

Detection of Excess Arsenic-Related Cancer Risks

Morales et al. (1) reanalyzed data from a study in an arseniasis-endemic area of Taiwan (2–5). Cancer risks for low-level waterborne arsenic exposures were estimated using a variety of statistical models with and without a comparison population. Morales et al. (1) concluded that although the shape of the exposure–response curve is uncertain at low levels of arsenic exposure, over a lifetime, one out of every 100–300 people who consume drinking water containing 0.050 mg/L arsenic may suffer an arsenic-related cancer (lung, bladder, or liver cancer) death. Smith et al. (6) predicted similar levels of arsenic risk.

Morales et al. (1) noted that despite the considerable uncertainties in the underlying data, the risks are “sobering.” However, they also concluded that the low concentrations of waterborne arsenic in the United States make it unlikely that such risks would be detected by epidemiologic studies (1), although they presented no calculations to support this conclusion. In reviewing the results of Lewis et al. (7) in the Millard County, Utah, study, the U.S. Environmental Protection Agency made a similar statement to the National Research Council Subcommittee to Update the 1999 Arsenic Report (8), although without listing their assumptions or showing a power calculation.

In the Millard County, Utah, study, Lewis et al. (7) followed a cohort of 4,058 individuals exposed to waterborne arsenic at levels of 0.014–0.166 mg/L. Expected death rates were calculated using Utah death rates for the same periods. No elevated death rates from bladder, lung, or liver cancers were observed for those who died through November 1996, and death rates were not higher in people with the highest levels of drinking water arsenic. In fact, for bladder and lung cancers, two cancer sites thought to have the strongest association with arsenic exposure, the authors observed 39 deaths when 63.5 were expected ($p < 0.05$). These findings are not consistent with the postulated excess risk for lung and bladder cancers, nor do they support the concerns that epidemiologic studies in the United States are not sufficiently powerful to detect the postulated arsenic-related health risks.

One of the problems in interpreting claims that studies in the United States lack the power to detect expected health risks is that these claims are made without presenting the assumptions and power calculations. Authors may assume that compliance

with the 1946 drinking water arsenic standard for interstate carrier water systems of 0.050 mg/L (9) is complete and that no populations consume water above that level. This is unfortunately not correct. Several scientists have claimed that arsenic health effects studies cannot be conducted in the United States because of high rates of migration; however, critics do not generally consider the assumed latency of the effect. For example, if the latency is 20–30 years, as might be expected if arsenic is a primary cause of cancer, the effect of migration is likely to be large. Alternatively, if only exposures that occur late in life are important and the latency is 10–15 years, as might be expected if arsenic is a late-stage promoter of cancer (10–12), the effect of latency might be small. Older people have lower rates of migration than younger people.

Our goal in this letter was to estimate the sample size required to test the arsenic risk predicted by Morales et al. (1) in the United States. According to the National Cancer Institute Surveillance, Epidemiology, and End Results Program (13), the average lifetime risk of dying from lung cancer for males and females in the United States is approximately 6.2%, whereas the average lifetime risk of dying from bladder cancer is approximately 0.46%. We made the following assumptions for two hypothetical studies—one with a population exposed to 0.100 mg/L and one with a population exposed to 0.050 mg/L:

- The added lifetime risk of death is 1 in 100 from consuming 0.050 mg/L and 1 in 50 from consuming 0.100 mg/L arsenic in drinking water.
- The arsenic-related cancer death risks are equally divided between added bladder and lung cancer death risks.
- We assume that there is an equal number of people in the cohort at background arsenic levels (0.050 mg/L) and at the high waterborne arsenic concentration (0.100 mg/L).

We calculated sample sizes for a cohort study using a published computer program for power and sample size calculation (14). Based on the above lifetime risks of death from bladder and lung cancer, a power of 0.80, and a p -value of 0.05, we calculated the sample sizes presented in Table 1. The sample sizes were estimated based on relative risks presented by Morales et al. (1).

Table 1. Sample sizes for each cohort.

Exposure level	RR bladder cancer	Sample size	RR lung cancer	Sample size
0.050 mg/L	1.72	7,600	1.05	77,228
0.100 mg/L	3.17	1,371	1.16	8,051

RR, relative risk.

The sample sizes presented in Table 1 are based on an assumed lifetime cancer death risk for the general population. Lewis et al. (7), in their Utah study, included a cohort of presumed nonsmokers. Whether or not arsenic health risks are higher for smokers (15) is an important consideration when designing a study. The required sample size is smaller if the added risks are the same for smokers and nonsmokers, and the study could be restricted to nonsmokers, as was generally the case for Lewis et al.’s Utah cohort (7). Alternatively, if a study of smokers is required (15), the background risk of cancers is much higher and the required sample size is much larger.

In addition, small relative risks, such as those for lung cancer, are difficult to study because of the potential effects of uncontrolled confounding. Investigators who believe that U.S. populations cannot be studied may have reached that conclusion because they considered the combined risks of bladder and lung cancer. We would agree that a study of current lung cancer risks in the general population could be problematic for water arsenic exposures of ≤ 0.050 mg/L. However, at higher arsenic exposures a study might be feasible because the sample size would be considerably less.

As mentioned above, specific assumptions about the magnitude of migration are important because loss of cohort members through migration would require an increased sample size to offset the expected losses. It is also essential to clearly specify the goal of the epidemiologic study and the outcomes of interest. Studies to better understand the underlying mechanisms for how arsenic causes or promotes the risk of cancer may require a different design than a study to validate or test the predicted increased health risks from waterborne arsenic exposure.

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Carcinogenicity of Trichloroethylene

The possible carcinogenicity of trichloroethylene (TCE) remains a controversy.

Over the past year the National Toxicology Program's *Tenth Report on Carcinogens* (1) reaffirmed the classification of TCE as "reasonably anticipated to be a human carcinogen." In a recent review (2), we summarized the results of published studies and concluded that the evidence suggested an association between TCE exposure and kidney and liver cancers, with somewhat weaker evidence of an association between TCE exposure and both Hodgkin's disease and non-Hodgkin's lymphoma. We pointed out that the data also suggested an association between TCE exposure and cervical cancer, although these data may have reflected exposure to tetrachloroethylene rather than to TCE because the observations of these cancers were mainly among dry cleaners. Others challenged our findings (3,4).

Two studies, an occupational cohort study by Hansen et al. (5) and a case-control study by Pesch and et al. (6), have appeared in the literature since the publication of our study (2); their findings provide further support for the carcinogenicity of TCE. The cohort study by Hansen et al. (5) is similar to two other studies identified in Tier I of our analysis (6,7); biomonitoring of urinary trichloroacetic acid was used to assess exposure to TCE. In Table 1 we present the standardized incidence ratios (SIRs) reported by Hansen et al. (5) for those cancers we identified as most likely associated with TCE exposure. Table 1 also includes recalculated average relative risks, which update the results of our previous review (2).

For kidney cancer, Hansen et al. (5) report a deficit of cases for males, an excess for females, and a slight decrement for both sexes combined. However, the relatively small number of cases does not affect the overall average relative risk in a substantial way. As noted by Hansen et al. (5), among others, the significance of the results hinge on the inclusion or exclusion of the study by Henschler et al. (11).

The report of five liver and biliary cancers and an elevated SIR in this newest study results in a larger and more precise estimate of the average risk. Our follow-up with Hansen revealed that the excess is largely due to the three cases of biliary cancer observed (10). Only two other studies reported on biliary cancer (7,12), and both reported an excess among TCE exposed workers: Anttila et al. (7), SIR = 1.6; 95% CI, 0.4–4.0; four cases; and Spirtas et al. (12): male; SMR = 2.4; 95% CI, 0.9–5.2; six cases. For liver cancer alone, the observation of Hansen et al. (5) is consistent with our previous study (2) and increases our concern that TCE may cause liver cancer.

A similar observation was seen for non-Hodgkin's lymphoma and cervical cancer. The inclusion of the findings of Hansen et al. (5) results in stronger and statistically significant average relative risks for each of these sites. Cervical cancer has a relatively high 5-year survival rate (> 67%) (13), particularly if diagnosed early, and suggests incidence as a more relevant end point. The studies reviewed, including that of Hansen et al. (5), show elevated incidence but not elevated mortality for this cancer, supporting the possible carcinogenicity of TCE. No cases of Hodgkin's disease were observed by Hansen et al. (5), and the average risk was largely unchanged.

The other notable finding in the study by Hansen et al. (5) is the elevated and statistically significant risk for esophageal cancer. Previous studies have not reported on the incidence of esophageal cancer, although the reported risks for esophageal cancer mortality are slightly elevated, particularly among dry cleaners.

A second European study also is consistent with a weak association between kidney cancer (specifically, renal cell carcinoma) and TCE exposure (6). In this multicenter case-control study with 935 incident cases, Pesch et al. (6) used a job exposure matrix

Table 1. Mean SIRs, 95% CIs, and number of observed cases for selected cancer sites.

Cancer type	Hansen et al. (5)		Wartenberg et al. (2)		New calculations ^a		Conclusions
	Mean SIR (95%CI)	No.	Mean SIR (95%CI)	No.	Mean SIR (95%CI)	No.	
Kidney cancer	0.9 (0.2–2.6) 2.4 (0.03–14)	3 M 1 F	1.7 (1.1–2.7)	21	1.6 (1.1–2.4)	25	Small change; still strongly suggestive
Liver cancer	1.8 0.1 Expected	2 M ^b F ^b	1.9 (1.0–3.4)	12	1.9 (1.1–3.2)	14	New data provide additional support
Liver/biliary cancer	2.6 (0.8–6.0) 0.4 Expected	5 M F	1.1 (0.3–4.8)	4	2.0 (1.0–4.3)	9	New data provide additional support
Hodgkin's disease	0.5 Expected 0.1 Expected	M F	1.5 (0.6–3.7) —	4	1.5 (0.6–3.7)	4	Small change; still suggestive overall
Non-Hodgkin's lymphoma	3.5 (1.5–6.9) 0.3 Expected	8 M F	1.5 (0.9–2.3) —	22	1.9 (1.3–2.8)	30	New data provide additional support
Cervical cancer	3.8 (1.0–9.8)	4 F	2.4 (1.2–4.8)	8 F	2.7 (1.6–4.8)	12 F	New data provide additional support

Abbreviations: CI, confidence interval; F, female; M, male.

^aAll average risks except those for kidney cancer are homogeneous based on the Q-test for homogeneity ($p > 0.2$). ^bData from Hansen (10).

and a job task exposure matrix to categorize exposure. Odds ratios were 1.1–1.3 for men and 0.8–1.8 for the smaller population of women.

In summary, we see the findings of Hansen et al. (5) and Pesch et al. (6) as providing additional support of our previous findings, which suggest that TCE exposure causes cancer in humans. Although alternative explanations, such as confounding and chance due to multiple comparisons, are possible explanations for this set of studies, as noted by Hansen et al. (5), we find it unlikely in light of the number of cases of cancer and sizes of the relative risks. Moreover, only a small number of subjects in the study by Hansen et al. (5) experienced TCE exposures at levels higher than the current permissible level (14), suggesting that excess cancer risks observed in this cohort study may be associated with low-level exposures to TCE.

The views in this letter are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.

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***N*-Acetyltransferase 2 Polymorphism in Patients with Spanish Toxic Oil Syndrome**

Ladona et al. (1) reported on the possible relationship between several genetic polymorphisms that regulate enzymatic activities involved in the processing of multiple xenobiotics and the risk of Spanish toxic oil syndrome (TOS). TOS, a disease that affected several thousand Spaniards 20 years ago, was attributed to the ingestion of adulterated rapeseed oil. Ladona et al. (1) reported an excess of *N*-acetyltransferase 2 (*NAT2*) slow-acetylation alleles and, consequently, an excess of slow acetylator genotypes in long-term survivors of this syndrome when they were compared to a group of “friends” (i.e., not consanguineous subjects living in the same area) but not when they were compared to siblings who were not affected by the syndrome.

In this setting, I find that the frequency of *NAT2**4 (wild type) normal allele reported in the “friends” control group is exceedingly high (0.38) when compared to that found in previous studies in Spanish and in other Caucasian populations (2,3); the results in these studies are in concordance with the proportion of slow acetylator phenotypes found in the same populations (4,5). Could this difference be due to the small number of subjects included in the control groups studied by Ladona et al. (1)?

In contrast, if the differences between patients and controls were actually due to an excess of slow acetylators among patients, this could reflect that slow acetylation serves a protective role. These subjects

Table 1. Acetylator phenotype in patients with toxic oil syndrome and in normal controls.

Group (n)	Slow acetylators (%)	Rapid acetylators (%)
Patients (83)	55	45
Controls (157)	57	43

are long-term survivors of the syndrome, and it is possible that rapid acetylators had a higher risk of dying in the acute phase of the disease.

In 1981 we used sulfamethazine as an enzyme-specific substrate (6) to determine the acetylator phenotype in 83 Spanish patients (36 males, age 46.8 ± 16.7 years, mean \pm SD) suffering TOS in its acute phase and in 157 normal controls (7). All subjects were from the same geographic area (Madrid, Spain) and ethnic origin (white Spaniards) as those studied by Ladona et al. (1). Results of our study (7) are shown in Table 1. We found no differences in the distribution of both phenotypes between cases and controls, with almost identical frequencies for slow acetylator individuals in both subgroups. These frequencies are also consistent with those reported for the Spanish and other Caucasian populations (4,5).

Ladona et al. (1) reported that the frequency of the *m2* allele (i.e., *NAT2**6) in patients (0.32) was higher than that found in both control groups. Fretland et al. (8) have demonstrated that there are quantitative and qualitative differences in the metabolic activity of the enzymatic proteins coded by *NAT2*-mutated slow-acetylator alleles. Thus, it may be hypothesized that a selective affinity exists between any toxic component of rapeseed oil and any of the mutated slow-acetylator alleles.

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Use of Reporter Genes and Vertebrate DNA Motifs in Transgenic Zebrafish as Sentinels for Assessing Aquatic Pollution

In a recent paper in *EHP*, Mattingly and co-workers (1) showed that dioxin-treated transgenic zebrafish [having 1,905 bp of 5' flanking region of the human *CYP1A1* gene, driving the jelly fish green fluorescent protein (GFP) reporter gene] displayed inducible GFP in the eye, nose, and vertebra of embryos 48 and 72 hr after fertilization. They first confirmed in zebrafish liver cells that zebrafish transcription factor proteins [e.g. the aromatic hydrocarbon receptor (AHR) and its heterodimeric binding partner the AHR nuclear transporter (ARNT)] must be capable of recognizing and binding to human aromatic hydrocarbon response elements (AHREs) present in the *CYP1A1* 5' flanking region.

We wish to point out that we have done similar experiments, although they were not cited by Mattingly et al. (1). We first made plasmid constructs using mammalian or trout response elements to drive the firefly luciferase (LUC) reporter gene and showed that transient transfection of the zebrafish ZEM2S cell line with these reporter constructs imparts dose-dependent gene induction upon exposure to a variety of chemicals (2,3). Using the golden mutant zebrafish, which has a decrease in interfering pigmentation, we then developed transgenic fish in which vertebrate DNA motifs that respond to selected environmental pollutants are capable of activating a reporter gene that can be easily assayed; details of our successes and failures in trying to generate stably transformed transgenic zebrafish cell lines have been reported (4). The expression of transgenes in zebrafish in our hands has been quite difficult to maintain past the F₁ generation, although some laboratories have been more successful (5). However, the short generation time (12 weeks), long life span (2–3 years), and relatively small diploid genome make the zebrafish a very attractive experimental model system.

We began with three DNA motifs that recognize three important classes of foreign chemicals (2). AHREs respond to numerous polycyclic hydrocarbons and halogenated coplanar molecules such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; dioxin) and polychlorinated

biphenyls. Electrophile response elements (EPREs) respond to quinones and numerous other potent electrophilic oxidants. Metal response elements (MREs) respond to heavy metal cations such as mercury, copper, nickel, cadmium, and zinc. We first established that zebrafish transcription factors are able to recognize mammalian or trout AHRE, EPRE, or MRE sequences in a dose-dependent and chemical-class-specific manner, and that expression of both the GFP and LUC reporter genes are easily detected in zebrafish cell cultures (2) and in intact live zebrafish (4). As anticipated, some agents gave a response to only one of the three classes, whereas others gave a mixed (AHRE- plus EPRE-mediated or MRE- plus EPRE-mediated) response. We are extending these studies to include estrogen response elements (EREs) to detect the effects of environmental endocrine disruptors, and retinoic acid response elements (RARE, RXRE) to detect the possible effects of retinoids in the environment.

A very important aspect of this assay is its sensitivity due to the property of bioconcentration that is exhibited to varying degrees in all fish species. Each environmental pollutant is known to be bioconcentrated; for example, 10⁻¹⁷ M TCDD in a body of water is concentrated 100,000 times (6) to approximately 10⁻¹² M TCDD in a fish, where it would act upon the AHRE motif and turn on the GFP or LUC reporter gene. Variations in sensitivity of this model system can also be achieved by increasing the copy number of response elements and perhaps by altering the sequence of each core consensus response element and flanking

regions. This transgenic technology should allow for a simple, exquisitely sensitive, and inexpensive assay for monitoring aquatic pollution.

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CLARIFICATION

In the December 2001 Forum article “Old Pesticides Pose New Problems for Developing World” [*EHP* 109:A578–A579], the partnership behind the Africa Stockpiles Project was not clearly defined. The Africa Stockpiles Project is an initiative of the World Wildlife Fund and Pesticide Action Network UK that is now being developed by a strategic partnership consisting of African regional organizations (the African Union, the United Nations Economic Commission for Africa, the African Development Bank, and Pesticide Action Network Africa), United Nations agencies (the Food and Agriculture Organization of the United Nations, the United Nations Environment Programme, the United Nations Industrial Development Organization, the Secretariat of the Basel Convention), the World Bank, and the industry association CropLife International.