Issues in Arsenic Cancer Risk Assessment

We respond to Mushak and Crocetti's recent publication (*1*), which criticizes several of our recently published analyses related to arsenic cancer risk assessment (*2–5*). Basically, their criticism is that evidence of an overestimate of arsenic risk using the present cancer slope factor (CSF) is poorly supported. We present here additional information which demonstrates flaws in these arguments showing that 1) considerable amounts of inorganic arsenic are present in rice and yams; 2) a water ingestion rate of 4.5 l/day for Taiwanese farmers is scientifically justifiable; 3) consideration of these alternative estimates of arsenic intake from food and water, when calculating a cancer slope factor, will yield lower cancer risks; 4) analysis of monomethyl arsenic/dimethyl arsenic (MMA/DMA) ratios supports a threshold for arsenic methylation and detoxification; and 5) dietary protein deficiencies, even in the range experienced by the Taiwanese, have been shown to affect methylation efficiencies. Thus, application of cancer risk estimates derived from Taiwanese populations may indeed overestimate risks to U.S. populations.

Inorganic arsenic intake from food. Accurate assessment of arsenic intake from food is critical in quantifying total exposure of the Taiwanese farmers. In their commentary, Mushak and Crocetti critique the methods used by Yost et al. (*4*) to analyze speciated arsenic content of rice and yams in the Taiwanese diet. They assert that Yost et al. (*4*) noted that their methods "...required chemically forcing conditions," suggesting that observed inorganic arsenic was due to breakdown of organic arsenic during sample digestion. The available evidence indicates that methods used by Yost et al. (*4*) are no more likely to break down organic arsenic species than other analytical methods used to examine arsenic speciation. For example, the Ontario Ministry of the Environment (OMOE) (*6*) reports high concentrations of organic arsenic in seafood despite use of concentrated nitric acid (15.9 M) and sulfuric acid (18 M) digestions, which constitute a much more chemically forcing method than the hydrochloric acid (2 M) used by Yost et al. (*4*). Sanders (*7*) also reports high concentrations of organic arsenic in algae following digestion with concentrated nitric acid.

In contrast to Mushak and Crocetti's assertion, the work of Pyles and Woolson (*8*) and the OMOE (*6*) are consistent with the findings of Yost et al. (*4*), demonstrating that inorganic arsenic accounts for a large fraction of total arsenic in some foods (58–61% in polished rice, 83% in rice grain, and 48–88% in yams). Pyles and Woolson (*8*) recovered 40% of the total arsenic in potato flesh as inorganic arsenic, whereas only a trace of organic arsenic was detected in analyses of these tissues. Mushak and Crocetti (*1*) mischaracterize their data in stating that inorganic arsenic comprised only 8% of total arsenic. OMOE studies found that inorganic arsenic makes up 42% of total arsenic in rice, 50% in whole wheat bread, and 73% in apple juice (*6,9*). Mushak and Crocetti incorrectly cite OMOE data as the basis for stating that 10% of arsenic in potatoes occurs in inorganic forms, when in fact, OMOE has not conducted a speciation analyses of arsenic in potatoes. [OMOE staff were not aware of the report cited by Mushak and Crocetti entitled "Percentage of Inorganic Arsenic in Food: A Preliminary Analysis," document number 87-48-45000-057; the authors appear to be referring to a report with that number titled "Organic vs. Inorganic Arsenic in Selected Food Samples" (*6*).]

Inorganic arsenic intake from water. There is considerable evidence to suggest that a total fluid intake rate of 4.5 l/day (EPA's current assumption) represents a conservative estimate for an agrarian Taiwanese population working outdoors in a warm climate. In their commentary, Mushak and Crocetti criticize this value, suggesting that Taiwanese adult males and females require about 2 liters of total water, based on an estimated fluid requirement of 38 ml/kg body weight for an adult in a 37.8°C environment (*10*). However, this value represents a minimum requirement to maintain fluid balance and assumes "the most favorable conditions of low solute load, minimal physical activity, and absence of sweating" (*10*), and does not account for the impact of physical activity on fluid requirements.

Sweat loss rates of 1 l/hr are not uncommon for individuals performing moderate physical activity in a hot climate (*11–13*). For example, Szlyk et al. (*14,15*) monitored fluid balance in healthy men during consecutive cycles of 30 min walking/30 min rest at 40°C and a relative humidity (RH) of 40%. Water requirements, represented by the total volume of sweat lost, averaged 4.1 liters over 6 hr. Similar sweat loss rates (0.71–0.99 l/hr) were reported by Pitts et al. (*16*) for individuals marching in dry heat conditions (37.8°C, 35% RH); higher rates (1.17–1.48 l/hr) were reported in moist heat conditions (32–35°C, 80–83% RH).

The climate in Taiwan is mostly warm and humid, with temperatures averaging 30°C in the summer months. Even at light work intensity, the U.S. Army recommends consuming approximately 1 liter water/hr to sustain fluid balance at temperatures of 30°C and 20% RH (*17*). Assuming this recommendation is based on a 70-kg man, the corresponding drinking water requirements for a 55-kg Taiwanese farmer performing moderate physical activities would be about 0.8 l/hr, or roughly 5–6 l/day.

Mushak and Crocetti note that "children 9–10 years old would consume 1.3 l/day of water at an ambient temperature of 37.8°C," based on data presented by Galagan et al. (*18*). This statement is not completely accurate; the temperature cited represented the maximum temperature reached during the day, and water represented only 43% of the direct fluid intake. Thus, direct fluid intake would total 3 l/day for a 35-kg child, corresponding to 6 l/day for an adult.

Individuals acclimated to a hot environment do not ingest less water than those not acclimated, as Mushak and Crocetti suggest. Studies indicate an earlier onset of sweating, increased sweat volumes, and an improved relationship between thirst and body fluid needs, resulting in greater fluid intakes in acclimatized individuals (*19,20*).

Voluntary dehydration is primarily attributed to a sluggish thirst mechanism in humans (*11*) and is not a conscious decision by the individual as Mushak and Crocetti state. Dehydration commonly reaches 2–3% of body weight and may go as high as 5% (i.e., 3.5 liters for a 70-kg individual) (*21*). Thirst stimulation is triggered upon reaching a fluid deficit equivalent to a 0.5–1% loss in body weight (*11*). Although urine volume can decrease as a result of voluntary dehydration [from typical volumes of 1–2 l/day to as little as 0.3–0.5 l/day (*10,11*)], reductions in urine volume alone can only account for a water deficit of about 1.5 liters. Such reductions are insufficient to fully compensate for water losses due to voluntary dehydration (up to 3.5 liters) or to extensive sweat loss (estimated above to be roughly 5–6 liters for a Taiwanese farmer). Furthermore, water deficits resulting from voluntary dehydration are usually made up after one or two meals and a night's rest (*11,21*). Because an individual could not continually lose water without suffering the effects of dehydration, voluntary dehydration must be a transient phenomenon and has no effect on average total water consumption rates.

Mushak and Crocetti are mistaken in arguing that Beck et al. (*3*) and Yost et al. (*4*) have failed to consider arsenic intake from the preparation of rice and tea. In fact, EPA's recommended drinking water intake level of 4.5 l/day accounts for both direct water consumption (3.5 liters; including tap water and other beverages made from tap water, such as tea) and indirect water con-

sumption through the preparation of rice and dried sweet potatoes (1 liter) (*22*).

Implications of alternative estimates of arsenic intake from food and water on the arsenic cancer slope factor. Consideration of inorganic arsenic intake from food is important in estimating the arsenic CSF. Mushak and Crocetti incorrectly conclude that added inorganic arsenic from the diet would have no effect on the calculation of the arsenic CSF. In fact, as arsenic intakes increase and the dose–response curve shifts to the right, the y-intercept can not fall below zero because zero intake can not produce "negative cancers." Thus, the low-dose portion of the curve must flatten to keep the intercept zero or positive. Brown and Abernathy (*23*) have also recently completed an analysis of the impact of revised food and water intake rates on estimates of cancer risk and reached similar conclusions.

Evidence for nonlinearities in the arsenic dose response. Several lines of evidence (in particular, studies on the impact of high doses of arsenic on saturation of arsenic methylation) indicate that the dose–response relationship for arsenic may be nonlinear. Mushak and Crocetti argue that because the percentage of inorganic arsenic excreted in the urine often does not vary with increasing exposure [reviewed by Hopenhayn-Rich et al. (*24*)], the hypothesis that methylation becomes saturated at high doses is implausible. However, the percentage of arsenic excreted in the urine may not be the only valid indicator for methylation. Recent studies suggest that the ratio of MMA and DMA metabolites, which reflects the efficiency of MMA methylation to DMA, may be a more sensitive indicator of changes in the methylation process.

We have reviewed additional studies since our previous response (*3*) and found multiple study results, in addition to Froines et al. (*25*) and Del Razo et al. (*26*), which indicate higher MMA/DMA ratios in exposed groups compared to controls within a given study. For example, Farmer and Johnson (*27*) found MMA/DMA ratios of 0.007 in controls, compared to ratios of 0.016, 0.11, 0.22, 0.21, 0.26, and 0.29 in exposed populations, listed in order of low exposure to high exposure. Similarly, Hopenhayn-Rich et al. (*28*), Hseuh et al. (*29*), and Yamauchi et al. (*30*) have also found higher MMA/DMA ratios in exposed versus control populations. These findings indicate that humans may not able to convert MMA to DMA as efficiently at higher arsenic exposures and suggest a saturation of the methylation reaction at higher doses. Furthermore, additional evidence supporting this trend in MMA/DMA ratios comes from recent evidence that, even among a group of individuals with chronic arsenic exposure, those exhibiting cutaneous signs of arsenicism have greater MMA/DMA ratios than exposed individuals without cutaneous signs (*31*).

A more dramatic change in MMA/DMA ratios with dose was observed in a recent animal study; Hughes et al. (*32*) exposed mice to acute doses ranging from 0.5 to 5000 µg arsenate per kilogram body weight. The resulting MMA/DMA ratios were 10-fold higher in the highest exposure group compared to the lowest exposure group, suggesting that methylation of MMA to DMA was impaired with increasing exposure. While this study involved acute and not chronic exposures, it nonetheless provides valuable information about the saturation of arsenic methylation.

Mushak and Crocetti (*1*) question the method sensitivity of blood arsenic analyses by Valentine et al. (*33*), based on the practical quantitation limit (PQL) of 4 µg/l derived in Eaton's study of arsenic in water (*34*). However, in a single, well-controlled study where there is minimal variability in method, matrix type, instrument and operator performance, and quality control, the method detection limit is the appropriate measure of the level of detection, not the practical quantitation limit derived from a multi-laboratory study. Hydride generation–atomic absorption, the method used by Valentine et al. (*33*), can reach nanogram-per-liter levels of detection in biological samples including blood (*34*). At the time of Valentine's study (*33*), detection limits for arsenic in blood were about 0.1–1 µg/l for most techniques (*35*). Recently, Vahter et al. (*36*) achieved a method detection limit of 1 µg/l arsenic in blood using similar methods.

Effects of protein deficiencies. The impact of protein deficiency on arsenic methylation may be a factor in explaining differential susceptibility to arsenic across populations. Mushak and Crocetti (*1*) conclude that this is not likely to be an important factor based on a calculation of the molar ratio of arsenic to methionine and cysteine intake in the Taiwanese population, indicating that less than 1% of the daily donor methyl availability would be required to completely methylate total available arsenic. However, a study in rabbits suggests that even a 67- to 100-fold excess in the molar quantity of donor methyl compounds, as was estimated for the Taiwanese population, is within the range capable of affecting arsenic methylation.

Vahter and Marafante (*37*) measured the urinary excretion of speciated arsenic forms in rabbits fed a standard diet and in rabbits fed a methionine-restricted diet (containing 25% fewer methyl donor compounds). In control rabbits, 0.3% of the daily donor methyl availability would be required to completely methylate available arsenic; in rabbits fed the methionine-restricted diet, 1.2% of the daily donor methyl availability would be required. Rabbits fed the standard diet excreted 65% of an arsenic dose as DMA, compared to 39% in the rabbits fed the methionine-restricted diet. In other words, a methionine-restricted diet resulted in decreased methylation capacity, despite a 77-fold excess in the total molar quantity of donor methyl compounds. A possible explanation for this observation could be that arsenic and methionine stores are found in different subcellular compartments in the body. Thus, a simple consideration of the molar ratio of arsenic to methyl donor groups (methionine and cysteine) is not a good indicator of arsenic methylation capacity.

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Response: Accuracy, Arsenic, and Cancer

Slayton et al. (*1*) have attempted to rebut portions of our *EHP* commentary on arsenic (As) cancer risk assessment (*2*) and some of the debate that has emerged over such assessments. In responding to Slayton et al., we offer some additional information that interested readers may find helpful in comparing our commentary and these responses with Slayton et al. Our commentary drew particular attention to some obvious problems in reported criticisms of the Taiwanese As exposure and carcinogenesis data.

Dietary versus drinking water As in the Taiwanese study population. The cancer slope factors for ingested As based on well water As levels in Taiwanese studies were challenged by Beck et al. (*3*) and Yost et al. (*4*). They argued that their finding of inorganic As in some rice and yam samples from Taiwan required that diet As be factored into derivation of cancer dose–response curves. We noted some quantitative questions about their results. Our concerns are not trivial and the Yost et al. findings (*4*) require toxicological context and some mention of the wider implications. Nowhere in our paper did we argue that no inorganic As could be present in their few samples, merely that the As fractions in Yost et al. (*4*) appeared to differ in some cases from other reported values.

We suggested, as one possibility, use of different analytical methods. We also indicated that any analytical methodology involving use of strong acids merits scrutiny when speciating multiple forms of an element like As, especially when significant fractions of a carcinogenic form are being reported. How well do such measured levels of As forms reflect the original sample forms, and how well does *in vitro* chemical behavior reflect *in vivo* disposition of these forms when ingested? Inorganic As might be liberated in analysis but not *in vivo* when ingested. Water As does not offer this problem, being typically in the inorganic form. Such differences for rice, yams, and similar foods can be studied with controlled-diet feeding studies in the same way that seafood and other marine biota were studied.

There are several wider potential implications of the Yost et al. data (*4*), as reported. They may indicate the need for speciation analyses to detect variable inorganic As content when doing risk assessments at other As-impacted areas and communities. There may be a need to pay much more attention to food crops as exposure pathways for As in these communities. Use of analytical methods that do not methodologically alter original biochemical forms might be necessary.

Slayton et al. (*1*) note the well-known fact that strong acids do not materially break