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Urinary Creatinine and Arsenic Metabolism

Urinary creatinine is almost universally employed to adjust concentrations of urinary analytes for variations in hydration status. In the February 2005 issue of *EHP*, Barr et al. used data from the Third National Health and Nutrition Examination Survey (NHANES III) to establish reference ranges for urinary creatinine for specific age and demographic categories (Barr et al. 2005). They reported that the significant predictors of urinary creatinine concentrations include age, sex, race/ethnicity, body mass index, and fat-free mass. Although these indicators have been known for many years, the unintentional adjustment for these covariates when urinary metabolites are expressed per gram creatinine can have profound effects on the interpretation of data. The fact that these effects are often underappreciated or even unnoticed renders this paper highly relevant to exposure assessment and well worth revisiting. In our studies of arsenic methylation and one-carbon metabolism, we have noted several additional complications when expressing urinary arsenic as micrograms per gram creatinine. Note that one-carbon metabolism refers to the folate-dependent biochemical pathway responsible for methylation of DNA, arsenic, and hundreds of other substrates.

Our study in Bangladesh on 1,650 adults revealed that urinary creatinine concentrations are significantly correlated with plasma folate concentrations—particularly among males, who had a higher prevalence of folate deficiency than females in Bangladesh (Gamble et al. 2005). Although this association had not been previously reported, it is not surprising considering that the formation of creatine from methylation of guanidinoacetate accounts for approximately 75% of all folate-dependent transmethylation reactions (Mudd and Poole 1975) and that creatine is the direct precursor of creatinine.

In some analyses, adjusting urinary arsenic for creatinine obscured correlations between folate and arsenic metabolism. In other analyses, correlations between folate and arsenic/creatinine were due in part to the associations between folate and creatinine. Correct interpretation of the data would not be possible without considering the impact of the correlation between urinary creatinine and plasma folate. As did Barr et al. (2005), we decided to include urinary creatinine in the statistical models as a separate independent variable. However, because of the intimate link between creatine metabolism and one-carbon metabolism, inclusion of urinary creatinine in some models resulted in overcontrolling for the effects of folate and homocysteine, our variables of interest. Thus, expression of total urinary arsenic per gram creatinine runs the risk of confounding relationships between total urinary arsenic and arsenic metabolism. Adjusting for the specific gravity of urine was not useful because it is so highly correlated with urinary creatinine.

In summary, we concur with Barr et al. (2005) that urinary creatinine should be included in multiple regression models as a separate independent variable; in addition, the role of one-carbon metabolism as a predictor of urinary creatinine should also be considered in interpreting results. Specifically, we routinely test if urinary creatinine itself is a predictor of the outcomes of interest.

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Urinary Creatinine: Barr et al. Respond

Although individual predictors of urinary creatinine such as sex, body mass index, and age have been reported, no single research endeavor has documented the predictors in one study population as thoroughly as we reported in our recent article (Barr et al. 2005). The large volume of data available in the Third National Health and Nutrition Examination Survey (NHANES III; 1988–1994) [Centers for Disease Control and Prevention (CDC) 2003a] was ideal for examining and documenting these predictors. To date, our study provides the most concrete data in the literature demonstrating creatinine variation in diverse populations and the factors contributing to this variation. We agree with Gamble and Liu that although many research articles have recognized differences in creatinine concentrations within their study populations, few have attempted to correct for this variation. Our analysis of urinary creatinine concentration data in a large, representative segment of the U.S. population was intended to highlight the problems that can be encountered when routinely correcting urinary analyte concentrations for creatinine; however, Gamble and Liu point out in their letter yet another complication that may be encountered when evaluating urinary concentrations of chemicals that undergo a folate-mediated single-carbon metabolism. We are grateful that they alerted us of the possible complication of evaluating data for chemicals such as arsenic. Because folate is routinely measured in the ongoing NHANES cycles and speciated arsenic measurements have begun in the same samples, the role of one-carbon metabolism should certainly be considered in interpreting results for arsenic and other similarly metabolized chemicals for future editions of the CDC's National Report on Human Exposure to Environmental Chemicals (CDC 2001, 2003b).

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Ototoxicity

The January 2005 issue of *EHP* provided a much-needed overview of the prevalence of environment noise and its effects on health (Chepesiuk 2005; Manuel 2005; Schmidt 2005). Indeed, noise is pervasive and its adverse health effects are among the most common occupational injuries. Your consideration of noise-induced damage is especially welcome, given the strong focus of *EHP* on overexposure to chemical agents relative to overexposure to physical stimuli. Curiously absent from the discussion, however, was a review of the evidence that has accumulated over the past two decades concerning the ability of chemical agents to produce hearing impairment directly (ototoxicity) and to interact with noise exposure yielding either additive or synergistic impairment of the auditory apparatus. Research on such processes has received support in the United States from multiple agencies, including the National Institute of Environmental Health Sciences, the National Institute for Deafness and Other Communication Disorders, the National Institute for Occupational Safety and Health, and the U.S. Environmental Protection Agency.

Occupational epidemiology studies have demonstrated noise–chemical interactions in the workplace, and laboratory animal models have been effective in identifying ototoxicants, establishing dosimetry, identifying targets of toxicity, and determining the mechanisms for such ototoxicity. For example, occupational epidemiologic studies of Morata et al. (1997) demonstrated an excess risk of developing hearing loss among workers exposed to mixed solvents (mainly toluene) plus noise among printers compared with noise-exposed referent subjects or nonexposed matched controls. Similar studies have subsequently been published for styrene-exposed workers in the reinforced plastic industry (Morata et al. 2002; Sliwinska-Kowalska et al. 2003).

In laboratory animals, the pioneer experiments on the ototoxicity of solvents were initiated by Pryor and Rebert in the 1980s (e.g., Pryor et al. 1987; Rebert et al. 1983). Since these early studies, the ability of chemicals to directly disrupt auditory function has been established for trichloroethylene (Crofton et al. 1993; Fechter et al. 1998), toluene (Campo et al. 1999; Crofton et al. 1994; Johnson 1993), ethyl benzene (Cappaert et al. 2001), and styrene (Campo et al. 2001), among other agents. In addition, Lataye et al. (2001, 2003) have nicely identified the route by which solvents enter the cochlea and the pattern of damage that they produce in the inner ear.

Using developmental models, Rice and Gilbert (1992) demonstrated that methyl mercury exposure could impair auditory function in young primates. Also, hearing impairments have been reported for lead-exposed children (Osman et al. 1999; Schwartz and Otto 1987, 1991). Crofton and colleagues (Crofton et al. 1999, 2000; Lasky et al. 2002) demonstrated the ability of polychlorinated biphenyls to disrupt the development of the cochlea in rats by disrupting thyroid function.

In this laboratory, we have demonstrated that a series of chemical contaminants with potential to disrupt intrinsic antioxidant pathways or to enhance reactive oxygen species (ROS) generation can produce permanent hearing loss in the presence of noise. These agents include carbon monoxide (Fechter et al. 1987, 1988, 2000), hydrogen cyanide (Fechter et al. 2002), and acrylonitrile (Fechter et al. 2003; Pouyatos et al. 2005). This research provided evidence that intense noise can initiate ROS generation, resulting in cochlear damage. We hypothesized that even moderate noise levels, including noise close to permissible workplace exposure levels, may initiate ROS formation but that these are normally contained by antioxidant pathways. However, in the presence of pro-oxidant chemical agents, we demonstrated that even mild noise can yield oxidative stress leading to the death of sensory receptor cells for sound, the outer hair cells, and subsequent permanent impairment of auditory function (Fechter et al. 2000, 2002, 2003; Pouyatos et al. 2005). It is striking, although not surprising, that the auditory system is vulnerable to a range of chemical agents that initiate toxic processes that have been more fully studied in the brain and other organ systems.

The existing evidence has clear implications for both environmental and occupational health, and it highlights the continuing need for research on the issue. In Europe, the scientific information available has influenced public health policy. In February 2003, the European Parliament and the Council of the European Union (2003) published Directive 2003/10/EC on minimum safety requirements regarding the exposure of workers to noise. Ultimately, an increase in the awareness of the ototoxic potential of chemicals should improve preventive efforts and help reduce the risk of hearing loss.

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Bioremediation Monitoring

In their article published in the February issue of *EHP*, Ganey and Boyd (2005) made some excellent points about the potential pitfalls of simply assaying for the disappearance of an environmental pollutant during or as a result of bioremediation. This is important because it would be wrong to leave a metabolite that might pose as much or even more risk then the original chemical of interest.

Ganey and Boyd (2005) used the bioremediation of polychlorinated biphenyls (PCBs) as an example, which was an excellent choice. However, the subject of metabolism of the PCB bioremediation metabolites should also be considered. As chlorines are removed by bioremediation, the less-chlorinated products could be more readily metabolized by many species exposed to the bioremediated material. That is, less-heavily chlorinated products (or intermediates) of bioremediation may be less toxic because of shorter half-lives due to metabolism. This phenomenon can be exemplified by work we conducted years ago at Michigan State University. We showed that 3,4,3′,4′-tetrabromobiphenyl was less toxic than 3,4,5,3′,4′,5′-hexabromobiphenyl, even though it was bound at higher affinity by the dioxin receptors because it was more readily metabolized and eliminated (Millis et al. 1985).

Commercial preparations contain few or no strictly coplanar PCB or polybrominated biphenyl congeners. This fact does not seem to be appreciated, and the impression is sometimes given that those very toxic congeners are in the environment. In fact, the coplanar polyhalogenated biphenyls probably receive way too much attention, most likely because they were used rather extensively in research; however, they were used only as model toxic congeners. The synthesis of strictly coplanar halogenated biphenyls (i.e., 3,4,3′,4′-PCB) is much different from that of the commercial preparations (which was by simple halogenation of biphenyl). Phenyl is strongly *ortho-para* directing, leading to non-coplanar halogenated biphenyls. The initial *para* and/or *ortho* halogenation makes for an even stronger *ortho-para* directive. Thus, the major components will be non-coplanar halobiphenyls. Only very small amounts of single *ortho* halobiphenyls can be found in commercial mixtures, and these mixtures are quite ineffective in eliciting effects associated with binding by the dioxin receptor.

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Editor's note: In accordance with journal policy, Ganey and Boyd were asked whether they wanted to respond to this letter, but they chose not to do so.

Errata

In "Seasick Lungs: How Airborne Algal Toxins Trigger Asthma Symptoms" [Environ Health Perspect 113:A324 (2005)], the accompanying photograph of *Karenia brevis* should have been credited to Daniel Baden/University of North Carolina at Wilmington.

"Linking Toenail Arsenic Content to Cutaneous Melanoma" [Environ Health Perspect 113:A377 (2005)] should have clarified that Laura E. Beane Freeman received NIEHS funding while at the University of Iowa, before she joined the National Cancer Institute.

EHP regrets the errors.