Public Health Goals

Responses to Comments on Technical Support Document

Public Health Goals for Nitrate and Nitrite in Drinking Water

May 2018

Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

TABLE OF CONTENTS

INTRODUCTION

The draft technical support document *Public Health Goals for Nitrate and Nitrite in Drinking Water* was released by the Office of Environmental Health Hazard Assessment (OEHHA) for public comment on December 16, 2016, and a public workshop was held on February 13, 2017. The draft proposed an update to the 1999 public health goals (PHGs) assessment for nitrate and nitrite, and provided the scientific basis for the update. This draft also received formal external scientific peer review pursuant to Health and Safety Code Section 116365(c)(3)(D). The document was revised in response to public and peer review comments. The revised draft was released for public comment on February 9, 2018.

OEHHA's responses to comments received are summarized herein. Public and peer review comments are directly quoted (italicized), followed by OEHHA's responses. The full citations of journal publications and reports cited in the comments and responses are given in the PHG document.

The full text of the public and peer review comments is available on OEHHA's website. No public comments were received on the February 2018 draft. Public comments on the December 2016 draft were received from:

East Bay Municipal Utility District
The Environmental Justice Coalition for Water
Jean-Louis L'hirondel
Western Growers

External scientific peer review comments were received from:

Henry A. Anderson, MD
Chief Medical Office, Department of Health Services
Wisconsin Division of Public Health (retired)
Adjunct Professor
Department of Population Health Sciences
School of Medicine and Public Health
University of Wisconsin

Leslie T. Stayner, PhD
Professor and Director
Occupational and Environmental Epidemiology
School of Public Health
University of Illinois at Chicago

The public comment and response process is an important part of the overall PHG development process under Health and Safety Code Section 57003. They provide for

deliberation and in-depth consideration of the underlying scientific issues during PHG development. The document has now been finalized and is available at www.oehha.ca.gov.

For more information about the PHG process or to obtain copies of PHG documents, visit the OEHHA website. OEHHA may also be contacted at:

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California Environmental Protection Agency
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Attention: PHG Program

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RESPONSES TO EXTERNAL SCIENTIFIC PEER REVIEW COMMENTS

Henry A. Anderson, M.D.

Comment: I would suggest the work product would benefit from some process additions. The document is missing a detailed "methods" section. The literature review is quite comprehensive but no explanation is given for how the authors went about locating the published literature (key words searched for and in which data systems etc.). I have no doubt they used a systematic approach but that needs to be described.

Response: Additional information on OEHHA's literature search strategy has been added. As an example of the overall literature search strategy used, search terms and criteria for locating human cancer epidemiological studies have been added to Appendix I of the PHG document.

Comment: The methemoglobinemia section needs to have a definition of "clinically significant methemoglobinemia". It is mentioned that symptoms can occur at a level of 10% but the "normal" concentration is 2-3%. An increase in methemoglobin may be an "effect" but at what point does it become an "adverse effect"?

Response: Normal methemoglobin (metHb) levels in human blood range from 1% to 3% of total hemoglobin, and symptoms of methemoglobinemia are typically minimal until metHb levels exceed 10% to 20% (Wright et al., 1999; Greer et al., 2005; Manassaram et al., 2010). However, according to the differential diagnosis and treatment of methemoglobinemia presented in Knobelock and Proctor (2001), "Cyanosis becomes apparent when the methemoglobin level exceeds 1.5 g/dL (usually 1%-15% total hemoglobin)." Furthermore, cyanosis has been observed in infants with metHb levels as low as 3% (Greer et al., 2005). Because elevated metHb levels are a biomarker of methemoglobinemia, and because cyanotic symptoms can occur at modest elevations of metHb, an increase in infants and children that appears to be within the normal range in adults could have adverse effects. This is now noted on page 10 of the technical support document.

Comment: It would be useful in the summary introduction paragraph to add that the PHG's protect against infant methemoglobinemia as well as other toxic effects observed in adults – or some other mention that the health concern goes beyond just infant exposure.

Response: The summary paragraph now states, "While these PHGs are based on the occurrence of infant methemoglobinemia, a blood disorder that results in decreased oxygen distribution to tissues, they serve to protect against other health effects, such as liver toxicity, that have been shown to occur at higher levels of exposure and in the general population."

Comment: In the animal study section tables are provided which indicate NOELs, NOAELs etc. It is possible to use these figures as PODs and go through the exercise of calculating what PHG would result.

Response: While it is standard practice to present different NOAELs, LOAELs, and BMDLs (when available) for comparison and use in point of departure (POD) selection, OEHHA prefers not to present alternate calculations for the PHG using different PODs. This is to avoid any confusion as to what was selected as the critical study/endpoint, and what uncertainty factors were applied.

Leslie T. Stayner

Comment: The justification for the UF of 1 presented in the documents is that it was based on "human data" in the most sensitive population (i.e. infants and children). I believe this justification is inadequate. The U.S. EPA guidelines for assessing health risk of environmental exposures to children (U.S. EPA 2006) recommends that an intraspecies uncertainty factor (UF_H) of 10 be used to account for "variation in susceptibility among the members of the human population (i.e., interindividual or intraspecies variability)" (U.S. EPA 2006). The OEHHA guidelines (Appendix II) appear to allow for a UF_H factor of 1 when human study includes sensitive subpopulations (e.g. infants and children). However, even within children there is certainly variability in susceptibility. For example, the draft assessment states that "inherited metabolic disorders, such as a deficiency of NADH diaphorase (Kumar et al., 1989) and genetically controlled deficiencies of the enzymes glucose-6-phosphate dehydrogenase or metHb reductase have also been described to cause methemoglobinemia". It seems likely that children with these conditions might be particularly susceptible to the effects of nitrate in drinking water.

Response: OEHHA considers infants to be the most susceptible population, and the point of departure was determined from infant data. The critical and supporting studies used to derive the PHG encompassed a random sample of infants in a geographically diverse population. For example, Walton (1951) presented over 278 methemoglobinemia cases based on a questionnaire sent to all 48 states in the US at that time plus Alaska and Hawaii, to which all but one state replied, and found no reported cases were associated with drinking water containing less than 10 mg/L nitrate-nitrogen. There is diversity among the sampled populations, and this had no discernible impact on the results of the studies. No adverse effects were observed in any of the infants exposed to nitrate at the NOAEL, which suggests that human variability was not a major susceptibility factor for methemoglobinemia at this concentration. Therefore, OEHHA used an intraspecies uncertainty factor of 1 for PHG derivation.

Comment: A NOAEL of 45 mg/L also seems to be highly uncertain. The draft proposal indicates that the NOEAL was based on two small and very old case reports (Bosch 1950 and Walton 1951) and one more recent cross-sectional study (Sadeq 2008). However, several other case reports cited in the review appear to have observed cases at levels below 45 mg/L. The study by Gupta (1999) reported metHB levels of 12.7% in infants (≤ 1 year) exposed to 26 mg/L. The review dismisses these findings largely because there was no control group in this study. However, these levels appear to be remarkably high given background levels of metHB are generally < 2%. In the report by Knobeloch and Proctor (2001) one of the cases appears to have had exposure of < 25 mg/L. Given the limitations in the studies for methemoglobinemia, one could also argue that another uncertainty factor could be applied for uncertainty in the database. All in all, it seems that some margin of safety should be applied in developing the proposed (and current) PHG. Common sense suggests that setting a PHG (45 mg/L) so close to the level at which there are known effects (50 mg/L) is not wise.

Response: The findings in the Gupta et al. (1999) study presented in Table 3 of the PHG document, and reproduced below (Table 1), were not considered further because of shortcomings in the data. The population exposed to the lowest concentration of nitrate (26 mg/L) had surprisingly high percentages of metHb (normal range is 1-2%), even in older populations. There are no data reporting metHb levels in populations not exposed to nitrate, so it is unknown whether the high metHb levels in all groups were due to nitrate, some other unknown chemical contaminant in the water, or whether there was a problem with the testing methods. Furthermore, in all the age groups, including children ≤1 year of age, the dose-response in the Gupta et al. (1999) paper was non-monotonic, with lower metHb percentages at 222 mg/L than at other concentrations. These data limitations precluded this study from further consideration.

Table 1. Average methemoglobin levels (% metHb) in 178 people in five age groups exposed to different concentrations of nitrate in drinking water[a] (adapted from Gupta et al., 1999)

Nitrate concentration (mg/L)	Average % metHb in each age group				
	≤1 yr	>1 to ≤8 yrs	>8 to ≤18 yrs	>18 to ≤45 yrs	>45 yrs
26	12.69	8.94	4.81	9.33	7.90
45	19.52	15.17	16.52	19.01	10.72
95	26.99	15.13	9.69	12.73	7.00
222	7.06	8.07	5.76	6.93	7.06
459	15.38	15.46	11.06	9.87	10.0

[a]Correlation coefficients for nitrate concentration and metHb were: ≤1 yr = not reported; >1 to ≤8 yrs = 0.23; >8 to ≤18 yrs = 0.01; >18 to ≤45 yrs = -0.57; >45 yrs = -0.58

Knobeloch and Proctor (2001) reported a case of methemoglobinemia where the measured concentrations of nitrate were <25 mg/L nitrate-nitrogen, not <25 mg/L nitrate. Nitrate-nitrogen values measured from the reverse-osmosis water purification

system ranged from 9.9 to 23.5 mg/L (corresponding to 44 to 104 mg/L nitrate), which is in a range where toxicity may occur.

The NOAEL of 45 mg/L was determined from several human studies, and other examinations of the literature have not found any reports of infant methemoglobinemia with nitrate concentrations below that level (Fan et al., 1987; Fan and Steinberg, 1996). Therefore, OEHHA believes that 45 mg/L nitrate is a suitable NOAEL for infant methemoglobinemia.

Comment: I am also concerned that the review rejects all of the other endpoints (e.g. thyroid, reproductive and developmental effects and carcinogenicity) for consideration in developing the PHG. These endpoints were rejected due to "inconsistency among the studies, limitation of study designs, and the presence of confounding factors". In general, there are almost always inconsistencies and limitations in the epidemiologic literature of environmental hazards. A careful review tries to determine if these inconsistencies can be explained by the study characteristics. In this respect, I believe the report failed in some areas of its review of the human studies.

Response: All relevant toxicological endpoints, including thyroid toxicity, carcinogenicity, and developmental/reproductive toxicity, were considered. OEHHA expanded its review of developmental toxicity (see comment below), and updated the cancer analysis to include additional studies that were not included in the first draft (Fathmawati et al., 2017; Espejo-Herrera et al., 2016a; Espejo-Herrera et al., 2016b; Jones et al., 2016; Jones et al., 2017; Drozd et al., 2015; Xu et al., 2015). However, without being able to rule out major biases and confounding factors within the studies reviewed, OEHHA maintains that thyroid toxicity and carcinogenicity studies do not provide clear and consistent causal evidence of an association with nitrate. Developmental toxicity is addressed below.

Comment: Of particular concern, is its review on developmental and reproductive toxicity. I believe that there is now very strong evidence of an association between nitrate in drinking water and the risk of central nervous system (CNS) birth defects and particularly for neural tube defects (NTD). An increased risk of CNS birth defects has been reported in four of the studies summarized in Table 9 (Arbuckle et al. 1988, Brender et al. 2004a, Croen et al. 2001 and Dorsch et al. 1984). … I would strongly urge the Cal EPA to reconsider the evidence for this endpoint and whether this endpoint might be used as an alternative basis for their assessment for risk.

Response: OEHHA amended its review of developmental and reproductive toxicity to include several publications that were not included in the first draft (Bove et al., 1992; Klotz et al., 1998; Migeot et al., 2013; Brender et al., 2013; Huber et al., 2013; Vuong et al., 2016; Weyer et al., 2014; Holtby et al., 2014; Stayner et al., 2017; Li et al., 1996), and a much more thorough analysis of the risk of neural tube defects. A section on neural tube defects has been added to the revised PHG document, starting on page 54. Overall, some of the results of some of the studies that evaluated the association

between nitrates and CNS malformations provide limited evidence of an association between NTDs and drinking water nitrate levels below the current MCL. However, several inconsistencies exist within and between studies. For example, Croen et al. (2001) reported that nitrate in groundwater, but not mixed water (groundwater and surface water combined), was associated with an increased risk of NTDs at the same concentration. Brender et al. (2013) reported a strong association between daily intake of nitrate ≥5 mg/day from water, but not for nitrate water concentrations >15 mg/L. Migeot et al. (2013) and Holtby et al. (2014) reported that the highest nitrate concentrations examined were not significantly associated with developmental toxicity, but that lower concentrations were. Furthermore, both research groups showed no association between NTDs and dietary nitrate intake, which typically greatly exceeds nitrate intake from water (Croen et al., 2001; Huber et al., 2013). Given this, further research is needed to confirm the positive associations reported in some of these investigations.

Comment: The biologic plausibility of the observed increased risk of CNS BD is supported by several experimental animal studies in which an increased risk of CNS birth defects with exposure to N-nitroso compounds has been observed (Givelber and DiPaolo 1969, Koyama et al. 1970 and Pfaffenroth et al. 1974). These experimental studies are relevant and should have been discussed.

Response: The Givelber and DiPaolo (1969), Koyama et al. (1970), and Pfaffenroth et al. (1974) studies were not considered relevant to the PHG because the administered agents were nitrosoureas. (See response to comment that begins at the bottom of this page.)

Comment: I am also aware of a few relevant publications that were not discussed in the section on reproductive and developmental effects. Two of these are very recent publications one of which I authored (Stayner et al. 2017) and the other that is a doctoral dissertation by one of my students (Almberg et al. 2017).

Response: OEHHA evaluated and incorporated the study from Stayner et al. (2017). OEHHA was unable to obtain the dissertation by Almberg et al. (2017), as the link provided in the references would not connect. While the Stayner et al. (2017) paper provides evidence of an association between nitrates in drinking water and adverse birth outcomes, the evidence base as a whole contains inconsistencies. As noted above, OEHHA believes further investigation is warranted into the associations between nitrate exposure and adverse birth outcomes, including NTDS.

Comment: The third study is a publication by Migeot et al. (2103) which reported an association between nitrate in drinking water and the risk of being small for gestational age. While it is understandable how the review could have missed the most recent papers from my research group it is more difficult to understand how it missed the paper by Migeot et al. (2013), and the other papers on birth defects cited above (Brender et al.

2013, Givelber and DiPaolo 1969, Koyama et al. 1970 and Pfaffenroth et al. 1974). This raises questions about the thoroughness of the literature search and the approach used for this systematic review.

Response: The Migeot et al. (2013) and Brender et al. (2013) studies were evaluated and incorporated into the PHG document. The Givelber and DiPaolo (1969), Koyama et al. (1970), and Pfaffenroth et al. (1974) studies were not considered relevant to the PHG because the administered agents were nitrosoureas. In the PHG document, OEHHA states that nitrosamines, nitrosamides, and nitrosoureas can be carcinogenic and teratogenic. Although these compounds are derived from nitrite/nitrate, additional amines or amides are necessary for their formation. However, whether formation of nitrosamines would pose a significant cancer risk or reproductive and developmental hazard under actual food or water intake conditions is still not clear. Confounders include the fact that there are a variety of nitrosating agents, several nitrosation pathways, and many different precursors and scavengers of N-nitroso compounds that may be present in the diet. The potential risk to human health from nitrate-derived, endogenously formed N-nitroso compounds cannot be accurately estimated.

Comment: The document should at least mention the literature search strategy and criteria used for selecting papers for review.

Response: As an example of the overall literature search strategy used, search terms and criteria for locating human cancer epidemiological studies have been added to Appendix I of the PHG document.

Comment: I am aware of a few recent epidemiologic studies on bladder cancer (Jones et al. 2016 and 2017) and colon cancer (Espejo-Herrera et al. 2016) that showed a positive association with nitrate in drinking water that were not included in this review.

Response: The Jones et al. (2016, 2107) and Espejo-Herrera et al. (2016) studies have been evaluated and incorporated into the PHG document.

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Brender JD, Weyer PJ, Romitti PA, et al. (2013). Prenatal nitrate intake from drinking water and selected birth defects in offspring of participants in the national birth defects prevention study. *Environ Health Perspect* 121(9): 1083-9.

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RESPONSES TO PUBLIC COMMENTS, FIRST COMMENT PERIOD

East Bay Municipal Utility District

Comment: [T]he discussion on drinking water only considers nitrate and nitrite contamination in the source water. The document should also include a discussion on nitrification in the distribution system.

Response: Information about nitrification was added to page 5 in the PHG document. As the primary focus of this document is on the health effects of nitrate and nitrite, nitrate occurrence in environmental media is only briefly described.

Reference

AWWA (2013). M56 Nitrification Prevention and Control in Drinking Water, Second Edition. American Water Works Association.

The Environmental Justice Coalition for Water

Comment: We urge the Office to take notice of studies on the linkage between prenatal nitrate intake and birth defects. … [A]*t least one available human study not cited in the Draft PHG shows a significantly higher* [probability] *of birth defects from mothers who ingest ≥ 5 mg of nitrate per day, compared to control groups that consumed < 4.9 mg nitrate per day. Tested birth defects include, neural tube defects, isolated limb deficiencies, oral cleft defects, and congenital heart defects. Specifically, mothers who ingest ≥ 5 mg of nitrate per day were 1.5-2 times more likely to give birth to a child with neural tube defects, isolated limb deficiencies, or oral cleft defects when compared to the control group.*

Response: In revising the PHG document, OEHHA evaluated the epidemiological developmental toxicity studies cited by The Environmental Justice Coalition for Water and expanded the analysis of epidemiological data linking nitrate in drinking water to neural tube defects.

There are several studies that report positive associations of nitrate exposure in drinking water and developmental toxicity endpoints (Croen et al., 2001; Bukowski et al., 2001 Brender et al., 2013; Migeot et al., 2013; Holtby et al., 2014; Stayner et al., 2017). However, other studies showed no significant associations (Super et al., 1981; Aschengrau et al., 1989; Ashengrau et al., 1993; Cedergren et al., 2002; Brender et al., 2004). Of the positive studies, some had limited exposure information, and did not include information about bottled water or private well water (Migeot et al., 2013; Stayner et al., 2017).

Migeot et al. (2013) and Holtby et al. (2014) reported that the highest nitrate concentrations examined were not significantly associated with developmental toxicity, but that lower concentrations were. This lack of dose-response makes it difficult to interpret these data and suggests that nitrate may not be the sole causative factor.

Croen et al. (2001) and Brender et al. (2013) presented some evidence of an association between drinking water nitrate and neural tube defects (NTDs). However, inconsistencies within these studies suggest that nitrate may not be solely responsible for the increased risk of NTDs. Croen et al. (2001) reported that nitrate in groundwater, but not mixed water (groundwater and surface water combined), was associated with an increased risk of NTDs at the same concentration. Brender et al. (2013) reported a strong association between daily intake of nitrate ≥5 mg/day from water, but not for nitrate water concentrations >15 mg/L. OEHHA calculated that a pregnant woman exposed to 15 mg/L nitrate in drinking water would have a daily exposure of 49 mg/day nitrate from water, which is substantially higher than the 5 mg/day that was positively associated with NTDs. Furthermore, both research groups showed no association between NTDs and dietary nitrate intake, which typically greatly exceeds nitrate intake from water (Croen et al., 2001; Huber et al., 2013).

Inconsistencies in the available studies, as noted above, make it difficult to interpret the results regarding the developmental toxicity of nitrate or to use these studies in evaluating dose-response of nitrate exposure.

Comment: If the current PHGs are maintained this would, in effect, require mothers to consume less than .5L of tap water per day to avoid causing or contributing to an adverse health effect. We believe this is an untenable standard that would violate the Human Right to Water by recommending an unreasonably low daily water consumption rate.

Response: OEHHA is unclear how the value of 0.5 L/day for mothers was derived, but ingestion of water containing levels at or below the PHG (45 mg/L) showed no adverse effects in the most sensitive subpopulation, infants. Epidemiology studies linking nitrate exposure in water at low levels (≥5 mg/day) to birth defects did not provide sufficient evidence that nitrate was the sole causative agent (see comment above). Therefore, OEHHA concludes that the updated PHGs for nitrate and nitrite are sufficiently health protective for all age groups.

Comment: We urge the Office to give due weight to the linkages found between nitrate/nitrite ingestion and cancer in postmenopausal women. Statistically significant associations have been found between high nitrate drinking water levels and ovarian and bladder cancer in postmenopausal women. … Again, the Office is required to set a PHG that takes into account adverse health effects on members of a subgroup that comprises a meaningful portion of the general population, including postmenopausal women. We believe the Draft PHG fails to adequately consider and give due weight to all currently available scientific data on this issue.

Response: OEHHA acknowledges that several studies have reported associations between cancer and nitrate or nitrite in food or water, but consistent findings have not been seen across, and sometimes within, studies. For example, Jones et al. (2016) reported an increased risk for bladder cancer in post-menopausal women whose water supplies contained >5 mg/L nitrate-nitrogen for ≥4 years. However, the same study found no association between dietary nitrate and bladder cancer. While Inoue-Choi et al. (2015) did show an association between drinking water nitrate and ovarian cancer in post-menopausal women, the authors also noted the need for additional confirmatory studies with a larger number of ovarian cancer cases. Overall, the current cancer epidemiology studies are not adequate for establishing a PHG and further investigation of associations between nitrates and nitrites in drinking water and human cancer is warranted.

Comment: We further urge the Office to consider the synergistic effects resulting from exposure to multiple contaminants, which commonly co-occur with nitrate/nitrite, when setting the PHG. For example, the University of Nebraska found that where naturally-occurring uranium co-occurs with nitrate, the nitrate and uranium interact such that the uranium becomes more water-soluble and, thus, more bioavailable. These two constituents commonly co-occur in California's San Joaquin Valley. These and other known and likely synergistic effects should be taken into account in lowering the PHG for nitrate and nitrite in drinking water.

Response: OEHHA could not locate any information concerning the University of Nebraska finding cited in the comment. OEHHA is not aware of any toxicity studies on the synergistic effects of nitrate and uranium. Without such data OEHHA cannot evaluate health effects of the co-exposure described.

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Jean Louis L'hirondel

*Comment: I disagree with your presentation of the nitrate-induced infant methemoglobinemia, for three reasons: 1) You write: "*Methemoglobinemia was not observed in any cases where the drinking water nitrate levels were ≤ 45 mg/L." … the early epidemiology is not really satisfactory… *First, the limit of 10 mg NO_3^--N l^{-1} , i.e. 45 mg NO_3^- l^{-1}, suggested by Comly (1945) in potable water, was proposed at a rough guess [Cf. Box 6.1, p. 75].*

Secondly, the diagnostic criteria (or inclusion criteria) adopted by Bosch et al. *(1950) were curiously founded on the only suggestion from Comly. So, cases with less than 10 mg NO_3^--N l^{-1} were not included in the review.*

Response: Bosch et al. (1950) credit Comly for discovering nitrate as the etiologic factor for non-idiopathic infant methemoglobinemia but they do not attribute any diagnostic criteria or levels of nitrate-nitrogen to the Comly (1945) publication. According to Bosch et al. (1950), the first case of methemoglobinemia in Minnesota was reported in January 1947. A total of 139 cases were voluntarily reported to the Minnesota Department of Health in the years 1947-1949. Detailed data on the 129 wells involved in the 139 reported cases indicated none of the wells contained less than 10 ppm nitrate-nitrogen and only two wells contained 10-20 ppm nitrate-nitrogen. Although Bosch et al. (1950) indicate the use of a nitrate-nitrogen level of 10-20 ppm in water in the presumptive diagnosis of methemoglobinemia, they also note that no cases were known to have occurred in Minnesota from using water containing less than 30 ppm. This is supported by Walton (1951), who reported zero cases of infant methemoglobinemia associated with water nitrate below 10 mg/L nitrate-nitrogen.

Comment: Thirdly, Walton (1951) drew up a table. Of course, the number of cases of infant methemoglobinemia with water nitrate content below 10 mg NO_3^--N l^{-1} was equal to zero.

OEHHA is not aware of any infant methemoglobinemia studies that were excluded from Walton (1951) or the American Public Health Association (1949-1950, as cited by Walton, 1951) because the nitrate concentration was <10 mg/L nitrate-nitrogen. Subsequent examinations of infant methemoglobinemia in the United States by Fan et al. (1987) and Fan and Steinberg (1996) did not find any cases associated with nitrate-nitrogen levels <10 mg/L.

Comment: You do not make the distinction between <u>well water</u> and <u>municipal drinking water</u>, an essential distinction.

- First, well water may be filthy, with bacterial contamination. On the contrary, municipal drinking water is always controlled, with less than 10^2 germs ml^{-1}.
- Secondly, in a feeding bottle, nitrate is only reduced into nitrite when bacterial population exceeds 10^6 germs ml^{-1} [Cf. L'hirondel J. and L'hirondel, J.-L. (2002), p.40

and Tamme, T. et al. *(2010)]. There is no reduction at all of nitrate into nitrite, whatever the nitrate concentration may be, when bacterial population is below 10^6 germs ml^{-1}.*
- Thirdly, in 1985 the WHO stated that at that time some 2000 cases of infant methemoglobinemia had been reported since 1945 in the world medical literature [L'hirondel, J. and L'hirondel, J.-L. (2002), p.46]. 100% of the 2000 cases came from well water, 0% from municipal drinking water.

Response: The PHG document does report a case of infant methemoglobinemia from consuming municipal water (Vigil et al., 1965). In this case report, a cyanotic infant was diagnosed with methemoglobinemia, which was attributed to nitrate contamination in the tap water used to reconstitute evaporated milk (63 mg/L nitrate in tap water and 73 mg/L nitrate in formula). Furthermore, bacteriological samples from water supply wells were negative for coliform organisms and the water used for preparing the formula was boiled for approximately 20 minutes, which would have effectively killed any bacteria present. It is apparent that this case of methemoglobinemia was not the result of contaminated well water, and was most likely the result of nitrate exposure.

OEHHA acknowledges that bacterial contamination of water is a major confounding factor when evaluating nitrate and methemoglobinemia. However, the PHG document also cites several additional studies where methemoglobinemia occurred in the absence of contaminating bacteria (Cornblath and Hartmann, 1948; Knobeloch et al., 2000; Knobeloch and Proctor, 2001; Abu Naser et al., 2007). Cornblath and Hartmann (1948) showed that exposure to nitrate-contaminated water, both in the presence and absence of bacteria, increased metHb levels and induced signs of cyanosis. Furthermore, Craun et al. (1981) reported that bacterial contamination of rural wells did not increase metHb levels, and was not associated with methemoglobinemia. Therefore, OEHHA concludes that while bacterial contamination of drinking water may play a role in infant methemoglobinemia, it is not the sole causative factor. The evidence suggests that nitrate exposure is the primary underlying cause of infant methemoglobinemia.

Comment: My book: "L'hirondel J. and L'hirondel J.-L. (2002)" (pp. 26-27) mentioned the results of 7 experimental studies (Avery et al., *1966; Reed, 1996; Luhby* et al., *1954; Agunod* et al., *1969; Jean-Louis* et al., *1993; Harries and Fraser, 1968; Sondheimer* et al*., 1985). After day 1, infant gastric pH is acidic (from 2.5 to 5.5). It is also acidic (< 3) in premature and very low birth weight infants.*

*These results do not arrest your attention. On the contrary, you write: "*Although gastric pH drops within hours after birth, infants subsequently go through a period of relative achlorhydria, where the pH slowly increases over 10 days. The gastric pH will eventually drop to adult levels by 2 years of age (Kenner and Lott, 2007), but remains more alkaline than the adult gastric pH during infancy*".*

No figure of infant gastric pH supports the text. And the book from Kenner and Lott is not at my disposal. Some questions occur to me: Did Kenner and Lott carry out their own experiments in infant gastric juices, or did they only recopy what other authors

themselves said or recopied? If Kenner and Lott carried out their own experiments, what were exactly the figures?

Response: The citation of Kenner and Lott refers to a neonatal nursing textbook that presents the most common view of infant gastric pH in the scientific/medical literature: that pH is around 6-8 at birth, drops to more acidic levels after several hours, and slowly increases over a period of 10 days. The gastric pH level eventually falls to adult levels at approximately 2 years of age. This view has been cited in many publications, a selection of which are cited here (Bartelink et al., 2006; Fernandez et al., 2011; Morselli et al., 1980; OEHHA, 2001; Stewart and Hampton, 1989). Harries and Fraser (1968) also use the term achlorhydria to describe gastric pH >2.8.

OEHHA carefully examined the issue of infant gastric pH, evaluating the available literature on the subject. However, OEHHA was unable to locate the Reed (1996) reference mentioned in the comment. Below is a brief review of the pertinent literature.

Avery (1966) reported that the average pH from 32 infants at birth was >5, but this value fell to <4 at 2 hours after birth, and <3 at 24 hours. There is no pH data beyond 24 hours, so it is unknown if pH levels remained <3.

Harries and Fraser (1968) reported that the pH was 7.7 at birth in premature babies, but dropped to 2.6 after 6 hours. The pH rose to 3.7 at 24 hours, and a slight upward trend in pH was observed to postnatal day 14. The authors reported variability in acidity, with 71% of babies having a pH >2.8 at the first reading (1-3 hours after birth). At 24 hours, 69% of babies had a gastric pH >2.8. At 14 days, the mean pH was 4.8, high enough for nitrate-reducing bacteria to survive.

Agunod et al. (1969) reported that the pH range at birth was 2.5-5.5, with a median value of 3.7 (n=10). They also reported that acid output in the first month remained well below adult levels due to reduced volume of gastric juice and lower acid concentrations, and that acid concentration and output through the first 110 days of life was substantially lower than in adults and children aged 4-9 years. Only two of the 10 infants examined had a high acid concentration on postnatal day 1.

Luhby et al. (1954) reported in an abstract that newborn acid concentration was almost at adult levels, but the gastric juice volume was much smaller (n=8). There was no indication of when the readings were taken, and no specific details about pH were provided.

Jean-Louis et al. (1993) reported in an abstract a mean gastric pH of 4.1 at birth, which dropped to 2.6 on postnatal day 7 (n=26). No information after 7 days was presented.

Sondheimer et al. (1985) reported that 2- to 6-day-old healthy preterm infants (n=13) had a pH range of 4.6-5.8. Additionally, 7- to 15-day-old infants (n=10) had a mean fasting pH of 2.9, which increased to 5.6 directly after formula feeding. Feeding infants

a clear liquid meal did not alter the pH range in 2- to 6-day-old infants (4.5-5.3), but the range in 7- to 15-day-old infants was 2.2-3.9.

Miller (1941) reported that gastric acidity (levels of free and total acid) decreased after postnatal day 1, and remained low until postnatal day 30 (the end of the observation period). This suggests that the gastric environment is less acidic in young infants than in adults, although specific pH values were not reported.

Miclet et al. (1979) reported that pH at birth ranged from 1.4-7.8, with the majority of pH values clustered around 7 (n=130).

Maffei and Nobrega (1975) reported that breast fed infants had a mean pH >4 (n=14), up to a maximum of 7. Bottle-fed infants had a mean pH between 2-3 (n=14), with a maximum pH slightly greater than 5. Infants were 1-12 months old.

Omari et al. (2003) reported that healthy preterm infants (36 weeks postmenstrual age, 2.6 kg average body weight) had a baseline pH ranging from 1.8-2.6 based on different gastric depths measured. The pH increased after feeding (up to a maximum of 6.6-7.3), and returned to baseline after a few hours.

Many of the studies are consistent with the commonly accepted view that gastric pH is higher than adult levels at birth (Avery, 1966; Harries and Fraser, 1968; Miclet et al., 1979), drops shortly after birth (Avery, 1966; Harries and Fraser, 1968; Sondheimer et al., 1985), and that the pH remains higher than adult levels for an extended period (Harries and Fraser, 1968; Agunod et al., 1969; Miller, 1941; Maffei and Nobrega, 1975). The abstracts by Luhby et al. (1954) and Jean-Louis et al. (1993) did not provide enough information to draw conclusions, and it appears that the data were not published elsewhere. Based on the evidence, OEHHA concludes that while infant gastric pH appears to be somewhat variable, it appears to be higher than in adults for the majority of the period of concern for nitrate exposure, from birth to 6 months of age, thus making infants more susceptible to nitrate-induced methemoglobinemia.

*Comment: In infants, **in the mouth**, dietary nitrate (and also salivary nitrate, after enterosalivary circulation of nitrate) cannot be reduced into salivary nitrite, because nitrate-reducing oral microflora is absent. Before the age of 6 months, nitrite concentration in saliva is very low, even zero, though salivary nitrate concentration is high, up to 250 mg NO_3^- l^{-1} (Eisenbrand* et al., *1980) [Cf. L'hirondel, J. and L'hirondel, J.-L., 2002, p.25].*

Response: OEHHA acknowledges that infants have lower levels of salivary nitrite than adults due to reduced bacterial nitrate reductase activity, and this is described in the PHG document. It is unknown whether the nitrate-reducing capability of the bacteria in the neonatal/infant oral cavity would be affected by larger concentrations of nitrate from exogenous sources like drinking water. Additionally, nitrate-reducing bacteria in the infant gut may still convert nitrate to nitrite.

*Comment: In infants (like in adults), **in the stomach**, dietary nitrate (and also salivary nitrate after enterosalivary circulation of nitrate) cannot be reduced into nitrite. Unless contrary arguments from experiments by Kenner and Lott, 2007, the infant gastric pH is acidic. Virtually sterile, the infant gastric juice contains less than 10^6 germs ml^{-1}. [Cf. L'hirondel, J. and L'hirondel, J.-L., 2002, p.26].*

Response: See statement above regarding infant gastric pH.

*Comment: In infants (like in adults), **in the colon**, dietary nitrate (and also salivary nitrate after enterosalivary circulation of nitrate) cannot be reduced into nitrite, because nitrate is rapidly and almost immediately absorbed. It passes from the upper small intestine (the duodenum and the jejunum) to the blood stream, where it mixes with endogenously synthesized nitrate. Nitrate is not detected in feces [Cf. L'hirondel, J. and L'hirondel, J.-L., 2002, p.21].*

Response: OEHHA agrees that nitrate is rapidly absorbed from the duodenum and the jejunum.

*Comment: In infants, **in the feeding bottle**, nitrate from municipal drinking water, whatever its concentration, cannot be reduced into nitrite. Municipal drinking water is bacterially controlled. It always contains less than 10^6 germs ml^{-1}, really less than 10^2 germs ml^{-1}.*

*In infants, **in the feeding bottle**, on the contrary, nitrate from well water can be reduced into nitrite when the well is filthy, and when well water contains more than 10^6 germs ml^{-1}. There is a risk of methemoglobinemia. In infants, it is the only (and real) danger with dietary nitrate.*

Response: See statement above regarding municipal vs. well water, and the relevance of bacterial contamination of well water to methemoglobinemia induction.

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Western Growers

Comment: In the case of the nitrate PHG review, we believe OEHHA's process was sound and the conclusions warranted based on existing science and data.

Response: OEHHA acknowledges the comment.