EVIDENCE ON THE DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF

Phenol

DRAFT

July 2003

Reproductive and Cancer Hazard Assessment Section
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq.*) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that one of the mechanisms by which "a chemical is known to the state to cause cancer or reproductive toxicity [is] if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principles to cause cancer or reproductive toxicity" (Health and Safety Code Section 25249.8(b)). The "state's qualified experts" regarding findings of reproductive toxicity are identified as members of the Developmental and Reproductive Toxicant (DART) Identification Committee of the Office of Environmental Health Hazard Assessment's Science Advisory Board (Title 22, California Code of Regulations, Section 12301 (22 CCR 12301)). The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency.

Phenol was selected for review by the DART Identification Committee because of its assignment of a final priority of "High" under the "Procedure for Prioritizing Candidate Chemicals for Consideration Under Proposition 65 by the State's Qualified Experts", adopted by OEHHA in May, 1997. A notice announcing that prioritization and initiating a 60-day data call-in period was published in the California Regulatory Notice Register on February 14, 2003.

A public meeting of the Committee will be held on October 16, 2003 in Sacramento, California. Following discussion and Committee deliberation, the Committee will determine if phenol has been "clearly shown through scientifically valid testing according to generally accepted principles" to cause reproductive toxicity.

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A. ABSTRACT

Phenol (CAS No. 108-95-2) is a monohydroxy derivative of benzene, with a molecular formula of C_6H_5OH, and a molecular weight of 94.1. It is a high production volume chemical, with a reported 3.72 billion pounds total U.S. production in 1993. In the year 2000, combined environmental releases in the state of California totaled 68,237 pounds. Exposure to phenol can occur in the workplace, from environmental media, from contaminated food or water, or from the use of phenol-containing consumer products.

Phenol is readily absorbed by all routes, and rapidly distributes throughout the body. Following dermal or inhalation exposure, the half-life of phenol in the human body is approximately 3.5 hours. Unchanged phenol and its metabolic products are primarily excreted in the urine.

The minimum reported lethal oral dose in humans was approximately 70 mg/kg-bw. Oral and dermal LD_{50} values for laboratory animals have been reported to range from 300-600 mg/kg-bw, and 670-1400 mg/kg-bw, respectively. Phenol is highly irritating to the skin, eyes, and mucous membranes. Systemic effects in humans include gastrointestinal irritation, dermal necrosis, and cardiac arrhythmias. Lethal concentrations of phenol produce symptoms of muscle weakness, convulsions, and coma. Central or peripheral nervous system toxicity is presumed to be the primary cause of death in cases of acute phenol poisoning.

Chronic and subchronic studies in laboratory animals given phenol-containing drinking water have shown reductions in fluid consumption at phenol concentrations of 2500 and 5000 (chronic), or 10000 ppm (subchronic). In subchronic studies, body weight gain of rats and mice was also reduced at 10000 ppm phenol, but survival, feed consumption, and organ pathology were not affected. In chronic studies, body weight and body weight gain were affected, but there was no evidence for enhanced cancer risk. Two-stage carcinogenicity studies, however, have indicated that phenol has promoting activity.

Two epidemiological investigations found no meaningful associations between workplace exposure to phenol and adverse pregnancy outcome. It is possible, however, that due to the design and limitations of these studies, they were unable to detect an association between phenol and adverse pregnancy outcomes if such an association were present.

Findings of studies using rats and mice exposed to phenol *in utero* have included statistically significant reductions in fetal weight and viability, as well as increased frequencies of malformations. Reduced fetal or birth weight was the most frequently reported effect. In one study using rats, statistically significant decreases in mean fetal weights were observed in the absence of any evidence for treatment-related maternal toxicity. In other rat studies, as well as in phenol-exposed CD-1 mice, decreases in fetal or birth weights were associated with concomitant decreases in maternal weight.

No studies were identified on the potential of phenol to cause reproductive harm in human males.

A two-generation reproductive toxicity study in rats reported significant reductions in feed and water consumption, reductions in mean body weights, and changes in the patterns of body weight gain at a phenol concentration of 5000 ppm in drinking water. No significant changes were noted in fertility or reproductive function, excepting statistically-significant increases in testicular sperm counts and production rates for F1 males of the 5000 ppm group. For this group, the average age at preputial separation was significantly delayed, and body weight at preputial separation was significantly reduced relative to controls. Absolute prostate weights were significantly reduced at all three concentrations of phenol, while relative prostate weight was significantly reduced compared to controls for the 1000 ppm group.

Human data on the potential female reproductive toxicity of phenol includes epidemiological investigations of workplace exposures, and studies of placentas from women considered to have had high environmental exposures. No meaningful associations were found between workplace phenol exposures and adverse pregnancy outcome. It is possible that due to the design and limitations of these studies, they were unable to detect an association between phenol exposure and adverse pregnancy outcomes if an association were indeed present. Placentas from Polish women considered to have had high environmental exposures to phenol showed changes consistent with impairments of placental function.

A two-generation reproductive toxicity study reported consistent reductions in fluid consumption for female rats in the 5000 ppm phenol group. Feed consumption and weight gain for F1 females was only sporadically affected by treatment, although body weights were consistently, and significantly, reduced. Relative, as well as absolute, uterine weights were significantly reduced at all three concentrations of phenol. For F1 females of the 5000 ppm group, the average age at vaginal opening was significantly delayed, and body weight at vaginal opening was significantly reduced relative to controls. No significant, treatment-related changes were noted in fertility or reproductive function. There were no significant differences among groups for live litter size at birth in either generation, although pup viability on postnatal day 4 was significantly decreased for 5000 ppm pups in both the F1 and F2 generations. For animals exposed to 5000 ppm phenol, litter weights were significantly lower than control values for both generations on each of postnatal days 0, 4, 7, 14, and 21.

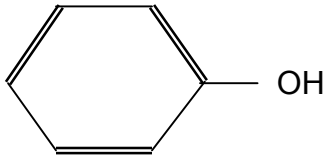
Developmental toxicity studies in rats and mice did not provide evidence for phenol effects on parameters such as implantation frequency. Some evidence was found for increases in resorption and decreases in fetal viability with phenol exposure.

B. INTRODUCTION

B.1. Chemical structure and main physical characteristics

Phenol (CAS No. 108-95-2) is a monohydroxy derivative of benzene, sometimes known as carbolic acid (Bruce et al., 1987). The compound has a molecular formula of C_6H_5OH, and a molecular weight of 94.1. The molecular structure of phenol is shown in Figure 1. Phenol's solubility in water is variable between 0-65° C, but it is highly soluble in most organic solvents.

Figure 1. Structure of Phenol



B.2. Use and exposure information

Phenol is a high production volume chemical. For 1993, total U.S. production of phenol was reported to be 3.72 billion pounds (HSDB, 1997). The housing and construction industries are considered to account for about half of the phenol used in the U.S., with an additional 10-15% attributed to automotive applications. Manufacture of phenolic resins is the largest single use of phenol, reported to be 1.182 billion pounds in 1987 (HSDB, 1997). With 68,237 pounds total environmental releases of phenol reported for the year 2000, Toxic Release Inventory (TRI) data rank California as 28th among U.S. states for releases of this chemical (Scorecard, 2003).

Exposures to phenol can occur in the workplace, from environmental media, from contaminated drinking water or foodstuffs, or from use of consumer products containing phenol (ATSDR, 1998). Phenol has been identified in the exhaust gases of private automobiles, in cigarette smoke, and in emissions from incinerators and wood fires (IPCS, 1994). As a by-product of the smoking process, phenol is also present in smoked meat and fish. Phenol may be formed endogenously from metabolism of other xenobiotics, notably benzene, and by catabolism of protein and other compounds by gut bacteria (McDonald et al., 2001).

Human taste and odor thresholds for phenol are low. A taste threshold of 0.3 mg/l water has been reported in the literature, and values ranging from 0.021 to 20 mg/m^3 air have been reported as an odor threshold (IPCS, 1994).

The U.S., Germany, and The Netherlands, all have an 8-hour time-weighted average occupational exposure limit of 5ppm (19 mg/m^3); all of these countries also advise a skin notation (Dutch Expert Committee, 1996).

U.S. EPA's Integrated Risk Information System (IRIS) has established the chronic oral Reference Dose (RfD) for phenol at 3×10^{-1} mg/kg-day on the basis of reduced maternal weight gain in rats at a Bench Mark Dose Level (BMDL) of 93 mg/kg-day (U.S. EPA, 2002a). The critical study used in deriving this RfD was a developmental toxicity study conducted in rats (Argus, 1997); the maternal NOAEL was 60 mg/kg-day, based on small decreases in maternal body weight gain at 120 mg/kg-day.

B.3. Metabolism and Pharmacokinetics

Phenol is readily absorbed by all routes: oral, inhalation, and dermal (see Bruce et al., 1987; Dutch Expert Committee, 1996; and U.S. EPA, 2002 for reviews). Symptoms of acute toxicity develop within minutes of administration by any route, demonstrating the rapidity with which absorption occurs. Animal experiments have shown that absorbed phenol distributes rapidly throughout the body. Lung, intestine, and liver are the main sites for conjugation of phenol *in vivo*.

In a study reported in abstract form (Gray and Kavlock, 1990), the disposition of orally administered phenol was studied in pregnant rats. Radiolabeled phenol was given at doses of 10, 100, or 1000 mg/kg (100 μCi/kg), and radioactivity measured at time points between 0.5 and 48 hours following treatment. Maternal C^{14} levels were found to peak in both blood and tissues at 0.5 hours post-dosing. With treatment on gestation day 11, levels of C^{14} in embryos and placenta were found to be comparable to the free fraction of C^{14} in maternal serum.

Metabolism of phenol in the body is primarily by Phase II conjugation reactions, secondarily by Phase I oxidative reactions to dihydroxy products, and thirdly by urinary excretion of unchanged phenol (Bruce et al., 1987; IPCS, 1994). There are inter-species differences in the relative importance of these elimination processes, particularly in the nature of the specific conjugates produced. On a percentage basis, urinary metabolite profiles appear to be similar for humans and rats. Rabbits and mice produce an additional metabolite, a sulfated quinol, which was not identified in humans or rats.

In both humans and experimental animals, elimination of phenol and its metabolites appears to be primarily though urinary excretion (Bruce et al., 1987; IPCS, 1994; Dutch Expert Committee, 1996; U.S. EPA, 2002). Only minimal amounts are excreted in feces or exhaled air. Data from humans as well as animals indicate a rapid clearance rate of phenol from the body. Results from controlled dermal and inhalation exposures to phenol correspond to a half-life in the human body of approximately 3.5 hours.

B.4. Non-DART toxicities

Human deaths have occurred following intentional ingestion of phenol, as well as by accidental dermal exposures of large areas of skin (> 25% of total) (ATSDR, 1998). The minimum reported lethal oral dose in humans was 4.8 g, or approximately 70 mg/kg (IPCS, 1994). Death occurred within 10 minutes. For laboratory animals, oral and dermal LD_{50} values have been reported to range from 300-600 mg/kg bw, and 670-1400 mg/kg bw, respectively (IPCS, 1994).

The symptoms of acute phenol toxicity are similar in humans and experimental animals, and are independent of the route of exposure (Bruce et al., 1987). In all cases, lethal concentrations of phenol produce symptoms of muscle weakness, convulsions, and coma. Central or peripheral nervous system toxicity is presumed to be the primary cause of death, although injury to other organ systems may contribute (ATSDR, 1998).

Spiller et al. (1993) published a retrospective review of phenol poisonings reported to a regional poison center. Ninety-six cases were identified, 52 of which were evaluated in a hospital. None of the included patients died. Some form of CNS depression was observed in 11 patients, with coma occurring in 2 patients. Where the timing was known, the onset of CNS effects was reported to have been rapid, occurring within 15 minutes to 1 hour of phenol exposure. No significant correlation was found between CNS depression and the extent of oral/esophageal burns. No seizures, cardiac dysrhythmias, or respiratory depression were noted in this series of cases.

Exposure to phenol by the inhalation or dermal route is highly irritating to skin, eyes, and mucous membranes. Systemic effects of phenol poisoning in humans include gastrointestinal irritation, dermal necrosis, and cardiac arrhythmias (ATSDR, 1998). Lung, myocardial, hepatic, and renal effects have been reported in phenol-exposed experimental animals. Muscle tremors, loss of coordination, paralysis, and convulsions have also been observed in experimental animals exposed to lethal doses of phenol.

Exposure of infants to phenolic disinfectants has been associated with a significantly ($p < 0.005$) increased mean third-day microbilirubin level, and an increase in the proportion of infants with a microbilirubin level of greater than 10 mg/dl (Doan et al., 1979). No cases of severe jaundice were seen in any of the 2,075 infants evaluated in the study. Phenolic disinfectants were used as recommended to clean hospital nursery surfaces; as infants did not come into direct contact with these surfaces, exposure was presumed to have occurred by inhalation of fumes.

A ten-day, nose-only, inhalation toxicity study in Fischer 344 rats did not find evidence of adverse effects (Hoffman et al., 2001). The target concentrations used were: 0, 0.5, 5.0, or 25 ppm phenol in air, 6 hours/day, 5 days/week, for 2 weeks. The authors estimated that the high concentration of 25 ppm was equivalent to an oral dose of approximately 28 mg/kg-day.

Oral subchronic studies in rats and mice demonstrated reductions in water consumption and body weight gain at a concentration of 10,000 ppm phenol in drinking water (NCI, 1980). This concentration was determined to have resulted in doses of 780 mg/kg-day for rats, and 1700 mg/kg-day for mice (U.S. EPA, 1992). Survival and feed consumption were not affected, and no effect on weight gain was seen at lower concentrations of 100-3,000 ppm. Organ weights were not reported, but gross appearance and histopathology of all organs was reported to give no indication of treatment-related effects in either species.

Chronic studies used concentrations of 2,500 and 5,000 ppm phenol in drinking water for both rats and mice (NCI, 1980). As in the subchronic studies, feed consumption and survival rates remained similar among groups within each species. In rats, water consumption was reduced from control levels by 10-20% at both concentrations of phenol, and body weight gain showed significant reductions with exposure to 5,000 ppm phenol. In mice, water consumption was reduced to 75% or 50-60% of control values at 2,500 and 5,000 ppm phenol, respectively. Mean body weights of both treated groups of both sexes were lower than control weights throughout most of the study. Organ weights were not reported for either species, but the types and frequencies of non-neoplastic lesions were considered to be appropriate for ageing animals of the species and strains used.

U.S. EPA has classified phenol in Group D, "not classifiable as to human carcinogenicity" (U.S. EPA, 1992). Although the authors of the NCI bioassay (1980) concluded that under the conditions of their study phenol was not carcinogenic in F344 rats or B6C3F1 mice of either sex, U.S. EPA (1992) considered the data inadequate for determining cancer risk. Two-stage carcinogenicity studies, however, have shown that when applied repeatedly to mouse skin, phenol has promoting activity (IPCS, 1994).

C. DEVELOPMENTAL TOXICITY

C.1. Human studies

An abstract by Hernberg et al. (1983) reports on data obtained from the Finnish Register for Congenital Malformations. Personal interviews of 1,047 Registry mothers were compared to an equal number of referents. Information on maternal occupational exposures to disinfectants during early pregnancy was classified blindly as to the case-referent status of pairs. The case-referent distribution was 51/55 for discordant pairs exposed to surface disinfectants (including phenol). In matched case control studies a discordant pair is one in which the two subjects had different exposures to the risk factor of interest. These are the only pairs that are informative about the association between exposure and disease. Overall, the study did not indicate meaningful associations between maternal exposure to disinfectants and the occurrence of congenital defects.

Phenol was among the chemical exposures included in a retrospective study of pregnancy outcome among university laboratory employees (Axelsson, et al., 1984). The population was divided into those with and those without occupational exposure to organic solvents. With exposure to organic solvents during laboratory work (576 pregnancies), there was a slight, but nonsignificant increase in the rate of miscarriage as compared to nonexposed pregnancies (576 pregnancies). The relative risk (RR) was 1.31, with a 95% confidence interval of 0.89 - 1.91. There were no differences between groups in perinatal death rates, or in the prevalence of malformations. Exposure to phenol was specifically reported in only five cases, all of which were normal deliveries.

C.2. Developmental toxicity in animals

While there are not large numbers of published papers on the developmental toxicity of phenol in experimental animals, phenol has been the subject of regulatory developmental toxicity studies using rats and mice (RTI, 1983 a and b; Argus 1997). In addition, phenol has been evaluated in *in vivo* studies using specialized protocols (IIT Research Institute, 1999; Ryan et al., 2001; Bishop et al., 1997; IRDC, 1993; Kavlock, 1990; Narotsky and Kavlock, 1995; Minor and Becker, 1971). *In vitro* studies of phenol have used whole embryo, cell culture, or developing *Xenopus* to examine the potential developmental toxicity of phenol and related compounds (Kavlock et al., 1991 a and b; Oglesby et al., 1988 and 1992; Fischer et al., 1993; Chapman et al., 1994; Mayura et al., 1991; Zhao et al., 1995; Dumpert, 1987). Information relevant to the potential for male-mediated developmental toxicity or transplacental mutagenicity of phenol is provided by the papers of Olshan and Faustman (1993) and Ciranni et al. (1988).

C.2.1. Studies conducted by the Research Triangle Institute (RTI), on behalf of the National Center for Toxicological Research (NCTR)

C.2.1.1. Rats

C.2.1.1.1. Pilot studies (RTI, 1983a)

A series of substudies were performed in order to set appropriate dose and dose-volume levels for the full-scale study. The study used Sprague-Dawley rats supplied by Charles River Laboratories, Inc., and referred in the document as “CD rats.” In the first substudy, doses of 0, 125, 160, 200, and 250 mg/kg-day were administered to timed-pregnant females in a gavage volume of 1ml/kg. Among the dose groups of 9-10 animals each, mortality rates were 0%, 70%, 78%, 100%, and 100% for the control through high dose, respectively. Clinical signs observed in these animals included tremors, twitching, convulsions, salivation, and respiratory distress. Necropsy findings included mottled liver, congested lungs, and an abnormally high content of wood-chip bedding in the gastrointestinal tract of treated animals.

Subsequent experiments (substudies II-V) employed higher dosage volumes of 5-7.5 ml/kg, which increased the animals' tolerance of phenol. Doses in these experiments were 0, 60, 80, 100, 125, 160, 200, 250, or 320 mg/kg-day, given to groups of 5-8 timed-pregnant females on each of gestation days 6-15.

In order to identify dose-response trends, differences among treatment groups, and differences between control and treated groups, nonparametric statistics were applied to data from the four subgroups (II-V). Differences among groups were tested for by Kruskal-Wallis one-way ANOVA by ranks. In cases of a significant Kruskal-Wallis, a two-tailed Mann-Whitney U test was applied to compare control data to results for each treated group. Jonckheere's test for k independent samples was applied to detect significant dose-response trends. Fisher's Exact Test (two-tailed) was applied to nominal scale data for pairwise comparisons between controls and each treated group.

Maternal mortality was 17% at doses of 160 or 200 mg/kg-day phenol (1/6 animals in each case), 71% at 250 mg/kg-day, and 88% at 320 mg/kg-day phenol. Analysis of the pooled data for substudies II-V revealed statistically significant, non treatment-related, decrements in maternal weight for the 100 and 125 mg/kg-day groups, which were evident on gestation day 0, and consistent throughout gestation ($p < 0.01$ or 0.001). Maternal liver weights were correspondingly low in these animals ($p < 0.01$). No other groups showed significant differences from controls for these parameters.

There were no differences among groups for total gestational weight gain, gravid uterine weight, or net maternal weight gain (gestational weight gain minus gravid uterine weight). Considering the treatment period alone, maternal weight gain showed a significant dose-response trend ($p < 0.001$) toward decreased gain with increasing dose of phenol. Two individual dose groups, 160 and 250 mg/kg-day, were significantly below controls for weight gain during the treatment days ($p < 0.05$ or 0.01).

There were no statistically significant effects of phenol on fetal viability, sex ratio, or morphology identified in the pilot study. No statistically significant differences were observed among groups for fetal weight, though there was a significant trend for decreasing average fetal body weight per litter with increasing dose ($p < 0.05$).

Table 1. Dose of phenol (mg/kg-day)

Weight (g)	**0**	**60**	**80**	**100**	**125**	**160**	**200**	**250**
Maternal weight gain (gd 6-15)	27.57## ± 2.52	27.01 ± 1.63	20.80 ± 3.00	17.27 ± 5.57	21.62 ± 2.64	7.22* ± 6.75	13.80 ± 5.71	-0.10** ± 0.30
Mean fetal weight/litter	3.80# ± 0.06	3.83 ± 0.11	3.87 ± 0.13	3.67 ± 0.07	3.79 ± 0.16	3.41 ± 0.11	3.48 ± 0.07	3.75 ± 0.08

significant trend: # $p < 0.05$, ## $p < 0.001$
significant difference from control group: * $p < 0.05$, ** $p < 0.01$

C.2.1.1.2. Main study (RTI, 1983a)

Phenol was given by oral gavage to groups of timed-pregnant female rats. Doses of 0, 30, 60, or 120 mg phenol/kg bw-day, were given on each of gestation days 6 - 15. The study was conducted in a set of two replicate experiments, one with 13 animals assigned to each dose group, and the other with 10 animals per group.

Data for all measures were pooled across replicates and nonparametric statistics were applied as described above for the pilot study. To evaluate replicate-based variation, ANOVA was performed on selected response variables using General Linear Model (GLM) procedures from the Statistical Analysis System (SAS) Library. Prior to GLM analysis, an arcsine-square root transformation was performed on maternal or fetal percentage data, and Bartlett's test for homogeneity of variance was performed on all data to be analyzed by GLM. Average body weight per litter and percent malformed fetuses per litter were analyzed in a three-way ANOVA design (dose x replicate x sex), followed by tests of specific interactions against the general error term where appropriate. A Test of Linear Trends (alpha level = 0.05) was performed for treatment effects on each measure subjected to two-way ANOVA. Duncan's Multiple Range Test was used for pairwise comparisons between dose groups (alpha level = 0.05).

All treated females survived until scheduled sacrifice. In each dose group, at least 20-22 females were found to be pregnant at sacrifice on gestation day 20. Body weights of pregnant females did not differ significantly among groups on any of gestation days 0, 6, 11, 15 or 20. Total maternal weight gain over the gestation period and net maternal weight gain (total weight gain minus gravid uterine weight) did not differ significantly among treatment groups. Maternal weight gain over the treatment period showed no statistically significant differences among groups, excepting a significant increase over controls in the 30 mg/kg-day group ($p < 0.01$). The authors concluded that this difference was at least partially accounted for by a single outlying dam.

No clinical signs of toxicity were observed during the treatment period, excepting sporadic “clinical weight loss” ($\geq$ 5 g in 24 hours) in all groups, including controls. Clinical weight loss observed in Replicate I animals on gestation day 13 was considered possibly related to the finding that most of the animals in one room had been allowed to run out of food on that date.

When uterine contents were examined on gestation day 20, there were significant increases in the proportion of litters with resorption sites in the 30 and 60 mg/kg-day dose groups ($p < 0.05$ or 0.01, respectively), but not at 120 mg/kg-day. There were no differences among groups in the number or percentage of resorptions per litter, or in the number of implantation sites per litter. No dead fetuses were observed in any group.

Mean weights of live fetuses were decreased in a dose-related manner, with high-dose fetuses significantly lower than controls ($p < 0.01$). Application of Jonckheere's Test demonstrated a significant ($p < 0.001$) trend for decreasing fetal body weight with increasing dose. Multifactorial analysis of variance was also applied to the weight data,

in order to consider the effects of dose, replicate, and fetal sex as potential sources of variation. Results of this procedure supported a dose effect similar to that observed using analysis of data pooled across replicates and/or fetal sex.

Table 2. Dose of phenol (mg/kg-day)

Parameter	0	30	60	120
Litters with resorptions	0/22	5/22*	7/22**	3/20
Mean live fetuses/litter	12.23 ± 0.51^	13.32 ± 0.51^	12.14 ± 0.56^	13.75 ± 0.62^
Mean fetal wt/litter (g)	4.14# ± 0.07^	4.10 ± 0.05^	4.03 ± 0.07^	3.84** ± 0.05^

significant trend: # $p < 0.001$
significant difference from control group: * $p < 0.05$, ** $p < 0.01$
^ S.E.M.

An unexplained result of this study was the finding of an unusually high frequency of malformed female fetuses (12.6%) among the vehicle control group in replicate II of the study. The authors were unable to identify factors in the study records to explain this increase in malformations among control fetuses, but did not find evidence of treatment-associated morphological alterations in fetuses.

Overall, the authors concluded that their results indicated a selective fetal toxicity in response to phenol as evidenced by a significant reduction in average fetal body weight per litter at a dose producing no distinctive signs of maternal toxicity.

C.2.1.2. Mice

C.2.1.2.1. Range-Finding Study (RTI, 1983b)

Timed-pregnant CD-1 mice were given doses of 0, 100, 200, 230, 260, 275, 300, or 400 mg phenol/kg bw-day, by gavage, daily on each of gestation days 6 - 15. The study was conducted as a set of four sub-studies, with 5-7 animals assigned to each test group. Several test conditions (0, 275, and 300 mg/kg-day) were repeated in multiple sub-studies, resulting in a total of 5-27 animals per group.

In order to identify dose-response trends, differences among treatment groups, and differences between control and treated groups, nonparametric statistics were applied to data from the four subgroups. Differences among groups were tested for by Kruskal-Wallis one-way ANOVA by ranks. In cases of a significant Kruskal-Wallis, a two-tailed Mann-Whitney U test was applied to compare control data to results for each treated group. Jonckheere's test for k independent samples was applied to detect significant

dose-response trends. Fisher's Exact Test (two-tailed) was applied to nominal scale data for pairwise comparisons between controls and each treated group.

Excess maternal mortality (15% or greater) was observed at the 3 highest doses (275, 300, or 400 mg phenol/kg bw-day); no maternal animals died at any of the lower doses. Clinical symptoms of toxicity, such as tremors and weight loss ($\geq$ 1 g/24 hours), were observed at doses $\geq$ 200 mg phenol/kg bw-day. There were no statistically significant treatment-related differences among groups in maternal body weight. Measures of maternal weight gain during the treatment period, however, were significantly lower than controls at doses of 230, 260, 275, and 400 (but not at 300) mg/kg-day. There was also a statistically significant trend for decreasing maternal weight gain with increasing dose ($p < 0.05$ or 0.01). Gravid uterine weights did not differ significantly among groups, and maternal liver weights were significantly ($p < 0.05$) lower than controls only at doses of 230 and 260 mg/kg-day.

Table 3. Maternal parameters by dose of phenol (mg/kg-day)

Parameter	**0**	**100**	**200**	**230**	**260**	**275**	**300**	**400**
Maternal mortality	0/27	0/6	0/6	0/6	0/6	2/13	5/20	2/5
Maternal weight gain gd 6-15 (g)	12.52# $\pm$ 0.57	10.96 $\pm$ 0.87	11.28 $\pm$ 1.78	4.35* $\pm$ 3.88	7.43* $\pm$ 2.83	10.09* $\pm$ 1.13	10.37 $\pm$ 1.38	6.90** $\pm$ 0.89

significant trend: # $p < 0.01$
significant difference from control group: * $p < 0.05$, ** $p < 0.01$

There were no differences between dose groups in the number of implantation sites per dam, or in the number of live fetuses per live litter (litters having at least one live fetus). The sex ratio was not affected.

Significantly increased frequencies of resorptions per litter were found only at doses of 230 and 260 mg/kg-day ($p < 0.01$ and 0.05, respectively). The numbers and percentages of dead fetuses per litter were significantly increased at 260 ($p < 0.001$) and 400 mg/kg ($p < 0.01$). The numbers and percentages of "nonlive" (dead plus resorbed) fetuses were significantly increased at 230 ($p < 0.01$ and 0.05, respectively) and 260 mg/kg-day ($p < 0.01$). There were no significant differences among groups for live litter size. The numbers and percentages of "affected" (nonlive plus malformed) fetuses were significantly increased at 230 ($p < 0.01$), 260 ($p< 0.01$ and 0.05, respectively), 300 ($p < 0.05$ for number, no significant effect on percentage) and 400 mg/kg-day ($p < 0.05$). Average fetal body weights per litter was significantly lower than controls at doses $\geq$ 260 mg/kg-day ($p < 0.05$-0.001).

Table 4. Fetal parameters by dose of phenol (mg/kg-day)

Parameter	0	100	200	230	260	275	300	400
Nonlive/litter	1.62 ± 0.60	1.80 ± 0.80	0.80 ± 0.49	7.25** ± 2.75	5.00** ± 1.41	1.10 ± 0.28	2.33 ± 0.89	1.00 ± 0.58
Live/litter	11.77 ± 0.59	12.20 ± 1.16	11.00 ± 1.18	10.67 ± 3.84	11.40 ± 1.94	12.50 ± 0.62	12.50 ± 1.23	11.00 ± 1.00
bw/litter (g)	0.964# ± 0.025	0.929 ± 0.020	0.885 ± 0.055	0.752 ± 0.124	0.690** ± 0.020	0.831** ± 0.038	0.777*** ± 0.032	0.715* ± 0.081

significant trend: # $p < 0.001$
significant difference from control group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

No malformations were observed in control litters, or in litters exposed to 100 mg phenol/kg-day. No significant increase in malformations was observed in fetuses exposed to 275 mg/kg-day. Significant increases in the numbers and percentages of malformed fetuses per litter were observed at 200 ($p < 0.05$), 230 ($p < 0.001$), 260 ($p < 0.05$), 300 ($p < 0.01$), and 400 mg/kg-day ($p < 0.001$). Cleft palate was the single most commonly observed type of malformation.

C.2.1.2.2. Main study (RTI, 1983b)

Phenol was given to timed-pregnant CD-1 mice, by gavage, at doses of 0, 70, 140, or 280 mg/kg-day. Dosing was performed once daily on each of gestation days 6-15. A total of thirty-one to 36 plug-positive females, from four rounds of mating, were assigned to each treatment group. One non treatment-related death occurred at 280 mg/kg-day, and another such death occurred at 140 mg/kg-day; both of these deaths were attributed to dosing error. Four additional deaths, which were thought to be treatment related, occurred in the 280 mg/kg-day group, resulting in a mortality rate of 4/35, or 11%, in that dose group.

In order to identify dose-response trends, differences among treatment groups, and differences between control and treated groups, nonparametric statistics were applied to data from the four breeding groups. Differences among groups were tested for by Kruskal-Wallis one-way ANOVA by ranks. In cases of a significant Kruskal-Wallis, a two-tailed Mann-Whitney U test was applied to compare control data to results for each treated group. Jonckheere's test for k independent samples was applied to detect significant dose-response trends. Fisher's Exact Test (two-tailed) was applied to nominal scale data for pairwise comparisons between controls and each treated group.

At the time of necropsy on gestation day 17, there were 22-29 pregnant females in each dose group, and weight and litter data discussed below pertain to these animals only. On gestation days 11, 15, and 17, statistically significant trends for decreased maternal body weight with increasing dose of phenol were identified ($p < 0.05$). An adverse effect of treatment was also evidenced by significant dose-related trends for reductions in weight gain over the whole of gestation, with or without correction for uterine weight ($p < 0.01$ or 0.001, respectively), as well as over the treatment period alone ($p < 0.001$). By pair-wise comparisons, maternal body weight on gd 17, and body weight gain during treatment only, or over gestation (corrected or uncorrected for uterine weight) were significantly lower in the 280 mg/kg-day dose group than among controls (see Table 5 below). The 280 mg/kg-day group also exhibited clinical signs of toxicity, including: clinical weight loss ($\geq$ 1 g/24 hr), tremors, ataxia, lethargy and irritability. Females exposed to 140 mg/kg-day were reported as having exhibited "mild tremors after dosing on gd 6-8, but this response was not observed on subsequent days of treatment." Gravid uterine weights were significantly reduced at 280 mg/kg-day ($p < 0.05$), with a significant trend effect ($p < 0.05$). Maternal liver weights showed no significant differences among groups, but a trend test (Jonckheere's) was significant at $p < 0.01$.

Table 5. Maternal weight parameters (g)

parameter	**0**	**70**	**140**	**280**
Body weight day 11	34.69 ± 0.45#	34.38 ± 0.63	34.32 ± 0.36	32.91 ± 0.72
Body weight day 15	42.86 ± 0.78#	43.01 ± 0.98	42.75 ± 0.51	39.70 ± 1.08
Body weight day 17	48.03 ± 1.03#	48.65 ± 1.24	48.00 ± 0.63	43.28 ± 1.70*
Weight gain (treatment)	11.79 ± 0.66###	11.89± 0.84	12.09± 0.43	8.12 ± 0.93**
Weight gain (gestation)	19.76 ± 0.99##	20.15 ± 1.22	19.34 ± 0.64	14.19 ± 1.63**
Weight gain (minus uterus)	4.18 ± 0.50###	4.02 ± 0.52	3.02 ± 0.44	1.42 ± 0.67***
Gravid uterus	15.80 ± 0.82#	16.13 ± 0.87	16.32 ± 0.62	12.77 ± 1.16*
Liver weight	2.49 ± 0.06##	2.46 ± 0.06	2.33 ± 0.03	2.26 ± 0.08

significant trend: # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$
significant difference from control group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

There were no significant differences between dose groups in the incidence of resorptions, dead fetuses, or malformed fetuses, although two females at 280 mg/kg-day had all resorptions. There were no significant differences among groups in mean live litter size. A trend for decreasing average fetal body weight per litter was statistically significant ($p < 0.001$). By pair-wise comparison, fetal body weights were significantly lower in the 280 mg/kg-day group than among controls ($p < 0.001$).

Table 6. Mean fetal body weight (g) per litter by dose of phenol (mg/kg-day)

0	70	140	280
1.026 ± 0.022#	0.989 ± 0.022	0.996 ± 0.013	0.842 ± 0.028*

significant trend: # $p < 0.001$
significant difference from control group: * $p < 0.001$

There were no significant differences among groups in the number or percent of total or male or female malformed fetuses per litter. There was an apparent, but not statistically significant trend for an increasing frequency of cleft palate with increasing dose of phenol. On the basis of affected fetuses/total fetuses/dose group, the frequencies of cleft palate were: 0/308, 1/290, 1/280, and 8/214 (for controls through high-dose groups, respectively). The authors compared these frequencies to an incidence among historical controls of 1/1580 (for the same strain of mouse in their laboratory). They concluded that the apparent increase in the frequency of cleft palate seen in this study might reflect the compromised status of the high-dose group maternal animals, rather than representing a direct effect of prenatal exposure to phenol.

C.2.2. The Argus Studies

Argus laboratories completed an oral developmental toxicity study of phenol in rats. The main study was preceded by a pilot, dose range finding study, both of which are discussed below.

C.2.2.1. The pilot study (Argus, 1997)

In the pilot study (Argus, 1997, Appendix H), eight timed-pregnant Sprague-Dawley rats, obtained from Charles River Laboratories, Inc., were randomly assigned to each of 6 dose groups. On each of gestation days 6-15, solutions of phenol were given by gavage, in three daily dosings separated by intervals of approximately 4.25 hours. Total daily doses were 0, 60, 120, 180, 270, and 360 mg phenol/kg bw-day.

There were no maternal deaths during the course of the range-finding study, and no necropsy findings for maternal animals were attributed to the test substance. Clinical observations of "fewer than normal feces in pan" were made for most phenol-treated animals. Other clinical findings considered to be possible results of treatment were excess salivation and localized alopecia in a few animals in the 360 mg/kg-day group.

Maternal weight gain during gestation, or during the treatment period alone, was somewhat reduced at the higher dose levels of phenol, but the data do not appear to have been evaluated statistically. Absolute and relative feed and water consumption by maternal animals showed the same pattern.

On evaluation of litters at gestation day 20, there were no differences between groups in the numbers of corpora lutea, implantations, live fetuses, or resorptions. There were no changes in the sex ratio. Fetal weights were reduced by approximately 5% in the 120, 180, and 360 mg phenol/kg bw-day dose groups, but no statistical analysis was performed. The authors conclude that this decrease was "correlated with decreased maternal weight gain over the period of treatment," but no correlation coefficient or analysis of sources of variation in fetal weight is provided in support of this claim.

Table 7. Maternal and Fetal Weight Data (g) at Doses of Phenol (mg/kg-day)

Parameter	0	60	120	180	270	360
Maternal body weight	418.6 ± 31.9	419.0 ± 18.5	409.0 ± 26.8	406.3 ± 36.2	408.9 ± 15.4	405.8 ± 16.8
Maternal gestation weight gain	138.6 ± 22.1	136.7 ± 5.4	127.9 ± 17.3	127.6 ± 26.9	128.9 ± 10.9	124.5 ± 9.1
Fetal body weight/litter	3.30 ± 0.25	3.37 ± 0.22	3.13 ± 0.28	3.15 ± 0.21	3.31 ± 0.17	3.18 ± 0.20

A single micrognathic fetus was reported for the 180 mg/kg-day dose group. The malformation was not considered related to treatment, as the authors stated that micrognathia is known to occur spontaneously in this strain of rat. Fetuses were examined for external abnormalities only; no examinations of internal organs or skeletal elements were performed.

No explanation is given for dividing the doses into three separate treatments. Nor were any pharmacokinetic data presented concerning the effect this regimen may have had on steady-state versus peak plasma phenol levels.

C.2.2.2. The main study (Argus, 1997)

The full-scale developmental toxicity study conducted by Argus Laboratories used total doses of 0, 60, 120, and 360 mg phenol/kg bw-day. As in the pilot study, doses were administered on gestation days 6-15 as three separate daily gavage treatments. Twenty-five timed-mated females were assigned to each group.

One female in the 360 mg/kg-day group died during the course of the study. This death was attributed to the test substance, although no clinical or necropsy evidence was provided to support this conclusion. Maternal body weights for the 360 mg/kg-day group were significantly below control values for most time points measured, and on each day subsequent to gestation day 9 ($p < 0.05$ or 0.01). Significant reductions in maternal weight-gain during the treatment period occurred at the 120 and 360 mg/kg-day dose levels ($p < 0.05$ and 0.01). At 360 mg/kg-day, maternal weight gain for the entire

gestation period was significantly reduced ($p < 0.01$). It should be noted, however, that weight gain for the 360 mg/kg-day group was already significantly lower than controls ($p < 0.05$) between gestation days 0 and 6 (before treatment commenced). Maternal feed consumption showed significant reductions over at least some intervals at all three doses of phenol, but most consistently at the two higher doses of 120 and 360 mg/kg-day. The frequencies of excess salivation and tachypnea were significantly ($p < 0.01$ for both endpoints) increased at 360 mg/kg-day, but no other clinical observations or necropsy results were considered to be related to treatment.

Table 8. Maternal weight parameters by dose of phenol (mg/kg-day)

Parameter (mean ± SD)	**0**	**60**	**120**	**360**
Day 0 weight (g)	251.1 ± 11.2	251.2 ± 10.6	250.6 ± 10.4	251.8 ± 10.6
Day 20 weight (g)	440.4 ± 29.0	435.1 ± 28.2	429.2 ± 25.1	412.3 ± 29.1**
0-6 weight gain (g)	48.7 ± 10.1	49.8 ± 6.6	46.9 ± 8.2	43.4 ± 8.1*
6-16 weight gain (g)	64.0 ± 10.7	58.0 ± 9.4	56.8 ± 10.8*	39.8 ± 5.8 **
0-20 weight gain (g)	189.3 ± 23.1	184.0 ± 22.6	178.6 ± 21.6	161.0 ± 23.8**

Significant difference from controls: * $p < 0.05$, ** $p < 0.01$

Fetal body weights were significantly reduced at the high dose of 360 mg phenol/kg-day. The average number of ossification sites per fetus per litter for the metatarsals was significantly reduced ($p \leq 0.05$) at 360 mg/kg-day. Neither litter size nor any other litter or fetal observations were considered to have been affected by treatment.

Table 9. Fetal weight by dose of phenol (mg/kg-day)

Parameter	**0**	**60**	**120**	**360**
Fetal weight	3.62 ± 0.30	3.63 ± 0.24	3.60 ± 0.30	3.41 ± 0.38*

Significant difference from controls: * $p < 0.05$

It is interesting to note, however, that two severely malformed fetuses were observed among the treated groups. One was a case of conjoined twins with exencephaly, seen in the 360 mg/kg-day group. In the 120 mg/kg-day dose group there was one fetus that had micrognathia and missing tongue. Examination of historical control data for Argus labs between June 1992 and June 1995 (26818 fetuses/1840 litters) revealed no other cases of conjoined twins. Three fetuses from three different litters were reported as having exencephaly, and six fetuses from six different litters had micrognathia. No cases of "missing tongue" were reported in the historical data set.

C.2.3. Additional Studies

C.2.3.1. In vivo

A two-generation reproductive toxicity study conducted in rats exposed via drinking water (IIT Research Institute, 1999; Ryan et al., 2001) is described and discussed in detail in section D.2.1. of this document (pair-based animal reproductive toxicity studies). The concentrations of phenol used were 0, 200, 1000, or 5000 ppm. Exact phenol consumption varied with sex and time point evaluated, but for P1 and F1 females at week 10 of direct exposure, the average doses were 0, 20, 94, and 351 mg/kg-day. There were no significant differences among groups for live litter size at birth in either generation. Litter weights on post-natal day 0, however, were significantly reduced in offspring of both generations exposed to 5000 ppm phenol ($p \leq 0.05$). Maternal body weights during gestation, as well as gestational weight gain also showed significant decreases in both generations at 5000 ppm phenol ($p \leq 0.05$ in all cases).

Table 10. Maternal and newborn endpoints by ppm phenol in drinking water

Parameter (mean ± SD)	**0**	**200ppm**	**1000ppm**	**5000ppm**
P1 postpartum maternal weight	293 ± 25.0	294 ± 26.8	288 ± 23.3	250* ± 27.6
F1 live pups/litter on PND 0	11.0 ± 2.2	11.3 ± 3.1	12.1 ± 2.4	10.6 ± 2.5
F1 PND 0 pup weight^	6.73 ± 0.419	6.85 ± 0.510	6.63 ± 0.513	6.38* ± 0.257
F1 postpartum maternal weight	288 ± 24.5	300 ± 24.5	299 ± 21.1	246* ± 21.5
F2 live pups/litter on PND 0	10.7 ± 2.5	11.6 ± 3.0	11.3 ± 3.3	11.2 ± 1.8
F2 PND 0 pup weight^	6.67 ± 0.375	6.80 ± 0.442	6.58 ± 0.417	6.20* ± 0.475

^Male and female pups combined, mean newborn weights per litter
Significant difference from controls: * $p < 0.05$

In a large study of reproduction in female mice exposed to xenobiotics (Bishop et al., 1997), treated females were single-pair mated with untreated males for a minimum of 347 days post-treatment. The protocol of this study involved a single intraperitoneal injection of one of 29 different chemicals (including phenol) given prior to mating and gestation. Thirty to 36 females were used in each treatment and control group. The exact dose of

phenol is not presented, but all doses were said to have been determined on the basis of the maximum tolerated dose (MTD) over a 30-day survival experiment. In the reproductive experiments, data were collected on litter size and the total number of litters produced during the reproductive lifespan of the treated animals. No other endpoints, such as offspring weights, were evaluated. In this test system, phenol was not found to have an effect on litter size or the number of litters produced.

The Proctor and Gamble Company provided U.S. EPA with a study conducted on their behalf by a contract laboratory, International Research and Development Corporation (IRDC). The IRDC (1993) study was a range finding study for maternal toxicity in pregnant rats. Groups of 10 time-mated Sprague-Dawley-derived rats supplied by Charles River Laboratories, Inc., were given phenol by gavage, daily on each of gestation days 6-15. The doses (in mg/kg) and volumes (ml/kg, in parentheses) used were: 0 (10), 60 (2.5), 60 (5.0), 60 (10.0), 120 (2.5), 120 (5.0), 120 (10.0), 180 (15.0 split into two 7.5 ml doses, with only the first dose given on day 15). Necropsies, including uterine examinations, were performed on gestation day 15. Gravid uteri were removed, weighed, and examined for the presence and locations of viable and nonviable fetuses, early and late resorptions, and the numbers of total implantations and corpora lutea.

One animal, which had been given 120 mg phenol/kg-day in a volume of 2.5 ml, died during the course of the study. Ninety percent of control animals, as well as 80-100% of treated animals from all groups, were considered to have shown no clinical signs of toxicity. Maternal body weights were not found to differ among groups on any of the gestation days for which weights were taken (0, 6, 9, 12, and 15 with and without the gravid uterus). There were no differences among groups for maternal body weight gain over the whole of gestation (with or without the gravid uterus), or for the intervals gestation day 0-6, 6-9, 9-12, or 12-15. For the treatment period (gestation days 6-15), however, significant decreases in maternal weight gain were noted for animals given 120 mg/kg-day phenol in a 5.0 ml volume, or 180 mg/kg-day phenol in two daily doses of 7.5 ml volume ($p < 0.01$ in both cases). A significant decrease in food consumption was seen only among animals given 120 mg/kg-day phenol in a 5.0 ml volume during the interval between gestation days 12-15 ($p < 0.01$). No differences among groups were found for any of the litter parameters evaluated, or for the weights of gravid uteri. Individual fetal weights were not determined.

Table 11. Maternal Body Weight and Weight Gain in Response to Phenol

Treatment: mg/kg (ml/kg)	Day 15 bw (g; adjusted for uterine weight)	Day 6-15 weight gain (g)
0 (10.0)	309 ± 16.9	42 ± 8.0
60 (2.5)	290 ± 21.7	34 ± 8.2
60 (5.0)	289 ± 22.6	33 ± 5.3
60 (10.0)	306 ± 28.3	36 ± 7.9
120 (2.5)	293 ± 17.6	32 ± 9.9
120 (5.0)	289 ± 19.3	29 ± 8.6*
120 (10.0)	287 ± 13.7	35 ± 10.0
180 (7.5 X 2)	287 ± 17.8	27 ± 7.4*

* significant difference from controls, $p < 0.01$

Kavlock (1990) conducted a large-scale study of the structure activity relationships of the developmental toxicity of substituted phenols *in vivo*. Groups of 12-15 timed-mated female Sprague-Dawley rats were given a single treatment with phenol or one of its congeners, on gestation day 11 (sperm-positive day = day 1). Dosing was performed by gastric intubation, and the doses used were 0, 100, 333, 667, or 1000 mg/kg. Pregnant females were allowed to deliver their litters, and raise the offspring until weaning. Maternal animals were observed for any clinical symptoms of toxicity, and weighed at specified intervals. Developmental toxicity was evaluated by determining viability and weight of offspring on postnatal days 1, 3, and 6. Any gross physical malformations were also noted at these times.

No maternal animals died at any dose of phenol. Weight changes for dams in the 1,000 mg phenol/kg bw group were significantly different from controls at 24 and 72 hours post dosing ($p < 0.05$ at both time points). At a phenol dose of 667 mg/kg, maternal weight change was significantly ($p < 0.05$) lower than in controls at 24, but not 72, hours post dosing. Maternal weight data for later time points were not provided.

In offspring of animals exposed to phenol at doses of 667 or 1000 mg/kg, a syndrome of limb paralysis and/or short or kinky tails was observed postnatally. Limb function of affected animals would alternate between apparent normality, and "a several second period of tetany." The syndrome was not evident at birth, but required a week to ten days to become manifest. At 667 mg/kg phenol, this effect was seen in offspring from 21.4% of litters. At the higher dose of 1000 mg/kg, 27.3% of litters had affected pups. No other measures of postnatal growth or viability were affected.

Narotsky and Kavlock (1995) used phenol as one of the chemicals employed in validating an experimental screening protocol for systemic toxicity, neurotoxicity, and developmental toxicity. Sixteen to 23 pregnant Fischer 344 rats were assigned to each dose group and given phenol, by gavage, at a dose of 0, 40, or 53.3 mg/kg, on each of gestation days 6-19. Maternal body weights were recorded at pre-determined time points, and animals were examined throughout the treatment period for clinical signs of toxicity. Dams were allowed to deliver their litters normally, and pups were examined and counted

on each of post-natal days (PND) 1, 3, and 6. Pups were weighed collectively on PNDs 1 and 6. Dams were sacrificed subsequent to the final litter evaluation, and their uteri examined to determine the number of implantation sites.

Maternal toxicity observed with phenol treatment in this study was considered to be "relatively mild." Statistically significant reductions in weight gain were noted in the text as occurring after four and ten treatments, but the data were not presented. Clinical symptoms of toxicity reported for treated dams included altered respiration (rales and dyspnea) at both doses. Phenol did not affect the number of implantation sites, but findings for pups included a statistically significant reduction from control values in the numbers of live pups in the 53.3 mg/kg-day dose group on both PND 1 and PND 6 ($p < 0.05$ in both cases). Developmental effects were apparently restricted to the offspring of four treated litters. One low-dose (40 mg/kg-day) and two high-dose (53.3 mg/kg-day) litters were completely resorbed. An additional 53.3 mg/kg-day group female was reported to have had significant loss of offspring perinatally, as well as reduced weights of surviving pups on PND 1. All four of these females were reported as having had severe respiratory symptoms in response to phenol exposure, although it is noted that other females with similar symptoms had apparently normal litters.

Two of the four surviving pups in one litter exposed to 53.3 mg phenol/kg-day had "kinked tails," a malformation also noted by Kavlock (1990). Overall, phenol was considered to pose a "slight" potential for causing developmental toxicity. While concurrent maternal toxicity was noted as possibly contributing to the observed developmental toxicity, it was also noted that similar levels of maternal toxicity were also observed with phenol or other congeners in the absence of any adverse effects on the offspring.

An abstract by Minor and Becker (1971) reported the results of teratology studies comparing the effects of sodium chloride, sodium salicylate, sodium benzoate, and phenol. The compounds were given by ip injection to timed-pregnant Sprague-Dawley rats on gestation days 9-11 or 12-14 (plug day = gestation day 1). Phenol was given at doses of 0, 20, 63, or 200 mg/kg. Whether given on gestation days 9-11 or 12-14, phenol was not found to be associated with reduced fetal weights, increased malformations, or increased *in utero* deaths. The numbers of pregnant animals per dose group was not reported, nor was any statistical analysis performed.

C.2.3.2. In vitro

Kavlock et al. (1991 a and b), Oglesby et al. (1988 and 1992), and Fisher et al. (1993) reported on *in vitro* studies comparing the relative potency of para-substituted phenols as developmental toxicants. The *in vitro* system employed by this lab consists of culturing intact rat embryos with or without the inclusion of metabolically-active primary hepatocytes. The embryos were explanted on gestation day 10, at the 9-13 somite stage of development, and maintained in culture for 42 hours. Endpoints evaluated included:

somite number; protein and DNA contents; and morphology of the fore- and hind-limb buds, the branchial arches, and the tail bud.

A previous *in vivo* study from the same laboratory (Kavlock, 1990) found few of the phenols tested produced developmental toxicity at a maximum maternal dose of 1000 mg/kg. In contrast, when tested *in vitro*, all 13 phenols had adverse effects on embryonic development (results summarized and discussed by Kavlock et al., 1991 a and b). Effective concentrations in culture media were generally less than 0.5 mM. The specific compounds tested in both systems were: *p*-amino, *p*-cyano, *p*-bromo, *p*-chloro, *p*-iodo, *p*-fluoro, *p*-methyl, *p*-hydroxy, *p*-methoxy, *p*-pentyloxy, *p*-heptyloxy, *p*-nitro, and phenol itself. Two of the 13 congeners, *p*-amino and *p*-hydroxy, were among the most potent of those tested in both assay systems. Otherwise, the potential of these phenols to induce developmental toxicity *in vitro* was not well correlated with their potential to produce developmental toxicity *in vivo*. Inclusion of metabolically-active hepatocytes in the *in vitro* test system reduced the potential of virtually all congeners to induce embryotoxicity. The one exception was phenol itself. This exception was explained on the basis of a slower rate of metabolic reactions involving the aromatic ring for the substituted phenols. Phenol, on the other hand, having no substituent group at the para position, is more readily available to undergo metabolism to a more reactive form such as *p*-hydroxyphenol.

Benzene and its metabolites, including phenol, were studied in a similar whole-embryo culture system (Chapman et al., 1994). For these experiments, rat embryos were explanted earlier in their development, on gestation day 9, and cultured for a total of 30 hours. Endpoints evaluated at the end of the culture period included embryonic viability, yolk sac diameters, maximal embryonic lengths, somite numbers, axial rotation, prosencephalic indices, anterior and posterior neuropore closure, and embryonic protein content. No statistically significant effects were seen on embryonic development with phenol added to the culture media at concentrations ranging from 0.1 to 1.6 mM. The addition of a metabolically-active hepatic preparation (S-9) to the culture system, however, resulted in significant manifestations of embryotoxicity that increased with increasing concentrations of phenol from 0.01 to 0.4 mM. One hundred percent of all conceptuses exposed to concentrations of 0.2 mM or above were scored as nonviable.

The toxicity of a series of chlorinated phenols was evaluated in culture systems using either intact postimplantation rat embryos, or *Hydra attenuata* (Mayura et al. 1991). Cultured rat embryos were explanted on gestation day 10, and exposed for 45 hours to a concentration of 0.6 mM phenol. No metabolically-active hepatic material from adult animals was incorporated into these experiments. Both test systems demonstrated a linear relationship between toxicity and the degree of chlorine substitution, with pentachlorophenol being the most, and phenol the least, toxic of the compounds tested. Phenol had no observed effects on any measure of embryonic growth or development, specifically: yolk-sac diameter, crown-rump length, somite number, morphological score, protein content, DNA content, or percent malformed.

Another structure-activity study of chlorophenols was performed using human embryonic palatal mesenchyme (HEPM) cells (Zhao et al., 1995). HEPM cells were seeded onto 24-well culture plates, and cultured for 24 hours before exposure to chemical-containing media; cells were cultured an additional 72 hours in the presence of test chemicals. The IC_{50} (concentration that inhibited cell growth by 50%) was determined for each of the various chlorophenols tested (phenol through pentachlorophenol). All of the chlorophenols tested, including phenol, caused significant inhibition of cell growth in a concentration-dependent fashion. Furthermore, the results indicated a linear relationship between IC_{50} values and the degree of chlorine substitution. Phenol was the least toxic in this system, with an IC_{50} value of 470.0 μM, and pentachlorophenol was the most toxic, with an IC_{50} value of 18.8 μM.

Phenol has also been tested for embryotoxic effects in the South African Clawed Toad (*Xenopus laevis*) (Dumpert, 1987). Phenol was added to the water in which these animals developed from spawn into tadpoles at concentrations of 0, 0.1, 1.0, 5.0, or 50.0 ppm. Offspring contained in one basin treated with 50 ppm phenol all died within six days of completing embryonic development. Offspring in two other basins treated with 50 ppm phenol died within a maximum of three weeks following completion of embryonic development. Other test animals developed without obvious abnormalities. Measurements of body lengths after a test period of five weeks revealed that animals exposed to 5 or 10 ppm phenol were on average 8% shorter than controls or test animals exposed to only 0.1 or 1.0 ppm phenol. The difference was not statistically significant.

C.2.4. Other Relevant Data

In their table of mammalian germ cell assay results pertinent to potential paternally-mediated developmental toxicity, Olshan and Faustman (1993) noted phenol as having negative results in one or more dominant lethal assays conducted in mice.

Ciranni et al. (1988) gave benzene and some of its metabolites, including phenol, to pregnant mice in a study of transplacental mutagenesis. Phenol was given by gastric intubation at a dose of 40 mg/kg. Male, virgin female, and pregnant CD-1 mice were sacrificed and their tissues harvested for analysis at intervals ranging from 0 to 40 hours following treatment. Fetal livers and adult bone marrow were scored for polychromatic and normochromatic erythrocytes (PCEs and NCEs). The PCE/NCE ratios were also calculated as an index of cell toxicity. Benzene (880 mg/kg) was found to be a strong inducer of micronuclei in maternal bone marrow (more than 6X control values), as well as an inducer of micronuclei in fetal liver (3-6X control values). There was no identified benzene toxicity to maternal bone marrow cells, but more than 60% of fetal liver cells were scored as showing effects of toxicity. Phenol, in contrast, showed some degree of micronucleus induction in maternal bone marrow (1-3X controls), as well as 0-30% cellular toxicity. No effect of phenol was shown on fetal liver cells.

C.3. Integrative Evaluation

Few reports on the potential developmental toxicity of phenol in humans were identified in the literature. In contrast, available data on the developmental toxicity of phenol in experimental animals are fairly extensive, including large-scale regulatory developmental toxicity studies in two species (RTI, 1983 a and b; Argus, 1997; IRDC, 1993) as well as peer-reviewed, published papers on research conducted in *in vivo* and *in vitro* test systems (Bishop et al., 1997; IRDC, 1993; Kavlock, 1990; Narotsky and Kavlock, 1995; Minor and Becker, 1971; Kavlock et al., 1991 a and b; Oglesby et al., 1988 and 1992; Fischer et al., 1993; Chapman et al., 1994; Mayura et al., 1991; Zhao et al., 1995; Dumpert, 1987; Olshan and Faustman, 1993; Ciranni et al.,1988), and a standard two-generation reproductive toxicity study (IIT Research Institute, 1999; Ryan et al., 2001).

Epidemiological investigations into the effects of phenol on pregnancy have been conducted in women who were exposed to mixtures of disinfectants or solvents at their place of work (Axelsson et al., 1984; Hernberg et al., 1983). While no meaningful associations between phenol and adverse pregnancy outcome were identified, it is possible that due to the design and limitations of these studies, they were unable to detect an association between phenol and adverse pregnancy outcomes if such an association were present.

Findings of studies on rats and mice exposed to phenol *in utero* have included statistically significant reductions in fetal weight and viability, as well as increased frequencies of malformations. Reduced fetal or birth weight was the most frequently reported effect (RTI, 1983 a and b; Argus, 1997; IIT Research Institute, 1999; Ryan et al., 2001). Results reported only in abstract form (Minor and Becker, 1971), for rats treated by the i.p. route, are also consistent with an effect of phenol on fetal weight. In one study using rats, statistically significant decreases in mean fetal weights were observed in the absence of any evidence for treatment-related maternal toxicity (RTI, 1983a). In CD-1 mice, as well as in additional rat studies, decreases in fetal or birth weights were observed at doses of phenol that also resulted in decreased maternal weights (RTI, 1983b; Argus 1997; IIT Research Institute, 1999; Ryan et al., 2001).

With respect to the general issue of considering reduced fetal weight as evidence for the developmental toxicity of phenol, U.S. EPA's published "Guidelines for Developmental Toxicity Risk Assessment" (U.S. EPA, 1991) notes: "A change in offspring body weight is a sensitive indicator of developmental toxicity, in part because it is a continuous variable. In some cases, offspring weight reduction may be the only indicator of developmental toxicity. While there is always a question as to whether weight reduction is a permanent or transitory effect, little is know about the long-term consequences of short-term fetal or neonatal weight changes. Therefore, when significant weight reduction effects are noted, they are used as a basis to establish the NOAEL."

In the particular case of the RTI rat study (1983a), "Average fetal body weight per litter showed a dose-related trend ($p<0.001$, Jonckheere's Test) toward decreasing fetal body weight as dosage increased, with the high-dose group significantly below controls on this

measure ($p<0.01$, Mann-Whitney U Test)." This analysis demonstrated a clear dose-response relationship between fetal weight and phenol exposure. Multifactorial analysis of variance failed to support experimental replicate or fetal sex as alternative determinants of variation in fetal weight. The use of replicate dose groups as a feature of the experimental design used by RTI (1983a) further strengthens confidence in these data on a statistical basis. As noted by U.S. EPA (1991), "Studies that employ a replicate experimental design (e.g., two or three replicates with 10 litters per dose per replicate rather than a single experiment with 20 to 30 litters per dose group) allow broader interpretation of study results since the variability between replicates can be accounted for using ANOVA techniques. Replication of effects due to a given agent within a study, as well as among studies or laboratories, provides added strength in the use of data for the estimation of risk."

One variable, which has a known tendency to be inversely correlated with fetal weight, is litter size (U.S. EPA, 1991). In the RTI rat study (1983a), mean litter size was larger among high-dose group animals than among controls, raising the possibility that increased litter size might have contributed to the observed fetal weight reductions at the high dose of phenol. On the other hand, there is no evidence to support litter size as a causal factor in this study. Litter size was *not* found to differ significantly between dose groups, whereas reductions in fetal weight demonstrated a statistically significant dose-effect relationship among all doses tested, and (by pair-wise comparison) were significantly lower than controls at the high dose.

Control fetuses from the RTI (1983a) rat study were heavier than the 95% upper confidence bound published for historical control data for the same laboratory over the years 1980-1983 (Kimmel and Price, 1990). The phenol-study control fetuses did, however, fall within the reported historical range. Conversely, the mean maternal body weight for control dams on gestation day 0 was lighter than the published historical 95% lower confidence bound. By gestation day 10, mean maternal body weight, weight gain, and absolute weight gain (corrected for the weight of the gravid uterus) for control dams were greater than the published 95% upper confidence bounds for these measures. While these departures from historical averages are interesting to note, it is not clear that they provide a basis for discounting the statistical comparisons between contemporaneous treated and control animals in the RTI (1983a) study. As part of the review generated from the 1986 "Consensus Workshop on the Evaluation of Maternal and Developmental Toxicity," Kavlock and Palmer (1987) reported on the discussion of historical data. While historical data were considered particularly useful in providing a context for rare events (i.e. malformations), they urged care in using historical data for quantitative purposes, and warned against "the inherent danger of using them to prove whatever point of view the investigator may possess." The U.S. EPA (1991) "Guidelines for Developmental Toxicity Risk Assessment" note, "Comparison of data from treated animals with concurrent study controls should always take precedent [sic] over comparison with historical control data."

In the RTI (1983b) study, which was conducted in mice, as well as in the Argus (1997) rat study and the rat reproductive toxicity study (IIT Research Institute, 1999; Ryan et al.,

2001), statistically significant reductions in fetal or birth weight were identified only at doses that also produced evidence of maternal toxicity. If the developmental toxicity observed in these studies was not directly related to phenol exposure, but rather resulted strictly from generalized impairment of the maternal animals, then developmental toxicity following phenol exposure of the pregnant animal should always and *only* be observed in the presence of maternal toxicity. Such a cause-effect relationship is not borne out by the RTI (1983a) rat study, which demonstrated an association between prenatal exposure to phenol and a statistically significant reduction in fetal weight in the absence on any adverse effects on maternal animals.

Furthermore, as the U.S. EPA "Guidelines for Developmental Toxicity Risk Assessment" (1991) state, "Current information is inadequate to assume that developmental effects at maternally toxic doses result only from maternal toxicity; rather, when the LOAEL is the same for the adult and developing organisms, it may simply indicate that both are sensitive to that dose level." In general, the occurrence of developmental toxicity at doses which also cause minimal maternal toxicity--defined by U.S. EPA (1991) as a slight maternal weight loss up to no more than 10% maternal mortality--does not, in and of itself, constitute evidence that the development toxicity is secondary to the observed maternal toxicity. The extent of maternal toxicity observed at the developmental LOAEL in the Argus 1997 study falls within the bounds defined as "minimal" by U.S. EPA (1991). There are no mechanistic or other data available supporting a conclusion that the observed developmental toxicity was solely the secondary result of maternal toxicity.

Finally, it should be noted that while both the RTI (1983a) and the Argus (1997) studies were conducted in rats, it is difficult to compare the results on a dose basis, because of the multiple dosings per day treatment regimen employed in the Argus studies. While on a mg/kg-day basis, the high dose in the Argus studies (360 mg/kg-day) was three times that used in the RTI study (120 mg/kg-day), no blood levels or other pharmacokinetic data were provided. Given the known rapidity with which phenol is absorbed and eliminated from the body, it is unclear how maximum circulating levels of phenol at a single dose of 120 mg/kg-day would have compared to those reached with three doses per day of 120 mg/kg, spaced several hours apart.

D. REPRODUCTIVE TOXICITY

D.1. Human studies

No data were identified that pertained to the potential reproductive toxicity of phenol in human males, and only limited human data are available on females. Two of the studies on human females (Hernberg et al., 1983; and Axelsson et al., 1984) are also summarized in Section C.1. above, "Developmental Toxicity; Human studies." A total of eight Polish language papers (Radowicki and Wierzba, 1997a, 1997b, and 1997c; Wierzba et al. 1997; Wierzba and Radowicki, 1997a and 1997b; Liebhart et al., 1979 and 1980) have also been obtained and are summarized below.

Phenol was among the chemical exposures included in a retrospective study of pregnancy outcome among university laboratory employees (Axelsson, et al., 1984). A total of 1160 pregnancies to 743 women were included in the study. With exposure to organic solvents during laboratory work, there was a slight but non-significant difference in the rate of miscarriage as compared to non-exposed pregnancies. The relative risk (RR) was 1.31, with 95% confidence interval of 0.89-1.91. There were no differences between groups in perinatal death rates, or in the prevalence of malformations. Exposure to phenol was specifically reported in only five cases, all of which were normal deliveries.

The eight studies published in the Polish language were screened for relevant data and the four most relevant studies were translated into English. Those studies report on aspects of placental structure and histopathology for pregnant women having increased levels of phenols in their urine (Radowicki and Wierzba, 1997a; Radowicki and Wierzba, 1997b; Wierzba et al. 1997; Wierzba and Radowicki, 1997a).

Placentas were obtained between the years of 1986 and 1989, from a population of healthy women who gave birth to normal infants in good condition (normal birth weight and an Apgar score of 9 or 10). Participants did not smoke, drink excessively, or depend upon drugs. One hundred forty-six of the mothers in the study were from a "threatened ecological region" (TER), while 80 others were considered to come from a non-TER (the area around Kutno). The TER was defined as an area subject to airborne toxic substances originating from petrochemical plants surrounding the city of Plock. Aromatic hydrocarbons, phenol among them, were considered to be the major hazardous pollutants. Urine samples for phenol measurements were taken from these women at 16, 28, and 36 weeks of pregnancy, as well as upon admission to the hospital for labor and birth.

TER mothers were found to have urinary phenol levels almost twice that of non-TER mothers: 8.61 ± 0.74 mg/l and 4.89 ± 0.23 mg/l, respectively ($p < 0.05$). Placental weights did not differ significantly between the TER and non-TER groups of mothers. Linear measures indicated that placentas from the two groups tended to differ in shape (oval for TER, round for non-TER), with some differences in placental thickness between primiparas and multiparas for the non-TER group only. The growth curve for placental thickness also differed between the two groups, indicating that non-TER placentas reached their maximum thickness a full two weeks before TER placentas did so. There was a statistically-significant correlation between placental thickness and gestation length in the two groups (for non-TER, Spearman = 0.3788, $p = 0.012$; for TER, Spearman = 0.1984, $p = 0.032$).

The distance of membrane rupture from the edge of the placenta (MREP) was influenced by TER status as well as parity. The mean MREP for non-TER pregnancies was 91.02 mm, which was considered to be within the normal range (70-100 mm). In the TER population, this measurement had an average of 44.66 mm, which was significantly ($p < 0.001$) smaller than the non-TER group.

The quality of the amniotic epithelia (AE) was also considered to be poor in TER pregnancies, as it was more frequently separated from the placental tissue (10.28% of AE separated, as compared to no cases of separation in the non-TER pregnancies; significance level of $p = 0.003$). Separation most commonly occurred in patients giving birth at 39 or 40 weeks. Within the TER group, there was a significant correlation between the quality of the AE and the duration of pregnancy (Spearman = 0.499; $p < 0.01$). The correlation in the non-TER group ($r^2=0.37$, $p>0.2$) was not statistically significant; however, there were fewer women in this group (n = 80) compared with the TER group (n = 146).

Microscopic assessment of placental tissue was performed on hematoxylin-eosin stained slides. The proportion of either hyperplastic or necrotic epithelial membranes of the external membranes of the placenta (EMEP) was considered to differ significantly between TER and non-TER placentas ($p < 0.05$ in both cases). Evidence for hyperplasia of the EMEP, inflammation, infiltration, poor quality of the epithelium covering terminal villi, and other changes were all found with increased frequency in the TER group (e.g. inflammation was present in 18.5% of TER placentas, but only 5% of non-TER placentas). An increased frequency of microinfarctions in terminal villi (MTV), as well as increased quantities of fibrin tightly surrounding changed villi were also observed in TER placentas ($p = 0.007$ and $p = 0.005$, respectively). All of these changes were considered to be consistent with impairments of placental function, such as abnormal generation and resorption of the amniotic fluid.

Translations were not obtained for four other Polish-language papers discussing aspects of data originating from the Plock region (Liebhart et al., 1979 and 1980; Wierzba and Radowicki, 1997b; Radowicki and Wierzba, 1997c). According to the brief English abstract, Liebhart et al. (1979 and 1980) found pathological changes in chorionic tissues collected from pregnant subjects having high urinary excretion of phenol. Wierzba and Radowicki (1997b) demonstrated significant changes in both PAS (periodic acid-Schiff staining for polysaccharides and mucopolysaccharides) and van Gieson (acid fuchsin in saturated picric acid staining for collagen) activities in the stroma of placental villi (X^2 = 9.36 and 8.48, respectively) for pregnant women from the polluted area. Radowicki and Wierzba (1997c), however, found no significance changes in gestation length between 146 pregnant women from the polluted area and 80 controls.

D.2. Animal studies

Information relevant to female reproductive toxicity is available from studies on the developmental toxicity of phenol (RTI, 1983 a and b; Argus, 1997), and from pair-based studies of the reproductive effects of phenol (Heller and Pursell, 1938; IIT Research Institute, 1999; Ryan et al., 2001). One study (Bishop et al., 1997) included phenol as one of 29 compounds tested for effects on reproduction in female mice.

Information relevant to the potential male reproductive toxicity of phenol is available from pair-based animal studies of reproductive toxicity (Heller and Pursell, 1938; IIT Research Institute, 1999; Ryan et al., 2001), from a multi-generation study of the effects

of phenol on the chromosomes of reproductive cells in male mice (Bulsiewicz, 1977; Allen et al., 1986), and from a study of dominant lethality in male mice (Shelby et al., 1993).

D.2.1. Pair-based studies

Apart from an early study that included a reproductive component (Heller and Pursell, 1938), only one multi-generation reproductive toxicity study of phenol was identified (IIT Research Institute, 1999; Ryan et al., 2001). These studies provide information of relevance to both male and female reproductive toxicity.

Heller, VG and Pursell, L. (1938) Phenol-contaminated waters and their physiological action

Groups of rats (strain not specified) were maintained through several generations on drinking water containing phenol at one of a series of dilutions. No data were provided on the numbers of animals in each group, or on the quantity of water consumed at each dilution. Nor were data provided on feed consumption, body weight, or any quantitative measurement of reproductive success. Concentrations ranging from 15 to 1,000 ppm phenol in drinking water were reported to have had no adverse effects on the exposed animals. Growth and reproduction--including the rearing of young--over as many as five generations, were said to have been completely normal.

Dilutions ranging between 1,000 and 5,000 ppm were tolerated without noticeable difficulty for three generations. At a concentration of 7,000 ppm phenol in drinking water for two generations, growth was characterized as "below normal," and the young were said to have exhibited "stunted growth." At 8,000 ppm phenol for two generations, growth was characterized as "fair," and "many young" were reported to have died. At 10,000 ppm phenol, water intake was considered to have been reduced, growth and reproduction were characterized as "retarded," and offspring were "not cared for." At 12,000 ppm (the highest concentration tested) growth was retarded, there was no reproduction, and adult animals exhibited increased mortality during hot weather.

Ryan, BM et al. (2001) Two-generation reproduction study and immunotoxicity screen in rats dosed with phenol via the drinking water

IIT Research Institute (1999) Two-generation oral (drinking water) reproductive toxicity study of phenol in rats

Methods:

This study was conducted as part of an Enforceable Consent Agreement between U.S. EPA and the Phenol Panel of the Chemical Manufacturers Association (CMA). As such, the study protocol was stated to have been modeled after the revised draft testing

guidelines set forth under EPA's Office of Prevention, Pesticides and Toxic Substances (OPPTS), which combines the testing guidance and requirements of OPPTS with those of the Office of Pesticide Programs (OPP) and the Organization for Economic Cooperation and Development (OECD). In addition, the potential immunotoxicity of phenol was examined in male rats following 13 weeks of phenol exposure.

One hundred percent phenol was purchased, and stored under conditions designed to minimize oxidation/degradation. Formulations of phenol in the drinking water were prepared weekly, and concentrations confirmed using reverse-phase high-performance liquid chromatography.

Parental (P1) rats of the Sprague-Dawley strain (obtained from Taconic Farms, Germantown, NY) were randomly assigned to four groups of 30 animals per sex per group, and given phenol in drinking water at concentrations of 0, 200, 1000, or 5000 ppm. According to the authors, the corresponding average daily intake of phenol for an adult rat at 1000 ppm was equivalent to about 70 mg/kg-day for males, and 93 mg/kg-day for females. Treatment of P1 animals began at approximately six weeks of age, at ten weeks prior to mating. Treatment was continued throughout mating, gestation, and lactation, until sacrifice for necropsy.

Starting at three weeks prior to mating, female cyclicity was evaluated by examination of daily vaginal lavage specimens. Animals were caged for mating on a one female to one male basis. Daily lavage was continued during the mating period, until a sperm positive smear was detected (gestation day 0). Dams were allowed a natural parturition.

P1 males no longer needed for mating were randomly assigned (10 males per group) to undergo clinical toxicology evaluation and immunotoxicity screening. Five extra male rats, which had not been otherwise assigned to a study group, were randomly selected as positive controls for the immunotoxicity screening.

On postnatal day (PND) 0, the day of observed parturition, the pups were counted, sexed, and examined grossly. On PND 4, the F1 litters were culled to four pups per sex per litter. Pups were weaned on postnatal day 22, and selected F1 offspring (at least one per sex per litter) were housed singly, and continued on the same concentration of phenol in water as their parents. This treatment continued for 11 weeks prior to mating. F1 adults of approximately 14 weeks of age were then paired for mating as described above, with care taken to avoid sibling matings. Treatment was continued throughout mating, gestation, and lactation, and until sacrifice.

Immediately after the mating phase of the study for the P1 and F1 generations, at least 20 males per group were sacrificed and their reproductive organs prepared for evaluation. The right testis and epididymis from each selected male were removed, trimmed of excess fat, and weighed. Sperm samples taken from the right caudal epididymis, at the origin of the vas deferens, were videotaped for motility analysis. Total and progressive motility were assessed for each of two epididymal sperm samples per animal from the control and 5000 ppm groups. Sperm count and sperm morphology were evaluated from

macerated cauda epididymides from males of all groups. Homogenization-resistant testicular sperm counts were determined for P1 and F1 controls and 5000 ppm group males, as well as for F1 males of the 1000 ppm group.

At least 20 rats per sex per group in the P1 and F1 generations were subjected to gross necropsy. Weights were taken for: uterus, ovaries with oviducts, testes, epididymides (left, total, and left caudal), prostate, brain, liver, kidneys, adrenals, spleen, thymus, and seminal vesicles. Histological assessments were performed on selected tissues from 20 randomly selected rats in the control and 5000 ppm groups from P1 and F1 generations. Tissues prepared for histology included: vagina, uterus, ovaries and oviducts, cervix, testes, prostate, and seminal vesicles. Spleen, thymus, liver, and kidneys from 10 randomly-selected rats/sex in the control and 5000 ppm groups of both generations were also evaluated histologically.

Litters of F2 pups were evaluated and culled as described above for the F1 generation. F2 animals were not intentionally exposed to the test substance in drinking water, although pre-weaning pups may have drunk some of their dam's water supply. F2 rats and culled pups were euthanized and discarded without necropsy.

Results:

Phenol consumption:

F1 weanlings (treated from approximately three weeks of age) consumed proportionately far more water, and hence phenol, during the first week of treatment than did the P1 animals (started on treatment at approximately six weeks of age), but by week 10, the amounts consumed were similar for the generations.

Table 12. Phenol consumption (mg/kg-day) of P1 and F1 males at weeks 1 and 10

Group	0	200 ppm	1000 ppm	5000 ppm
P1 week 1	0	24	118	476
P1 week 10	0	15	71	301
F1 week 1	0	32	171	860
F1 week 10	0	14	70	319

Table 13. Phenol consumption (mg/kg-day) of P1 and F1 females at weeks 1 and 10

Group	0	200 ppm	1000 ppm	5000 ppm
P1 week 1	0	17	87	353
P1 week 10	0	20	93	321
F1 week 1	0	35	172	824
F1 week 10	0	21	94	380

Mortality:

Three P1 male rats (one control and two 5000 ppm group animals) died during the study. The authors did not consider these deaths to have been treatment related. Shortly after weaning, three F1 pups from the 5000 ppm group died. The authors attributed these deaths to aversion to the taste of phenol, because the animals were not drinking the phenol-containing water. However, a toxic effect of the test chemical was not ruled out. Three F1 females also died spontaneously later on during the course of the study: a control female died during gestation, and one female each from the low and high concentration groups died during parturition. Again, the authors did not consider the deaths to be treatment related.

Water consumption:

For both sexes in both generations, water consumption by animals given phenol at concentrations of 200 or 1000 ppm phenol did not differ from that of control animals.

For P1 animals of both sexes, water consumption was significantly reduced in the 5000 ppm group at virtually all time points measured (weekly for males, except during cohabitation; and weekly prior to mating for females, then at specified intervals during gestation and lactation. $P \leq 0.05$ in all cases). For males of the 5000 ppm phenol group, the average daily liquid intake ranged between 71% and 86% of control values for any given study week. For high concentration group females, the corresponding range was 61% to 82% of control levels. A generally similar pattern of water consumption was reported for the F1 generation.

Food consumption:

Food consumption by the P1 generation was reduced in the 5000 ppm group males at weeks 1 and 14, and for 200 ppm concentration group males at week 10. Similar reductions were seen in P1 generation, 5000 ppm group females at weeks 1, 2, 3 and 5, as well as throughout lactation ($P \leq 0.05$ in all cases). For the F1 generation, daily food consumption by 5000 ppm group males was significantly decreased at all time points measured (study weeks 1-17), but for F1, 5000 ppm dams, significant reductions in food consumption were seen only at weeks 2, 3, and 5, with no significant differences from controls during gestation and lactation. Feed consumption by 1000 ppm, F1 females was significantly increased over controls ($p \leq 0.05$) for study week 11.

Body weight:

The mean body weight of P1 animals was significantly reduced in 5000 ppm group males at weeks 4-15, and in 5000 ppm group females during the premating, gestation, lactation, and the post-lactation time points at study weeks 17 and 18 ($p \leq 0.05$ in all cases). Female weights in the 5000 ppm group remained at about 90% of control weights during the premating period and gestation; during lactation, mean body weights for this group

dropped to about 85% of control values, but rose back to 90% at the postlactation time points.

Body weights of F1 males and females exposed to 5000 ppm phenol were consistently significantly lower than respective controls at all of the time points measured ($p \leq 0.05$ in all cases). At week 0 of the premating period, body weights for this group averaged 60% of control values. Treated animals generally continued gaining on their control counterparts, such that by termination at study week 21, body weights of 5000 ppm-exposed, F1 females were approximately 89% of the corresponding controls. Body weight gain:

For both males and females of the P1 generation, body weight gain was significantly reduced at 5000 ppm phenol only at week 1 and when summed over the entire premating period ($p \leq 0.05$ in all cases). At week 8 of the premating period, males of the 200 ppm phenol group showed significantly *greater* weight gain than control males ($p \leq 0.05$). Considering only the postmating period (study weeks 13, 14, and 15), control males gained 26 ± 9.9 g during these weeks, while 5000 ppm group males are reported to have gained only 15 ± 24.4 g. While the extreme variability seen within the 5000 ppm group seems to have arisen between weeks 14 and 15, potential explanations are not addressed in the study report or the published paper.

For females, pre-mating body weight gain at 5000 ppm was 82% of that observed for untreated control animals. On gestation days 7 and 14, maternal body weight gain was similar among all groups. By gestation day 20, however, a significant decrease in body weight gain was observed in 5000 ppm group dams, with the total gain by the end of gestation also significantly reduced in the 5000 ppm group ($p \leq 0.05$ in both cases). Maternal body weight gain was not affected by treatment during the lactation phase of the study, although, as already noted above, mean maternal body weight was significantly reduced in 5000 ppm group animals throughout these time points.

F1 body weight gain was significantly decreased in the 5000 ppm groups of both sexes during the first week of post-weaning treatment ($p \leq 0.05$). However, weight gain approached control values after study week 3 for males and study week 1 for females. Summed over the premating phase, total weight gain in males was significantly decreased relative to controls ($p \leq 0.05$). At week 16, however, the first week of the postmating phase, body weight gain of F1 males was significantly *greater* for animals in all three treated groups (200, 1000, or 5000 ppm phenol) than in control animals ($p \leq 0.05$ in all cases).

For F1 females exposed to 5000 ppm phenol, significant decreases in weight gain were seen only during week 1 of the premating period, and for total gestational weight gain ($p \leq 0.05$ in both cases). A significant increase in weight gain was seen for F1, 5000 ppm group females during study week 3 ($p \leq 0.05$). While total gestational weight gain for the 5000 ppm group was reduced to 89% of the control value, no significant differences were seen between treated and control animals at any of the interim weighings (gestation days 7, 14, and 20).

Mating and fertility:

There were no indications of fertility problems in either generation. Mating performance and fertility in both generations were similar to controls and there were no observed effects on vaginal cytology or male reproductive function. The average gestation length for all groups for both generations was approximately 22 to 22.5 days.

Litter size and postnatal mortality

There were no significant differences among groups for live litter size at birth in either generation. Ninety percent of 5000 ppm F1 pups were alive on PND 4, as compared to 99% of controls. This difference was statistically significant at $P \leq 0.05$. For F2 pups, only 67% of high dose pups were still alive on PND 4, as compared to 93% for controls (significant at $P \leq 0.05$). After culling, 96% of F1 high-concentration group pups survived the interval between postnatal days seven and 21, but in the F2 generation, only 74% of 5,000 ppm group pups survived that same interval ($P \leq 0.05$).

Litter weights and growth

For each of postnatal days 0, 4, 7, 14, and 21, litter mean weights of the 200 and 1000 ppm group pups did not differ significantly from control values for either the F1 or F2 generations. For the 5000 ppm group, pup weights were significantly lower than control values for both generations at all time points measured ($P \leq 0.05$). The authors presume that the reduced birth weights of 5000 ppm group F1 offspring were at least partially the direct result of the reduced maternal body weights in this group. The data do not address causal relationships, however, and so an independent effect of treatment on the offspring cannot be ruled out.

The authors note that the difference in body weight between control and high-concentration group pups continued to increase from approximately 5% on day 0 to 30% by PND 21. A particular decrease stated to have occurred between PNDs 7 and 21 was attributed to the pups' aversion to the taste of phenol in the drinking water, which they were beginning to consume directly. No data on pup water consumption are presented to demonstrate the pups' reliance on water, as opposed to milk, as a source of fluid. For F2 offspring, a similar pattern of reductions in body weight was reported, and the same explanation offered.

Developmental landmarks and organ pathology

For F1 males the average time to preputial separation was 47.8 days in the 5000 ppm group, as compared to 44 days for the control group. Similarly, vaginal patency in F1 5000 ppm group females occurred at 38.3 days, as compared to 34.6 days for controls. These differences were statistically significant at $P \leq 0.05$ for both sexes.

Table 14. Attainment of Developmental Landmarks by F1 Offspring (age in days; weight in g)

	0	200 ppm	1000 ppm	5000 ppm
Age at preputial separation	44.0 ± 2.16	45.4 ± 2.05	44.4 ± 20.2(?)	47.8* ± 3.13
Body weight at onset (male)	229 ± 23.0	236 ± 23.4	227 ± 19.7	195* ± 23.6
Age at vaginal opening	34.6 ± 1.85	34.5 ± 1.83	34.1 ± 2.25	38.3* ± 2.21
Body weight at onset	126 ± 13.7	127 ± 13.5	121 ± 12.6	112* ± 7.8

* Significant difference from controls a $P \leq 0.05$.
(?) Probably a misprint that should be 2.02

For both sexes in both generations several absolute organ weights were found to be significantly reduced in the 5000 ppm group relative to controls. For example, in 5000 ppm group P1 females, the absolute weights of adrenals, brain, ovaries, and spleen were all found to be significantly reduced ($P \leq 0.05$). On the other hand, when considered relative to total body weights, brain, kidney, and liver weights for these same animals were all significantly increased as compared to control animals ($P \leq 0.05$). With the exception of the uterus, relative weights of other organs did not differ significantly from controls. Significant reductions in both absolute and relative uterine weights were noted in all three phenol-treated groups in the F1 generation, but not the P1 generation.

The study's authors noted an increased incidence of uterine dilatation in the control group (7/20) as compared to the high-concentration group (2/20). They contend that this uterine dilatation was related to the significantly lower absolute and relative uterine weights in the phenol-treated groups. The authors also note higher numbers of control females in estrus or proestrus at necropsy, as compared to 5000 ppm group females, although there were no significant differences among groups in cycle length. The average the length of the estrous cycle for females in this study ranged from 3.8-4.7 days and was similar for all groups for both generations.

Significant decreases in absolute prostate weights were noted in all three phenol treated groups in the F1, but not in the P1 generation. Relative prostate weights were reduced in both the 200 and 1000 ppm groups, but only reached statistical significance ($p \leq 0.05$) for the 1000 ppm group. Absolute weights of testes and epididymides were significantly reduced ($p \leq 0.05$) at 5000 ppm only for F1, not P1, animals. Absolute weights of the seminal vesicles were significantly decreased ($p \leq 0.05$) in both generations exposed to 5000 ppm phenol. Relative seminal vesicle weights did not differ significantly among groups in either generation or any concentration group. Absolute epididymis weight showed some reduction at 1000 ppm in F1 males, but did not reach statistical

significance. Relative testes, epididymides, and right cauda epididymides weights were all significantly increased ($p \leq 0.05$) in F1 animals of the 5000 ppm group.

Table 15. Reproductive Organ Weights of F1 Offspring (g)

	0	200 ppm	1000 ppm	5000 ppm
Prostate (absolute)	0.896 ± 0.197	0.765* ± 0.165	0.742* ± 0.166	0.755* ± 0.155
Final body weight (male)	515 ± 39.6	511 ± 31.4	503 ± 35.2	423* ± 31.0
Prostate (relative)	0.175 ± 0.039	0.150 ± 0.031	0.147* ± 0.027	0.179 ± 0.036
Uterus (absolute)	0.806 ± 0.332	0.621* ± 0.134	0.604* ± 0.169	0.533* ± 0.168
Final body weight (female)	294 ± 22.3	303 ± 23.0	304 ± 21.3	262* ± 17.6
Uterus (relative)	0.27 ± 0.106	0.21* ± 0.044	0.20* ± 0.058	0.20* ± 0.068

* Significant difference from controls a $P \leq 0.05$.

Epididymal sperm counts (cells/g cauda) were not affected by phenol treatment in either generation. Neither testicular sperm count (cells/g testis), nor sperm production rate (cells/per day) were affected by phenol exposure in the P1 generation. However, relative to controls, statistically significant increases in testicular sperm count and production rate were noted in the 5000 ppm group of the F1 generation.

Studies of sperm motility and morphology did not reveal any differences between control and high-concentration group animals.

Microscopic examination failed to show any adverse treatment related lesions in the reproductive or other organs of either sex of either generation, for any of the treatment groups.

Immune system, clinical chemistry, and hematology:

Evaluation of immune system endpoints revealed no significant changes in spleen weight, cellularity (cells/spleen), or antibody-forming cells (per/spleen or per 10^6 cells) for any phenol treated group compared to controls. Effects were seen in a positive control group, which was treated with cyclophosphamide.

Clinical chemistry and hematology parameters were unaffected by phenol treatment apart from a minimal, but statistically significant, increase in blood urea nitrogen in the 5000 ppm group following 13 weeks of treatment.

D.2.2. Male reproductive toxicity

Apart from the pair-based reproductive toxicity studies (Heller and Pursell, 1938; Ryan et al., 2001; IIT Research Institute, 1999) described above in Section D.2., two additional studies address endpoints of male reproductive toxicity (Bulsiewicz 1977; Shelby et al., 1993). As the pair-based reproductive toxicity studies are described in detail in the preceding Section D.2.1. (Pair-based studies) of Section D. (Reproductive Toxicity), they will not be described again here; implications of data from the pair-based studies will be discussed in detail in the integrated evaluation for male reproductive toxicity below (Section D.3.1.).

Bulsiewicz, H. (1977) The influence of phenol on chromosomes of mice (Mus musculus) in the process of spermatogenesis

Bulsiewicz (1977) studied the effects of phenol on the chromosomes of germ cells in five generations of male mice. Sexually mature male and female Porton mice, approximately 30-days of age, were divided into groups of six animals/sex/dose, with five animals/sex for the control group. Animals were given 2 ml of water containing phenol at concentrations of 0, 0.08, or 0.8 mg/l, or 8.0 g/l by gavage on a daily basis for 30 days prior to mating. Based on an average body weight of 25 g, as reported by the authors for sexually mature males, these concentrations corresponded to doses of 0, 6.4, 64, and 640 mg/kg bw.

The duration of the mating period was not reported. Males were sacrificed after mating; females continued to receive phenol treatment throughout pregnancy and lactation. Ten days after birth, one male and one female pup were selected from each litter (total of 5 or 6 animals per sex per group) and given phenol solution for 30 days. At the end of this 30-day period, the animals were mated to produce the F_2 generation. The protocol was repeated to produce a total of five generations. A total of 30 (control group) or 36 males sacrificed immediately after mating (5 or 6 males per group from each generation of a total of five generations) were included for chromosome evaluation.

Germ cells from the testes of these males were prepared for chromosome evaluation by methods that were not reported in detail, but rather cited to publications from other laboratories. Chromosomes in twenty spermatogonia and 120-150 primary spermatocytes in metaphases from each mouse were stained and examined on a light microscope at X1500 magnification. Detailed criteria for identification of chromosomal aberrations were not reported by the authors, but were cited to the published literature. According to the authors, chromosomal anomalies (aberrations) were identified according to the nomenclature for either human or mouse chromosomes. Three major anomalies presented in the tables of the original report included fractures and breaks of chromatid, aneuploidy, and polyploidy. The authors did not report use of any statistical analysis.

Over the course of the study, no animals died at either 0.08 or 0.8 mg/ml in any generation. Among animals of the 8.0 g/l group, eight mice died in the F_3 generation, 16 in the F_4 generation, and 22 in the F_5 generation; however, the total number of mice included for observation was not reported. Three additional mice from the 8.0 g/l group were sacrificed prior to schedule, due to their moribund condition. The authors did not report any information on the causes of death. No information on treatment-related effects on body weights or other general toxicity was provided.

The major findings as reported by the authors are summarized in Tables 16 and 17 below. Data presented in these tables are based on those from the tables in the original publication. However, OEHHA staff noticed that the total number of metaphase plates examined (for spermatogonia from the control group and primary spermatocytes from the 8.0 mg/l group) or the number of metaphase plates without chromosomal aberrations (for spermatogonia from the 0.8 and 8.0 mg/l group) reported in the original tables were slightly different from those stated in the text. These inconsistencies, marked in the tables below, do not appear to alter overall patterns or trends in the findings.

Table 16. Chromosomal aberrations in spermatogonia of Porton mice based on data presented in the Table 1 and 2 of original report by Bulsiewicz (1977)

Treatment	Generation of animals	No. of metaphase plates examined	No. of metaphase plates without aberration	No. of chromosomal aberrations observed		
				Fractures or breaks of chromatid or chromosome	Aneuploidy	Polyploidy
Control	P	120	118	1	0	1
	F1	120	114	0	3	3
	F2	120	118	2	0	1
	F3	120	117	0	1	2
	F4	120	118	1	1	1
	F5	120	118	0	2	0
	Total	**720[A]**	**703**	**4**	**7**	**8**
0.08 mg/l	P	120	110	6	2	4
	F1	120	88	16	14	2
	F2	120	85	21	11	6
	F3	120	84	13	16	10
	F4	120	85	18	12	8
	F5	120	70	20	16	14
	Total	**720**	**522**	**76**	**71**	**44**
0.8 mg/l	P	120	90	13	6	13
	F1	120	80	31	18	19
	F2	120	55	37	21	17
	F3	120	50	30	27	19
	F4	120	38	43	25	28
	F5	120	23	49	31	26
	Total	**720**	**336[B]**	**203**	**128**	**122**
8.0 mg/l	P	120	75	20	12	16
	F1	120	50	32	21	23
	F2	120	32	44	23	27
	F3	100	12	20	36	32
	F4	120	14	54	33	37
	F5	80	0	71	30	45
	Total	**660**	**183[C]**	**241**	**155**	**180**

Note: The numbers marked are different from those stated in the text. The numbers stated by the authors in the text are: A = 750, B=340, and C=123.

Table 17. Chromosomal aberrations in primary spermatocytes of Porton mice based on data presented in the Table 3 of the original report by Bulsiewicz (1977)

Treatment	Generation of animals	No. of metaphase plates examined	No. of metaphase plates without aberrations	No. of chromosomal aberrations observed		
				Fractures or breaks of chromatid or chromosome	Aneuploidy	Polyploidy
Control	P	698	695	2	0	1
	F1	745	743	2	3	0
	F2	873	868	0	0	2
	F3	718	714	0	1	4
	F4	700	699	0	1	0
	F5	595	590	1	2	2
	Total	**4329**	**4309**	**5**	**7**	**9**
0.08 mg/l	P	650	635	2	6	10
	F1	760	720	3	13	18
	F2	720	680	0	17	22
	F3	700	661	4	21	17
	F4	740	692	6	24	31
	F5	620	553	13	17	42
	Total	**4190**	**3941**	**28**	**98**	**140**
0.8 mg/l	P	640	611	9	13	28
	F1	670	613	6	17	39
	F2	740	620	12	64	53
	F3	770	621	7	103	50
	F4	760	403	9	96	174
	F5	850	500	11	180	140
	Total	**4430**	**3368**	**54**	**473**	**484**
8.0 mg/l	P	640	610	16	18	86
	F1	700	540	34	13	85
	F2	770	585	29	29	101
	F3	520	326	45	37	74
	F4	740	510	49	35	103
	F5	500	202	71	74	132
	Total	**3870[A]**	**2773**	**244**	**206**	**581**

Note: A: The author stated in the text that this number is 3970.

In the control group, chromosomal aberrations were observed in 17 out of 720 (2.4%, from table) or 750 (2.3%, from text) spermatogonia at metaphase. Among 4,329 diakinetic-metaphasal plates of primary spermatocytes, 20 (0.4%) of them contained chromosomes with aberrations.

At the lowest concentration of phenol tested (0.08 mg/l), chromosomal changes were observed in 198 out of 720 (27.8%) spermatogonia and 249 of 4190 (5.9%) primary spermatocytes. At the middle concentration (0.8 mg/l), chromosomal aberrations were observed in 384 (53.3%, from table) or 380 (52.7%, from text) spermatogonia and 1062 of 4430 (24.0%) diakinetic-metaphasal plates of spermatocytes.

In the high-dose group (8.0 g/l), 477 (72.3%, from table) or 537 (81.4%, from text) of 660 spermatogonia showed abnormalities, as did 1097 of 3870 (28.3%, from table) or 1097 of 3970 (30.2%, from text) primary spermatocytes. In addition, testes from three mice sacrificed moribund showed a total absence of primary and secondary spermatocytes, spermatids, and spermatozoa. Large numbers of proliferating spermatogonia were observed in these tissues. All metaphasal plates of spermatogonia from these three animals showed chromosomal abnormalities. Data from these animals were not included with the rest of their dose group.

Based on the findings summarized above, the author of the study concluded that phenol produced dose-related chromosomal changes in mouse spermatogonia and primary spermatocytes, and that the phenol-induced chromosomal aberrations increased in intensity with succeeding generations.

Shelby, M.D. et al. (1993) Fertility, reproduction, and genetic disease: studies on the mutagenic effects of environmental agents on mammalian germ cells

The National Toxicology Program (NTP) tested phenol for mammalian germ-cell mutagenicity due to concerns for potential occupational exposure (Shelby et al., 1993). The methods and results of this test are reported in a very general fashion as part of a larger review of mutagenicity in mammalian germ cells. Phenol was listed as "negative," based on unpublished NTP data on dominant lethality in the male mouse; details of methods and data were not reported.

D.2.3. Female reproductive toxicity

Information relevant to female reproductive toxicity is available from the pair-based reproductive toxicity studies conducted in rats (Heller and Pursell, 1938; Ryan et al., 2001; IIT Research Institute, 1999), a reproductive study in mice in which only females were treated (Bishop et al., 1977), and studies of developmental toxicity conducted in rats and mice (RTI, 1983 a and b; Argus, 1997).

As the pair-based reproductive toxicity studies are described in detail above in Section D.2.1. (Pair-based studies) of Section D. (Reproductive Toxicity), they will not be described again here; implications of data from the pair-based studies will be discussed in detail in the integrated evaluation for female reproductive toxicity below (Section D.3.2.).

Standard, regulatory developmental toxicity studies of phenol have been conducted in rats and mice (RTI, 1983 a and b; Argus, 1997). The protocols and results of these studies are described in detail in section C.2. of this document, under the heading "Developmental Toxicity in Animals." Only the aspects of these studies pertaining to the potential female reproductive toxicity of phenol will be reiterated below.

Bishop, JB et al. (1997) Alterations in the reproductive patterns of female mice exposed to xenobiotics

Bishop et al. (1997) gave a single, i.p., injection of phenol to female mice that were then single-pair mated with untreated males for a minimum of 347 days post-treatment. The protocol of this study involved a single intraperitoneal injection of one of 29 different chemicals (including phenol) given prior to mating and gestation. Thirty to 36 females were used in each treatment and control group. The exact dose of phenol is not presented, but all doses were said to have been determined on the basis of the maximum tolerated dose (MTD) over a 30-day survival experiment. In the reproductive experiments, data were collected on litter size and the total number of litters produced during the reproductive lifespan of the treated animals. No other endpoints, such as offspring weights, were evaluated. As phenol had no effect on litter size or the total number of litters produced in this test system, it was scored as a negative.

Research Triangle Institute (RTI) (1983a) Pilot study

In a pilot developmental toxicity study performed in rats (Sprague-Dawley rats obtained from Charles River Laboratories, Inc., and referred to in the document as "CD rats"), doses were 0, 60, 80, 100, 125, 160, 200, 250, or 320 mg/kg-day, with maternal mortality increasing from 0% to $\geq$ 17% at doses $\geq$ 160 mg/kg-day (RTI, 1983a). One hundred percent of females (7-8 females/dose group; 25 controls) were found to be pregnant at sacrifice at doses of 60, 80, 100, 125, 200, and 250 mg/kg-day. Pregnancy rates were 95.8% at 0 mg/kg-day, 80% at 160 mg/kg-day, and 0% at 320 mg/kg-day (out of only one surviving animal at this dose). For the remaining pregnant animals, there were no statistically significant differences among dose groups in the number of implantation sites per litter, the numbers or frequencies of resorptions per litter, or the numbers or percentages of litters having at least one resorption. There were no dead fetuses found in any of the groups, and no significant differences between groups in the number of live fetuses per litter.

Research Triangle Institute (RTI) (1983a) Main study

In the full-scale follow-up study using doses of 0, 30, 60, and 120 mg/kg-day, at least 95% of females out of each group of 23 were found to be pregnant on gestation day 20 (RTI, 1983a). There were no differences among dose groups in the number or percentage of resorptions or implantation sites per litter; no dead fetuses were observed in any of the dose groups. However, there were significant increases in the proportion of litters with

resorption sites in the 30 and 60 mg/kg-day dose groups ($p < 0.05$ or 0.01, respectively), but not at 120 mg/kg-day.

Argus (1997) Pilot and main studies

In another range-finding study performed in rats (Sprague-Dawley rats obtained from Charles River Laboratories, Inc.), no differences were found between dose groups (eight pregnant animals each at 0, 60, 120, 180, 270, and 360 mg/kg-day) in the numbers of corpora lutea, implantations, live fetuses, or resorptions (Argus, 1997). In the full-scale study (Argus, 1997), 25 timed-mated females were assigned to each dose group (0, 60, 120, and 360 mg/kg-day). The numbers of corpora lutea or implantation sites, resorption frequency, or live litter size were not affected by treatment.

Research Triangle Institute (RTI) (1983b) Pilot study

Doses of 0, 100, 200, 230, 260, 275, 300, or 400 mg phenol/kg-day were used in a pilot developmental toxicity study performed in pregnant CD-1 mice (RTI, 1983b). Group sizes ranged from five to 27. There were no significant differences among groups for maternal weights through the time of sacrifice on gestation day 17. Nor were there differences among groups in gestational weight gain or absolute weight gain. Maternal weight gain over the treatment period (gestation days 6-15), however, showed significant differences from controls at 230, 260, 275, and 400 mg/kg-day ($p < 0.05$ or 0.01); no differences were seen at 100, 200,or 300 mg/kg-day. Two females, one out of six in each of the 230 and 260 mg/kg-day dose groups, were found to have totally resorbed litters, a finding which appears to have led to a statistically significant increase in the average number of resorptions per litter for these two dose groups ($p < 0.01$ or 0.001). The number and percentage of dead fetuses per litter were significantly increased at 260 and 400 mg/kg-day ($p < 0.01$ or 0.001). The number, but not the percentage, of litters having at least one dead fetus was significantly increased at 260 mg/kg-day ($p < 0.01$). The number and percentage of nonlive fetuses per litter were significantly increased at 230 and 260 mg/kg-day ($p < 0.05$ or 0.01). However, no differences were found among dose groups in the number of implantation sites, or in the number of live fetuses (in litters having at least one live fetus).

Research Triangle Institute (RTI) (1983b) Main study

In the full-scale follow-up study (RTI, 1983b), there were at least 22-29 pregnant females in each group of 31-36 plug-positive females originally assigned to each treatment group (0, 70, 140, and 280 mg/kg-day). There were no significant differences among groups in the numbers of females pregnant at sacrifice, the number of implantation sites per litter, the number or percentage of resorptions per litter, the number or percentage of litters having at least one resorption, the number or percentage of dead fetuses per litter, or the number or percentage of litters having at least one dead fetus, the number or percentage of nonlive fetuses per litter, the number or percentage of litters having at least one nonlive fetus, or the mean live litter size. One dam at 70 mg/kg-day and two dams at 280 mg/kg-day had totally resorbed litters.

D.2.4. Other relevant data

Veeramachaneni, DNR et al. (2001) Long-term effects on male reproduction of early exposure to common chemical contaminants in drinking water.

Forty pregnant Dutch-belted rabbits were divided into three groups and given either plain deionized water, or chemical-containing drinking water at 1X or 3X. At 1X, the mixture contained the following chemicals in the specified ppm amounts: 7.75 arsenic, 1.75 chromium, 9.25 lead, 12.5 benzene, 3.75 chloroform, 8.5 phenol, and 9.5 trichloroethylene. Exposure was begun on gestation day 20 and continued after delivery through the end of lactation. Male offspring continued to be exposed via drinking water from the time of weaning until 15 weeks of age, when they were provided with untreated water. Reproductive parameters were assessed at 57-61 weeks of age.

On this treatment regimen, pregnant dams on gestation day 25 received an average of 0.52 mg phenol/kg bw, and pups of 8-14 weeks of age received daily averages of 0.86-1.40 mg/kg bw (1X) or 1.79-2.79 mg/kg bw (3X).

Although total sperm per ejaculate and daily sperm production were unaffected by treatment, the frequency of abnormal sperm increased significantly ($p < 0.05$) in the treated groups. Weights of testis and epididymis were not significantly affected by treatment. Baseline serum LH levels were significantly ($p < 0.05$) lower in treated than control animals; testosterone secretion in response to injection of human chorionic gonadotrophin was low ($p < 0.04$). Sexual behavior, as assessed by ejaculation in response to a teaser animal, was impaired in the chemical-exposed animals.

D.3. Integrative evaluation

D.3.1. Male reproductive toxicity

No studies were identified on the potential of phenol to cause reproductive harm in human males. Two pair-based studies (Hellar and Pursell, 1938; IIT Research Institute, 1999; Ryan et al., 2001) have investigated the effects of phenol on reproduction in experimental animals, with some additional relevant data provided by Bulsiewicz (1977). Veeramachaneni et al. (2001) studied rabbit reproduction after early exposure to a mixture of chemicals that included phenol. In their table of mammalian germ cell assay results pertinent to potential paternally-mediated developmental toxicity, Olshan and Faustman (1993) noted phenol as having negative results in one or more dominant lethal assays conducted in male mice.

The Veeramachaneni et al. (2001) study using a mixture of chemicals that included phenol found increased frequencies of abnormal sperm, and adverse effects on male hormone levels and sexual behavior in treated animals relative to controls. While it is not

possible to attribute the effects observed in this study directly to phenol, as opposed to other chemicals in the mixture, this study demonstrates the potential for early exposures to low doses of chemical agents to have lasting effects on the male reproductive system.

In the Bulsiewicz (1977) study, dose-dependent chromosomal aberrations were reported in spermatogonia and primary spermatocytes of male mice treated by gavage with a solution of phenol in water. The frequency of phenol-induced aberrations appeared to increase with successive generations. Three males exposed to approximately 640 mg phenol/kg bw-day were sacrificed ahead of schedule due to their moribund condition. These animals showed a complete absence of primary and secondary spermatocytes, spermatids, and spermatozoa. However, it should be noted that in a review of the literature on chemical-induced aneuploidy in mammalian male germ cells (Allen et al., 1986), the Bulsiewicz (1977) study was classified as inconclusive. The reasons given for this classification were protocol uncertainties, indirect or questionable criteria for polyploidy, and the lack of separate data on hyperploidy.

Heller and Pursell (1938) described adverse effects on offspring growth and viability following consumption of phenol at concentrations of 7,000 ppm and above in drinking water, over several generations of rats. As neither effects nor doses of phenol were quantified, these data remain merely suggestive of a possible effect of phenol on reproduction.

A two-generation reproductive toxicity study in rats exposed to phenol-treated drinking water was published by Ryan et al. (2001) and submitted in complete form (IIT Research Institute, 1999) to U.S. EPA as part of an Enforceable Consent Agreement with that agency. Findings for the highest phenol concentration of 5000 ppm included significant reductions in feed and water consumption, reductions in mean body weight and changes in the patterns of body weight gain. F1 males of the 5000 ppm group were found to have reductions in absolute weights of several non-reproductive organs: adrenals, brain, liver, and spleen. Absolute adrenal and spleen weights were also significantly reduced at 1000 ppm phenol.

With respect to male reproductive organs, absolute weights of seminal vesicles, testes, and epididymides were all significantly reduced for F1 males exposed to 5000 ppm phenol. Absolute seminal vesicle weight was also significantly reduced in P1 males exposed to 5000 ppm phenol. Absolute prostate weights were significantly reduced at all three concentrations of phenol in the F1 generation, and at 5000 ppm phenol in the P1 generation. When evaluated relative to total body weight, only prostate weight in F1 males at 1000 ppm was significantly reduced as compared to controls. No phenol-associated histopathological changes in reproductive or other organs were reported for any exposure group of either generation. For F1 males of the 5000 ppm group, the average age at preputial separation was significantly delayed, and body weight at preputial separation was significantly reduced relative to controls.

No significant changes were noted in fertility or reproductive function for either generation at any phenol concentration. Neither sperm morphology, nor sperm motility

differed between control and 5000 ppm group animals. Epididymal sperm counts (cells/g cauda) were not affected by phenol treatment in either generation. Neither testicular sperm count (cells/g testis), nor sperm production rate (cells/per day) were affected by phenol exposure in the P1 generation. However, relative to controls, statistically significant increases in testicular sperm count and production rate were observed in the 5000 ppm group of the F1 generation. No data were presented on total sperm counts per epididymis or testis. Since epididymal and testicular weights in 5000 ppm, F1 males were significantly reduced, and sperm contribute to the weights of these organs, total sperm counts on a per organ basis might have revealed meaningful differences from controls.

Ryan et al. (2001) ascribe essentially all of their findings to taste aversion to the phenol-containing drinking water, rather than to any toxic effect of phenol. The authors state that, "Persistent and significant reductions in water consumption, and transient effects of food consumption were observed in the 5000-ppm group of both generations. The decreased water consumption was believed to be due to the animals' aversion to the flavor of phenol in the drinking water." However, the authors present no data pertaining to the hydration state of their experimental animals at the high phenol concentration of 5000 ppm. With respect to prostate weights, which were significantly decreased at phenol concentrations not affecting water consumption, the authors did not consider the findings to be adverse. Relevant considerations for interpreting the results reported by IIT Research Institute, 1999 and Ryan et al., 2001 include: the nature of the aversion response to contaminated water or feed, the fluid requirements of rats, the relationship between body weight and organ weights, physiological triggers for the onset of puberty, and the biological significance of prostate weight.

Animals can learn to avoid toxic food or water by availing themselves of sensory cues such as taste or smell (Forbes and Kyriazakis, 1995; Lamprecht et al., 1997; Berman et al., 1998; Ratcliffe et al., 2003). Rats are particularly adept at this type of "conditioned taste aversion" learning (Lamprecht et al., 1997; Berman et al., 1998). In the case of phenol-treated drinking water (IIT Research Institute, 1999; Ryan et al., 2001), it is not clear whether unpalatability alone prevented animals from drinking a minimal amount required to maintain health. Alternatively, the rats may have learned to associate the taste of phenol with the sensations of phenol-induced toxicity. Since phenol-treated water was the only fluid source, the rats' long-term daily fluid intake may have represented a compromise position between the toxic effects of phenol and the adverse effects of dehydration.

The mammalian system requires water for: 1) adjusting body temperature, 2) excreting the end products of digestion, 3) excreting drugs and drug residues, 4) achieving a sensation of satiety, 5) satisfying behavioral drives, and 6) lubrication of food in the mouth and stomach (Kyriazakis and Savory, 1997). Ambient temperature, level of physical activity, diurnal rhythms, protein, mineral and moisture content of the diet, as well as water cleanliness or adulteration with toxic substances can all influence fluid consumption (Kyriazakis and Savory, 1997; Ang et al., 2001; Fitzsimons and Magnen, 1969; Bolles, 1961). Rats (like other animals) have some potential to physiologically

adapt to reduced fluid intake by decreasing fluid loss by means such as increasing urine concentration (Dicker and Nunn, 1957; Fitzsimons and Magnen, 1969). Conversely, under *ad lib* conditions, rats drink a greater volume of water than the minimum required to maintain fluid balance (Fitzsimons, 1957; Barboriak and Wilson, 1969; Rowland and Flamm, 1977). Because of these potential influences and adaptations, fluid intake alone is not sufficient information to accurately assess the hydration state of experimental animals.

In a recent "Toxicological Review of Phenol," U.S. EPA (2002b) describes an unpublished 13-week neurotoxicity study that was conducted using the same dosing regimen as applied in the reproductive toxicity study reported by IIT Research Institute (1999) and Ryan et al. (2001). Groups of 15 male and 15 female Sprague-Dawley rats were given phenol in drinking water at concentrations of 0, 200, 1000, or 5000 ppm. In this study, dehydration was assessed by grabbing the scruff of each animal's neck. If the raised skin showed a delay in returning to its normal position on one or more study days, the animal was scored as dehydrated. None of the animals exposed to 200 ppm phenol were considered to be dehydrated. At 1000 ppm phenol, two out of 15 animals for each sex were considered to be dehydrated on one or more study days. At 5000 ppm phenol, most of the animals were scored as dehydrated for at least one of the study days. These animals appear to have been consuming somewhat less fluid than those exposed to the 5000 ppm phenol in the study reported by IIT Research Institute (1999) and Ryan et al. (2001) (see Table 18 below). Furthermore, as U.S. EPA (2002b) noted, "basing the analysis on the clinical sign of dehydration may not appropriately reflect whether the animals were dehydrated, because no objective measure of dehydration was used and because decreased water consumption in this group occurred throughout the study" [whether or not animals were scored as dehydrated].

Table 18. Average water consumption by animals exposed to 5000 ppm phenol in drinking water as percentage of concurrent control

Neuro study*, males	Repro study**, P1 males	Neuro study*, females	Repro study**, P1 females
60%	71-86%	40-55%	61-82%

* As reported by U.S. EPA, (2002b)
** As reported by IIT Research Institute (1999) and Ryan et al. (2001)

It should also be remembered that phenol has been demonstrated to exert toxic effects in humans and experimental animals by routes other than drinking water. Inhalation, dermal, and gavage exposures to phenol have all been associated with evidence of toxicity (ATSDR, 1998; IPCS, 1994; Bruce et al., 1987; Doan et al., 1979; RTI 1983a and 1983b; Argus, 1997; Kavlock, 1990; Narotsky and Kavlock, 1995; Bulsiewicz, 1977). The findings of such studies would not appear to support aversion to the taste of treated drinking water as the only mechanism by which phenol exposure results in toxicity.

A number of studies present information relevant to understanding the relationships between feed-restriction-induced decrements in body weight or growth rate and reproductive endpoints such as organ weights, and age at onset of puberty (Fan et al., 1997; Engelbregt et al., 2000; Carney et al., 1998; Merry and Holehan, 1981; Grewal et al., 1971; Howland, 1975; Abdo et al., 1991; Scharer, 1997; Glass and Swerdloff, 1980; Oishi et al., 1979).

Scharer (1977) notes the importance of, as well as the difficulties inherent in, distinguishing changes in organ weights attributable to generalized growth retardation from those resulting directly from test compound toxicity. Experiments with feed deprivation in rats (Grewal et al., 1971; Howland, 1975; Scharer, 1977; Oishi et al., 1979) or mice (Abdo et al., 1991) have typically resulted either in no change, or else in reduced absolute organ weights of deprived animals as compared to controls. In these same studies, however, organ weights relative to body weight may be unchanged or significantly increased over control values. Along with brain and adrenals, testes weights seem to be particularly conserved, and increase in weight in underfed groups largely as a function of age rather than body weight (Scharer, 1977). Considered in such context, a finding of significantly reduced relative prostate weight, such as was observed at a concentration of phenol below that associated with a significant reduction in body weight (IIT Research Institute, 1999 and Ryan et al., 2001), may represent a compound-specific effect.

U.S. EPA's published "Guidelines for Reproductive Toxicity Risk Assessment" (1996) include the prostate, testes, epididymides, and seminal vesicles among the male reproductive organs for which weights may be useful for reproductive risk assessment. They state that, "Significant changes in absolute or relative male reproductive organ weights may constitute an adverse reproductive effect," and expand upon the interpretation of absolute versus relative organ weights by noting:

Evaluation of data on absolute organ weights is important, because a decrease in a reproductive organ weight may occur that was not necessarily related to a reduction in body weight gain. The organ weight-to-body weight ratio may show no significant difference if both body weight and organ weight change in the same direction, masking a potential organ weight effect.

U.S. EPA's guidelines also note that "prostate and seminal vesicle weights are androgen-dependent, and therefore may reflect changes in the animal's endocrine status or testicular function."

Apart from organ weights, studies have evaluated the effects of feed restriction on age at puberty in rats (Carney et al., 1998; Merry and Holehan, 1981; Engelbregt et al., 2000; Glass and Swerdloff, 1980). Normally, preputial separation occurs prior to the appearance of mature sperm in the caput epididymis, as well as in advance of a significant increase in circulating androgen levels (Kornenbrot et al., 1977). The triggering event for preputial separation does not appear to be attainment of some minimal body weight (Carney et al., 1998; Engelbregt et al., 2000; Korenbrot et al., 1977;

Glass and Swerdloff, 1980). Rather, interference with maturational processes normally established during the perinatal period can delay preputial separation (Engelbregt et al., 2000), as can castration as late as postnatal day 35 in the absence of replacement androgen (Korenbrot et al., 1977). Korenbrot et al. (1977) concluded that preputial separation depends upon the continued presence of low levels of androgen. As noted by Clark (1993), "delays in preputial separation in a developmental or reproductive toxicity study should be considered as adverse for human health risk assessment, particularly if the effect is irreversible such as results from a permanent malformation of the prepuce and/or glans penis."

In their published "Guidelines for Reproductive Toxicity Risk Assessment" (1996), U.S. EPA discuss alterations in the onset of puberty as potential evidence of reproductive system toxicity. The guidelines note:

In males, preputial separation or appearance of sperm in expressed urine or ejaculates can serve as markers of puberty. Body weight at puberty may provide a means to separate specific delays in puberty from those that are related to general delays in development. Agents may differentially affect the endpoints related to puberty onset, so it is useful to have information on more than one marker.

...Delays in pubertal development in rodents are usually related to delayed maturation or inhibition of function of the hypothalamic-pituitary axis. Adverse reproductive outcomes have been reported in rodents when puberty is altered by a week or more, but the biologic relevance of a change in these measures of a day or two is unknown....

In the case of phenol, a mean delay of almost four days in preputial separation was seen for F1 males exposed to 5000 ppm (IIT Research Institute, 1999 and Ryan et al., 2001). At the time of preputial separation, these animals also showed an average weight decrement of 15% relative to controls. No assessment was made for the presence of sperm in urine or ejaculates at around the time the puberty, so no second marker of puberty onset is available. Nor were data on nonreproductive developmental landmarks, such as pinna unfolding, included for comparison.

D.3.2. Female reproductive toxicity

Human data on the potential female reproductive toxicity of phenol includes epidemiological investigations of workplace exposures (Axelsson et al., 1984; Hernberg et al., 1983), and studies of placentas from women considered to have had high environmental exposures (Radowicki and Wierzba, 1997a, 1997b, and 1997c; Wierzba et al. 1997; Wierzba and Radowicki, 1997a and 1997b; Liebhart et al., 1979 and 1980). Two pair-based studies have investigated the effects of phenol on reproduction in experimental animals (Hellar and Pursell, 1938; IIT Research Institute, 1999; Ryan et al., 2001). Additional relevant information comes from an injection study performed in

pregnant mice (Bishop et al., 1997), and from oral developmental toxicity studies performed in rats and mice (RTI, 1983a and b; Argus 1997).

An epidemiological investigation into the effects of phenol on pregnancy was conducted in women exposed to mixtures of disinfectants or solvents at their place of work (Axelsson et al., 1984). While no meaningful associations between phenol and adverse pregnancy outcome were identified, it is possible that due to the design and limitations of this study, it was unable to detect an association between phenol and adverse pregnancy outcomes if such an association were present.

A total of eight Polish language papers (Radowicki and Wierzba, 1997a, 1997b, and 1997c; Wierzba et al. 1997; Wierzba and Radowicki, 1997a and 1997b; Liebhart et al., 1979 and 1980) were also obtained and considered. Four of these were translated into English for OEHHA, while the others had English language abstracts available. These studies report on aspects of placental structure and histopathology for women believed to have had high environmental exposures to aromatic hydrocarbons, including phenol, and showing excessive levels of phenol in their urine as compared to women from a non-threatened area. Placentas from women having high environmental exposures showed changes consistent with impairments of placental function.

Hellar and Pursell (1938) described adverse effects on offspring growth and viability over several generations of rats consuming phenol at concentrations of 7,000 ppm and above in drinking water. As neither effects nor doses of phenol were quantified, these data remain merely suggestive of a possible effect of phenol on reproduction.

A two-generation reproductive toxicity study in rats exposed to phenol-treated drinking water was published by Ryan et al. (2001) and submitted in complete form by IIT Research Institute (1999) to U.S. EPA as part of an Enforceable Consent Agreement with that agency. Findings for the highest phenol concentration of 5000 ppm included consistently significant reductions in fluid consumption. F1 females showed significant reductions in feed consumption for weeks 2, 4, and 5 out of the 11-week premating period; there were no significant differences among groups in feed consumption during gestation and lactation. Body weights of these animals were significantly reduced throughout the premating period, gestation, and lactation; body weight gain was only significantly reduced for week 1 of the premating period, and when considered as total weight gain over the entire experimental period.

Of the organ weights evaluated, only absolute spleen and uterus weights were significantly reduced as compared to controls for F1 females exposed to 5000 ppm phenol. Relative brain, kidney, and liver weights were significantly increased for this same concentration group. Relative, as well as absolute, uterine weights were significantly reduced at all three concentrations of phenol. Absolute uterine weights were clearly decreased in a concentration-dependent manner; assessment of concentration effects on relative uterine weights is complicated by the abrupt decrease in body weight at the highest phenol concentration.

No significant changes were noted in fertility or reproductive function. There were no significant differences among groups for live litter size at birth in either generation, although pup viability on PND 4 was significantly decreased at 5000 ppm phenol, for both the F1 and F2 generations. For animals exposed to 5000 ppm phenol, litter weights were significantly lower than control values for both generations on each of postnatal days 0, 4, 7, 14, and 21, ($P \leq 0.05$). The authors presume that the reduced birth weights of high-concentration group F1 offspring were at least partially the direct result of the reduced maternal body weights in this group. The data do not address causal relationships, however, and so an independent effect of treatment on offspring cannot be ruled out.

Estrous cycle length did not differ among groups for either the P1 or the F1 generation. For F1 females of the 5000 ppm group, the average age at vaginal opening was significantly delayed, and body weight at vaginal opening was significantly reduced relative to controls.

Ryan et al. (2001) ascribe essentially all of their findings to taste aversion to the phenol-containing drinking water, rather than to any toxic effect of phenol. However, the authors present no data pertaining to the hydration state of their experimental animals at the high phenol concentration of 5000 ppm. Relevant considerations for interpreting the results reported by IIT Research Institute, 1999 and Ryan et al., 2001 include: the nature of the aversion response to contaminated water or feed, the fluid requirements of rats, the relationship between body weight and organ weights, physiological triggers for the onset of puberty, and the biological significance of uterine weight. Several of these issues have been discussed in detail in the preceding section (D.3.1. Male reproductive toxicity), and will not be repeated here. The authors also conclude that the decreased uterine weights, which were seen at phenol concentrations below that associated with decreased fluid consumption, were an artifact of stage of estrus at the time of sacrifice, and not indicative of female reproductive toxicity.

U.S. EPA's published "Guidelines for Reproductive Toxicity Risk Assessment" (1996) note the fluctuation in normal uterine weights as a function of the estrous cycle: "...uterine weights taken from cycling animals have a high variance, and large compound-related effects are required to demonstrate a significant effect unless interpreted relative to that animal's estrous cycle stage." Nonetheless, the Agency states:

Effects on the uterus that may be considered adverse include significant dose-related alteration of weight, as well as gross anatomic or histologic abnormalities. In particular any of the following effects should be considered as adverse.
Infantile or malformed uterus or cervix
Decreased or increased uterine weight [emphasis added]
Endometrial hyperplasia, hypoplasia, or aplasia
Decreased number of implantation sites

In the study reported by IIT Research Institute (1999) and Ryan et al. (2001), phenol exposure was associated with concentration-dependent decreases in uterine weight that

were of a magnitude to reach statistical significance. Decreases were evident at all three concentrations of phenol, including the two levels (200 and 1000 ppm) that were not associated with decreased fluid consumption or reductions in body weight (Fig.2).

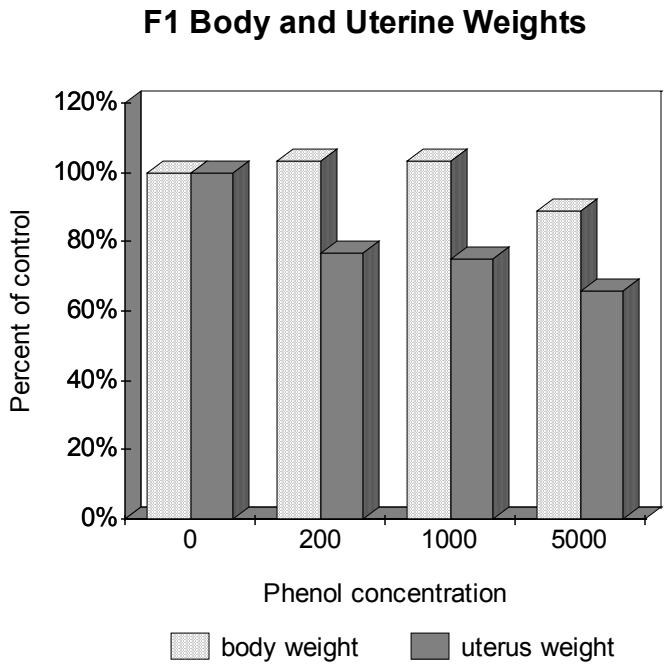


Figure 2

There were no significant differences among groups in length of the estrous cycle, so the stage of estrus at sacrifice must be presumed to have been random with respect to treatment group. Based on vaginal cytology data, Ryan et al. (2001) reports that numbers of F1 females in proestrus/estrus at necropsy were 16/24, 15/24, 13/25, and 9/22 (for controls, 200 ppm, 1000 ppm, and 5000 ppm phenol, respectively).

With respect to the issue of delayed puberty, U.S. EPA's guidelines (1996) include "altered timing of vaginal opening" among "significant effects on the vagina that may be considered adverse." Puberty in female rats does not appear to be triggered by attainment of a critical body weight, a critical total body fat, or a critical percentage of body fat (Glass et al., 1979; Glass and Swerdloff, 1980; Engelbregt et al., 2000). On the other hand, energy intake may affect the timing of puberty onset (Carney et al., 1998; Fan et al., 1997; Messer and Anson, 2000), particularly when restricted during the perinatal period (Engelbregt et al., 2000).

In the case of phenol, a mean delay of almost four days in vaginal opening was seen for F1 females exposed to 5000 ppm (IIT Research Institute, 1999 and Ryan et al., 2001). At the time of vaginal opening, these animals also showed an average weight decrement of 11% relative to controls. Vaginal cytology was not performed at the time of puberty, so

no second marker of puberty onset is available. Nor were data on nonreproductive developmental landmarks, such as pinna unfolding, included for comparison.

In a study by Bishop et al. (1997), female mice were given a single intraperitoneal injection of phenol prior to mating and gestation. Data were collected on litter size and the total number of litters produced during the reproductive lifespan of these animals. No other developmental endpoints, such as offspring weights, were evaluated. In this narrowly-focused system, phenol was scored as negative. These data do not contradict findings from other studies reporting adverse effects, however, as those studies were conducted using different dosing regimens and evaluated more comprehensive suites of reproductive and developmental endpoints.

The protocols and results of standard, oral, regulatory developmental toxicity studies of phenol conducted in rats and mice (RTI, 1983 a and b; Argus, 1997) are discussed in detail in section C.2. of this document, under the heading "Developmental Toxicity in Animals." Only those aspects potentially indicative of the female reproductive toxicity of phenol will be discussed below.

In a pilot developmental toxicity study performed in rats (RTI, 1983a), doses ranged from 0 to 320 mg/kg-day. For surviving animals, there were no statistically significant differences among dose groups in the frequency of pregnancy, the number of implantation sites per litter, or in resorption frequency. There was no evidence for adverse effects on fetal viability. Similar results were obtained in the full-scale follow-up study (RTI, 1983a), as well as in another set of pilot and full-scale studies performed in rats (Argus, 1997). The full RTI study (1983a), however, found significant increases in the proportion of litters with resorption sites in the 30 and 60 mg/kg-day dose groups ($p < 0.05$ or 0.01, respectively), but not at 120 mg/kg-day.

Doses in a pilot developmental toxicity study performed in pregnant CD-1 mice (RTI, 1983b), ranged from 0 to 400 mg phenol/kg-day. There were no significant differences among groups for maternal weights throughout the study; decreases in maternal weight gain over the treatment period was seen at doses of 230 mg/kg-day and above, with the exception of 300 mg/kg-day. No differences were found among dose groups in the number of implantation sites, or in the number of live fetuses (in litters having at least one live fetus). Mean resorptions per litter were increased in the 230 and 260 mg/kg-day groups, with one female in each of these two groups having totally resorbed litters. Adverse effects on fetal viability were seen at doses of 230, 260, and 400 mg/kg-day.

Doses in the full-scale follow-up study (RTI, 1983b) ranged from 0-280 mg/kg-day. No adverse effects on reproductive parameters were reported, although one dam at 70 mg/kg-day and two dams at 280 mg/kg-day had totally resorbed litters.

E. SUMMARY TABLES

Table 19. Animal studies of the developmental toxicity of phenol

Reference	Study design	Maternal effects	Developmental Effects
RTI, 1983a	Pilot study, CD rats 0, 60, 80, 100, 125, 160, 200, 250, 320 mg/kg-day, gds 6-15; gavage 5-8 females/group	Maternal mortality in excess of 10% at doses ≥ 160 mg/kg-day Decreased weight gain during treatment period at 160 and 250 mg/kg; trend over all doses	Trend for decreased average fetal body weight per litter with increasing dose
	Full study, CD rats 0, 30, 60, 120 mg/kg-day, gds 6-15; gavage 20-22 females/group Two replicate experiments	No evidence of treatment-related maternal toxicity	Increased number of litters with resorptions at 30 and 60 mg/kg-day, but not at 120 Significant decrease in mean fetal weight at 120 mg/kg-day; significant trend for decreased fetal weight over all doses
RTI, 1983b	Pilot study, CD-1 mice 0, 100, 200, 230, 260, 275, 300, 400 mg/kg-day, gds 6-15; gavage 5-27 females/group	≥ 15% maternal mortality at doses ≥ 275 mg/kg-day Clinical symptoms observed at doses ≥ 200 mg/kg-day Weight gain during treatment reduced at 230, 260, 275, and 400 (but not 300) mg/kg/day; significant trend Liver weights lower than controls at 230 and 260 mg/kg-day	Increased resorptions and "nonlive" fetuses at 230 and 260 mg/kg-day only Increased dead fetuses at 260 and 400 mg/kg-day only Increased "affected" fetuses at 230, 260, 300, and 400 mg/kg-day Mean fetal body weight per litter decreased at doses ≥ 260 mg/kg-day Increased malformed fetuses per litter at 200, 230, 260, 300, and 400 mg/kg-day
	Full study, CD-1 mice 0, 70, 140, 280 mg/kg-day, gds 6-15; gavage 22-29 pregnant females/group Four replicate experiments	Maternal mortality at 280 mg/kg-day Reduced body weights and weight gain at 280 mg/kg-day; significant trend effects Tremors and other clinical signs at 140 and 280 mg/kg-day	Decreased fetal body weight at 280 mg/kg-day; significant trend for decreased weight with increasing dose Nonsignificant trend for increased fetal incidence of cleft palate

Table 19. Continued

Reference	Study design	Maternal effects	Developmental Effects
Argus, 1997	Pilot study, Sprague-Dawley rats 0, 60, 120, 180, 270, and 360 mg phenol/kg bw-day, divided into 3 gavage dosings/day, gds 6-15 8 pregnant females/group	No statistically significant changes reported Apparent increase in clinical symptoms at 360 mg/kg-day Apparent decreases in body weight and weight gain at doses ≥ 120 mg/kg-day	No statistically significant changes reported Apparent decreases in fetal weight per litter at doses 120, 180, and 360 (but not 270) mg/kg-day
	Full study, Sprague-Dawley rats 0, 60, 120, 360 mg/kg-day, divided into 3 gavage dosings/day, gds 6-15 25 pregnant females/group	One maternal death at 360 mg/kg-day Reduced body weight and total weight gain at 360 mg/kg-day Reductions in body weight gain during treatment at 120 and 360 mg/kg-day Increased salivation and tacypnea at 360 mg/kg-day	Decreased fetal body weight at 360 mg/kg-day Decreased metatarsal ossification per fetus/litter at 360 mg/kg-day One fetus at 120 mg/kg-day, and one at 360 mg/kg-day had rare malformations
Kavlock, 1990	Sprague-Dawley rats 0, 100, 333, 667, or 1000 mg/kg by gavage on gd 11 12-15 pregnant animals/group	Reduced weight gain at 24 hours post-dosing at 667 and 1000 mg/kg; also reduced at 72 hours at the higher dose	Kinked tails and limb paralysis at 667 and 1000 mg/kg
Narotsky and Kavlock, 1995	Fischer 344 rats 0, 40, or 53.3 mg/kg, by gavage on gds 6-19 16-23 animals/group	Altered respiration and weight gain noted at both doses; data not presented	Reduced live pups/litter on pds 1 and 6 at 40 and 53.3 mg/kg-day 2 cases of kinked tail in one litter at 53.3 mg/kg-day
U.S. EPA, 1999; Ryan et al., 2001	Sprague-Dawley rats 0, 200, 1000, or 5000 ppm in drinking water for two generations ~ 0, 20, 94, or 351 mg/kg ~ 30 animals/sex/group	Reduced body weight and gestational weight gain at 5000 ppm (~351 mg/kg) Reduced water consumption at 5000 ppm Reduction in feed consumption at some time points at 5000 ppm	Reduced litter weight on pnd 0 at 5000 ppm (~351 mg/kg)

Table 20. Animal studies of the male reproductive toxicity of phenol

Reference	Study design	Systemic effects	Reproductive Effects
U.S. EPA, 1999; Ryan et al., 2001	Sprague-Dawley rats 0, 200, 1000, or 5000 ppm in drinking water for two generations ~ 0, 20, 94, or 351 mg/kg ~ 30 animals/sex/group	Reduced body weights and weight gain at some time points at 5000 ppm (~351 mg/kg) Reduced water consumption at 5000 ppm Reduction in feed consumption at some time points at 5000 ppm	No effects on mating or fertility Increased age at preputial separation at 5000 ppm (~351 mg/kg) Decreased absolute prostate weight at all doses Decreased relative prostate weight at 1000 ppm (~94 mg/kg)
Bulsiewicz, 1977	Mice ~ 0, 6.4, 64, or 640 mg/kg by gavage to both sexes for 5 generations	At 640 mg/kg: 8 F_3, 16 F_4, and 22 F_5 mice died 3 males at 640 mg/kg sacrificed moribund	Dose-dependent increases in frequency of chromosomal changes in spermatogonia and primary spermatocytes at all doses Moribund males had no primary or secondary spermatocytes, spermatids, or spermatozoa; excess spermatogonia

Table 21. Animal studies of the female reproductive toxicity of phenol

Reference	Study design	Systemic effects	Reproductive Effects
U.S. EPA, 1999; Ryan et al., 2001	Sprague-Dawley rats 0, 200, 1000, or 5000 ppm in drinking water for two generations ~ 0, 20, 94, or 351 mg/kg ~ 30 animals/sex/group	Reduced body weight and gestational weight gain at 5000 ppm (~351 mg/kg) Reduced water consumption at 5000 ppm Reduction in feed consumption at some time points at 5000 ppm	No effects on mating or fertility Increased age at vaginal opening at 5000 ppm (~351 mg/kg) Decreased absolute and relative uterine weights at all doses
RTI, 1983a	Pilot study, CD rats 0, 60, 80, 100, 125, 160, 200, 250, 320 mg/kg-day, gds 6-15; gavage 5-8 females/group	Maternal mortality in excess of 10% at doses ≥ 160 mg/kg-day Decreased weight gain during treatment period at 160 and 250 mg/kg; trend over all doses	100% pregnant at 60, 80, 100, 125, 200, and 250 mg/kg 80% pregnant at 160 mg/kg 0% pregnant at 320 mg/kg (1 surviving female not pregnant at sacrifice) No effect on litter frequencies of implantation, resorption, or fetal viability
	Full study, CD rats 0, 30, 60, 120 mg/kg-day, gds 6-15; gavage 20-22 females/group Two replicate experiments	No evidence of treatment-related maternal toxicity	≥ 95% pregnant at all doses Increased number of litters with resorptions at 30 and 60 mg/kg-day, but not at 120
Argus, 1997	Pilot study, Sprague-Dawley rats 0, 60, 120, 180, 270, and 360 mg phenol/kg bw-day, divided into 3 gavage dosings/day, gds 6-15 8 pregnant females/group	No statistically significant changes reported Apparent increase in clinical symptoms at 360 mg/kg-day Apparent decreases in body weight and weight gain at doses ≥ 120 mg/kg-day	No effect on per litter frequencies of corpora lutea, implantation, live fetuses, or resorptions
	Full study, Sprague-Dawley rats 0, 60, 120, 360 mg/kg-day, divided into 3 gavage dosings/day, gds 6-15 25 pregnant females/group	One maternal death at 360 mg/kg-day Reduced body weight and total weight gain at 360 mg/kg-day Reductions in body weight gain during treatment at 120 and 360 mg/kg-day Increased salivation and tacypnea at 360 mg/kg-day	No effect on per litter frequencies of corpora lutea, implantation, live fetuses, or resorptions

Table 21. Continued

Reference	Study design	Systemic effects	Reproductive Effects
RTI, 1983b	Pilot study, CD-1 mice 0, 100, 200, 230, 260, 275, 300, 400 mg/kg-day, gds 6-15; gavage 5-27 females/group	≥ 15% maternal mortality at doses ≥ 275 mg/kg-day Clinical symptoms observed at doses ≥ 200 mg/kg-day Weight gain during treatment reduced at 230, 260, 275, and 400 (but not 300) mg/kg/day; significant trend Liver weights lower than controls at 230 and 260 mg/kg-day	Increased resorptions and "nonlive" fetuses at 230 and 260 mg/kg-day only; 1/6 females in each of these dose groups had totally resorbed litters Increased dead fetuses at 260 and 400 mg/kg-day only No effects on implantation frequency or number of live fetuses (in litters having at least one live fetus)
	Full study, CD-1 mice 0, 70, 140, 280 mg/kg-day, gds 6-15; gavage 22-29 pregnant females/group Four replicate experiments	Maternal mortality at 280 mg/kg-day Reduced body weights and weight gain at 280 mg/kg-day; significant trend effects Tremors and other clinical signs at 140 and 280 mg/kg-day	No effects on frequency of pregnancy, implantation sites, resorptions, or dead fetuses. 1 totally resorbed litter at 70 mg/kg; 2 totally resorbed litters at 280 mg/kg

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