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Manganese as a Potential Confounder of Serum Prolactin

de Burbure et al. (2006) elegantly demonstrated that dopaminergic markers in the serum, namely prolactin and homovanillic acid, are affected in children exposed to cadmium, lead, mercury, and arsenic. These findings, at low environmental exposure levels, reinforce the potential of these metals to perturb dopaminergic function and optimal development.

In spite of the strengths of the article, de Burbure et al. (2006) overlooked an important potential confounder. Specifically, the authors should consider the possibility that manganese confounded their data; if so, the data set should be reexamined. A strong relationship between manganese exposure and serum prolactin levels has been raised in multiple studies. Although prolactin levels serve as a direct measurement of monoamines or their metabolites in peripheral tissues (e.g., blood platelets, plasma, urine), plasma prolactin is also an indirect indicator of dopaminergic functioning, a target for excessive exposure to manganese (Mutti and Smargiassi 1998; Smargiassi and Mutti 1999). A concordance between neurocognitive deficits and manganese exposure also exists, including a recent study in children exposed to water manganese concentrations exceeding 300 µg/L (Wasserman et al. 2006). A significant and positive correlation between blood manganese concentrations and prolactin levels in cord blood has also been established (Tasker et al. 2004). Other examples abound, although negative relationships between manganese and prolactin have also been reported (Roels et al. 1992).

The potential that exposure to manganese contributed to or confounded the effects of the four metals on serum prolactin levels in the cohorts studied by de Burbue et al. (2006) should be considered. If samples are available for additional analysis, correlations between manganese exposure and prolactin would be beneficial and welcomed by various health forums as the debate on safe manganese exposure levels and sensitive health effect biomarkers continues.

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Potential Confounder: Bernard and de Burbure Respond

We thank Aschner for his positive comments and interesting suggestion regarding a possible influence of manganese on serum prolactin levels. We fully acknowledge that the four elements we studied (de Burbure et al. 2006) are probably not the only determinants of serum prolactin levels, especially because variations in blood or urinary levels explained only a few percent of the total variance. Other factors, including perhaps some other metals, most probably also contribute to modulate dopaminergic function and serum prolactin levels. Because the epidemiologic evidence suggesting a link between manganese and serum prolactin in children is recent, we did not measure this metal in our study. However, if we had measured blood manganese in the children in our study, it is far from certain that this factor would have emerged as a significant determinant, possibly confounding the relationships between neurologic markers and blood or urinary levels of cadmium, mercury, and lead.

In the recent study by Wasserman et al. (2006), neurocognitive deficits were indeed not related to blood concentrations of manganese. As to the correlation between blood manganese at birth and cord blood prolactin levels reported by Takser et al. (2004), this association was not adjusted for possible exposure to lead, an indisputable confounder in this sort of study. This being said, we agree of course with Aschner that exposure to manganese should be considered in future studies investigating the developmental effects of heavy metals in the environment.

The authors declare they have no competing financial interests.

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Dietary Nitrate: Where Is the Risk?

Links between nitrate and health risk have been studied for > 50 years, resulting in a large body of research. As two book-length reviews of the issue (Addiscott 2005; L'hirondel and L'hirondel 2001) tried to show, none of the health claims against dietary nitrate have been substantiated.

If there was not already an established maximum contaminant level (MCL) of 10 ppm for nitrate in drinking water in the United States [U.S. Environmental Protection Agency (EPA) 1991; World Health Organization (WHO) 1958], it would be extremely difficult, if not impossible, to justify one based on the extensive evidence gathered to date.

The conclusion of Ward et al. (2005) in their article "Drinking-water Nitrate and Health—Recent Findings and Research Needs" (Ward et al. 2005) is somewhat different. Although they were not able to reasonably conclude that dietary nitrates elevate a single health risk, the authors insisted that possible risks "must be more thoroughly explored before changes to nitrate water quality standards are considered." Why?

Examining methemoglobinemia, cancer, reproductive, and other potential risks, Ward et al. (2005) presented the extensive body of

research demonstrating only very slight negative, very slight positive, or no correlation (and usually all three). This is exactly what one would expect if there were no actual correlation. Yet instead of reexamining the MCL, Ward et al. recommended further searching for a justification of the 50-year-old regulation (WHO 1958).

We would like to draw attention to a few key additional points.

First, although the U.S. MCL for drinking water nitrate is 45 mg/L (U.S. EPA 1991), nitrate concentrations in vegetables may be > 50 times higher; vegetables often contain > 2,000–3,000 mg nitrate per kilogram. Yet nitrate-rich vegetables are good for health.

Ward et al. (2005) seem aware of this point, as they stated that "intake of dietary nitrate is less likely to increase nitrosation, because of the presence of nitrosation inhibitors in vegetables." However, they forgot the metabolism of nitrate in humans. Salivary nitrate (not dietary nitrate) is reduced to nitrite in the mouth. In fact, plasma nitrate is extracted by the salivary glands and secreted at high concentrations in saliva; in adults and children > 6 months of age, a fraction of this salivary nitrate is converted in the mouth to nitrite. Nitrite levels in saliva are maximal 20–60 min after nitrate intake. Also, because of the acidity of the gastric juice (Dang Vu et al. 1994), nitrite concentrations in gastric juice are extremely low; 15-fold to several hundred-fold less than that of salivary nitrite.

Regarding the cancer risks of nitrate, if drinking water with 10–20 ppm nitrate-nitrogen (nitrate-N) were toxic, vegetables (with their comparatively high nitrate levels) would likely also be toxic, in spite of the presence of reputed nitrosation inhibitors.

Second, during the last 12 years, several works have indicated beneficial effects of nitrate due to its conversion in the body into nitrite (NO^{2-}), nitric oxide, and diverse reactive compounds. The studies carried out since 1994 by the teams of Benjamin and Duncan are worth noting (Benjamin 2000; McKnight et al. 1999). Also, a meeting was held in Bethesda, Maryland, 8–9 September 2005 under the aegis of the National Institutes of Health and devoted to the "Role of Nitrite in Physiology, Pathophysiology and Therapeutics" (Gladwin et al. 2006).

The current MCL for nitrates of the United States, Europe, and World Health Organization are all based on the flawed American Public Health Association survey from 1948 in which "special emphasis was placed on restricting the data to those [infantile methemoglobinemia cases] definitely associated with nitrate-contaminated water" (Walton 1951). This requirement ensured the inclusion of any suspected (although not proven) case of infantile methemoglobinemia where nitrate levels were even slightly above background (~2–5 ppm). A mere five suspected cases in that survey were reported at 10–20 ppm nitrate-N , and in some cases the water was tested months after the cyanotic episode.

The societal costs of complying with the current MCL are growing, especially in rural communities least economically capable of shouldering the high costs per person of nitrate-ion removal. This economic burden imposed with questionable medical basis seems to have completely escaped Ward et al. (2005).

Although we are not against continued study to ensure adequate protection of public health, it seems to us that more than enough evidence has been gathered to confidently say that nitrates are not the threat they were once thought to be. Raising the drinking water MCL for nitrates to 20 ppm nitrate-N to reflect the extensive body of research would relieve many small rural communities of a significant economic burden without adding appreciably to any known health risks.

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Dietary Nitrate: Ward et al. Respond

We read with interest the letter by L'hirondel et al. regarding our workgroup report (Ward et al. 2005). L'hirondel et al. describe the research on methemoglobinemia, cancer, adverse reproductive, and other health outcomes as "extensive" and state that the range of results found is what would be expected if there were no correlation between these health outcomes and drinking water nitrate exposure. We disagree with their assessment of the literature. The etiologies of specific cancers and adverse reproductive outcomes are likely to be different from each other, and there are too few well-designed studies of any particular health outcome to draw conclusions about risk.

L'hirondel et al. correctly point out that nitrate levels are higher in certain vegetables than in most drinking water sources. Indeed, when nitrate levels are below the regulatory limit of 10 mg/L nitrate-nitrogen (nitrate-N), the majority of nitrate intake comes from vegetables (Chilvers et al.1984; Levallois et al. 2000). Ingested nitrate from diet and drinking water is secreted at high concentrations by the salivary glands and is reduced to nitrite by bacteria in the mouth. In the acidic stomach, the nitrite is rapidly converted to nitrous acid and then to nitric oxide and nitrosating species, which can react with amines and amides to form *N*-nitroso compounds (NOC), the potential causative agents in the etiology of specific cancers, adverse reproductive outcomes, and diabetes. Low gastric nitrite concentrations, as reported by Vu et al. (1994) and McColl (2005), do not mean that nitrite is not involved in endogenous nitrosation, as implied by L'hirondel et al.

Human studies have shown that water nitrate exposure above the regulatory limit increases urinary excretion of NOC (Mirvish et al. 1992; Moller et al. 1989; Vermeer et al. 1998). NOC formation also increased after a meal of vegetables high in nitrate and low in ascorbic acid (e.g. beets, celery); however, NOC formation was inhibited after a meal of these vegetables together with vegetables and fruits containing ascorbic acid and nitrate (Knight et al. 1991). Numerous studies have shown that the formation of NOC in the stomach is inhibited by dietary antioxidants found in vegetables and fruits (Bartsch et al. 1988; Mirvish et al. 1998; Vermeer et al. 1999).

Therefore, inhibition of endogenous NOC formation may account for some of the observed inverse associations between vegetable intake and many cancers and adverse reproductive outcomes.

To adequately evaluate the risk associated with consumption of nitrate in drinking water at the regulatory limit of 10 mg/L nitrate-N [background levels are typically < 1 mg/L (Nolan and Hitt 2003)], studies must account for the potentially different effects of dietary and water sources of nitrate. Well-designed studies include the assessment of exposure for individuals (e.g., case–control, cohort studies) in a time frame relevant to disease development, and the evaluation of factors affecting nitrosation. Estimating NOC formation via nitrate ingestion requires information on diet and drinking water nitrate, inhibitors of nitrosation (e.g., vitamin C, polyphenols), nitrosation precursors (e.g., red meat, nitrosatable drugs), and medical conditions that may increase nitrosation (e.g., inflammatory bowel disease).

Only a few such studies evaluated risk among potentially susceptible groups (reviewed by Ward et al. 2005), and two studies found significantly elevated risks associated with water nitrate levels below the regulatory limit (Brender et al. 2004; De Roos et al. 2003). Higher nitrate levels in drinking water were associated with an increased risk of colon cancer among individuals with high red meat or low vitamin C intakes (De Roos et al. 2003). Higher water nitrate ingestion was linked with neural tube defects in the offspring of women who used nitrosatable drugs during the periconceptional period (Brender et al. 2004).

We agree with L'hirondel et al. that diarrhea, in addition to high water nitrate exposure, can cause methemoglobinemia in infants; in our article (Ward et al. 2005) we stressed the need for further studies to clarify the role of drinking water nitrate exposure. Nevertheless, it is important to note that the regulatory limit does not include a safety factor; rather, it is based on available data supporting no observed adverse effect for methemoglobinemia in infants (the most sensitive subpopulation) [U.S. Environmental Protection Agency (EPA) 1991]. Therefore, we do not agree that the regulatory limit is overprotective as suggested by L'hirondel et al.

Until more well-designed studies are conducted and evaluated, we reject the conclusions by L'hirondel et al. that enough evidence has been gathered to safely raise the drinking water limit for nitrate. Raising the regulatory limit, and thereby allowing the increased intake of drinking water nitrate, would likely result in increased exposure to endogenously formed potentially carcinogenic and neurotoxic *N*-nitroso compounds and possibly result in new cases of methemoglobinemia.

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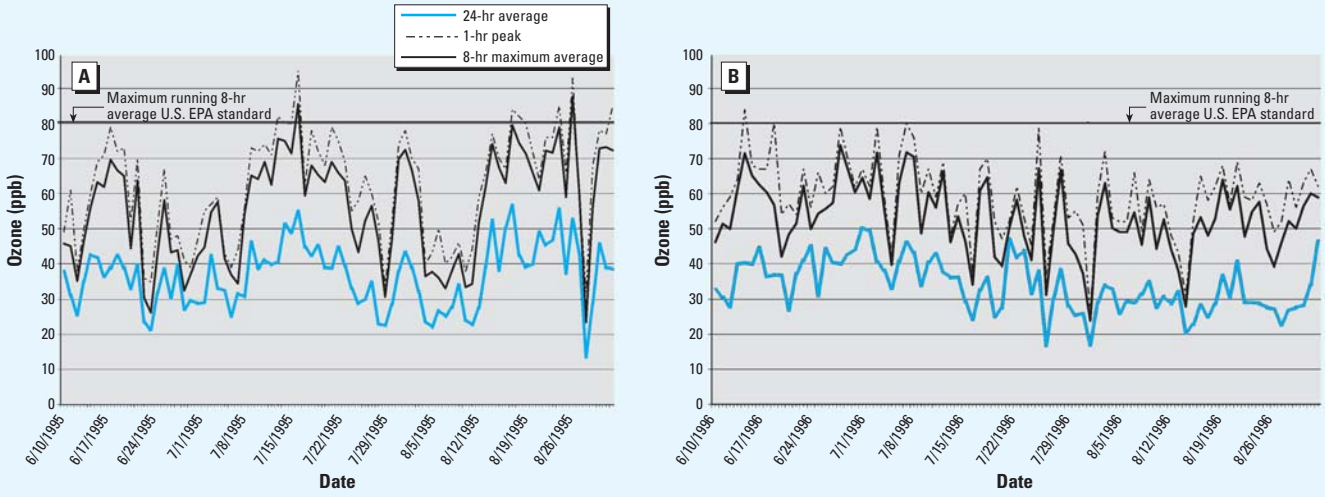
Errata

In Figure 1 of the article by Triche et al. [Environ Health Perspect 114:911–916 (2006)], the 24-hr average and the 8-hr maximum average were labeled incorrectly. The corrected figure appears below:



On page 873 of the article by Sirivelu et al. [Environ Health Perspect 114:870–874 (2006)], two sentences were incorrect: "IL-1" was omitted from the first sentence and placed incorrectly in the second. The corrected sentences are as follows:

> We have previously shown that NE levels in the AN are elevated after an immune stressor such as IL-1 (MohanKumar et al. 1998). AN has also been implicated in autonomic functions such as respiratory processing mediated by carotid body receptor (Banks and Harris 1988), suggesting that apart from the PVN, the AN also may be involved in stress-induced autonomic alterations.

EHP regrets the errors.

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