 and 68 **[66?]** days post-fertilization). Froglets were killed and observed at completion of metamorphosis (Stage 66). Total length was measured, sex was determined, and testes and ovaries were assessed for abnormalities such as asymmetry, complete absence, presence of melanocytes, irregular shape, segmentation or fragmentation, vacuoles, and ambiguous sexual morphology. Data were analyzed by Fisher exact test, ANOVA, Wilcoxon rank sum test, *G* test, and χ^2 test. Following exposure to bisphenol A, there were no significant differences in survival, distribution of developmental stages on Day 32 or 62, time to completion of metamorphosis (Stage 66), or length of Stage 66 froglets. Bisphenol A exposure did not affect sex ratio or abnormalities in testis or ovary **[data were not shown by authors for testis and ovary effects]**. In contrast, exposure to 17β-estradiol resulted in an increase in ratio of females to males and testicular and ovarian abnormalities. The study authors identified a no-observed-effect concentration of 500 μg/L for bisphenol A.

Strengths/Weaknesses: The use of a wide range of exposure levels is a strength, but the incomplete data presentation with missing organ weight data and the lack of histological evaluations are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study may have utility for environmental assessment, but is not useful for human risk assessment.

Levy et al. (2004), supported by the Ministry of Environment and Traffic of Baden-Württemberg, evaluated the effect of bisphenol A on gonad development in *Xenopus laevis* tadpoles. Tadpoles ($n = 40$/group) were exposed beginning at Stages 42/43 to ethanol vehicle or to bisphenol A (>99% purity) or 17β-estradiol, both at concentrations of 10^{-8} or 10^{-7} M **[bisphenol A concentrations 2.3 and 23 μg/L. Actual concentrations were 90–105% of target concentrations after addition of bisphenol A to the media but decreased to low levels by the end of the 48-hr period between media changes. Culture ware was not discussed.]** After completion of metamorphosis, froglets were killed for examination of gonads. Tadpoles not completing metamorphosis were killed after 120 days of chemical exposure for examination of gonads. In a second experiment, bisphenol A concentrations were 10^{-8}, 10^{-7}, or 10^{-6} M **[2.3, 23, or 228 μg/L]** and the 17β-estradiol positive control used a concentration of 10^{-7} M. In a third experiment, 50 tadpoles/group were treated for 2 weeks with ethanol vehicle, bisphenol A 10^{-7} M **[23 μg/L]**, or 10^{-7} M 17β-estradiol after which whole-body homogenates were

used for extraction of RNA and determination of *ER* by RT-PCR. Statistical analyses were performed with Kruskal–Wallis *H* test followed by Mann–Whitney U test. The gonadal sex of control animals was 56% male and 44% female. 17β-Estradiol treatment increased the female ratio to 81% at 10^{-7} M and 84% at 10^{-8} M. Bisphenol A treatment resulted in a significant increase in females (69%) at 10^{-7} M **[23 μg/L]**. At 10^{-8} M bisphenol A, there were 65% females, which did not reach statistical significance. In the second experiment, a significant increase in females was seen after treatment with 10^{-7} M **[23 μg/L]** (70%, compared to 48% in controls and 96% with 17β-estradiol treatment). There was no significant effect of bisphenol A at 10^{-8} M **[2.3 μg/L]** (51% female) or 10^{-6} M **[228 μg/L]** (53% female). Bisphenol A and 17β-estradiol both resulted in increased *ER* mRNA. The authors concluded that bisphenol A affects the sexual development of *Xenopus laevis*, probably through an estrogenic mechanism.

Strengths/Weaknesses: The measurement of bisphenol A in the media is a strength, but its lack of stability is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation process.

Yang et al. (2005), supported by the Chinese Ministry of Science and Technology, examined the effects of bisphenol A exposure in black-spotted pond frog tadpoles. Thirty tadpoles/tank were exposed in duplicate to bisphenol A (≥95% purity) at concentrations of 0, 0 (+DMSO vehicle), 2, 20, or 200 μg/L **[ppb]** for up to 60 days **[culture ware not discussed]**. Tadpoles were also exposed to mixtures containing bisphenol A+ nonylphenol at 2+2, 20+20, or 200+200 μg/L. Additional tadpoles were exposed to mixtures containing the same bisphenol A/nonylphenol mixtures in addition to *p,p′*-DDE 2+2+0.5, 20+20+5, or 200+200+50 μg/L. Five tadpoles/tank were pooled at 15, 30, 45, and 60 days. The tadpoles were homogenized for measurement of testosterone and thyroxin levels by radioimmunoassay. Alkaline-labile phosphate was measured as a biomarker for vitellogenin. Data were analyzed by ANOVA.

Malformations of tail flexure were observed in 10% of tadpoles exposed to 200 μg/L bisphenol for 45 days, and similar rates of malformation (13.3%) were observed in the mixtures containing 200 μg/L bisphenol A. A "decrease" (not statistically significant) in thyroxine levels was observed following 60 days of exposure to all bisphenol A doses (≥2 μg/L). "Increases" (not statistically significant) in testosterone levels were reported with all bisphenol A doses at 30 days of exposure. *p,p′*-DDE at ≥5 μg/L inhibited increases in testosterone level observed with mixtures of bisphenol A and nonylphenol **[not statistically analyzed]**. "Increases" (not statistically significant) in alkaline-labile phosphate levels were reported following 30 or more days of exposure to all bisphenol A doses. In animals exposed to bisphenol A and nonylphenol in combination compared to either compound alone, alkaline-labile phosphate levels were increased at 15 days of exposure but decreased at 60 days of exposure **[not statistically analyzed]**. *p,p′*-DDE inhibited the increase in alkaline-labile phosphate levels induced by the bisphenol A+ nonylphenol mixture on Day 15 of exposure **[not statistically analyzed]**.

Strengths/Weaknesses: The lack of attention to statistical analysis is a weakness and makes the authors' conclusions unreliable.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Imaoka et al. (2007), supported by the Japanese Ministry of Education, Science, Culture, Sports, and Technology, evaluated the effects of bisphenol A on development of the African clawed frog, *Xenopus laevis*. Embryos were cultured with bisphenol A from Stage 10.5, formation of the neural plate, to Stage 35 at a bisphenol A (in DMSO) concentration of 25, 50, or 100 μM **[5.8, 11, or 23 mg/L]**. Tadpoles were morphologically evaluated at Stages 28–35. Total RNA was extracted and reversed transcribed and RT-PCR used to quantify the expression of specific genes. Expression levels relative to β-actin or histone H4 were compared with Student *t*-test. Abnormalities in the head and eye region were described with a "minor effect" at 25 μM and a "major effect" at 50 μM bisphenol A. **[Data were not shown.]** There were no treatment-related effects on expression of *sox-2*, *nrp-1*, *myoD*, *sox17α*, or notch. Relative expression levels of *pax-6* declined in a concentration-related manner to about 56% if control at the high concentration **[estimated from a graph]**. Relative expression levels of *esr-1* decreased in a concentration-dependent manner to about 22% of control at the high concentration **[estimated from a graph]**. Microinjection into blastomeres of plasmids containing NICD (the intracellular domain of notch), but not of X-delta-1 (a notch ligand) corrected the decreased expression of *esr-1*. The authors concluded that bisphenol A decreased *esr-1* expression by disrupting notch signaling.

Strengths/Weaknesses: This is an interesting study on the molecular alterations induced in frog embryos exposed to BPA. The study demonstrated alterations in several key developmental genes and malformed development at high concentrations. The high concentrations are, however, weaknesses and the effects of uncertain concern to human health because humans would not be exposed in this manner.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

3.2.10.3 Fish: Kishida et al. (2001), supported by the National Science Foundation and U.S. EPA, included bisphenol A in a study to test the utility of changes in CYP450 aromatase mRNA expression as a marker of xenoestrogen effects in the CNS of zebrafish (*Danio rerio*). Fish embryos were incubated in solutions containing bisphenol A **[purity not indicated]** at 0 (DMSO vehicle), 0.01, 0.1, or 10 μM **[0, 2.3, 23, or 228 μg/L]** from 2–48 hr post-fertilization **[culture ware not discussed]**. Expression of the CYP450 aromatase gene was determined in 50 embryos/treatment group using an RT-PCR/Southern blot technique. **[There was no mention of statistical analyses of data.]** The Southern blot analysis revealed a ~3-fold increase in the band intensity of CYP450 aromatase at the high concentration (10 μM) of bisphenol A. The potency of bisphenol A was determined to be lower than those of 17β-estradiol and diethylstilbestrol, which induced ~3–4-fold increases in band intensity at concentrations up to 3 orders of magnitude lower than bisphenol A. In additional experiments with exposure to bisphenol at 2–48 hr post-fertilization, embryo mortality was increased by exposure to 10 and 20 μM **[228 and**

457 μg/L] bisphenol A and malformations (curved tails) were increased by exposure to 20 μM. The effects were similar to those observed with 17β-estradiol, but bisphenol A was less potent. **[Very few protocol details were provided, and no data were shown by study authors for mortality and malformation endpoints.]** The study authors concluded that bisphenol A could act as a developmental neurotoxicant by upregulating CYP450 aromatase expression but that further studies were needed to determine if there are changes in neural estrogen biosynthesis or CNS development.

Strengths/Weaknesses: A weakness of this study for the current evaluation is the lack of morphometric data. The significance of the observed change in aromatase is not clear.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Segner et al. (2003), supported by the European Commission, examined estrogenicity responses and in vivo life cycle effects in zebrafish exposed to bisphenol A. Estrogenicity studies are discussed in Section 2. One hundred fertilized eggs/vessel were exposed to bisphenol A (98% purity) at 0, 94, 188, 375, 750, or 1500 μg/L under semistatic conditions **[culture ware not discussed]**. Exposures were continued until fish became sexually mature. The numbers of fish/vessel were adjusted to 50 following 42 days of exposure and 30 following 75–78 days of exposure. Two replicates were examined. Bisphenol A concentrations were confirmed by GC/MS. Endpoints evaluated included survival, behavior, growth, time to first spawning, egg production, and fertilization success (percent fertilized eggs/vessel/day). Statistical analyses included ANOVA and William test. EC_{50}-values were calculated by probit analysis and analyzed by Kruskal–Wallis and Mann–Whitney *U* tests. 17β-Estradiol, ethinyl estradiol, and 4-*tert*-octylphenol were also examined using similar protocols. The authors only discussed results for reproductive success because they stated that it was the most consistent and reproducible effect following exposure of the fish to estrogenic substances. An EC_{50}-value of 6140 nM **[1.4 mg/L]** bisphenol A was obtained for fertilization success, and the study authors stated that the value exceeded concentrations typically found in the environment. Bisphenol A had a relative potency of 0.0000006 compared to17β-estradiol and was 45 times less potent than 4-*tert*-octyl-phenol. The study authors concluded that the in vivo potency of the compounds was overestimated by in vitro estrogenicity assays (described in Section 2).

Strengths/Weaknesses: This study was well performed.

Utility (Adequacy) for CERHR Evaluation Process: This study is useful in showing a lack of effect on fertilization at environmentally relevant concentrations of bisphenol A, but not useful to the evaluation process.

Metcalfe et al. (2001), supported by the Environmental Science and Technology Alliance Canada, the Natural Sciences and Engineering Research Council of Canada, and Health Canada, in glass jars, exposed medaka (*Oryzias latipes*) from 1 day after hatching until 85–110 days after hatching to bisphenol A **[purity not indicated]** at 0, 10, 50, 100, or 200 μg/L ($n = 60$ fish/treatment). Over the 48 hr between media change, actual concentrations were a mean 59.6% of nominal concentrations. Fish were killed and embedded in paraffin for section. Gonads were evaluated to determine the sex of the fish and whether testes contained ova, an intersex condition. Length and weight of the animals and sex ratio were not altered by treatment **[statistical methods not reported]**. There were 2 instances of intersex gonads in males exposed to bisphenol A 10 μg/L and no instances at higher concentrations. Histologic changes in testes including a reduction in germ cells were noted at 50 μg/L and higher. At 200 μg/L, oogenesis in females was more advanced than in controls.

Strengths/Weaknesses: Strengths of this study are the step-sectioning of gonads and the use of several positive control estrogens, which worked as expected.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful to the evaluation process.

Yokota et al. (2000), supported by the Japanese Environment Agency, exposed medaka (*Oryzias latipes*) to bisphenol A (>99% purity) at 0, 3.2, 16, 80, 400, or 2000 μg/L from fertilization until 60 days after hatching ($n = 60$/treatment) **[culture ware not discussed]**. Actual bisphenol A concentrations were generally within 3% of nominal concentrations before hatching. After hatching, the lower 2 concentrations were ~70–80% of nominal and the higher concentrations were ~90% of nominal. Fish were assessed for survival, time to hatching, and growth. Sixty days after hatching, 19 or 20 fish/treatment were killed and sectioned for examination of the gonads using hematoxylin and eosin staining of fixed specimens. Statistical analysis was performed using ANOVA and nonlinear regression. Hatchability was >90% in all treatment groups. Time to hatch and mortality were not affected by treatment, although there was a non-concentration dependent delay in hatching at 13 μg/L. Body length and weight 60 days after hatching were negatively correlated with bisphenol A concentration, and length and weight at 2000 μg/L were significantly lower than control values on pair-wise comparison. Based on external appearance and gonad examination, there were more females than males at 400 μg/L and there were no males at 2000 μg/L. Control sex ratio was 2:1 (male:female). There were 6 fish with intersex gonads among the 19 examined in the 2000 μg/L group. The authors concluded that bisphenol A adversely affects the early life stage of medaka with alteration of sexual differentiation.

Strengths/Weaknesses: This study was well performed.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful to the evaluation process.

Pastva et al. (2001), support not indicated, examined the effects of bisphenol A exposure on development of medaka (*Oryzias latipes*). In a study examining abnormalities in embryos, 5 eggs were placed in individual glass vials containing bisphenol A **[purity not indicated]** at 0, 20, or 200 μg/L. There were 5 glass vials/exposure concentration, for a total of 25 embryos/group. The exposure period began 5 hr following fertilization and was continued for 9 days. Embryos were examined for malformations daily by observing them through the clear protective membrane of the egg. The severity of malformations was scored and severity indices were determined. In a second study examining mortality, newly hatched larvae were exposed for 96 hr to a method control solution, ethanol vehicle control solution, or 200 μg/L bisphenol A. Ten larvae were added to each

jar, and there were 3 replicates/test solution (i.e., 30 larvae/concentration). Data were analyzed by *t*-test. The malformation severity index was increased significantly at 5–8 days following fertilization in embryos exposed to 200 μg/L bisphenol A, but the severity index did differ significantly from the control value on Day 9. Abnormalities consisted of pericardial edema, hemorrhage, and hemostasis. Larval mortality was not affected by exposure to 200 μg/L bisphenol A. The study authors concluded that exposure to environmentally relevant concentrations of bisphenol A resulted in embryonic deformities in medaka, but that the embryos were able to repair the abnormalities before hatching.

Strengths/Weaknesses: This study using medaka is similar in design to the FETAX assay, which uses *Xenopus*. These types of assays have not been demonstrated to have relevance for human risk assessment.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Lee et al. (2003b), supported by Jeonnam Regional Environment Technology Development Center, exposed 51-day-old Korean rockfish (*Sebastes schlegeli*) fry to bisphenol A in feed at 0, 0.05, 0.5, 5, 50, and 100 mg/kg diet for 29 days **[purity of bisphenol A, stability in feed, and culture ware not indicated]**. At the end of the experiment, gonads were removed and sex determined by light microscopy of stained sections. There was no effect of bisphenol A on sex ratio compared to controls. **[The data presentation and statistical analysis are unclear: the number of female fish and number of male fish in each dose group are presented as averages with an unspecified error and analyzed by Student *t*-test. Whole numbers would have been expected with χ^2 analysis.]** The authors concluded that there was no estrogenic effect of bisphenol A on sex differentiation in the Korean rockfish.

Strengths/Weaknesses: The use of a positive control, which worked as expected, is a strength of this study. The inadequate presentation of data and statistical analysis is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Honkanen et al. (2004), supported by the Finnish Graduate School of Environmental Science and Technology and the Academy of Finland, examined the effects of bisphenol A exposure on yolk-sac fry of landlocked salmon. Ten 8-day-old fry/beaker were exposed to bisphenol A **[99% pure]** at concentrations of 0, 10, 100, or 1000 μg/L for 42 days, in glass beakers. The ethanol vehicle and pure tap water were used as negative controls. There were 3–4 replicates/dose. One fry/beaker was photographed and killed following 6 days of exposure. After 6 weeks of exposure, all remaining fry were blotted and weighed. Three fry/beaker were photographed and 3 fry/beaker were examined histologically. Statistical analyses included ANOVA and Tukey test. Effects observed in fry exposed to the highest bisphenol A concentration included: yolk sac edema and hemorrhaging around gill arches and the front part of the yolk sac at 6 days of exposure; phlegmatic behavior (lack of activity during siphoning to renew solutions) on Day 8 of exposure; and darkening of color at Day 17 of exposure. No increases in mortality were observed. At the end of the exposure period, wet weights were increased in fry exposed to the highest concentration, and the study authors stated that the effect was due to fluid accumulation. In fry exposed to the mid- and high-concentration of bisphenol A, strongly stained fragments were observed in nuclei and storage substances in liver were decreased. No abnormalities were observed in histological examinations of heart, kidney, and thyroid gland. The study authors concluded that bisphenol A induced toxicity in fry at concentrations rarely found in the environment.

Strengths/Weaknesses: The range of concentrations used in this study is a strength.

Utility (Adequacy) for CERHR Evaluation Process: The finding of an effect only at a high concentration of bisphenol A may have importance for environmental assessments but is not of utility in the current evaluation process.

3.2.10.4 Reptile and bird: Stoker et al. (2003), supported by the Argentine National Agency for the Promotion of Science and Technology and Argentina Ministry of Health, examined the effects of in ovo bisphenol A exposure on sexual development of the crocodilian reptile *Caiman latirostris*. A preliminary experiment was conducted to determine the effects of temperature on sex determination, and it was established that incubation at 30°C resulted in production of females while incubation at 33°C resulted in the production of males. In the main experiment, eggs were collected from 5 nests in Argentina. Half the eggs were incubated at 30°C and the other half at 33°C. Care was taken to avoid exposing eggs to putative sources of estrogens such as spray paint, plastic, and nesting materials. At each incubation temperature, eggs from each nest were distributed among treatment groups. Twenty days following collection, 1 egg/nest/incubation temperature was opened for stage determination. At developmental stage 20, bisphenol A **[purity not indicated]** was applied topically to the eggshell at concentrations of 1.4 or 140 ppm (0.09 or 9 mg/egg). Other eggs were treated with 0.014 or 1.4 ppm 17β-estradiol. Control eggs were left untreated or exposed to the ethanol vehicle. Hatchlings were weighed and measured at birth. At 10 days of age, 4 animals/group/incubation temperature were killed for determination of sex by examination of internal genitalia. Sex determination was confirmed by histological evaluation of organs, which were fixed in 10% buffered formalin. Morphometric analysis of seminiferous tubules was also conducted in 10-day-old animals. The remaining animals (6–11/group/incubation temperature) were raised until 6 months of age, at which time they were killed, measured, and sexed by examination of external genitalia. Evaluators were blinded to treatment conditions. Statistical analyses included Kruskal–Wallis ANOVA and Mann–Whitney *U* test.

At 33°C, there was 100% sex reversal in the high-dose bisphenol A and high-dose 17β-estradiol groups at 10 days and 6 months of age. Whereas 100% of control and low-dose animals in the 33°C group were male, 100% of animals in the high-dose bisphenol A and 17β-estradiol group were female. Although there was no sex reversal in the low-dose bisphenol A or 17β-estradiol groups incubated at 33°C, morphometric evaluations at 10 days of age revealed significantly increased perimeter of seminiferous tubules, which had empty lumens. There

were no significant effects reported for bisphenol A following incubation at 30°C. The study authors concluded that bisphenol A induced estrogenic effects in caiman as evidenced by reversed gonadal sex and disrupted gonadal histoarchitecture.

Strengths/Weaknesses: This study appears to have been well performed and the use of a positive control is a strength. A weakness is the expression of exposure level in terms of total egg weight, which precludes easy comparison to human exposure levels.

Utility (Adequacy) for CERHR Evaluation Process: This study has no utility in the evaluation process.

Berg et al. (2001), supported by the Foundation for Strategic Environmental Research and the Swedish Council for Forestry and Agricultural Research, examined the effects of bisphenol A exposure on development of sex organs in quail and chicken embryos. The effects of tetrabromobisphenol A were also examined but will not be discussed. Bisphenol A (99.4% purity) was injected into yolk of Japanese quail eggs on the third day of incubation and into chicken (domestic fowl) eggs on the fourth day of incubation at doses of 0 (propylene glycol vehicle), 67, and 200 μg/g egg. Eggs were also injected with diethylstilbestrol at doses of 2, 20, and 200 ng/g egg **[culture ware not discussed]**. Two days before the anticipated hatching date, embryos were examined for mortality (32–43 quail embryos and 34–91 chicken embryos/group examined) and müllerian duct abnormality or testicular histopathology (8–15 quail embryos/group and 7–30 chicken embryos/group examined). Testes were fixed in 4% formalin. Data were analyzed by Fisher exact probability test.

Exposure to bisphenol A did not increase mortality in quail embryos. Incidence of females with abnormal müllerian ducts was increased in quail embryos exposed to the high bisphenol A dose but the incidence of ovotestis in males was not increased by bisphenol A exposure. Mortality of chicken embryos was increased following exposure to both bisphenol A dose levels. The incidence of male chicken embryos with ovotestis was increased at the high-dose of bisphenol A but there was no effect on females with abnormal müllerian ducts. Effects observed in one or more diethylstilbestrol groups included increased incidence of females with abnormal müllerian ducts in quail embryos and males with ovotestis in quail and chicken embryos. Based on study findings, the study authors concluded that bisphenol A can cause estrogen-like malformations in reproductive organs of birds.

Strengths/Weaknesses: The detailed evaluation of genital tract morphology is a strength, but the expression of exposure level in μg per g egg makes it difficult to compare to human exposure levels.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful to the evaluation process.

Halldin et al. (2001, 2005), supported by the European Union and numerous Swedish agencies, examined the effect of in ovo exposure to bisphenol A on sexual behavior of male Japanese quail. On Day 3 of incubation, the yolks of an unspecified number of quail eggs were injected with vehicle (emulsion of peanut oil, lecithin, and propylene glycol) or Bisphenol A **(>99% purity)** at 67 or 200 μg/g egg, and eggs were incubated at 37.5°C at 60% relative humidity. After hatching, male and female chicks were housed together. Males were individually housed at 7 weeks of age. At 9 weeks of age, 17 control and 4–7 treated males/group were examined for sexual behavior. Behavior with a sexually receptive female was evaluated by observing actions such as neck grab, mount attempt, mounts, and cloacal contact movement. Testing was conducted for 2 min/day over 5 consecutive days. At the completion of testing, testis weight was measured, gonado-somatic index was determined, and plasma testosterone levels were measured by RIA. Females exposed in ovo ($n = 5$–8/group) were evaluated for numbers of eggs laid over 5 days and oviduct morphology. Statistical analyses included Kruskal–Wallis test, or χ^2 test for trend. No effects of bisphenol A exposure were reported for any of the effects examined including sexual behavior of males, testicular weight, gonado-somatic index in males, plasma testosterone levels, or numbers of eggs produced. Numbers of females with retained right oviduct were increased in the bisphenol A groups (2 of 5 and 4 of 7 in each respective bisphenol A group vs. 1 of 8 in controls) but the effect did not achieve statistical significance. Sexual behavior was reportedly affected at an ethinyl estradiol dose of 0.006 μg/g egg and diethylstilbestrol doses of 0.019 and 0.057 μg/g egg. The study authors concluded that, with the possible exception of a trend for retained right oviduct in females exposed to 200 μg/g egg, bisphenol A was not shown to affect any of the endpoints examined in Japanese quail, which were demonstrated to be a well-suited model for studying effects of estrogenic compounds.

Strengths/Weaknesses: The use of 2 positive controls and the attention to sexual behavior are strengths. Weaknesses are the expression of exposure level in μg per g egg, making it difficult to compare to human exposure levels, the lack of detail in the reporting of methods and results, and the lack of apparent statistical analysis.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful to the evaluation process.

Panzica et al. (2005), supported by the University of Torino and Region Piemonte, conducted a study that intended to examine the effects of in ovo bisphenol A exposure on the vasotocin system and sexual behavior of Japanese quail. In 2 sets of experiments, quail eggs were injected with bisphenol A **[purity not indicated]** at 50, 100, or 200 μg/egg following 3 days of incubation **[culture ware not discussed]**. Exposure to bisphenol A resulted in a dramatic decrease in the number of live chicks hatching (8–11% vs. 55–60% in controls). Chicks that hatched survived less than a week. Dissection of non-hatched embryos indicated that development was blocked immediately following injection in most embryos. A high rate of malformations was observed in chicks that died following hatching. **[No further information was presented for methods, and no data were presented for individual doses.]**

Strengths/Weaknesses: Weaknesses are the expression of exposure level in μg per g egg, making it difficult to compare to human exposure levels, and the lack of data presentation.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Furuya et al. (2002), supported by the Japanese Ministry of Education, Science, Sports, and Culture, examined the effects of bisphenol A exposure on growth

of testes and combs of male chickens. Beginning at 2 weeks of age, male white Leghorn chicks were orally dosed weekly with corn oil vehicle ($n = 5$) or 200 mg bisphenol A **[purity not indicated]** ($n = 12$). **[The specific method of oral dosing was not reported. It is assumed that birds were dosed until they were killed.]** Chickens were killed at 16 weeks of age. Combs and testes were weighed. Testes were fixed in 4% paraformaldehyde and examined histologically. **[Statistical methods were not discussed, and the levels of statistical significance were not reported.]** Bisphenol treatment did not affect body weight, but comb and testis weight were significantly lower in the chickens exposed to bisphenol A. Spermatogenesis was disturbed in the chickens of the bisphenol A group, as observed by small seminiferous lumen and scarcity of spermatids and mature sperm. Diameter of seminiferous tubules and incidence of seminiferous tubules with mature sperm were significantly lower in the bisphenol A group. The study authors concluded that bisphenol A might disturb the growth of comb and testes in male chickens, possibly through an endocrine mechanism.

Strengths/Weaknesses: The study of male puberty in chickens is a strength. Weaknesses are the use of a single dose level and the lack of information on dosing and statistical analysis. The study would have been strengthened by measurement of hormone levels.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful to the evaluation process.

Sashihara et al. (2001), supported by the Japan Ministry of Education, Science, and Culture and the Uehara Memorial Foundation, examined the effects of early life exposure to bisphenol A on growth and behavior in male chicks. Layer type (Julia) chicks were obtained from a local hatchery, housed in windowless rooms **[no further housing details provided]**, given ad lib access to water and feed (Toyohashi Feed and Mills Co.), and provided continuous lighting. Birds were group housed based on weight. At 4 days of age, 0, 100, or 200 μg of bisphenol A **[purity not given]** dissolved in 10% ethanol and sesame oil, was injected into the brain ($n = 12$ or 13 per group). Chicks were followed for growth up to 20 days after treatment. A subset of 7 chicks/group was used for behavioral testing 8 days after treatment. Birds were placed under isolation distress condition and for a 5-min period were observed in a cage for motor activity and vocalization. At 20 days of age, birds were killed and liver, kidney, testis, and brain were weighed. Statistical analyses were performed using ANOVA and Duncan multiple range tests.

There were no treatment effects on food intake 6 hr after injection or on body weight gain measured 3 days after exposure. In the behavioral test, there were no treatment effects on jumping, locomotor activity, and duration of crouching. There was a statistically significant dose-dependent increase in the frequency of distress vocalizations. There were no treatment effects at 20 days on body or organ weights. The authors concluded that an acute early life exposure of the chick brain to 100 or 200 μg bisphenol A may affect stress-induced behavior, which may involve an estrogen-mediated pathway.

Strengths/Weaknesses: The rationale for the selection of the test animal and dosing procedures are not provided. Given that acute doses were injected directly into the brain, specific rationale for the method and selection of dose are critical to understanding the relevance of the study to human health or to wildlife or livestock concerns. This provides a vacuum for the interpretation of the dose-related increase in vocalizations that were reported.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful to the evaluation process.

Furuya et al. (2006), supported by the Japanese Ministry of Education, Science, Sports, and Culture, examined the effects of bisphenol A exposure on development of male chicks. Beginning at 2 weeks of age, male white Leghorn chicks were orally dosed every 2 days with bisphenol A at 0 (alcohol/corn oil vehicle) 0.002, 0.020, 0.200, 2, or 200 mg/kg bw. The high-dose level was considered to be a positive control based on previous observations in the laboratory. **[No information was provided about the specific method of oral dosing, number of birds treated, purity of bisphenol A, or the type of feed or caging and bedding materials used. It was implied but not clearly stated that exposures were continued until the birds were killed.]** The birds were killed at 5, 10, 15, 20, and 25 weeks of age. The comb, wattle, and testes were weighed. Part of the testicular tissue was used to isolate mRNA for evaluation of *ERα* and aromatase expression by RT-PCR. Additional testicular tissue was fixed in 10% buffered formalin for histopathology analysis and assessment of spermatogenesis by using immunohistochemistry techniques to measure proliferating cell nuclear antigen levels. **[Methods for statistical analyses were not reported.]**

Although responses were not dose-related, significant decreases in weight (doses at which effects were observed) were reported for comb and wattle at 10 weeks of age ($\geq$0.002 mg/kg bw), testis at 10 weeks of age (200 mg/kg bw), comb and testis at 15 weeks of age ($\geq$0.020 mg/kg bw), wattle at 15 weeks of age ($\geq$ 0.2 mg/kg bw), comb at 20 weeks of age ($\geq$0.200 mg/kg bw), testis at 20 weeks of age (200 mg/kg bw), and comb and testis at 25 weeks of age (200 mg/kg bw). There were no effects on body weight. Histopathological observations in testis (doses at which effects were observed) included significant and dose-related reductions in the number of spermatogonia at 5 weeks of age ($\geq$ 2 mg/kg bw) and number of spermatogonia, spermatocytes, and spermatids at 10–25 weeks of age ($\geq$0.02 mg/kg bw, except for decreases in spermatocytes at 10 weeks of age, which occurred at $\geq$0.200 mg/kg bw). Seminiferous tubule diameter was significantly reduced at all ages in groups exposed to $\geq$0.020 mg/kg bw. Significant and dose-related reductions in testicular proliferating cell nuclear antigen levels were observed at $\geq$0.200 mg/kg bw at 10 weeks of age and $\geq$0.020 mg/kg bw at 15–25 weeks of age. *ERα* mRNA was significantly increased according to dose (doses at which effects were observed) at 10 weeks of age ($\geq$0.020 mg/kg bw), 15 and 20 weeks of age ($\geq$0.200 mg/kg bw/day), and 25 weeks of age (200 mg/kg bw). Significant and dose-related increases were also observed for aromatase mRNA expression (doses at which effects were observed) at 5 weeks of age ($\geq$0.002 mg/kg bw), 10 weeks of age (0.200 mg/kg bw), and 15 weeks of age (200 mg/kg bw). The study authors concluded that exposure to bisphenol A at environmentally relevant levels may affect male chicken phenotypes and result in unbalanced gene expression in the testis.

Strengths/Weaknesses: This study is a more detailed follow-up of the previous study by these authors (Furuya et al., 2002), and replication of these results is a strength. Additional strengths are the use of multiple exposure levels and the oral route of administration. The lack of information on statistical methods is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful to the evaluation process. While the in vitro studies are useful for mechanistic insights, cellular evaluation, and endpoint identification, *inter alia*, the studies as a group were considered not useful for the evaluation process.

3.2.11 In vitro. **Takai et al. (2000)**, supported by the Japanese Ministry of Education, Science, and Culture, the Ministry of Health and Welfare, and the Science and Technology Agency, examined the effects of in vitro bisphenol A exposure on preimplantation mouse embryos. Two-cell embryos were obtained from B6C3F$_1$ mice and incubated for 48 hr in media containing bisphenol A **[purity not indicated]** at concentrations ranging from 100 pM to 100 μM **[23 ng/L to 23 mg/L] [culture ware not discussed]**. A negative control group was exposed to the ethanol vehicle and the effects of tamoxifen were also tested. Cell numbers were counted, and trophoblast spreading was evaluated in blastocysts. Statistical analyses included χ^2, Fisher post-hoc, and Student *t*-tests. The number of embryos or samples/group ranged from 14–400 for each endpoint evaluated. Significant effects observed with bisphenol A exposure (percent change vs. control) included increased rate of development from 2- to 8-cell embryos following 24 hr exposure to 3 nM **[0.68 μg/L]** (94% vs. 88%), increased development to the blastocyst stage following 48 hr exposure to 1 and 3 nM **[0.23 and 0.68 μg/L]** (69% in both dose groups vs. 58.7%), and decreased development to the blastocyst stage following 48 hr exposure to 100 μM **[23 mg/L]** bisphenol A (31.2 vs. 58.7%). No effects were observed at concentrations between 10 nM and 10 μM **[23 μg/L and 2.3 mg/L]** bisphenol A. **[Data were not shown by study authors.]** Addition of 100 nM tamoxifen to cultures decreased development to the blastocyst stage at 1 and 3 nM **[0.23 and 0.68 μg/L]** bisphenol A and increased development to blastocyst stage at 100 μM **[23 mg/L]** bisphenol A. Trophoblast spreading was increased in blastocysts exposed to 100 μM **[23 mg/L]** bisphenol A. Bisphenol A exposure did not affect morphology of or cell numbers in blastocysts. The study authors concluded that environmentally relevant concentrations of bisphenol A may affect early embryonic development through the ER and may also affect subsequent development.

Strengths/Weaknesses: The wide range of bisphenol A concentrations is a strength. The postulated involvement of the ER in bisphenol A activity could have been more convincingly demonstrated with a positive control such as 17β-estradiol and with a more specific estrogen antagonist than tamoxifen. The use of serum-free and phenol red-free media is an appropriate way to avoid estrogenic contamination but is an artificial environment compared to the estrogen-rich milieu in which preimplantation embryos normally develop.

Utility (Adequacy) for CERHR Evaluation Process: This study provides some mechanistic information but is not useful in the evaluation process.

Takai et al. (2001), supported by the Japanese Ministry of Education, Science, Sports, and Culture, the Ministry of Health and Welfare, and the National Institute for Environmental Studies, examined the effects of in vitro preimplantation exposure of mice to bisphenol A. Two-cell embryos were obtained from B6C3F$_1$ mice and incubated for 48 hr in media containing bisphenol A **[purity not indicated]** at 0 (ethanol vehicle), 1 nM **[0.23 μg/L]** or 100 μM **[23 mg/L] [culture ware not discussed]**. Embryos were assessed for number developing to the blastocyst stage, and then blastocysts were transferred to uterine horns of pseudopregnant mice (7/mouse). The dams were allowed to deliver and nurse the litters until weaning on PND 21 (day of birth not defined). Pups were randomly culled to maintain litter sizes at no more than 6. Body weight of pups was measured at birth and at weaning. Litters and pups were considered the experimental unit for statistical analyses. Statistical analyses included χ^2 and Fischer protected least significant difference tests. The number of embryos developing to the blastocyst stage was significantly increased by exposure to bisphenol A at 1 nM **[0.23 μg/L]** but decreased by exposure to 100 μM **[23 mg/L]** (72.2 and 33.3% at each respective concentration vs. 62.1% in controls). Developing embryos appeared morphologically normal and there were no significant differences in the numbers of cells. Birth weight, number of pups/litter, and sex ratio were not affected by treatment. At weaning, pups in both dose groups weighed more than controls (34–39% greater) and the effect was significant on a litter and pup basis. The study authors concluded that bisphenol A may affect early embryonic and postnatal development at low, environmentally relevant concentrations.

Strengths/Weaknesses: This study was cleverly designed as a follow-up to the previous study and appears to show that a low concentration of bisphenol A stimulates early embryo development while a high concentration inhibits early embryo development. This study did not evaluate the effect of exogenous bisphenol A under physiologic conditions. The use of serum-free and phenol red-free media is an appropriate way to avoid estrogenic contamination but is an artificial environment compared to the estrogen-rich milieu in which preimplantation embryos normally develop. The trophic effects of bisphenol A at low concentration may have been compensating for the estrogen deprivation of the control culture. It would have been interesting to compare physiologic concentrations of 17β-estradiol to the control culture conditions.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Li et al. (2003), support not indicated, examined the effect of in vitro bisphenol A exposure on post-implantation mouse and rat embryos. A limited amount of information was available for the study, which was published in Chinese, but included an abstract and data tables presented in English. GD 8.5 mouse embryos and GD 9.5 rat embryos were cultured for 48 hr in media containing bisphenol A **[purity not indicated]** at 0, 40, 60, 80, or 100 mg/L **[culture ware not discussed]**. Exposure of rat embryos to bisphenol A concentrations ≥60 mg/L resulted in reduced crown-rump length and yolk sac diameter and affected yolk sac circulation and morphologic

differentiation of the nervous system, heart, and forelimbs. Additional effects observed in rats at ≥80 mg/L included reductions in head length, number of somites, and flexion and changes in morphologic differentiation of the otic and optic system and tail. Exposure of mouse embryos to ≥60 mg/L bisphenol A resulted in reductions in flexion, yolk sac diameter, and yolk sac circulation and changes in morphologic differentiation of the olfactory system and branchial arches. In mouse embryos exposed to ≥80 mg/L bisphenol A, there were reductions in head and crown-rump length and number of somites and changes in morphologic differentiation of the visual system, heart, brain, auditory system, and fore- and hindlimb buds. The study authors concluded that high concentrations of bisphenol A are toxic to rat and mouse embryos in vitro.

Strengths/Weaknesses: The use of excessively high concentrations of bisphenol A is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Monsees et al. (2000), supported by the Federal Environmental Agency of Germany, examined the effects of bisphenol A exposure on rat Sertoli cell cultures. Sertoli cell cultures were prepared using testes from 18–21-day-old Sprague–Dawley rats. The cultures were exposed for 24 hr to bisphenol A or ethinyl estradiol at 0 or 10–50 μM **[2.3–11 mg/L] [culture ware not discussed]**. The effects of pesticides and heavy metals were also examined but will not be discussed. Endpoints assessed following the incubation period included viability by measurement of mitochondrial enzyme activity and lactate and inhibin B production. There were 8 replicates/experiment, and the experiment was repeated 3 times. Data were analyzed by Student *t*-test or unpaired Mann–Whitney test. Exposure of cells to bisphenol A resulted in increased lactate production (up to 30%) at ~25 μM **[5.7 mg/L]** bisphenol A and increased inhibin B production at ~10 μM **[2.3 mg/L]** and greater. There was no effect on cell viability following exposure to bisphenol A. Effects of ethinyl estradiol included increased mitochondrial dehydrogenase activity and a biphasic effect on inhibin B production, with an increase at ~10 μM and decreases at higher doses. The study authors concluded that secretion of lactate and inhibin B by Sertoli cells appeared to be sensitive markers for exploring possible Sertoli cell toxicants.

Strengths/Weaknesses: The use of high concentrations of bisphenol A is a weakness. It is not clear how the increased lactate and inhibin B production would correlate with reproductive capacity.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

Iida et al. (2003), supported by an unnamed grantor and by Takeda Science Foundation, examined the effects of in vitro bisphenol A exposure on cultured rat Sertoli cells. The cell cultures were prepared using testes of 18-day-old rats and were exposed for up to 48 hr to bisphenol A **[purity not indicated]** at concentrations ranging from 50–100 μM **[11–23 mg/L] [culture ware not discussed]**. Control cells were incubated in the DMSO-containing media. Morphology was examined by phase-contrast microscopy, and viability was assessed using the CellTiter 96 system in cells exposed to 0, 50, 100, 150, 200, and 300 μM **[0, 11, 23, 34, 46, and 68 mg/L]**. Immunochemistry analyses were conducted to detect transferrin and caspase-3 and apoptosis was assessed using a TUNEL method in cells exposed to 0, 100, and 200 μM **[0, 23, and 46 mg/L]** bisphenol A for 48 hr. A fluorescence staining technique was used to examine actin structure in cells incubated with 200 μM **[46 mg/L]** bisphenol A. Experiments were performed in triplicate and repeated at least three times. Data were analyzed by ANOVA.

Bisphenol A concentrations of ≥150 μM **[34 mg/L]** increased detachment of Sertoli cells from substrate and reduced viability. In a time-response study, cell viability was reduced following exposure to 200 μM **[46 mg/L]** bisphenol A for ≥12 hr. Transferrin secretion by Sertoli cells was decreased following incubation with bisphenol A **[apparently at ≥100 μM (23 mg/L); statistical significance not indicated]**. Following incubation with 200 μM **[46 mg/L]** bisphenol A, observations included solitary cells with a cortical ring of actin filaments and underdeveloped stress fibers, cells with membrane blebs consisting of protruding actin filaments, and round cells with a disorganized actin cytoskeleton and chromatin condensation. The study authors indicated that the observations were consistent with apoptosis. Expression of capsase-3 was observed in the round Sertoli cells. Capsase-3-positive cells were rarely observed in control cells, but were observed at incidences of <1% in the 100 μM **[23 mg/L]** group and ~9% in the 200 μM group. Further examinations revealed that most and possibly all of TUNEL-positive cells were stained with the caspase-3 antibody. The study authors concluded that decreased viability of Sertoli cells was most likely due to apoptosis and not necrosis.

Strengths/Weaknesses: The evaluation of multiple endpoints is a strength; however, the concentrations of bisphenol A were much higher than are likely to be achieved with human exposures.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful for the evaluation process.

Miyatake et al. (2006), supported by the Japanese Ministry of Health, Labor, and Welfare, and the Ministry of Education, Culture, Sports, Science, and Technology, conducted a series of studies to examine the effect of bisphenol A exposure on cultures of mouse neuron/glia cells and astrocytes. Cell cultures were obtained from midbrains of ICR mice on PND 1. Statistical analyses included ANOVA followed by Student *t*-test.

In the first two studies, astrocyte and neuron/glia cultures were incubated for 24 hr in media containing bisphenol A **[purity not indicated]** or 17β-estradiol at 0 or 10 fM to 1 μM **[bisphenol A concentrations of 2.3 pg/L–0.23 mg/L]** for 24 hr, and intensity of glial fibrillary acidic protein immunoreactivity was measured **[culture ware not discussed]**. In astrocyte cultures activation of cells, as determined by stellate morphology and significantly increased glial fibrillary acidic protein, occurred with exposure to bisphenol A at 100 fM **[23 pg/L]**, 1 pM **[0.23 ng/L]**, 10 pM **[2.3 ng/L]**, 10 nM **[2.3 μg/L]**, 100 nM **[23 μg/L]**, and 1 μM **[0.23 mg/L]**, but the effect was not observed in cells exposed to bisphenol A at 10 fM **[2.3 pg/L]**, 100 pM **[23 ng/L]**, or 1 nM **[0.23 μg/L]**. In neuron/glia cultures, a significant increase in glia fibrillary acidic protein was observed at bisphenol A concentrations of 100 fM **[23 pg/L]**, 1 pM **[0.23 ng/L]**, 10 pM **[2.3 ng/L]**,

100 nM **[23 ng/L]**, and 1 μM **[0.23 mg/L]**, but not at bisphenol A concentrations of 10 fM **[2.3 pg/L]**, 100 pM **[23 ng/L]**, 1 nM **[0.23 μg/L]** or 10 nM **[2.3 μg/L]**. Increases in glial fibrillary acidic protein immunoreactivity were not observed in astrocyte or neuron/glia cultures following treatment with 17β-estradiol. The study authors concluded that exposure of cell cultures to bisphenol A results in biphasic activation of astrocytes.

In a third study, the role of steroid hormone receptors in bisphenol A-induced astrocyte activation was examined. Astrocyte and neuron/glia cell cultures were pretreated with an ER antagonist (ICI 182,780), an ER agonist/antagonist (tamoxifen), a progesterone receptor antagonist (mifepristone), or an androgen receptor antagonist (flutamide) for 24 hr. The cultures were then incubated with bisphenol A at 0, 1 pM **[0.23 ng/L]**, or 1 μM **[0.23 mg/L]**, with and without the receptor ligands, for another 24 hr. None of the ligands attenuated astrocyte activation, and the study authors concluded that bisphenol A-induced activation of astrocytes was not mediated by estrogen, progesterone, or androgen receptors.

In a fourth study, mouse midbrain astrocyte or neuron cultures were incubated for 24 hr in media containing bisphenol A at 0, 1 pM **[0.23 ng/L]**, 1 nM **[0.23 μg/L]**, or 1 μM **[0.23 mg/L]**. A fluorescent technique was used to measure calcium levels following treatment of cells with 1–100 μM dopamine. In astrocyte and neuron cultures, dopamine-induced increases in intracellular calcium were enhanced following pretreatment with bisphenol A at 1 pM [0.23 ng/L], but not at 1 nM **[0.23 μg/L]** or 1 μM **[0.23 mg/L]**. In neuron cells, pretreatment with 1 μM **[0.23 μg/L]** bisphenol A suppressed dopamine-induced increases in intracellular calcium. The study authors concluded that in vitro bisphenol A exposure results in altered dopamine responsiveness in astrocytes and neurons.

In a fifth study, neuron/glia cultures were incubated in media containing bisphenol A or 17β-estradiol at 1 pM, 1 nM, or 1 μM for 24 hr **[bisphenol A concentrations of 0.23 ng/L, 0.23 μg/L, and 0.23 mg/L]**. An immunohistochemistry technique was used to identify apoptotic cells by the presence of caspase-3. Treatment with 1 μM **[0.23 μg/L]** bisphenol A activated caspase-3 in neurons. No increase in caspase 3 was observed following exposure to cells to 17β-estradiol. The study authors concluded that high in vitro exposures to bisphenol A may result in toxicity to neurons.

Strengths/Weaknesses: The use of multiple concentrations of bisphenol A over a wide range, the evaluation of multiple endpoints, and the comparison to known receptor ligands are strengths.

Utility (Adequacy) for CERHR Evaluation Process: This study is interesting in suggesting a non-hormonal mechanism of bisphenol A activity. Although the study contains suggestive mechanistic information, it is not useful for the evaluation process.

Yamaguchi et al. (2006), supported by the Promotion and Mutual Aid Corporation for Private Schools of Japan, examined the effects of low-level bisphenol A exposure on the differentiation of serum-free mouse embryo astrocyte progenitor cells into astrocytes. Astrocyte progenitor cells were grown on fibronectin-coated Petri dishes under standard incubator conditions. Differentiation of astrocyte progenitor cells was induced with leukemia inhibitory factor (LIF) and bone morphogenetic protein-2 (BMP2) **[culture ware not discussed]**. Cells were additionally exposed to bisphenol A **[purity not provided]** at concentrations of 0.1 ng/L to 100 mg/L with or without tamoxifen for 24, 48, 72, or 120 hr, to establish optimal experimental parameters. A tetrazolium salt based colorimetric assay was used to assess cell viability and dot-blot or Western blot detection of glial fibrillary acidic protein production was used as a marker of differentiated astrocytes. Subsequent assays were performed using bisphenol A treatments of 0.1 ng/L **[4 pM]** or 1 mg/L **[40 mM]**. Controls were treated with LIF and BMP-2 for 48 hr. ANOVA and Tukey test were used for statistical analyses.

Bisphenol A 0.11 ng/L had no effect on astrocyte progenitor differentiation; However, bisphenol A at 1, 10, and 100 ng/L induced significant differentiation compared to controls based on dot-blot assays of glial fibrillary acidic protein production. The highest glial fibrillary acidic protein levels were induced with 10 ng/L bisphenol A exposure. At bisphenol A concentrations $\geq$1 μg/L, there were no differences in astrocyte progenitor differentiation compared to control. Bisphenol A 10 ng/L induced significantly higher levels of phosphorylated signaling transducer and activator protein 3 (pSTAT3) and phosphorylated mothers against *decapentaplegic* homolog 1 (pSmad1), the activated forms of both proteins, which are induced to form a protein complex by BMP-2 and LIF, and in turn, promote glial fibrillary acidic protein expression. Addition of 10^{-6} M tamoxifen resulted in glial fibrillary acidic protein, pSTAT3, and pSmad1 comparable to control levels. Bisphenol A at 10 ng/L and 1 μg/L only marginally increased levels of Smad6 and oligodendrocyte lineage transcription factor 2, inhibitors of pSTAT3-p300 and pSmad1–Smad4 protein complex formation, which induce glial fibrillary acidic protein expression.

The authors suggested that low levels of bisphenol A may alter brain development through a mode of action involving elevated levels of glial fibrillary acidic protein production through estrogen receptor regulation of glial fibrillary acidic protein expression and through a stimulatory BMP-2/LIF signaling pathway that induces the formation of pSmad and pSTAT3 coactivator complexes of glial fibrillary acidic protein expression.

Strengths/Weaknesses: This study is interesting, but the in vitro system is not useful for predicting in vivo effects in humans.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation process.

3.3 Utility of Developmental Toxicity Data

3.3.1 Human. There are no human data on developmental effects of bisphenol A.

3.3.2 Experimental animals. There are 21 studies in which bisphenol A was given at a single dose level to rats and six studies in which bisphenol A was given at a single dose level to mice. These studies explored various aspects of bisphenol A developmental effects but are not useful in establishing dose–response relationships. The lowest dose level evaluated in these studies was 0.0024 mg/kg bw/day in rats (Akingbemi et al., 2004) and 0.002 mg/kg bw/day in mice (Nishizawa et al., 2003). There are 25 rat and 30 mouse studies in which

Table 81
Summary of High Utility Developmental Toxicity Studies (Single Dose Level)

Model (route)	Dose and dosing period (mg/kg bw/day)	Significant developmental findings	Reference
Rat			
Sprague–Dawley (oral by pipette)	0.04, PND 23–30 and animals evaluated at PND 37 or 90	↑ ERα expression in females vs. males in medial pre-optic area (also seen with positive control)↓ Testosterone in males at PND 37 but not PND 90	Ceccarelli et al. (2007)
Sprague–Dawley males (oral by pipette)	0.040, PND 23–30	↓ Investigation of new object, ↓ intromission latency, ↓serum testosterone	Della Seta et al. (2006)
F344/N dams (gavage)	0.1, GD 3–PND 20	↓ Correct avoidance responses and ↑ failure of avoidance in active avoidance testing; no ↑ in locomotion following trans-2-phenylcyclopropylamine hydrochloride challenge in males	Negishi et al. (2004a)
Sprague–Dawley males (gavage)	100, PND 23–53	↑ Age of preputial separation; ↑ kidney and thyroid weights; ↓ liver weight; ↓ cortical thickness of the kidney; ↑ hydronephrosis; ↑ multinucleated giant cells in seminiferous tubules; ↓no. undergoing spermatogenesis	Tan et al. (2003)
Mouse			
CD-1 dam (oral)	0.050, GD 16–18	↑ Anogenital distance adjusted for body weight on PND60; ↑ prostate weights on PND 3, 21, and 60; ↓ relative (to body weight) epididymis weight in the bisphenol A group on PND 60; ↑ androgen receptor binding on PND 21 and 60	Gupta (2000)
CD-1 dam (oral from syringe)	0.010, GD 11–18	↓ Place preference associated with *d*-amphetamine in females	Laviola et al. (2005)
CD-1 dam (oral by pipette)	0.010, GD 14–18; offspring mated and dosed with 0 or 0.010 on GD 14–18	In mice exposed only during gestational development or in adulthood during pregnancy: ↓ time nursing and in nest and ↑ time nest building, resting alone, grooming, and out of nest In mice exposed during both gestational development and in adulthood during pregnancy: ↑ time resting alone	Palanza et al. (2002)
CD-1 dam (oral by pipette)	0.010, GD 14–18	↑ No. of prostate ducts and proliferating cell nuclear antigen staining in dorsolateral prostate; ↑ prostate duct volume in dorsolateral and ventral prostate	Timms et al. (2005)

↑, ↓ Statistically significant increase, decrease compared to controls; ↔no statistically significant effects compared to controls.

bisphenol A was given at multiple dose levels. These studies included oral and subcutaneous administration routes; due to pharmacokinetic considerations, studies using the oral route are of greater utility in estimating human risk.

3.4 Summary of Developmental Toxicity Data

The studies summarized here are those considered by the Panel to be the most important and relevant for the assessment of the effects of Bisphenol A on the human population. Evaluation of the scientific literature was made on the scientific quality of the study and also on its relevance to the assessment of the level the concern about potential effects of BPA on human health. The judgment was based on the criteria the Panel adopted which focused on the potential for providing information for the evaluation process. Several excellent studies have been placed in the ''adequate-but-limited-utility'' category with regard to the evaluation process. The Panel did not consider the source of funding of any of the studies in any of their deliberations.

It is highly unlikely that humans would ever experience the very high internal levels of bisphenol A that are produced after an injection of bisphenol A. While it would be possible to measure levels of parent compound and metabolite after injections, no parenteral exposure studies in this data set have done so. Section 1 and (Wilson et al., 2006) indicate that ca. 99% of human exposure comes from dietary sources, and bisphenol A is subject to efficient first-pass metabolic conversion in the gut and liver to the inactive glucuronide conjugate in humans and rats (Pottenger et al., 2000; Völkel et al., 2002; Inoue et al., 2003b). In contrast, bisphenol A injected subcutaneous or i.p. circulates as much higher proportion of the unconjugated parent compound (Pottenger et al., 2000). Because oral exposure is so relevant to the human situation, and the uncertainties associated with the altered internal metabolite profile and the abundant data from oral studies, the Panel puts greater weight on studies using the oral route of exposure for formulating levels of concern about human exposures.

The hypothesis has been advanced that the Charles River SD rat is insensitive to estrogens and other EDCs and therefore it should not be used for developmental studies of potential endocrine disruptors, and the studies of the effects of BPA that used this strain should be discounted. In order to address this important issue the

Table 82
Summary of High Utility Developmental Toxicity Studies (Multiple Dose Levels)

		Bisphenol A dose level (mg/kg bw/day)						Reference
Model (treatment)	Endpoint	NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	
Rat								
Han–Wistar (drinking water from before mating through gestation and lactation)	Male reproductive organ weights, sperm production, testicular histopathology	≥0.775–4.022 (high dose)						Cagen et al. (1999b)
CD (gavage, 2-generations exposure including pre-and postnatal development periods)	Prenatal or postnatal growth or survival, developmental landmarks, anogenital distance, age of puberty, fertility, estrous cyclicity, or sperm counts	≥0.2 (high dose)						Ema et al. (2001)
Sprague–Dawley dam (gavage GD 1–20)	↓ Live fetuses/litter	300	1000	929	348	982	713	Kim et al. (2001b)
	↓ Male body weight	100	300	456	339	694	497	
	↓ Female body weight	300	1000	439	328	682	490	
	↓ Ossification	300	1000					
Sprague–Dawley dam (gavage GD 11–PND 20)	Volume of SDN-POA, age or weight at vaginal opening or first estrous, estrous cyclicity, mean lordosis intensity, prostate weight, or histopathology in ventral prostate, ovary, or uterus	≥320 (high dose)						Kwon et al. (2000)
CD dams (gavage GD 6–15)	Implantation sites, resorptions, body weight, viability, sex ratio, and malformations	≥640 (high dose)						Morrissey et al. (1987)
Sprague–Dawley dam (gavage GD6–PND 21)	↑ Uterine epithelial cell nuclei		0.1 (low dose)					Schönfelder et al. (2004)
	↑ Uterine epithelial nuclei with condensed chromatin		0.1 (low dose)					
	↑ Uterine epithelial cells with cavities		0.1 (low dose)					
	↓ ERβ-positive cells in uterine tissue		0.1 (low dose)					
	↓Thickness of uterine luminal epithelium	0.1	50					
	↑ ERα-positive cells in uterine epithelium	0.1	50					
Wistar-derived Alderley–Park dams (gavage GD 6–21)	Delayed vaginal opening	0.1	50	68	51	35	16	Tinwell et al. (2002)
	↓ Sperm count/testis	0.1	50	55	30	57	31	
	↓ Sperm count/g testis	0.1	50	81	41	68	34	
	↓ Daily sperm count/testis	0.1	50	56	31	59	31	
	↓ Daily sperm count/g testis	0.1	50	83	42	70	34	
Sprague–Dawley (dietary, multiple generations with exposure during pre-and post natal development)	Live F1 pups/litter	47.5	475	268	192	559	394	Tyl et al. (2000b, 2002b)
	Live F2 pups/litter	47.5	475	422	152	459	294	
	Live F3 pups/litter	47.5	475	236	174	376	286	
	F1 body weight, PND 4	47.5	475	406	283	561	400	
	F1, F2, or F2 body weight, PND 7	47.5	475	217–328	183–257	265–410	218–313	
	F1, F2, or F2 body weight, PND 14	47.5	475	183–243	163–209	177–227	153–191	
	F1, F2, or F2 body weight, PND 21	47.5	475	208–252	166–226	223–267	175–220	
	↑ Age at F1 vaginal opening	47.5	475	394	343	206	176	
	↑ Age at F2 vaginal opening	47.5	475	404	336	277	228	
	↑ Age at F3 vaginal opening	47.5	475	471	401	396	203	
	↑ Age at F1 preputial separation	4.75	47.5	466	411	188	163	
	↑ Age at F2 preputial separation	47.5	475	300	255	241	203	
	↑ Age at F3 preputial separation	47.5	475	547	473	222	189	
	Mating, fertility, pregnancy, or gestational indices; precoital interval, postimplantation loss, estrous cyclicity, and reproductive organ histopathology; sperm count, morphology or motility;	≥475 (high dose)						

Table 82
Continued

Model (treatment)	Endpoint	Bisphenol A dose level (mg/kg bw/day) NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	Reference
	anogenital distance in males or females; areolas/nipples in males							
Mouse								
CD-1 dam (gavage GD 6–15)	↑ Resorptions/litter	1000	1250	817	377	1245	1162	Morrissey et al. (1987)
	↓ Fetal body weight/litter	1000	1250	1079	785	1249	1024	
C57BL/6N males (gavage GD 11–17 or PND 21–43)	Sperm density or lesions in reproductive organs	≥0.200 (high dose)						Nagao et al. (2002)
	↓ Absolute seminal vesicle weight in group exposed during gestation		≤0.002 (low dose)[a,b]					
CF-1 (oral by pipette, GD 11–17)	↑ Prostate weight		≤0.002 (low dose)					Nagel et al. (1997)
C57BL/6 dam (gavage GD 3–PND 21)	no effect AGD or AGD corrected for body weight	≥0.2 (high dose)						Ryan and Vandenbergh (2006)
	No effect on errors in radial arm and Barnes mazes	≥0.2 (high dose)						
	↓ Time in open arms of plus maze	0.002	0.2					
	↓ Time in light part of light/dark preference box	0.002	0.2					
CD-1 (dietary, multiple generations with exposure during pre- and post-natal development)	↓ F1 body weight on PND 7, 14, and 21	50	600	548–560	267–313	580–617	370–506	Tyl et al. (2006)
	↓F1 male body weight at PND 21 necropsy	50	600	564	313	640	599	
	↓F1 female body weight at PND 21 necropsy	50	600	387	254	776	598	
	Hepatic cytoplasmic variation, F1 male	5	50	124	92.5			
	Hepatic cytoplasmic variation, F2 male	50	600	224	178			
	Hepatic cytoplasmic variation, F1 female	5	50	333	200			
	Seminiferous tubule hypoplasia, F1 male	50	600	591	406			
	Seminiferous tubule hypoplasia, F2 male	5	50	283	233			
	Age of preputial separation, F1 parental or non-mated males	50	600	727–754	572–576	491–551	364–414	
	Anogenital distance per body weight, F1 male on PND 21	5	50	1373	607	1769	616	
	Postnatal survival; daily sperm production; efficiency of daily sperm production; sperm motility or morphology; estrous cyclicity; numbers of ovarian primordial follicles; mating or fertility indices; or adult prostate weight	≥600 (high dose)						

[a]There was little to no evidence of a dose-response relationship.
[b]No effects were observed at one or more higher dose levels.

Table 83
Summary of Limited Utility Developmental Toxicity Studies (Single Dose Level)

Model (route)	Dose and dosing period (mg/kg bw/day)	Significant developmental findings	Reference
Rat			
Long–Evans male offspring (gavage) Experiment 3	0.0024, PND 21–90	↑ Serum LH level; ↓ weight of seminal vesicles; ↓ Testicular testosterone level; and ↓ basal and LH-induced ex vivo testosterone production	Akingbemi et al. (2004)
Wistar male pup (s.c. injection)	100, PND 2–12	Advanced testicular lumen formation, ↑testis weight, ↑ Sertoli cell volume/testis, ↑ spermatocyte nuclear volume/ unit Sertoli cell, and ↑ plasma FSH on PND 18; ↑ plasma FSH on PND 25; ↑ testicular weight in adulthood	Atanassova et al. (2000)
Wistar dam (drinking water)	~2.5, gestation[a]–PND 21	In rats 4–7 months of age: no effect on the number of corticotropin-releasing hormone neurons in the preoptic areas of males, a loss in sex difference in the anterior and posterior bed nuclei of the stria terminalis	Funabashi et al. (2004a)
Sprague–Dawley male pup (s.c. injection)	0.010, PND 1, 3, and 5; half the rats exposed to 17β-estradiol and testosterone in adulthood	In rats with no 17β-estradiol and testosterone exposure in adulthood: no effects on dorsal prostate weight, histopathology alterations, proliferation index, or apoptotic index In rats with 17β-estradiol and testosterone exposure in adulthood: ↑ incidence and severity of prostatic intraepithelial neoplasia; ↑ proliferation and apoptosis in regions of prostatic intraepithelial neoplasia	Ho et al. (2006b)
Sprague–Dawley pup (s.c injection)	300, PND 1–5	No effects on age of vaginal opening or preputial separation, copulation or fertility indices, sexual behavior of males, histopathologic alterations in males, or female reproductive organs, or effects on SDN-POA. **[Panel noted possible ↑ number of apically located nuclei in prostate, but a definitive conclusion could not be made based on 1 photograph]**	Nagao et al. (1999)
Wistar. male pup (s.c injection)	50, PND 22–32	↑ Serum prolactin levels on PND 29 but not PND 120; ↑ lateral but not ventral prostate weight; ↑ focal luminal polymorphonuclear cellular infiltrate in prostate No histological evidence of prostate inflammation	Stoker et al. (1999)
Mouse			
CF1 (oral)	0.0024, GD 11–17	↑ Body weight at weaning; ↓ postnatal pup survival; ↓ period between vaginal opening and first estrus No effect on age of vaginal opening	Howdeshell et al. (1999)
ICR/Jc1 mouse dams (s.c. injection)	0.02, GD 0 to GD 10.5, GD 12.5, GD 14.5, or GD 16.5	↑ Tuj1 in the intermediate zone at GD 14.5 and GD 16.5; ↑ PDI immunoreactivity in the neocortex from GD12.5 until GD16.5 and in subplate cells at GD 14.5; variable changes in BrdU labeling depending on when labeled and location; ↑ gene expression of *Math3*, *Ngn2*, *Hes1*, *LICAM*, and *THR-alpha*a at GD14.5; ↓ gene expression of *Hes1* and *Hes5* at GD 12.5 No effect on immunoreactivity pattern for KI-67, nestin, Musashi and histone H_3.	Nakamura et al. (2006)
ICR (oral)	0.002 mg/kg bw/day from 6.5–11.5, 6.5–13.5, 6.5–15.5, and 6.5–17.5 days post-coitum	Variable changes in retinoic acid retinoid X receptors á mRNA expression in brain, ovary, and testis, depending on brain region and day of exposure	Nishizawa et al. (2003)
Other			
Prepubertal Poll Dorset female lambs (i.m. injection)	3.5 biweekly, at 4–11 weeks of age (ovariectomy at 9 weeks of age)	↔ On blood levels during treatment; ↔ on body, kidney, adrenal, or ovarian weights; ↓ pulsatile LH secretion	Evans et al. (2004)
Prepubertal Poll Dorset female lambs (i.m. injection)	3.5 biweekly, at 4–11 weeks of age (ovariectomy at 9 weeks of age)	↑ Uterine/cervical tract weight, endometrial area, and endometrial/myometrial ratio Qualitative observations included endometrial edema, decreased endometrial gland density, crowding of cells in the uterine epithelium, keratinized cervical epithelium, ↑ intracellular staining for ERα and ERβ in the uterine subepithelium	Morrison et al. (2003)
Suffolk ewes (s.c. injection)	5 GD 30–GD 90	↓ Birth weight, height and chest circumference in female offspring at birth ↑ Anoscrotal:anonavel ratio in male offspring at birth ↑ LH and first breeding season in female offspring at PND 60	Savabieasfahani et al. (2006)

[a]Implied but not stated that exposure occurred during the entire gestation period.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

Panel members reviewed the literature on estrogen-sensitivity across rat strains and suppliers, the following is a summary of our findings.

Different strains of rats show clear, robust reproducible differences in response to potent estrogens and antiandrogens. Several traits have been shown to be

Table 84
Summary of Limited Utility Developmental Toxicity Studies (Multiple Dose Levels)

Model (treatment)	Endpoint	Bisphenol A dose level (mg/kg bw/day) NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	Reference
Rat								
Long–Evans males (gavage PND 21–35) Experiment 1	↓ Serum 17β-estradiol		0.0024 (low dose)[a,b]					Akingbemi et al. (2004)
	↓ Serum LH and testosterone		0.0024 (low dose)[a,b]					
Sprague–Dawley (dietary for 17 weeks)	↓ pup weight at weaning (PND 21)	70	200					General Electric, (1976)
Sprague–Dawley (dietary for 18 weeks)	No adverse effects reported	60 (high dose)						General Electric (1978)
Sprague–Dawley female pups (sc injection PND 0–9)	↓Body weight in lactation period	105	427	286	200	233	156	Kato et al. (2003)
	↑ Age of vaginal opening	26	105	345	267	159	116	
	↓ No. with normal estrous cycles	105	427	81	28			
	↑ No. with cleft clitoris	26	105	299	failed			
	↓ Ovary weight	105	427	85	59	140	93	
	↓ Uterus, wet weight	105	427	66	55	128	96	
	↓ Uterus, blotted weight	105	427	273	128	318	168	
	↓ Uterine fluid weight	26	105	42	34	139	104	
	↑ No. with polycystic ovaries		≤105 (lowest dose examined)	81	24			
	↓ No. corpora lutea	105	427	238	90			
	↓ No. with corpora lutea	105	427	65	38	137	83	
	↓Corpora lutea area		≤105 (lowest dose examined)	42	37	84	66	
Sprague–Dawley female pups (sc injection PND 0–9)	No adverse effects reported	≥97 (high dose)						Kato et al. (2006)
Sprague–Dawley (feed GD15–PND 10)	No adverse effects reported	3000 ppm [c]						Masutomi et al. (2004)
Mouse								
CF-1 (oral by pipette, GD 11–17)	Prostate weight and sperm production	≥0.020 (high dose)						Ashby et al. (1999)
ICR/Jcl (s.c. GD 11–17)	↓ Female body weight at weaning		≤0.002 (low dose)[a]	0.065	0.017	0.088	0.021	Honma et al. (2002)
	↓ Male body weight at birth	0.002	0.020	0.054	0.020	0.031	0.015	
	↑ Anogenital distance of females at weaning		≤0.002 (low dose)[a,b]					
	↑Anogenital distance of males on PND 60		≤0.002 (low dose)	0.035	0.020	0.035	0.020	

	↓ Age at vaginal opening	0.002	0.020				
	↓ Body weight at vaginal opening		≤0.002 (low dose)				
	↓ Age at 1st estrus	0.002	0.020				
	↑ Estrous cycle length		≤0.002 (low dose)[a]	0.021	0.007	0.12	0.021
	↑ Cornified cells		≤0.002 (low dose)[b]	0.17	0.020	0.44	0.021
	↓ Lymphocytes in vaginal smear		≤0.002 (low dose)[b]	0.26	0.020	0.26	0.020
ICR (oral GD 6.5–13.5 or 6.5–17.5)	↑ mRNA expression for arylhydrocarbon receptor in brain, testis, and ovary		≤0.00002 (low dose)[b]				Nishizawa et al. (2005b)
	↑ mRNA expression for retinoic acid α receptor in brain and ovary		≤0.00002 (low dose)[b]				
	↑ mRNA expression for retinoic acid α receptor in testis	0.20	20				
	↑ mRNA expression for retinoid X α receptors in brain		≤0.00002 (low dose)[b]				
	↑ mRNA expression for retinoid X α receptor in testis and ovary	0.002	0.020[b]				
ICR (oral GD 6.5–13.5 or 6.5–17.5)	↑ mRNA expression for arylhydrocarbon receptor, arylhydrocarbon receptor repressor, and arylhydrocarbon receptor nuclear translocator in brain, testis, and ovary		≤0.00002 (low dose)[b]				Nishizawa et al. (2005a)
ICR/Jcl (s.c. GD 10–18; female offspring ovariectomized)	↑ No. of vaginal epithelial layers		≤10 (low dose)				Suzuki et al. (2002)
	↓ No. with corpora lutea		≤10 (low dose)[b]				
ICR/Jcl (s.c. for 5 days beginning at birth; mice later ovariectomized except those used to monitor estrous cycles)	↑ Mitotic rate in uterine stromal cells and vaginal epithelial cells	10	100				Suzuki et al. (2002)
	↑ Vaginal epithelial layers	10	100				
	↑ No. with polyovular follicles and no. polyovular follicles/mouse	10	100				
	Estrous cyclicity	100 (high dose)					
CF-1 (oral by pipette, GD 11–17)	↓ Body weight		≤0.002 (low dose)[b]				vom Saal et al. (1998)

[a]There was little-to-no evidence of a dose-response relationship.
[b]No effects were observed at one or more higher dose levels.
[c]Feed consumption and dam weight not reported-dose not calculable.

Table 85
Summary of Behavioral Studies in Rats and Mice Treated With Bisphenol A

Treatment, mg/kg bw/day	Treatment age	Age at assessment	Results	Reference
High Utility				
Rat				
Treatment of dam				
3.2, 32, or 320, gavage	GD 11–PND 20	6 months	Lordosis behavior not affected by treatment	Kwon et al. (2000)
0.1, gavage	GD 3–PND 20	Open field: 8 weeks	Open field: No treatment effect	Negishi et al. (2004a)
		Spontaneous motor activity: 12 weeks	Spontaneous motor activity: No treatment effect	
		Passive avoidance: 13 weeks	Passive avoidance: No treatment effect	
		Elevated plus maze: 14 weeks	Elevated plus maze: No treatment effect	
		Active avoidance: 15 weeks	Active avoidan: Fewer correct avoidance responses	
Treatment of offspring				
0.04, micropipette	PND 23–30	45 days	No treatment effect on environmental exploration, social investigation, play, or social interaction ↓ Response to novel object ↓ Intromission latency	Della Seta et al. (2006)
Mouse				
0.010, syringe feeding	GD 11–18	60 days	↓ Conditioned place preference (reinforced with amphetamine) in females	Laviola et al. (2005)
0.010, micropipette (treatment of F_0 and F_1 females)	GD 14–18	Maternal behavior of F_1 assessed	Altered maternal behaviors when exposure was either prenatal or as an adult; however, exposure prenatally plus as an adult was not effective	Palanza et al. (2002)
2 or 200, placed in back of dam's throat	GD 3–PND 21	5 weeks, ovariectomized female offspring	No effect on errors in radial arm and Barnes mazes	Ryan and Vandenbergh (2006)
			Effects in high dose group:Puberty advanced ↓ Time in open arms of plus maze ↓ Time in light part of light/dark preference box	

↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

estrogen sensitive in rats including prolactin regulation in the pituitary, thymic involution, uterine pyometra, and liver carcinogenesis to name a few. It is evident that there are strain differences in respect to specific estrogen induced endpoints. However, there is no clear pattern in which one strain can be considered to be more or less sensitive than another. The results of BPA studies with the SD rat cannot therefore be ignored.

3.4.1 Human. There are no human data on developmental effects of bisphenol A. A study of the association between miscarriage and mean serum bisphenol A levels is discussed in Section 4.4.1.

3.4.2 Experimental animal. Studies considered by Expert Panel members to be of utility in evaluating developmental toxicity in mice are summarized in Tables 81, 82, 83, 84. Rat and mouse studies with behavioral endpoints are summarized in Table 85. The discussion of developmental toxicity is arranged according to general endpoints evaluated.

General developmental toxicity (growth, survival, malformations).

Rat Studies: Prenatal studies with oral dosing of rats consistently demonstrated an absence of malformations at doses up to 1000 mg/kg bw/day (Morrissey et al., 1987; Kim et al., 2001b). Reduced fetal survival and body weights at birth or during the postnatal period were reported in studies with oral exposures occurring throughout the entire gestation and/or lactation periods (Tyl et al., 2000a, 2002b; Kim et al., 2001b). LOAELs for decreased numbers of live fetuses or pups ranged from 475–1000 mg/kg bw/day (Tyl et al., 2000a, 2002b; Kim et al., 2001b). LOAELs for decreased pup body weight at birth were estimated at 300–1000 mg/kg bw/day (Tyl et al., 2000a, 2002b; Kim et al., 2001b). The LOAEL for reduced body weight during the postnatal period was 475 mg/kg bw/day (Tyl et al., 2000a, 2002b).

Mouse Studies: No increase in malformations was observed in mice with oral gavage of bisphenol A at doses of $\leq$1250 mg/kg bw/day (Morrissey et al., 1987). Prenatal developmental toxicity reported for mice included increased resorptions (LOAEL 1250 mg/kg bw/day) and decreased fetal body weight (LOAEL 1250 mg/kg bw/day) (Morrissey et al., 1987). Decreased body weight during the postnatal period was also reported in offspring of mouse dams exposed to bisphenol A during the entire gestation and lactation period (LOAEL

Table 86
Adult Body Weights of Offspring Exposed During Gestation or Lactation

Strain	Period of dosing	Route	Dose (mg/kg/day)	Measured on PND	Finding	Weights	SE	Sample size	Reference
CR:Long–Evans	GD 12–PND 21	Gavage	0.0, 2.4	90	M: ↑ 90 days at 2.4	450, 494	~14	12–14/group	Akingbemi et al. (2004)
CR:Long–Evans	GD 21–PND 90	Gavage	0.0, 2.4	90	NE	407, 412	~11	12–14/group	Akingbemi et al. (2004)
F344/N	GD 10–PND 20	Gavage	0.0, 4.0, 40.0, 400.0	(7, 21, 28, 56), 84	M: ↓ 7 days, 28 days at 40, ↓ 7 days, 21 days, 28 days, 56 days at 400 F: ↓ 7 days, 28 days at 4 and 40, ↓ 7 days, 21 days, 28 days at 400	M 303, 303, 303, 297 F: 186, 187, 185, 184	~3	27+, 27+, 27+, 15+, 9+	Negishi et al. (2003)
Fisher	GD 1–PND 21	Gavage	0.0, 7.5, 120	(23, 28) 91	NE	259, 267, 259	~9	5, 5, 5	Yoshino et al. (2002)
Fisher	GD 1–PND 21	Gavage	0.0, 0.05, 7.5, 30, 120	455	NE	427, 427, 420, 428	~21	12,12, 12, 12	Ichihara et al. (2003)
SD	GD 6–GD 21	Gavage	0.0, 0.1, 50	44, 50, 44	F: ↓ ~47 at 0.1	F: 246, 227, 242	~15	20, 20, 20	Talsness et al. (2000)
SD	GD 6–PND 21	Water	0.0, 0.1, 1.2	(22, 28, 37, 56, 87) 110	M: ↑ 28 days, 37 days, 56 days at 0.1 and 28 days, 37 days, 560 days at 1.2 F: ↑ 22 days, 28 days, 37 days, 56 days, 87 days, 110 days at 0.1	M: ~510, ~540, ~540; F: ~310, ~325, ~310 (from graph)	M: ~12 F: ~12	M: 27, 19, 19 F: 23, 18, 19	Rubin et al. (2001)
SD	GD 6–PND 20	Gavage	0.0, 4.0, 40.0	63	NE	M: ~380, ~385, ~381 F: ~250, ~245, ~237	M: ~4 F: ~3	M: 5, 4, 4, 0 F: 5, 5, 5, 0	Kobayashi et al. (2002)
SD	GD 6–GD 21	Gavage	0.0, 0.023, 0.049, 0.108	98	NE	F: 260, 261, 258, 250	~17.5	31, 21, 25, 25	Tinwell et al. (2002)
SD CD All F1 generations	Mating 1–PND 21	Diet	0.0, 0.015, 0.3, 4.5, 75, 750, 7500	>168	M: ↓ 84 days at 7500 F: ↓ 84 days at 7500	M: 501, 505, 493, 506, 518, 476, 369 F: 290, 383, 287, 294, 295, 283, 234	M: ~8 F: ~5	30,10,10,10, 10,10,10	Tyl et al. (2002b)
SD Crj IGS- F1 generation	GD 11–GD 17	Gavage	0.0, 0.0002, 0.002, 0.02, 0.2	M: 40; F: 30	NE	M: 241,237, 245, 228, 236 F: 114, 120, 113, 114, 113	M: ~18 F: ~12	25, 25, 25, 25, 25	Ema et al. (2001)
SD Crj IGS- F2 generation	GD 11–GD 17	Gavage	0.0, 0.0002, 0.002, 0.02, 0.2	M: 41; F: 31	NE	M: 240, 241, 237, 236, 237 F: 116, 115, 113, 113, 117	M: ~18 F: ~13	25, 25, 25, 25, 25	Ema et al. (2001)
SD Crj:CD IGS	GD 15–PND 10	Diet	0.0, 60, 3000	77	M: ↓ 77 days at 3000	M: 465, 452, 468, 421 F: 279, 272, 299, 254	M: ~33 F: ~25	8 litters	Takagi et al. (2004)
SD Crl:CD BR	GD 11–PND 20	Gavage	0.0, 3.2, 320	~47	NE	F: 691, 736, 683, 668, 697	~19	44, 51, 47, 28, 38	Kwon et al. (2000)
Wistar–Hans	14 days before mating–PND 21	Water	0.0, 0.01, 0.1, 1.0, 10.0	(22, 29, 36, 43, 50, 57, 64, 71, 78, 85) 90	NE	331, 321, 328, 328, 328, 332	~20	51, 26, 26, 28, 27, 25 (litters)	Cagen et al. (1999b)
Wistar	GD 1–PND 21	Gavage	0.0, 0.03, 0.3	~87	NE	455.2, 460.8, 454.1	~7	13, 15, 13	Kubo et al. (2003)
Wistar–AP	GD 6–GD 21	Gavage	0.0, 0.024, 0.051, 0.109	98	NE	F: 228, 241, 237, 237	~15	26, 26, 27, 26	Tinwell et al. (2002)

NE, no effect.

600 mg/kg bw/day), but the effect was not observed in a second generation exposed according to the same protocol (Tyl et al., 2006). An increase in hepatic histopathologic findings (cytoplasmic variation) at weaning was also observed in offspring of mouse dams exposed during gestation and lactation (LOAEL 50–600 mg/kg bw/day) (Tyl et al., 2006). A single dose level study with gestational exposure in mice reported increased lactational body weight gain and decreased postnatal pup survival at 0.0024 mg/kg bw/day (Howdeshell et al., 1999).

Reproductive system development.

Rat studies: Delays in vaginal opening were observed in offspring of rat dams receiving high oral doses of bisphenol A on GD 6–15 or during the entire gestational and lactational period (Tyl et al., 2000a, 2002b; Tinwell et al., 2002). No delays in vaginal opening were observed with doses of bisphenol A $\leq$1.2 mg/kg bw/day administered to dams during gestation or lactation (Tyl et al., 2000a, 2002b; Ema et al., 2001).

Estrous cycle alterations were not reported in rat oral exposure studies covering a wide range of doses (<1–475 mg/kg bw/day) administered during all or part of the gestational or lactational periods (Kwon et al., 2000; Tyl et al., 2000a, 2002b; Ema et al., 2001).

Studies suggest that preputial separation is delayed following oral administration of high bisphenol A doses (LOAELs = 47.5–475) to male rat offspring in the post weaning period (Tyl et al., 2000a, 2002b; Tan et al., 2003). No effects on preputial separation were observed when treatment of rat dams with high-doses (50–384 mg/kg bw/day) ended during the gestation or lactation period (Tinwell et al., 2002) Oral doses of bisphenol A $\leq$1 mg/kg bw/day also had no effect on preputial separation (Ema et al., 2001; Tyl et al., 2000a, 2002b).

Effects on rat sperm parameters were inconsistent. Decreased sperm count and daily sperm production were reported in offspring of dams exposed during gestation (LOAEL 50 mg/kg bw/day for sperm count/g testis, LOAEL 50 mg/kg bw/day for daily sperm count/g testis) (Tinwell et al., 2002). A single dose level study reported decreased numbers of rats undergoing spermatogenesis following post-weaning exposure of males to 100 mg/kg bw/day (Tan et al., 2003). In contrast, no consistent effects on sperm parameters were observed in rats following exposures with up to 475 mg/kg bw/day during the prenatal, lactational, and post-weaning periods (Tyl et al., 2000a, 2002b). Other rat studies with gestational and lactational doses ranging from <1–4 mg/kg bw/day also reported no effects on sperm parameters (Cagen et al., 1999b; Ema et al., 2001). Testicular histopathology (multinucleated giant cells in seminiferous tubules and absent spermatogenesis) was only reported in a single dose level study at a bisphenol A dose of 100 mg/kg bw/day administered in the post-weaning period (Tan et al., 2003).

Although some sporadic effects were reported for anogenital distance in male and female rats, study authors concluded that the endpoint was not affected by prenatal, lactational, and/or post-weaning exposure to bisphenol A (Ema et al., 2001; Tinwell et al., 2002; Tyl et al., 2000b, 2002b).

No effects on rat prostate weight were observed with bisphenol A doses of <1–475 mg/kg bw/day administered during the gestational, lactational, and/or post-weaning periods (Cagen et al., 1999b; Kwon et al., 2000; Tyl et al., 2000b, 2002b; Tinwell et al., 2002). The study of Timms et al. (2005) in mice raise a level of concern.

Mouse studies: Exposure of mice to bisphenol A during pre- and post-natal development delayed preputial separation (LOAEL 600 mg/kg bw/day) (Tyl et al., 2006). Effects reported for anogenital distance were inconsistent. A single dose study reported an increase in anogenital distance in male mice at 0.050 mg/kg bw/day (Gupta, 2000). A second study with a wide dose range (0.003–600 mg/kg bw/day) reported no consistent or dose-related effects on anogenital distance (Tyl et al., 2006).

One group of investigators reported increased prostate weight at 0.002 and 0.020 mg/kg bw/day in offspring of mouse dams exposed during pregnancy (Nagel et al., 1997). These prostate effects were consistent with findings in single dose level studies with gestational exposure of mice, however, it is noted that the studies had differing periods of exposure and ages of evaluation. One of these studies demonstrated increased prostate weight at 0.050 mg/kg bw/day (Gupta, 2000). Another study demonstrated increased numbers of prostate ducts and proliferating cell nuclear antigen staining in dorsolateral prostate and increased prostate duct volume in dorsolateral and ventral prostate at 0.010 mg/kg bw/day (Timms et al., 2005). However, no effects on prostate or sperm production were observed in more robust studies with multiple dose levels and larger group sizes. A third mouse study with exposures occurring during gestation, lactation, and post-lactational periods also reported no effects on prostate weight, daily sperm production, or efficiency of daily sperm production at doses of 0.003–600 mg/kg bw/day (Tyl et al., 2006). A fourth mouse study demonstrated no effect on sperm density following low-dose exposure ($\leq$0.200 mg/kg bw/day) during gestation or the post-weaning period (Nagao et al., 2002).

Seminiferous tubule hypoplasia in association with undescended testes in mouse weanlings was reported following exposure during pre- and post-natal development (LOAEL 50–600 mg/kg bw/day; BMD_{10} 283–591 mg/kg bw/day) but the effect was not observed in mice examined in adulthood (Tyl et al., 2006). The findings were similar to those in studies reporting no testicular histopathology or lesions in reproductive organs following pre- and post-natal exposure to bisphenol A at $\leq$0.2 mg/kg bw/day (Nagao et al., 2002).

Following exposure of mice during pre- and postnatal development; no effect on age of vaginal opening, estrous cyclicity, or numbers of ovarian primordial follicles were observed at doses ranging from 0.003–600 mg/kg bw/day (Tyl et al., 2006). No effect on age of vaginal opening was reported but there was a shortened period between vaginal opening and first estrus following gestational exposure to 0.0024 mg/kg bw/day in a single dose level study (Howdeshell et al., 1999).

Body Weight: All rat and mouse multigenerational studies have measured body weight as an endpoint. No consistent differences have been detected in the weights of offspring of animals exposed to low to moderate doses of BPA (Table 86).

Hormone Levels: Several studies have measured testosterone and LH levels in rats, there have also been investigations of thyroid hormone (T4) levels. No

consistent effects on the levels of these hormones have been seen (Ema et al., 2001).

Fertility and ability to raise pups to weaning following developmental exposure: Multigenerational studies in both rats and mice have shown that BPA over a wide dose range does not compromise the ability of animals exposed during development to successfully produce offspring, raise them to weaning and for those offspring to successfully give rise to a subsequent generation of animals (Ema et al., 2001; Tyl et al., 2002b, 2006).

Neural and Behavioral Endpoints Following Oral Administration: Several studies addressing effects on neural and behavioral endpoints have been conducted following gestational and lactational exposure [rats: (Funabashi et al., 2004a; Negishi et al., 2004a; Della Seta et al., 2005)]; mice: [(Palanza et al., 2002; Nishizawa et al., 2003, 2005b; Laviola et al., 2005; Ryan and Vandenbergh, 2006)], pubertal exposure [rat: (Akingbemi et al., 2004; Della Seta et al., 2006; Ceccarelli et al., 2007)], and exposure during adulthood [gerbils: (Razzoli et al., 2005)].

Gestational and lactational exposures in rats have reported subtle effects on sexually-dimorphic brain nuclei (Funabashi et al., 2004a), hormonal receptors in brain (Nishizawa et al., 2003, 2005b), and certain sexually-dimorphic or reproductively relevant behaviors (Negishi et al., 2004a; Ryan and Vandenbergh, 2006). Most of this work has utilized single doses across the range of 2–40 μg/kg, and none has been confirmed or linked to other functional or clearly adverse effects. No effects on the volume of the SDN-POA of the hypothalamus were observed in offspring of rats orally exposed to bisphenol A doses ranging from 3.2–320 mg/kg bw/day during the gestation and lactation period (Kwon et al., 2000). Single dose level rat studies demonstrated reduced sexually dimorphic difference in corticotropin-releasing hormone neurons in anterior stria terminalis at 2.5 mg/kg bw/day (Funabashi et al., 2004a). No changes in sexual behavior were reported for female rats exposed to 0.3–320 mg/kg bw/day or males exposed to $\leq$0.3 mg/kg bw/day during the gestation and/or lactation period (Kwon et al., 2000).

Maternal behavior of dams has also been suggested to be altered in two studies of dams exposed during gestation and lactation (Palanza et al., 2002; Della Seta et al., 2005).

One study involving exposures during puberty (Della Seta et al., 2005) suggested alterations in exploratory and sexual behavior of males following 40 μg/kg on PND 23–30. Certain changes in hypothalamic estrogen receptors (Ceccarelli et al., 2007) following 40 μg/kg exposures on PND 23–30 have been reported. Akingbemi et al. (2004) reported effects on gonadal hormonal and receptor endpoints in the pituitary following 2.4 μg/kg/day on PND 21–35.

Other endpoints: Following oral exposure of mice to bisphenol A during gestation, changes were observed for mRNA expression of arylhydrocarbon receptors, receptor repressor, or nuclear translocator and retinoic acid and retinoid X receptors in brain, testes, and/or ovary at 0.00002–20 mg/kg bw/day (Nishizawa et al., 2003, 2005a,b). The strongest effects were found at the lowest doses following exposures during organogenesis (GD 6.5–13.5 or 6.5–17.5) (Nishizawa et al., 2005a,b). The study authors suggested those changes as possible mechanisms for bisphenol A-induced toxicity.

A summary of LH and testosterone effects observed in humans and in bisphenol A-exposed experimental animals is included in Section 4.4.

Summary and Conclusion of Developmental Hazards: There are sufficient data to conclude that bisphenol A does not cause malformations or birth defects in fetuses exposed during gestation at levels up to 640 mg/kg/day (rats) and 1000 mg/kg/day (mice) (Morrissey et al., 1987). This is consistent with the lack of malformations seen in offspring in multigenerational studies (Tyl et al., 2002b, 2006).

There are sufficient data to conclude that bisphenol A does not alter male or female fertility in rats or mice after gestational exposure up to doses of 450 mg/kg/day (Cagen et al., 1999b; Tyl et al., 2000a, 2002b; Ema et al., 2001).

There are sufficient data to conclude that bisphenol A does not change the age of puberty in male or female rats [NOAELs of 0.2 mg/kg/day (Ema et al., 2001) and 1823 mg/kg/day (Tyl et al., 2002b)]. While limited data available suggest an effect on the onset of female puberty in mice [LOAEL 0.2 mg/kg/day (Ryan and Vandenbergh, 2006), 0.002 mg/kg/day, (Howdeshell et al., 1999)], the data are insufficient to conclude that bisphenol A accelerates puberty in female mice. The limited data available suggest, but are insufficient to conclude, that bisphenol A slightly delays the age of puberty in male mice at a LOAEL of ca. 550–800 mg/kg/day (Tyl et al., 2006).

There are sufficient data to conclude that bisphenol A exposure during development does not permanently affect prostate weight in adult rats or mice [NOAELs of: 1823 mg/kg/day (Tyl et al., 2002b), 600 mg/kg/day (Tyl et al., 2006), 4 mg/kg/day (Cagen et al., 1999b), 0.2 mg/kg/day (Ema et al., 2001), 50 mg/kg/day (Tinwell et al., 2002), and 320 mg/kg/day (Kwon et al., 2000). There are sufficient data to conclude that bisphenol A does not cause prostate cancer in rats or mice after adult exposure [calculated dose ranges of 25–400 mg/kg/day for rats, 600–3000 mg/kg/day, mice (NTP, 1982)]. There are slight suggestions, but insufficient data to conclude, that bisphenol A might predispose toward prostate cancer in rats in later life following developmental exposure [at 10 μg/kg (Ho et al., 2006a)]. There are slight suggestions, but insufficient evidence to conclude, that fetal exposure to bisphenol A can contribute to urinary tract deformations in mice [10 μg/kg (Timms et al., 2005)].

There are sufficient data to suggest that developmental exposure to bisphenol A causes neural and behavioral alterations related to sexual dimorphism in rats and mice (ca. 2.5 mg/kg/day, gestation and lactation in rats, (Funabashi et al., 2004a); LOEL 0.00002 mg/kg/day, fetal mice, (Nishizawa et al., 2005a); 0.0002 mg/kg/day, fetal mice, (Nishizawa et al., 2003), 0.04 mg/kg/day, weaning to puberty, rats, (Ceccarelli et al., 2007); 0.1 mg/kg/day, GD 3–PND 20, rats, (Negishi et al., 2004a); 0.2 mg/kg/day, GD 3–PND 20, mice, (Ryan and Vandenbergh, 2006); 0.01 mg/kg/day, GD 11–18, mice, (Laviola et al., 2005), although other studies report no change in a related measure, the size of the sexually dimorphic nucleus of the pre-optic area (SDN-POA) [300 μg/kg/day, rats

(Nagao et al., 1999); NOEL of 320 mg/kg/day, rats, (Kwon et al., 2000)].

4.0 REPRODUCTIVE TOXICITY DATA

4.1 Human

4.1.1 Female. **Takeuchi and Tsutsumi (2002)**, supported by the Japanese Ministry of Education, Science, Sports, and Culture, the Ministry of Health and Welfare, and the Science and Technology Agency, measured bisphenol A in the blood serum of 14 healthy women, 11 healthy men, and 16 women with polycystic ovary syndrome **[diagnostic criteria not discussed]**. The healthy women were evaluated in the mid-follicular phase of the menstrual cycle. Bisphenol A was measured using a competitive ELISA. Serum was also evaluated for total and free testosterone, 17β-estradiol, androstenedione, dehydroepiandrosterone sulfate, LH, FSH, and prolactin. Statistical analysis was by ANOVA. Correlation coefficients were obtained from a linear regression analysis. Mean ± SEM bisphenol A serum concentrations (ng/mL) were 0.64 ± 0.10 in normal women, 1.49 ± 0.11 in normal men, and 1.04 ± 0.10 in women with polycystic ovary syndrome. Bisphenol A serum concentrations were correlated significantly with total testosterone ($r = 0.595$) and free testosterone ($r = 0.609$) in all subjects and in all female subjects ($r = 0.559$ for total testosterone and 0.598 for free testosterone). Bisphenol A serum concentrations were not significantly correlated with any other hormone measures. The authors concluded that either bisphenol A stimulates testosterone production or metabolism of bisphenol A is inhibited by testosterone. They further suggested that displacement of sex steroids from sex-hormone binding globulin by bisphenol A might disrupt the estrogen-androgen balance.

Strengths/Weaknesses: Quality assurance for the hormone radioimmunoassays appeared adequate; however, there was no standardization for time of day for the serum samples, which may result in variable testosterone levels. ELISA has not been standardized for human sera, and may overestimate bisphenol A due to nonspecific binding (see Section 1.1.5). Very little descriptive information was given on any of the groups beyond mean age and body-mass index. No information was given on recruitment methods and participation rates/exclusions. The lack of diagnostic criteria for polycystic ovary syndrome is a weakness. No potential confounders or effect modifiers were identified except mean age and body-mass index. Mean values appear to have been similar between groups. The positive correlations between bisphenol A level and total/free testosterone levels in all women and in entire study group were noted, but these analyses were not adjusted for potential confounders or effect modifiers. No information was given on whether the data were normally or lognormally distributed. The study was limited by small numbers in each group and the results should be regarded as descriptive epidemiology.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but has limited utility given its small size, and limited design. The study provides some insight for potential mechanisms affecting the levels of bisphenol A in the body.

Takeuchi et al. (2004a), supported by the Japanese Ministry of Education, Science, Sports, and Culture, the Ministry of Health, Labor, and Welfare, the National Institute for Environmental Studies, and the Science and Technology Agency, examined relationships between serum sex hormone and bisphenol A concentrations in women with ovarian dysfunction and obesity. Fasting blood samples were collected during the midfollicular phase from 19 non-obese and 7 obese healthy women with normal menstrual cycles. Blood samples were also obtained from 7 women with hyperprolactinemia, 21 patients with hypothalamic amenorrhea, and 13 non-obese and 6 obese patients with polycystic ovary syndrome. **[It not known whether any of these subjects were the same as those reported earlier by this group (Takeuchi and Tsutsumi, 2002).]** Mean ages for the subgroups ranged from 25–29 years old. Blood serum was analyzed for bisphenol A levels using an ELISA technique, and total and free testosterone, 17β-estradiol, androstenedione, dehydroepiandrosterone sulfate, LH, FSH, prolactin, and insulin levels were measured using by RIA. Statistical analyses included ANOVA and linear regression analysis.

Compared to non-obese healthy women, concentrations of bisphenol A in serum were significantly higher in non-obese women with polycystic ovary syndrome **[48% higher]**, obese women with polycystic ovary syndrome **[65% higher]**, and obese healthy women **[46% higher]**. Statistically significant positive correlations were found between bisphenol A level in serum and body mass index ($r = 0.500$) and serum levels of total testosterone ($r = 0.391$), free testosterone ($r = 0.504$), androstenedione ($r = 0.684$), and dehydroepiandrosterone sulfate ($r = 0.514$). The study authors concluded that there is a strong relationship between serum levels of bisphenol A and androgens, possibly due to androgen effects on metabolism of bisphenol A.

Strengths/Weaknesses: Quality assurance for the hormone radioimmunoassays appears adequate. In contrast to the 2002 article by these authors (Takeuchi and Tsutsumi, 2002), blood draws were time-standardized to 9:00–10:00 AM after overnight fasting. As noted in Section 1.1.5, ELISA may overestimate bisphenol A. It was not clear whether any of the women in this study were also included in their 2002 publication. No potential confounders or effect-modifiers were identified except mean age and body-mass index, and neither of these was controlled in the analyses. Positive correlations were observed for bisphenol A level with body-mass index, total testosterone, free testosterone, androstenedione, and dehydroepiandrosterone sulfate for all study groups. These correlations are also found (with the exception of total testosterone) in the control (''normal women'') group as well. Normality of the distributions of the hormones were not reported, and not transformed before analysis. The study was limited by small numbers and results should be regarded as descriptive epidemiology.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but has limited utility in assessing possible relationships of bisphenol A with androgens (testosterone, free testosterone, androstenedione, dehydroepiandrosterone sulfate) and conditions that may promote hyperandrogenism (obesity, polycystic ovarian syndrome).

Hiroi et al. (2004), supported by the Japanese Ministry of Health, Labor, and Welfare, the National Institute for Environmental studies, and the Japan Science and Technology Agency, compared blood bisphenol A levels in women with and without endometrial hyperplasia. Volunteers were recruited from an outpatient clinic in Japan. Women included in the study consisted of 11 controls with normal endometrium, 19 with endometrial hyperplasia, and 7 with endometrial carcinoma. The hyperplasia group was further divided according to severity: 10 with simple hyperplasia and 9 with complex hyperplasia. Mean ages were 48.4–48.9 years in groups without cancer, and the mean age was 63.1 years in the group with endometrial cancer. Blood samples were collected at the time of endometrial examination. Serum bisphenol A levels were measured by ELISA. Data were analyzed by Student *t*-test, with the exception of gravidity and parity, which were analyzed by χ^2 test. There were no significant differences in age, gravidity, parity, or body height, weight, or mass index between the groups without endometrial cancer. Women with endometrial cancer were significantly older and had significantly lower values for gravidity, parity, height, and weight. Mean $\pm$ SD serum bisphenol A levels were reported at 2.5 ± 1.5 ng/mL in controls, 2.2 ± 1.6 ng/mL in women with hyperplasia, and 1.4 ± 0.5 ng/mL in women with endometrial cancer. When the group with hyperplasia was divided according to severity, serum bisphenol A blood levels were reported at 2.9 ± 2.0 ng/mL in the group with simple hyperplasia and 1.4 ± 0.4 ng/mL in the group with complex hyperplasia. Serum bisphenol A levels were significantly lower in women with complex endometrial hyperplasia or endometrial cancer than in controls. The study authors concluded that their preliminary findings demonstrated a possible link between bisphenol A exposure and endometrial hyperplasia or cancer. It was noted that modes of action for bisphenol A may be more complex than expected and that these contradictory results might provide a clue about mechanisms of production of estrogen-dependent diseases.

Strengths/Weaknesses: Because this was a small, cross-sectional study, it is not possible to determine whether this association preceded disease, or could have been associated with the disease process. As noted in Section 1.1.5, ELISA may over estimate bisphenol A.

Utility (Adequacy) for CERHR Evaluation Process: The cross-sectional study design is adequate but of limited utility for this evaluation, but raises research questions regarding mechanisms of production of estrogen-dependent diseases.

Sugiura-Ogasawara et al. (2005), supported by the Japanese Ministry of Health, Labor, and Welfare, conducted a study to determine if there is an association between recurrent miscarriage and bisphenol A levels in blood. The cases in this study were 45 patients with a history of 3 or more (3–11) consecutive first trimester miscarriages. Mean $\pm$ SD age of the cases was 31.6 ± 4.4. None of the cases had a history of live birth. All were seen at a Japanese hospital between August, 2001–December, 2002. Half of the cases were housewives and half were employed in various occupations. A hysterosalpingography analyses was conducted in cases, and chromosome analyses were conducted for both cases and their partners. Women were excluded from the study if uterine anomalies were observed or chromosomal abnormalities were detected in either partner. Serum bisphenol A levels were determined by ELISA. Immunological endpoints examined included antinuclear antibodies, antiphospholipid antibodies, and natural killer cell activity. Blood testing for hypothyroidism, diabetes mellitus, and hyperprolactinemia was conducted. Blood samples were obtained 5–9 days following ovulation in at least 2 cycles. Blood samples to determine progesterone and prolactin levels were taken at 3 months following the last miscarriage and before the next conception. For subsequent pregnancies, ultrasounds were conducted, and spontaneously aborted embryos/fetuses were karyotyped. Serum levels of bisphenol A in cases were compared to those of 32 healthy non-pregnant hospital employees with no history of live birth, infertility, or miscarriage. Mean $\pm$ SD age of controls was 32.0 ± 4.8. None were taking oral contraceptives. Like the cases, the controls lived near Nagoya City. Statistical analyses included Welch test, Mann–Whitney test, and Pearson correlation coefficient.

Bisphenol A levels (mean $\pm$ SD) were reported to be significantly higher in women with recurrent miscarriages (2.59 ± 5.23 ng/mL) compared to healthy controls (0.77 ± 0.38 ng/mL). In the 45 cases, incidences of abnormal conditions were 15.6% for hypothyroidism, 13.3% for antiphospholipid antibodies, 22.2% for antinuclear antibodies, 11.1% for hyperprolactinemia, and 20.5% for luteal phase defect. Serum levels of bisphenol A were significantly higher in patients who tested positive versus negative for antinuclear antibodies (mean $\pm$ SD = 7.382 ± 9.761 vs. 1.222 ± 1.54 ng/mL). Thirty-five of the patients became pregnant and 48.6% had another miscarriage. Serum bisphenol A levels in patients who miscarried were 4.39 ± 8.08 ng/mL, and serum bisphenol A in patients with successful pregnancies were 1.22 ± 1.07 ng/mL (not statistically significant). The study authors concluded that exposure to bisphenol A is associated with recurrent miscarriage.

In a letter to the editor, Berkowitz (2006) stated that this study did not support an association between bisphenol A blood levels and recurrent miscarriage. Several limitations were noted for the study. Timing and numbers of blood samples collected were not defined clearly. It was noted that because bisphenol A has a short half-life, it would be critical to know if blood samples were obtained in a timeframe relevant to the occurrence of miscarriage. Although differences in serum bisphenol A levels in cases compared to controls achieved statistical significance, it was noted that median levels of bisphenol A in serum were nearly identical in patients with recurring miscarriages (0.71 ng/mL) and controls (0.705). The similarities in median values suggested there were no differences between the two groups, and it was suggested that apparent differences in mean serum levels of bisphenol A were due to a few individuals, as was demonstrated in Figure 1 of the Sugiura-Ogasawara et al. (2005) report. Berkowitz (2006) stated that the Welch test was inappropriate for statistical analyses and noted that the two evaluation groups could not be considered comparable because of differences in occupation (housewives compared to medical workers) and unknown fertility of controls. Because the controls were not evaluated for factors such as hypothyroidism and systemic lupus erythematosus (associated with

antinuclear antibodies), the conditions may have been over represented in cases and may have been the cause of the reported differences between the 2 groups. Although mean bisphenol A levels were (non-significantly) lower in women who subsequently became pregnant and had a successful pregnancy compared to those who miscarried, Berkowitz (2006) noted that the median level of bisphenol A was actually higher in women with the successful pregnancies. Last, the ELISA method for measuring bisphenol A levels has not been validated and is subject to inaccuracy due to extensive cross-reactivity.

In a response to the comments by Berkowitz (2006), Sugiura-Ogasawara (2006) stated that although measurement of bisphenol A levels at various time points would have been ideal, obtaining samples every day during pregnancy would have been difficult. Sugiura-Ogasawara (2006) clarified that bisphenol A values were based on a single sample in each individual, but that similar tendencies were observed for a second blood sample. With respect to the use of women with live births as controls, Sugiura-Ogasawara (2006) explained that the same blood samples were used for measurements of other environmental compounds, some of which are known to decrease after delivery. It was noted that none of the cases had systemic lupus erythematosus, and that use of controls with hypothyroidism or antinuclear antibodies was not considered important for the study. Superiority of the HPLC method compared to the ELISA method for measuring serum bisphenol A levels was acknowledged, but the authors stated that the ELISA method was used because of limited funding, reiterated that the study was preliminary and used a small number of volunteers, and that additional studies using a larger sample and more appropriate analytical methods were needed.

Strengths/Weaknesses: The letter from Berkowitz (2006) summarizes many of the weaknesses of this study. No quality assurance information was given for the biomarker/hormone measurements. As the Berkowitz letter points out, the ELISA method is not standardized for human sera (and may overestimate bisphenol A due to nonspecific binding), the distribution of exposure was not normal, and median values of the two groups were similar, with two women skewing the mean. Little information was provided on the characteristics of the two study groups or response rates. Age and body-mass index were controlled in the analyses, but other potential confounders and effect modifiers were not. The time between exposure and observation was not appropriate. Spontaneous abortions have been associated with many factors which have not been addressed here. The authors' conclusions require the assumption that bisphenol A measurement levels represent those present during the important time frame for the spontaneous abortion. The authors do not report the time frame for collection of the blood samples. Nonnormal data were not appropriately transformed for analysis. Welch's test was used "…to compare bisphenol A levels…because the distribution of the two groups might have differed." Welch's test is a *t*-test for groups with unequal variance, not different distributions (both should be normal, which was probably not the case).

Utility (Adequacy) for CERHR Evaluation Process: Because of limitations in the design and analysis of this work, this study is inadequate has no utility in this evaluation.

Yang et al. (2006), supported by the Korean FDA, measured urine bisphenol A in 172 Korean men and women and evaluated the relationship of these values with UDP-glucuronosyl- and sulfotransferase polymorphisms, with sister-chromatid exchange testing, and with self-reported symptoms of possible endocrine origin. First-morning urine samples were collected at the time of a routine physical examination, as was a blood sample, and a questionnaire was completed. Urine bisphenol A was measured using reverse phase HPLC. DNA was isolated from blood samples and polymorphisms were determined at *SULT1A1* and *UGT1A6*. Sister chromatid exchange in response to *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) was evaluated in blood cells **[not otherwise specified]**. The relationship between urine bisphenol A and continuous variables was assessed with simple or multiple regression analysis and the relationship with categorical variables assessed with the Wilcoxon test.

None of the subjects reported occupational exposure to bisphenol A. The median urine bisphenol A concentration was 7.86 μg/L. Urine bisphenol A was not different in men and women. Urine bisphenol A was associated with body-mass index ($P = 0.06$) and self-reported frequency of alcohol consumption ($P = 0.08$). *SULT1A1* and *UGT1A6* polymorphisms were not associated significantly with urine bisphenol A concentrations. No significant associations were observed between urine bisphenol A and MNNG-induced sister-chromatid exchange, although they were associated when lower levels of MNNG were used. There were no significant associations between urine bisphenol A and self-reported symptoms of possible endocrine origin, including thirst/frequent urination, dizziness, neck mass, heat intolerance, sweating, hot flashes, swelling of lymph nodes, dysmenorrhea, menstrual irregularity, or menorrhagia. The authors concluded that even though they had been unable to associate an endocrine disorder with urine bisphenol A, continuous biologic monitoring of bisphenol A would be prudent.

Strengths/Weaknesses: Bisphenol A was measured in urine using HPLC. No information was given regarding any selection criteria or response rates and some outcome measures were self-reported.

Utility (Adequacy) for CERHR Evaluation Process: While small, this study is useful for providing descriptive exposure information on BPA urinary levels (see Section I). This study does not have utility for evaluation of reproductive endpoints.

4.1.2 Male. **Luconi et al. (2001)**, supported by the Italian Public Health Project, examined the effects of in vitro exposure of human spermatozoa to bisphenol A. Semen was collected from normozoospermic men, and spermatozoa were separated. Intracellular calcium was measured using a spectrofluorometric method in cells treated with 1 μM bisphenol A, 1 μM 17β-estradiol, 10 μM progesterone, **[17 β-estradiol is noted as 10μM in Figure 6, text states 1μM]** or the same concentrations of bisphenol A in combination with 17β-estradiol or progesterone. Effects on acrosome reaction were examined using a fluorescent staining method in cells exposed to 1 μM **[0.23 mg/mL]** bisphenol A for 2 hr, with and without exposure to 10 μM progesterone. **[In the study figures summarizing results, sample numbers in studies involving bisphenol A were listed at 5–11. It**

is not known if the sample numbers represented total numbers of sperm donors. Very few protocol details were provided in the Methods section and many of the limited details presented above were obtained from the Results section.] Data were analyzed by Student *t*-test and 1-way ANOVA. Treatment of spermatozoa with bisphenol A resulted in a modest influx of calcium, but bisphenol A had no effect on calcium responses induced by 17β-estradiol or progesterone. Bisphenol A exposure did not affect basal acrosome reaction or acrosome reaction induced by progesterone. Results were in contrast to those observed with 17β-estradiol, which inhibited the acrosome reaction induced by progesterone. The study authors concluded, BPA did not exert any direct effect on calcium fluxes and acrosomal reaction in human spermatozoa either in basal conditions or in response to progesterone challenge.

Strengths/Weaknesses: Strengths of this study include examining human spermatozoa and use of a concurrent control (E2) to demonstrate the responsiveness of the system. Weaknesses include limited information on the spermatozoa samples, the single concentration of BPA used, and lack of clarity of concentrations of E2 versus bisphenol A administered.

Utility (Adequacy) for CERHR Evaluation Process: This study did not demonstrate that BPA-altered progesterone-mediated acrosomal reaction and is not useful in the evaluation process.

Hanaoka et al. (2002), supported by the Japanese Ministry of Health and Welfare and Ministry of Education, Science, Sports, and Culture, examined possible relationships between bisphenol A exposure and hormone levels in male workers. Exposed workers included 42 men in 3 Japanese plants who sprayed an epoxy hardening agent consisting of a mixture of bisphenol A diglycidyl ether (10–30%), toluene (0–30%), xylene (0–20%), 2-ethoxyethanol (0–20%), 2-butoxyethanol (0–20%), and methyl isobutyl ketone (0–30%). The workers were said to wear "protection devices" during spraying. Controls consisted of 42 male assembly workers from the same plants who did not use bisphenol A diglycidyl ether, were within 3 years of age to exposed workers (37 years vs. 38 years), and smoked the same number of cigarettes/day as exposed workers (21/day). Percentages of smokers were 86% in both groups, but percentages of alcohol drinkers were significantly lower in the exposed workers (43%) than in controls (57%) ($P = 0.03$). Urine and blood samples were obtained during periodic health examinations performed in June and July, 1999. Urinary bisphenol A was measured by HPLC, and urinary organic solvent metabolites were measured by GC or HPLC. Plasma LH, FSH, and free testosterone levels were measured by immunosolvent assay in a commercial laboratory. Data were log transformed and compared by paired *t*-test, Pearson correlation coefficient, and χ^2 test. Adjustments were made by linear regression for age and drinking habits, which were considered possible confounders.

Urinary bisphenol A concentrations were significantly higher in exposed workers (median: 1.06 μmol/mol creatinine **[0.043 μg/kg bw]**; range: <0.05 pmol to 11.2 μmol/mol creatinine) than in controls (median: 0.52 μmol/mol creatinine **[0.021 μg/kg bw]**; range: <0.05 pmol to 11.0 μmol/mol creatinine). Average difference was reported as 2.5 (95% CI = 1.4–4.7; $P = 0.002$). Bisphenol A was not detected in 3 exposed workers and 1 control. Urinary solvent metabolites were detected more frequently in exposed workers than controls. No differences in plasma testosterone or LH concentrations were observed between exposed workers and controls. Plasma FSH concentrations were significantly lower in exposed workers (median = 5.3 mIU/mL; range = 4.0–8.3 mIU/mL) than in controls (median = 7.6 mIU/mL; range = 5.4–11.0 mIU/mL; average difference = 1.3; 95% CI = −1.5 to −1.0). A "mild correlation" was reported between urinary bisphenol A and FSH ($r = -0.20$, $P = 0.071$) but was not observed for urinary solvent levels. A statistically significant relationship was observed between FSH and bisphenol A following adjustment for alcohol intake ($r = -0.23$; $P = 0.045$). The study authors concluded that bisphenol A may be generated endogenously following exposure to bisphenol A diglycidyl ether, and bisphenol A may disrupt gonadotropic hormone secretion in men.

Strengths/Weaknesses: Quality assurance for the hormone radioimmunoassays appeared adequate. Blood draws and urine samples were time standardized between 10:00 AM and 12:00 PM. Reference values were given and population values were considered in the discussion. Use of HPLC for bisphenol A and standard methods for the other urinary metabolites with creatinine-adjustment are strengths. The epoxy sprayer workers were matched to coworkers from other parts of the process. All selected workers participated in the study. Analyses were adjusted for age and alcohol use, and workers were matched on age (±3 years) and cigarette use. A plausible ($P = 0.07$) correlation between bisphenol A and decreasing FSH was reported. The authors took care to note that all FSH levels were within the clinical normal range. Correlations between other workplace exposures and hormones were not observed. Blood and urine samples were collected concurrently, but not on the first day of the week. Statistical methods were appropriate to the study size and distribution of the data. Nonnormal distributions were transformed or treated as nonnormal. Biomarker data were handled appropriately in analysis.

Utility (Adequacy) for CERHR Evaluation Process: This survey was methodologically sound and mechanistically thoughtful. This study is adequate and of high utility for the evaluation.

4.2 Experimental Animal

Studies in this section examine reproductive endpoints after administration of bisphenol A to sexually mature animals. Reproductive endpoints after administration of bisphenol A during pregnancy, the neonatal period, or puberty are discussed in Section 3.2.

4.2.1 Female

4.2.1.1 Rat: Goloubkova et al. (2000), supported by the Brazilian National Council of Scientific and Technological Development and the National University of Rio Grande Do Sul, examined the effects of bisphenol A exposure on the uterus and pituitary of ovariectomized rats. Wistar rats (60–67 days old) were fed a standard certified rodent diet. **[No information was provided on housing or bedding materials.]** Rats were subjected to bilateral ovariectomy or sham surgery. At 14 days post-surgery, rats were randomly assigned to groups of at

least 6 animals. Rats were s.c. injected with bisphenol A in DMSO vehicle (>99% purity) at doses of 11, 78, 128, or 250 mg/kg bw/day for 7 days. An ovariectomized vehicle control group was exposed to the 50% DMSO vehicle. A sham-operated control group was not exposed to the vehicle. Rats were killed following the dosing period, and body and uterine weight were measured. Trunk blood was collected for measurement of serum prolactin level by RIA. The anterior pituitary was weighed and preserved in 10% formalin. An immunohistochemical technique was used to identify pituitary cells expressing prolactin. A total of 3 or 4 rats per group were evaluated for prolactin-positive cells in the pituitary and 6–8 rats were evaluated for the other endpoints. Data were analyzed by ANOVA followed by post-hoc Student-Neuman–Keuls test or Kruskal–Wallis ANOVA followed by post-hoc Dunn test.

In the 250 mg/kg bw/day group, final body weight was 7% lower than in the ovariectomized vehicle control group, and body weight gain was lower compared to the ovariectomized vehicle and sham controls. There was no effect of treatment on food intake. A dose-related increase in uterine weight occurred in all groups of rats exposed to bisphenol A compared to the ovariectomized vehicle controls, but uterine weight in the bisphenol A groups was lower than in the sham controls. Ovariectomy resulted in decreased pituitary weight in ovariectomized vehicle controls and in the bisphenol A 11 and 78 mg/kg bw/day dose groups compared to sham controls. Pituitary weight did not differ from sham controls after 128 mg/kg bw/day bisphenol A and was greater than in sham controls after 250 mg/kg bw/day bisphenol A. Basal prolactin levels did not differ between the sham and ovariectomized vehicle controls. Serum prolactin levels were increased in the 128 and 250 mg/kg bw/day bisphenol A groups compared to the ovariectomized vehicle controls. Ovariectomy reduced the numbers of prolactin-positive cells in the pituitary. The number of prolactin positive cells in the pituitary was increased by 64% in the 250 mg/kg bw/day group compared to the ovariectomized controls. The study authors concluded that the reproductive tract and neuroendocrine axis of Wistar rats can respond to bisphenol A.

Strengths/Weaknesses: This study represents a comprehensive neuroendocrine assessment across multiple doses. Weaknesses are the absence of a positive control to demonstrate maximal response in endpoints examined, high-dose levels required to induce response, and the s.c. route of administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility for the evaluation process.

Funabashi et al. (2001), supported by Yokoyama City University, examined the effects of bisphenol A exposure on expression of progesterone receptor mRNA in the brain of ovariectomized rats. The effects of butylbenzyl phthalate were also examined but will not be discussed. **[No information was provided on feed, caging, or bedding materials.]** Wistar rats were ovariectomized at 7–8 weeks of age. Ten days following ovariectomy, 6 rats/group were s.c. injected with sesame oil vehicle, 10 mg bisphenol A **[purity not reported]**, or 10 μg 17β-estradiol. Rats were killed 24 hr later and the preoptic area, medial basal hypothalamus, and anterior pituitary were removed. Expression of mRNA for progesterone receptor, preproenkephalin, and neurotensin were assessed by Northern blot. Data were analyzed by ANOVA followed by Fisher protected least significant difference test. Exposure to bisphenol A resulted in increased expression of progesterone receptor mRNA in the preoptic area and anterior pituitary. Bisphenol A did not affect expression of mRNA for neurotensin in the preoptic area or preproenkephalin in medial basal hypothalamus. 17β-Estradiol increased expression of mRNA for progesterone receptor in the preoptic area, medial basal hypothalamus, and anterior pituitary and increased preproenkephalin mRNA expression in medial basal hypothalamus. The study authors concluded that bisphenol A increases expression of progesterone receptor mRNA in the preoptic area of adult ovariectomized rats.

Strengths/Weaknesses: Strengths are the use of a positive control and the biological plausibility of the model. Weaknesses include subcutaneous administration of a single high-dose level.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion but of limited utility.

Yamasaki et al. (2002a) conducted a 28-day exposure study that provided some information on the reproductive organs of male and female rats. **[Complete details of this study are included in Section 2. Results for females are discussed in this section, and results for males are discussed in Section 4.2.2.1.]** CD rats were fed a commercial diet (MF Oriental Yeast Co.) and housed in stainless steel wire mesh cages. Ten 7-week-old rats/sex/group were gavaged with bisphenol A **[98% purity]** at 0 (olive oil vehicle), 40, 200, or 1000 mg/kg bw/day for 28 days. Due to the death of 1 animal exhibiting clinical signs in the 1000 mg/kg bw/day group, the high-dose was reduced to 600 mg/kg bw/day on Day 8 of the study. In an additional study, rats were exposed to ethinyl estradiol at 0, 10, 50, or 200 μg/kg bw/day for 28 days. There were no treatment-related alterations in blood levels of thyroid hormones, FSH, LH, 17β-estradiol, prolactin, or testosterone. The numbers of females with diestrus lasting 4 or more days was increased in the high-dose group. Relative weights of ovary and uterus were unaffected. No gross or histopathological alterations were reported for reproductive organs. The study authors concluded that change in estrous cyclicity was the only useful endpoint for evaluating the endocrine-mediated effects of bisphenol A. In comparison, females from the mid- and/or high-dose ethinyl estradiol group experienced alterations in estrous cyclicity, decreased ovarian weight, increased uterine weight, and histopathological changes in the ovary, uterus, and vagina.

Strengths/Weaknesses: This study was well-conducted, used an appropriate route of administration, a positive control group, adequate sample sizes, a range of doses, and evaluations of both sexes. Weaknesses include failure to define the criteria for an abnormal estrous cycle, female necropsy at a point unrelated to stage of estrous.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Spencer et al. (2002), supported by NIH, evaluated the uterine response to bisphenol A before and after

deciduoma formation in pseudopregnant Sprague–Dawley rats. **[Cage and bedding materials and feed were not indicated.]** Adult females underwent mechanical cervical stimulation to induce pseudopregnancy **[pseudopregnancy day not indicated]**. On pseudopregnancy day 4, deciduoma formation was induced under ether anesthesia by antimesometrial uterine epithelial trauma, applied through a laparotomy under ether anesthesia. Rats were treated with s.c. bisphenol A **[97% purity]** 0 or 200 mg/kg bw in alcohol/saline on pseudopregnancy days 1–4 and killed on pseudopregnancy day 5, or treated on pseudopregnancy days 5–8 and killed on pseudopregnancy day 9. Uteri and pseudopregnancy day 9 endometria were harvested. Uteri were weighed and homogenized for measurement of protein and DNA content. Inducible nitric oxide synthase activity, decidual prolactin-related protein mRNA, *ER* mRNA, and cytosolic ER binding sites were measured in uteri and/or endometria. Blood was obtained for determination of serum 17β-estradiol and progesterone. **[*n* = 5 was indicated for some of the data presentations.]** Results are summarized in Table 87. The authors called attention to the difference in bisphenol A effect depending on whether exposure was before or after deciduoma induction. They concluded that there was a decrease in proliferation when bisphenol A was given during deciduoma induction, with a decrease in decidual proteins, in spite of a lack of differential effect on *ER* mRNA or cytosolic ER binding sites. The authors also concluded that bisphenol A activity appeared to be antagonized by progesterone **[although they probably meant that bisphenol A antagonized the action of progesterone]**.

Strengths/Weaknesses: These data are intriguing, but the functional consequences of bisphenol A administration on decidual formation were not assessed and the s.c. route of administration and the use of a single high-dose are a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility to the evaluation process.

Table 87
Bisphenol A Effects on Pseudopregnant Rats[a]

Endpoint	Treatment period, pseudopregnancy day 1–4	Treatment period, pseudopregnancy day 5–8
Uterus		
Wet weight	↑ 1.4-fold	↓ 63%
Protein content	↑ 1.4-fold	↓ 64%
DNA content	↔	↓ 53%
Decidual prolactin-related protein mRNA[b]	↔	↓ 44%
ER mRNA[b]	↓ 29%	↓ 50%
Cystosolic ER-binding sites	↓ 57%	↓ 37%
Nitric oxide synthase activity[b]	↔	↓ 50%
Pseudopregnancy day 9 endometrium		
Decidual prolactin-related protein mRNA[b]	Not applicable	↓ 48%
ER mRNA[b]	Not applicable	↓ 43%
Nitric oxide synthase activity[b]	Not applicable	↓ 40%
Serum		
17β-Estradiol	↔	↔
Progesterone	↔	↓ 49%

[a]Spencer et al. (2002).
[b]Estimated from a study graph by CERHR.
↑, ↓, ↔ Statistically significant increase, decrease, or no change compared to vehicle control.

Funabashi et al. (2003), supported by Yokohama City University, examined the effects of bisphenol A exposure on sexual behavior and progesterone receptor expression in adult rats. Wistar rats were ovariectomized at 7–8 weeks of age. **[No information was provided on feed, caging, or bedding materials.]** In two sets of experiments, an immunohistochemistry technique was used to measure expression of progesterone receptor in the preoptic area and ventromedial hypothalamus following bisphenol A exposure. In the first experiment, 3–5 rats/group were s.c. injected with sesame oil vehicle, 10 mg bisphenol A (~40 mg/kg bw) **[purity not reported]**, or 10 μg 17β-estradiol (~40 μg/kg bw) 2 weeks following ovariectomy. In the second experiment, ovariectomized rats (3–4/group) were s.c. injected with bisphenol A at 0.001, 0.010, 0.1, or 1 mg (~0.004, 0.040, 0.4, or 4 mg/kg bw). Rats were killed the day following dosing, and brains were removed and fixed in 2% paraformaldehyde. Statistical analyses included ANOVA followed by Scheffé post-hoc test and Kruskall–Wallis test. Sexual behavior was examined in a third experiment. Ovariectomized rats were s.c. injected with sesame oil vehicle, 10 mg bisphenol A, or 10 μg 17β-estradiol. The next day, rats were injected with 1 mg progesterone or vehicle to generate 4 treatment groups: sesame oil+progesterone ($n = 5$), bisphenol A+sesame oil ($n = 5$), bisphenol A+progesterone ($n = 8$), or estradiol+progesterone ($n = 6$). Examination of behavior with a sexually receptive male was conducted 5–7 hr following progesterone or vehicle injection. Statistical analyses included ANOVA followed by Scheffé post-hoc test.

In the first experiment, injection of rats with 10 mg bisphenol A increased progesterone-positive cells in both the preoptic area and ventromedial hypothalamus. The dose–response experiment demonstrated that dose-related increases in progesterone-positive cells in both brain regions occurred following exposure to ≥0.1 mg bisphenol A. In sexual behavior testing, treatment with bisphenol A had no effect on lordosis quotient. Rejection quotient was significantly higher in rats exposed to 10 mg bisphenol A and primed with 1 mg progesterone than in the vehicle control rats primed with progesterone. Treatment with 17β-estradiol resulted in increased numbers of progesterone positive cells in the preoptic area and ventral medial hypothalamus and increased lordosis quotient. The study authors concluded that the findings suggest that bisphenol A influences sexual behavior by altering the progesterone receptor system in the hypothalamus.

Strengths/Weaknesses: This study appears to have been relatively well conducted with the incorporation of a positive control group and examination of anatomical and functional endpoints. The number of animals per group is sufficient given the nature of this study design. However, the route of administration was sc.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for the evaluation process but of limited utility due to the route of administration.

Funabashi et al. (2004b), supported by Yokohama City University and the Japanese Ministry of Education, Culture, Sports, Science, and Technology, examined the effects of bisphenol A exposure on expression of progesterone receptor mRNA in brain of adult ovariectomized rats. *p*-Nonylphenol and 4-*tert*-octyl phenol were also examined, but will not be discussed. **[No information was provided on feed, housing, or bedding materials.]** Wistar rats were ovariectomized at 7 weeks of age, and experiments were conducted 10 days following ovariectomy. In the first experiment, 6 rats/group were s.c. injected with sesame oil vehicle or 10 mg bisphenol A (~40 mg/kg bw) **[purity not reported]**. Rats were killed 24 hr following injection, and frontal, parietal, and temporal cortex were removed. In a second experiment, frontal, temporal, and occipital cortex were collected from rats at 0, 6, 12, or 24 hr following injection with 10 mg bisphenol A; 5–6 rats were killed and examined at each time point. In both experiments, progesterone receptor mRNA expression was determined by Northern blot in each area of the cortex. Data were analyzed by ANOVA followed by Fisher protected least significant difference post-hoc test. At 24 hr following bisphenol A exposure, expression of progesterone receptor mRNA was increased in the frontal cortex and decreased in the temporal cortex. In the time-course experiments, expression of progesterone receptor mRNA was increased in the frontal cortex and decreased in the temporal cortex from 6–24 hr following exposure. Bisphenol A had no effect on expression of progesterone receptor mRNA in the parietal or occipital cortex. The study authors concluded that bisphenol A can alter the neocortical function through the progesterone receptor in adult rats, but the physiological significance of the effect is not known.

Strengths/Weaknesses: This study links relatively high single-dose (10 mg) s.c. bisphenol A administration to the induction of progesterone receptor mRNA, an estrogenic response. Weaknesses is the absence of a positive control to demonstrate maximal response in estrogen-mediated increases in progesterone mRNA and the failure to examine any physiological or functional endpoints. It was also not determined if increases in mRNA were associated with increases in progesterone receptor protein. There was only one dose level administered at a single time point. The s.c. route of dose administration is a weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion but of limited utility.

Della Seta et al. (2005), supported by a grant from MURST, Italy, examined the effects of bisphenol A exposure on maternal behavior in rats. **[No information was provided in the manuscript on the type of chow, bedding, and caging used. The Expert Panel has been informed that Harlan Teklad 2018 chow, Lignocel bedding, and polysulfone cages were used (F. Farabolli et al., personal communication, March 1, 2007).]** Female Sprague–Dawley rats were trained to ingest peanut oil from a micropipette. At 14 weeks of age, female rats were mated for 48 hr. On the day following mating, females were randomly assigned to groups administered peanut oil ($n = 23$) or 0.040 mg/kg bw/day bisphenol A **[purity not indicated in the manuscript; ≥95% according to the authors (F. Farabollini et al., personal communication, March 1, 2007)]** ($n = 17$) through a micropipette. Dosing was continued through the gestation and lactation periods. Two days following delivery, litters were culled to 4 male and 4female pups and were cross-fostered within treatment groups. Pups were weighed on Days 2, 7, and 21 following birth. Maternal behavior was tested at 3 and 4 days and at 8 and 9 days following delivery. In 30-min test sessions, frequency, duration, and latency of behaviors such as retrieving pups, licking pups, postures, and nest building were evaluated with pups of the same sex. Behavior with pups of the opposite sex was evaluated on the second day of the test period, and the order of testing with male and female pups was reversed during each testing period (Days 3–4 and 8–9). Data were analyzed by general linear model, Duncan multiple range test, and/or Mann–Whitney *U* test. The numbers of females giving birth were 9 of 17 in the bisphenol A group and 18 of 23 in the control group. Nine dams in the control group and 7 in the bisphenol A group were evaluated for maternal behavior. The only significant effect reported for bisphenol A was reduced duration of licking-grooming pups, which occurred with both sexes of pups during both observation periods **[~25–50 % decrease as estimated from a graph]**. Effects reported to be marginally significant were decreased frequencies of licking-grooming of pups ($P < 0.09$), anogenital licking of pups ($P < 0.08$), and arched back posture ($P < 0.07$). The study authors concluded that maternal behavior in rats is influenced by prolonged exposure to low bisphenol A doses during pregnancy and lactation.

This behavioral study suggested that a low, oral dose of bisphenol A (0.040 mg/kg bw/day) affects pregnancy and maternal behavior.

Strengths/Weaknesses: Weaknesses include the use of a single dose level and an unusually low pregnancy rate in the controls (18/23) as well as the authors emphasis on marginally significant bisphenol A effects.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

4.2.1.2 Mouse: Park et al. (2004), support not indicated, examined the effects of bisphenol A exposure on the reproductive and hematological systems of male and female mice. **[Results for females are discussed here, and results for males are discussed in Section 4.2.2.2.]** Adult ICR mice were fed mouse formulation feed (Cheil Feed). **[No information was provided about caging or bedding materials.]** Fifteen mice/sex/group were i.p. injected with bisphenol A **[purity unknown]** in an ethanol/corn oil vehicle at 0.05, 0.5, or 5.0 mg/kg bw on 5 occasions (every 3 days over a 14-day period). One control group received no treatment and a second control group was i.p. injected with corn oil. Females were examined 7 days following administration. Reproductive organs were weighed and fixed in Bouin solution, and histopathological examination was conducted. Hematological and clinical chemistry endpoints were also assessed. Data were analyzed by least significant difference test.

Exposure to bisphenol A had no effect on body weight. Significant decreases were observed for right ovary weight in the mid- and high-dose group

and left ovary weight in the mid-dose group **[25–27% lower]**. No treatment effects were observed for uterine or ovarian histology. There were no effects of bisphenol A treatment on hematological endpoints in females. Blood urea nitrogen levels were decreased significantly **[by 28–32%]** in females of all dose groups. The study authors did not report conclusions regarding study findings.

Strengths/Weaknesses: The study design regarding frequency and route of administration and the lack of an appropriate positive control are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate though of limited utility for the evaluation process.

Berger et al. (2007), supported by the Natural Sciences and Engineering Research Council of Canada, examined the effect of bisphenol A exposure on blastocyst implantation in mice. CF-1 mice were housed in polypropylene cages and were fed Harlan Teklad 22/5 rodent chow, which was stated to contain soy. **[No information was provided about bedding materials.]** On GD 1–4 or 5 **[described as GD 1–5 in Methods section and GD 1–4 in study figures and tables]** (GD 0 = day of vaginal plug), 8–9 mice/group were s.c. injected with peanut oil vehicle or bisphenol A (97% purity) at 10.125 mg/animal/day. **[Assuming that the mice weighed 0.02 kg at the start of gestation (USEPA, 1988), CERHR estimated bisphenol A intake at 500 mg/kg bw/day.]** Mice were killed on GD 6 for an examination of implantation sites. Data were analyzed by χ^2 test or 2-sample *t*-test. The number of implantation sites was reduced significantly in the treated animals (mean of ~2.5 compared to ~15 in controls). Implantation sites were observed in 8 of 8 control females at a range of 12–17/female. Six of 9 females in the bisphenol group had no implantation sites. The study authors concluded that pregnancy disruption occurred during the period of implantation.

Strengths/Weaknesses: Weaknesses include lack of experimental details for examining the uteri, use of a single high-dose, number of corpora lutea were not recorded.

Utility (Adequacy) for CERHR Evaluation Process: Due to the absence of key information and faulty methodology, this study is inadequate for evaluation process.

Al-Hiyasat et al. (2004), supported by Jordan University of Science and Technology, examined the effect of bisphenol A and dental composite leachate on fertility of female mice. In this study, Swiss mice were fed a standard laboratory feed containing soy protein. **[No information was provided on caging and bedding materials.]** At 60 days of age, 11 mice/group were gavaged with distilled water or composite leachate for 28 days. Components of the composite leachate were identified by HPLC and included tri-(ethylene glycol)-dimethacrylate (5945 mg/L), bisphenol A glycerolate dimethacrylate (2097 mg/L), and bisphenol A (78 mg/L). **[Based on the reported volume of administration of 0.2 mL and a body weight of 34.4 g, CERHR estimated bisphenol A intake from leachate at 0.45 mg/kg bw/day.]** Additional 60-day-old mice ($n = 15$/group) were gavaged with bisphenol A (97% purity), at doses of 0 (ethanol/distilled water vehicle), 0.005, 0.025, or 0.1 mg/kg bw/day for 28 days. Five mice/group in the bisphenol A study were killed at the end of the dosing period for measurement of body, uterus, and ovary weights. All mice in the leachate study and 10 mice/group in the bisphenol A study were mated to untreated males (2 females to 1 male) for 10 days. One week following the end of the mating period, the mice were killed and examined for pregnancy, implantations, viable fetuses, and resorptions. Body, ovary, and uterus weights were measured in mice from the leachate study. Data were analyzed by Student *t*-test or Fisher exact test.

Effects in the leachate group included increased relative (to body weight) ovarian weight and decreased percentages of pregnant mice. In mice exposed to bisphenol A, body weights were decreased at all dose levels. Effects observed in mice exposed to the mid- and high-dose of bisphenol A included increased uterine weight, increased percentages of resorptions/implantations, and increased percentages of mice with resorptions. Ovarian weight was increased in mice of the high-dose bisphenol A group. **[Although the effects were not statistically significant, the percentages of pregnant females were 90, 77.7, 80, and 60% pregnant mice in the control and each respective dose group.]** In both the composite leachate and bisphenol A groups, there were no statistically significant effects on implantations or viable fetuses. The study authors concluded that bisphenol A and components leached from dental composite have adverse effect on fertility and the reproductive system of mice.

Strengths/Weaknesses: With only 5–10/group, this study was underpowered for determination of potential bisphenol A-related effects on fertility and other endpoints. Confirmation of mating was not performed (cohabitation was for 10 days; if the mice mated on day 10, the necropsy would have been performed on GD 7. Mean body weight and reproductive organ weights of bisphenol A-treated animals were only collected from 5 mice/dose level. Moreover, the normal body weight range for 10-week-old female Swiss mice is 28–35 g. Given that there are only 5 mice/group, it is hard to draw any meaningful conclusions from these data.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation based on small sample size.

Matsumoto et al. (2004), support not indicated, examined the effect of maternal bisphenol A exposure on growth of offspring in mice; this study was discussed in Section 3.2.7. Because the results of this study bear on lactation competence in treated dams, the study will also be considered here. Mice were fed standard rodent chow (CE-2, Japan Clea). **[No information was provided on caging and bedding materials.]** Mice of the ddY strain were exposed to bisphenol A (≥97% purity) through feed at 0 or 1% from GD 14–PND 7. The study authors stated that the bisphenol A dose was equivalent to 1000 mg/kg bw/day. **[The numbers of dams treated was not indicated. Day of vaginal plug and day of birth were not defined.]** Mice delivered pups on PND 21. Body weight of pups were monitored during the postnatal period in 31 pups from the control group and 61–89 pups from the bisphenol A group. Serum prolactin levels were measured by RIA in 3 dams/group 4 days following delivery. Pups were killed on PND 7, and

stomach weight was measured. Data were analyzed by Student *t*-test.

No differences were reported for live pups at birth. During the postnatal period, body weights of pups in the bisphenol A group were significantly lower **[by ~40%]** than control group pups. No deaths were reported for pups in the control group, but 30% of pups in the bisphenol A group died before PND 7. On PND 1, milk could be seen in stomachs of pups from the control group, but not the bisphenol A group. **[The number of pups evaluated for milk in stomach was not reported]**. On PND 7, stomach weight was significantly lower **[by 40%]** in pups from the bisphenol A compared to control group. Serum prolactin level was significantly reduced **[by 46%]** in dams from the bisphenol A group. The authors concluded that administration of a high bisphenol A dose to mice resulted in suppressed postnatal growth of offspring which probably resulted from an insufficient supply of milk, which might have been due to decreased prolactin secretion.

Strengths/Weaknesses: This study was conducted at a single high-dose that likely induced maternal toxicity (which was not assessed); therefore, it is difficult to delineate if the findings in the mouse pups are the result of potential bisphenol A-related effects of maternal toxicity or an effect on the pup.

Utility (Adequacy) for CERHR Evaluation Process: Given the likely confounding effects of maternal toxicity, this study is considered inadequate and of no utility.

4.2.1.3 Other mammals: Nieminen et al. (2002a), support not indicated, examined the effects of bisphenol A exposure on hormone levels in the European polecat (*Mustela putorius*). Five animals/group/sex **[age not reported]** were administered bisphenol A **[purity not reported]** in feed at concentrations providing doses of 0, 10, 50, or 250 mg/kg bw/day for 2 weeks. Body weight and length were measured during the study. Animals were killed at the end of the exposure period, with sampling conducted in random double-blinded order. Liver and kidney were weighed. Blood samples were obtained for measurement of hormone levels by RIA. Microsomal enzyme activities were determined. Statistical analyses included ANOVA, post-hoc Duncan test, Student *t*-test, Spearman correlation coefficient, Kolmogorov–Smirnov test, and/or Levene test.

There were no clinical signs of toxicity and no effects on body weight or body mass index following bisphenol A exposure. Absolute and relative liver weight were significantly increased in females of the high-dose group. Plasma cortisol levels were significantly reduced in females of the mid-dose group. Bisphenol A exposure had no significant effects on plasma levels of testosterone, estradiol, FSH, or thyroid hormones. Glutathione-*S*-transferase (GST) activity was significantly increased in females of the high-dose group. UDPGT activity was significantly higher in females of the mid- and high-dose group and males of the high-dose group. There was no effect on 7-ethoxyresorufin O-deethylase (EROD) activity. The study authors concluded that the endocrine effects in this study were not as remarkable as the effects on liver enzymes.

Strengths/Weaknesses: A strength of this study is the use of a non-rodent species and multiple doses. Weaknesses include small sample size and absence of reproductive endpoints.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful due to small sample size and absence of reproductive endpoints.

Nieminen et al. (2002b), support not indicated, examined the effects of bisphenol A exposure on endocrine endpoints in field voles (*Microtus agrestis*). Animals were housed in plastic cages with wood shavings and fed R36 diet (Lactamin, Sweden). Sexually mature field voles were randomly assigned to groups that received bisphenol A **[purity not reported]** in propylene glycol by s.c. injection for 4 days. Doses of bisphenol A (numbers of females in each group) were 0 ($n = 5$), 10 ($n = 7$), 50 ($n = 5$), and 250 ($n = 8$) mg/kg bw/day. Animals were killed the day following the last dose. Body and liver weights were measured. Blood was drawn for measurement of sex steroids, thyroxine, and weight-regulating hormone levels in plasma using RIA or immunoradiometry methods. The activities of EROD, UDPGT, and GST were measured in hepatic and renal microsomes using appropriate substrates. Statistical analyses included ANOVA, post-hoc Duncan test, Student *t*-test, Kolmogorov–Smirnov test, Levene test, Mann–Whitney *U* test, χ^2 test, and Spearman correlation. **[Results for males are discussed in Section 4.2.2.3.]**

Mortality was significantly increased by bisphenol A treatment, with incidences of 18, 36, and 20% in the low- to high-dose groups. No mortality was observed in the control group. Bisphenol A treatment did not significantly affect body or liver weight. Plasma testosterone levels increased with dose, and statistical significance was attained in high-dose females compared to control females. 17β-Estradiol levels decreased with dose in females. Pooled (male+female) LH levels were not significantly altered by treatment. Liver EROD activity **[apparently combined for males and females]** was significantly decreased at the mid- and high-dose, and liver GST activities **[not clear if for males or females or both]** was significantly decreased at the highest dose level. There were no other significant effects on microsomal enzymes examined. The study authors concluded that wild mammals such as field voles could be more susceptible to bisphenol A-induced toxicity than laboratory rodents.

Strengths/Weaknesses: A strength is the use of another species**.** The small number of voles/dose level, the subcutaneous route of administration, and questionable statistical procedures are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Razzoli et al. (2005), supported by the Ministry of University Education and Research and the University of Parma, examined the effects of bisphenol A on sociosexual and exploratory behavior in female Mongolian gerbils, a monogamous species. Animals were fed Mil Morini Rodent Chow (Reggio Emilia, Italy) and housed in Plexiglas cages with wood shaving/cotton nesting material. At 11–12 weeks of age, female gerbils were trained to drink corn oil from a syringe, and 1 week later, they were paired with a male. From Days 1–21 of cohabitation, 12 females/group were fed 0 (corn oil vehicle), 0.002, or 0.020 mg/kg bw/day bisphenol A **[purity not indicated]** from a syringe. A group of 12 females received ethinyl estradiol, the positive control, 0.04 μg/kg bw/day during the same time period. During the cohabitation period, social behavior (e.g., agonism,

social investigation, huddling, and nest sharing) was observed and body weights of females were measured. A free exploratory test, which measured the amount of time females spent in an area of a cage with home nesting material compared to the time spent in an unfamiliar area of a cage, was conducted following the 21-day cohabitation period. Exploratory behavior was evaluated by an observer blinded to treatment groups. Statistical analyses included ANOVA and Duncan test for multiple comparisons.

Bisphenol A treatment did not affect body weight. Social sniffing was increased significantly **[by 60%]** in the low-dose bisphenol A group. Significant effects **[percent changes compared to control]** observed in the exploratory test were decreased time in the unfamiliar area at the low **[60%]** and high **[44%]** dose, fewer transitions to the unfamiliar area at the low **[60%]** and high **[50%]** dose, fewer transitions to the home cage at the high-dose **[29%]**, and less time in the unfamiliar area at the low dose **[46%]**. Similar results for both social sniffing and exploratory behavior were observed in the positive control group. According to the study authors, this study demonstrated that chronic exposure of adult female gerbils to environmentally relevant doses of bisphenol A during the hormonally sensitive period of cohabitation resulted in subtly altered social and exploratory behavior.

Strengths/Weaknesses: This study examined behavioral endpoints in gerbils, and included a positive control (ethinyl estradiol) and 2 doses of bisphenol A. It appears to be well conducted using oral dosing, respectable sample size (given study complexity), and use of a positive control. Weaknesses include failure to account for temporally repeated measures in statistical analyses.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion but of limited utility for the evaluation process.

4.2.1.4 Invertebrates: Although studies in invertebrates may be important for understanding mechanisms of action and environmental impact, the Panel views these studies as not useful for the evaluation process.

Oehlmann et al. (2000), supported by the Berlin Federal Environmental Agency, reported the effects of bisphenol A on reproductive organs in the freshwater ramshorn snail (*Marisa cornuarietis*) and the marine dog whelk (*Nucella lapillus*). In the first experiment, adult ramshorn snails were exposed for 5 months to bisphenol A in ethanol at 0, 1, 5, 25, or 100 μg/L. Thirty snails/group were removed every month for evaluation of reproductive organs. **[Culture ware type not indicated. The purity of bisphenol A and its stability during the exposure period were not reported. The snails removed for evaluation were adults; this species requires 8 months to attain sexual maturity. Octylphenol was also evaluated, but is not discussed here.]** In the second experiment, ramshorn snails were exposed to bisphenol A in ethanol at 0, 1, or 100 μg/L for 1 year. Thirty F_1 snails per time point were removed for evaluation at 6, 8, and 12 months. In the third experiment, dog whelk were exposed to bisphenol A in glacial acetic acid at 0, 1, 25, or 100 μg/L for 3 months. Thirty specimens were removed for evaluation each month. Evaluations included measurements of sex organs and the identification of sperm or oocytes in the genital tract. Statistical analyses included ANCOVA followed by Tukey or Student-Newman–Keuls test, Kruskal–Wallis test, χ^2 test, and Weir test.

Adult ramshorn snails were reported to show increases in volume of the capsule and albumen glands (portions of the oviduct). **[Apparently, the increase in volume was based on appearance rather than measurements. The measured lengths of the sex organs were not affected by treatment.]** Occasional specimens that had been exposed to bisphenol A showed rupture of the oviduct with protrusion of the egg mass. Enumeration of spawning masses and eggs showed statistically significant time-dependent increases in all bisphenol A groups. Histologic examination of the gonads did not suggest abnormalities of spermatogenesis or oogenesis. The F_1 snails also demonstrated a statistically significant increase in spawning mass and oocyte production at the 100 μg/L bisphenol A concentration, and some specimens showed rupture of the oviduct at 12 months of age in both bisphenol A groups. An increase in imposex **[the presence of vas deferens tissue]** was noted significantly more often in snails exposed to bisphenol A 100 μg/L than controls. Adult dog whelk demonstrated a significant increase in the length and weight of the sex glands and an increase in number of females with oocytes in the oviduct. The authors concluded that invertebrates are sensitive to bisphenol A toxicity at environmentally relevant concentrations.

Strengths/Weaknesses: The study appears to be well conducted and suggests that bisphenol A has stimulatory (17β-estradiol-like) effects on the spawning masses and eggs of snails. These changes occurred in the absence of a histological correlate. The potential stability/biotransformation was discussed in the introduction but not determined during the exposure period.

Utility (Adequacy) for CERHR Evaluation Process: This study is not considered useful for the evaluation process.

Forbes et al. (2007), supported by the Bisphenol A Global Industry Group, evaluated the effects of bisphenol A on reproduction in the freshwater ramshorn snail (*Marisa cornuarietis*). Bisphenol A **[purity not indicated]** concentrations in test water were 0, 0.10, 1.0, 16, 160, and 640 μg/L. Concentrations were periodically checked. Thirty breeding pairs per treatment level were observed for a 12-week period. The number of egg masses and number of eggs/egg mass were recorded. Hatchability was evaluated using 5 consecutive egg masses collected from 5females/replicate (75 egg masses/treatment). Juvenile growth rates were calculated for a subset of the offspring. Nested ANOVAs were used for data analysis. All snails survived. There were no significant treatment-related differences in adult egg production, hatchability, or juvenile growth rate. Interindividual variability in these parameters was prominent, and the authors concluded that a large number of replicates would be necessary using this animal model to detect reproductive effects.

Strengths/Weaknesses: This study examined dose response over a 12-week exposure of freshwater snails to bisphenol A with egg masses and number of eggs/egg mass as endpoints. Although no treatment-related effects were observed, interindividual variability was high.

Utility (Adequacy) for CERHR Evaluation Process: This study is not considered useful for the evaluation process.

Schirling et al. (2006), supported by the county of Baden-Württemberg, examined the effects of bisphenol A on embryo development in the apple snail, *Marisa cornuarietis*. Stocks of 150 adult snails were maintained in a glass aquarium containing tap water and sea salt, exposed to a 12/12 hr light/dark cycle, and fed fish flake food, carrots, and cucumbers. Fifteen to twenty eggs/exposure group were placed in a glass Petri dish with bisphenol A **[purity not indicated]** 50 or 100 μg/L **[11.4 or 22.8 mM]**, ethinyl estradiol 10 μg/L, DMSO 0.005‰ (solvent for ethinyl estradiol), or water (solvent for bisphenol A). From embryo visibility (~3.5 days after egg laying) to ~day 14, eggs were evaluated daily for formation of eyes, tentacles, heart rate, and hatching. Statistical analyses were performed using Student *t*-test or Kruskal–Wallis test.

There were no differences in formation of eyes and tentacles between treatments groups Heart rate was significantly decreased on Day 9 for bisphenol A 100 μg/liter compared to the water control group with description of "a similar trend" in hatching. **[The data figure does not show a statistically significant effect of bisphenol A treatment on hatching.]** There was a significantly higher hatching weight in the 100 μg/L bisphenol A group compared to the water control group. Ethinyl estradiol treatment significantly decreased embryo heart rate compared to the water control group but not compared to the DMSO control. No statistically significant effects of ethinyl estradiol on time to hatch or hatching weight were demonstrated. The authors concluded that bisphenol A and ethinyl estradiol had similar effects on snail development.

Strengths/Weaknesses: Weaknesses include the lack of evaluation of the achieved concentration and stability of bisphenol A in water and the comparison of ethinyl estradiol to the water control instead of the DMSO control. The authors' conclusions are weakened by the lack of statistical significance of most of their findings.

Utility (Adequacy) for CERHR Evaluation Process: This study is not considered useful for the evaluation process.

4.2.1.5 In vitro: Although in vitro studies may be important for understanding mechanisms of action and cellular and subcellular events, the Panel views these studies as not useful for the evaluation process.

Xu et al. (2002), supported by the Japan Society for the Promotion of Science, examined the effects of bisphenol A exposure on mouse ovarian granulosa cells in a series of experiments. Ovarian granulosa cells were obtained from 4-week-old $B6C3F_1$ mice. Following incubation of cells with 0 or 100 fM **[23 pg/L]** to 100 μM **[23 mg/L]** bisphenol A **[purity not indicated]** in ethanol vehicle for 72 hr, the CellTiter 96 assay was used to evaluate cell viability, and the TUNEL assay and 4′,6-diamidino-2-phenyllindole staining were used to evaluate apoptosis. In cells that were incubated in 100 μM **[23 mg/L]** bisphenol A for 24, 48, or 72 hr, the TUNEL method was used to evaluate apoptosis and a flow cytometry technique was used to assess apoptosis and the cell cycle. Bcl2 and Bax protein expression was examined by Western blot, and mRNA expression was assessed by RT-PCR in cells that were exposed to 100 μM **[23 mg/L]** bisphenol A for 72 hr. Experiments were repeated a minimum of 3 times. Statistical analyses included ANOVA followed by Fisher protected least significant difference test. **[Statistical significance was not clearly indicated for some endpoints.]**

A dose-related reduction in cell viability was observed at bisphenol A concentrations ≥100 pM **[23 ng/L]**. Examination of cells by the TUNEL method indicated a concentration-related increase in apoptosis at bisphenol A concentrations ≥100 pM **[23 ng/L]**. Features noted in apoptotic cells included cellular shrinkage, membrane blebbing, and nuclear condensation. Apoptotic cells, as determined by TUNEL and the presence of sub-G_1 cells were increased in a time-related manner following incubation with 100 μM **[23 mg/L]** bisphenol A from 24 to 72 hr. An increase in G_2-M arrest was also observed and reached a maximum value following a 48-hr incubation of cells with 100 μM **[23 mg/L]** bisphenol A (18 vs. 12% in controls). Expression of Bax protein was increased and Bcl2 protein was decreased following incubation with 100 μM **[23 mg/L]** bisphenol A for 72 hr. Similar expression patterns were observed for *Bax* and **Bcl2** mRNA expression **[data were not shown by study authors]**. The study authors concluded that bisphenol A at doses of 100 pM **[23 ng/L]** and higher, presumably relevant to environmental concentrations, decreases viability and increases apoptosis in granulosa cells. The study authors postulated that apoptosis may have been induced by decreases in the anti-apoptotic protein Bcl2 and increases in the pro-apoptotic protein Bax.

Strengths/Weaknesses: Because this study used in vitro study PMSG-stimulated murine cells, metabolism is likely to have been minimal (if present at all) and the in vitro dosimetry of bisphenol A is difficult to extrapolate to in vivo dosimetry. Bisphenol A is known to induce reactive oxygen species, which may influence the tetrazolium salt-based assay. Moreover, based on the data presented the mechanism by which bisphenol A may be inducing cell cytotoxicity/apoptosis is likely not "endocrine disruptor" mediated.

Utility (Adequacy) for CERHR Evaluation Process: This study is not considered useful for the evaluation process.

Mlynarcíková et al. (2005), supported by the European Union, examined the effects of bisphenol A exposure on hormone production by porcine ovarian granulosa cells. Granulosa cell cultures were prepared from porcine ovaries collected from a slaughter house. The cells were incubated for 72 hr in media containing bisphenol A **[purity not indicated]** at 10^{-8}–10^{-4} M **[2.3 μg/L to 23 mg/L]** or the DMSO vehicle, with or without addition of 1 μg/mL FSH or LH. Following the incubation period, media were collected for measurement of progesterone and 17β-estradiol concentrations by RIA. Experiments were replicated 5–8 times. Data were analyzed by ANOVA and Bonferroni post test. Significant changes in progesterone production, included an increase at 10^{-5} M **[2.3 mg/L]** and decrease at 10^{-4} M **[23 mg/L]** bisphenol A. Bisphenol A significantly increased FSH-stimulated progesterone synthesis at 10^{-6} M **[0.23 mg/L]** and inhibited FSH-stimulated progesterone production at 10^{-4} M **[23 mg/L]**. LH-induced progesterone production was inhibited by 10^{-4} **[23 mg/L]** bisphenol A. FSH-induced 17β-estradiol production was also inhibited by bisphenol A at all concentrations tested, but statistical significance was only attained at doses ≥10^{-6} M

[0.23 mg/L]. Bisphenol A dimethylacrylate was also tested, and most results were similar to those observed with bisphenol A. The study authors concluded that ovarian steroidogenesis might be a target of bisphenol A toxicity.

Strengths/Weaknesses: Potential estrogenic effects were observed at 10^{-5} M bisphenol A. Decreases in responses observed at the 10^{-4} M concentration are likely due to nonspecific cytotoxicity. Bisphenol A-mediated responses in progesterone endpoints appeared to reach a near maximum at the lowest dose level examined. There was no mention of whether phenol red-free media were used or whether fetal bovine serum was charcoal-stripped. The serum likely contained steroids, which would have been potential confounding factors. Also, it appears that cell viability was not examined after the incubation period. With exception of the highest dose level, there was no dose response (inconsistent trends); the statistical flags are potentially due to random chance. Since this was an in vitro study, the potential effects of metabolism could not be assessed.

Utility (Adequacy) for CERHR Evaluation Process: Due the weaknesses and limitation in the experimental design, this study is considered inadequate.

Mohri and Yoshida (2005), supported by the Japanese Ministry of Education, Science, Sports, and Culture, examined the effects of bisphenol A and 17β-estradiol exposure on calcium oscillations in immature mouse oocytes. Immature oocytes with intact germinal vesicles were obtained from 8–12-week-old CD-1/ICR mice and incubated in bisphenol A **[purity not indicated]** in a DMSO vehicle at concentrations of 0 or 10 nM **[2.3 μg/L]** to 100 μM **[23 mg/L]** for 60 min. Calcium oscillations were measured using a Fura-2 dye and image analyzer. Data were analyzed by Student *t*-test. At 100 μM **[23 mg/L]** bisphenol A, the duration of calcium oscillations was significantly shortened and the oscillations became irregular. The same findings were observed following exposure to 17β-estradiol at concentrations that were 10,000-fold lower than that of bisphenol A, producing the same effect. The study authors stated that estrogens may affect the oocyte by regulating calcium oscillations and that bisphenol A could affect oocyte maturation.

Strengths/Weaknesses: This study appears to have been well conducted; however, because this study used an in vitro system, metabolism could not be assessed. It is unclear if calcium oscillations play a role in oocyte maturation in other species, including humans.

Utility (Adequacy) for CERHR Evaluation Process: This study is not considered useful for the evaluation process.

4.2.2 Male.. Studies on the androgenicity of bisphenol A, including Hershberger assays, are discussed in Section 2.2.3.

4.2.2.1 Rat: Yamasaki et al. (2002a), support not indicated, conducted a 28-day exposure study that provided some information on the reproductive organs of male and female rats. **[Complete details of this study are included in Section 2. Results for males are discussed in this section, and results for females are discussed in Section 4.2.1.1.]** CD rats were fed a commercial diet (MF Oriental Yeast Co.) and housed in stainless steel wire mesh cages. Ten 7-week-old rats/sex/group were gavaged with bisphenol A **[98% purity]** at 0 (olive oil vehicle), 40, 200, or 1000 mg/kg bw/day for 28 days. Due to the death of 1 animal exhibiting clinical signs in the 1000 mg/kg bw/day group, the high-dose was reduced to 600 mg/kg bw/day on Day 8 of the study. In an additional study, rats were exposed to ethinyl estradiol at 0, 10, 50, or 200 μg/kg bw/day for 28 days. There were no treatment-related abnormalities in sperm or alterations in blood levels of thyroid hormones, FSH, LH, 17β-estradiol, prolactin, or testosterone. Changes in relative reproductive organ weights **[assumed to be relative to body weight]** included a 28% decrease in relative ventral prostate weight and 21% increase in relative testis weight in the high-dose group. No gross or histopathological alterations were reported for reproductive organs. The study authors concluded that change in estrous cyclicity was the only useful endpoint for evaluating the endocrine-mediated effects of bisphenol A. In comparison, male rats exposed to the mid and/or high doses of ethinyl estradiol experienced decreased prostate, seminal vesicle, and pituitary weights; increased testis weight; and histopathological alterations in prostate, seminal vesicle, mammary gland, and testis.

Strengths/Weaknesses: This study was well-conducted, used an appropriate route of administration, a positive control group, adequate sample sizes, a range of doses, and evaluations of both sexes. A weaknesses include an insufficient duration of exposure to examine the full spermatogenic cycle.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Takahashi and Oishi (2001), support not indicated, examined the effects of bisphenol A exposure on testis of rats. F344 rats were fed standard, soy-containing diet (CE-2; Clea Japan) and housed in stainless steel suspended cages. Four-week-old male rats ($n = 8$/group) were administered bisphenol A (99.0% purity) through diet at concentrations of 0, 0.25, 0.5, or 1.0% for 44 days. The study authors estimated bisphenol A intake at 235, 466, and 950 mg/kg bw/day. The stability of bisphenol A in the diet was verified. Food intake was measured, and animals were weighed and observed daily for clinical signs. Rats were killed when mean body weight of controls reached ~200 g. Testosterone levels were measured in serum using an ELISA method. Preputial gland, testes, epididymides, prostate, seminal vesicles, kidneys, and liver were weighed. The testis was fixed in buffered 6% formaldehyde and examined histologically. Statistical analyses included Bartlett test, ANOVA, Dunnett or Scheffé parametric test, Kruskall–Wallis test, Dunnett non-parametric test, Wilcoxon rank sum test, χ^2 test, Mantel–Haenzel test, and Fisher exact test.

Statistically significant findings are summarized in Table 88. Body weight gain and terminal body weights were reduced in males of the mid- and high-dose groups. Food intake was said to be slightly decreased according to dose. Absolute organ weight effects included decreased weight of preputial glands at all doses; liver in the mid- and high-dose group; and seminal vesicles with coagulation glands, dorsal and lateral prostate, and hypophysis at the high-dose. **[The Expert Panel assumes that by coagulation gland, the authors mean the anterior prostate or coagulating gland.]** Significant organ weight effects relative to body weights are

Table 88
Effects Observed in Male Rats Exposed to Bisphenol A Through Diet[a]

Endpoint	Dose, % in diet **[mg/kg bw/day]**						
	0.25	0.5	1.0	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Terminal body weight	↔	↓ 13%	↓ 18%	0.55 **[522]**	0.42 **[399]**	0.41 **[389]**	0.30 **[285]**
Relative weight							
Dorsal and lateral prostate	↔	↔	↓ 32%	0.29 **[276]**	0.22 **[209]**	0.52 **[494]**	0.36 **[342]**
Preputial gland[b]	↓ 22%	↓ 26%	↓ 25%	0.13 **[124]**	0.09 **[86]**	0.18 **[171]**	0.12 **[114]**
Liver	↔	↓ 10%	↓ 14%	0.69 **[656]**	0.56 **[532]**	0.30 **[285]**	0.23 **[218]**
Kidney	↑ 8%	↑ 8%	↑ 12%	0.99 **[940]**	0.69 **[656]**	0.50 **[475]**	0.34 **[323]**
No. rats with:							
Seminiferous tubule degeneration[c]	↔	↑ to 6/8	↑ to 5/8				
Disorganization of Stage I–VI spermatids (+ severity)[c]	↑ to 4 of 8	↔	↔				
Disorganization of Stage I–VI spermatids (2+ severity)[c]	↔	↔	↑ to 6 of 8	0.36 **[342]**	0.22 **[209]**		
% Seminiferous tubules in stages							
I–VI	↓ 59%	↓ 70%	↓ 53%				
IX–XI	↑ 3.4-fold	↑ 5.2-fold	↑ 4-fold				
XII–XIV	↑ 3.2-fold	↑ 3.6-fold	↑ 3-fold				

[a]Takahashi and Oishi (2001).
[b]Benchmark doses were estimated using a polynomial model.
[c]Control value = 0 of 8.
↑,↓ Statistically significant increase, decrease compared to controls; ↔no statistically significant effect compared to controls.

summarized in Table 88. Changes in relative organ weights included decreased preputial gland weight and increased kidney weights at all doses, decreased liver weight at the mid- and high-dose, and decreased dorsal and lateral prostate weight at the high-dose. Testicular lesions observed with bisphenol A treatment included seminiferous tubule degeneration at the mid and high-dose, disorganized spermatids at all dose levels, and differences in percentages of seminiferous tubules in spermatogenic stages at all dose levels. Although it does not appear that statistical significance was attained, dose-related increases in arrested spermatogenesis and disappearance of elongated spermatids were also reported. There were no significant effects on serum testosterone concentrations. The study authors concluded that bisphenol A was toxic to the testis and accessory sex organs of F344 rats at a minimum toxic dose of 235 mg/kg bw/day.

Findings suggest a hormonal effect on hormone-dependent reproductive tissues at all doses examined. The lowest dose level, 0.25% in diet, exhibited histopathological changes in the testes, most strikingly described as a large alteration in the relative frequency of the different stages of the seminiferous epithelium. Due to techniques used for fixation and embedding of the testes, the histopathological analyses may be of limited value.

Strengths/Weaknesses: This study reports a relatively well conducted study with a relevant route of administration. General toxicity was demonstrated. Formalin produces excessive shrinkage of testes when followed by paraffin embedding and is inappropriate especially when staging will be conducted.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Sakaue et al. (2001), supported by the Japanese Science and Technology Agency, examined the effect of bisphenol A exposure on spermatogenesis in the adult rat. Animals were fed CE-2 chow (CLEA Japan) and housed in stainless steel wire caging. Thirteen-week old male Sprague–Dawley rats (5/group) were gavaged for 6 days with the ethanol/corn oil vehicle or bisphenol A (99.6% purity) at doses 0.020, 0.200, 2, 20, or 200 mg/kg bw/day. The high-dose was based on a preliminary experiment that demonstrated reduced daily sperm production in a Holtzman rat gavaged with 200 mg/kg bw/day bisphenol A for 6 days. In this study, rats were killed 2 days following dosing (at 14 weeks of age) or at 18 weeks of age. Testes were weighed. Sperm endpoints were measured from one testis. Histopathological examinations were conducted on the other testis after fixation in Bouin fluid, paraffin embedding, and staining with hematoxylin and eosin. Statistical analyses included Student *t*-test, ANOVA, and Fisher protected least significant difference test.

There were no changes in daily sperm production/g testis at 14 compared to 18 weeks of age. **[No data were shown for 14-week-old rats, and results of bisphenol A treatment were not discussed.]** Bisphenol A did not significantly affect body or testis weight at 18 weeks of age. In the 18-week-old rats, daily sperm production and daily sperm production/g tissue were significantly reduced **[by ~25%]** in all bisphenol A treatment groups. The study authors noted the lack of a dose–response relationship and that daily sperm production in treated groups at 18 weeks of age was comparable to that of 14-week-old controls. Histopathological evaluations of testis revealed no evidence of atrophy or disrupted spermatogenesis in the seminiferous tubules. **[Data were not shown by study authors.]**

Table 89
Conditions Used in Experiments to Study Bisphenol A Effects on Sperm Production in Rats[a]

Experiment	Bisphenol A doses mg/kg bw/day	No. rats/group	Diet/water	Caging
1	0, 0.020, 2, or 200	10	RM3/Automatic system	Stainless steel, unspecified bedding
2	0, 0.020, 2, or 200	10	5002/Glass bottles	Stainless steel, no bedding
3	0, 0.020, 2, or 200	10/bisphenol A group 20/control group	5002/ Glass bottles	Stainless steel, no bedding
4	0, 0.020, 2, or 200	10/bisphenol A group 20/control group	CE2/ Glass bottles	Stainless steel, no bedding
5	0	10	RM3, 5002, or CE2/not specified	Not specified

[a]Ashby et al. (2003).

To obtain more dose–response information, Sakaue et al. (2001) repeated the study in 8 rats/group dosed **[assumed by gavage as in the first study]** with 0.000002, 0.00002, 0.0002, 0.002, 0.020, 0.200, or 2 mg/kg bw/day bisphenol A. **[It is assumed that ages of rats, treatment period, and observation periods were the same as in the first study.]** Body and testis weights were not affected by bisphenol A treatment at Week 18. At Week 18, significant decreases in daily sperm production and daily sperm production/g tissue were observed at 0.020, 0.200, and 2 mg/kg bw/day. **[The decrease compared to control was estimated from a graph. For daily sperm production, the decreases were ~30% at 0.020 mg/kg bw/day, ~34% at 0.200 mg/kg bw/day, and ~32% at 2 mg/kg bw/day. For daily sperm production/g tissue, the decreases were ~24% at 0.020 mg/kg bw/day, ~32% at 0.200 mg/kg bw/day, and ~28% at 2 mg/kg bw/day.]**

In a third experiment, rats were given a single oral dose of 0.020 mg/kg bw bisphenol A. Six hours later, the rats were killed, the right testis was homogenized, and the cytosol was examined for protein expression using two-dimensional polyacrylamide gel electrophoresis. Changes in intensity and mobility were noted for 3 unidentified proteins. The study authors concluded that the dose–response curve for bisphenol A affects on spermatogenesis in the adult rat was monotonic rather than having an inverted U-shape.

Strengths/Weaknesses: This study used a relevant route of administration and multiple doses. A weakness is the brief exposure period. Variability in control daily sperm production between the first and second study is disturbing; given the small sample (5 or 8/group), this variability severely decreases confidence in the data. No histopathologic correlate was presented.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility due to small sample and variable control values between experiments.

Ashby et al. (2003), support not indicated, examined the effects of bisphenol A exposure on sperm production in rats. The study attempted to replicate earlier findings from Sakaue et al. (2001). Five independent experiments were conducted, and the conditions for each experiment are summarized in Table 89. Some of the experiments used the same conditions as the Sakaue et al. (2001) study, including stainless steel cages with no bedding, CE2 diet (CLEA, Tokyo, Japan), and glass water bottles. In the first 4 studies, 10–20 adult (~13-week-old) Sprague–Dawley rats/group were gavaged with bisphenol A (99% purity) at 0 (ethanol/corn oil vehicle), 0.020, 2, or 200 mg/kg bw/day for 6 days. Concentrations of dosing solutions were verified. In the fifth study, rats fed different diets were gavaged with vehicle for 6 days. Rats were fed 1 of 3 diets as indicated in Table 89. Phytoestrogen aglycone content of the feed was measured. Respective concentrations of daidzein, genistein, and coumestrol in each feed were reported at 94, 62, and 0.6 μg/g diet for Rat and Mouse No. 3 (RM3; Special Diet Services Ltd.); 40, 23, and 0.1 μg/g diet for 5002 (Purina Mills); and 157, 106, and 2.2 μg/g CE2 diet. Ten rats were used in each group, except in third and fourth studies, where 20 control rats were split into 2 groups before dosing. Rats were administered drinking water through an automatic system in the first study and via glass bottles in the other studies. In the first study, rats were housed 3/cage at the start of the study and 2/cage later in the study. In the other 4 studies, rats were housed 2/cage. Rats were weighed during the study. Animals were killed at 18 weeks of age, 5 weeks after the start of dosing. Liver, kidney, and reproductive organs were weighed, and sperm counts were obtained. In the first 4 studies, data were analyzed by ANOVA, ANCOVA for organ and body weights, and Dunnett test. Results from all 4 studies were also analyzed by ANOVA in an attempt to increase study power. Data from the fifth study were analyzed by Fisher least significant difference test.

In the 4 studies that compared the effects of bisphenol A exposure to a vehicle control group, there were no significant effects of bisphenol A exposure on sperm count, daily sperm production, or weights of body, liver, kidney, testis, prostate, epididymis, or seminal vesicle. One animal exposed to 200 mg/kg bw/day bisphenol A in the third study was reported to have unexpectedly small testes and epididymides, but the study authors indicated that inclusion of this animal in later statistical analyses had no effect on outcome. One animal in the 200 mg/kg bw/day group in the fourth study had a small testis. No significant effects were observed when data from the first 4 experiments were pooled and analyzed. The study authors noted that some endpoints were variable from one experiment to the other. It was noted that prostate weights were 10% lower in animals from Experiment 1 than from Experiments 2–4. Sperm counts and daily sperm production were reportedly different in control animals from Experiment 1 compared to Experiment 2. It was noted that rats were fed different diets in Experiment 1 (RM3) and Experiment 2 (5002), and a study to examine the effects of feed was conducted. In the study examining effects in rats fed

different diets but exposed to vehicle, no effects of diet on daily sperm production were observed. The only significant effect reported was a 9% lower weight of right epididymis in rats fed CE2 compared to RM3 or 5002 feed. The study authors stated that the effect was likely spurious due to lack of effect on other endpoints, no effect on left or total epididymis weight, and lack of the effect in the first 4 experiments. The study authors concluded that there was no evidence in their study that bisphenol A affected reproductive organ weights or daily sperm production. Lack of bisphenol A-induced effect on daily sperm production was in contrast to observations of the Sakaue et al. (2001) study, which reported a decrease in this endpoint. Subtle genetic differences in the rats were suggested as a possible reason for differences in results between the 2 studies.

Given the robustness and comprehensiveness of this study, it is highly useful. It strongly suggests that the NOAEL for potential bisphenol A-mediated effects on the adult rat reproductive system exceeds 200 mg/kg/day. Absence of confirmation of the work of Sakaue et al. (2001) led to an extensive study of the potential variables (e.g., diet, housing, etc.) that might account for the discrepancies. These data suggests that subtle changes in study endpoints, especially daily sperm production and organ weights, may occur by random chance or genetic differences in the respective lab's supplier of rats may play a role. These data also strongly suggest bisphenol A administered orally has no effect on sperm production albeit following only 6 days of administration.

Strengths/Weaknesses: This study reports a well conducted, comprehensive assessment of the potential effects of bisphenol A delivered by 6 daily doses on daily sperm production. The 6-day treatment period is a (understandable) weakness.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and useful for the evaluation process.

Tohei et al. (2001), supported in part by the Japan Society for the Promotion of Science, examined the effects of bisphenol A exposure on testicular function of Wistar–Imamichi rats. **[No information was provided about composition of chow, bedding, or caging.]** In a series of studies, rats were dosed with bisphenol A **[purity not indicated]** in sesame oil by s.c. injection for 2 weeks. Bisphenol A doses were 0.1 or 1 mg/day **[∼0.3 or 3 mg/kg bw/day based on the reported body weights of 300–350 g]**. The dose of 1 mg/day bisphenol A was stated to be similar to the highest exposures reported in humans, which were based on saliva levels measured in patients receiving composite dental sealants. Doses and exposure duration were based on results of preliminary studies. Five or 6 animals/dose group were used in each experiment. Statistical analyses included ANOVA, Fisher protected least significant difference test, and Mann–Whitney *U* test.

In the first study conducted to examine testicular and pituitary function, LH, FSH, prolactin, testosterone, and inhibin were measured in plasma, pituitary, and/or testis by RIA in rats s.c. dosed with 1 mg/day bisphenol A for 2 weeks. Statistically significant effects **[percent differences compared to controls, as estimated from a graph]** included increases in plasma levels of LH **[150%]** and prolactin **[1067%]** and decreases in levels of plasma testosterone **[29%]** and testicular inhibin **[36%]**.

In a second experiment to examine testicular response, rats were s.c. dosed with 0.1 or 1 mg/day bisphenol A for 2 weeks. The rats then received 10 IU hCG through an atrial cannula. Blood samples were drawn for measurement of progesterone and testosterone levels before and at various time intervals between 30 and 180 min following the hCG challenge. Plasma progesterone and testosterone levels were increased following the hCG challenge in control rats. In the bisphenol A-treated rats, only a slight increase in progesterone levels occurred 30 min following challenge, and plasma progesterone levels were significantly lower compared to the control group at 60–150 min following challenge. There was an increase in plasma testosterone level following challenge of the bisphenol A group, but values were significantly lower than control values at 90–120 min following the challenge.

In a third experiment examining pituitary response, adult male rats were castrated 5 days before bisphenol A treatment. Castrated rats were s.c. injected with 1 mg/day bisphenol A and 75 μg/day testosterone propionate for 2 weeks. The rats then received 250 ng gonadotropin-releasing hormone by s.c. injection. Plasma LH was measured before and at various time intervals between 0.25 and 4 hr following the gonadotropin-releasing hormone challenge. No statistically significant effects were observed.

In a fourth study, males were dosed with 1 mg/day bisphenol A for 2 weeks and then paired with females in proestrus. Sexual function was evaluated by scoring mounts, intromissions, and ejaculations. No significant effects were observed for sexual function. Based on the findings reported in all studies, the study authors concluded that "The testis is probably a more sensitive site for [bisphenol A] action than the hypothalamus-pituitary axis."

Strengths/Weaknesses: RIAs appear to have been conducted competently. Subcutaneous is not a relevant route of exposure, and the sample size was limited. Blood collection via decapitation is not appropriate, because decapitation stress affects plasma prolactin and LH secretion. No mention is made of the order of killing. If controls were killed first and the guillotine was not cleaned between uses (and animals were not in separate rooms), there may be serious confounding of the data. Because rat plasma testosterone levels are normally highly variable, the low degree of variability in this study, given the small sample size, is remarkable (∼ ±0.12 ng/mL). No functional consequence of the alterations in hormone levels were described. Weaknesses include use of two doses delivered subcutaneously, critically small sample sizes, use of an inappropriate method of plasma collection, the stressful nature of cannula insertion just one day before measurement, and inappropriate statistical analyses that did not account for temporally repeated measures.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation process.

Kim et al. (2002b), supported by the Korean Ministry of Health and Social Welfare, examined the effects of bisphenol A exposure on the male reproductive system. A translation of the study was provided by the American Plastics Council. Four-week-old male F344 rats (7/group) were given bisphenol A in drinking water at 0 (ethanol vehicle), 0.1, 1, 10, or 100 ppm for 13 weeks. According to the study authors,

Table 90
Reproductive Effects in Male Rats Orally Dosed With Bisphenol A[a]

Endpoint	Dose, mg/kg bw/day 0.0002	0.002	0.020	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	BMD_{1SD}
Relative organ weight							
Testis	↓ 5%	↓ 6%	↓ 7%	0.056	0.021	0.014	0.0087
Epididymis	↓ 13%	↓ 17%	↓ 26%	0.011	0.0082	0.0069	0.0050
Ventral prostate	↑ 13%	↑ 34%	↑ 29%	0.014	0.0083	0.015	0.0089
Epididymal sperm motility[b]	↓ 23%	↓ 37%	↓ 41%				
Epididymal sperm count	↔	↓ 18%	↓ 27%				

[a]Chitra et al. (2003a).
[b]Values estimated from a graph by CERHR; data estimated from graphs were not modeled.
↑,↓ Statistically significant increase, decrease; ↔ no statistically significant effect.

these values were equivalent to 0.011, 0.116, 1.094, and 11.846 mg/kg bw/day. **[No information was provided about bisphenol A purity, or feed, caging, or bedding materials.]** Body weight and food and water consumption were measured during the study. Urine was collected for 24 hr following completion of dosing, and then animals were killed. Blood was collected. Organs, including those of the male reproductive system, were weighed. Parts of organs were preserved in formalin and examined histologically. Testes and epididymides were preserved in liquid nitrogen to obtain sperm counts and for measurement of levels of testicular enzymes. Data were analyzed by ANOVA.

Bisphenol A treatment had no significant effect on body weight or food or water intake. There were no effects on absolute or relative weights of the testis, epididymis, prostate, seminal vesicle, liver, kidney, heart, lung, spleen, or brain. Daily sperm production and number of sperm heads were unaffected by bisphenol A treatment. No significant effects were observed for activities of testicular γ-glutamyl transpeptidase, sorbitol dehydrogenase, acid phosphatase, or β glucuronidase. No histopathological alterations were reported for the testis, epididymis, seminal vesicle, prostate, spleen, or brain. Bisphenol A levels in urine are reported in Section 2. The study authors concluded that sperm density and the male reproductive system do not appear to be affected in F344 rats exposed to bisphenol A.

Strengths/Weaknesses: Strengths include a wide range of doses, use of an appropriate route of exposure, and the use of Fischer 344 rats. Weaknesses include marginal sample size and the absence of information about certain study design features.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility in the evaluation process.

Chitra et al. (2003a), supported by the Lady Tata Memorial Trust, Indian Council of Medical Research, and the Population Council, examined the effects of bisphenol A on the reproductive system of male rats. Animals were given "standard commercial laboratory chow." **[Bedding and caging materials were not reported.]** Six 45-day-old male Wistar rats/group were orally dosed **[gavage assumed]** with bisphenol A (97% purity) in olive oil at 0, 0.0002, 0.002, and 0.020 mg/kg bw/day for 45 days. Rats were killed 24 hr following the last treatment. Testes, epididymides, seminal vesicles, and ventral prostate were weighed. Epididymal sperm counts and motility were assessed. Antioxidant enzyme activities were measured in sperm. Statistical analyses included ANOVA followed by Student *t*-test. Significant effects on organ weights and sperm endpoints are summarized in Table 90. Bisphenol A treatment did not affect body weight. Absolute and relative (to body weight) weights of testis and epididymis and were reduced, and absolute and relative ventral prostate weights were increased at all dose levels. Effects on relative organ weights are summarized in Table 90. Sperm motility was decreased at all dose levels, and sperm counts were reduced at the mid and high-dose. There were dose-related decreases in activity of superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase in sperm at all dose levels. Hydrogen peroxide generation and lipid peroxidation in sperm increased dose-dependently at all dose levels. The study authors concluded that adverse effects of bisphenol A on the male reproductive system may be due to oxidative stress.

Although these studies have a limited number of animals per group, they appear to be relatively well conducted, and there are apparently consistent dose-dependent changes in testis and epididymis weights and sperm parameters. The epididymal (portion not mentioned) sperm numbers measured in this study are consistent with the daily sperm production measured by Sakaue et al. (2001). A potential significant concern in this study is the use of olive oil as the vehicle. The stability/reactivity of bisphenol A was not determined and it is possible that bisphenol A interacted with olive oil, resulting in the observed findings. This study provides suggestive data that bisphenol A induces oxidative stress in epididymal sperm and alters testis and epididymis weights at low doses.

Strengths/Weaknesses: Strengths include the use of oral and low multiple doses and appropriate measures. A weakness includes the marginal sample size.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion but of limited utility based on small group size.

Chitra et al. (2003b), supported by the Population Council, New York, examined the effects of bisphenol A and vitamin C exposure on epididymis and sperm counts in rats. Wistar rats (45-days old) were fed standard commercial laboratory chow and housed in plastic cages. **[No information was provided about bedding.]** Four rats/group were orally dosed with bisphenol A (97% purity) at 0 (olive oil vehicle), 0.0002, 0.002, or 0.020 mg/kg bw/day for 60 days. Additional rats received the same bisphenol A doses in conjunction

with 40 mg vitamin C. **[The specific method of oral dosing was not stated. A vehicle control group administered vitamin C was not included.]** Rats were killed 24 hr following the last dose. Epididymides were fixed in Bouin solution and examined histologically. Sperm were counted and examined for viability and motility. Levels of antioxidant enzymes were measured in sperm and epididymis. Data were analyzed by ANOVA followed by Student *t*-test.

There was no effect on sperm viability, but significant dose-related reductions were observed in sperm motility and count in all dose groups. **[In the low- to high-dose group, sperm motility was reduced to ~70, 60, and 55% of control levels. Sperm counts in the low to high-dose group were ~12, 30, and 40% lower than control values.]** Complete degeneration of epithelia of caput, corpus, and cauda epididymis was reported at all dose levels. **[It was not clear if the effect occurred in every rat of each dose group.]** Significant dose-related decreases in glutathione peroxidase and superoxide dismutase activity and increased lipid peroxidation were observed in sperm and epididymis of rats from each bisphenol A treatment group. No changes in sperm motility, sperm count, antioxidant enzyme activity, or lipid peroxidation were observed when bisphenol A was administered with vitamin C. The study authors concluded that bisphenol A induced oxidative stress and degeneration of epididymal epithelium, and vitamin C protected against those effects.

Strengths/Weaknesses: A critical weakness is the use of only 4 animals per dose group.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for inclusion due to concerns with group size.

Saito et al. (2003a), support not indicated, examined the effects of bisphenol A exposure on sex hormone levels in male rats. Wistar rats were fed MF feed (Oriental Yeast Co.). **[No information was provided about bedding and caging materials.]** Eight or 9 rats/group were s.c. injected with bisphenol A **[purity not reported]** at 0 (corn oil vehicle), 0.005, or 5 mg every 2 days from 3–11 weeks of age. **[Based on a graph showing body weights of ~50 g at the beginning of treatment and ~300 g at the end of treatment, the bisphenol A doses would have been 0.1 and 100 mg/kg bw at the beginning of the treatment period and 0.017 and 17 mg/kg bw at the end of the treatment period.]** Additional groups of 8–9 rats were injected with 5 μg/day 17β-estradiol or diethylstilbestrol. Rats were killed at 13 weeks of age, 2 weeks following the last treatment. Body, testes, and other reproductive organs were weighed. Levels of 17β-estradiol and testosterone were measured in plasma by RIA. Data were analyzed by Student *t*-test and Dunnett test. No clinical signs of toxicity or changes in behavior were observed. Exposure to bisphenol A did not affect body weight gain or absolute or relative testis weight. No effects were observed for weights of prostate, preputial gland, or epididymis. **[Data were not shown by study authors.]** Plasma testosterone levels were significantly reduced in the low bisphenol A group **[by ~1.5 fold]** and plasma estradiol levels were significantly increased in the high bisphenol A dose group **[by ~8-fold]**. Effects observed with 17β-estradiol and diethylstilbestrol exposure included decreased body weight gain, reduced absolute and relative testis weight, decreased plasma testosterone levels, and increased plasma 17β-estradiol levels. The study authors concluded that bisphenol A disturbed sex steroid production in male rats.

Single point testosterone measurements are normally highly variable; the apparent significant decrease in testosterone observed in this study may be spurious and due to the small group size, an unusual low variability in testosterone, and the use of the Student *t*-test, an inappropriate statistical test for this analysis. There is some concern with the dynamic range of the 17β-estradiol RIA as 17β-estradiol is normally measured in pg/mL.

Strengths/Weaknesses: Weaknesses include the s.c. route of exposure, the use of an inappropriate method of anesthesia when measuring hormone levels, inadequate sample sizes for highly variable testosterone endpoint, and inappropriate statistical tests on hormone data.

Utility (Adequacy) for CERHR Evaluation Process: Based on experimental design concerns, this study is inadequate for the evaluation.

Takahashi and Oishi (2003), support not indicated, examined species, strain, and route differences in reproductive systems of male rodents exposed to bisphenol A. The studies in rats are discussed in this section, and the studies in mice are discussed in Section 4.2.2.2. Animals were housed in stainless steel suspended cages or "chip-bedded" plastic cages. **[No information was provided about the type of chow used.]** Animals used in this study were 4 weeks old at the start of dosing. In the dietary portion of the study, male Wistar rats or Holtzman SD rats were given feed containing 0 or 0.25% bisphenol A (>99.0% purity) for 2 months. There were 8 animals in each dose group. The 0.25% dose group was reported to produce minimal testicular effects in a previous study. Mean bisphenol A intakes were estimated by study authors at ~200 mg/kg bw/day in rats. In parenteral exposure studies, 4-week-old male Wistar rats were s.c. dosed with bisphenol A in propylene glycol at 0 or 200 mg/kg bw on 4 days/week for 1 month. Additional male Wistar rats were given i.p. injections of bisphenol A in propylene glycol at 0, 2, or 20 mg/kg bw 4 days/week for 1 month. An i.p. dose of 200 mg/kg bw was originally administered but resulted in death. There were 5–6 animals/group in the parenteral exposure studies. In both the dietary and parenteral exposure studies, animals were observed daily for clinical signs, and body weight and food intake were measured. Animals were killed at the end of the dosing period. Liver, kidney, and reproductive organs were weighed. Testes were fixed in formaldehyde solution and examined histologically. The study authors noted that the appropriate fixative for the testis is Bouin solution but that obvious and severe injuries could be detected with the method used in the present study. Testosterone was measured in serum by ELISA. Daily sperm production and efficiency and epididymal sperm reserves were evaluated. Statistical analyses included *F* test, Student *t*-test, Aspin–Welch test, Bartlett test, ANOVA, Dunnett test, Kruskall–Wallis test, Dunnett non-parametric test, Wilcoxon rank-sum test, χ^2 test, Mantel–Haenzel test, and Fisher exact test.

In rats exposed through diet, there was no effect on body weight or absolute organ weight. Relative liver weight was significantly increased in Wistar rats exposed to bisphenol A. **[Data were not shown by study**

Table 91
Effects in Rats Given Bisphenol A by Intraperitoneal Injection[a]

Endpoint	Dose, mg/kg bw 2	20	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{SD}$
Weight						
Terminal body	↔	↓12%	19	12	17	5
Ventral prostate	↔	↓29%	7	5	9	6
Liver	↔	↓18%	14	8	12	6
Kidney	↔	↓12%	20	11	19	6
Serum testosterone	↔	↓69%	3	2	16	9

[a]Takahashi and Oishi (2003).
↑,↓ Statistically significant increase, decrease; ↔ no statistically significant effect.

authors.] The study authors indicated that they forgot to weigh seminal vesicles and prostate glands. No effects were reported for reproductive organ histopathology, daily sperm production or efficiency of production, epididymal sperm reserves, or serum testosterone levels in rats exposed to bisphenol A through diet. **[Data were not shown by study authors.]**

In the portion of the study where rats were administered 200 mg/kg bw bisphenol A, stiffness was observed at the injection site. Terminal body weight was lower **[by 20%]** in treated rats. Treatment resulted in **[∼20%]** decreases in absolute liver, kidney, preputial gland, and testis weight and **[∼40–80%]** decreases in epididymis, seminal vesicle, and prostate weight. The study authors also reported decreases in relative weights of epididymis, seminal vesicle and coagulation gland, and prostate. **[Data were not shown. The Expert Panel assumes that by coagulation gland, the authors mean the anterior prostate or coagulating gland.]** No histopathological alterations were observed in the seminiferous tubules of control animals. In the bisphenol A group, histopathological observations (incidence) in seminiferous tubules included focal atrophy (60%), exfoliation (60%), detachment (20%), missing Stage VII/VIII spermatids (40%), retention of Stage IX/XI spermatids (60%), and loss of basement membrane (20%). Bisphenol A treatment reduced daily sperm production **[by ∼25%, as estimated from a graph for total production but not per g testis.]** Reserves in head and body of the epididymis and the cauda epididymis were also reduced/g of tissue in bisphenol A-treated rats **[by ∼43 % in the head and body of epididymis and 63% in the cauda epididymis, as estimated from a graph]**. There was no significant effect on serum testosterone level.

Effects in rats administered bisphenol A by i.p. injection are summarized in Table 91. At 20 mg/kg bw, terminal body weight and prostate, liver, and kidney weight were reduced. Serum testosterone levels were also reduced in rats from the 20 mg/kg bw/day group. There were no effects on testicular histopathology or sperm endpoints. **[Data were not shown by study authors.]** Enlarged ileum was observed at necropsy in the 20 mg/kg bw group and histopathological examination revealed mucosal degeneration and hyperplastic duodenum, jejunum, ileum, and cecum. The study authors concluded that bisphenol A is more toxic through s.c. and i.p. exposure routes than by oral exposure in the diet.

This study reports a comprehensive study comparing 2 mouse and 2 rat strains using minimal numbers of animals per group. The data suggest that systemic exposure is necessary for bisphenol A estrogenic activity to be exhibited and strongly indicate that route of administration (oral vs. i.p.) is an important consideration. A minimal exposure range; the study did not explore low doses.

Due to differences in strain sensitivities, a NOAEL was not established. Nevertheless, it is likely to be near 0.25% in the diet.

Strengths/Weaknesses: Strengths include multiple routes of exposure, use of two strains of mice and rats, and a comparison of the oral, i.p., and subcutaneous routes. Weaknesses include use of single high doses administered for different durations across groups using minimal sample sizes.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility.

Herath et al. (2004), supported by Japan Society for Promotion of Science and the Japanese Ministry of Education, Culture, Sports, Science, and Technology, examined the effects of bisphenol A exposure on reproductive hormones and sperm endpoints in male rats. Octylphenol was also examined in this study, but results will not be discussed. Wistar–Imamichi rats were fed a soy-containing commercial feed (Nosan, Japan) and housed in metal cages. Rats were randomly assigned to groups and beginning at 50 days of age, 10–11 rats/group were s.c. injected with bisphenol A (≥95% purity) at 0 (DMSO vehicle) or 3 mg/kg bw/day for 5 weeks. Rats were weighed during the study. LH, testosterone, and progesterone concentration were measured in blood after 2 weeks of treatment and on the following day, 1 hr after a challenge with gonadotropin-releasing hormone. Rats were killed after 5 weeks of treatment. Blood was obtained for measurement by RIA of LH, progesterone, testosterone, immunoreactive inhibin, and insulin growth factor 1 levels. The testis, seminal vesicle, epididymis, and prostate were weighed, and sperm counts and motility were determined. A total of 5–11 rats/group were examined for each endpoint. Statistical analyses included ANOVA and Duncan Multiple Range test.

No statistically significant effects on baseline LH, testosterone, or progesterone levels were observed following 2 weeks of bisphenol A treatment. Following injection with gonadotropin-releasing hormone, LH levels were significantly increased in the bisphenol A group and progesterone levels were significantly increased in the vehicle control group. In the bisphenol A group compared to the control group, incremental increases following injection with gonadotropin-releasing hormone were smaller for testosterone **[∼410 vs. 875%]** and progesterone **[∼75 vs. 510%]**; statistical significance was reported for the progesterone effect. Following 5 weeks of bisphenol A treatment, significant effects on plasma hormone levels compared to controls included decreased testosterone **[by ∼55%]** and increased insulin-like growth factor 1 **[by ∼20%]**. Ventral prostate weight was significantly higher **[by ∼29%]** in

the bisphenol A versus control group, but there were no effects on testis, seminal vesicle, or right epididymis weight. **[Relative reproductive organ weights were not reported.]** Epididymal sperm counts were reduced significantly **[by ~10%]** in the bisphenol A group, but there was no significant effect on sperm motility. The study authors concluded that bisphenol A exposure can affect basal and gonadotropin-releasing hormone-stimulated LH production and reduced daily sperm production in rats.

Strengths/Weaknesses: This study appears to have been relatively well conducted. A major weakness of this study is the inconsistency in the hormone data (control data after 2 weeks were dramatically different than after 5 weeks even though both are from sexually mature rats). The subcutaneous route of administration with the use of DMSO as vehicle are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful for the evaluation process primarily due to the significant inconsistencies in the hormone data from control animals.

Toyama et al. (2004), supported in part by the Japanese Ministry of Environment and Ministry of Education, Science, Sports, and Culture, examined the effects of bisphenol A exposure on the reproductive system of male rats and mice. **[No information was provided about feed, caging, or bedding materials. The mouse portion of the study is discussed in Section 4.2.2.2.]** Adult male Wistar rats ($n = 12$/group) were s.c. injected with bisphenol A **[purity not indicated]** at 0.020 or 0.200 mg/kg bw/day for 6 consecutive days. Three control animals were s.c. injected with the DMSO/olive oil vehicle for 6 days. Ten animals/bisphenol A group and 2 controls were killed the day following treatment and perfused with glutaraldehyde. Testes were weighed and examined by light and electron microscopy. Epididymis, preputial gland, ventral prostate, and seminal vesicle with coagulating glands were also weighed. The remaining animals, 2 in each bisphenol A group and 1 in the control group, were held an additional 2 months and then subjected to fertility tests. In fertility testing, each male was mated to 2 untreated females. One of the 2 mated females was kept until parturition. **[The males were apparently killed for an examination of reproductive organs following fertility testing.]** Results were qualitatively reported, and statistical analyses were not conducted.

The description of the results was limited primarily to rats in the 0.020 mg/kg bw/day group. Body and male accessory reproductive organ weights were not affected by bisphenol A treatment. **[Data were not shown by study authors.]** In the bisphenol A group, examination by light microscopy revealed exfoliation of round spermatids, deformed heads of mature spermatids, and multinucleated giant cells in seminiferous epithelium. Testicular effects observed by electron microscopy included abnormal acrosomal caps and invagination and/or vacuole formation in nuclei of spermatids beyond Step 1. Ectoplasmic specialization around Sertoli cells was also affected by bisphenol A treatment. No histological or ultrastructural abnormalities were observed in the testis 2 months following exposure. Sexual behavior was observed to be normal in treated males. Females delivered normal pups and litter sizes were similar between groups. The study authors concluded that bisphenol A exposure did not affect fertility in rats and that adverse effects were transient.

Strengths/Weaknesses: Definite conclusions cannot be drawn from such a limited data set; the fertility assessment was not meaningful due to the sample size (2/group). The background incidence of the electron microscope findings was not discussed. Another weakness is the subcutaneous route with DMSO as a vehicle.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful in the evaluation.

4.2.2.2 Mouse: Takao et al. (1999), support not indicated, examined the effects of bisphenol A exposure on the reproductive system of mice. Five-week-old male C57BL/6 mice were exposed to bisphenol A **[purity not indicated]** in drinking water at 0 (0.005% ethanol in water vehicle), 0.0005, or 0.050 g/L for 4 or 8 weeks. **[Based on daily water intakes and body weights reported in the study, bisphenol A intake was estimated by CERHR at 0.14 and 13 mg/kg bw/day.]** To maintain bisphenol A at a stable concentration, drinking water was changed twice a week, but the stability of bisphenol A was not verified. Mice were killed, and both testes and spleen were removed and weighed. One testis was processed for histopathological evaluation. Plasma testosterone, corticosterone, and LH levels were measured in 7 mice/group using RIA or enzyme immunoassay. **[No information was provided on the purity of bisphenol A, time between last dose and sacrifice, or the type of chow, caging, or bedding materials used. Very few details were provided on the methods, including histopathological evaluation.]** Statistical analyses included ANOVA followed by Fisher protected least significant difference test.

Water intake was reduced significantly **[by 8%]** in the high-dose group exposed for 4 weeks. There were no effects on body weight or absolute or relative (to body weight) testis or spleen weight. Plasma testosterone levels were reduced **[by 87–89%]** in the high-dose group, but statistical significance was attained only in the group exposed for 8 weeks. No statistically significant changes were reported for plasma corticosterone or LH levels. The number of multinucleated cells in the seminiferous tubules was increased in high-dose mice treated for 8 weeks. The study authors concluded that exposure to bisphenol A around the peripubertal period may disrupt the reproductive tracts of male mice.

Strengths/Weaknesses: This study lacks important experimental details on methodology, including numbers of treated animals. Although it appears that bisphenol A in the drinking water results in a dose-related decrease in plasma testosterone, this endpoint is highly variable because testosterone is secreted in a pulsatile manner, and controls for Weeks 4 and 8 varied by ~30%.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful for the evaluation process due to the paucity of important experimental details and the variability of the testosterone data.

Al-Hiyasat et al. (2002), supported by the Deanship of Scientific Research at Jordan University of Science and Technology, examined the effect of bisphenol A exposure on fertility of male mice. **[No information was provided about composition of chow, bedding, or caging.]** Ten 60-day-old male Swiss mice/group were gavaged with the

Table 92
Effects Observed Following Gavage of Male Mice with Bisphenol A and Mating With Untreated Females[a]

Endpoint	Dose, mg/kg bw/day						
	0.005	0.025	0.1	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Body weight	↓18%	↓ 21%	↓13%				
Relative weight							
Testis	↔	↑ 26%	↔				
Seminal vesicle	↔	↓ 27%	↔				
No. sperm/testis	↔	↓ 17%	↓29%	0.035	0.029	0.036	0.028
No. sperm/mg testis	↔	↓ 16%	↓37%	0.027	0.023	0.029	0.023
Daily sperm production	↔	↓ 17%	↓29%	0.035	0.029	0.036	0.028
Efficiency of sperm production	↔	↓ 16%	↓37%	0.027	0.023	0.029	0.023
No. sperm/epididymis	↓ 14%	↓ 25%	↓35%	0.033	0.026	0.040	0.030
Sperm/mg epididymis	↔	↓ 17%	↓31%	0.033	0.025	0.053	0.038
Percent pregnant females	↔	↓ 40%	↓33%				
Resorptions/implantation site (3% control rate)	13%	15%	13%				
Percent females with resorption sites	↑ 2.5-fold	↑ 3.8-fold	↑ 3.4-fold				

[a]Al-Hiyasat et al. (2002).
↑,↓ Statistically significant increase, decrease, ↔ no statistically significant effect.

ethanol/distilled water vehicle or bisphenol A (97% purity) for 30 days. **[The study listed the bisphenol A doses as 5, 25, and 100 ng/kg bw. An erratum was later released that indicated the correct units were µg/kg bw (0.005, 0.025, and 0.1 mg/kg bw/day).]** Following the dosing period, each male was mated for 10 days with 2 untreated female mice, who were placed inside the cage of the male during the same time period. The males were then killed for an evaluation of testes, seminal vesicles, and preputial gland weights. Sperm counts and daily sperm production were determined. Mated females were killed 10 days later to determine numbers of pregnancies, implantation sites, viable fetuses, total resorptions, and females with resorptions. **[There was no indication that mating was confirmed by checking for sperm in the vagina.]** Data were analyzed by Student *t*-test or Fisher exact test.

Results that obtained statistical significance are summarized in Table 92. Body weights were lower in all dose groups compared to controls. There were no evident dose–response relationships for organ weights. Absolute testis weight was decreased at the low dose, and absolute seminal vesicle weight was reduced at the mid and high-dose. Effects on relative organ weights are summarized in Table 92. Decreases in testicular sperm counts and daily sperm production were observed at the mid and high-dose. Total sperm counts in the epididymis were decreased at all dose levels, and sperm counts/mg epididymis were decreased at the mid and high-dose. The total number of resorptions and females with resorptions were increased at all dose levels. The percentage of pregnant females was reduced at the mid and high-dose. The study authors concluded that bisphenol A could adversely affect fertility and reproduction of adult male mice.

The number of animals per group was too small ($n = 10$) for a definitive assessment of study endpoints. The method of randomization (or initial body weights) was not presented. There is also an absence of a dose response in several of the endpoints assessed. Given that mice usually have poor (relative to rats) fertility rates, the confidence in control data is limited. The male mice were killed shortly after the mating period, which may have influenced/confounded the number of sperm in the epididymis. Student *t*-test is an inappropriate analysis for organ weights (ANOVA with appropriate post-hoc test would be appropriate). Statistical significance is suspect, and the changes in organ weights are minimal in magnitude.

Strengths/Weaknesses: Weaknesses include small sample sizes for endpoints, inadequate coverage of the full spermatogenesis cycle in dosing duration, measurement of sperm counts without allowing adequate time following mating, and inappropriate accounting of sire influences on resorption rates in statistical analyses. Sample sizes are small for fertility assessments.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate for inclusion. Data on tissue weights are of limited utility for the evaluation process, however fertility data are not useful.

Nagao et al. (2002), support not indicated, examined the effects of bisphenol A in mice following exposure during different life stages. An initial experiment, described in more detail in Section 3.2.7, found that C57BL/6N mice were more sensitive to 17β-estradiol than ICR mice, and the study authors therefore used C57BL/6N mice to examine the effects of bisphenol A. Life stages examined included prenatal development, adolescence, and adulthood. The study conducted in adult mice is described here, while the studies conducted during prenatal development and adolescence are described in Section 3.2.7. C57BL/6N mice were fed PLD (phytoestrogen-low diet; Oriental, Japan). They were housed in polycarbonate cages with wood bedding. Daidzein and genistein levels were analyzed in diet, tap water, and bedding and found to be below 0.5 mg/100 g. At 10 weeks of age, 20 male mice/group were gavaged with bisphenol A (99.0% purity) at 0.002, 0.020, or 0.200 mg/kg bw/day for 6 days. Twenty control males/group were given 0.5% carboxymethyl cellulose **[assumed to be the vehicle]**. Six weeks after the final dose was administered, the mice were weighed and 15 males/group were killed and necropsied. The testis, epididymis, and seminal vesicles with coagulating glands were weighed. The ventral prostate was not weighed due to difficulties in obtaining only prostate

and determining the precise weight. Epididymal sperm counts were obtained. Histopathological examinations were conducted for organs fixed in Bouin solution. Data were analyzed by Bartlett test to determine homogeneity of variance, followed by ANOVA when homogeneity of variance was confirmed or Kruskal–Wallis analysis of ranks when variance was not homogenous. Dunnett test was used for multiple comparisons.

In the bisphenol A group, there were no significant differences in body weight gain or terminal body weights. **[Data were not shown.]** There were no significant differences in absolute or relative (to body weight) weights of the testis, epididymis, or seminal vesicles. There were no significant effects on sperm count. No histopathological alterations in reproductive organs were reported. The study authors concluded that low-dose bisphenol A exposure of mice did not reduce sperm density.

Strengths/Weaknesses: This study was well conducted and adds to the understanding of the potential effects of low doses of bisphenol A administered by a relevant route of exposure. Strengths are an appropriate number of mice per group, the use of response to 17β-estradiol in 2 strains of mice to identify the most sensitive strain, and the presentation of sperm data in light of historical control data.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Peknicová et al. (2002), supported by the Czech Republic and EU, examined the effects of bisphenol A exposure on mouse sperm. CD-1 mice were given ST1 feed (Velaz a.s., Prague). Three generations of mice were exposed to bisphenol A **[purity not indicated]** through drinking water at doses of 0.000002 and 0.000020 mg ''/animal's weight/day.'' It was stated that there were 6 pairs of mice in the control group. Litter size was evaluated in 3 generations; 1 litter was examined in the first and second generation and 2 litters were examined in the third generation. In each generation, samples of sperm were collected from all males and a histopathological investigation of testes was conducted in $\geq$3 males/group. Sperm acrosomal status was assessed using an immunohistochemical and Western blot method. Statistical analyses included ANOVA and Newman–Keuls test. **[Very few experimental details were provided. No information was provided on bedding and caging materials, bisphenol A purity, the numbers of mice in each treatment group, treatment of the control group, ages of mice during treatment, durations of treatment, sample sizes and litter representation for sperm effects, and mating procedures. It was not clear if female rats were also treated.]** Litter sizes were significantly reduced in the first and second generation of mice treated with the low dose (5–6.7 pups/litter vs. 11.5–12 pups/litter in controls). There were no effects of bisphenol A treatment on testes weight. **[Data were not shown by authors.]** Pathological changes observed in testes from the low-dose group included damaged seminiferous tubule and reduced spermatogenesis. Acrosome integrity, evaluated as percent cells binding monoclonal antibodies to acrosin and intra-acrosomal proteins, was significantly reduced in all 3 generations of the low-dose group (48.5–57.7 compared to 93.3–95% integrity in controls) and the third generation of the high-dose group (62.5 compared to 93.3% integrity in controls). **[While the text of the study stated that acrosomal integrity was significantly affected only in the third generation of the high-dose group, the caption for Figure 7 of the study stated that both the second and third generations were significantly affected. Based on findings reported in the figure, it appears that the description in the text is correct.]** The study authors concluded that bisphenol A exposure negatively impacts fertility, spermatogenesis, and sperm quality in mice.

Strengths/Weaknesses: Although potentially interesting findings are presented, the study lacks many important details and sample sizes are critically inadequate.

Utility (Adequacy) for CERHR Evaluation Process: Due to study design concerns, this study is inadequate and has no utility for the evaluation.

Takahashi and Oishi (2003), support not indicated, examined species, strain, and route differences in reproductive systems of male rodents exposed to bisphenol A. Studies on mice are discussed here, and studies on rats are discussed in Section 4.2.2.1. Animals were housed in stainless steel suspended cages or ''chip-bedded'' plastic cages. **[No information was provided about the type of chow used.]** Animals used in this study were 4 weeks old at the start of dosing. In the dietary portion of the study, CD-1 (ICR) mice and C57BL/6CrSlc mice were given feed containing 0 or 0.25% bisphenol A (>99.0% purity) for 2 months. There were 8 animals in each dose group. The 0.25% dose was reported to produce minimal testicular effects in a previous study. Mean bisphenol A intakes were estimated by study authors at ~400 mg/kg bw/day in mice. The parenteral exposure studies were performed only in rats. Animals were observed daily for clinical signs, and body weight and food intake were measured. Animals were killed at the end of the dosing period. Liver, kidney, and reproductive organs were weighed. Testes were fixed in formaldehyde solution and examined histologically. The study authors noted that the appropriate fixative for the testis is Bouin solution, but that obvious and severe injuries could be detected with the method used in the present study. Testosterone was measured in serum by ELISA. Daily sperm production and efficiency and epididymal sperm reserves were evaluated. Statistical analyses included *F* test, Student *t*-test, Aspin–Welch test, Bartlett test, ANOVA, Dunnett test, Kruskall–Wallis test, Dunnett non-parametric test, Wilcoxon rank-sum test, χ^2 test, Mantel–Haenzel test, and Fisher exact test.

There were no significant effects on organ or body weights in C57BL/6CrSlc mice exposed through diet. In CD-1 (ICR) mice exposed through diet, there were increases in absolute testis **[16%]**, liver **[12%]**, and kidney **[20%]** weights and a decrease in absolute epididymis **[12%]** weight. The study authors reported that relative testis weight was not significantly affected, but when the value from 1 mouse with a high relative testis weight was deleted, the effect attained statistical significance. **[Data were not shown by study authors.]** No effects were reported for testis histopathology, daily sperm production or efficiency of production, epididymal sperm reserves, or serum testosterone levels in mice exposed to bisphenol A through diet. **[Data were not shown by study authors.]** The study authors

concluded that the testicular toxicity of bisphenol A is "relatively weak," based on the co-occurrence of liver and kidney toxicity at exposure levels causing testicular effects.

Strengths/Weaknesses: A strength is the use of dietary exposure and the examination of strain differences in mice. Weaknesses include use of a single very high-dose level.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility.

Park et al. (2004), support not indicated, examined the effects of bisphenol A exposure on the reproductive and hematological systems of male and female mice. **[Results for males are discussed here, and results for females are discussed in Section 4.2.1.2.]** Adult ICR mice were fed mouse formulation feed (Cheil Feed). **[No information was provided about caging or bedding materials.]** Fifteen mice/sex/group were i.p. injected with bisphenol A **[purity not indicated]** in an ethanol/corn oil vehicle at 0.05, 0.5, or 5.0 mg/kg bw on 5 occasions (every 3 days over a 14-day period). One control group received no treatment, and a second control group was i.p. injected with corn oil. Males were examined 2 days following administration. Semen was collected and assessed for sperm number, viability, and motility. Reproductive organs were weighed and fixed in Bouin solution, and histopathological examination was conducted. Hematological and clinical chemistry endpoints were also assessed. Data were analyzed by least significant difference test.

Exposure to bisphenol A had no effect on body weight or on weights of male reproductive organs including testis, epididymis, vesicular gland, or coagulating gland. Reductions in sperm concentrations **[by 18%]** and increases in sperm abnormalities **[by 28%]** were significant in the high-dose group. There were no treatment effects on testicular histology. There were no significant effects on hematological or clinical chemistry endpoints in males treated with bisphenol A. The study authors did not report conclusions regarding study findings.

Strengths/Weaknesses: Weaknesses include the i.p. route. Frequency of administration was every 3 days and, given the half-life of the chemical, it is unlikely that sufficient blood chemical levels were sustained to induce "maximal" bisphenol A-mediated responses.

Utility (Adequacy) for CERHR Evaluation Process: Given the dosing paradigm (i.p. injection every 3 days) this study is adequate but of limited utility in the evaluation process.

Toyama et al. (2004), supported in part by the Japanese Ministry of Environment and Ministry of Education, Science, Sports, and Culture, examined the effects of bisphenol A exposure on the reproductive system of male rats and mice. **[No information was provided about feed, caging, or bedding materials. The mouse study is discussed here, and the rat study is discussed in Section 4.2.2.1.]** Adult male ICR mice ($n = 12$/group) were s.c. injected with bisphenol A **[purity not indicated]** at 0.020 or 0.200 mg/kg bw/day for 6 consecutive days. Three control animals were s.c. injected with the DMSO/olive oil vehicle for 6 days. Ten animals/bisphenol A group and 2 controls were killed the day following treatment and perfused with glutaraldehyde. Testes were weighed and examined by light and electron microscopy. Epididymis, preputial gland, ventral prostate, and seminal vesicle with coagulating glands were also weighed. The remaining animals,2 males in each bisphenol A treatment group and 1 control male, were held an additional 2 months and then subjected to fertility tests. In fertility testing, each male was mated to 2untreated females. One of the 2 mated females was kept until parturition. **[The males were apparently killed for an examination of reproductive organs following fertility testing.]** Results were qualitatively reported, and statistical analyses were not conducted.

The study authors noted that all effects were similar between rats and mice and between dose groups, and their description of results was primarily limited to rats in the 0.020 mg/kg bw/day group. Body and male accessory reproductive organ weights were not affected by bisphenol A treatment. **[Data were not shown by study authors.]** In the bisphenol A group, examination by light microscopy revealed exfoliation of round spermatids, deformed heads of mature spermatids, and multinucleated giant cells in seminiferous epithelium. Testicular effects observed by electron microscopy included abnormal acrosomal caps and invagination and/or vacuole formation in nuclei of spermatids beyond Step 1. Ectoplasmic specialization around Sertoli cells was also affected by bisphenol A treatment. No histological or ultrastructural abnormalities were observed in testes 2 months following exposure. Sexual behavior was observed to be normal in treated males. Females delivered normal pups and litter sizes were similar between groups. The study authors concluded that bisphenol A exposure did not affect fertility in mice and that adverse effects were transient.

Strengths/Weaknesses: It is not possible to draw definite conclusions from such a limited data set; the fertility assessment was not meaningful due to the small sample size (2/group). The background incidence of the electron microscopy findings was not discussed. An additional weakness is the subcutaneous route with the use of DMSO as vehicle.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful due to the limited number of animals per group.

Anahara et al. (2006), supported by the Japanese Ministry of Environment and Ministry of Education, Culture, Sports, Science, and Technology, examined the effects of bisphenol A exposure on expression of cortactin protein in the mouse testis. Cortactin is an actin binding protein that makes up the apical ectoplasmic specialization between Sertoli cells and spermatids and the basal ectoplasmic specialization between Sertoli cells. Cortactin is one of several proteins that control spermatid development. Adult (12-week-old) male ICR mice ($n = 5$–7/group) were s.c. injected with corn oil vehicle, 0.0024 mg/kg bw/day bisphenol A, 2.5 μg/kg bw/day diethylstilbestrol, or 1.2 μg/kg bw/day 17β-estradiol for 5 days. **[No information was provided on purity of bisphenol A or the types of feed, caging, or bedding used.]** Animals were killed on the day following the last injection. Testes were homogenized and expression of cortactin protein was determined in testes from 5–7 rats/group by Western blot, immunohistochemistry, and immuno electron microscopy techniques. Data were analyzed by *t*-test. Exposure to bisphenol A resulted in a significant decrease in testicular cortactin protein

expression **[to ~60% of control levels]**. Immunohistochemical analysis revealed that cortactin staining was reduced in the apical ectoplasmic specialization but not in the basal ectoplasmic specialization. Examination by immuno electron microscopy revealed no expression of cortactin around heads of spermatid and deformation of nuclei and acrosomes. Effects observed with 17β-estradiol and diethylstilbestrol were similar to those observed with bisphenol A, with the exception that diethylstilbestrol also reduced cortactin protein expression in the basal ectoplasmic specialization and did not result in deformation of spermatids. The authors concluded that exogenous chemicals can damage junctional proteins like cortactin and have adverse effects on Sertoli cell protein regulation.

Strengths/Weaknesses: The subcutaneous route of administration of a single dose was a weakness as were suboptimal sample sizes. Western blot analysis of cortactin was inappropriately presented as a function of the control value with no variability in the control sample. There were no apparent differences in levels of protein expression between various estrogenic agents/treatments. No adverse outcomes of the changes in cortactin were explored.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful for the evaluation process.

4.2.2.3 Other mammals: Moon et al. (2001), supported by Korea University Medical Science Research Center and the Korean Ministry of Education, examined the effects of bisphenol A exposure on penile function in rabbits. **[No information was provided on feed or caging and bedding materials.]** Male, 8–12 week-old New Zealand white rabbits were i.p. injected with corn oil vehicle or 150 mg/kg bw bisphenol A **[purity not reported]**, every other day for 12 days to a cumulative dose of 900 mg/kg bw **[75 mg/kg bw/day]**. Rabbits were killed at 4 weeks ($n = 15$/group) and 8 weeks ($n = 15$/group) following bisphenol A treatment. In 5 rabbits/group, the penis was removed and fixed in 10% neutral buffered formalin for histological examination. In 10 rabbits/group, the corpora cavernosa were removed from the penis, and in vitro responses to norepinephrine, acetylcholine, sodium nitroprusside, and L-arginine were studied. Data were analyzed by Student *t*-test. Treatment with bisphenol A significantly suppressed contraction of corpora cavernosa in response to norepinephrine and relaxation in response to acetylcholine, sodium nitroprusside, and L-arginine at both stages of evaluation. Histopathological observations in the bisphenol A-treated rabbits but not control rabbits at both ages included intracavernosal fibrosis in conjunction with decreased sinusoidal spaces. Compared to rabbits in the control group, both age groups of rabbits exposed to bisphenol A had significantly increased trabecular smooth muscle content (73.3–83.2 vs. 33.2% in controls) and a non-significant difference in thickness of tunica albuginea (0.93–1.12 mm vs. 0.32–0.43 mm in controls). The study authors concluded that bisphenol A may affect erectile responses by inducing histological alterations in the penis.

Strengths/Weaknesses: There is no evidence that bisphenol A has any effect on the ability to attain an erection resulting in copulation in mice or rats. The lack of a plausible rationale is a weakness. This study does not have a concurrent control (e.g., 17β-estradiol) to ascertain if the observed effects are the result of estrogenic responses in the penis. The route of administration and use of a single dose are weaknesses.

Utility (Adequacy) for CERHR Evaluation Process: Due to the weakness identified above and the nature of the endpoints examined, this study is inadequate and of no utility for human risk assessment.

Nieminen et al. (2002a), support not indicated, examined the effects of bisphenol A **[purity not indicated]** exposure on hormone levels in the European polecat (*Mustela putorius*). There were no significant effects on plasma levels of testosterone, estradiol, FSH, or thyroid hormones. Details of this study are discussed in Section 4.2.1.3.

This study provides evidence that the bisphenol A administered to polecats increases GST and UDPGT activity. Since these findings were dose-related it appears that in the polecat bisphenol A increases Phase 2 metabolism but has minimal effects on hormone levels. Due to the limited number of animals and the absence of a dose–response relationship, the hormonal changes in this study are difficult to interpret.

Strengths/Weaknesses: Strengths include the use of a non-rodent species and multiple doses. Weaknesses include small sample sizes and the limited nature of reproductive endpoints.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate and not useful for the evaluation process.

Nieminen et al. (2002b), support not indicated, examined the effects of bisphenol A exposure on endocrine endpoints in field voles (*Microtus agrestis*). Animals were housed in plastic cages with wood shavings and fed R36 diet (Lactamin, Sweden). Sexually mature field voles were randomly assigned to groups that received bisphenol A **[purity not reported]** in propylene glycol by s.c. injection for 4 days. Doses of bisphenol A (numbers of males in each group) were 0 ($n = 6$), 10 ($n = 4$), 50 ($n = 6$), and 250 ($n = 7$) mg/kg bw/day. Animals were killed the day following the last dose. Body and liver weights were measured. Blood was drawn for measurement of sex steroids, thyroxine, and weight regulating hormone levels in plasma using RIA or immunoradiometry methods. The activities of EROD, UDPGT, and GST were measured in hepatic and renal microsomes using appropriate substrates. Statistical analyses included ANOVA, post-hoc Duncan test, Student *t*-test, Kolmogorov–Smirnov test, Levene test, Mann–Whitney *U* test, χ^2 test, and Spearman correlation. **[Results for females are discussed in Section 4.2.1.3.]**

Mortality was significantly increased by bisphenol A treatment, with incidences of 18, 36, and 20% in the low- to high-dose groups. No mortality was observed in the control group. Bisphenol A treatment did not significantly affect body, liver, or testis weight. Plasma testosterone levels increased with dose, and statistical significance was attained in high-dose males and females. Pooled (male+female) LH levels were not significantly altered by treatment. Liver EROD activity **[apparently combined for males and females]** was significantly decreased at the mid and high-dose and liver GST activities **[not clear if for males or females or**

both] was significantly decreased at the highest dose level. There were no other significant effects on microsomal enzymes examined. The study authors concluded that wild mammals such as field voles could be more susceptible to bisphenol A-induced toxicity than laboratory rodents.

Strengths/Weaknesses: Strengths include the use of a non-rodent species and multiple doses. Weaknesses include small sample sizes and limited nature of reproductive endpoints as well as the use of the subcutaneous route of administration.

Utility (Adequacy) for CERHR Evaluation Process: This study is inadequate for the evaluation.

4.2.2.4 Fish and invertebrates:: Although studies in fish and invertebrates may be important for understanding mechanisms of action and environmental impact, the Panel views these studies as not useful for the evaluation process.

Shioda and Wakabayashi (2000), supported by the Japanese Ministry of Education, examined the effects of bisphenol A exposure on reproductive capability of male medaka (*Oryzias latipes*). Adult male medaka were housed for 2 weeks in glass beakers containing distilled water and bisphenol A **[purity not indicated]** at 0, 0.3, 1, 3, or 10 μM **[0, 0.07, 0.23, 0.69, or 2.3 mg/L]. [The number of male fish treated was not reported. Though not specifically stated, it was suggested that fish in the negative control group were exposed to the acetone vehicle.]** Following exposure, each male was housed with two females in beakers containing distilled water. The numbers of eggs spawned, fertilized, and hatched were determined. Statistical analyses included *F* test followed by *t*-test or Welch test. Exposure to bisphenol A 10 μM **[2.3 mg/L]** significantly reduced the number of eggs produced and hatched compared to the negative control group. Additional compounds were also examined, and it was reported that eggs and hatchings were significantly reduced following exposure to 17β-estradiol ($\geq$ 3 nM), but not nonylphenol or diethylhexyl phthalate. The study authors concluded that the reproductive effects induced by bisphenol A in this study occurred at a higher concentration than results observed in a yeast estrogen screen.

Strengths/Weaknesses: This study appears to have been well conducted study and suggests that bisphenol A 2.3 mg/L in water decreases the number of medaka eggs produced and hatched.

Utility (Adequacy) for CERHR Evaluation Process: This study was not considered useful for the evaluation process.

Kinnberg and Toft (2003), supported by the Danish Environmental Research Programme, examined the effects of bisphenol A exposure on the reproductive system of male guppies (*Poecilia reticulata*). Thirty sexually mature male guppies/group were exposed for up to 30 days to bisphenol A **[purity not indicated]** at nominal concentrations of 0 (acetone vehicle) 5, 50, 500, or 5000 μg/L. Levels of bisphenol A in water were verified. Exposure to the 5000 μg/L concentration was stopped after 21 days because of a high mortality rate. All fish in the high-dose group and 6 fish/group in the lower dose groups were killed and fixed in neutral buffered formalin. Histopathological examination was conducted in whole fish. The mortality rate in the 5000 μg/L group was 77%, but no increase in mortality was observed in the lower concentration groups. Testes of fish from the high-dose group contained spermatozeugmata (bundles of spermatozoa with heads pointing outward and tails in the center) in ducts, and the authors stated the effect indicated blocked spermatogonial mitosis. **[No information was provided on incidence or severity of testicular lesions, and it does not appear that statistical analyses were conducted.]** Additional compounds were also tested, and it was indicated that effects induced by flutamide, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene, and 4-*tert*-octylphenol were similar to those observed with bisphenol A exposure. In contrast, exposure to 0.03 and 0.1 μg/L 17β-estradiol resulted in hypertrophy of Sertoli cells and efferent duct cells. The study authors concluded that a high bisphenol A concentration induced adverse effects on testicular structure.

Strengths/Weaknesses: This study appears to have been well conducted. The metabolism of bisphenol A in fish is unknown. It appears the bisphenol A does not exhibit the typical 17β-estradiol-like effect on the testis. Findings occurred at high relative exposures. There was no apparent low-dose effect.

Utility (Adequacy) for CERHR Evaluation Process: This study was not considered useful for the evaluation process.

Oehlmann et al. (2000), supported by the Berlin Federal Environmental Agency, reported the effects of bisphenol A on reproductive organs in the freshwater ramshorn snail (*Marisa cornuarietis*) and the marine dog whelk (*Nucella lapillus*). Details of this study are discussed in Section 4.2.1.4, and most of the findings pertained to female snails. Adult ramshorn snails did not show abnormalities of male sexual organs or gonads after exposure to bisphenol A **[purity not indicated]** concentrations up to 100 μg/L for 5 months or after exposure for the first year of life. In the dog whelk, a 1-month exposure to 1, 25, or 100 μg/L bisphenol A significantly decreased the proportion of males with sperm in the seminal vesicles compared to the vehicle-exposed control. The length of the penis and prostate gland were also reduced by all concentrations of bisphenol A in this animal. The authors concluded that bisphenol A toxicity occurs in invertebrates at environmentally relevant concentrations.

Strengths/Weaknesses: The study appears to have been well conducted and suggests that bisphenol A has an effect on the dog whelk. The potential stability/biotransformation was discussed in the introduction but not determined during the exposure period.

Utility (Adequacy) for CERHR Evaluation Process: This study was not considered useful for the evaluation process.

4.2.2.5 In vitro: While cell culture studies can provide useful insights into cellular and subcellular mechanisms, most of these studies are considered of no utility for the evaluation process. The study by Akingbemi et al. (2004) should nevertheless be considered for mechanistic value, and is considered adequate but of limited utility by the Panel for the evaluation process.

Nikula et al. (1999), support not indicated, examined the in vitro effects of bisphenol A on steroidogenesis in mouse Leydig tumor cell cultures. Octyl phenols were also examined in this study, but results will not be discussed. In the first experiment, cells were incubated for 48 hr in media containing bisphenol A **[purity not**

indicated] at 0 (ethanol vehicle) or 10^{-7}–10^{-4} M **[0.023–23 μg/L]** or estradiol at 10^{-8} M **[culture ware type not indicated]**. Production of cyclic adenosine monophosphate (cAMP) and progesterone was measured following the incubation period and at 1 and 3 hr following a challenge with 10 ng/mL hCG. In additional experiments, the cells were exposed to bisphenol A at 0 or 10^{-6} M **[0.23 μg/L]** or 17β-estradiol or diethylstilbestrol at 10^{-8} M. Production of cAMP and progesterone was measured following the incubation period and at 1 and/or 3 hr following challenge with hCG, forskolin, cholera toxin, or 8-bromo-cAMP. An additional study measured binding of ^{125}I-hCG to the LH receptor following a 48-hr exposure to bisphenol A at 0 or 10^{-6} M **[0.23 μg/L]**. Each experiment contained 5–8 replicates, and results from 3 independent experiments were pooled. Data were analyzed by ANOVA followed by Fisher test.

Bisphenol A had no effect on basal cAMP or progesterone production. At 3 hr following the hCG challenge, the increase in cAMP production was attenuated following previous exposure to bisphenol A at concentrations $\geq 10^{-7}$ M **[0.023 μg/L]** and increase in progesterone production was reduced at bisphenol A concentrations $\geq 10^{-6}$ M **[0.23 μg/L]**. At 3 hr following challenge, 10^{-6} M **[0.23 μg/L]** bisphenol A decreased hCG-induced cAMP production but had no effect on forskolin- or cholera toxin-induced cAMP production. Following 3-hr challenges, hCG-induced progesterone production was reduced following exposure to 10^{-6} M **[0.23 μg/L]** bisphenol A, but there were no effects on forskolin-, cholera toxin-, or 8-bromo-cAMP-induced progesterone production. Generally, 17β-estradiol and diethylstilbestrol attenuated hCG-, forskolin, and 8-bromo-cAMP-induced progesterone production. Bisphenol A exposure had no effect on binding of ^{125}I-hCG to the LH receptor. The study authors concluded that bisphenol A appears to inhibit cAMP formation and steroidogenesis in rat Leydig tumor cells by preventing coupling between the LH receptor and adenylate cyclase. Because no inhibition of cAMP production was observed following incubation of cells with 17β-estradiol, the study authors concluded that the effects of bisphenol A may not be estrogen-related.

Strengths/Weaknesses: This appears to be a well conducted in vitro study. Stimulation occurred in the absence of steroid-rich fetal bovine serum. There was no mention of whether phenol red-free media were used. Cell viability does not appear to have been determined. Because this study used an in vitro system, the effects of metabolism were limited. Nonetheless, this study provides compelling evidence that the actions of bisphenol A may be non-estrogen mediated.

Utility (Adequacy) for CERHR Evaluation Process: This study was not considered useful for the evaluation process.

Murono et al. (2001), from the Centers for Disease Control and Prevention, examined the effects of bisphenol A exposure on steroidogenesis in cultured rat Leydig cells. Leydig cell cultures were prepared from testes of 55–65-day-old Sprague–Dawley rats ($n = 8$–10). Cells were incubated in 0 or 1–1000 nM **[0.23–230 μg/L]** bisphenol A **[purity not indicated]** in DMSO vehicle, with and without 10 mL U/mL hCG for 24 hr **[culture ware not indicated]**. Following the incubation period, testosterone level was measured by RIA and ^{125}I-hCG binding to LH receptors was assessed. Media containing hydroxycholesterol was then added to the cultures, and testosterone production following a 4-hr incubation period was measured. The effects of 17β-estradiol and 4-*tert*-octylphenol were also examined, but will not be discussed. Cell viability was evaluated by trypan blue exclusion and found to be unaffected at the bisphenol A concentrations used in this study. Three experiments with 4 samples/experiment were conducted. Data were analyzed by ANOVA and Student-Newman–Keuls test. Bisphenol A had no effect on basal or hCG-induced testosterone production or hCG binding to LH receptors. **[Data were not shown by study authors.]** Conversion of hydroxycholesterol to testosterone was also unaffected by exposure of Leydig cells to bisphenol A. No effect on testosterone production was observed following exposure of cells to 17β-estradiol. The study authors noted the similarity of effect between bisphenol A and 17β-estradiol, which differed from the modest effects observed with 4-*tert*-octylphenol exposure.

Strengths/Weaknesses: This study appears to have been well conducted. Phenol red-free media were used and cell viability after treatment was assessed. There was likely limited metabolism of bisphenol A, and the activity of metabolites cannot be assessed.

Utility (Adequacy) for CERHR Evaluation Process: This study was not considered useful for the evaluation process.

Akingbemi et al. (2004), supported by NIEHS, U.S. EPA, NICHHD, and NIH, conducted in vitro studies to examine the effects of bisphenol A exposure on Leydig cell cultures. In vivo studies were also conducted and are described in Section 3 because exposures were commenced in immature animals. In a series of studies, testosterone production by Leydig cells was assessed following incubation of cells with various doses of bisphenol A or bisphenol A in combination with other compounds. Leydig cells were obtained from 90-day-old rats. In a dose–response study, testosterone and 17β-estradiol levels were measured in Leydig cells that were incubated with bisphenol A **[purity not indicated]** at 0 (ethanol vehicle), 0.01, 0.1, 1, 10, 100, or 1000 nM **[0, 0.0023, 0.023, 0.23, 2.3, 23, and 230 μg/L]** bisphenol A for 18 hr **[culture ware not indicated]**. To determine if bisphenol A induces estrogenic effects on Leydig cells, testosterone production was also measured in cells incubated with diethylstilbestrol or 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane, a metabolite of methoxychlor, at the same concentrations as bisphenol A. In mechanistic studies, Leydig cells were incubated with 0.01 nM **[0.0023 μg/L]** bisphenol A, with and without the addition of LH or the antiestrogenic compound ICI 182,780. Endpoints assessed included testosterone and 17β-estradiol production and expression of mRNA for steroidogenic metabolizing enzymes, ER, and steroidogenic acute regulatory protein, a substance that transports the cholesterol used in testosterone synthesis. Levels of hormones in media were measured using RIA methods, and mRNA expression was evaluated using RT-PCR techniques. Statistical analyses included ANOVA and the Duncan multiple range test.

In the concentration–response study, production of testosterone by Leydig cells was decreased following exposure to bisphenol A at 0.01 nM **[0.0023 μg/L]** but not at higher doses. Diethylstilbestrol reduced testosterone

production at all dose levels, and 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane reduced testosterone production at concentrations ≥100 nM. Some statistically significant effects were observed in the mechanistic studies in which cells were exposed to 0.01 nM bisphenol A. In one study, LH-stimulated but not basal testosterone production was reduced by bisphenol A exposure. A second study demonstrated a decrease in basal testosterone production following bisphenol A exposure, but no decrease in testosterone level was observed following incubation of cells with bisphenol A in combination with ICI 182,270. 17β-Estradiol production was decreased in cells exposed to bisphenol A. Changes in mRNA expression following bisphenol A exposure included reduced expression of mRNA for the steroidogenic enzymes P45017β-hydroxylase and aromatase. ERβ was not detected in Leydig cells, and expression of *ER*β mRNA was not affected. The study authors concluded that environmentally relevant concentrations of bisphenol A act directly on Leydig cells to inhibit steroidogenesis, presumably via the ER.

Strengths/Weaknesses: This study appears to have been very well-conducted. The study used a wide dose range and showed decreased testosterone production in in vitro Leydig cell cultures at low (0.1 nM) but not at higher concentrations. The response of multiple endpoints provides compelling evidence of a biological effect at 0.01 nM. An explanation for the selective effect of bisphenol A at this single low concentration (0.1 nM) was not provided, nor was the dose range of this effect explored.

Utility (Adequacy) for CERHR Evaluation Process:. This study is adequate but of limited utility for the evaluation process.

Song et al. (2002), supported by the Hormone Research Center and the Korean Andrological Society, examined the role of bisphenol A in inducing expression of orphan nuclear receptor *Nur77*, a receptor that plays an important role in the regulation of LH-induced steroidogenesis in Leydig cells. Methods used in this study are described in conjunction with the results. **[It does not appear that statistical analyses were conducted in this study.]** Following treatment of the mouse Leydig cell line K28 with bisphenol A **[purity not indicated]** at ≥0.01 μM, expression of *Nur77* mRNA was increased in a dose-related manner, with saturation of expression observed at 1 μM **[0.23 mg/L] [culture ware not indicated]**. In a time-response study with 1 μM **[0.23 mg/L]** bisphenol A, maximal expression of *Nur77* mRNA was observed at 30 min following treatment, basal levels of expression were observed from 2–12 hr following treatment, and expression was again increased at 24 hr following treatment. When K28 cells were pretreated with the protein kinase inhibitor H89 or the mitogen-activated protein kinase (MAPK) inhibitor PD98059, induction of *Nur77* mRNA by bisphenol A was reduced by 40–45%. Induction of *c-fos* and *c-jun* mRNA occurred concurrently with induction of *Nur77* mRNA. Bisphenol A-induced increases in *Nur77* promotor activity were greater following transfection of cells with *Nur77* promoter reporter and c-jun but not with *c-fos*. Possible activation of MAPK by bisphenol A was examined using an immunoblot method with an antibody specific for phosphorylated MAPK. Phosphorylation of MAPK reached a maximum level at 10 min following bisphenol A treatment. No changes in bisphenol A-induced induction of *Nur77* were observed following pretreatment with a protein kinase C inhibitor or P13 K inhibitor. The study authors stated that together these results suggest possible involvement of the protein kinase A and MAPK pathways in bisphenol A-induced induction of Nur77.

In K28 cells transfected with Nur77 promoter or monomer binding site-luciferase reporters, gene promoter activities and transactivation were increased following treatment with ≥0.1 μM **[0.023 mg/L]** bisphenol A, thus suggesting similar responses between promotor activity and mRNA induction. In a yeast assay, bisphenol A had no effect on interactions between *Nur77* and its corepressor, silencing mediator of retinoid and thyroid receptor.

Exposure of K28 cells to 1 μM **[0.23 mg/L]** bisphenol A resulted in increased progesterone production, which was inhibited 25% by the overexpression of dominant negative *Nur77*, which reduces the transactivation activity of *Nur77*. Expression of mRNA for steroidogenic enzymes was investigated and it was found that bisphenol A treatment increased expression of steroidogenic acute regulatory mRNA, cholesterol side-chain cleavage enzyme, and 3β-hydroxysteroid dehydrogenase. Effects of bisphenol A on expression of mRNA for *Nur77* and steroidogenesis enzymes was tested in prepubertal mice (18 days old). Injection of 5 mice/group with 125 mg/kg bw/day bisphenol A resulted in increased expression of *Nur77* mRNA and testosterone levels in mouse testis from 1–6 hr following exposure. **[Very few details were provided for the in vivo experiment.]** The study authors concluded that the results of these studies indicate that bisphenol A induces *Nur77* gene expression and alters steroidogenesis in Leydig cells, indicating a possible novel mechanism of toxicity.

Strengths/Weaknesses: This study appears to have been well conducted and links in vitro bisphenol A administration to dose-related (classic, not inverted) activation of *Nur77* and subsequent downstream signal transducing proteins. Various confirmatory experiments supported this relationship. These data strongly suggest that bisphenol A (>0.1 μM) activates *Nur77*. The toxicological implications of these findings were not addressed.

Utility (Adequacy) for CERHR Evaluation Process: This study was not considered useful for the evaluation process.

Hughes et al. (2000), supported by the Medical Research Council, the British Heart Fund, and the European Chemical Industry Council, examined the effects of bisphenol A on rat testicular calcium pumps. Other phenolic compounds were examined, some in greater detail than bisphenol A, but this discussion is limited to bisphenol A. Studies were conducted to determine the effects of bisphenol A exposure on calcium ATPase pump activity, calcium uptake in testicular microsomes, calcium levels in the TM4 Sertoli cell line, and TM4 cell viability **[culture ware not indicated]**. In the cell-viability study, cells were exposed to bisphenol A **[purity not indicated]** at 0, 100, 300, or 600 μM **[0, 23, 68, or 137 mg/L]** for 16 hr. In each study, 2–12 samples/group were analyzed. **[For most studies, very few details were provided about procedures such as exposure**

concentrations used and time that cells were incubated. There was no discussion of statistical procedures, and it was not clear if statistical analyses were conducted for some endpoints.]

Bisphenol A inhibited calcium ATPase activity in rat testis microsomes. Mean ± SEM median inhibitory concentration (IC_{50}) values were reported at 0.40 ± 0.15 μM **[91 ± 34 μg/L]** for inhibition of calcium ATPase activity and 2.5 ± 1.0 μM *[571 ± 228 μg/L]* for calcium uptake. Exposure to 200 μM **[47 mg/L]** bisphenol A increased intracellular calcium levels in TM4 cells. A viability study was conducted to determine if increased intracellular calcium levels resulted in cell death. Bisphenol A exposure resulted in reduced TM4 cell viability (percent viability compared to control cells was 93, 64, and 17% at concentrations of 100, 300, and 600 μM). The study authors concluded that these results provide evidence that environmental estrogens may induce toxicity in male reproductive development by disrupting calcium homeostasis.

Strengths/Weaknesses: This interesting mechanistic study examined the role of bisphenol A in modulating intracellular calcium levels. It is difficult to interpret the relationship between microsomal and intact cell effects of bisphenol A given the large difference in concentrations needed to produce an effect. Moreover, it is not clear if bisphenol A caused cytotoxicity by a calcium-dependent or non-calcium-mediated process.

Utility (Adequacy) for CERHR Evaluation Process: This study was not considered useful for the evaluation process.

Tabuchi et al. (2002), supported by the Japanese Ministry of Education, Culture, Sports, Science, and Technology and Takeda Science Foundation, examined the effects of bisphenol A exposure on viability and gene expression in TTE3 cells, a mouse Sertoli cell line. The cells were incubated for 24 hr in media containing 0 or 24–400 μM **[5.5–91 mg/L]** bisphenol A (99.7% purity) in a DMSO vehicle **[culture ware not indicated]**. Cell viability was determined, and gene expression changes were examined using microarray and PCR techniques. Data were analyzed by Dunnett multiple conversion test or Student *t*-test. Compared to values in control cells, bisphenol A exposure reduced cell viability by 25% at 100 μM **[23 mg/L]**, 33% at 200 μM **[46 mg/L]**, and 96% at 400 μM **[91 mg/L]**. Based on the results of the cell-viability studies, a bisphenol A concentration of 200 μM **[46 mg/L]** was selected for the gene expression studies. Of 1081 genes examined by microarray, mRNA was downregulated in 3 cases and upregulated in 10 cases. Six genes were selected for evaluation of mRNA expression by PCR, and of those genes, 1 was downregulated (*ERα*) and 5 were upregulated (*iNOS*, *chop-10*, *odc*, *BipGRP78*, and *osip*). The study authors concluded that microarray analysis is a useful tool for investing molecular mechanisms of bisphenol A-induced toxicity in testicular cells.

Strengths/Weaknesses: This interesting mechanistic study appears to have been well conducted, but it is unclear from the data if bisphenol A-related changes in *chop-10* are a primary (or secondary) effect or are the result of cytotoxicity.

Utility (Adequacy) for CERHR Evaluation Process: This study is not useful in the evaluation.

Tabuchi and Kondo (2003), supported by Japanese Ministry of Education, Culture, Sports, Science, and Technology, Takeda Science Foundation, and Toyama Daiichi Bank Foundation, conducted a series of experiments to examine the effects of in vitro bisphenol A exposure on gene expression in mouse Sertoli cells. The experiments used TTE3 cells, an immortalized Sertoli cell line established from transgenic mice expressing temperature-sensitive simian virus large T-antigen. Cells were exposed to bisphenol A (99.7% purity) in a DMSO vehicle **[culture ware not discussed]**. The majority of experiments were repeated 2–4 times, and data were analyzed by Student *t*-test. **[Statistical significance was not reported in the results section of the study.]** Before conducting gene expression studies, cells were exposed to 25–400 μM **[5.7–91 mg/L]** bisphenol A for 3–24 hr, and viability was determined using a tetrazolium compound. Cell viability was reduced at bisphenol A concentrations ≥ 200 μM **[46 mg/L],** and reductions in viability were increased with longer durations of exposure. Intracellular calcium levels were measured using a fluorescence imaging technique over a 15-min period in cells exposed to 0–400 μM **[0–91 mg/L]** bisphenol A, and a dose-related increase in calcium influx was observed at ≥ 100 μM **[23 mg/L]**. Based on results for cell viability and calcium influx studies, a concentration of 200 μM **[46 mg/L]** was selected for the gene-expression experiments.

Using a PCR technique, it was determined that expression of mRNA for transferrin was decreased and glucose-regulated protein mRNA was increased by bisphenol A exposure of up to 24 hr. Observations of increased intracellular calcium concentration and upregulated glucose-regulated protein mRNA expression led the study authors to conclude that bisphenol A stresses the endoplasmic reticulum. Gene expression was analyzed by a cDNA microarray technique after exposure for 3, 6, 12, and 24 hr, and it was determined that 31 of 865 genes examined were upregulated by exposure to bisphenol A; no downregulation of genes was observed. The greatest change in gene expression was observed for *chop-10*, a stress-response gene. Upregulation of 4 genes, *c-myc*, *fra-2*, *odc*, and *chop-10*, were confirmed by quantitative PCR. *Chop-10* was determined to be the most responsive gene. To determine if *chop-10* was required for development of endoplasmic reticulum stress and cell injury, a stably transfected cell line expressing *chop-10* antisense RNA (*chopR14*) was developed. Mock cells were used as negative controls in studies where cells were exposed to 200 μM **[46 mg/L]** bisphenol A for up to 24 hr. Production of chop-10 protein, as determined by Western blot analysis, was reduced in the *chopR14* cells compared to the mock cells following exposure to bisphenol A. In contrast to the mock cells, no reductions in cell viability or transferrin mRNA expression were observed in the *chopR14* cells following bisphenol A exposure. There were no changes in glucose-regulated protein mRNA expression in *chopR14* versus mock cells. The study authors postulated that bisphenol A may disrupt the male reproductive system by altering calcium homeostasis in Sertoli cell endoplasmic reticulum without interacting with the ER and that genes such as *chop-10* may be involved in the process.

Strengths/Weaknesses: This mechanistic study appears to have been well conducted, but it is unclear from the data if bisphenol A-related changes in *chop-10*

are a primary (or secondary) effect or are the result of cytotoxicity. Calcium levels were also affected and collectively these changes may be the result of apoptosis initiated by some other mechanism.

Utility (Adequacy) for CERHR Evaluation Process: This study was not considered useful for the evaluation process.

Tabuchi et al. (2006), supported in part by the Japanese Ministry of Education, Culture, Sports, Science, and Technology, examined the effects of bisphenol A on gene expression in mouse Sertoli cell cultures. TTE3 cells were incubated in media containing bisphenol A **[purity not reported]** at 0 (DMSO vehicle) or 200 μM **[46 mg/L]** for up to 12 hr **[culture ware type not discussed]**. Cells were examined for viability using dye exclusion assays and for apoptosis by formation of DNA ladders. RNA was extracted from cells, and gene expression was determined by PCR and microarray analyses. Data were analyzed by Student *t*-test. Cell viability was decreased in a time-related manner between 3–12 hr of bisphenol A exposure, but there was no evidence of apoptosis. PCR analysis indicated that bisphenol A exposure significantly and time-dependently increased mRNA transcripts for 2 endoplasmic reticulum stress markers, *hspa5* and *ddit3*. Microarray analysis demonstrated that 661 sets of genes were downregulated and 604 sets of genes were upregulated >2-fold following bisphenol A exposure. Pathway analysis of decreased gene clusters revealed 2 significant genetic networks associated with the cell cycle or cell growth and proliferation. In increased gene clusters, two genetic networks were associated with cell death, DNA replication, recombination and repair, or injuries and abnormalities. The study authors concluded that the genes, genetic clusters, and genetic networks identified in this study are likely involved in Sertoli cell injury following bisphenol A exposure.

Strengths/Weaknesses: State-of-the-art technology was used in this study to examine gene expression changes after in vitro bisphenol A exposure of a Sertoli cell line. Only one dose level was examined. The use of hormone rich fetal bovine serum in the media may be a confounder. The absence of DNA laddering is not conclusive evidence of the absence of apoptosis (e.g., adherent cells undergoing apoptosis often are released into the culture media). Moreover, it is not surprising that given this ''high'' bisphenol A concentration, ''novel'' and likely non-specific gene changes were noted.

Utility (Adequacy) for CERHR Evaluation Process: This study was not considered useful for the evaluation process.

4.2.3 Male and female.

4.2.3.1 Rat: Two unpublished studies performed by the International Research and Development Corporation for General Electric (General Electric, 1976, 1978) provided some information on reproductive toxicity in rats orally exposed to bisphenol A. The studies are described in detail in Section 3.2.3.1. There was no effect on fertility in male and female rats given feed containing up to 9000 ppm bisphenol A (~650 mg/kg bw/day in males and 950 mg/kg bw/day in females) for an unspecified period before mating (General Electric, 1976). A second study reported no effects on estrus cyclicity or gestation length **[data not shown by study authors]** or male or female fertility in rats given feed containing bisphenol A at up to 1000 ppm (~60 mg/kg bw/day in males and 100 mg/kg bw/day in females) for ~70 days before mating (General Electric, 1978).

Ema et al. (2001), supported by the Japanese Ministry of Health and Welfare, conducted a multigeneration reproductive toxicity study of bisphenol A in CD rats. Animals were housed in suspended stainless steel cages at the beginning of the study. From GD 17, wood chips were used as bedding. Rats were fed CRF-1 chow (Oriental Yeast Co). In the study that was conducted according to GLP, F_0 male rats and female rats with 4–5-day estrous cycles were randomly assigned to groups of 25/sex. Five-week-old males and 10-week-old females were gavaged with 0 (distilled water vehicle), 0.0002, 0.002, 0.020, or 0.200 mg/kg bw/day bisphenol A (99.9% purity). Males were dosed for 10 weeks before mating and during the mating period, which lasted up to 2 weeks. Females were dosed from 2 weeks before mating, and during the mating, gestation, and lactation periods. Doses were based on results of studies by Nagel et al. (1997) and vom Saal et al. (1998). Stability and concentration of dosing solutions were verified. Dams delivered and nursed their pups. At weaning on PND 22 (day of birth defined as PND 0), 1 or 2 F_1 weanlings/litter/sex (25/sex/group) were selected to continue in the study. Dosing of F_1 animals began on PND 23 and continued for 10 weeks before mating and through the mating period, which lasted up to 3 weeks. Dosing was continued through the gestation and lactation periods. Twenty-five F_2 weanlings/sex/group were selected on PND 22. Beginning on PND 22, male F_2 rats were dosed for 4 weeks and females were dosed for 11 weeks before being killed.

Endpoints examined in adult rats included clinical signs, body weight, and food intake. Fertility, copulation, and gestational indices were examined in mating rats. Vaginal smears were evaluated for 2 weeks before mating in F_0 and F_1 females and at 9–11 weeks of age in F_2 females. Dams were killed and necropsied following weaning of their pups, and uterine implantation sites were examined. Males were killed following mating. Organs were weighed and histopathology examinations were conducted in control and high-dose animals. Sperm endpoints were measured in F_0 and F_1 adult males. Serum hormone levels were measured in 6 adult F_0 and F_1 males and proestrous females. At birth, pups were counted, sexed, and examined for viability and external malformations. On PND 4, litters were culled to 4 male and 4 female pups. At weaning, 1 male and female F_1 and F_2 weanling was killed for organ weight measurement; histopathology exams were conducted in seminal vesicles and coagulating glands of F_2 weanlings. Survival and growth were monitored during the postnatal period. Pups were examined for developmental landmarks and attainment of vaginal opening or preputial separation. Anogenital distance in pups was examined at numerous time points during the lactation period and through adulthood. Behavioral testing was conducted at 5–7 weeks of age. The litter was considered the experimental unit in data obtained before weaning. Statistical analyses included Bartlett test for homogeneity of variance, ANOVA, and/or Dunnett multiple comparison, Kruskal–Wallis, Mann–Whitney U, χ^2, or Fisher exact tests.

In F_0 and F_1 adult animals, there were no treatment-related effects on clinical signs, body weight gain, or death. The only significant reproductive effects reported in adult

animals were non-dose-related decreases in percentages of females with normal estrous cycles (76 vs. 96% in controls) and reduced gestation duration (by 0.5 days) in the F_1 group treated with 0.020 mg/kg bw/day. Bisphenol A did not significantly affect the precoital interval, copulation index, fertility index, gestation index, number of implantations, or delivery index. There were no adverse effects on sperm endpoints such as count, motility, or morphology in F_0 or F_1 males. A significant decrease in abnormal and tailless sperm was observed in F_1 males of the 0.020 mg/kg bw/day group. There was no evidence of histopathological effects in reproductive organs of F_0 animals that did not copulate or had totally resorbed litters or in F_1 animals of the high-dose group. **[Data were not shown by study authors.]** In F_0 females, there were significant decreases in serum LH concentrations at 0.0002, 0.002, and 0.020 mg/kg bw/day and in serum triiodothyronine levels at 0.200 mg/kg bw/day. **[Data were not shown by study authors.]** Organ weight changes in F_1 adult males included decreased absolute weights of lung at 0.0002 and 0.200 mg/kg bw/day, kidney at 0.2 mg/kg bw/day, and testis at 0.020 mg/kg bw/day. Absolute ovarian weight was decreased in females of the 0.0002 mg/kg bw/day group. Seminal vesicle weight was decreased in F_2 males of the 0.200 mg/kg bw/day group. **[Data were not shown by study authors]**.

There were no significant effects on number of F_1 or F_2 pups delivered, sex ratio, or pup survival during the lactation period. Body weights of F_1 pups in the 0.020 mg/kg bw/day group were significantly lower **[by 6–7%]** on PND 14 and 21. Testicular descent was delayed by 0.7 days in F_2 offspring from the 0.020 and 0.200 mg/kg bw/day groups. There were no significant effects on age of pinna detachment, incisor eruption, or eye opening. Some significant but non-dose-related effects on reflex development were observed. Day of mid-air righting reflex was accelerated by 1.2 days in F_1 males and 1.5 days in F_1 females of the 0.020 mg/kg bw/day group. In F_2 males, negative geotaxis was delayed by 0.8 days at 0.0002 mg/kg bw/day, 0.5 days at 0.002 mg/kg bw/day, and 0.8 days at 0.020 mg/kg bw/day. Bisphenol A treatment did not significantly affect age of vaginal opening or preputial separation in F_1 or F_2 offspring. Some sporadic and small (within 5% of control values) changes in anogenital distance were observed in F_1 and F_2 offspring. In F_1 males, decreased anogenital distance was observed in the 0.0002 mg/kg bw/day group on PND 57 and in the 0.020 mg/kg bw/day group on PND 106, 113, and on the day of sacrifice. In F_1 females, anogenital distance was decreased in the 0.200 mg/kg bw/day group on PND 4 and increased in the 0.002 and 0.020 mg/kg bw/day group on PND 7. Decreases in anogenital distance of F_2 females were observed in the 0.020 mg/kg bw/day group on PND 64, 71, 85, 92, and on the day of sacrifice and in the 0.200 mg/kg bw/day group on PND 57, 64, and on the day of sacrifice. In F_1 offspring, there were no significant effects on behavior, as determined by open-field testing and performance in a T-maze. **[Data were not shown by study authors.]** There was no evidence of histopathological effects in seminal vesicle or coagulating gland of F_2 pups from the high-dose group. **[Data were not shown by study authors.]** Organ weight changes in F_1 male weanlings included decreased absolute lung weight at 0.020 and 0.200 mg/kg bw/day group and decreased kidney weight at 0.020 mg/kg bw/day. In male F_2 weanlings, significant decreases were observed in absolute and relative seminal vesicle weight and absolute thyroid weight at 0.002 mg/kg bw/day, absolute lung weight at 0.020 mg/kg bw/day, and relative heart weight at 0.200 mg/kg bw/day; relative liver weight was significantly increased in F_2 males of the 0.002 mg/kg bw/day group. The study authors concluded that oral administration of bisphenol A at 0.0002 to 0.200 mg/kg bw/day to 2 generations of rats did not cause changes in reproduction or development.

[The NTP Statistics Subpanel (NTP, 2001) reviewed an unpublished study that appeared to be the same study later published as Ema et al. (2001). The subpanel noted that in general they agreed with the statistical methodology used in the study but stated that the Dunnett test does not require significance of ANOVA. It was noted that the anogenital distance findings were the most difficult to interpret. The Subpanel noted that many of the anogenital distance effects remained statistically significant when analyzed by ANCOVA, a method they considered superior to adjustment by body weight. The NTP Subpanel agreed with the author's conclusion that effects on anogenital distance were not biologically significant. They noted an error in the unpublished study abstract that described increases in anogenital distance in F_1 and F_2 females in the 0.020 and 0.2 mg/kg bw/day groups when actually the effect should have been decreased anogenital distance. [It was not clear to CERHR if this error was carried forward to the published report.]

Strengths Weaknesses: This well-designed comprehensive low-dose assessment of potential bisphenol A-related effects on multiple generations of rats examined a wide variety of hormonally sensitive endpoints. The study had appropriate power with an appropriate number of rats per group. Route of administration (oral) was appropriate. The concentrations of the dosing solutions were verified (both prior and after). It would have been helpful if a dose level that caused maternal toxicity was also used; however, given the objective of this study it is a minor point. This thorough multiple generation rat study is highly valuable for human risk assessment of low dose oral exposure to bisphenol A. This study indicates that the NOAEL for bisphenol A exceeds 0.2 mg/kg bw/day under the conditions of this study.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

Tyl et al. (2000a, 2002b), sponsored by The Society of the Plastics Industry, Inc., conducted a multigeneration study of bisphenol A in rats. In the study that was conducted according to GLP, Sprague–Dawley rats were fed Purina Certified Rodent Chow 5002. F_0 rats (30/sex/group) were exposed to bisphenol A (99.5% purity) in feed for 10 weeks before mating. **[Age at start of exposure was not reported, but based on information provided in the discussion, it appears that the animals were adults at the start of exposure.]** Vaginal smears were evaluated during the last 3 weeks of the prebreeding period. Exposure continued through a 2-week mating period. Males were exposed an additional 3 weeks following mating, and females were exposed through gestation and lactation. Concentrations of bisphenol A added to feed were 0, 0.015, 0.3, 4.5, 75, 750, or 7500 ppm.

Target intakes were ~0, 0.0009, 0.018, 0.27, 4.5, 45, and 450 mg/kg bw/day in males and 0.001, 0.02, 0.30, 5, 50, and 500 mg/kg bw/day in females. Actual intakes were 0.0007–0.003, 0.015–0.062, 0.22–0.73, 4.1–15.4, 37.6–167.2, and 434–1823 mg/kg bw/day. The study was designed to include low-dose exposures reported to increase prostate weights (Nagel et al., 1997; vom Saal and Sheehan, 1998) and maximally tolerated doses expected to result in toxicity. Concentration, stability, and homogeneity of bisphenol A in feed were verified. During the study, body weight and food intake were measured and animals were examined for clinical signs. F_0 males were killed and necropsied following delivery of the F_1 litter. Histopathological evaluation of organs was conducted in all control animals and 10 animals/bisphenol A dose group. Reproductive organs were weighed and sperm endpoints were evaluated. F_0 females were killed and necropsied following weaning of their litters. Selected organs were weighed and ovarian primordial follicles were counted.

On PND 4, F_1 litters were culled to 10 pups, with equal numbers of each sex when possible. Endpoints examined in pups included growth and survival in the prenatal period and retained areolae or nipples on PND 11–13. At weaning on PND 21, 30 F_1 offspring/sex/group were randomly selected and exposed to bisphenol A in the diet according to the same protocol as F_0 rats. Those selected offspring were monitored for vaginal opening and preputial separation and later mated. Up to 3 F_1 weanlings/sex/litter were killed for organ weight measurement. Mating and evaluation of F_1 offspring were conducted according to the same procedures described for F_0 rats. The same procedures were repeated in F_2 rats and F_3 litters during the lactation period. Anogenital distance was measured in F_2 and F_3 rats at birth. Following weaning of F_3 offspring, up to 3/sex/litter were randomly selected for necropsy. Thirty/sex/dose were selected for evaluation of vaginal patency, preputial separation, and estrous cyclicity. Bisphenol A exposure was continued in those offspring until they were killed ~10 weeks following weaning. F_3 offspring were not mated, but necropsy evaluations were conducted as described above for previous generations.

Statistical analyses for quantitative continuous data included Bartlett test for homogeneity of variances, ANOVA, Dunnett, linear trend, Kruskal–Wallis, or Mann–Whitney *U* tests. Frequency data were analyzed by χ^2, Fisher exact, and Cochran–Armitage tests. Covariance and correlations analyses were also conducted.

Treatment-related systemic findings with available quantitative information in adult rats are summarized in Table 93. Body weights and body weight gain were consistently lower in F_0, F_1, F_2, and F_3 adult rats of the 750 and 7500 ppm dose groups, including during gestation and lactation periods. Terminal body weight effects are summarized in Table 93. Terminal body weight was reduced in all generations at 7500 ppm and in F_1 females and F_1 and F_2 males at 750 ppm. There were no consistent or clearly treatment-related effects on feed intake. No treatment-related clinical signs were reported. In the 7500 ppm group, absolute weights of the liver in males and the kidney in both sexes were decreased across generations. Relative weights were either increased or did not attain statistical significance. **[According to Table 2 of the study, absolute liver weights were also decreased in males of the 750 ppm group. The study authors also mentioned reductions in weights of adrenal glands, spleen, pituitary, and brain at the high-dose, but there were no data shown in the report for those endpoints.]** Other changes in non-reproductive organ weight occurred sporadically at lower dose and were not dose-related or consistent across generations. Relative organ weight changes that consistently attained statistical significance at the highest dose are summarized in Table 93. Histopathological analyses revealed a higher incidence of mild renal tubular degeneration and chronic hepatic inflammation in F_0, F_1, and F_2 but not F_3 females of the 7500 ppm group.

Treatment-related effects on reproductive endpoints in adult animals are summarized in Table 93. In evaluating organ weights, the study authors only considered organ weight effects to be biologically significant if statistically significant results were obtained in the same direction for absolute and relative weights. Therefore, the study authors concluded that the only treatment-related organ weight effects were reduced absolute and relative ovary weights. **[Numerous statistically significant effects on reproductive organ weights were reported in Table 2 of the study. Reductions in testes, epididymides, prostate, and seminal vesicle weights were observed in most generations of the 7500 ppm group. When adjusted for body weight, organ weights were either increased or did not differ significantly from controls.]** Relative reproductive organ weight changes that consistently attained statistical significance at the highest dose are summarized in Table 93. The authors reported no effect on mating, fertility, pregnancy, or gestational indices. **[With the exception of gestational length, data were not shown by study authors.]** Precoital interval, post-implantation loss, estrous cyclicity, and reproductive organ histopathology were also unaffected by bisphenol A treatment. In the high-dose group, there was no adverse effect on paired ovarian primordial follicle counts but counts were significantly increased by 43% in the F_0 generation. Implantation sites were decreased in F_0, F_1, and F_2 dams of the 7500 ppm group. The only significant effects on sperm endpoints were decreased epididymal sperm concentration in F_1 males and decreased daily sperm production in F_3 males of the 7500 ppm dose group. There were no effects on sperm morphology or motility. The study authors considered sperm to be unaffected by treatment.

Treatment-related effects observed in developing rats are summarized in Table 94. The number of live pups/litter was reduced in F_1, F_2, and F_3 litters of the 7500 ppm group. Body weights of F_1, F_2, and F_3 pups of the 7500 mg/kg bw/day groups were lower during the lactation period. Some small (~5%) decreases in pup body weight during the lactation period at lower doses were apparently not considered treatment-related by study authors. Postnatal survival was unaffected by bisphenol A treatment. In male rats, there were no effects on anogenital distance or the presence of areolas or nipples. Anogenital distance was significantly increased in F_2 females at all doses except 75 and 7500 ppm; there was no affect on anogenital distance in F_3 females. The study authors did not consider anogenital distance effects to be biologically or toxicologically significant. Vaginal patency was delayed in F_1, F_2, and F_3 females, and the effect remained significant

following adjustment for body weight. Preputial separation was delayed in F_1 males of the 750 and 7500 ppm groups, F_2 males in the 0.3, 75, 750, and 7500 ppm groups, and F_3 males of the 7500 ppm group. When adjusted for body weight, the effect remained significant in F_1 males of the 750 and 7500 ppm groups and F_2 and F_3 males of the 7500 ppm group. The study authors stated that reduced body weights were the most likely cause of puberty delay in males and females. **[In rats killed at weanling, absolute organ weights were said to be decreased at the high-dose but increased when adjusted for body weight. The specific organs affected were not reported and no data were presented. The exception was ovarian weights, which were reported to parallel effects observed in adult females with decreases in both absolute and relative weight at 7500 ppm.]**

The study authors concluded that there was no evidence of low-dose bisphenol effects (1 μg to 5 mg/kg bw/day) at any stage of the life cycle. They identified NOAELs of 75 ppm (~5 mg/kg bw/day) for adult systemic toxicity and 750 ppm (~50 mg/kg bw/day) for offspring and reproductive effects. The study authors concluded that bisphenol A should not be considered a selective reproductive toxicant.

[The NTP Statistics Subpanel (NTP, 2001) stated that the study by Tyl et al. (2000a) apparently lacked a check for outliers, but noted that the study was in draft form at the time of review. The NTP subpanel agreed with most author conclusions but disagreed with a conclusion that relative uterine weights were equivalent across all groups. The unnecessary use of ANOVA with Dunnett test was noted. Some possible outliers and 10-fold errors in data points that could have affected conclusions were observed. Overall, the NTP Subpanel concluded that Tyl et al. (2000a) study was the most comprehensive of the studies reviewed. They stated that the statistical methods were well thought out and appropriate.]

Strengths/Weaknesses: This assessment of potential bisphenol A-related effects on multiple generations of rats was well-designed and comprehensive. The large number of rats/group (30), the multiple endpoints examined, and the oral route of administration (diet) are strengths. The concentration of bisphenol A in the test diet was verified, and maternal and paternal toxicity was identified. This study explored a wide dose range and demonstrates an absence of adverse effects on reproductive function at very low bisphenol A dose levels. This study is highly valuable for human risk assessment for oral exposure to bisphenol A.

This study identified a NOAEL of 75 ppm (for general toxicity) and 750 ppm (for reproductive toxicity).

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

4.2.3.2 Mouse: NTP (1984) and **Morrissey et al. (1989)** sponsored a continuous breeding study in CD-1 mice exposed to bisphenol A through s.c. implants. Mice were fed Purina certified ground rodent chow (#5002) and housed in polypropylene or polycarbonate cages containing Ab-Sorb-Dri bedding. Silastic implants were used for s.c. dosing of mice with bisphenol A (~95% purity) in corn oil vehicle. Stability and weight of bisphenol A in pumps was verified. In the dose-range finding portion of the study (Task 1), 8 mice/sex/group (8 weeks old) received implants containing vehicle or bisphenol A. Dosages were estimated by determining the difference in bisphenol A weight at the start and end of the 14-day dosing period. It was estimated that mice received 0, 6.25, 12.5, 25, 50, or 100 mg bisphenol A. Endpoints examined included body weight changes, survival, and uterine weight. Blood was collected to determine plasma bisphenol A levels. Data were analyzed by ANOVA, Duncan Multiple Range Test, χ^2 test, and Fisher exact test. The goal of Task 2 was to determine a maximum tolerated dose that produced signs of toxicity but did not reduce body weight or increase lethality by >10% and to identify a low dose that did not result in toxicity. Concentrations of bisphenol A in plasma were below the detection limit (3 ng/mL) in the 6.25 mg group but were reported at 7.0–7.7 μg/L in the 12.5 mg group, 8.4 μg/L in the 25 mg group, 13.1–18.5 μg/L in the 50 mg group, and 31.5–56.2 μg/L in the 100 mg group. In mice treated with bisphenol A, there were no increases in death or effects on body weight gain. The study authors noted that reproductive tract weight in the high-dose group was greater **[by 52%]** than in the control group but statistical significance was not achieved because of high variability.

In the continuous breeding portion of the study (Task 2), mice were 11 weeks old at the start of dosing. Forty mice/sex/group received implants containing the vehicle and 20/sex/dose received implants containing bisphenol A at 25, 50, or 100 mg. Over a dosing period of 18 weeks, it was estimated that animals in each treatment group received 11.65, 20.05, and 38.60 mg bisphenol A. **[Assuming body weights of ~38 g, as indicated in the study report, doses would have been ~306, 527, and 1015 mg/kg bw over 18 weeks or 2.4, 4.2, and 8.1 mg/kg bw/day.]** Mice were 11 weeks old at the start of dosing, which began during a 7-day premating period. The mice were then randomly paired with animals from the same dose group and housed together during a 98-day breeding period. Litters born during the breeding period were examined for viability, weighed, sexed, and discarded. Following the 98-day mating period, mice were separated for 21 days to allow for the birth of the last litter. Dosing was continued throughout the breeding and separation periods. However, implants were often expelled through cutaneous lesions or the incision site. When animals expelled their implant, a new one was inserted but pregnant mice were allowed to complete their pregnancy before insertion of the new implant. Therefore dosing was not uniform. Endpoints examined in adult mice included body weight, number of litters/pair, and fertility. Following delivery of the final litter, parental animals were killed and animals in the 0 and 100 mg group were necropsied. Liver, brain, and reproductive organs were weighed. Data were analyzed by χ^2 test, Fisher exact test, Kruskal–Wallis test, Jonckheere test, and Mann–Whitney *U* test.

With the exception of cutaneous lesions at the implantation site, there were no clinical signs of toxicity. In parental mice, there were no effects on body weight, mortality, fertility, or number of litters born. There were no changes in weights of organs including, liver brain, pituitary, the female reproductive tract, testis, epididymis, prostate, or seminal vesicles. Statistically

Table 93
Treatment-Related Effects in Adult Rats Fed Bisphenol A Through Diet in a Multigeneration Reproductive Toxicity Study[a]

	Dose, ppm diet **[mg/kg bw/day[b]]**													
Endpoint	0.015 **[0.0095]**	0.3 **[0.019]**	4.5 **[0.285]**	75 **[4.75]**	750 **[47.5]**	7500 **[475]**	BMD_{10}		$BMDL_{10}$		BMD_{1SD}		$BMDL_{1SD}$	
Terminal body weight														
F_0 males	↔	↔	↔	↔	↔	↓ 22%	3554	**[225]**	3137	**[199]**	3133	**[198]**	2701	**[171]**
F_1 males	↔	↔	↔	↔	↓ 6%	↓ 26%	2811	**[178]**	2548	**[161]**	2443	**[155]**	2153	**[136]**
F_2 males[c]	↔	↔	↔	↔	↓ 12%	↓ 29%	733	**[46]**	554	**[35]**	648	**[41]**	484	**[31]**
F_3 males[c]	↔	↔	↔	↔	↔	↓ 26%	1456	**[92]**	913	**[58]**	1260	**[80]**	786	**[50]**
F_0 females	↔	↔	↔	↔	↔	↓ 13%	5722	**[362]**	4753	**[301]**	4741	**[300]**	3876	**[245]**
F_1 females	↔	↔	↔	↔	↓ 6%	↓ 16%	4600	**[291]**	3950	**[250]**	3730	**[236]**	3142	**[199]**
F_2 females[c]	↔	↔	↔	↔	↔	↓ 14%	3863	**[245]**	1576	**[100]**	3115	**[197]**	1291	**[82]**
F_3 females	↔	↔	↔	↔	↔	↓ 20%	3664	**[232]**	3194	**[202]**	3456	**[219]**	2949	**[187]**
Relative paired kidney weight														
F_0 males	↔	↔	↔	↔	↔	↑ 14%	5903	**[374]**	4555	**[288]**	6536	**[414]**	5035	**[319]**
F_1 males	↔	↔	↓ 5%	↔	↔	↑ 10%	5729	**[363]**	4662	**[295]**	5053	**[320]**	4088	**[259]**
F_2 males	↔	↔	↔	↔	↑ 5%	↑ 18%	4524	**[287]**	3893	**[247]**	3471	**[220]**	2950	**[187]**
F_3 males	↔	↔	↔	↔	↔	↑ 16%	6986	**[442]**	4319	**[274]**	6720	**[426]**	3403	**[216]**
F_0 females	↔	↔	↔	↔	↔	↑ 7%	8008	**[507]**	7521	**[476]**	7712	**[488]**	6578	**[417]**
F_2 females	↔	↔	↔	↔	↔	↑ 6%	7930	**[502]**	7515	**[476]**	7621	**[483]**	6247	**[396]**
Relative paired testis weight														
F_0 males	↔	↔	↔	↔	↔	↑ 27%	2924	**[185]**	2567	**[163]**	2998	**[190]**	2596	**[164]**
F_1 males	↔	↔	↔	↔	↔	↑ 18%	3287	**[208]**	2763	**[175]**	4106	**[260]**	3428	**[217]**
F_2 males	↔	↔	↔	↔	↔	↑ 24%	3086	**[195]**	2874	**[182]**	3245	**[206]**	2779	**[176]**
F_3 males	↔	↔	↔	↔	↔	↑ 19%	4329	**[274]**	2593	**[164]**	5010	**[317]**	3298	**[209]**
Relative paired epididymis weight														
F_0 males	↔	↔	↔	↔	↔	↑ 19%	3804	**[241]**	3072	**[195]**	5044	**[319]**	4068	**[258]**
F_1 males	↔	↔	↔	↔	↔	↑ 19%	2963	**[188]**	2566	**[163]**	3255	**[206]**	2786	**[17]**
F_2 males[c]	↔	↔	↔	↔	↑ 8%	↑ 24%	884	**[56]**	596	**[38]**	951	**[60]**	641	**[41]**
F_3 males	↔	↔	↔	↔	↔	↑ 22%	3449	**[218]**	2516	**[159]**	4117	**[261]**	3095	**[196]**
Relative liver weight														
F_0 females	↔	↔	↔	↔	↔	↑ 11%	7663	**[485]**	5848	**[370]**	7965	**[504]**	7439	**[471]**
F_2 females	↑	↔	↔	↔	↔	↑ 19%	6912	**[438]**	3650	**[231]**	7454	**[472]**	5533	**[350]**
Relative paired ovary weight]														
F_0 females	↔	↔	↔	↔	↔	↓ 19%	4103	**[260]**	3149	**[199]**	7126	**[451]**	5387	**[341]**
F_1 females	↔	↔	↔	↔	↔	↓ 15%	5754	**[364]**	3964	**[251]**	10,237	**[648]**	6966	**[441]**
F_2 females	↓ 15%	↔	↓ 15%	↓ 11%	↔	↓ 24%	7053	**[447]**	3520	**[223]**	7646	**[484]**	6360	**[403]**
No. with renal tubule degeneration														
F_0 females	0/12	0/12	0/12	0/14	0/12	4/13	6491	**[411]**	3848	**[244]**				
F_1 females	0/10	0/10	0/10	0/10	0/10	8/11	5498	**[348]**	2470	**[156]**				
F_2 females	0/11	0/10	0/12	0/11	0/12	7/13	5884	**[373]**	3018	**[191]**				
No. females with chronic liver inflammation														
F_0 females	0/12	1/12	0/12	0/14	1/12	3/13	4867	**[308]**	3214	**[204]**				
F_1 females	0/10	0/10	3/10	1/10	1/10	3/11								
F_2 females	1/11	0/10	2/12	2/11	2/12	5/13	3029	**[192]**	1856	**[118]**				
No. of implantation sites														
F_0 dams	↔	↔	↔	↔	↔	↓16%	4088	**[259]**	3021	**[191]**	8020	**[508]**	5832	**[369]**
F_1 dams[c]	↔	↔	↔	↔	↔	↓26%	6120	**[388]**	2383	**[151]**	7000	**[443]**	4713	**[298]**
F_2 dams	↔	↓8%	↔	↔	↔	↓18%	4917	**[311]**	3597	**[228]**	7679	**[486]**	5631	**[357]**
Epididymal sperm concentration, F_1	↔	↔	↔	↔	↔	↓18%	5012	**[317]**	3407	**[216]**	11,050	**[700]**	7407	**[469]**
Daily sperm production, F_3	↔	↔	↔	↔	↔	↓19%	7399	**[469]**	4025	**[255]**	8279	**[524]**	7596	**[481]**

[a]Tyl et al. (2002b).
[b]Based on target doses provided by the study authors and expressed as an average of the dose for males and females.
[c]Benchmark dose values were estimated using a polynomial model.
↑,↓ Statistically significant increase, decrease, ↔ no statistically significant effect.

significant effects observed in pups included increased numbers of live male and total pups and increased adjusted (for litter size) pup weight in the mid-dose group. Unadjusted and adjusted male and female pup weights were significantly increased at the high-dose. The study authors noted that the effects observed in this study were random and most likely due to chance. They concluded that bisphenol A did not induce adverse effects on fertility in male or female mice. It was noted that further studies using a better route of exposure are needed for bisphenol A.

Strengths/Weaknesses: This study appears to have been well conducted. When compared to studies that used the oral route of exposure, this study provides evidence that the manifestation of maternal toxicity is dependent on the route of administration and that route-dependent metabolism may be important for toxicity. However, the administration of bisphenol A via silastic

Table 94
Treatment-Related Effects in Developing Rats in a Multigeneration Reproductive Toxicity Study of Bisphenol A[a]

	Dose, ppm diet **[mg/kg bw/day[b]]**											
Endpoint	0.015 **[0.0095]**	0.3 **[0.019]**	4.5 **[0.285]**	75 **[4.75]**	750 **[47.5]**	7500 **[475]**	BMD_{10}		$BMDL_{10}$		BMD_{1SD}		$BMDL_{1SD}$	
Live pups/litter														
F_1	↔	↔	↔	↔	↔	↓ 20%	4232	**[268]**	3033	**[192]**	8823	**[559]**	6225	**[394]**
F_2	↔	↔	↔	↔	↔	↓ 26%	6661	**[422]**	2405	**[152]**	7241	**[459]**	4645	**[294]**
F_3	↔	↓ 11%	↔	↔	↔	↓ 26%	3733	**[236]**	2742	**[174]**	5943	**[376]**	4518	**[286]**
Pup body weight														
F_1, PND 4	↔	↔	↔	↔	↔	↓ 11%	6412	**[406]**	4473	**[283]**	8860	**[561]**	6317	**[400]**
F_1, PND 7	↔	↔	↔	↔	↔	↓ 23%	3432	**[217]**	2891	**[183]**	4179	**[265]**	3448	**[218]**
F_2, PND 7	↔	↔	↔	↔	↔	↓ 15%	5179	**[328]**	4059	**[257]**	6023	**[381]**	4653	**[295]**
F_3, PND 7	↔	↔	↔	↔	↔	↓ 13%	4976	**[315]**	3854	**[244]**	6474	**[410]**	4940	**[313]**
F_1, PND 14	↔	↔	↔	↔	↔	↓ 27%	2890	**[183]**	2570	**[163]**	2789	**[177]**	2415	**[153]**
F_2, PND 14	↔	↔	↔	↔	↔	↓ 20%	3840	**[243]**	3302	**[209]**	3579	**[227]**	3013	**[191]**
F_3, PND 14	↔	↔	↔	↔	↔	↓ 20%	3704	**[235]**	3224	**[204]**	3323	**[210]**	2827	**[179]**
F_1. PND 21	↔[c]	↔	↔	↔[c]	↔[c]	↓ 27%	3284	**[208]**	2621	**[166]**	3523	**[223]**	2763	**[175]**
F_2, PND 21	↔[c]	↔	↔	↔[c]	↔[c]	↓ 20%	4253	**[269]**	3566	**[226]**	4219	**[267]**	3473	**[220]**
F_3, PND 21	↔[c]	↔	↔	↔[c]	↔[c]	↓ 19%	3972	**[252]**	3423	**[217]**	3575	**[226]**	3016	**[191]**
Anogenital distance, F_2 females	↑ 3%	↑ 3%	↑ 3%	↔	↑4%	↔								
Age of vaginal opening adjusted for body weight														
F_1	↔	↔	↔	↔	↔	↑ 3.6 days	6225	**[394]**	5422	**[343]**	3248	**[206]**	2786	**[176]**
F_2	↔	↔	↔	↔	↔	↑ 4 days	6381	**[404]**	5307	**[336]**	4367	**[277]**	3600	**[228]**
F_3	↔	↔	↔	↔	↔	↑ 3.2 days	7444	**[471]**	6325	**[401]**	6249	**[396]**	3198	**[203]**
Age of preputial separation adjusted for body weight														
F_1	↔	↔	↔	↔	↑ 1.7 days	↑ 4.9 days	7350	**[466]**	6485	**[411]**	2974	**[188]**	2580	**[163]**
F_2	↔	↔	↔	↔	↔	↑ 7.4 days	4740	**[300]**	4025	**[255]**	3809	**[241]**	3201	**[203]**
F_3	↔	↔	↔	↔	↔	↑ 4 days	8637	**[547]**	7466	**[473]**	3503	**[222]**	2984	**[189]**

[a]Tyl et al. (2002b).
[b]Based on target doses provided by the study authors and expressed as an average of the dose for males and females.
[c]A significant (~5%) decrease in pup body weights observed only in F_1 and/or F_2 litters was apparently not considered treatment-related by study authors.
↑,↓ Statistically significant increase, decrease, ↔ no statistically significant effect.

implants makes the extrapolation for human risk assessment difficult in the absence of an improved pharmacokinetic understanding.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate but of limited utility for the evaluation process.

NTP (1985a) and **Morrissey et al. (1989)** sponsored a continuous breeding study in CD-1 mice exposed to bisphenol A (98% purity). Additional information on ovarian follicle counts in F_0 and F_1 females was published in a report by Bolon et al. (1997). In this study, mice were fed NIH-07 open formula rodent chow and housed in polypropylene or polycarbonate cages containing Ab-Sorb-Dri litter. The laboratory at which the study was conducted was stated to be in full compliance with GLP regulations. In the preliminary study (Task 1), 8 mice/sex/group (8 weeks old) were fed diet containing bisphenol A at 0, 0.3125, 0.625, 1.25, 2.5, or 5.0% for 14 days. By assuming that a 40-g mouse ingests 7 g feed/day, the study authors estimated bisphenol intake at 0, 437.5, 875.0, 1750.0, 4375.0, 8750.0 mg/kg bw/day. The aim of the preliminary study was to determine a maximum tolerated dose that induced significant toxicity but resulted in ≥90% survival and ≤10% decrease in weight gain. Statistical analyses included ANOVA, and χ^2 test. Lethality was significantly increased in the high-dose group. Body weight gain was depressed in groups exposed to ≥1.25% bisphenol A. Clinical signs of toxicity were observed in the 2.5 and 5.0% dose groups and included dehydration, dyspnea, lethargy, tremors, ptosis, piloerection, and diarrhea.

In the reproduction and fertility study (Task 2), 11-week-old mice were randomly assigned to treatment groups according to body weight. The mice were fed diets containing 0, 0.25, 0.5, or 1.0% bisphenol A. The NTP stated that a 40-g mouse consuming 7 g of feed/day would be exposed to bisphenol A at 437.5, 875, and 1750 mg/kg bw/day. **[Based on body weight and feed intake values reported for males at ~3 week intervals, CERHR estimated mean bisphenol A intake at ~365, 740, and 1630 mg/kg bw/day. Feed intakes were reported only at Week 1 and 18 for females, and Week 18 most likely represented the lactation period. For Week 1, bisphenol A intake by females was estimated at 410, 890, and 1750 mg/kg bw/day. At Week 18, bisphenol A intake by females was estimated at 1090, 1785, and 3660 mg/kg bw/day.]** There were 40 mice/sex in the vehicle control group and 20/sex in each bisphenol A group. Exposure to bisphenol A began during a 7-day premating period. Following the premating period, males and females from the same treatment group were randomly paired and housed together for 98 days and following the mating period, each male and female was housed separately for 21 days. Bisphenol A dosing was continued throughout the mating and separation period. Concentration and stability of bisphenol A in feed were verified. During the 98-day cohabitation period, pups born were counted, sexed, and weighed. All litters

Table 95
Effects Observed in Adult Mice Dosed With Bisphenol A in a Continuous Breeding Study[a]

Endpoint	Dose, % in diet **[mg/kg bw/day]**						
	0.25 **[437.5]**	0.5 **[875]**	1.0 **[1750]**	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
F_0 males and females							
Litters/pair	↔	↓ 5%	↓ 9%	1.0 **[1750]**	0.74 **[1295]**	0.96 **[1680]**	0.66 **[1155]**
Postpartum dam weight[b]	↔	↔	↓ 6–9%	1.0 **[1750]**	0.83 **[1452]**	0.87 **[1522]**	0.66 **[1155]**
Necropsy dam weight	No data	No data	↓ 4%				
Percent motile sperm	No data	No data	↓39%				
Relative organ weight, males[c]							
Liver	No data	No data	↑ 29%				
Kidney/adrenal	No data	No data	↑ 16%				
Seminal vesicle	No data	No data	↓ 19%				
Relative organ weight, females[c]							
Liver	No data	No data	↑ 27%				
Kidney/adrenal	No data	No data	↑ 10%				
Liver lesions, males and females[d]	No data	No data	↑[e]				
Kidney lesions, males and females[d]	No data	No data	↑[e]				
F_1 males and females							
Relative organ weight, males[c]							
Liver	↑ 7%	↑ 7%	↑ 29%	0.62 **[1085]**	0.42 **[735]**	0.59 **[1032]**	0.39 **[682]**
Kidney/adrenal[f]	↑ 16%	↑ 20%	↑ 20%	0.18 **[315]**	0.14 **[245]**	0.15 **[262]**	0.12 **[210]**
Left testis/epididymis[f]	↔	↓ 10%	↓ 9%	0.64 **[1120]**	0.32 **[560]**	0.53 **[928]**	0.27 **[472]**
Right testis[g]	↔	↓ 13%	↔				
Right epididymis[f]	↓ 11%	↓ 16%	↓ 18%	0.24 **[420]**	0.15 **[262]**	0.46 **[805]**	0.25 **[438]**
Seminal vesicle	↓ 11%	↔	↓ 28%	0.40 **[700]**	0.29 **[508]**	0.66 **[1155]**	0.47 **[822]**
Relative organ weight, females[c]							
Liver	↑ 6%	↑ 13%	↑ 20%	0.49 **[858]**	0.38 **[665]**	0.45 **[788]**	0.35 **[612]**
Kidney/adrenal[g]	↑ 13%	↑ 15%	↑ 13%				
Percent motile sperm[g]	↔	↓ 31%	↔				
Liver lesions, males[d]	↑[e]	↑[e]	↑[e]				
Liver lesions, females[d]	↔	↑[e]	↑[e]				
Kidney lesions, males and females	↑[e]	↑[e]	↑[e]				

[a]NTP (1985a).
[b]Values were reported following the birth of 5 litters, the benchmark doses are for values reported following the birth of the fifth litter because the greatest magnitude of effect was observed at that time point.
[c]Relative organ weights were adjusted for body weight; when absolute and relative organ weights changed in the same direction, only the relative organ weights were listed in this table.
[d]See text for a description of the types of lesions observed.
[e]It does not appear that statistical analyses were conducted for histopathology data, but incidence was increased compared to controls.
[f]Benchmark doses were estimated using a polynomial model.
[g]Benchmark doses were not estimated for endpoints without dose-response relationships.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

Table 96
Effects in Immature F1 Mice in a Continuous Breeding Study With Bisphenol A

Endpoint	Dose, % in diet **[mg/kg bw/day]**						
	0.25 **[437.5]**	0.5 **[875]**	1.0 **[1750]**	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Live pups/litter	↔	↓ 20%	↓ 48%	0.30 **[525]**	0.20 **[350]**	0.43 **[752]**	0.30 **[525]**
Proportion pups born alive	↔	↔	↓ 4%	3.0 **[5250]**	0.79 **[1382]**		
Live birth weight[b]	↔	↑5%	↑ 6%	0.43 **[752]**		0.34 **[595]**	
Mortality by PND 21[c]	↔	↔	↑ to 37.5%	0.48 **[840]**	0.40 **[700]**		

[a]NTP (1985a).
[b]Hill model used for benchmark dose calculations.
[c]Control mortality was 6.3%. Mortality was reported on a per pup basis, which limits the utility of the benchmark dose model.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

excluding the last one born were killed on the day of birth so that animals could continue mating. The last litter was raised by the dam and weaned on PND 21 (day of birth not defined). Birth weight and weight gain were recorded in the last litter. Reproductive endpoints in parental rats included the number of litters born and fertility. Statistical analyses included Kruskal–Wallis ANOVA on ranks, Mann–Whitney *U* test, χ^2 test, one-way ANOVA, arc sine square-root transformation, and Duncan multiple range test.

Table 97
Effects Observed in Mice Fed Bisphenol A-Containing Feed for One Generation[a]

Endpoint	Dose, % in diet (mg/kg bw/day)					
	0.5 (840–1055)[b]	1 (1669–1988)[b]	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
F_0 females body weights and feed intake						
GD 17 body weight[c,d]	↓ 8%	↓ 11%	1292	646	742	404
PND 0 body weight[d]	↔	↓ 7%	2130	1675	1813	1193
GD 0–17 body weight change[c,d]	↓ 16%	↓ 19%	472	283	701	387
Study day 0–7 feed intake	↑11%	↔				
GD 14–17 feed intake (g/day)	↔	↓ 13%	1454	898	1840	1172
GD 0–17% food efficiency	↓ 16%	↓ 16%				
Relative (to body weights) organ weights in F_0[e]						
Liver, male	↑ 22%	↑ 24%	706	561	705	555
Liver, female	↑ 27%	↑ 29%	615	484	746	586
Kidney, female	↑ 8%	↑ 24%	973	529	1309	863
Clinical chemistry effects in F_0 females, not examined in males						
Blood urea nitrogen	↔	↑ 43%	628	266		
Sodium	↓ 9%	↔				
Potassium	↓ 18%	↔				
Chloride	↓ 8%	↔				
Histopathology in F_0 females (not examined in males)[f]						
Renal tubule epithelium degeneration (control: 0/20)	9/20	9/20				
Renal tubule epithelium necrosis (control: 0/20)	6/20	8/20	663	480		
Renal tubule regeneration (control: 2/20)	12/20	20/ 20	223	151		
Centrilobular hepatocyte hypertrophy (control 0/20)	2/20	11/20	902	612		
Diffuse hepatocyte hypertrophy (control 0/20)	6/20	6/20				
Reproductive/developmental effects						
Gestational length	↑ 2%	↑ 2%				
No. of live pups	↔	↓ 15%	1116	727	1925	1189
Total no. of pups	↔	↓ 15%	1116	727	1925	1189
Female pup body weight	↓0.6%[g]	↓4%[g]	2281	1728	2332	1733

[a]Tyl et al. (2002a).
[b]Bisphenol A intakes included values estimated for males and females during prebreeding or gestation; intake values for the appropriate sex were used in benchmark dose analyses; intakes during gestation were used for females.
[c]The effect was reported at earlier time period but is shown here only for the latest or longest time period evaluated.
[d]Benchmark doses were estimated using the polynomial model.
[e]Only effects on relative organ weights were shown.
[f]Histopathology data were not statistically analyzed.
[g]By trend test.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effect.

In the cross-over trial (Task 3), ~20 males and females from the high-dose group were randomly paired with control mice for 7 days in order to determine the affected sex. Twenty control males and females were also paired. The animals were not exposed to bisphenol A during the 1-week mating period, but in animals from the high-dose group, dosing with bisphenol A was continued for 21 days on separation of the mating pairs. Vaginal smears were obtained from females that did not mate or did not appear to be pregnant. Fertility and offspring survival were determined. Parental mice from the control ($n = 38$/sex) and high-dose groups ($n = 19$/sex) were necropsied within a week following completion of the cross-over trial. Body, liver, kidney, and reproductive organ weights were obtained, and sperm count, morphology, and motility were determined. Testes, ovaries, and oviducts were fixed in Bouin solution and prostate, seminal vesicles/coagulating glands, uterus, liver, and kidney were fixed in 10% neutral buffered formalin for histopathological evaluation.

In Task 4 of the study, 20 F_1 mice/sex/group (at least 2/sex from 10 randomly selected litters/group) were mated within dose groups for 7 days and examined for reproductive function. Because fewer F_1 mice in high-dose group were available as a result of increased mortality, only 11 mice/sex were mated. The animals continued to receive the same diet given to their parents. Vaginal smears were obtained from females that did not mate or did not appear to become pregnant. One litter/pair was examined for sex, body weight, and viability. The parental F_1 animals from all dose group were killed and examined as described for Task 3 of the study.

Treatment-related effects observed in adult rats are summarized in Table 95, and effects occurring in immature rats are summarized in Table 96. Bisphenol A treatment had no effect on mating or fertility index in F_0 or F_1 mice. Postpartum body weights were reduced in F_0 dams of the high-dose group. In F_0 mice, the number of litters produced/pair and numbers of live F_1 pups/litter were reduced at the mid- and high-dose level. A decrease in the proportion of pups born alive occurred in F_0 mice of the high-dose group. No effects were observed on sex ratios of F_1 or F_2 pups. Weights of live F_1 pups were increased at the mid and high-dose. There were no significant effects when pup weights were adjusted for total numbers of live and dead pups in the litter.

Table 98
Treatment-Related Effects in Adult Mice Fed Bisphenol A Through Diet in a Multigeneration Reproductive Toxicity Study

	Dose, ppm diet **[mg/kg bw/day based on target intakes provided by study authors]**													
Endpoint	0.018 [0.003]	0.18 [0.03]	1.8 [0.3]	30 [5]	300 [50]	3500 [600]	BMD_{10}		$BMDL_{10}$		BMD_{1SD}		$BMDL_{1SD}$	
Body weight gain during lactation, F_0	↔	↔	↔	↔	↔	↑2.2–fold	249	[42.2]	150	[25.4]	4258	[722]	2941	[498]
Terminal body weight F_1 retained males	↔	↔	↔	↔	↔	↓10%	3455	[586]	2388	[405]	3503	[594]	2608	[442]
Relative liver to body weight														
F_0 males	↔	↔	↔	↔	↔	↑17%	2189	[371]	1820	[308]	2021	[343]	1668	[283]
F_1 parental males	↔	↔	↔	↔	↑5%	↑22%	1662	[282]	1425	[242]	1637	[277]	1389	[235]
F_1 retained males	↔	↔	↔	↔	↔	↑23%	1584	[268]	1383	[234]	1685	[286]	1405	[238]
F_0 females	↔	↔	↔	↔	↔	↑17%	2524	[428]	1595	[270]	3014	[511]	2155	[365]
F_1 females	↔	↔	↔	↔	↔	↑10%	3424	[580]	2438	[413]	3551	[602]	3024	[513]
Relative right kidney to body weight														
F_0 males	↔	↔	↔	↔	↑8%	↑20%	1861	[315]	1536	[260]	2100	[356]	1723	[292]
F_1 parental males	↔	↔	↔	↑11%	↑10%	↑21%	2079	[352]	1913	[324]	862	[146]	773	[131]
F_1 retained males	↔	↔	↔	↔	↔	↑27%	1501	[254]	1229	[208]	1978	[335]	1610	[273]
F_0 females	↔	↔	↔	↔	↔	↑13%	3568	[605]	2504	[424]	4326	[733]	3041	[515]
F_1 females	↔	↔	↔	↔	↔	↑8%	3629	[615]	2976	[504]	3702	[627]	3393	[575]
Relative left kidney to body weight														
F_0 males	↔	↔	↔	↔	↑9%	↑19%	1899	[322]	1548	[262]	2249	[381]	1825	[309]
F_1 parental males	↔	↔	↔	↑13%	↑10%	↑22%	2074	[352]	1650	[280]	2547	[432]	2020	[342]
F_1 retained males	↔	↔	↔	↔	↑11%	↑28%	1466	[248]	1205	[204]	1937	[328]	1582	[268]
F_0 females	↔	↔	↔	↔	↔	↑11%	3746	[635]	2550	[432]	4773	[809]	3258	[552]
Relative pituitary to body weight														
F_1 parental males[a]	↔	↔	↔	↔	↔	↑10%	3413	[578]	2087	[554]	3627	[615]	3182	[539]
F_1 retained males[a]	↔	↔	↔	↔	↔	↑16%	2678	[454]	1934	[328]	3476	[589]	2512	[426]
Relative brain to body weight F_1 retained males[a]	↔	↔	↔	↔	↔	↑9%	2678	[454]	1934	[328]	3476	[589]	2512	[426]
Hepatocyte centrilobular hypertrophy incidence (control incidence in parentheses)														
F_0 males (6/56)	1/10	2/10	2/10	0/10	4/10	10/10	122	[20.7]	70	[11.8]				
F_1 parental males (7/55)	0/10	0/10	4/10	2/10	1/10	6/10	879	[149]	578	[98.0]				
F_1 retained males (4/50)	1/10	3/10	2/10	2/10	5/10	7/10	656	[111]	442	[74.9]				
F_0 females (1/56)	0/10	0/10	0/10	0/10	1/10	6/10	1348	[228]	947	[161]				
F_1 females (2/55)	0/10	0/10	0/10	0/10	3/11	7/10	962	[163]	679	[115]				
Renal nephropathy incidence (control incidence in parentheses)														
F_0 males (12/56)	0/10	3/10	2/10	2/10	1/10	4/10	1556	[264]	750	[127]				
F_1 parental males (6/55)	2/10	0/10	1/10	2/10	0/10	4/10	1418	[240]	838	[142]				
F_1 retained males (8/50)	1/10	0/10	0/10	2/10	0/10	3/10	1991	[337]	992	[168]				
F_1 females (10/55)	1/10	3/10	0/10	1/10	1/11	4/11	1646	[279]	847	[144]				
Paraovarian cysts (control incidence in parentheses)														
F_0 (9/56)	1/11	2/12	1/11	1/12	3/14	7/17	1328	[225]	833	[141]				
F_1 (14/55)	1/11	1/11	1/10	2/10	2/11	7/15	1193	[202]	708	[120]				
Epididymal sperm concentration, F_0[a]	↔	↔	↔	↔	↔	↓15%	3343	[567]	1884	[319]	3581	[607]	3241	[549]
Gestational length														
F_0	↔	↔	↔	↔	↔	↑2%	21,351	[3619]	3770	[639]	6749	[1144]	3536	[599]
F_1	↔	↔	↔	↔	↔	↑2%	17,820	[3020]	3784	[641]	4552	[772]	3134	[531]

[a]Not considered a treatment-related effect by study authors.
↑,↓ Statistically significant increase, decrease; ↔ no statistically significant effect compared to controls.

Table 99
Treatment- or Dose-Related Effects in Developing Mice in a Multigeneration Reproductive Toxicity Study With Bisphenol A

	Dose, ppm diet **[mg/kg bw/day based on target intakes provided by study authors]**									
End point[a]	0.018 **[0.003]**	0.18 **[0.03]**	1.8 **[0.3]**	30 **[5]**	300 **[50]**	3500 **[600]**	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$
Body weight										
F_1, PND 7	↔	↔	↔	↔	↔	↓13%	3304 **[560]**	1849 **[313]**	3433 **[582]**	2403 **[407]**
F_1, PND 14	↔	↔	↔	↔	↔	↓11%	3453 **[585]**	2256 **[382]**	3639 **[617]**	2988 **[506]**
F_1, PND 21	↔	↔	↔	↔	↔	↓17%	3236 **[548]**	1577 **[267]**	3421 **[580]**	2342 **[370]**
F_1 male, PND 21 necropsy	↔	↔	↔	↔	↔	↓12%	3325 **[564]**	1845 **[313]**	3776 **[640]**	3536 **[599]**
F_1 female, PND 21 necropsy	↔	↔	↔	↔	↔	↓18%	2284 **[387]**	1501 **[254]**	4577 **[776]**	3529 **[598]**
Lactational survival indices (control index, %, in parentheses)										
F_2 PND 21 survival (100%)[c]	↔	↔	↔	↔	↓ to 86.6%	↔				
F_2 Lactational index (97.2%)[c]	↔	↔	↔	↔	↓ to 86.6%	↔				
Relative thymus to body weight, F_1 male, PND 21[b]	↔	↔	↔	↔	↑ 13%[b]	↑ 10%[b]				
Relative spleen to body weight										
F_1 male, PND 21	↔	↓ 12%	↔	↔	↔	↓ 30%	3123 **[529]**	1074 **[182]**	3538 **[600]**	3148 **[534]**
F_2 male, PND 21	↔	↔	↔	↔	↔	↓ 20%	2148 **[364]**	1425 **[242]**	7013 **[1189]**	3560 **[603]**
F_1 female, PND 21	↔	↔	↔	↔	↔	↓ 23%	3168 **[537]**	647 **[110]**	4571 **[775]**	3677 **[623]**
F_2 female, PND 21	↔	↔	↔	↔	↔	↓ 21%	1787 **[303]**	1311 **[222]**	5022 **[851]**	3517 **[596]**
Relative paired testes weight to body or brain weight										
F_1, PND 21 (body weight)	↔	↔	↔	↔	↔	↓ 8%	3578 **[606]**	2720 **[461]**	3861 **[654]**	3550 **[602]**
F_2, PND 21 (brain weight)	↔	↔	↔	↔	↔	↓ 11%	3316 **[562]**	2003 **[339]**	5342 **[905]**	3571 **[605]**
Relative paired epididymides to body weight, F_1[b]	↔	↑ 18%	↔	↔	↔	↔				
Relative brain to body weight F_1 female, PND 21[b]	↔	↔	↔	↔	↔	↑ 17%[b]	2219 **[376]**	1415 **[240]**	3576 **[606]**	2825 **[479]**
Relative left kidney to body weight, F_2 male, PND 21	↔	↔	↔	↔	↔	↑ 6%	6664 **[1129]**	3540 **[600]**	8501 **[1441]**	3589 **[608]**
Relative seminal vesicles with coagulating gland to brain weight, F_2 [b]	↔	↔	↔	↔	↓ 15%	↓ 16%	2389 **[405]**	1315 **[223]**	11,294 **[1914]**	3631 **[615]**
Uterus with cervix and vagina weight relative to bodyweight, F_2 PND 21[b]	↔	↔	↔	↔	↓16%	↔				
Relative paired ovary weights, F_1[b]	↔	↔	↑	↔	↔	↔				

Hepatic cytoplasm alteration (control incidence in parentheses)										
F_1 males (6/44)	1/26	0/17	1/22	6/24	10/20	13/20	732 **[124]**	546 **[92.5]**		
F_2 males (6/54)	1/25	1/25	1/25	1/24	2/20	9/23	1442 **[244]**	1050 **[178]**		
F_1 females (2/46)	1/27	2/21	3/24	4/26	8/16	6/22	1966 **[333]**	1182 **[200]**		
Unilateral hydronephrosis, F_1 males (0/44)[b]	0/26	0/17	0/21	0/24	0/21	3/21b				
Seminiferous tubule hypoplasia (control incidence in parentheses)										
F_1 (1/96)	0/54	0/37	1/45	3/51	2/45	5/43	3485 **[591]**	2398 **[406]**		
F_2 (5/114)	1/53	2/61	2/55	0/51	5/49	20/57	1670 **[283]**	1377 **[233]**		
Undescended testis, F_1 PND 21 (control 11/135)	5/79	5/54	10/70	5/78	7/50	12/600	2694 **[462]**	1755 **[301]**		
Anogenital distance adjusted for body weight F_1 male, PND 21[b]	↔	↔	↔	↔	↓4%	↓5%	8099 **[1373]**	3582 **[607]**	10,436 **[1769]**	3632 **[616]**
Age of preputial separation (adjusted per body weight)										
F_1 parental males	↔	↔	↔	↔	↔	↑2 days	4450 **[754]**	3397 **[576]**	3252 **[551]**	2445 **[414]**
F_1 retained males	↔	↔	↓0.6 days	↔	↔	↑1.8 days	4288 **[727]**	3375 **[572]**	2897 **[491]**	2145 **[364]**
Body weight on day of vaginal opening in F_1	↔	↔	↔	↔	↔	↓22%	3076 **[521]**	1281 **[217]**	3294 **[558]**	1972 **[334]**
Age of vaginal opening adjusted for PND 21 body weight[b]	↔	↔	↔	↔	↔	↓2.4	3501 **[593]**	2953 **[501]**	3404 **[577]**	2419 **[410]**

[a]Based on numbers of animals listed in data tables, it appears that statistical analyses in live animals before or on PND 21 considered the litter as the statistical unit, but statistical analyses conducted at PND 21 necropsy considered the individual pup as the statistical unit.
[b]Not considered treatment related by study authors.
[c]Effect was not discussed by study authors but it is unlikely to be related to treatment.

Therefore the NTP concluded that the increased pup weights resulted from the smaller litter size. Body weights were evaluated through PND 21 in F_1 pups, and no effects were found on pup body weight gain during the lactation period. Mortality in F_1 offspring during the postnatal period was increased in the high-dose group.

The cross-over test revealed no effect on mating or fertility in either males or females exposed to bisphenol A. Postpartum body weight was not affected in the treated females. The number of live pups/litter was significantly reduced **[by 26%]** in the group containing treated males and **[by 51%]** in the group containing treated females. Live pup weight was increased in the group containing treated females, but there was no significant effect following adjustment for litter size. There were no effects on the proportion of pups born alive or on sex ratio.

In sperm analyses conducted in high-dose F_0 males and all dose groups of F_1 males, sperm motility was reduced in high-dose F_0 males and mid-dose F_1 males. There were no effects on sperm count or morphology in either generation. Effects were observed on organ weights, which were examined in F_0 adults of the high-dose group and F_1 animals from each treatment group. Effects on absolute reproductive organ weights of F_1 mice included decreased right epididymis weight at all doses, decreased left testis/epididymis weight at the mid and high-dose, and decreased seminal vesicle weight at the high-dose. Significant effects on relative organ weights adjusted for body weight in F_1 rats included decreased right epididymis weight at all doses, decreased seminal vesicle weight at the low and high-dose, and decreased relative left testis and epididymis weight at the mid and high-dose. Reproductive organ weight effects observed in high-dose F_0 males included decreased absolute and relative seminal vesicle weight. There were no effects on prostate weight. No effects were reported for estrous cyclicity of F_0 females. There were no gross or histopathological alterations in F_0 or F_1 reproductive organs including testis, epididymis, prostate, seminal vesicles, ovary, vagina, and uterus. Effects observed in high-dose F_0 animals were also summarized in a report by Morrissey et al. (1988).

Effects were observed on non-reproductive organ weights, which were examined in F_0 adults of the high-dose group and F_1 animals from each treatment group. In the F_1 mice, dose-related effects on absolute organ weights included increased kidney/adrenal weight at all doses in both sexes and increased liver weight in mid- and high-dose females and high-dose males. Significant effects on relative organ weight adjusted for body weight in F_1 rats included increased liver and kidney/adrenal weights at all doses in both sexes. Organ weight effects observed in high-dose F_0 males included increased absolute and relative liver and kidney/adrenal weight. In F_0 female rats of the high-dose group, absolute and relative liver weight and relative kidney weights were increased. Body weights of high-dose F_0 females were reduced at necropsy. Histopathology was examined in F_0 rats of the high-dose group and F_1 rats from all dose groups. Treatment-related hepatic lesions observed in both generations included multifocal necrosis, multinucleated giant hepatocytes in males and females, and centrilobular hepatocytomegaly in males. Multifocal mineralization of liver cells was also observed in F_1 females of the high-dose group. Hepatic lesions were observed at all dose levels for F_1 males and in F_1 females of the mid- and high-dose group. Treatment-related renal lesions were observed in both generations and described as tubular cell nuclear variability, increased severity of spontaneous tubular interstitial lesions, cortical tubular dilatation, mineralization of renal cells, and micro-calculi in tubular epithelium that sometimes occurred with effaced tubular epithelium, tubular regeneration, and/or dilated tubules containing casts. **[It appears that the incidence of renal lesions was increased at all doses in F_1 rats.]** Renal lesions were stated to generally be more prominent in females than males. The study authors concluded that exposure of mice to bisphenol A resulted in toxicity to the reproductive system, kidney, and liver. The possibility was noted that some or all effects on reproductive performance may have been secondary to the generalized toxicity of bisphenol A.

This study demonstrates changes in F_1 male absolute reproductive weights (seminal vesicle with coagulating gland as well as epididymis; the testis and prostate appear not to have been appreciably affected). This study also suggested that reproductive toxicity and general toxicity occurred at similar dose levels. Bisphenol A-mediated general toxicity may have contributed to the observed female fertility effect, because this effect was noted with dosed females cohabiting with non-dosed males. In the male, however, the effect on motility is likely bisphenol A-related, resulting in the observed fertility deficits.

In Task 2, a clear effect on fertility was found with a NOAEL of 0.25% bisphenol A in the diet.

Strengths/Weaknesses: This comprehensive toxicology study was well-conducted. General toxicity was clearly demonstrated at all F_1 dose levels, and histopathological findings appear to be a sensitive indicator of effect. As a limitation of this design, because bisphenol A was in the diet, exposure to bisphenol A did not occur during cohabitation; therefore, direct exposure to bisphenol A was minimal or nonexistent during sperm maturation, capacitation and ovulation.

Utility (Adequacy) for CERHR Evaluation Process: These data are adequate and of high utility for the evaluation process.

Tyl et al. (2002a), sponsored by the Society of the Plastics Industry, conducted a one-generation reproductive toxicity study in mice. The study was conducted to verify the findings of reduced pup numbers at birth in a continuous breeding study conducted by the NTP (NTP, 1985a). GLP guidelines were applied in the conduct of the study. CD-1 mice were fed Purina Certified Rodent Diet Meal and housed in polycarbonate cages containing Sani-chip bedding. Mice were stratified according to body weight and randomly assigned to treatment groups. Starting at 9 weeks of age, 20 mice/sex/group were given feed containing bisphenol A (99.36% purity) 0, 5000, or 10,000 ppm. Males and females were fed the bisphenol A-containing diets during a 2-week prebreeding period and a 1-week mating period. The day of vaginal plug detection was defined as GD 0. Exposures in females continued through the gestation period of ~19 days. The study authors reported bisphenol A intakes of 0, 840, and 1669 mg/kg bw/day in males during the prebreeding period; 0, 1055, and

1988 mg/kg bw/day in females during the prebreeding period, and 0, 870, and 1716 mg/kg bw/day in females during the gestation period. **[Intake values were obtained from the Results section and study summary tables. They differed from values reported in text Table C, which were assumed to be in error.]** Homogeneity and stability of bisphenol A in feed were verified. Parameters evaluated during the study included clinical signs, body weight, and feed intake. Reproductive endpoints evaluated included implantation loss and indices of mating, fertility, pregnancy, and gestation. F_0 Males were killed at the end of the breeding period; liver and kidney were weighed. At birth, pups were counted, sexed, weighed, and evaluated for viability and external alterations. F_0 females and F_1 pups were killed on the day of parturition (PND 0). Dams were assessed for clinical chemistry parameters of liver and kidney function; corpora lutea and implantation sites; uterus, ovary, kidney, and liver weight; and liver and kidney histopathology. The male, female, pregnant female, or the litter were considered statistical units. Statistical analyses included ANOVA, Levene test, GLM procedure, Dunnett test, χ^2 test, Cochran–Armitage test, and Fisher exact probability test.

Treatment-related effects in F_0 animals are summarized in Table 97. There were no treatment-related changes in clinical signs, body weight gain, feed intake, or food efficiency in males or in females during the prebreeding period. A transient increase in food intake occurring in females of the low-dose group on study stays 0–7 did not appear to be treatment-related. Gestational body weight gain was decreased in the high-dose group, beginning on GD 7 and in the low dose group beginning on GD 10. Body weights of live F_0 females were significantly lower in the high-dose group on PND 0, but no significant differences were observed during necropsy conducted later in the day. A significant decrease in feed intake was reported for the high-dose group on GD 14–17, only when the values were expressed as g/day. **[The results section indicated that food efficiency during gestation was not significantly affected, but a downward trend was observed. Table 10 of the study reported a significant decrease in food efficiency.]** Significant necropsy findings observed in males included increased absolute and relative liver weight at both doses and increased absolute paired kidney weight at the low dose. Absolute and relative liver and paired kidney weight were increased significantly in females from both dose groups. Histopathological observations in females included dose-related increases in incidence and severity of hepatocyte hypertrophy and increased kidney lesions (renal tubular epithelial necrosis, degeneration, and regeneration) in both dose groups. Significant clinical chemistry findings in females included increased blood urea nitrogen in the high-dose group and decreased sodium, potassium, and chloride levels in the low-dose group.

Treatment-related reproductive or developmental effects are summarized in Table 97. No significant effects were observed for mating, fertility, or pregnancy indices; time to insemination; numbers of ovarian lutea or implantation sites; or implantation loss. Gestation duration was extended by ~10 hr in both dose groups; the study authors stated that the biological significance of the finding is not known. Total and live pup numbers were decreased in the high-dose group. No significant effects on pup weight were observed but a downward trend was statistically identified for female pup weight.

The study authors concluded that their study confirmed the NTP (1985a) finding of reduced litter size in mice fed 10,000 ppm bisphenol A in feed. The NTP finding of decreased litter size at 5000 ppm bisphenol A was not confirmed in this study, likely due, according to the authors, to the shorter exposure duration in the current study than in the NTP study. The study authors concluded that the litter size decreases in their study were likely caused by the compromised status of dams.

Strengths/Weaknesses: Strengths of this report include the comprehensive design with the assessment of multiple relevant endpoints. There were adequate numbers of animals, the doses and stability of the compound were verified, and the oral route of exposure was used. Weaknesses include the limited number of doses examined and the relatively high-doses studied.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and useful for the evaluation process.

Tyl et al. (2006), sponsored by the American Plastics Council, conducted a two-generation study of bisphenol A in mice. The study was conducted accorded to GLP. CD-1 mice were received in two cohorts approximately 2 weeks apart and data from the two cohorts were combined. Mice were fed Purina Certified Ground Rodent Diet No. 5002. The supplier provided information about phytoestrogen content of feed (177–213 ppm genistein, 173–181 ppm daidzein, and 39–55 ppm glycitein). Mice were housed in polypropylene cages with Sani-Chip bedding. Assignment of F_0 animals to groups involved randomization stratified by weight. F_0 and F_1 mice (28 sex/group/generation) were fed diets containing bisphenol A (99.70–99.76% purity) at 0.018, 0.18, 1.8, 30, 300, or 3500 ppm. Target intakes were 0.003, 0.03, 0.3, 5, 50, or 600 mg/kg bw/day, respectively. Based on measured feed intake, the study authors estimated bisphenol A intake in males at 0.0024–0.0038, 0.024–0.037, 0.24–0.37, 3.98–6.13, 39.1–60.8, or 529–782 mg/kg bw/day. Bisphenol A intakes (in mg/kg bw/day) by females were estimated at 0.0030–0.0041, 0.030–0.042, 0.32–0.43, 5.12–7.12, 54.2–67.8, 653–910 during the premating period; 0.0027–0.0029, 0.027–0.028, 0.28–0.29, 4.65–4.80, 47.0–48.6, 552–598 during the gestation period; and 0.0063–0.0087, 0.062–0.091, 0.61–0.89, 10.4–15.1, 103.2–146.4, 1264–1667 during the lactation period. In each generation, there were 2 vehicle control groups with 28 mice/sex/group. A positive control group was given feed containing 17β-estradiol at 0.5 ppm (target intake of 0.08 mg/kg bw/day). Estimated intakes for 17β-estradiol (in mg/kg bw/day) were 0.074–0.104 in males, 0.093–0.12 in females during the pre-mating period, 0.08–0.081 in females during the gestation period, and 0.160–0.25 in females during the lactation period. Dose selections were based on observations from several studies. **[The Expert Panel notes that a separate 2-generation study was used to characterize the dose–response relationship for 17β-estradiol.]** Homogeneity, stability, and concentration of bisphenol A in feed were verified. Exposure of F_0 mice began at ~6 weeks of age. Exposure of F_1 animals began at weaning, although it was noted that pups began eating the dosed feed in the late lactation period. F_0 and F_1 mice were fed the bisphenol A-containing diets for a minimum of 8

weeks before mating and during a 2-week mating period. Exposures of males continued through the gestation period of the litters they sired. Exposures of females continued through the gestation and lactation period. During the study, adult animals were monitored for clinical signs of toxicity, body weight, and food intake.

Estrous cycles were evaluated in F_0 and F_1 females during the last 3 weeks of the pre-breeding exposure period. Day of vaginal plug was defined as GD 0 and day of birth was considered PND 0. F_1 and F_2 pups were counted, sexed, weighed, and assessed for viability and physical abnormalities at birth and throughout the lactation period. Anogenital distance was measured in F_1 and F_2 pups at birth and on PND 21. On PND 4, F_1 and F_2 litters were standardized to 10 pups, with equal numbers per sex when possible. Pups removed on PND 4 were killed and examined for visceral alterations, with a focus on the reproductive system. The remaining pups were maintained and weaned on PND 21. At weaning, 28 F_1 pups/sex/group (1 per sex per litter) were randomly selected for mating and those animals were referred to as parental mice. An additional F_1 male/litter was selected for a 3-month exposure (referred to as retained males). Two F_1 pups/sex/litter were selected for gross necropsy and organ weight measurement at weaning. Histopathological examination of reproductive organs was conducted in one PND 21 pup/sex/litter. Histopathological evaluation of reproductive and systemic organs were conducted in the second F_1 pup from each group at weaning. All F_2 pups were killed at weaning and organ weights were measured. Vaginal opening and preputial separation were monitored in parental and retained F_1 mice. Parental F_0 and F_1 males were killed following delivery of the litters they sired. Retained F_1 males were killed at the same time as the parental F_1 males. Parental F_0 and F_1 females were killed after their pups were weaned. Organs, including those of the reproductive system, were weighed in adult F_0 and F_1 animals. Histopathological evaluations were conducted in all animals from the vehicle control group, in 10 F_0 and F_1 parental animals from each treatment group, in all F_1 retained males, and 10 animals from the 17β-estradiol positive control group. Histopathological evaluation of reproductive organs was also conducted in animals with suspected reduced fertility. Testes were preserved in Bouin fixative. Daily sperm production, efficiency of daily sperm production, and epididymal sperm count, motility, and morphology, were evaluated in F_0 and F_1 males. Data from the 2 control groups were analyzed separately and then pooled for statistical analysis of treatment groups. Statistical analyses included ANOVA, Levene test, robust regression methods, Wald χ^2 test, *t*-test, Dunnett test, Fisher exact probability test, and ANCOVA.

Treatment- or dose-related results and observations in reproductive organs of adult animals are summarized in Table 98. There were no consistent effects on body weight or body weight gain in F_0 males. Body weight gain during lactation was increased in F_0 females from the 3500 ppm group. During the premating period, body weights were decreased by $\leq 10\%$ in F_1 parental animals from the 3500 ppm group (study days 0, 7, 49, and 56 in males and study 0 in females). In retained F_1 males from the 3500 ppm group, body weights were decreased at most time periods between study days 7 and 84 and at necropsy. No consistent or dose-related changes in feed intake or efficiency were observed throughout the study in F_0 or F_1 animals. There were no clinical signs of toxicity or treatment-related deaths in F_0 or F_1 males or females. Increases in absolute and relative to body or brain weights of kidney and liver were consistently observed in F_0 and F_1 adults. Significant and dose-related organ weight changes relative to body weight are summarized in Table 98. Other effects on organ weight (e.g., seminal vesicles, epididymides, coagulating glands, and pituitary) were not considered to be treatment-related by study authors due to factors such as lack of a dose–response relationship, no consistency between absolute and relative weights, no histopathology, or no consistency across generations. Absolute and relative prostate weights were unaffected by bisphenol A exposure. There were no treatment-related gross systemic findings in F_0 or F_1 adults. Incidence of minimal to mild hepatocyte centrilobular hypertrophy was increased in both generations at 300 and/or 3500 ppm (Table 98). Renal nephropathy incidence was increased in F_0 males and in F_1 males and females of the 3500 ppm group. **[It did not appear that histopathological data were statistically analyzed.]**

Treatment- or dose-related reproductive effects in adult animals are summarized in Table 98. Bisphenol A exposure had no effect on numbers of implantation sites or resorptions or on mating, fertility, or gestational indices in F_0 or F_1 mice. Gestational length was increased in F_0 and F_1 females from the 3500 ppm group; the study authors stated the effect was of unknown biological significance. Epididymal sperm concentration was decreased in F_0 males of the 3500 ppm group but no effect was observed in F_1 parental or retained males. There was no effect on daily sperm production, efficiency of daily sperm production, or sperm motility or morphology in either generation. The study authors did not consider the decrease in sperm concentration in F_0 animals to be treatment-related based on lack of consistency between generations, no effect on any other andrological endpoint, and no effect on fertility. Estrous cyclicity and numbers of ovarian primordial follicle counts were not affected by bisphenol A exposure in F_0 or F_1 females. The only gross observation in reproductive organs was a slightly increased incidence of gross ovarian cysts in F_0 females from the 3500 ppm group. The incidence of paraovarian cysts was increased in F_0 and F_1 females from the 3500 ppm group. **[It did not appear that histopathological data were statistically analyzed.]**

Significant findings in developing mice are summarized in Table 99. Live F_1 and F_2 pups and litters at birth, sex ratio, and survival during the lactation period were not affected and there were no clinical or gross signs of toxicity in F_1 or F_2 offspring. A non-dose-related decrease in PND 21 survival index and lactational index (pups surviving on PND 21/PND 4) was described in F_2 pups of the 300 ppm group. **[The biological significance of the effect was not discussed by the study authors, but because the effect was not dose-related it is unlikely to be of biological significance.]** In F_1 pups from the 3500 ppm group, body weights were reduced during PND 7, 14, and 21 in F_1 females and both sexes combined and on PND 7 and 21 in F_1 males. Body weight results for both sexes combined are summarized in Table 99. An increase in male pup body weight observed on PND 7 in

the 1.8 ppm group was not considered to be treatment-related by the study authors because no dose–response relationship was observed. There was no effect on anogenital distance in F_1 or F_2 males or females on PND 0. Anogenital distance was also unaffected in F_2 males and F_1 and F_2 females on PND 21. Anogenital distance adjusted for body weight was reduced in F_1 males from the 300 and 3500 ppm groups on PND 21. Based on the lack of effect on anogenital distance at birth and inconsistencies between generations, the study authors did not consider the decreases in anogenital distance in F_1 males to be treatment-related. An increase in anogenital distance in F_2 females from the 0.018 ppm group on PND 0 was not considered to be treatment-related by the study authors. Preputial separation (absolute age and adjusted for body weight on day of acquisition) was delayed in parental and retained F_1 males of the 3500 ppm group. When adjusted for PND 30 body weight, preputial separation was delayed in retained but not parental F_1 males from the 3500 ppm group. Data for preputial separation adjusted for body weight on day of acquisition are shown in Table 99. Body weights on day of vaginal opening were lower in F_1 females from the 3500 ppm group. Day of vaginal opening was accelerated in the 3500 ppm group if adjusted for PND 21 body weight, but not body weight on the day of acquisition. Due to the lack of effect when adjusted for body weight on day of acquisition, the study authors did not consider effects on vaginal opening to be treatment-related.

Shown in Table 99 are significant organ weight effects relative to body weight. Dose-related organ weight changes in F_1 weanlings that were considered to be treatment-related by study authors included decreased absolute and relative (to body or brain weight) spleen and paired testes weights at 3500 ppm. Treatment-related absolute organ weight changes in F_2 weanlings included decreased weights of spleen, paired testes, and seminal vesicles with coagulating glands in the 3500 ppm group. Changes in organ weights relative to body weight in F_2 weanlings included decreased spleen weight in males and females and increased relative left kidney weight in 3500 ppm males. Treatment-related changes in organ weight relative to brain weight in F_2 weanlings were decreased spleen weight in both sexes and decreased paired testes weight at 3500 ppm and seminal vesicles with coagulating glands at 300 and 3500 ppm. Other organ weight effects (e.g., affecting epididymides, thymus, brain, ovaries, and/or uterus with cervix and vagina weights) were not considered to be dose-related due to lack of dose–response relationships or no consistent effects across generations. Included in Table 99 are significant organ weight effects relative to body weight. Significant organ weight effects relative to brain weight were included in Table 99 when the organ weight effect was significant only when normalized for brain weight. The study authors reported no gross findings in F_1 or F_2 weanlings. The incidence of undescended bilateral testes was increased in F_1 and F_2 weanling males of the 3500 ppm group. The incidence of hepatic cytoplasm alteration (clear hepatocellular cytoplasm, slightly more basophilic cytoplasm, and/or minute vacuoles) was apparently increased in F_1 males from the 300 and 3500 ppm groups and F_1 females and F_2 males from the 3500 ppm group. The incidence of seminiferous tubule hypoplasia was increased in F_1 and F_2 weanlings from the 3500 ppm group. **[Another histopathological finding that appeared to be possibly increased in weanlings from the 3500 ppm group was unilateral hydronephrosis in F_1 males. It did not appear that histopathological data were statistically analyzed.]**

Effects of 17β-estradiol in males were delayed preputial separation, reduced anogenital distance at weaning but not at birth, decreased weights of testes, epididymides, and seminal vesicles with coagulating gland, and increased incidence of seminiferous tubule hypoplasia and undescended testis. Effects of 17β-estradiol in female mice were accelerated vaginal patency, increased uterus with cervix and vagina weight, fluid filled/enlarged uterus, enlarged/thickened vagina, increased vaginal epithelial keratinization, and prolonged gestation. Reproductive effects in the 17β-estradiol group included decreased fertility, increased stillbirth, reduced live pups per litter, and increased dead pups.

The study authors identified bisphenol A NOELs of 30 ppm (~5 mg/kg bw/day) for systemic effects, 300 ppm (~50 mg/kg bw/day) for developmental toxicity, and 300 ppm (~50 mg/kg bw/day) for reproductive toxicity.

Strengths/Weaknesses: Strengths include the large number and range of doses examined, the rigor with which the study was performed, the large sample size in each group, the number of additional animals per litter that were retained and examined, the use of a concurrent estrogenic positive control group, and the thoroughness of the histologic evaluation.

Utility (Adequacy) for CERHR Evaluation Process: This study is adequate and of high utility for the evaluation process.

4.2.3.3 Fish and invertebrates: Although studies in fish and invertebrates may be important for understanding mechanisms of action and environmental impact, the Panel views these studies as not useful for the evaluation process.

Kwak et al. (2001), supported by the Korean Ministry of the Environment, exposed adult male swordtail fish (*Xiphophorus helleri*) to bisphenol A 0, 0.4, 2, or 10 ppm **[mg/L]** for 72 hr ($n = 20$ fish/group). **[No information on purity or culture ware was provided.] [Nonylphenol was also studied but will not be discussed here.]** At the end of the exposure period, the fish were killed and livers were removed for measurement of vitellogenin. Testes of 10 fish/group were processed for flow cytometry by preparation of single cell suspensions stained with annexin V-fluorescein isothiocyanate and propidium iodide to detect necrosis and apoptosis. TUNEL staining was used to confirm apoptosis in testis sections. In a second experiment, juvenile male fish (30 days old) were exposed to bisphenol A in water at 0, 0.2, 2 and 20 ppb **[μg/L]** for 60 days, after which body length and sword length were measured. **[The sword is a portion of the caudal fin that elongates as a secondary sex characteristic.]** Statistical analysis used ANOVA followed by least significant difference test. Hepatic vitellogenin was increased by bisphenol A **[data were not shown]**. Apoptosis was increased in testes from fish exposed to bisphenol A at 10 ppm **[mg/L]** by TUNEL assay. **[Flow cytometry was said to be more sensitive, but data did not appear to have been statistically analyzed.]** Sword growth was decreased by bisphenol A

Table 100
Summary of Serum Hormone Changes in Human Studies

Study members	Hormone effects	Other	Reference
High utility			
Urine in male workers 42 exposed 42 non-exposed	↓ FSH (exp median 5.3 mIU/ml vs. 7.6 in controls) No difference LH, free testosterone	BPA exposure Exposed men: 1.06 μmol/mol creatinine [0.043 μg/kg bw) Non-exposed men:	Hanaoka et al. (2002)
Limited utility			
Serum samples from: 14 healthy women 11 healthy men 16 women with PCOS	↑ total testosterone ($r = 0.595$, all subjects) ↑ free testosterone ($r = 0.609$, all subjects) No difference LH	0.52 μmol/mol creatinine [0.02 μg/kg bw)	Takeuchi and Tsutsumi (2002)
Serum samples from: 26 healthy women 19 women with PCOS 28 women with other conditions	↑ total testosterone ($r = 0.391$) ↑ free testosterone ($r = 0.504$) ↑ androstenedione ($r = 0.684$) ↑ dehydroepiandrosterone sulfate (DHEAS) ($r = 0.514$) No difference LH		Takeuchi et al. (2004a)
Serum samples from women: 11 controls 10 simple hyperplasia (HP) 9 complex hyperplasia (HP) 7 endometrial cancer (EC)		dec BPA in complex HP and EC patients compared to controls	Hiroi et al. (2004)

exposure in a concentration-dependent manner, with statistically significant decreases from control at 2 and 20 ppb **[μg/L]**. The authors concluded that bisphenol A at 20 ppb decreases sword growth and that reproductive impairment occurs in a concentration-dependent manner.

Strengths/Weaknesses: This study of bisphenol A is consistent with previous reports on the effects of estrogenic compounds in fish (vitellogenin production and changes secondary sex characteristics). It is unclear exactly how these fish were maintained before exposure and during the long-term exposure. Bisphenol A concentrations in the test waters were not determined and only 3 concentrations of bisphenol A were used.

Utility (Adequacy) for CERHR Evaluation Process: Of note is the classic dose response obtained in this apparently sensitive model. Given the absence of confirmation of exposure conditions and that this is a fish species immersed in the test agent, this study is not useful in the evaluation.

Sohoni et al. (2001), supported by the Society of the Plastics Industry, exposed adult (122-day-old) fathead minnows (*Pimephales promelas*) to bisphenol A in water at 0, 1, 16, 160, and 640 μg/L ($n = 60$/group) **[No information on purity or culture ware was provided]**. Actual concentrations were 70–96% of nominal concentrations. After 42 days of exposure, 15 fish/group were killed for evaluation of somatic growth, relative gonad weight, plasma vitellogenin, and histologic assessment of the testis. Eight breeding pairs/group were segregated for continued exposure for 123 days. Eggs were removed and counted daily. On 2 occasions, eggs were continued in the same bisphenol A concentration as their parents and the percent hatching was assessed 4 days after fertilization. The remaining adult fish were killed after 71 days of exposure for evaluation of somatic growth, relative gonad weight, and histologic assessment of the gonad. Data were analyzed using 2-way ANOVA and Dunnett test or Kruskal–Wallis and Dunn multiple method test. Linear regression was used to evaluate the relationship between bisphenol A concentration and growth. There were no significant long-term effects of treatment on growth of female fish, but male fish showed a inverse relationship between bisphenol A concentration and growth with significant decrements in length and weight on pairwise comparison at bisphenol A concentrations of 640 and 1280 μg/L. Relative gonad weight was also decreased in males and females at these bisphenol A concentrations. Plasma vitellogenin was increased in females beginning at bisphenol A concentrations of 640 μg/L and in males beginning at 160 μg/L. A delay in spermatogenesis was suggested by an increase in spermatogonia or spermatocytes and a decrease in spermatozoa in testes beginning at a bisphenol A concentration of 16 μg/L. There were no intersex gonads and no treatment-related changes in ovarian histopathology. The number of eggs spawned per female was lower in the control than the treatment groups and attributed by the authors to an unexplained problem in one of the control tanks. The 1280 μg/L bisphenol A concentration resulted in failure of 7 out of 8 females to produce any eggs. Hatching was impaired in eggs exposed to bisphenol A concentrations of 640 and 1280 μg/L. The authors noted that the bisphenol A concentrations resulting in impairment of somatic growth and

reproductive success were only 7-fold lower than the 96-hr median lethal concentration, and concluded that the reproductive effects may have been the result of sublethal generalized toxicity rather than effects mediated through the endocrine axis.

Strengths/Weaknesses: This study was well-conducted with multiple dose levels and concentrations in the test water were confirmed. "General toxicity" was identified and good histology was used. The conclusions regarding weak estrogenic activity were appropriate at 160 μg/L and higher. Other effects were likely due to general toxicity. A classic dose response was noted.

Utility (Adequacy) for CERHR Evaluation Process: Fish are apparently a sensitive model for assessment of responses to weak estrogenic compounds. Given that this study evaluated a fish species, it is not useful in the evaluation.

Kang et al. (2002), supported by the Japanese Ministry of the Environment, exposed adult (4-month-old) breeding pairs of medaka (*Oryzias latipes*) to bisphenol A (>99% purity) in the water at 0, 1000, or 4000 μg/L for 3 weeks **[culture ware not discussed]**. Bisphenol A concentrations during the exposure period were 78–86% of nominal concentrations. Thirty-two pairs of fish had been selected for exposure during an acclimatization period based on their capacity to spawn daily, with the production of ≥15 eggs/day and 90% fertility. During the exposure period, eggs were collected daily and assessed for fertility. Fertilized eggs collected on the last 3 days of the exposure period were permitted to develop in untreated water, and 60 larvae/group were grown for 60 days after hatching to assess normalcy of development. The parent fish were killed at the end of the treatment period for evaluation of external sex characteristics and for histologic assessment of the gonads. Hepatic vitellogenin was also assessed. Statistical comparisons of egg number were made using ANCOVA with female body weight as a covariate. Fertility, growth endpoints, and hepatic vitellogenin data were analyzed with ANOVA or Kruskal–Wallis test with post-hoc Dunnett or Mann–Whitney *U* test. There were no treatment effects on egg number, fertility, mortality, relative gonad weight, or relative liver weight in the adult fish. Ovarian tissue was found in the testis in some males in all bisphenol A-treated groups, although normal testicular tissue with apparently normal spermatogenesis was also found. Hepatic vitellogenin was increased in male fish in the high-dose group to control female levels. There were no treatment-related alterations in hepatic vitellogenin in female fish. Offspring at 60 days of age did not demonstrate treatment-related alterations in survival, growth, or secondary sex characteristics. The sex ratio was not significantly different in offspring of parents exposed to bisphenol A, although the authors noted that the low-dose group had a numerical deficit of males (41% males compared to 50% in the controls). The authors concluded that although bisphenol A increased hepatic vitellogenin in males and produced an intersex gonad, there were no adverse effects on reproductive capacity or the normalcy of offspring.

Strengths/Weaknesses: This appears to have been a well conducted study. The bisphenol A findings are consistent with the work of others, using sensitive endpoints in fish such as vitellogenin production. Given the nature of the intersex gonad observation, it should be considered as adverse even though the severity was not sufficient to induce decreases in reproductive capacity under the conditions tested.

Utility (Adequacy) for CERHR Evaluation Process: This study indicates that bisphenol A is able to induce vitellogenin in male fish and intersex gonads. This study exhibited classic dose responses in the affected endpoints. Because this study was conducted in fish, it is not useful in the evaluation.

Lahnsteiner et al. (2005), supported by the Austrian Federal Ministry of Agriculture, Forestry, Environment, and Water Management, examined the effects of bisphenol A exposure on reproduction of male and female brown trout (*Salmo trutta f. fario*). Fish were caught and acclimated for 2 weeks before starting the study. Ten males/group and 6 females/group were exposed in a flow-through system to bisphenol A at 0 (DMSO vehicle), 1.75, 2.4, or 5.00 μg/L beginning in the late prespawning period and continuing through the remainder of the spawning season **[No information on purity or culture ware was provided]**. The bisphenol A concentrations selected were said to occur in the Austrian water system. Endpoints examined included time point of spawning, sperm count and motility, ability of sperm to fertilize eggs from non-treated females, and numbers and viability of eggs produced by treated females. Statistical analyses included ANOVA and Tukey *b* post-hoc test.

Throughout the entire spawning period, only 1 male in the high bisphenol A dose group produced semen and it was of low quality as indicated by significantly reduced sperm density, motility rate, swimming velocity, and fertility. In the low- and mid-dose groups, sperm density was significantly reduced in the early spawning period but was not affected in the mid or end part of the spawning period. Additional significant effects observed in the low-dose group included decreased sperm motility in the early spawning period, reduced swimming velocity in the early and middle spawning period, and increased circular motion and decreased linear motion in the middle of the spawning period. In the mid-dose group, sperm motility and swimming velocity were significantly decreased in the early and mid-spawning period, and a significant increase in circular motion and a decrease in linear motion occurred in the mid and late part of the spawning period. The study authors interpreted the sperm effects as representing a 4-week delay in spawning. Fertility of males in the low- and mid-dose group was not affected by bisphenol A treatment. In females, no eggs were produced by fish in the high-dose group. In all other dose groups, there were no significant effects on egg volume, viability, mass, mass increase during hardening, or on numbers of eggs produced by females. However, ovulation was delayed by 2 weeks in the low-dose group and by 3 weeks in the mid-dose group. The study authors concluded that exposure of trout to bisphenol A resulted in negative effects on semen and egg quality.

Strengths/Weaknesses: In this study of fish, alterations in sperm motility were observed consistent with those observed in mice. Fertility effects in the female were also similar to those observed in other species. Weaknesses include a failure to determine the actual bisphenol A concentrations in the test system, the narrow dose range examined (1.75 to 5 μg/L), and the small number of fish/dose level assessed.

Table 101
Summary of High Utility Reproductive Toxicity Studies (Single and Multiple Dose Levels)

Model and treatment (doses in mg/kg bw/day)	Endpoint	Bisphenol A dose level (mg/kg bw/day)						Reference
		NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	
Female								
Sprague–Dawley rat (fed during pregnancy and lactation)	↓Duration of licking/grooming pups		0.04 (single dose)	Single dose study	Della Seta et al. (2005)			
CD rat (gavage, 40, 200, or 600/1000 × 28 days)	Altered estrous cycle	Unclear	≤1000/600	Data presentation does not permit modeling	Yamasaki et al. (2002a)			
Male								
Sprague–Dawley rat (gavaged, 0.020, 0.200, 2, 20, or 200 × 6 days)	No effect on daily sperm production, sperm count or reproductive organ weight	200	>200					Ashby et al. (2003)
F344 rats (drinking water with 0.011, 0.116, 1.094 or 11.846 × 13 weeks)	No adverse effects reported	11.846	-					Kim et al. (2002b)
C57BL/6N mouse (gavaged with 0.002, 0.020, or 0.200 × 6 days)	No effect on reproductive organ weight or epididymal sperm count	≥0.200	-					Nagao et al. (2002)
F344 rat (diet 235, 466, 950)	Histologic alterations in testis	<235	235	No dose response	Takahashi and Oishi (2001)			
CD rat gavaged with 40, 200, or 600/1000 × 28 days	↓ Relative ventral prostate weight	200	600/1000	Data presentation does not permit modeling	Yamasaki et al. (2002a)			
	↑ Relative testis weight	200	600/1000	Data presentation does not permit modeling				

Multigeneration								
CD rat (gavaged with 0.0002, 0.002, 0.020, or 0.200 before mating and × 2 generations)	No significant or dose-related reproductive effects	≥0.200	-					Ema et al. (2001)
Sprague–Dawley rat (dietary with ~0.0009, 0.018, 0.27, 4.5, 45, or 450 (male) and ~0.001, 0.02, 0.3, 5, 50, or 500 (female) × 3 filial generations)	↓ F1 epididymal sperm concentration	47.5a	475	317	216	700	469	Tyl et al. (2000b, 2002b)
	↓ F_3 daily sperm production	47.5	475	469	255	524	481	
	↓ Live pups/litter[b]	47.5	475	236	174	376	286	
	↓ Pup body weight[b]	47.5	475	183	163	177	153	
	Advanced vaginal opening[b]	47.5	475	394	343	206	176	
	Advanced F_1 preputial separation	4.75	47.5	466	411	188	163	
CD-1 mouse [F0 diet with ~840 or 1669 (male) and ~1055 or 1988 (female)]	↓ No. of live pups	840/1055	1669/1988	1116	727	1925	1189	Tyl et al. (2002a)
	↓ Female pup body weight (trend test)			2281	1728	2332	1733	
CD-1 mouse (diet with ~0.003, 0.03, 0.3, 5, 50, or 600 from 6 weeks of age × 2 filial generations)	↓ F_0 epididymal sperm concentration	50	600	567	319	607	549	Tyl et al. (2006)
	↑ Gestation length[b]	50	600	3619	639	1144	599	
	↓ Relative testis weight[b]	50	600	562	339	905	605	
	↓ Seminal vesicle weight relative to brain weight, F_2	30	50	405	223	1914	615	
Reproductive assessment by continuous breeding								
CD-1 mouse (diet with ~437.5, 875, or 1750 over 14-week continuous breeding period)	↓ Litters/breeding pair	437.5	875	1750	1295	1680	1155	NTP (1985a)

[a]Dose levels expressed as a mean of the estimated male-female target dose levels.
[b]Benchmark doses are shown for the generation with the lowest values.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

Table 102
Summary of Limited Utility Reproductive Toxicity Studies (Single and Multiple Dose Levels)

Model & treatment (doses in mg/kg bw/day)	Endpoint	Bisphenol A dose level (mg/kg bw/day) NOAEL	LOAEL	BMD_{10}	$BMDL_{10}$	BMD_{1SD}	$BMDL_{1SD}$	Reference
Female								
Wistar rat (ovariectomized; s.c. dosed with ~40 × 1 day)	Altered progesterone receptor mRNA in different brain regions		~40 (single dose)	Single dose study				Funabashi et al. (2001, 2004b)
Wistar rat (ovariectomized; s.c. dosed with ~0.004, 0.04, 0.4, or 4 s.c., single dose)	↑ Progesterone receptor in brain regions	0.04	0.4	Data				presentation does not permit modeling
			Funabashi et al. (2003)					
Wistar rat (ovariectomized; s.c. dosed with 11, 78, 128, or 250 × 7 days)	↓ Body weight and body weight gain		250					Goloubkova et al. (2000)
	↑ Blotted uterine weight (compared to ovariectomized controls)		11 (low dose)					
	↑ Pituitary weight (compared to ovariectomized controls)		128					
	↑ Serum prolactin (compared to ovariectomized controls)		128					
	↑ Prolactin immunopositive cells in anterior pituitary		250					
ICR mice, i.p. every 3 days over 2 wks	No effect on body weight, uterine or ovarian histology, or hematological endpoints	5	> 5					Park et al. (2004)
	↓ Blood urea nitrogen		0.05 (low dose)					
	↓ Ovarian weight (right)		0.5					
	↓ Ovarian weight (left)		0.5 only					
Mongolian gerbil, fed 0.002 or 0.02 from Days 1–21 of cohabitation	↑Social sniffing	< 0.002	0.002	No dose response				Razzoli et al. (2005)
Sprague–Dawley (pseudopregnant; s.c.)	↑ Uterine wet weight and protein content on Days 1–4 with ~60% ↓ on Days 5–8		20 (single dose)	Single dose study				Spencer et al. (2002)
Male								
Long–Evans rat Leydig cells (cell culture)	↓ Testosterone production; various effects on mechanistic endpoints (e.g., LH-stimulated and basal testosterone production, mRNA expression)		0.0023 μg/L	No dose response				Akingbemi et al. (2004)
Swiss mouse, gavaged with 0.005, 0.025, and 0.1 × 30 days	↓ Body weight		0.005					Al-Hiyasat et al. (2002)
	↑ Relative testis weight[b]	0.005	0.025					
	↓ Seminal vesicle weight[b]	0.005	0.025					
Wistar rat, gavage for 45 days with 0.0002, 0.002, or 0.02	↓ Relative testis weight		0.0002	0.056	0.021	0.014	0.0087	Chitra et al. (2003a)

	↓ Relative epididymis weight		0.0002	0.011	0.0082	0.0069	0.0050	
	↓ Relative ventral prostate weight		0.0002	0.014	0.0083	0.015	0.0089	
ICR mice, i.p. every 3 days over 2 weeks	↓ Sperm concentration	0.5	5.0					Park et al. (2004)
	↑ Sperm abnormalities	0.5	5.0					
Sprague–Dawley rat, gavaged with 0.020, 0.200, 2, 20, or 200 × 6 days	↓ Daily sperm production (absolute and per g testis)	<0.020	0.020	No dose response				Sakaue et al. (2001)
Sprague–Dawley rat, gavaged with 0.000002, 0.00002, 0.0002, 0.002, 0.020, 0.200, or 2 × 6 days	↓ Daily sperm production (absolute and per g testis)	0.002	0.020	Data				presentation does not permit modeling.
			Sakaue et al. (2001)					
Wistar or Holtzman SD rat (diet)	No effect on reproductive organ histopathology, daily sperm production, epididymal sperm reserves, or serum testosterone			Single dose study				Takahashi and Oishi (2003)
Wistar rat (s.c.)	↓ Terminal body weight, absolute and relative reproductive organ weight; altered testicular histopathology		~200 (single dose)	Single dose study				Takahashi and Oishi (2003)
CD-1 (ICR) mouse (diet)	↑ Absolute testis weight, ↓absolute epididymis weight; no effect on testis histopathology, epididymal sperm reserves, daily sperm production, or serum testosterone		~400 (single dose)	Single dose study				Takahashi and Oishi (2003)
C57BL/6CrSlc mouse (diet)	No effect on reproductive organ weights; no effect on testis histopathology, epididymal sperm reserves, daily sperm production, or serum testosterone	~400 (single dose)		Single dose study				Takahashi and Oishi (2003)
Wistar rat, 2 or 20 i.p. 4 days/week × 1 month	↓ ventral prostate weight	2	20	7	5	9	6	Takahashi and Oishi (2003)
	↓ Serum testosterone	2	20	3	2	16	9	
	↓ Preputial gland relative weight	<235	235	124	86	171	114	
Reproductive assessment by continuous breeding								
CD-1 mouse, ~2.4, 4.2, or 8.1 over 18-week continuous breeding period, s.c. implant	No adverse effects on fertility	≤8.1						NTP (1984); Morrissey et al., (1989)

[a]Dose levels expressed as a mean of the estimated male-female target dose levels.
[b]Benchmark doses are shown for the generation with the lowest values.
↑,↓ Statistically significant increase, decrease compared to controls; ↔ no statistically significant effects compared to controls.

Table 103
Summary of Blood LH and Testosterone Changes in Experimental Animal Studies

Endpoints/protocol	LH effects[a]	Testosterone effects[a]	Reference
High Utility			
Experimental animal studies with oral exposure			
Adult male and female rats gavaged for 28 days	↔ at 40–1000 mg/kg bw/day	↔ at 40–1000 mg/kg bw/day	Yamasaki et al. (2002a)
4-week-old male rats fed bisphenol A in diet for 44 or 60 days	Not examined	↔ at 235–950 mg/kg bw/day or 200 mg/kg bw/day	Takahashi and Oishi (2001, 2003)
Multiple generation gavage dosing study in rats	↓ in F_0 adult females at 0.0002, 0.002, and 0.020 mg/kg bw/day but not at high dose (0.2 mg/kg bw/day); not considered treatment-related.	↔	Ema et al. (2001)
Experimental animal studies with parenteral exposure			
Female lambs i.m. injected at 4–11 weeks of age; ovariectomy at 9 weeks of age	↔ on blood levels during treatment; ↓ pulsatile secretion after treatment with 3.5 mg/kg bw biweekly	Not examined	Evans et al. (2004)
Limited utility			
Experimental animal studies with oral exposure			
Male rats gavaged from PND 21–35	↓ at 0.0024 mg/kg bw/day but ↔ at higher doses (0.010–200 mg/kg bw/day)	↓ at 0.0024 mg/kg bw/day but ↔ at higher doses (0.010–200 mg/kg bw/day)	Akingbemi et al. (2004)
Male rats gavaged from PND 21–90	↑ at 0.0024 mg/kg bw/day	↔ at 0.0024 mg/kg bw/day	Akingbemi et al. (2004)
4-week-old mice fed bisphenol A through diet for 2 months	Not examined	↔ at 400 mg/kg bw/day	Takahashi and Oishi (2003)
Experimental animal studies with parenteral exposure			
4-week-old male rats s.c. dosed on 4 days/week for 1 month	Not examined	↔ at 200 mg/kg bw	Takahashi and Oishi (2003)
4-week-old male rats i.p. injected for 1 month	Not examined	↓ at 20 mg/kg bw	Takahashi and Oishi (2003)

[a]Unless otherwise stated, animals were examined immediately after the treatment period.
↑,↓ Statistically significant increase/decrease compared to controls; ↔ no statistically significant effects compared to controls.

Utility (Adequacy) for CERHR Evaluation Process: This study suggests that fish are sensitive to bisphenol A-induced abnormalities in reproductive endpoints. Because this study was conducted in fish, it is not useful in the evaluation.

Ortiz-Zarragoitia and Cajaraville (2006), supported by the European Commission, examined the effects of bisphenol A exposure on the reproductive and digestive systems of adult blue mussels. For a period of 3 weeks, mussels were exposed to bisphenol A in acetone vehicle at 0 or 50 ppb **[μg/L] [no information on purity or culture ware was provided]**. Additional compounds were also tested but will not be discussed. Ten mussels/sex/group were examined at the end of the exposure period. The digestive gland was examined for volume of peroxisomes and peroxisomal proliferation. Gonads were histologically evaluated and assessed for alkali-labile phosphate level, a vitellogenin-like protein that is a possible biomarker of endocrine disruption. Statistical analyses included ANOVA followed by Duncan post-hoc test, Kruskall–Wallis, and Mann–Whitney *U* test. Bisphenol A had no effect on gonadal development, gonadal alkali-labile phosphate levels, or digestive gland peroxisomal proliferation or peroxisomal volume. However, observations of follicular brown cell aggregates and gonadal hemocyte infiltration in 35% of male and female mussels indicated severe gamete resorption.

Strengths/Weaknesses: This study evaluated bisphenol A-induced alterations in several reproductive endpoints in adult mussels. Severe gamete resorption was observed. Weaknesses include the failure to confirm bisphenol A concentrations in the test water and the use of only 1 concentration.

Utility (Adequacy) for CERHR Evaluation Process: Because this study was conducted in the mussel, it is not useful in the evaluation.

4.3 Utility of Reproductive Toxicity Data

4.3.1 Human. One high utility study of 42 men occupationally exposed to bisphenol A diglycidyl ether and 42 unexposed men evaluated the relationship

Table 104
Estimates of U.S. General Population Intake of Bisphenol A

Exposure source	Population	BPA mg/kg bw/day	Notes	Source
Formula	Infant	0.001	Assumes 4.5 kg bw, 700 ml formula at 6 μg/L BPA (U.S. canned formula max)	Expert Panel
Breast milk	Infant	0.001	Assumes 4.5 kg bw, 700 ml at 6.3 μg/L (U.S. breast milk max)	Expert Panel
Food	Infant (0–4 months old)	0.0016	European Commission	Table 11 Table 14
	Infant (6–12 months old)	0.0008–0.00165		
	Child (4–6 years old)	0.0012	European Commission	Table 11 Table 14
	Adult	0.00037 (canned food) 0.00048 (canned food + wine)	European Commission	Table 11 Table 14
Aggregate	Child (1.5–5 years old)	0.00004-0.00007	Max = 0.00007–0.00157 Assumes 50% absorption	Wilson et al. (2003, 2006)
Estimates based on urinary metabolites				
Aggregate	Child	0.00007	U.S. 6–8-year-old girls (max = 0.00217)	Table 15
	Adult	0.000026	U.S. population 95th percentile 0.0.00159	Table 15

between urinary levels of bisphenol A and plasma LH, FSH, and free testosterone, found reduced FSH levels among the exposed men. No fertility endpoints were evaluated. Three studies were considered to have low utility in the evaluation process due to limitations in design and analysis but suggest directions for future research. Two of these studies measured serum bisphenol A in healthy women, women with polycystic ovary syndrome and healthy men and evaluated correlations with serum gonadotropins, prolactin, testosterone, and other androgens. No fertility endpoints were included in these studies. The third study of 37 women found significantly lower bisphenol A concentrations among women with endometrial cancer and complex endometrial hyperplasia compared to healthy women and women with simple hyperplasia.

4.3.2 Experimental animal. Female reproductive toxicity testing using multiple dose levels has been evaluated in 2 rat, 1 mouse, and 1 gerbil study. Endpoints affected in these studies included brain progesterone receptor, estrous cyclicity, resorptions, and social sniffing. Male reproductive toxicity testing using multiple dose levels has been evaluated in 7 rat and 2 mouse studies. Affected endpoints in males included reproductive organ weight and histology, serum testosterone, daily sperm production, sperm motility, sperm concentration, percent pregnant females after mating, and females with resorptions after mating. There are 4 multigeneration tests, 2 in rats and 2 in mice, involving gavage or dietary treatments with bisphenol A with dose levels as low as 0.0009 mg/kg bw/day. There are also 2 reproductive assessments by continuous breeding, 1 of which involved subcutaneous implants for bisphenol A delivery and 1 of which used dietary administration in which the lowest dose level was ~437.5 mg/kg bw/day.

4.4 Summary of Reproductive Toxicity Data

The hypothesis has been advanced that the Charles River Sprague-Dawley (SD) rat is insensitive to estrogens and other EDCs and therefore it should not be used for developmental EDC studies and the studies of the effects of BPA that used this strain should be discounted. In order to address this important issue Expert Panel members reviewed the literature on estrogen-sensitivity among rat strains and the following is a summary of our findings.

Different strains of rats show clear, robust reproducible differences in responses to potent estrogens and antiandrogens. Several traits have been shown to be estrogen sensitive in rats including prolactin regulation in the pituitary, thymic involution, uterine pyometra, and liver carcinogenesis to name a few. It is evident that the SD rat and other rat strains are less sensitive to the effects of estrogens than the F344 rat. However, for some traits, the reverse is true. In addition, while the SD was less sensitive than the F344 to estrogen, the reverse was true for sensitivity to tamoxifen.

The sensitivity to estrogens has been mapped to specific chromosomes for several traits. In no case has it been demonstrated that the SD strain is completely insensitive to any known estrogen. It is evident that different traits map to different chromosomes and the degree of estrogen sensitivity varies from tissue to tissue, likely depending on the tissue-specific gene regulated by ER on the chromosome.

Therefore, one cannot conclude that the SD is insensitive to estrogens and the results of BPA studies with BPA should be ignored. In fact, there are several studies reporting low dose effects that used the SD rat. A comparison of the uterotrophic data from the OECD study with EE, BPA, and other estrogens does not indicate that the SD rat is less sensitive to any estrogen versus the Wistar. In this study, oral EE at 1 μg/kg/day for 3 days stimulated uterine weight whereas 0.3 μg/kg/day was uterotrophic when administered s.c. In addition, in the pubertal female rat assay, EE, the antiestrogen tamoxifen and the estrogenic pesticide methoxychlor produced equivalent responses in the Long–Evans and SD female rats.

Although some have hypothesized that the Crl: CD (SD) rat is more insensitive to estrogens than SD rats

from other suppliers, there are no data supporting this assertion.

4.4.1 Human. Human reproductive studies are summarized in Table 100. A study of 42 men occupationally exposed to an epoxy hardening agent containing bisphenol A diglycidyl ether found higher urinary bisphenol A concentrations, corrected for creatinine, than were found in 42 men who worked in the same factory but did not have known exposure to the hardening agent (Hanaoka et al., 2002). Differences were not detected between the worker groups in plasma testosterone or LH, but plasma FSH was significantly lower in exposed workers **[BPA: 0.043 μg/kg bw]** than in workers not exposed to the hardening agent **[BPA: 0.021 μg/kg bw]**. A significant correlation was noted between total urinary bisphenol A concentration and decreased FSH when adjusted for age and alcohol intake ($r = 0.23$, $P = 0.045$).

Two studies from Takeuchi and Tsutsumi (2002) and Takeuchi et al. (2004a) suggested a relationship between serum bisphenol A concentration and serum testosterone (total and free). The first study (Takeuchi and Tsutsumi, 2002) included women with and without polycystic ovary syndrome (POS), and healthy men. Statistically significant positive correlations were observed for women with and without POS (0.559 for total testosterone and 0.598 for free testosterone, $P < 0.01$), and with all participants (0.595 and 0.609, respectively, $P < 0.001$). The second study (Takeuchi et al., 2004a) reported only cycling women with and without obesity and women with POC, with and without obesity, hyperprolactinemia and hypothalamic amenorrhea. Statistically significant positive correlations were found for bisphenol A and total testosterone ($r = 0.391$, $P < 0.001$), free testosterone ($r = 0.504$, $P < 0.001$), androstenedione ($r = 0.684$, $P < 0.001$), and dehydroepiandrosterone sulfate (DHEAS, $r = 0.514$, $P < 0.001$). Although these studies used ELISA, which may overestimate bisphenol A compared to HPLC, significant correlations between bisphenol A levels and higher serum testosterone levels were found. The authors speculated that androgens either may affect bisphenol A metabolism or the reverse.

A study of 37 women found differences in bisphenol A concentrations by health status. Significantly lower mean bisphenol A concentrations were found among women with endometrial cancer (1.4 ng/ml, $n = 7$) and complex endometrial hyperplasia (1.4 ng/ml, $n = 9$) compared to healthy women (2.5 ng/ml, $n = 11$) and women with simple hyperplasia (2.9 ng/ml, $n = 10$) (Hiroi, 2004).

4.4.2 Experimental Animal.

Reproductive toxicity studies of high and limited utility are summarized in Table 101 and Table 102 respectively.(single and multiple dose level studies in the same utility category are combined within a table). Based on reproductive studies using a single dose level, the lowest dose level at which an effect was seen in these studies was 0.04 mg/kg/day fed to female rats during pregnancy and lactation and resulting in a decreased duration of licking/grooming pups (Della Seta et al., 2005). This study of neural and behavioral effects is shown here for convenience but has been included with other studies focused on these endpoints for further discussion in Section 3.

For high utility female reproductive studies using multiple doses, the lowest effect level, for altered estrous cycle, was ≥600 mg/kg bw/day by gavage in rat for 28 days (Yamasaki et al., 2002a). For high utility male reproductive studies, the lowest effect level, for histologic alterations in the testis, was 235 mg/kg bw/day by gavage in rat for 28 days (Takahashi and Oishi, 2001). The value of the histologic observations may be limited due to the fixation and embedding techniques employed, raising some concern over the validity of this endpoint.

The reproductive assessments by continuous breeding included a study using very high-dose levels (NTP, 1985a), and this study is not the most informative for reproductive risk assessment. In a multigeneration study, CD rats did not show statistically significant or dose-related reproductive effects over 2 generations with bisphenol A gavage doses of 0.0002, 0.002, 0.020, or 0.200 mg/kg bw/day (Ema et al., 2001). In Sprague–Dawley rats treated for 3 generations, adverse reproductive effects consisted of decreased F_1 epididymal sperm concentration, decreased F_3 daily sperm production, decreased live pups/litter, decreased pup body weight, and delayed vaginal opening at an average dose level of 475 mg/kg bw/day. Delayed preputial separation was seen in F_1 and F_2 males at an average dose level of 47.5 mg/kg bw/day (Tyl et al., 2000a, 2002b). In CD-1 mice given bisphenol A for 2 generations in the diet at dose levels as low as ~0.003 mg/kg bw/day, the most sensitive effect was a reduction in F_2 seminal vesicle weight relative to brain weight at 50 mg/kg bw/day. Effects on F_0 epididymal sperm concentration, gestation length, and relative testis weight occurred at 600 mg/kg/day, the next highest dose level (Tyl et al., 2006).

A summary of LH and testosterone effects observed in bisphenol A-exposed experimental animals and in humans are included in Table 103.

Data sufficiency statement for human data: In summary, there are insufficient data to evaluate whether bisphenol A causes male or female reproductive toxicity in humans. However, several studies collectively suggest hormonal effects, including one study of exposed male workers likely to have multiple routes of exposure including inhalation (Hanaoka et al., 2002).

Data sufficiency statement for animal data: In summary, the experimental animal literature was assessed for its utility (high utility, limited utility, or no utility) based on the criteria established by this Expert Panel, including an evaluation of experimental design and statistical procedures. Studies with high and limited utility were further grouped according to female and male reproductive toxicity, their use of single or multiple dose levels, a multigenerational exposure paradigm, and the measurement of various hormonal endpoints. Greater weight was given to studies using the oral route of exposure, because of evidence that oral exposure predominates in humans and that target tissue exposure to parent compound (bisphenol A) is very low after oral exposure and first-pass metabolism as compared to subcutaneous or other routes of exposure.

There is sufficient evidence in rats and mice that bisphenol A causes female reproductive toxicity, characterized as delayed vaginal opening with subchronic or chronic oral exposure NOAELs of 47.5 mg/kg bw/day and a LOAEL of 475 mg/kg bw/day (Tyl et al., 2002b).

There is sufficient evidence in rats and mice that bisphenol A causes male reproductive toxicity, characterized as delayed preputial separation, with subchronic or chronic oral NOAEL of 4.75 mg/kg bw/day and a LOAEL of 47.5 mg/kg bw/day (Tyl et al., 2002b).

There is inconsistent evidence in rats and mice that bisphenol A alters testosterone and gonadotropin levels in males after oral postnatal exposure.

There is inconsistent evidence in male and female mice that bisphenol A produces aneugenic effects in germ cells after exposure.

5.0 SUMMARIES, CONCLUSIONS, AND CRITICAL DATA NEEDS

5.1 Developmental Toxicity

No data on the effects of human developmental exposure to bisphenol A are available. There is a large literature describing studies in rodents and some work in other species. A large experimental animal literature was reviewed, assessed for its utility, and weighed based on the criteria established by this Panel.

From the rodent studies we can conclude that bisphenol A:

- Does not cause malformations or birth defects in rats or mice at levels up to the highest doses evaluated: 640 mg/kg/day (rats) and 1250 mg/kg/day (mice).
- Does not alter male or female fertility after gestational exposure up to doses of 450 mg/kg bw/day in the rat and 600 mg/kg bw/day in the mouse (highest dose levels evaluated).
- Does not permanently affect prostate weight at doses up to 475 mg/kg/day in adult rats or 600 mg/kg/day in mice.
- Does not cause prostate cancer in rats or mice after adult exposure at up to 148 or 600 mg/kg/day, respectively.
- Does change the age of puberty in male or female rats at high doses (ca. 475 mg/kg/day).

Rodent studies suggest that bisphenol A:

- Causes neural and behavioral alterations related to disruptions in normal sex differences in rats and mice. (0.01–0.2 mg/kg/day).

The data on bisphenol A are insufficient to reach a firm conclusion about:

- A change in the onset of puberty in male rats or mice at doses up to 475–600 mg/kg/day.
- An acceleration in the age of onset of puberty at a low dose in female mice at 0.0024 mg/kg/day, the only dose tested.
- Whether Bisphenol A predisposes rats toward prostate cancer or mice toward urinary tract deformations.

5.2 Reproductive Toxicity

There are insufficient data to evaluate whether bisphenol A causes male or female reproductive toxicity in humans. A large experimental animal literature was reviewed, assessed for its utility, and weighted based on the criteria established by this expert panel, including an evaluation of experimental design and statistical procedures. These animal data are assumed relevant for the assessment of human hazard.

Female effects: There is sufficient evidence in rats and mice that bisphenol A causes female reproductive toxicity with subchronic or chronic oral exposures with a NOAEL of 47.5 mg/kg bw/day and a LOAEL of $\geq$475 mg/kg bw/day.

Male effects: There is sufficient evidence in rats and mice that bisphenol A causes male reproductive toxicity with subchronic or chronic oral exposures with a NOAEL of 4.75 mg/kg bw/day and a LOAEL of $\geq$47.5 mg/kg bw/day.

5.3 Human Exposures

Bisphenol A is FDA-approved for use in polycarbonate and epoxy resins that are used in consumer products such as food containers (e.g., milk, water, and infant bottles) food can linings (Staples et al., 1998; SRI, 2004) and in dental materials (FDA, 2006). Resins, polycarbonate plastics, and other products manufactured from bisphenol A can contain trace amounts of residual monomer and additional monomer may be generated during breakdown of the polymer (European-Union, 2003).

Environmental Exposures: Bisphenol A emitted from manufacturing operations is unlikely to be present in the atmosphere in high concentrations. However, it was found in 31–44% of outdoor air samples with concentrations of <LOD (0.9) to 51.5 ng/m^3 (Wilson et al., 2006). Indoor air samples found concentrations $\leq$29 ng/m^3 (Rudel et al., 2001, 2003; Wilson et al., 2003). Limited U.S. surface water sampling found bisphenol A in 0–41% of samples ranging from <0.1 to 12 ug/L (Kolpin et al., 2002; Boyd et al., 2003). Twenty-five to 100% of indoor dust samples contained bisphenol A with concentrations of <detectable to 17.6 μg/g (Rudel et al., 2001, 2003; Wilson et al., 2003, 2006).

Exposures Through Food: The highest potential for human exposure to bisphenol A is through products that directly contact food such as food and beverage containers with internal epoxy resin coatings and through the use of polycarbonate tableware and bottles, such as those used to feed infants (European-Union, 2003). Studies examining the extraction of bisphenol A from polycarbonate infant bottles in the U.S. found concentrations <5 μg/L. Canned infant formulas in the U.S. had a maximum levels of 13 μg/L in the concentrate that produced a maximum of 6.6 μg/L when mixed with water (FDA, 1996; Biles et al., 1997a). Breast milk studies in the U.S. have found up to 6.3 μg/L free bisphenol A in samples (Ye et al., 2006). Measured bisphenol A concentrations in canned foods in the U.S are <39 μg/kg (FDA, 1996; Wilson et al., 2006). Limited drinking water sampling in the U.S. indicates that bisphenol A concentrations were all below the limit of detection (<0.1 ng/L) (Boyd et al., 2003).

Biological Measures of Bisphenol A in Humans: The panel finds the greatest utility in studies of biological samples that use sensitive and specific analytical methods (LC-MS or GC-MS) and report quality control measures for sample handling and analysis. The panel further focused on biological monitoring done in

U.S. populations. In the U.S, adult urine concentrations of free bisphenol A are <0.6 μg/L and total bisphenol A concentrations are <19.8 μg/L (Calafat et al., 2005; Liu et al., 2005; Ye et al., 2005). The 95th percentile total bisphenol A concentration for 394 adult volunteers (males and females; 20–59 years old) from the NHANES III survey was 5.18 μg/L (Calafat et al., 2005). Girls age 6–9 in the U.S. have concentrations of total bisphenol A <54.3 μg/L, with median concentrations ranging from 1.8–2.4 μg/L (Liu et al., 2005; Wolff et al., 2006). No U.S. studies have examined blood or semen concentrations of bisphenol A. Amniotic fluid total bisphenol A concentrations in the U.S are <1.96 μg/L. Dental sealant exposure to bisphenol A occurs primarily with use of the dental sealant bisphenol A dimethylacylate. This exposure is considered an acute and infrequent event with little relevance to estimating general population exposures.

Bisphenol A Intake Estimates: The panel found that previous oral intake estimates for infants fed formula and breast milk did not use levels reported for the U.S. population, so the panel estimated intake based on typically-used parameters. The panel found the food intake estimates made by the European Commission (2002) used concentrations of bisphenol A comparable to U.S. food concentrations in their intake estimates, so have included these estimates as well (Table 104). Estimates from duplicate diets in U.S. children (Wilson et al., 2003, 2006) found lower bisphenol A concentrations in foods than those estimated by the European Commission, therefore the aggregate estimates of intake by Wilson et al. were somewhat lower than those estimated by the European Commission. However, the aggregate intake estimates by Wilson et al. (2003, 2006) are in line with the estimates based on urinary metabolite measurements for children described above.

Estimates of intake based on occupational air concentrations of bisphenol A from U.S. powder paint workers suggest exposures up to 100 ug/kg bw/day (USEPA, 1988). Estimates of intake based on urinary metabolite levels among Japanese workers spraying epoxy coatings resulted in a mean estimate of exposure of 0.043 μg/kg bw/day (<0.002 pg to 0.45 μg/kg bw/day) (Hanaoka et al., 2002).

5.4 Overall Conclusions

The panel spent a considerable amount of time attempting to interpret and understand the inconsistent findings reported in the ''low dose'' literature for bisphenol A. Conducting low dose studies can be challenging because the effects may be subtle and small in magnitude and therefore more difficult to statistically distinguish from background variability. The inherent challenge of conducting these types of studies may be exacerbated with bisphenol A because the endpoints of concern are endocrine-mediated and potentially impacted by factors that include phytoestrogen content of the animal feed, extent of bisphenol A exposure from caging or water bottles, and the alleged sensitivity of the animal model to estrogens. The Panel believed that high-dose studies are less susceptible to these types of influences because the toxicologic response should be more robust and less variable. While the Panel did not necessarily expect a specific effect to display a monotonic dose response (e.g., consistently increasing organ size), many members of the panel expected the high-dose studies with bisphenol A to detect *some* manifestation of toxicity (e.g., altered weight, histopathology) in tissues reported to be affected at low doses even if the study could not replicate the reported low dose effect. There are several large, robust, well designed studies with multiple dose groups using several strains of rats and mice and none of these detected any adverse reproductive effects at low to moderate dosage levels of BPA administered via the relevant route of human exposures. Further, none of these studies detected changes in prostate weight, age at puberty (rat), pathology or tumors in any tissue, or reproductive tract malformations. For this reason, Panel members gave more weight to studies that evaluated both low- and high-doses of bisphenol A compared to low-dose-only studies in cases where the target tissues were comparably assessed.

Every chemical that produces low dose cellular and molecular alterations of endocrine function also produces a cascade of effects increasing in severity resulting in clearly adverse alterations at higher doses, albeit the effects can be different from those seen at low doses. With these endocrine disrupters, but not BPA, the low dose effects are often causally linked to the high-dose adverse effects of the chemical. This is true for androgens like testosterone and trenbolone, estrogens like DES, 17β-estradiol and ethinyl estradiol, xenoestrogens like methoxychlor and genistein, and antiandrogens like vinclozolin, for example. Hence, the failure of BPA to produce reproducible adverse effects via a relevant route of exposure, coupled with the lack of robustness of the many of the low dose studies (sample size, dose range, statistical analyses and experimental design, GLP) and the inability to reproduce many of these effects of any adverse effect strains the credibility of some of these study results. They need to be replicated using appropriate routes of exposures, adequate experimental designs and statistical analyses and linked to higher dose adverse effects if they are to elevate our concerns about the effects of BPA on human health. The lack of reproducibility of the low dose effects, the absence of toxicity in those low-dose-affected tissues at high-doses, and the uncertain adversity of the reported effects led the panel to express ''minimal'' concern for reproductive effects.

In contrast, the literature on bisphenol A effects on neural and behavioral response is more consistent with respect to the number of ''positive'' studies although it should be noted that the high-dose studies that proved to be the most useful for evaluating reproductive effects did not adequately assess neural and behavioral responses. In addition, even though different investigators assessed different neural and behavioral endpoints, the Panel concluded that the overall findings suggest that bisphenol A may be associated with neural changes in the brain and behavioral alterations related to sexual dimorphism in rodents. For this reason, the Panel expressed ''some'' concern for these effects even though it is not clear the reported effects constitute an adverse toxicological response.

Concerns are expressed relative to current estimates of general population exposure levels in the U.S.

1. *For pregnant women and fetuses*, the Expert Panel has different levels of concern for the different

developmental endpoints that may be susceptible to bisphenol A disruption, as follows:

- For neural and behavioral effects, the Expert Panel has some concern;
- For prostate effects, the Expert Panel has minimal concern;
- For the potential effect of accelerated puberty, the Expert Panel has minimal concern; and
- For birth defects and malformations, the Expert Panel has negligible concern.

2. *For infants and children*, the Expert Panel has the following levels of concern for biological processes that might be altered by Bisphenol A, as follows:

   - Some concern for neural and behavioral effects; and
   - Minimal concern for the effect of accelerated puberty.

3. *For adults*, the Expert Panel has negligible concern for adverse reproductive effects following exposures in the general population to Bisphenol A. For highly exposed subgroups, such as occupationally exposed populations, the level of concern is elevated to minimal.

5.5 Critical Data Needs

1. *Neural and behavioral endpoints.* A concerted effort is needed to better understand the effects of gestational and lactational exposure to bisphenol A on maternal behavior and offspring brain structure and behavior. This effort should include molecular and cellular studies to ascertain the sensitivity of the developing brain to bisphenol A-induced structural and biochemical alterations. The association between bisphenol A and neural and behavioral endpoints should also be examined in longitudinal studies of pregnancy and child development in humans.
2. *Human exposure assessment.* Additional data are needed to clarify bisphenol A exposures and internal dosimetry in the general population, newborns, and occupationally-exposed individuals. Available data demonstrate that a large fraction of children and adults have detectable levels of bisphenol A metabolites in their urine. What are needed are duplicate diet studies to identify in detail the sources and routes of exposure of bisphenol A. For example, while research suggests diet is the major source of BPA for U.S. infants and young children, the detailed analysis of BPA levels has primarily focused on polycarbonate baby bottle leachates and canned food. The contributions of non-canned food and drinking water routes of exposure for U.S. youth and adults not occupationally-exposed to BPA remain unknown and in need of further study. Levels of BPA in residential drinking water wells and community water sources have not been systematically studied. Also unknown is the impact of landfill leachates on levels of bisphenol A in U.S. drinking well waters and whether chlorinated congeners of bisphenol A are found in U.S. municipal water supplies. Finally, more measurement are needed of free and total bisphenol A, its glucuronide conjugate, and other metabolite concentrations from maternal, fetal, and neonatal tissues or fluids (i.e., placenta, amniotic fluid, breast milk, urine, serum). These data would provide insight into the roles of metabolism and exposure route on internal dose.
3. Human studies relating adult exposure to reproduction and development, including effects on hormone levels.
4. *Physiologically-based pharmacokinetic (PBPK) models.* PBPK models are needed to facilitate the interpretation and applicability of animal studies, including rodents and nonhuman primates, for human risk assessment.
5. *Effects on prostate and mammary gland development.* Additional data are needed to understand the susceptibility to disruption of prostate and mammary gland development in humans and animals by bisphenol A exposure. Laboratory animal studies should initially focus on the oral route of exposure and should be informed by any new knowledge about human exposure and human internal dosimetry. A particular data need is an improved understanding of the biology of PIN (prostatic intraepithelial neoplasia) in animal models and its relationship to prostate cancer. Similarly, bisphenol A-induced alterations in mammary gland development and their potential relationship to mammary cancer should be investigated across a broad range of internal concentrations and external doses.
6. *Altered puberty.* The robustness and biologic basis for altered puberty following bisphenol A exposure should be evaluated in mouse, rat, and gerbil. In laboratory animals, this evaluation should be performed following combined gestational and lactational exposure, and following pubertal exposure alone, and should include an assessment of any changes in hormonal responsivity at later ages, and all related to internal and tissue concentrations of bisphenol A. In addition, longitudinal cohort studies examining the potential modulation by bisphenol A of the onset, progression, and control of puberty in humans should be performed.
7. *Biological mechanism for low-dose-only effects.* Most useful would be data that provided a biologically-plausible explanation for effects that appear at low doses but not higher doses. This might involve the membrane-bound estrogen receptor and its possible activation by bisphenol A.
8. More work directed toward urinary tract morphological and histologic changes after developmental exposure would be helpful to determine the robustness and relevance of the limited report of these effects in one study.
9. *Inter-laboratory replication of studies.* Inter-laboratory replication of critical findings is a sine qua non for enhancing confidence in experimental results. Such studies should be supported by funding agencies, and should be facilitated by the open sharing of experimental details and approaches. The future reproducibility should also be considered by investigators as they design their studies.

10. *Critical design components for all future research on BPA:*

a. Appropriate experimental design and statistical analysis, especially accounting for litter effects;
b. Appropriate route (oral) of exposure. Studies with non-oral route of administration should include internal dose measurements of free BPA;
c. Multiple dose groups ranging from low to high;
d. Linkage of effects to adverse effects; and
e. Relevant endpoints, with biologically plausible outcomes especially for estrogen-mediated effects on reproduction and behavior.

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APPENDIX III. PUBLIC COMMENTS AND PEER REVIEW REPORT

Public comments received during the NTP-CERHR evaluation of bisphenol A and the peer review report for the draft NTP Brief on Bisphenol A are available on the CERHR website at *http://cerhr.niehs.nih.gov/chemicals/bisphenol/bisphenol.html*.