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Human Toxicity of PBDDs and PBDFs

The brillant literature review and health risk assessment by Mennear and Cheng-Chung, "Polybrominated Dibenzo-*p*-dioxins and Dibenzofurans: Literature Review and Health Assessment" [*EHP* 102(suppl 1):265–274], states that "reports of human toxicity associated with exposure to PBDDs and PBDFs were not found" (p. 272). In fact, in their review, no references are discussed or quoted regarding human studies.

Two papers have been published on the human toxicology of these compounds. The first (*1*) is a recent report, previously presented at the Dioxin '90 Congress (*2*), about a chemist who was exposed to 2,3,7,8,-tetrabromodibenzodioxin (TBDD) and to 2,3,7,8-tetrachlorodibenzodioxin (TCDD) in March and September 1956, respectively, when synthesizing these chemicals. The chemist was defined as "in good health" in 1990, when determinations of chlorinated and brominated dioxins and dibenzofurans were performed on whole blood. High concentrations of several congeners were detected, and the results were used to discuss the half-life of the chemicals in humans. The subject presented a mild chloracne after an unspecified time from his exposure to bromodixoins in March, suggesting that TBDD could produce skin effects as chlorodioxins. Other more relevant symptoms occurred after the exposure to TCDD in September, and the patient was hospitalized for a short period.

The second was a study of subjects exposed to PBDDs and PBDFs as a result of working at a BASF factory in etrusion blending of polybutyleneterphthalate with decarbromodiphenyl ether, used as a flame retardant. The intensity of exposure was determined in 1989 through air monitoring (*3*). The paper presents blood levels of 2,3,7,8,-TBDF and TBDD and of total congener profiles for some exposed workers and the results of a comparison of several immunological tests in a population of exposed versus a population of unexposed deriving from the same working cohort. Workers had detectable blood levels of TBDD and TBDF; half-life estimates of these chemicals are presented. The results of immunological tests were described as "not adversely impacted at these burdens of PBDFs and PBDDs," even though the results of several tests showed a correlation with exposure, and in the subject having the highest blood levels of PBDFs and PBDDs, immunological changes were quite relevant. The authors stated that clinical examination did not reveal "skin lesions consistent with an acnegenic response."

It should be stressed that the results of the two quoted articles do not change the conclusions of Mennear and Cheng-Chung on the health risks of PBDDs and PBDFs. However, slightly different suggestions for future research can be derived. Human populations have been or are exposed to these chemicals because of their use in several work processes involving flame retardants, environmental exposures (mainly due to municipal incinerators), or because of accidents due to thermal decomposition of flame retardants. These exposed human populations can be suitable, at least in theory, for toxicological and epidemiological observations.

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Arsenic Risk Assessment

A commentary highly critical of two of our published studies was recently published in *Environmental Health Perspectives* (Carlson-Lynch et al., 102:354–356). One of our papers examines the epidemiological evidence for a methylation threshold for inorganic arsenic and concludes that there is no consistent evidence to support the hypothesis of such a threshold in humans (*1*). The second paper critiqued by Carlson-Lynch et al. estimates the human cancer risks of arsenic at internal sites (lung, liver, kidney, and bladder) using linear extrapolation from Taiwanese data (*2*). Carlson-Lynch et al. contend that the analyses conducted in our studies are flawed and that the conclusions reached in our publications are erroneous, rendering them unsuitable for use by the EPA in risk assessment.

Whether or not our studies are used by the EPA for risk assessment is of little concern to us, but we are certainly concerned about statements that they are flawed. Careful examination will show that all of the major points raised in the commentary are either incorrect or have no valid basis. We would like to respond to the criticisms made, point by point, in the order presented, beginning with the methylation paper (*1*).

Critique: The average arsenic exposures in almost all of the studies analyzed were too low to observe methylation saturation.

Response: The commentators base this statement on three issues. First, the authors state that evidence from an experimental study (of only four human volunteers each receiving only one dose level) suggests that methylation would be completely saturated at exposures greater than 500 µg/day (*3*). However, at the highest oral dose in this study, 1000 µg/day, the amount of urinary arsenic in the inorganic form was only 26%, hardly demonstrating methylation saturation even at this level. Buchet et al. (*3*) state in their paper that "speciation of the arsenic metabolites in urine indicated that the arsenic methylation capacity of the human body was not yet saturated, even with an oral daily dose of 1000 µg As." The evidence of *any* metabolic saturation from this study is not conclusive. Each of four arsenic dosing levels was assigned to a different individual subject, making it impossible to differentiate interindividual differences in methylation efficiency from dose-dependent effects that might apply to a general population.

Second, the authors state that we analyzed only two groups with average urinary arsenic levels at or above 190 µg/l, which they hypothesize corresponds to the concentration above which methylation saturation occurs. This statement obscures the fact that 1) the two groups combined had a total of 35 people, 2) our analysis of available individual data (see Figure 2 of our paper) included 14 persons with urinary arsenic levels >190 µg/l. No trend of higher relative proportions of unmethylated arsenic is suggested for those 14 individuals.

Third, the authors state: ". . . a regression analysis on the individual data within the Yamauchi et al. [*4*] population was borderline significant at $p = 0.10$. . ." (p. 354). However, this was just one of nine regression analyses we presented. The slopes were positive in four (including the Yamauchi study) but negative in five (*1*: Table 9).

As a matter of interest, in our more recent studies of chronically exposed popu-

lations in the United States and Chile, we have urinary speciation data for over 100 individuals exposed to average levels well above 1000 µg As/day and ranging above 2000 µg/day with no evidence of methylation saturation even at these levels. Our study in Nevada of 18 chronically exposed individuals also found no evidence of methylation saturation, even with an average estimated intake of 2260 µg As/day (*5*).

If a methylation threshold for arsenic does exist, the epidemiological and experimental evidence suggest that it must be at exposure levels well above 2000 µg/day, making it completely irrelevant to usual human exposures and adverse health outcomes.

Critique: We used urinary arsenic concentrations from grab samples as the basis for evaluating methylation capacity. However, the proportion of inorganic arsenic excreted in the urine varies with time; thus an individual grab sample is not representative of the degree of methylation that is occurring.

Response: This concern would apply to small studies of short-term exposures, such as those of experimental studies of human volunteers or incidents of accidental or self-induced poisoning. Our critics again cite as an example Buchet et al. (*3*), where volunteers ingested a single daily dose of arsenic for only five days, resulting in an elimination profile with varying proportions of the three urinary arsenic species over the time period following ingestion. However, for chronically exposed populations ingesting arsenic on a regular daily basis, grab samples do give a representative picture when averaged over the whole group. For example, grab samples taken from a large number of people should give the same methylation picture as 24- or 48-hour samples (which our critics claim are appropriate measurements) from randomly selected subpopulations. It should be noted that the epidemiological goal is often characterizations of the group, and not precise characterization of each individual. For example, epidemiological studies frequently use one or two casual blood pressure readings on individuals to characterize groups, rather than 24-hour monitoring of each person.

The following are points raised in criticism of our cancer risk assessment paper (*2*):

Critique: In deriving risk estimates associated with arsenic exposure using linear regression, Smith et al. assumed that the arsenic intake of the population was zero. This assumption would artificially increase the slope of the exposure–response curve.

Response: Relative to the exposed populations, the intake of the control populations was virtually zero. Nonetheless, examination of the graphs in Figure 1 of our paper would show that the dose–response slopes would actually increase rather than decrease if one assumed a higher exposure for the control population.

Critique: Smith et al. did not discuss the implications of detoxification in estimating potential risks from low-level exposures typical of the U.S. population.

Response: We did. A whole section of our paper is devoted to it: "Is There a Threshold?" Table 4 of our paper presents human methylation data.

Critique: Smith et al. do not consider key uncertainties in the use of the Taiwan data in their analysis. Reference is then made to fluorescent humic substances in Taiwanese drinking water.

Response: We have discussed this topic in detail in another paper published by our group (*6*) which we referenced in our methylation article. In brief, there is little, if any, valid evidence to support the claim that humic substances contribute to increased cancer occurrence in the arsenic-endemic areas of Taiwan.

Critique: We do not address the differences between the Taiwanese and U.S. populations that would reduce the accuracy of using exposure–response data from Taiwan for U.S. populations. We then make reference to protein and methionine intake.

Response: We did. A paragraph in the section titled "Risk Estimation for the U.S. Population" addresses this issue. For example, in reference to protein deficiency, we state: "If nutritionally inadequate diets among the Taiwanese population exposed to arsenic made them more susceptible to the carcinogenic effects of arsenic, the extrapolated risk estimates for the average U.S. population would be too high" (*2*: 264). However, a reanalysis of the data presented in the paper referenced by our critics (*7*) shows that by current standards the Taiwanese intake of protein and methionine was adequate (*8*).

Carlson-Lynch et al. further claim that our exposure parameters in terms of drinking water intake in Taiwan were too low. We would merely note that we used published EPA estimates of Taiwanese drinking water intake (*9*). If one used higher estimates, then cancer risk estimates for the U.S. would indeed be a little lower. However, the estimate of 4.5 liters of arsenic-containing water per day that they refer to comes from a speculative EPA staff discussion with virtually no data to support it (*10*). In our view, the previous estimate of 3.5 liters/day has just as much plausibility for an all-year average for males in a warm, but temperate, climate. However, it is a moot point since this variable has only a small effect on risk estimates.

Finally, in the brief paragraph of the article not devoted to criticism of our studies, the authors state that mechanistic or more refined epidemiological studies are needed to assess the relationship between internal cancers and arsenic ingestion. On this we can agree. They then cite as an example an article by Guo et al. (*11*). (Note: We have been informed that C.J. Chen was not one of the authors as cited by Carlson-Lynch et al., and that the paper will be published in *Journal of Geological Chemistry and Health*, not *Science and Technology Letters*). Since this interesting article is in press and may not be available at the time this letter is published, we will note that it contains no mechanistic data. Furthermore, it is an ecological study which hardly qualifies as a "refined epidemiological study." In addition, the Guo et al. paper uses a highly unusual form of regression analysis whose validity has not been established. Only well-designed epidemiological studies with individual exposure data will identify the true dose–response relationship between inorganic arsenic ingestion and the risk of internal cancers. At the present point in time, we know of no mechanistic data, nor firm epidemiological data, which allow one to conclude that the relationship between arsenic ingestion and cancer is sublinear, let alone confirm the existence of a threshold.

In conclusion, an evaluation of the commentary written by Carlson-Lynch et al. demonstrates that none of the criticisms is valid. It seems unfortunate that we were not invited to comment at the time of its publication.

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Response to Smith et al.

Smith et al.'s criticisms of our commentary in *EHP* (102:354–356) fall into four general categories: arsenic methylation and detoxification; the recalculation of the slope factor for ingested arsenic; differences between U.S. and Taiwanese populations; and recent epidemiological analyses. As discussed below, we stand by our earlier conclusion that the dose–response relationship for arsenic carcinogenicity is likely to be nonlinear, and we feel that there is no basis for dismissing the methylation saturation hypothesis as one possible explanation for nonlinearity. We offer the following response to Smith et al.'s criticisms.

Arsenic methylation: We acknowledge that the issue of arsenic methylation and detoxification is complex; however, we believe that the methodological limitations we observed in the Hopenhayn-Rich et al. (*1*) article on arsenic methylation weaken their argument that human studies do not support a methylation threshold hypothesis for arsenic. Moreover, we call readers' attention to several recent studies that suggest that percent inorganic arsenic in urine is not as sensitive an indicator of the saturation of the methylation pathway as is the ratio of the percentage of urinary metabolites, MMA to DMA. Hughes et al. (*2*) demonstrated an increase in the relative percentage of MMA to DMA (per administered dose of arsenic) excreted in the urine of mice, with increase in dose. In single oral doses ranging from 0.5 to 5000 µg/kg, the ratio of MMA to DMA increased by approximately a factor of 10 from the lowest to the highest group. The relative percent of inorganic arsenic bound to tissue also increased with dose. The authors propose that inorganic arsenic binds to macromolecules and does not appear in urine until the binding becomes saturated. Therefore, inorganic arsenic is not as sensitive an indicator of saturation of the methylation pathway as is the concentration of methylated metabolites. Similarly, in an epidemiological study of humans exposed to elevated levels of arsenic in drinking water in Mexico, Del Razo et al. (*3*) report that the MMA:DMA ratio in urine was significantly increased in the study group by a factor of 2.4 times relative to that in the control population. In another epidemiological investigation of increased levels of arsenic in drinking water in northeast Taiwan, Froines (*4*) observed a statistical increase of 1.5 times in the ratio of percentages of urinary MMA: DMA in the exposed population relative to the controls.

In our commentary, we stated that Smith et al. (*5*) did not discuss the implications of detoxification in estimating potential risks from low-level exposures typical of the U.S. population. To be more precise, we should have stated that although Smith et al. briefly discussed methylation, they neglected to adequately consider some of the evidence that suggests there may be a saturation level for the methylation reaction, and therefore discounted the role of detoxification in the dose–response relationship for arsenic carcinogenicity.

Smith et al. refer to studies in Chile and Nevada which further support their position regarding a lack of evidence for saturation of the methylation pathway. We recommend that these studies, as well as other published data, be reviewed to see whether MMA:DMA ratios change, on an individual subject basis, with increasing dose.

Issues concerning the recalculation of the slope factor: As Smith et al. have noted, we incorrectly stated in our original commentary that assuming a zero arsenic intake for the control population would artificially increase the slope of the exposure–response curve. By assuming a zero intake for the control population, Smith et al. did indeed calculate a shallower slope, using a simple linear regression, than would have been calculated if the control population alone had been assumed to have increased arsenic exposure (and all other assumptions and data points remained the same). We should have stated that a more accurate representation of the arsenic exposure for *all* exposure groups, including careful considerations of background dietary arsenic intake and water consumption rates in both exposed and control groups, as well as the use of a more appropriate dose–response model, would result in a significantly *decreased* cancer slope factor (CSF) and lower risks.

Background levels of arsenic in the diet should be considered as part of exposure in all groups, since elevated levels of inorganic arsenic have been shown to be present in the food supply in Taiwan. Recent analyses have suggested that the actual amount of inorganic arsenic in the Taiwanese diet may range from 62 to 290 µg per day (*6*). Thus, dietary intake of arsenic in control populations is unlikely to be "virtually zero," as Smith et al. have suggested, and estimates of dietary arsenic should be added to all exposure groups, including the control group.

As an example of the impact of background arsenic intake on estimates of cancer risk, Yost et al. (*6*) have shown that EPA's current CSF for arsenic, calculated using a linearized multistage model, would be lowered from 1.75 to as little as 0.13 kg-day/mg by correcting for background dietary sources of inorganic arsenic. Had Smith et al. (*5*) used these estimates of dietary arsenic intake in their analyses, an overall decrease in calculated risks for internal cancers would have been expected. However, this lowered risk would be reflected in Smith et al. (*5*) as a change in the intercept, but not the slope, of the exposure–response curve because they used simple linear regression to model total (as opposed to excess) mortality.

Water ingestion rates also can significantly affect total arsenic exposure and the calculation of cancer slope factors. As discussed in our commentary, EPA has recently approved an RfD for arsenic based on Taiwanese data and a water consumption rate of 4.5 l/day for males and females (*7*). While it is true that EPA had previously estimated Taiwanese water intake to be 3.5 l/day for males and 2 l/day for females (*8*), the characterization by Smith et al. that the more recent estimate of 4.5 l/day comes from "a speculative EPA staff discussion with virtually no data to support it" is inappropriate. Abernathy and his colleagues considered discussions of water consumption rates with several individuals from a Taiwanese Blackfoot treatment cen-