

Teratogen Update: Inorganic Arsenic

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ABSTRACT

Background: Inorganic arsenic has been used by many laboratories to study the pathogenesis of exencephaly in rodents. These studies, which used predominantly injection exposures, coupled with the paucity of epidemiology data, resulted in the erroneous inference that inorganic arsenic should be considered a human teratogen.

Methods: This study assembles and assesses literature analyses of older human and animal investigations together with the results of new experimental studies. These recent studies were performed according to modern regulatory guidelines, and relevant exposure routes (inhalation and ingestion) were used to evaluate the potential risk of developmental effects in humans.

Results: The existing epidemiological data are inadequate to support risk assessment because of the failure to confirm or measure arsenic exposure during early gestation and the deficiencies in accounting for potential confounding factors. The animal data revealed that inorganic arsenic caused malformations in offspring only when it was injected into the veins or peritoneal cavity of pregnant animals during early gestation. Exposure via inhalation or oral ingestion, even at concentrations that were nearly fatal to pregnant females, caused no arsenic-related malformations.

Conclusions: Inorganic arsenic poses virtually no danger to developing offspring when maternal exposure occurs by relevant routes (oral and inhalation) at concentrations that are likely to be experienced in the environment or in the workplace.

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Environmental exposures to inorganic arsenic compounds are highly unlikely to cause congenital malformations in humans. This conclusion has been drawn from a recent series of analytical (DeSesso et al., '98) and experimental studies (Nemec et al., '98; Stump et al., '99; Holson et al., '99a,b) that were designed specifically to provide input for risk assessments.

The initial piece of this body of work is a detailed analysis of the published data from human and animal developmental studies (DeSesso et al., '98). That review critically evaluated 13 studies of human populations that may have been exposed to inorganic arsenic, 32 *in vivo* laboratory animal investigations, and 11 *in vitro/in ovo* studies. As detailed below, DeSesso et al.

concluded that neither the human nor the animal studies were of sufficient robustness to support a risk assessment.

Definitive data for risk assessment should be gathered from well-designed epidemiology studies of populations that were exposed to known amounts of arsenic. Unfortunately, such data are not available. The human data set for inorganic arsenic consists predominantly of ecologic investigations of populations living near smelters (Nordström et al., '78a,b, '79a,b; Beckman and Nordström, '82; Tabacova et al., '94a,b) or industrial facilities that had processed arsenic (Ihrig, '97; Ihrig et al., '98), or of populations that were exposed to drinking water containing arsenic (Zierler et al., '88; Aschengrau et al., '89; Börzsönyi et al., '92). In-depth critiques of the epidemiology data set have been published (DeSesso et al., '98; Holson et al., '00). These studies failed to verify that arsenic exposure occurred prior to the adverse event; they did not measure arsenic exposure (or in the few studies with surrogate measurements, exposure during the critical period of gestation was not estimated); and they did not account for other potentially confounding risk factors (e.g., alcohol use, maternal age, socioeconomic/nutritional status). In addition, many of the investigations of human populations centered on occupations or locations where people were exposed to ill-defined mixtures of materials that were assumed to include inorganic arsenic. Quite simply, the human data set is inadequate (Golub et al., '98; National Research Council [NRC], '99).

In the absence of good quality epidemiology data, risk assessors revert to data from well-conducted experimental animal studies. In the case of inorganic arsenic, a large number of *in vivo* studies appeared in the peer-reviewed literature prior to 1995. For the most part, however, the goal of these studies was to investigate the pathogenesis of neural tube defects, which could be elicited by injection of maternally toxic (and often nearly fatal) doses of arsenic directly into the veins or abdominal cavities of mice, hamsters, or rats (for reviews, see DeSesso et al., '98; Golub et al., '98). These experiments had been designed to optimize the occurrence of neural tube defects, *not* to assess the

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risks to humans from environmental exposures to arsenic (Mottet and Ferm, '83; Willhite and Ferm, '84). Thus, when these experimental designs were compared to criteria that characterize study designs that are useful for risk assessment (i.e., dose-response design with at least three treated groups, documented presence of maternal toxicity at the high dose, exposure throughout organogenesis or from implantation through the remainder of gestation, route of administration that mimics the expected route of human exposure, at least 20 pregnant animals per group), they were found to be deficient and were deemed inappropriate for use in human risk assessment (Jacobson et al., '99; Holson et al., '00).

Because the database for assessing the potential developmental toxicity of inorganic arsenic was inadequate, a series of new laboratory studies (Nemec et al., '98; Stump et al., '99; Holson et al., '99a,b) was designed according to modern regulatory guidelines using relevant exposure routes and animals commonly used in reproductive toxicity tests (mice, rats, rabbits). In each of these studies, there were three or four arsenic-exposed groups of pregnant animals, with 20–25 mated/inseminated animals per group. The animals were exposed to one of several inorganic arsenic compounds (arsenic acid, sodium arsenate, or arsenic trioxide) by either a single oral dose early in gestation, repeated oral doses, or repeated inhalation exposures. None of the investigations reported arsenic-related structural malformations (including neural tube defects) in fetuses from dams receiving oral or inhalation exposures. In some studies, arsenic exposures, which also caused the deaths of some of the pregnant animals, induced low birth weights and resorptions. To confirm that the new experiments were consistent with older reports, some animals were injected intraperitoneally with sodium arsenate early in gestation. At term, the offspring of those litters exhibited high incidences of exencephaly and other craniofacial malformations.

The results of this series of experiments, along with the extensive previous experimental data on inorganic arsenic, were incorporated into a new risk assessment of the potential developmental toxicity of inorganic arsenic. This assessment was peer reviewed by a panel of experts from the government, academia, and industry (Toxicology Excellence for Risk Assessment [TERA], '99) and published by Holson et al. ('00). That risk assessment concluded that the production of structural malformations in experimental animals depends on both the dose and the route of administration of inorganic arsenicals. Based on the time course of experimentally measured arsenic concentrations in the blood after either oral or intraperitoneal injection of arsenic trioxide and the outcome of pregnancy in animals receiving similar doses in companion studies, Holson et al. ('00) suggested that structural malformations are induced only when maternal blood concentrations exceed a threshold that is capable of being exceeded only after intravenous or intraperitoneal injections. This leads to the conclusion that exposure to inorganic ar-

senic by environmentally relevant routes (oral and inhalation), and at concentrations normally encountered by people, poses little to no risk of causing structural malformations in offspring. Exposure would have to be of sufficient magnitude to cause concomitant, nearly fatal effects in the mothers to present a risk of developmental effects. Such effects do not occur at concentrations normally encountered by people. Indeed, measures taken to protect adults from the other adverse effects of arsenic, such as skin lesions and cancer, will protect the developing conceptus as well.

Because arsenic-containing substances occur naturally in soils, water, air, and food, and are also released into the environment by human activities (NRC, '77; Tamaki and Frankenberger, '92; Thornton and Farago, '97; Schoof et al., '99), exposure to arsenic is unavoidable. It is instructive to estimate the margin of exposure (the difference between the no observed adverse effect level in safety tests and the high end of potential exposure to arsenic). Holson et al. ('00) published such a margin of exposure estimate based on standard Environmental Protection Agency (EPA) assumptions for consumption of moderately contaminated soils (100 ppm) and drinking water (50 $\mu\text{g As/L}$). Under their assumptions, the margin of exposure for intake from these sources compared with animal developmental no observed adverse effect levels ranged from 270 to 2,700.

For most adults, the greatest background sources of inorganic arsenic intake are dietary intake and smoking (Vahter, '94; Valberg et al., '97). The average daily dietary inorganic arsenic uptake among adults in the United States is 11–14 μg (Borum and Abernathy, '94); total daily inorganic arsenic uptake from all sources is 16–19 μg (Valberg et al., '97). In addition, smoking can lead to the uptake of up to 5.4 $\mu\text{g As}$ per pack (Valberg et al., '97). Valberg et al. ('97) estimated the total inorganic arsenic uptake of a smoker (one pack per day) who drinks water containing 50 $\mu\text{g As/L}$, experiences occupational inhalation exposure of arsenic dusts, and has a high dietary intake of arsenic (including 100 ppm in soils) to be 138–156 $\mu\text{g As}$ per day. If the exposed individual were a pregnant woman who weighs 65 kg, this would amount to 2.12–2.40 $\mu\text{g As/kg}$ per day. When this extreme total daily intake is compared with the developmental no observed adverse effect levels reported for rats, mice, and rabbits, the calculated margins of exposure range from 170 to 1,900. This demonstrates a wide margin of safety for developing offspring.

If exposures to inorganic arsenic are so unlikely to cause structural malformations in humans, why did so many scientists believe that they could? For over three decades, many investigators injected high doses of inorganic arsenic into pregnant rodents during the critical period for neural tube closure in their investigations of the pathogenesis of the neural tube defect exencephaly (Ferm and Carpenter, '68; Hood, '72; Hood and Bishop, '72; Beaudoin, '74; Willhite, '81; Hood and Harrison, '82; Umpierre, '81; Carpenter, '87). The repetition by numerous laboratories of study protocols

that were designed specifically for the purpose of inducing exencephaly, and the subsequent reporting of the results of those studies, contributed to the misimpression that exposure of pregnant mammals to inorganic arsenic by any route and at any dose could be associated with neural tube defects in offspring (e.g., Barlow and Sullivan, '82; Schardein, '85; Golub, '94; Shepard, '95; Shalat et al., '96).

Additional information about inorganic arsenic contributed to the plausibility of such an erroneous interpretation. For instance, inorganic arsenic (like most chemicals) readily crosses the placenta of mammals (Hanlon and Ferm, '77; Dencker et al., '83; Lindgren et al., '84; Hood et al., '87, '88). Under the conditions of whole embryo culture (high, static exposure concentrations), arsenic is developmentally toxic (e.g., Chaîneau et al., '90) and has been associated with subcellular and metabolic changes in embryos. These changes include expression of stress proteins (Mirkes and Cornel, '92), methylation of DNA and subsequent alterations in gene expression (Włodarczyk et al., '96a,b), and possible formation of oxygen-free radicals (Tabacova et al., '96). These *in vitro* findings, however, are not useful for human risk assessment. In addition to the work in experimental animals, several epidemiological studies (mostly conducted in environs surrounding smelters or manufacturing facilities) had inappropriately alleged adverse pregnancy outcomes from maternal exposure to inorganic arsenic.

Taken together, (1) reproducibly induced exencephaly in laboratory animals after arsenic injection, (2) placental transfer of arsenic, (3) subcellular and molecular changes in cultured embryos, and (4) published allegations of adverse pregnancy outcomes among potentially exposed human populations raised concern among state and federal agencies. California listed inorganic arsenic as an agent that had the potential to be a human developmental toxicant (Donald et al., '92). This decision was apparently influenced by the many studies that had used high doses of arsenic as a tool to perturb developing systems, not by a critical evaluation of the studies to determine the quality of their data for risk assessment purposes.

Decisions based on the volume of citations concerning a substance, rather than on the quality and appropriateness of the data, can be unjustified. This has been the case for inorganic arsenic. Recent reviews of the supporting animal data (e.g., Golub et al., '98; DeSesso et al., '98; NRC, '99) have critically examined the data set for inorganic arsenic. They have pointed out that consistent production of malformations was reported only in experiments that exposed rodents by either intravenous or intraperitoneal injections. When exposures occurred by routes that are expected for humans under either occupational or environmental conditions (i.e., by oral or inhalational administration), malformations were not produced. Furthermore, a recent analysis of the literature (DeSesso et al., '99) has documented that many agents given during the time of neural tube closure can cause neural tube defects in surviving fetuses, thus repudiating

the notion that there exists a cadre of selective developmental toxicants that target only the brain. This suggests that the exencephaly reported in the older investigations using injected arsenicals at maternally toxic doses was likely due to nonspecific perturbations of the maternal/embryonal complex.

In summary, although there are few epidemiological data to support a developmental toxicity risk assessment, the extensive body of older animal studies demonstrated that arsenic could pose a hazard to prenatally developing offspring *only* under experimental conditions that are not relevant to human exposure. The more recent studies designed for the purpose of providing data for risk assessment clearly demonstrate that inorganic arsenic poses virtually no threat to developing offspring when maternal exposure occurs by relevant routes (oral and inhalation) at concentrations that are likely to be experienced occupationally or environmentally.

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