



May 8, 2014

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RE: Notice of Intent to List – Nitrite in Combination with Amines or Amides; February 7, 2014.

Dear Ms. Oshita:

The American Meat Institute (AMI) submits this letter in response to an invitation for comments in the above-referenced notice of intent to list published by the California Environmental Protection Agency's Office of Environmental Health Hazard Assessment (OEHHA or the agency).¹ AMI is the nation's oldest and largest trade association representing packers and processors of beef, pork, lamb, veal, turkey, and processed meat products, and AMI member companies account for more than 95 percent of United States output of these products. Many AMI members produce meat and poultry products that utilize nitrite and for that reason AMI has a direct interest in the published notice.

Nitrite is an important direct food ingredient to the meat and poultry industry. Nitrite is used in the production of cured meat and poultry products because it is very effective in inhibiting the growth of *Clostridium botulinum* and helps provide sufficient bacterial inhibition for pathogens that have food safety implications, such as *Listeria monocytogenes* or *Clostridium perfringens*. Nitrite has a synergistic food safety effect when used in combination with other antimicrobial treatments.

¹ The listing is proposed in accordance with California's Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65).

The public health concern regarding nitrites in meat and poultry products arose in the 1970s when certain meat products cooked at very high temperatures, e.g. bacon, were shown to produce carcinogenic *N*-nitrosamines. The meat industry and U.S. Department of Agriculture’s Food Safety and Inspection Service took this development very seriously and have enacted best practices and regulatory policies, such as limiting ingoing levels of nitrite and adding ascorbic acid to formulations. Well established in the scientific literature is the effectiveness of ascorbic acid or vitamin C in inhibiting the mechanism, both in humans and in cooking cured meat products, which produces *N*-nitroso compounds. It is the carcinogenic *N*-nitrosamines that are the public health concern not nitrite, nitrate, amines, and amides.

In the 1980s, the Food and Drug Administration nominated sodium nitrite for further carcinogenicity and genotoxicity evaluation to the U.S. National Toxicology Program (NTP) to better assess whether nitrite *per se* was a carcinogen. The two-year cancer bioassay study in rats and mice was commissioned based on nitrite’s use in cured meat and poultry products, and at that time, concerns regarding the formation of carcinogenic *N*-nitrosamines. The *NTP Technical Report No. 495* (2001) for sodium nitrite was the most definitive, chronic carcinogenic bioassay study ever conducted and the resulting conclusion went through extensive public peer review.²

The only adverse finding of this NTP “gold standard study” was “equivocal evidence” in the forestomach in female mice. Because humans do not have a forestomach, it is not considered to be an appropriate organ for human cancer hazard assessment.^{3,4} NTP’s bioassay study of sodium nitrite reported “None” for “neoplastic effects” in male and females rats and male and female mice and “no evidence” of carcinogenic activity in male and female rats and male mice.⁵ In short, NTP found no consequential toxicological hazard to humans through exposure to nitrite. Although, the proposed Proposition 65 listing is based on an authoritative body classification by the International Agency for Research on Cancer (IARC), NTP is also considered an authoritative body by OEHHA as well and AMI requests that

² National Toxicology Program. (2001). Toxicology and Carcinogenesis Studies of Sodium Nitrite (CAS NO. 7632-00-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies), Natl. Toxicol. Program. Tech. Rep. Ser., No. 495, United States, pp. 7–273.

³ Cohen, S., Arnold, L. (2011). Chemical carcinogenesis. *Toxicol Sci.* 120(suppl. 1): S76-92.

⁴ Hoenerhoff, M., Hong, H., Ton, T., Lahousse, S., Sills, R. (2009). A review of the molecular mechanisms of chemically induced neoplasia in rat and mouse models in National Toxicology Program bioassays and their relevance to human cancer. *Toxicol Pathol.* 37(7): 835-48.

⁵ National Toxicology Program. (2001). page 10.

OEHHA consider the NTP findings in its deliberations regarding this proposed Proposition 65 listing.

OEHHA referenced the 2010 IARC Monograph No. 94 on ingested nitrites and nitrates as the basis for the proposed Proposition 65 listing.⁶ IARC's overall evaluation was "*Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A).*"⁷ This conclusion was based primarily on findings of "*limited evidence in humans for the carcinogenicity of nitrite in food*" and "*sufficient evidence in experimental animals for the carcinogenicity of nitrite in combination with amines or amides.*"

IARC conducted its monograph review meeting in June 2006 and published the final monograph in July 2010. New scientific evidence, published since June 2006 and discussed in detail below, is now available. With this new scientific evidence available, AMI contends that IARC would today reach the determination of "*Not classifiable as to its carcinogenicity to humans - Group 3.*" Accordingly, based on the extensive discussion that follows, AMI urges OEHHA not to list nitrite in combination with amines or amides.

I. The Proposed Listing Does Not Accurately State the International Agency for Research on Cancer's Overall Evaluation.

As justification for the proposed listing OEHHA determined that "Nitrite in combination with amines or amides *meets the criteria for the listing as known to the State to cause cancer under Proposition 65, based on findings of the IARC (2010).*"⁸ That determination, however, is not an accurate interpretation of the IARC (2010) overall evaluation. That overall evaluation concluded "*Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A).*"⁹ Contrary to OEHHA's determination, IARC did not conclude

⁶ IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010). Ingested nitrate and nitrite and cyanobacterial peptide toxins. *IARC Monogr Eval Carcinog Risks Hum.* 94:1–464.

⁷ *Id.* at 323.

⁸ http://oehha.ca.gov/prop65/CRNR_notices/admin_listing/intent_to_list/noilpkg48cnitrite.html. Accessed February 20, 2014.

⁹ Grosse, Y., Baan, R., Straif, K., Secretan, B., Elghissassi, F., Coglianò, V. (2006). Carcinogenicity of nitrate, nitrite, and cyanobacterial peptide toxins. *The Lancet Oncology* 7, 628–629.; World Health Organization, (2006). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans Volume 94: Ingested nitrates and nitrites, and cyanobacterial peptide toxins Lyon, France. 14–21 June 2006, List of Participants.; and IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010). Ingested nitrate and nitrite and cyanobacterial peptide toxins. *IARC Monogr Eval Carcinog Risks Hum.* 94:1–464.

that nitrite *per se* was probably carcinogenic to humans. Instead, IARC found that the chemical products that result from endogenous nitrosation are probably carcinogenic to humans, but only under those dietary conditions where endogenous nitrosation in the human stomach is favored, e.g. when concurrent dietary antioxidant intake such as vitamin C or other antioxidants, is insufficient to limit or prevent such endogenous nitrosation.

IARC, in a 2008 review article in *Environmental and Molecular Mutagenesis*, emphasized that the classification of nitrite and nitrate was based not on the chemicals themselves but on the biological mechanism of endogenous nitrosation.

“This is, perhaps, the first time that a mechanism has been an intrinsic part of the definition of a suspected carcinogenic agent in humans. There were no separate assessments of nitrate or nitrite themselves, because nitrate and nitrite are interconvertible in vivo and the conditions leading to endogenous formation of N-nitroso compounds are typically present in the human stomach. If endogenous nitrosation occurs after exposure to nitrate or nitrite, then the exposure would be probably carcinogenic. If endogenous nitrosation does not occur, as would be the case with sufficient concurrent exposure to antioxidants such as vitamin C, then the classification would not apply.”¹⁰ (Emphasis added)

With the publication of the final monograph in 2010 IARC again stated it was the mechanistic event, not the chemicals (nitrite, nitrate, amines, amides) that was being classified.

“The cancer hazard from nitrate/nitrite ingestion cannot be determined without considering these other factors. Accordingly, the Working Group defined the agent not as ‘ingested nitrate or nitrite, but as ingested nitrate or nitrite under conditions that result in endogenous nitrosation’. This marks the first use of a mechanistic event (endogenous nitrosation) leading to carcinogenesis in the wording of an evaluation statement.”¹¹ (Emphasis added)

¹⁰ Cogliano, V., Baan, R., Straif, K., Grosse, Y., Secretan, B., Ghissassi, F. (2008). Use of mechanistic data in IARC evaluations. *Environmental and Molecular Mutagenesis*. 49: 100-109.

¹¹ IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010). page 39.

IARC consistently stated it is the “mechanism” or “mechanistic event” of endogenous nitrosation that is probably carcinogenic to humans, not nitrite or nitrate. OEHHA’s proposed listing of nitrite in combination with amines and amides as the agent is an inaccurate interpretation of IARC’s overall evaluation because if endogenous nitrosation does not occur under certain dietary intake conditions, then the classification would not apply. More specifically, in the “Overall evaluation” IARC explains

“There is an active endogenous nitrogen cycle in humans that involves nitrate and nitrite, which are interconvertible *in vivo*. Nitrosating agents that arise from nitrite under acidic gastric conditions react readily with nitrosatable compounds, especially secondary amines and amides, to generate *N-nitroso* compounds. These nitrosating conditions are enhanced following ingestion of additional nitrate, nitrite or nitrosatable compounds. Some of the *N-nitroso* compounds that could be formed in humans under these conditions are known carcinogens.”¹²

The formal identification and sufficiency of evidence section of the proposed listing provides that the IARC report “satisfies the formal identification and sufficiency of evidence criteria in the Proposition 65 regulations for *nitrite in combination with amines and amides*.” IARC concluded “There is *sufficient evidence* in experimental animals for the carcinogenicity of nitrite in combination with amines or amides” (emphasis in original). The agency relies on IARC’s discussion of data and conclusions in the report that nitrite in combination with amines or amides causes cancer in experimental animals.”¹³ The proposed listing, however, only focused on one component of IARC’s six component evaluation and rationale statement supporting IARC’s overall evaluation. IARC’s evaluation and rationale is provided below in its totality.

¹² IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010).

¹³ http://oehha.ca.gov/prop65/CRNR_notices/admin_listing/intent_to_list/noilpkg48cnitrite.html. Accessed February 20, 2014.

“There is *inadequate evidence* in humans for the carcinogenicity of nitrate in food.

There is *inadequate evidence* in humans for the carcinogenicity of nitrate in drinking-water.

There is *limited evidence* in humans for the carcinogenicity of nitrite in food. Nitrite in food is associated with an increased incidence of stomach cancer.

There is *inadequate evidence* in experimental animals for the carcinogenicity of nitrate.

There is *sufficient evidence* in experimental animals for the carcinogenicity of nitrite in combination with amines or amides.

There is *limited evidence* in experimental animals for the carcinogenicity of nitrite *per se*.

Overall evaluation

Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is *probably carcinogenic to humans (Group 2A)*.¹⁴

Because OEHHA did not base its listing on the entirety of the IARC’s six component rationale and did not interpret the authoritative body’s “Overall evaluation” accurately, pursuant to Title 27, California Code of Regulations Article 3 §25306(j), there is not substantial evidence that the proposed listing meets the requisite criteria and the proposed listing should be withdrawn.

II. IARC’s “Overall evaluation” on a Probable Carcinogenic Agent in Humans is a Mechanism Not a Specific Chemical(s).

The California code provides that a chemical known to the State of California to cause cancer or reproductive toxicity must be labeled or identified accordingly.¹⁵ Specifically, California regulations provide that “*the chemical has been included on a list of chemicals causing cancer or reproductive toxicity issued by the authoritative body; or is the subject of a report which is published by the authoritative body and which concludes that the chemical causes cancer or reproductive toxicity; or has otherwise been identified as causing cancer or reproductive toxicity by the authoritative body in a document that indicates that such identification is a final*

¹⁴ IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010).

¹⁵ CAL. HSC. CODE §25249.8(b).

action,” and that an authoritative body “specifically and accurately identifies the chemical.”¹⁶

The proposed listing identifies the chemical(s) or agent(s) at issue as “nitrite in combination with amines and amides.” This determination is overly broad and an inaccurate definition of a chemical. Indeed, the proposed listing ignores the totality of IARC’s determination, which is a broad biochemical mechanism and the products of said mechanism may be “probably carcinogenic to humans.”

More specifically, IARC stated it is the products of endogenous nitrosation mechanism that are probable carcinogens to humans -- not nitrite, not amines, and not amides. IARC also stated that if endogenous nitrosation does not occur then the classification would not apply. IARC’s inclusion of the statement “...*under conditions that result in...*” was a carefully crafted conclusion reached by the voting members of the Monograph Working Group and was an essential element of the “Overall evaluation” statement.

The combined consumption of nitrite with amines or amides is not carcinogenic to humans. This critical physiological certainty must be considered by OEHHA because listing the mechanism itself has considerable implications. For instance, a spinach salad with chopped egg – a common food dish containing nitrites and amines/amides – is not carcinogenic. That common food dish, however, when eaten under the certain dietary conditions that would favor endogenous nitrosation, *i.e.* diets limited in antioxidant vitamin intakes, may present a hazard. Similarly, taking an oral dose of nitric oxide-releasing non-steroidal anti-inflammatory drug and then eating any source of amines or amides, *i.e.* any food or beverage with a peptide bond or organic compound with a nitrogen atom, again, is not carcinogenic. However, if that drug and food are consumed under certain dietary conditions, *i.e.* diets limited in antioxidant intake, endogenous nitrosation may occur in the human stomach and pose a hazard. Likewise, a pregnant Californian may be told by her physician to take a folic acid supplement, a known amide, to prevent miscarriage and neural tube defects and is not currently considered carcinogenic. However, the proposed listing could prompt a warning if the supplement is consumed with any source of nitrites, such as spinach, beets, celery, cured meats, medicinal sources of nitrite, *etc.* In addition, many aquifers in California providing drinking water to their populations contain nitrate and nitrite. All of these examples conflict, however, with IARC’s overall evaluation that the hazard only exists when ingested nitrate or nitrite under specific dietary conditions result in endogenous nitrosation, *i.e.* may create compounds that are probably carcinogenic to humans.

¹⁶ Title 27, California Code of Regulations, Article 3 §25306(d)(1)-(2).

In short, listing “nitrite in combination with amines and amides” as the agent(s) is an inaccurate interpretation of IARC’s overall conclusion and the OEHHA notice should be withdrawn.

III. Significant New Scientific Evidence was Not Considered by IARC in June 2006 Monograph Meeting.

The carcinogenicity of numerous *N*-nitrosamines has been recognized for decades and human exposure to trace levels of these compounds can occur in foods, tobacco, certain consumer products, and the environment.¹⁷ Initially, the understanding of the mechanism in foods was unknown, which led to concerns regarding the reactants of nitrite, amines, and amino acids and spawned decades of research. This research has generated considerable scientifically valid evidence, which was not considered by IARC during its determination. Had this evidence been considered IARC’s rationale for its Group 2A classification would not be scientifically supportable.

For background information regarding the current state of understanding regarding nitrites, nitrates, nitric oxide, and their association with health outcomes and future research, OEHHA should consider the following selected chapters from the book *Nitrite and Nitrate in Human Health and Disease*. ^{18,19,20,21,22} These chapters are written by renowned experts in their respective fields.

¹⁷ Importantly, OEHHA has already listed dozens of these *N*-nitroso compounds consistent with Proposition 65.

¹⁸ Ignarro, L. (2011). Foreward. *In*: Bendich, A. (ed.), *Nitrite and nitrate in human health and disease*, Nutrition and Health. Humana Press, New York.

¹⁹ Lajous, M., and Willett, W. (2011). Chapter 6: Nutritional epidemiology of nitrogen oxide: what do the numbers mean? *In*: Bendich, A. (ed.), *Nitrite and nitrate in human health and disease*, Nutrition and Health. Humana Press, New York.

²⁰ Hord, N. (2011). Chapter 10: Regulation of dietary nitrate and nitrite: balancing essential physiological roles with potential health risks. *In*: Bendich, A. (ed.), *Nitrite and nitrate in human health and disease*, Nutrition and Health. Humana Press, New York.

²¹ Klurfeld, D. (2011). Chapter 16: Nitrites and Nitrates in Cancer. *In*: Bendich, A. (ed.), *Nitrite and nitrate in human health and disease*, Nutrition and Health. Humana Press, New York.

²² Bryan, N., and Loscalzo, J. (2011). Chapter 17: Looking forward. *In*: Bendich, A. (ed.), *Nitrite and nitrate in human health and disease*, Nutrition and Health. Humana Press, New York.

In 2012, a group of experts in nitrite physiology, toxicology, meat curing chemistry, and epidemiology published a review of the new and growing scientific body of evidence regarding nitrites, nitrates and cancer.²³ These scientists concluded that if the following information had been considered by IARC, the Group 2A classification would not have been scientifically supportable:

- the human nitrogen oxide metabolism was not addressed, specifically the importance of S-nitrosation;
- new epidemiological evidence shows no association between dietary intake of nitrite and stomach cancer, which was the only organ determined by the IARC Working Group to demonstrate an increased incidence of cancer; and
- quality of animal toxicology studies considered by IARC did not have the scientific rigor that other authoritative groups, *i.e.* NTP, used for its determinations.

This scientific review of the evidence was submitted to IARC in 2012.²⁴ The experts requested IARC reconsider its 2006 conclusion as they believed

*“...there is inadequate evidence for carcinogenicity in humans and also inadequate evidence in experimental animals for the carcinogenicity of nitrite per se. Therefore, according to IARC carcinogenicity criteria, the overall classification for ingested nitrite and nitrate would then be determined to be Group 3 **“The agent is not classifiable as to its carcinogenicity to humans.”**”²⁵*

To date, IARC has neither responded nor refuted the experts’ assessment of the scientific evidence submitted.

²³ Bryan, N., Alexander, D., Coughlin, J., Milkowski, A. and Boffetta, P. (2012a). Ingested nitrate and nitrite and stomach cancer risk: an updated review. *Food Chem Tox.* 50:3646-3665.

²⁴ Bryan, N., Alexander, D., Coughlin, J., Milkowski, A. and Boffetta, P. (2012b). Personal Correspondence to Dr. Christopher Wild, Director, International Agency for Research on Cancer.

²⁵ *Id.*

A. Nitrogen Oxide Physiology

For the last three decades, the carcinogenic nature of *N*-nitrosamines has been well reported in the scientific literature, but until the mid-1990s, what was not understood and reported was the discovery of the role nitrogen oxides (NO) have in human physiology and the profound importance of nitric oxide, nitrite, and nitrate in human homeostasis. Bryan *et al.* (2012a) provides an excellent summary of nitrosation, how physiologically it is critical to human health and its history as it relates to cancer. Bryan *et al.* (2012a) discusses the important differences between *S*-nitrosation and *N*-nitrosation that form potential carcinogenic *N*-nitroso compounds. *S*-nitrosation is the physical process to convey NO biochemistry, which has critical functions in many health outcomes, such as cardiovascular disease, diabetes, hypertension, neurotransmission, among others.^{26,27,28} OEHHA should consider the reviews of Bryan (2014; 2006) and Lundberg, *et al.* (2011), as well as the 2009 submission by Dr. Nathan Bryan to the Dietary Guidelines Advisory Committee summarizing the biomedical research of nitrite and nitrate regarding its safety and efficacy toward a variety of disease conditions.^{29,30,31,32}

The distinction between *S*-nitrosation and *N*-nitrosation is important as OEHHA considers the proposed Proposition 65 listing. IARC only evaluated the scientific evidence for the *N*-nitrosation pathway and did not include the fundamental role *S*-nitrosation has on human physiology. IARC's myopic focus on *N*-nitrosation does not accurately put into context the hazard-benefit role of endogenous nitrosation has in the human body. Had IARC evaluated the mechanistic evidence of both *S*-nitrosation and *N*-nitrosation the scientific evidence

²⁶ Bryan, N. (2014). Defining nitrite and nitrate as dietary nutrients. Under Review.

²⁷ Lundberg, J., Carström, M., Larsen, F., and Weitzberg, E. (2011). Roles of dietary inorganic nitrate in cardiovascular health and disease. *Cardiovascular Research*. 89:525-532.

²⁸ Bryan, N., Fernandez, B., Bauer, S., Garcia-Saura, M., Milsom, A., Rassaf, T., Maloney, R., Bharti, A., Rodriguez, J., and Feelisch, M. (2005). Nitrite is a signaling molecule and regulator of gene expression in mammalian tissues. *Nat Chem Biol*. 1(5): 290-7.

²⁹ Bryan, N. (2014).

³⁰ Bryan, N. (2006). Nitrite in nitric oxide biology: cause or consequence? A systems-based review. *Free radical biology & medicine*. 41(5): 691-701.

³¹ Lundberg *et al.* (2011).

³² Bryan, N. August 10, 2009. Dietary Guidelines Advisory Committee Submission. Committee ID: 000576.

would have overwhelmingly led to a lower classification or a more precise definition of nitrosation in regard to the human health hazard.

For these reasons, IARC's unduly broad, vague interpretation of nitrosation is not scientifically accurate, precluding its use as the basis for the proposed listing and necessitating that the OEHHA notice be withdrawn.

B. New Epidemiological Evidence Finds No Association Between Ingested Nitrite and Nitrate and Cancer.

IARC, as part of its overall conclusion, stated "*There is limited evidence for the carcinogenicity of nitrite in food. Nitrite in food is associated with increased incidence of stomach cancer.*"³³ Since the 2010 publication of IARC's Monograph, two major epidemiological studies found no association with nitrite and stomach cancer in addition to other epidemiological studies finding no association with consumption of nitrites and cancer.

Loh *et al.* (2011) examined the relationship between dietary intake of exogenous and endogenous *N*-nitroso compounds and cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study.³⁴ This large prospective cohort with more than 23,000 participants found neither dietary intake of exogenous *N*-nitroso compounds nor endogenous *N*-nitroso compounds were significantly associated with an increased cancer risk. Importantly, Loh, *et al.* (2011) came to this conclusion by factoring in the biological significance of the protective effect of vitamin C by inhibiting endogenous nitrosation process thus reducing endogenous nitrosation as well as the relationship with *Helicobacter pylori*. This work built on the 2006 cohort EPIC-EURGAST study that also found no association between dietary intake of preformed *N*-nitroso compounds and stomach cancer.³⁵ Significantly, EPIC studies are sponsored and coordinated by IARC.

³³ IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010).

³⁴ Loh, Y., Jakszyn, P., Luben, R., Mulligan, A., Mitrou, P. and Khaw, K. (2011). *N*-nitroso compounds and cancer incidence: the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk Study. *Am J Clin Nutr.* 93: 1053-61.

³⁵ Jakszyn, P., Bingham, S., Pera, G., Agudo, A., Luben, R., Welch, A., Boeing, H., Del Giudice, G., Palli, D., Saieva, C., Krogh, V., Sacerdote, C., Tumino, R., Panico, S., Berglund, G., Siman, H., Hallmans, G., Sanchez, M., Larranaga, N., Barricarte, A., Chirlaque, M., Quiros, J., Key, T., Allen, N., Lund, E., Carneiro, F., Linseisen, J., Nagel, G., Overvad, K., Tjonneland, A., Olsen, A., Bueno-de-Mesquita, H., Ocke, M., Peeters, P., Numans, M., Clavel-Chapelon, F., Trichopoulou, A., Fenger, C., Stenling, R., Ferrari, P., Jenab, M., Norat, T., Riboli, E., and Gonzalez, C. A. (2006). Endogenous versus exogenous exposure to *N*-nitroso compounds and gastric cancer risk in the European

Similarly, nitrite and nitrate were found by Cross *et al.* (2011) not to be associated with increased esophageal or stomach cancer.³⁶ Cross *et al.* utilized the National Institutes of Health-AARP Diet and Health study, which was a prospective cohort of more than 500,000 participants. This project was the largest study examining nitrite, nitrate, and stomach cancer among a United States population. Further, Cross *et al.* (2011) also confirmed other research that found no or null association with *N*-nitroso compounds and stomach cancer.³⁷

Many of the authors of the Cross *et al.* (2011) study are U.S. National Cancer Institute epidemiologists, including Dr. Mary H. Ward, who served as Chair of the “Cancer in Humans Subgroup” at the June 2006 Nitrite/Nitrate IARC Working Group meeting. In addition, Dr. Antonio Agudo, a Spanish epidemiologist of the 2006 EPIC-EURGAST study, was a voting member of the Nitrite/Nitrate IARC Working Group in June 2006 and also a member of the “Cancer in Humans Subgroup.” However, the 2006 EPIC-EURGAST cohort study was not considered by the IARC Working Group and does not appear as a reference in the monograph.³⁸ AMI contends that if Drs. Ward and Agudo had served on the IARC’s Humans Subgroup any time after the publication of their two large cohort studies (2006 and 2011), they would have reached an “inadequate evidence” determination for stomach cancer.

Other studies support this position. For example, the intake of fresh and processed red meat and nitrosamines, both endogenous and exogenous, was found to have no association with bladder cancer in a 2011 EPIC cohort study.³⁹

Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study. *Carcinogenesis*. 27(7): 1497-1501.

³⁶ Cross, A., Freedman, N., Ren, J., Ward, M., Hollenbeck, A., Schatzkin, A., Sinha, R., and Abnet, C. (2011). Meat consumption and risk of esophageal and gastric cancer in a large prospective study. *Am J Gastroenterol*. 106(3): 432-442.

³⁷ Jakszyn *et al.* (2006).

³⁸ *Id.*

³⁹ Jakszyn, P., Gonzalez, C., Lujan-Barroso, L., Ros, M., Bueno-de-Mesquita, H., Roswall, N., Tjonneland, A., Buchner, F., Egevad, L., Overvad, K., Raaschou-Nielsen, O., Clavel-Chapelon, F., Boutron-Ruault, M., Touillaud, M., Chang-Claude, J., Allen, N., Kiemeny, L., Key, T., Kaaks, R., Boeing, H., Weikert, S., Trichopoulou, A., Oikonomou, E., Zylis, D., Palli, D., Berrino, F., Vineis, P., Tumino, R., Mattiello, A., Peeters, P., Parr, C., Gram, I., Skeie, G., Sanchez, M., Larranaga, N., Ardanaz, E., Navarro, C., Rodriguez, L., Ulmert, D., Ehrnstrom, R., Hallmans, G., Ljungberg, B., Roddam, A., Bingham, S., Khaw, K., Slimani, N., Boffetta, P., Jenab, M., Mouw, T., Michaud, D., and Riboli, E. (2011). Red meat, dietary nitrosamines, and heme iron and risk of bladder cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Cancer Epidemiology, Biomarkers & Prevention*. 20(3): 555-9.

Additionally, researchers found no significant association between dietary exposure to nitrite, nitrate, and pancreatic cancer in the National Institutes of Health-AARP Diet and Health study.⁴⁰

Bryan *et al.* (2012a) succinctly summarized the current state of the epidemiologic evidence:

“...available epidemiologic evidence, and in particular the results of the large prospective studies reported after IARC’s review and evaluation in 2006, do not support the hypothesis of an association between ingestion of nitrate or nitrite, and resulting endogenous nitrosation, and stomach cancer. The fact that the results of methodologically weaker studies appear to support an association, which is not confirmed in the most rigorous and informative studies (in particular those of cohort design), strongly points towards bias and confounding as explanations for the former and towards the conclusion of lack of a causal association for stomach cancer. Based on this comprehensive review, the currently available epidemiologic evidence does not support an independent association between nitrate, nitrite or N-nitroso compound exposure and stomach cancer. This conclusion is supported by the fact that associations across the cohort studies are generally weak in magnitude, have relative risks above and below the null value with most associations being non-significant, show no consistent evidence of a dose–response relationship and show no associations (with some in the inverse direction) observed in two recently published large prospective studies (Cross et al., 2011; Loh et al., 2011).”⁴¹

⁴⁰ Aschebrook-Kilfoy, B., Cross, A., Stolzenberg-Solomon, R., Schatzkin, A., Hollenbeck, A., Sinha, R., and Ward, M. (2011). Pancreatic cancer and exposure to dietary nitrate and nitrite in the NIH-AARP Diet and Health Study. *American J Epi.* 174(3): 305-315.

⁴¹ Bryan *et al.* (2012a).

IARC determined there was “*limited evidence*” in humans for the carcinogenicity of nitrite in food, specifically regarding an increased incidence in stomach cancer.⁴² The findings of Loh *et al.* (2011) and Cross *et al.* (2011) demonstrate no association with nitrite, *N*-nitroso compounds and stomach cancer and using IARC classification methodology the total epidemiological scientific evidence would be classified as “*inadequate evidence.*” Had the above-discussed epidemiological evidence been considered by IARC, the Group 2A classification would not have been scientifically supportable. For that reason the proposed OEHHA listing should be withdrawn.

C. Animal Toxicology Evidence Finds No Link to Ingested Nitrite and Nitrate and Cancer.

The *NTP Technical Report No. 495* (2001) is the most definitive, chronic carcinogenicity bioassay study of sodium nitrite ever conducted and the resulting conclusion went through extensive peer review.⁴³ Bryan *et al.* (2012) succinctly summarized the findings of the NTP Technical Report No. 495 and the only adverse finding was as follows:

“...in this entire lifetime bioassay of sodium nitrite, fed in drinking water at three doses up to 3000 ppm to both rats and mice (equivalent to average daily doses of approximately 130 mg/kg in male rats, 150 mg/kg in female rats, 220 mg/kg in male mice, and 165 mg/kg to female mice), was the occurrence of combined benign and malignant forestomach tumors in female mice.” Further “the NTP peer review conducted by the Technical Reports Review Subcommittee concluded in their “Summary” Table (NTP, 2001, p. 10): “Neoplastic effects: None” observed in either male or female rats or mice. The Panel classified the female mouse forestomach tumor findings in their Table as “Uncertain findings.” Not only were these increased forestomach tumor incidences very weak as a function of dose (1/50, 0/50, 1/50, 5/50 in control, low, middle and high doses, respectively), but the forestomach is not considered to be an appropriate organ for cancer hazard assessment since humans do not even have this organ (Cohen and Arnold, 2011; Hoenerhoff et al., 2009).

⁴² IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. (2010).

⁴³ National Toxicology Program. (2001).

*The NTP peer review committee reached a unanimous decision to change the Draft NTP Technical Report’s “equivocal evidence” of female rat mammary tumors to “no evidence” and to change “some evidence” of forestomach carcinogenicity in female mice to “equivocal evidence” in the final Technical Report.”*⁴⁴

NTP found “Neoplastic effects – None” for male and female rats and mice due to exposure to nitrite. Had the above toxicological data in combination with new epidemiological and mechanistic evidence been available to be considered by the IARC in 2006, the Group 2A classification would not have been scientifically supportable. Using the IARC’s classification methodology the new scientific evidence for cancer in experimental animals would be classified as “*inadequate*” evidence, which would result in a Group 3 “*not classifiable*” conclusion.

Multiple nitrite studies conducted on rats since the NTP Technical Report was published in 2001, many of which were not available to IARC for consideration during its June 2006 deliberations, have shown no evidence that nitrite-only exposure has led to tumor formation. ^{45, 46, 47, 48, 49, 50} Likewise, recent animal

⁴⁴ Bryan *et al.* (2012a).

⁴⁵ Ishii, Y., Umemura, T., Kanki, K., Kuroiwa, Y., Nishikawa, A., Ito, R., Saito, K., Nakazawa, H., Hirose, M. (2006). Possible involvement of NO-mediated oxidativestress in induction of rat forestomach damage and cell proliferation by combined treatment with catechol and sodium nitrite. *Archives of Biochemistry and Biophysics*. 447: 127–135.

⁴⁶ Kitamura, Y., Umemura, T., Okazaki, K., Kanki, K., Imazawa, T., Masegi, T., Nishikawa, A., Hirose, M. (2006). Enhancing effects of simultaneous treatment with sodium nitrite on 2-amino-3-methylimidazo[4,5-f]quinoline-induced rat liver, colon and Zymbal’s gland carcinogenesis after initiation with diethylnitrosamine and 1,2-dimethylhydrazine. *International Journal of Cancer*. 118: 2399–2404.

⁴⁷ Kitamura, Y., Yamagishi, M., Okazaki, K., Furukawa, F., Imazawa, T., Nishikawa, A., Hirose, M. (2006). Lack of enhancing effects of sodium nitrite on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine(PhIP)-induced mammarycarcinogenesis in female Sprague-Dawley rats. *Cancer Letters*. 235: 69–74.

⁴⁸ Kuroiwa, Y., Ishii, Y., Umemura, T., Kanki, K., Mitsumori, K., Nishikawa, A., Nakazawa, H., Hirose, M. (2007). Combined treatment with green tea catechins and sodium nitrite selectively promotes rat forestomach carcinogenesis after initiation with N-methyl-N²-nitro-N-nitrosoguanidine. *Cancer Science*. 98: 949-957.

⁴⁹ Kuroiwa, Y., Okamura, T., Ishii, Y., Umemura, T., Tasaki, M., Kanki, K., Mitsumori, K., Hirose, M., Nishikawa, A. (2008). Enhancement of esophageal carcinogenesis in acid reflux model rats

mechanistic work of Van Hecke *et al.* (2014) demonstrated that certain *N*-nitroso compound formation was not supported by nitrite-cured meats.⁵¹

NTP is an authoritative body and OEHHA should consider the NTP findings in its deliberations for the proposed Proposition 65 listing. For these reasons, the proposed OEHHA listing should be withdrawn.

IV. The Proposed Listing Would Have Adverse Unintended Consequences.

The discussion above demonstrates that because there are flaws in the proposed listing's interpretation of IARC's conclusion and because a considerable body of new scientific evidence has been generated since IARC met in 2006 that the proposed listing is now scientifically unsupportable and should be withdrawn. In addition to those considerations, if the proposed listing is finalized, there will be significant unintended adverse consequences and these consequences should not be casually dismissed.

A. Emerging Research Demonstrates Positive Health Effects of Nitrite

OEHHA should have the entirety of the new scientific evidence regarding nitrite, nitrate, and NO, especially as it relates to positive human health outcomes. The discovery that NO was the endothelium-derived relaxing factor in the 1990s fundamentally changed how scientists viewed NO's importance in human physiology. Indeed, this work has been recognized, most notably by the 1998 Nobel Prize in Physiology or Medicine.

treated with ascorbic acid and sodium nitrite in combination with or without initiation. *Cancer Science*. 99: 7–13.

⁵⁰ Kuroiwa, Y., Yamada, M., Matsui, K., Okamura, T., Ishii, Y., Masumura, K., Tasaki, M., Umemura, T., Mitsumori, K., Nohmi, T., Hirose, M., Nishikawa, A. (2008). Combined ascorbic acid and sodium nitrite treatment induces oxidative DNA damage-associated mutagenicity in vitro, but lacks initiation activity in rat forestomach epithelium. *Toxicological Sciences*. 104: 274–282.

⁵¹ Van Hecke, T., Vanden Bussche, J., Vanhaecke, L., Vossen, E., Van Camp, J., and De Smet, S. (2014). Nitrite Curing of Chicken, Pork, and Beef Inhibits Oxidation but Does Not Affect N-Nitroso Compound (NOC)-Specific DNA Adduct Formation during in Vitro Digestion. *J Agric Food Chem*. 62: 1980-1988.

Since that time, the human medical field has been conducting human biochemical research and developing medical treatments that include nitrite-based drugs. A search of the National Institutes of Health ongoing projects reveals that more than 50 studies have been funded regarding the role of nitrite in human health -- with the goal of better understanding cardiovascular disease, treatment of foodborne illnesses, pulmonary disease, among others.⁵²

Lundberg *et al.* (2011) states

“...the nutritional implications of nitrate and nitrite biology are among the most intriguing in this area of research. The amounts of these anions needed for the effects on the cardiovascular system, described in this review, are readily achieved via our everyday diet, most easily via a rich intake of fruits and vegetables. If the cardiovascular benefits of this healthy diet turn out to be related to their high amount of nitrate, we have to reconsider our current thinking and realize that inorganic nitrate may not necessarily be a threat to human health. Instead, in some years, we might even consider this anion as an essential nutrient.”

This hypothesis is further supported by Bryan (2014). There are many other human medical research examples that demonstrate the importance of nitrate, nitrite, NO in the human body. AMI is hopeful medical research in this area continues. .

B. Nitrites, Amines, and Amides are Ubiquitous in Nature

Human exposure to nitrites, amines, and amides is high because each is ubiquitous in nature. In fact, saliva accounts for more than 90 percent of the total daily ingested nitrite exposure to humans. Nitrate absorbed from food such as green leafy vegetables is excreted in saliva and bacteria in the human mouth convert the nitrate to nitrite. Accounting for this physiological fact and considering almost all foods contain amines or amides, based on the proposed listing all foods would have to display the required warning, raising questions about the practical benefit of applying a warning label to all foods.

⁵² National Institutes of Health’s Research Portfolio Online Reporting Tools.
<http://projectreporter.nih.gov/reporter.cfm>. Accessed March 31, 2014.

For example, the proposed listing could have a significant impact on the produce industry. The health benefits of certain dietary patterns, such as the Mediterranean or Dietary Approaches to Stop Hypertension (DASH), are well established and recommended by health professionals (*i.e.* American Heart Association) and federal nutritional policy (*Dietary Guidelines for Americans*), to prevent or control adverse health outcome such as obesity, hypertension, among others. These dietary patterns recommend increased consumption of fruits, vegetables, and plant-based proteins. The work of Hord *et al.* (2009) demonstrate that individuals consuming a DASH diet could, if consumed at the high recommended levels, exceed “...*the World Health Organization’s Acceptable Daily Intake for nitrate by 550% for a 60-kg adult.*”⁵³ Nitrate is a physiological substrate of the reduction reaction resulting in nitrite and this reaction occurs within the human body. AMI agrees with Hord *et al.* (2009) the results from their study question the rationale to limit consumption of nitrate and nitrite when dietary patterns based in nitrate/nitrite rich foods are encouraged for positive human health outcomes.

OEHHA should carefully consider the unintended consequences of putting suspicion on vegetables high in nitrate, which would be a disservice to efforts to encourage consumers to eat more fresh fruits and vegetables.

V. Conclusion

AMI will continue to support the use of sound science as the foundation for regulatory requirements to achieve the intended human health and food safety objectives associated with any regulatory program. The aforementioned discussion demonstrates that the authoritative body OEHHA relied on, IARC, had not considered the totality of scientific evidence available during its determination, nor was IARC able to take into consideration the extensive scientific evidence that became available after the June 2006 determination.

Using the IARC’s classification methodology and evaluating the scientific evidence published since IARC’s determination, a Nitrite/Nitrate IARC Working Group meeting conducted today would reach a much different conclusion and classification for ingested nitrate and nitrite would be:

⁵³ Hord, N., Tang, Y., and Bryan, N. (2009). Food sources of nitrates and nitrites: the physiologic context for potential health benefits. *Am J Clin Nutr.* 90: 1-10.

- There is *inadequate evidence* in humans for the carcinogenicity of nitrate in food.
- There is *inadequate evidence* in humans for the carcinogenicity of nitrate in drinking-water.
- There is *inadequate evidence* in humans for the carcinogenicity of nitrite in food. Nitrite in food is not associated with an increased incidence of any human cancers.
- There is *inadequate evidence* in experimental animals for the carcinogenicity of nitrate.
- There is *inadequate evidence* in experimental animals for the carcinogenicity of nitrite when combined endogenously with amines or amides.
- There is *inadequate evidence* in experimental animals for the carcinogenicity of nitrite *per se*.

Overall Evaluation: Ingested nitrite and nitrate are *not classifiable as to its carcinogenicity to humans (Group 3)*.

IARC's incorrect interpretations and the subsequent scientific evidence demonstrate that IARC's rationale for its Group 2A classification is not scientifically supportable.

For foregoing reasons, AMI respectfully requests that the above-referenced notice be withdrawn.

* * * * *

If you have any questions regarding any aspect of these comments or would like to discuss them please contact me at 202-587-4249 or bbooren@meatami.com.

Respectfully submitted,



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