EVIDENCE ON DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF INORGANIC ARSENIC

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PREFACE

The Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65, California Health and Safety Code 25249.5 *et seq.*) requires that the Governor cause to be published a list of those chemicals "known to the state" to cause cancer or reproductive toxicity. The Act specifies that "a chemical is known to the state to cause cancer or reproductive toxicity … if in the opinion of the state's qualified experts the chemical has been clearly shown through scientifically valid testing according to generally accepted principals to cause cancer or reproductive toxicity." The lead agency for implementing Proposition 65 is the Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency. The "state's qualified experts" regarding findings of reproductive toxicity are identified as members of the Developmental and Reproductive Toxicant Identification Committee of the Office of Environmental Health Hazard Assessment's Science Advisory Board (22 CCR 12301).

During a public meeting held in Sacramento, California, on May 12, 1995 the Committee selected inorganic arsenic as a candidate for evaluation and requested that OEHHA staff prepare a review of the scientific evidence relevant to the reproductive toxicity of this agent. This draft document, which was released to the Committee and the public on October 4, 1996, responds to that request. While this hazard identification document does not provide dose-response evaluation, exposure assessment, or determination of allowable or safe exposure levels, the document does provide information which may be useful in such appraisals.

A public meeting of the Committee will be held December 4, 1996, in Sacramento, California. Following discussion and Committee deliberation, the Committee will determine whether inorganic arsenic "has been clearly shown through scientifically valid testing according to generally accepted principles" to cause reproductive toxicity.

CONTENTS

CONTENTS (continued)

I. Executive Summary

Arsenic is found in biological systems as inorganic oxy forms (oxides, arsenites and arsenates) and as organic arsenic compounds. Inorganic forms are considered in this document. Inorganic arsenic is used in wood preservative treatments and prior to this decade was widely used as a pesticide. Nonferrous smelters can contribute to arsenic contamination of air and soil. Arsenic found in soil and drinking water comes from geophysical as well as anthropogenic sources. The most common route of exposure for the general public is food and drinking water. Public health concerns have centered on carcinogenic, cardiovascular and nervous system effects seen in populations exposed to arsenic in drinking water.

The reproductive and developmental toxicity of inorganic arsenic has been studied primarily in animals using arsenite and arsenate salts and arsenic trioxide. Arsenic has been extensively studied as a teratogen in animals. Data from animal studies demonstrate that arsenic can produce developmental toxicity, including malformation, death, and growth retardation in four species (hamsters, mice, rats, rabbits). A characteristic pattern of malformations is produced, and the developmental toxicity effects are dependent on dose, route and the day of gestation when exposure occurs. Studies with gavage and diet administration indicate that death and growth retardation are produced by oral arsenic exposure. Significant increases in skeletal malformations were also seen by gavage, and characteristic malformations (although not at statistically significant rates) were reported in one gavage study. Arsenic is readily transferred to the fetus and produces developmental toxicity in embryo culture.

Animal studies have not identified an effect of arsenic on fertility in males or females. No dominant lethal effects were reported in several available animal studies. When females were dosed chronically for periods that included pregnancy, the primary effect of arsenic on reproduction was a dose dependent increase in conceptus mortality and in postnatal growth retardation. Human data are limited to a few studies of populations exposed to arsenic from drinking water or from working at or living near smelters. Associations with spontaneous abortion and stillbirth have been reported in more than one of these studies. Interpretation of most of these studies is complicated because study populations were exposed to multiple chemicals.

II. Introduction

A. Chemical Structure and Physical Characteristics

1. Definition

Arsenic (MW 75) is an element that can exist in biological systems in several forms:

- arsenite ($As^{III}O_3^{-3}$)
- arsenate ($As^{v}O_4^{-3}$)
- arsenide (As^{-3})
- organic arsenic compounds (As-C covalent bond)

This review is limited to inorganic arsenic, defined as arsenite and arsenate salts and arsenoxides. Arsenide (As^{-3}) is also an inorganic form of arsenic, but exposures usually occur via the gas arsine and result in a different, distinctive toxicological profile based on binding to hemoglobin and red blood cell lysis. Arsenate and arsenite compounds and alkylated arsenic species are used commercially. Inorganic arsenic predominates in environmental media (air, water, soil) and commercial uses and it is more toxic than organic arsenic.

Organic arsenic compounds include: methyl (cacodylate), dimethyl and trimethyl forms, commonly found in mammalian tissues; arsenobetaine, commonly found in fish and seafood; and arsenocholine, arsenosugars and arsenolipids, commonly found in plants.

2. Environmental sources and fate

Arsenic rarely occurs in metallic form. Arsenic from combustion sources (smelters, coal burning) is usually in the form of arsenic oxide in particulates and enters soils and water as arsenite and arsenate. Inorganic arsenic from commercial uses is also in this form, with the more stable arsenate form predominating. Organic arsenic compounds enter the environment from commercial uses in very small amounts and are typically not broken down to the inorganic form. Arsine is used commercially and produced by microbial degradation, but is rapidly oxidized to arsenate and arsenite. Arsenic adsorbs to soil, but can be leached into water. In areas with high geological arsenic content (typically from sulfide ores and volcanic soils), water and plant arsenic concentrations are elevated (USPHS/ATSDR, 1991).

3. Bioconcentration and biomagnification

Bioconcentration factors of arsenic in aquatic species are typically low (<20) (USPHS/ATSDR, 1991, p.103); and biomagnification is not significant.

4. Similarity to other toxic metals

Arsenic is a metalloid that is typically grouped with metals in considering its developmental and reproductive toxicity (Ferm, 1972; Domingo, 1994). Arsenic can be grouped with lighter metals like lithium, chromium, flouride, boron and aluminum which are commonly found in most organic matter in trace amounts and exhibit biological activity.

B. Regulatory Information and Background

Arsenic is recognized as a carcinogen by Proposition 65, the International Agency for Research on Cancer (Group 1, carcinogenic to humans; IARC, 1987) and the United States Environmental Protection Agency (US EPA) (Group A, carcinogenic to humans, IRIS, 1995). Several guidelines based on toxicity other than cancer have been developed. The federal Maximum Contaminant Level (a drinking water standard) was set at 50 μg/L (US EPA, 1977). Efforts are currently underway to develop a drinking water standard based on carcinogenicity. The US EPA RfD of 0.3 μg/kg/day is based on data from human studies demonstrating "hyperpigmentation, keratosis and possible vascular complications" (IRIS, 1995). Environment Canada has used reproductive toxicity data from an animal study (Schroeder and Mitchener, 1971) to estimate risk to Canadian wildlife (CEPA, 1993). This resulted in a Tolerable Daily Intake of 15 μg/kg-bw for wildlife.

Arsenic was the highest ranking of 164 developmental and reproductive toxicant (DART) candidates compiled for consideration under Proposition 65 by RCHAS in 1992 (Donald *et al.*, 1992). This ranking was based on production, usage, and exposure data, inclusion on published lists of reproductive toxicants, and nomination by experts in reproductive toxicity who responded to a mailing. Arsenic was also ranked first as the agent that "posed the greatest reproductive or developmental hazard to humans" based on responses of a Delphi committee of experts organized by RCHAS to prioritize candidate DARTs. At the Federal level, arsenic was 1 of 30 chemicals identified as of greatest concern for regulation as a reproductive toxicant (GAO, 1991).

C. Sources of Exposure in California

Several areas in California and Nevada have been identified as having high natural arsenic concentrations in groundwater due to high arsenic geological deposits. Elevated blood As concentrations have been observed in some local populations (Goldsmith *et al.,* 1972, Valentine *et al.,* 1979; Hopenhayn-Rich *et al.*, 1993). Statewide surveys of drinking water sources in California indicated that 200 small water systems and 2 large water systems exceeded the Federal Maximum Contaminant Level (MCL) for arsenic (CDHS, 1991). Another study (ACWA, 1994) found 1 of 180 water systems exceeded the MCL and that >65% of ground water samples and >50% of surface water samples had detectable arsenic (>1 ppb).

Additional sources of inorganic arsenic ingestion are dolomite and bone meal used as nutrient supplements, and folk medicine preparations (Kerr and Saryan, 1986).

Surveys conducted in 1990 by the California Air Resources Board (ARB) identified average outdoor air concentration of 1.9 ng/m^3 in high density population areas with no known sources of emissions (CDHS/ATES, 1990). Fumes and dusts from copper, lead and zinc smelters can be a major source of arsenic air and soil contamination if ores with high arsenic content are used; however, no copper, lead, or zinc smelters have been operated in California in recent years (NLM, 1994). Recently, elevated arsenic in soil contaminated with mine tailings have raised toxicity concerns in a residential development, Mesa d'Oro, in the Sierra Nevada foothills (Szymanski, 1994).

Data from 1986 (NLM, 1994) indicates that the major commercial uses of inorganic arsenic are in wood preservatives (68%), agricultural chemicals (herbicides and desiccants) (23%), glass (4%), alloys (3%) and "other" (2%).

In California in 1994 (latest available data), 110,753 pounds of arsenic pentoxide and arsenous acid were used commercially as wood protection treatment, and 3,262 pounds were used for fumigation (Cal/EPA/DPR, 1996). In addition, 16,691 pounds of sodium arsenite were used on grapes in California in 1994. All registrations of inorganic arsenic pesticides for food crops have been canceled by US EPA, and food tolerances expired in June, 1994. Thus use should decline and cease as existing stores are depleted. Cacodylic acid, an organic arsenic pesticide was reported at 45,943 lbs used in 1994. Toxic Release Inventory data for California in 1994 lists releases of "arsenic" of 3,930 lbs, the bulk of which went to offsite disposal, with 12 lbs released to water and 60 lbs to sewers. Arsenic trioxide was previously used widely as a rodenticide. However, domestic manufacture and use of arsenic trioxide has been discontinued (NLM, 1994, p.4); only 0.32 pounds were used in California in 1994. There is one processor of inorganic arsenic in California (NLM, 1994).

The major source of arsenic exposure is food, estimated at 20-50 μg/day average with higher intakes (190 μ/day) when diet contains a high proportion of seafood. However, assays of arsenic in food are usually for "total" arsenic and estimates do not separate organic and inorganic forms, although organic forms predominate (see Appendix I). Food tolerances formerly set in connection with arsenic acid use as a cotton defoliant (4 ppm for cottonseed) and sodium arsenite use on grapes (0.5 ppm) have expired. Arsenic acid was not widely used on cotton crops in California but sodium arsenite use on grapes was limited almost entirely to California. Data from 1968-1988 gave average residues of 0.006 ppm for grapes and 0.14 ppm for raisins.

D. Pharmacokinetics

1. Absorption, distribution and excretion

The cumulative absorption of water solutions of arsenite and arsenate from the gastrointestinal tract is >90% in humans and most experimental animals (Vahter, 1983). Lower values, between 40 and 50%, have been reported for hamsters (Odanaka *et al.*, 1980; Marafante and Vahter, 1987). Oral bioavailability studies in humans report values between 54 and 80% for inorganic arsenic (Buchet *et al.*, 1981). Oral bioavailability of arsenic from soil and house dust contaminated by smelter emissions was shown to be approximately 15% in monkeys (Freeman *et al.*, 1995). Gastrointestinal absorption of arsenate is inhibited in rats by the presence of phosphate (Gonzalez *et al.*, 1995). Absorption after intratracheal installation in animals is very high (90%) for soluble arsenic compounds (Marafante and Vahter, 1987). Vahter (1983) cites an early study (Holland *et al.*, 1959) in which 85-90% of arsenic deposited in the lung of human volunteers by smoking arsenic in cigarettes was absorbed within 14 days. In rhesus monkeys, 6.3% of labeled arsenic in water solution and 4.5% in soil was absorbed from skin (Wester *et al*., 1993). Vahter (1983) cites case reports in which clinical signs of arsenic poisoning were reported in humans after dermal exposure to arsenic trichloride or arsenic acid.

Arsenic is poorly bound to plasma proteins but can partition into red blood cells and bind intracellular proteins (Vahter, 1983). Arsenic appears to distribute readily to all tissues. The only differentially high concentrations have been reported in hair and nails (USPHS/ATSDR, 1992). Arsenite binds strongly to dithiol and vicinal thiol groups and is associated with protein to a greater degree than arsenate (Styblo *et al.*, 1995). Arsenic does not bind to metallothionein (Chen and Whanger, 1994), as was suggested by a protective role of zinc treatment in arsenic lethality (Kreppel *et al.*, 1994). Arsenic elimination occurs primarily by urinary excretion, with a small biliary contribution that varies with species (Vahter, 1983).

A pharmacokinetic study in humans (Pomroy *et al*., 1980) found the data best fit a 3 compartment model. The calculated half-lives for ^{74}As in three compartments were:

- compartment 1; 66 % of dose; $t_{1/2}$=2.1 days
- compartment 2; 30% of dose; $t_{1/2}$=9.5 days
- compartment 3; 4% of dose; $t_{1/2}$=38.4 days

The compartments with longer half-lives indicate a potential for bioaccumulation.

2. Metabolism

Arsenic metabolism varies between species, but the components of the pathways are very similar in mammals. Methylation is the major route of detoxification, although recent studies indicate that intracellular protein binding in the liver and gut are important in limiting toxicity. Recently, specific arsenite binding proteins have been identified in rabbit liver (Bogdan *et al.*, 1994).

Arsenite binds to glutathione and is methylated via s-adenosyl methionine. Arsenate can be converted to arsenite via intracellular redox cycling prior to methylation. Recently it has been recognized that glutathione also binds arsenate and organic arsenicals, which are reduced prior to methylation (Delnomdedieu *et al*., 1994). The mono- di- or tri-methylated species are excreted in urine.

There is very little metabolism of organic arsenicals. Humans do not appear to break down the As-C bond in arsenobetaine, which is excreted largely unchanged. Some metabolism of arsenosugars, however, appears to occur (Le *et al.*, 1994). Thus significant amounts of arsenite and arsenate are not produced from ingested organic arsenicals (Buchet *et al.*, 1994).

Species differ in the extent of methylation (amount of nonmethylated arsenic excreted), the methylated species produced (mono, di, tri-methyl arsenate), the importance of biliary excretion, and the extent of tissue binding prior to or after methylation (Vahter, 1994). Rats, mice and dogs methylate arsenic more extensively than hamsters, rabbits and humans. Interestingly, two nonhuman primate species studied, marmoset monkeys and chimpanzees, are unable to methylate arsenic (Vahter *et al.,* 1995), and this may also be true of the guinea pig (Healy *et al*., 1996). Liver is the main site of methylation with systemic dosing, but most cell types are thought to possess the ability to methylate arsenic (Fischer *et al*., 1985). Rabbits, hamsters and particularly rats, demonstrate significant biliary excretion whereas little is seen in mice and humans. Only humans excrete a significant amount of monomethylarsenate. Different methyl transferases are thought to be involved in the mono and dimethylation pathways. Marmoset monkeys sequester a significant portion of an administered dose in liver, whereas rats sequester dimethylarsenate in red blood cells. Attempts are underway to identify arsenic binding proteins that should help clarify comparative metabolism.

Most studies describing species differences in methylation are based on acute, single dose administration. However, metabolite profiles in chronically exposed humans (Foa *et al.*, 1984, Valentine *et al.,* 1979) suggest that chronic dosing does not alter metabolic processing of arsenic. At high acute doses in mice, methylation is slowed and intermediate products accumulate (Hughes *et al*., 1994). However, there is no evidence of a methylation threshold in humans beyond which arsenic toxicity increases disproportionally (Hopenhayn-Rich *et al*., 1993).

Physiologically based pharmacokinetic (PBPK) models hold the promise of cross species extrapolation of dose to tissue concentration at relevant sites of toxic actions. PBPK models have been developed for hamsters and rabbits, two species that most resemble humans with respect to methylation patterns (Mann *et al.*, 1996); however, neither a fetal compartment nor reproductive organs were included in these models.

E. Identity and Mechanism of Active Agent

Arsenate and arsenite are interchangeable through oxidation/reduction reactions. Arsenate is thought to be the principal form in circulation but may be converted to arsenite intracellularly (Wang *et al.*, 1996; Vahter and Envall, 1983). Mechanisms of chronic low level intoxication have not been specifically studied with the exception of carcinogenesis. A large database on genotoxicity (USPHS/ATSDR, 1992) suggests that inorganic arsenic shows very slight or no mutagenic activity but can produce chromosomal aberrations and sister chromatid exchange.

Other mechanism studies have focused on direct, acute cytotoxicity. Arsenite is thought to inhibit enzymes by binding to sulfhydryl groups, whereas arsenate "uncouples" oxidative phosphorylation by substituting for phosphate groups (Leonard and Lauwreys, 1980; Squibb and Fowler, 1983). The ability of arsenite to inhibit phosphatases also has implications for signal transduction. Recently, studies of keratinocyte and fibroblasts reported that arsenate altered phosphorylation and transcription factor activity (Kachinskas *et al.*, 1994; Huang *et al*., 1995), suggesting that specific effects on gene activation could occur during development.

Few mechanism studies have focused directly on developmental and reproductive effects. Intraperitoneal injection of arsenite (10 mg/kg) in pregnant mice has been shown to induce heat shock proteins in the embryo, an effect that can also be elicited in embryo culture (Honda *et al*., 1992; Mirkes and Cornel, 1992). Induction of heat shock proteins is thought to serve a protective function but has also been suggested to have implications for embryonic development (German, 1984). Free radical damage has been proposed as a mechanism of teratogenic effects (Tabacova *et al.*, 1994). Shalat *et al.*, (1996) suggested that effects on cell proliferation, cytoskeletal functions, cell death, placental and embryonic vasculature, and nutrient transport could be mechanisms of neural tube defects.

Because arsenic is detoxified by methylation pathways it could disrupt or compete with other methylation pathways. Effects on methyl group metabolism have been proposed for a number of teratogens and this may underlie the prophylactic effects of folate supplements. However, folate infusion was not found to protect against arsenic teratogenesis in hamsters (Ferm and Hanlon, 1986).

F. General Toxicity

Arsenic is well known as an acute toxicant due to homicidal poisoning uses. Target organs include the gastric mucosa, skin, and nervous system. Symptoms of acute toxicity are gastrointestinal distress, skin eruptions, severe diarrhea, kidney failure, convulsions, coma and death (Schoolmeester and White, 1980). In survivors, a severe peripheral neuropathy develops. The same syndrome has been reported in a poisoned pregnant woman (Bollinger *et al*., 1992). Characteristic symptoms of chronic poisoning are identified as skin hyperpigmentation, garlic odor of the breath, liver and kidney failure, lethargy and peripheral neuritis (USPHS/ATSDR, 1991).

High natural levels of arsenic in some areas of the world have provided information on characteristic toxic effects of long-term, low-level arsenic consumption. Symptoms include hyperpigmentation, peripheral neuropathy and vasoconstriction (blackfoot disease), and squamous cell cancers (Lianfang and Jianzhong, 1994; Cebrian *et al.*, 1994). Recently, the elevated arsenic in drinking water has also been associated with cardiovascular disease (Chen *et al.*, 1996).With the exception of DART endpoints, acute and chronic arsenic toxicity has been little studied in animal models. This is probably due to the existence of ample amounts of human data, and to the recorded species differences in arsenic pharmacokinetics (see Section II.D).

G. Essential Nutrient Status

Studies in chickens, rats, minipigs and goats indicate that inorganic arsenic is an essential trace element for these species (Uthus, 1992). The deficiency syndrome includes retarded growth, infertility and myocardial damage. Arsenic is thought to play a role in methionine, glutathione, taurine and/or polyamine metabolism due to its interaction with methyl groups. No studies of human deficiency syndromes were located. The average daily intake of arsenic from food in the US has been estimated at 46 μg in 1986 (USPHS/ATSDR, 1992). An estimated safe and adequate daily intake (ESADI) for arsenic of 12-40 μg/day based on animal and human studies has been proposed by Uthus (1994).

III. Developmental and Reproductive Toxicity Data

A number of recent reviews of arsenic reproductive toxicity are available both in the scientific literature and in regulatory documents (Ferm, 1972; Ferm, 1977; Hood, 1972; Hood, 1983; Hood, 1989; Leonard and Lauwerys, 1980; Barlow and Sullivan, 1982; Willhite and Ferm, 1984; Tabacova, 1986; Hardin-Barlow *et al*., 1989; CDHS/ATES, 1990; Cal/EPA/PETS, 1992; USPHS/ATSDR, 1992; Domingo, 1994; Golub, 1994; Shalat *et al.,* 1996). Data addressing arsenic reproductive toxicity include:

- human data on pregnancy outcome after smelter-related or drinking water exposures
- a few case reports of arsenic poisoning of pregnant women
- a large number (>40) of studies of arsenic teratogenesis in laboratory rodents from the basic science literature
- multigeneration and developmental toxicity studies conducted in connection with registration of arsenic acid as a pesticide
- dominant lethal studies in laboratory rodents
- a few recent articles in rats that studied postnatal growth and behavior

A. Developmental Toxicity

1. Human data

In a brief report of an ecological study, spontaneous abortion and stillbirth were examined in a Hungarian population (n=25,648) exposed to elevated arsenic in drinking water from regional wells (Borzsonyi *et al*., 1992). Data on live births, spontaneous abortions, and stillbirth in the arsenic exposed population over an 8 year period were compared to a population in a neighboring area with low drinking water arsenic concentrations. The arsenic exposed population was described as demonstrating increased incidence of hyperpigmentation and hyperkeratosis; however the incidence of cancer, blackfoot disease, cardiovascular or neurological disorders was not elevated. There was some indication of an association of elevated arsenic with spontaneous abortion and a stronger association with stillbirth; both effects were statistically significant. This study provides supportive evidence of an association between arsenic exposure and stillbirth.

Two case-control studies addressed the association between arsenic in community drinking water and adverse reproductive outcomes. In a study of 270 cases of children with congenital heart disease compared to 665 control patients, Zierler *et al,* (1988) examined the association between four cardiac defects and *in utero* exposure to nine metals. The authors found an increased frequency of coarctation of the aorta (Prevalence Odds Ratio = 3.4; 95% CI:1.3-8.9) among children born to mothers residing in areas with detectable levels of arsenic in the community water supply during the first trimester of pregnancy. No association was detected between arsenic levels in water and occurrence of the three other cardiac lesions investigated. Because of limitations in the study design, including assessment of multiple exposures and outcomes and lack of information regarding actual consumption of drinking water among the pregnant women, it is not possible to conclude from this study that the association with the observed heart defect is not explained by chance alone, or to eliminate the possibility that other malformations may be associated with exposure to inorganic arsenic.

In another study, Aschengrau *et al.,* (1989) evaluated exposure to arsenic in 286 women with evidence of spontaneous abortion compared to 1391 control women. The crude odds ratio of exposure to inorganic arsenic in drinking water among cases compared to controls was 1.3 (95% CI: 1.0-1.6). Exposure to water containing higher arsenic levels was more strongly associated with spontaneous abortion than exposure to lower arsenic levels. After adjustment for multiple cofounders using a multiple logistic regression model, only exposure to higher levels of arsenic (1.4-1.9 μg/L) was found to be associated with spontaneous abortion and the magnitude of association was small and not statistically significant (Exposure Odds Ratio 1.5; 95% CI: 0.4-4.7).

Several studies of workers and residents exposed to smelter emissions containing arsenic as well as lead, cadmium and mercury report an association between employment at or residence near the Rönnskar smelter in Sweden and multiple adverse reproductive outcomes including spontaneous abortion, low birth weight, congenital malformation and chromosomal damage (Beckman, 1978; Nordstrom *et al.*, 1978a,b and 1979a,b). Adjustment for multiple exposures was not feasible. It is not possible to determine from these studies whether the adverse reproductive outcomes observed were associated with exposure to inorganic arsenic or to other agents contained in the smelter emissions.

There are three case reports of inorganic arsenic poisoning in pregnant women. In all cases, the mothers survived under clinical care. In one case, the mother ingested arsenic at 30 weeks gestation and delivered a premature infant 3 days later which died the same day (Lugo *et al.,* 1969). In the second case, 28 weeks pregnant, intrauterine death occurred 4 days after poisoning and toxic levels of arsenic were found in fetal tissues (Bollinger, *et al.,* 1992). In the third case, 20 weeks pregnant, chelation therapy was immediately initiated and a healthy infant was delivered at 36 weeks (Daya *et al.*, 1989).

2. Animal data

Discussion of the animal data are organized by topic. More detailed information on the design of the studies reviewed is provided at the end of this section in Table 3.

Characterization of developmental toxicity from single dose injection and gavage studies

The majority of studies of arsenic developmental toxicity have administered single doses of arsenic by injection or gavage during embryogenesis and examined structural abnormalities (malformations). Most of these studies of arsenate and arsenite were directed at describing and understanding arsenic teratogenesis; some studies examined alleviation or prevention of arsenic teratogenesis. The studies consistently reported teratogenesis because they used doses and routes that were known to produce clear and abundant instances of malformation. Neural tube defects, anophthalmia and exophthalmia, renal and gonadal agenesis and fused ribs and vertebral and sternebral malformations were most frequently reported. Intrauterine mortality, growth retardation and maternal mortality were part of this syndrome. A summary of the earlier studies, as provided by their authors, are shown in Tables 1 and 2. Individual studies are outlined in the first section of Table 3, but are not described in detail here. Examples of data tables from studies using i.p., gavage and embryo culture administrations are provided in Appendix II.

Little work has been done on postnatal endpoints after prenatal exposure. In an abstract (Earnest and Hood, 1981), mice treated with sodium arsenite by gavage (5 mg/kg) or i.p. injection (2 or 4 mg/kg) on gestation day (gd) 1-17 were evaluated postnatally. No effect on postnatal weight or maturational indices were reported, but survival through 30 days of age was reduced. Forty day old mice from the 5 mg/kg p.o. group made more errors on the initial day of testing in a Lashley III maze. Some human studies suggesting that hearing can be affected in children exposed to arsenic are described in Appendix III. However, these exposures appear to have occurred postnatally. In an animal study of postnatal exposure, Nagaraja and Desiraju (1993 and 1994) administered arsenate (5 mg/kg/day by gavage) to rats from 2 to 60 days of age and found changes in both behavior

Table 1
THE MOST FREQUENT MALFORMATIONS FOLLOWING ARSENATE TREATMENT IN HAMSTERS, MICE AND RATS[1,2]

Hamsters[3]	Mice[4]	Rats
Exencephaly	Rib malformations	Vertebral defects
Rib malformations	Vertebral defects	Renal agenesis
Renal agenesis	Exencephaly	Rib malformations
Other urogenital abnormalities	Short jaw with protruding tongue	Anophthalmia
Cleft lip and palate	Hydrocephalus	Gonadal agenesis
Anophthalmia	Exophthalmia	Exencephaly

[1] In order of decreasing frequency.
[2] Table reproduced from Beaudoin (1974). All studies used injection routes
[3] Ferm *et al.,* 1971
[4] Hood and Bishop, 1972

Table 2
INFLUENCE OF MATERNAL ARSENIC EXPOSURE ROUTE OF MICE ON INSULT TO THE DEVELOPING OFFSPRING[1,2]

Treatment					
Agent	Route and Dose (mg/kg)	Prenatal Mortality	Fetal Stunting	Malformation	Maternal Mortality
Arsenate	ip:40	Moderate to Severe	Moderate	Moderate	16.2%
	po:120	Moderate to Severe	Moderate	Slight	16.1%
Arsenite	ip:10-12	Severe	Moderate	Moderate	6.67-24.4%
	po:40-45	Moderate	Slight	Slight	19-36%

[1] Reproduced from Hood, 1983. The abbreviation "ip" signifies intraperitoneal exposure; "po" signifies oral exposure.
[2] Data from Hood *et al.,* 1978, Hood, 1972, Baxley *et al.,* 1981.
(operant learning and extinction) and regional neurotransmitters (cholinergic and adrenergic) 100 days after discontinuation of treatment.

In addition to these studies of developmental toxicity, one study of transplacental carcinogenesis has been conducted in Swiss mice (Osswald and Goerttler, 1971). A translation of this article indicated an experimental design with three groups; control, prenatal treatment (0.5 mg/kg arsenic, 20 s.c. injections during pregnancy) and prenatal + postnatal treatment (20 prenatal injections, plus 20 weekly injections after weaning). Arsenic was stated to be a sodium salt but the chemical form (As^{+3} or As^{+5}) was not given. Mice were necropsied at the time of spontaneous death and the incidence of leukosis (lymphatic leukemias and lymphomas) was provided in the article as 3/36 for controls, 13/59 for prenatal group and 41/90 for the prenatal + postnatal group. Although this study was incomplete at the time of the report, the data are interesting because it has been difficult to demonstrate carcinogenicity of arsenic in adult animal models.

Comparison of species

The relative sensitivity of induction of developmental toxicity effects in commonly studied species appears to be rabbits/hamsters>mice>rats. In comparisons of mice and hamsters, the two most frequently studied species, hamsters appear more sensitive to arsenic-induced teratogenesis with single dose administration (Table 3). With multiple oral dosing throughout embryogenesis (WIL, 1988a and 1988b), rabbits appeared more sensitive than mice to both maternal and developmental toxicity. Only four studies in rats (Table 3) are available, generally indicating a higher required dose for teratogenic response. In a small study in sheep, potassium arsenate was administered in gelatin capsules to 4 pregnant ewes (James *et al.*, 1966). The first ewe received 0.75 mg/kg BW. Dosing was discontinued after 18 days due to maternal toxicity and a "very small" lamb was delivered at term. The other three ewes received 0.5 mg/kg for either 45, 140 or 147 days and gave birth to normal lambs. None of the limited studies of arsenic poisoning in pregnant women (discussed above) were able to provide an estimate of dosing. However, laboratory animals appear to be less susceptible to the acute toxicity of arsenic than humans (USPHS/ATSDR, 1991).

Dose-response relationships

Single dose administration studies indicate a steep dose response curve for severity of effect. Hood (1972) found a 4-fold greater incidence of malformations with 12 mg/kg as compared to 10 mg/kg injections of sodium arsenite on day 10 gestation. Baxley *et al.* (1981) found a 2-fold greater incidence of embryolethality with 45 mg/kg as compared to 40 mg/kg administered by gavage on day 10.

Dose response relationships for developmental endpoints were described in detail in a large study by Ferm and Hanlon (1985). Five doses of arsenate and four durations of exposure (6, 7, 8, or 9 days) were administered to hamsters (n=5-11/group) during embryogenesis, allowing consideration of total dose as well as daily dose. The study used implanted minipumps containing sodium arsenate to minimize maternal toxicity and avoid the short term, peak doses obtained with i.p. injection. All exposures ended on day

13 gestation, when malformations, conceptus death as indexed by resorption, and fetal body weights and lengths were determined. No maternal toxicity data were presented.

Figures 1-3 present the dose-response and duration-response data for three endpoints. Statistical comparisons were not conducted for these data. The graphs suggest the following interpretations concerning dose-response relationships:

- dose-response relationships exist for all endpoints.
- cumulative dose (dose x duration) is a more important determinant of fetal loss and growth retardation while peak dose is a more important determinant of malformation than is cumulative dose.

In a separate study, information relevant to identification of the active agent and the effective internal dose of the active agent were obtained (Hanlon and Ferm, 1986a). Arsenic doses in maternal blood peaked 2 days after implantation of a minipump containing 0.642 M arsenate in the pregnant hamster on gd 6. At that time, equivalent amounts of arsenic were found in plasma and red cells. Of the arsenic in plasma, 69% was arsenate, 7% was arsenite, and 26% was methylated arsenic. None of the plasma arsenic was found to be protein-bound. Hanlon and Ferm (1986b) estimated that a concentration of 4.3 μmol As/kg maternal blood "poses a minimal, but real, teratogenic threat in the hamster". They also estimated that at a blood level of 8.4 μmole As/kg blood 51% of surviving fetuses are malformed.

The oral dose of arsenic that would produce the minimally teratogenic dose level in hamster blood as determined by Hanlon and Ferm (4.3 μmol As/kg maternal blood for 24 hours) has been estimated at 2.8 mg As^{+5}/kg/day by OEHHA (Brown, 1992). This value was based on predictions of a multicompartment physiologically based pharmacokinetic model of arsenic disposition in the hamster. Unknown model parameters were fitted using the data of Yamauchi and Yamamura (1985) and the model was constructed with Stella v.2.1 software (High Performance Systems, Inc., Hanover, NH). This dose is comparable to the dose of 4 mg As/kg/day as arsenic acid (As^{+5}) which produced exencephaly with facial cleft by gavage administration to pregnant mice (WIL, 1988a). The complete anomaly data from this study is provided in Appendix II. In rabbits, a dose of 2 mg As/kg/day as arsenic acid (As^{+5}) was reported to produce developmental toxicity by gavage administration (WIL, 1988b); however, there was also maternal mortality at that dose.

Comparison of routes of administration

Hood (1972) and Baxley *et al.* (1981) conducted studies comparing single dose gavage and i.p. administration of sodium arsenite. Fetal effects (malformation and embryolethality) were higher by injection than by gavage. In contrast, maternal mortality was higher by the oral route than by injection. These data are summarized in Figure 4.

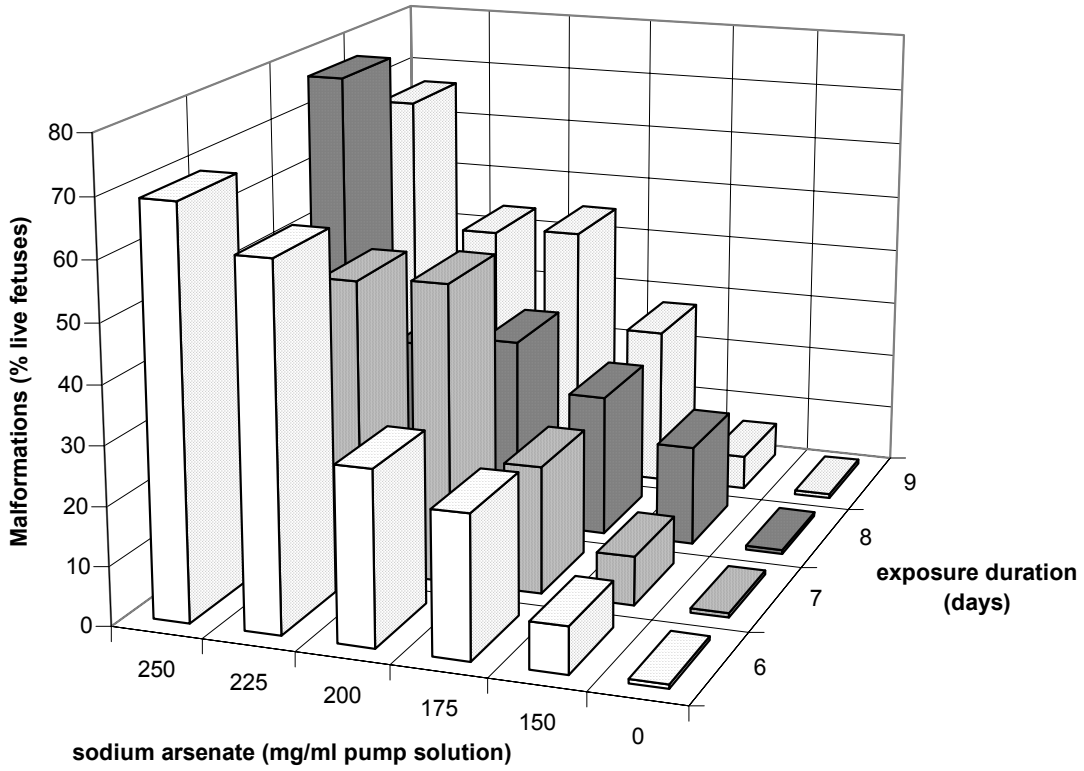

Figure 1. Malformation incidence as a function of dose and duration in hamsters exposed to sodium arsenate via minipump during embryogenesis. Data from Ferm and Hanlon (1985).

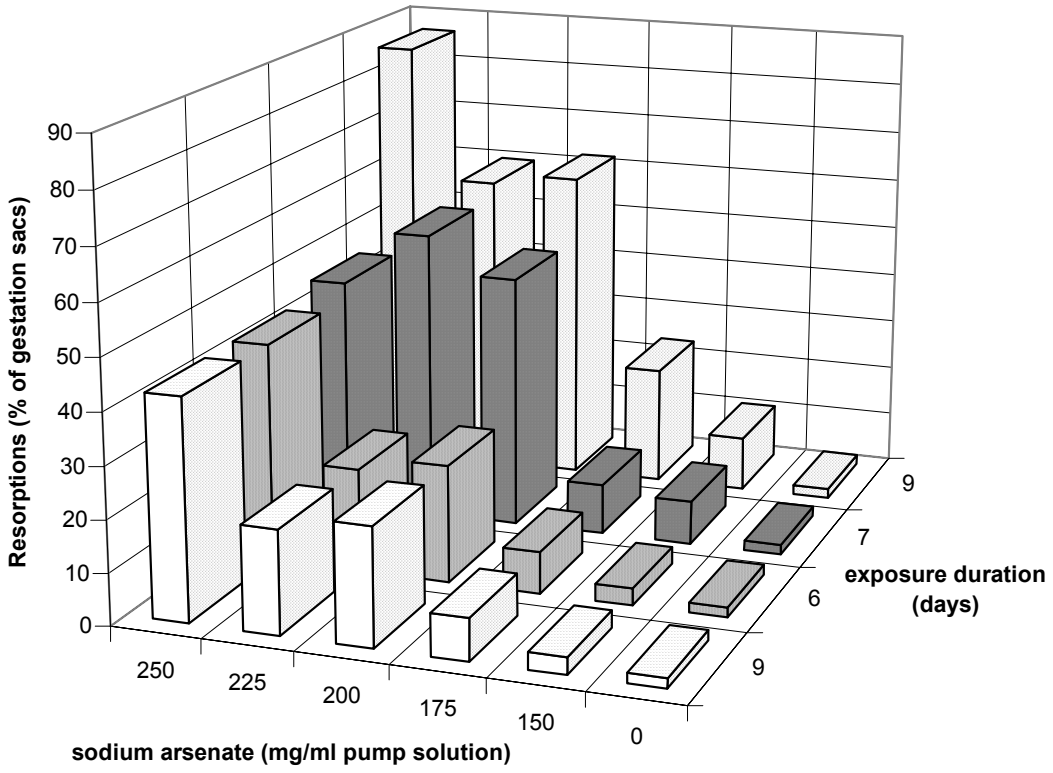

Figure 2. Incidence of embryolethality as a function of dose and duration in hamsters exposed to sodium arsenate via minipump during embryogenesis. Data from Ferm and Hanlon (1985).

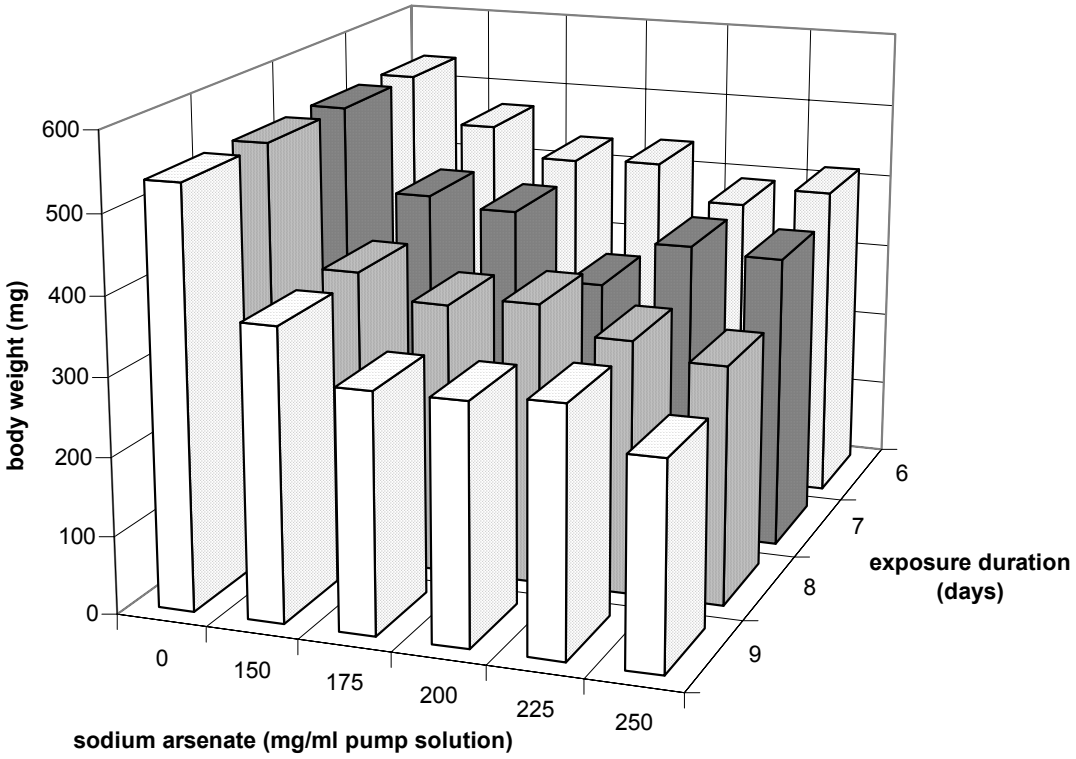

Figure 3. Fetal Body weight on gd 13 as a function of dose and duration of exposure in hamsters exposed to sodium arsenate via minipump during embryogenesis. Data from Ferm and Hanlon (1985).

Hood *et al.* (1978) compared doses of sodium arsenate considered to produce similar levels of maternal toxicity in mice by gavage (120 mg/kg) or i.p. injection (40 mg/kg) on single days between gd 7 and gd 15. With gavage, statistically significant increases were reported for *in utero* death and resorptions on gd 11, and for skeletal malformations on gd 9. There was no statistically significant increase in gross malformation on any day. Significant reductions in fetal weight were reported after treatment on gd 10, 11 or 15. By i.p. injection, statistically significant increases were reported for *in utero* death on gd 7, 11, 13, and 14, for skeletal malformations on gd 9, and for gross malformation on gd 9 or 10. Fetal weights were significantly affected on gd 7, 9, or 10. Although both routes led to increased incidence of skeletal malformation on day 9, the rate of malformation was 54% by injection and 14% by gavage.

Differences in effectiveness by route of administration may be due to the different amounts of agent reaching the fetus. Peak concentrations of arsenic in the fetus were about 4-fold higher after i.p. administration of 20 mg/kg sodium arsenate i.p. as compared to 40 mg/kg administered by gavage on gd 18 (Hood *et al.*, 1988). These doses were selected to produce equivalent maternal toxicity (no maternal mortality) on this gestation day. Detoxification via methylation in maternal liver may also be a factor reducing toxicity via enteral administration.

For chronic administration, most studies have used oral routes. Schroeder and Mitchener (1971) administered arsenite (salt unspecified) at a dose of 5 ppm in drinking water to mice for three generations. Measures relevant to developmental toxicity were average litter size, dead litters, "young deaths", and "runts". Average litter size (no statistics provided) was smaller in arsenic-treated groups than in controls in all three generations. In a two generation study conducted according to FIFRA guidelines, 0.53, 3.65, or 13.25 mg As/kg/day was administered in feed (Hazelton, 1990). As regards developmental toxicity, the high dose was associated with smaller litter sizes, increased rates of resorption, lower birth weights, postnatal growth retardation, and increased postnatal mortality. At the mid-dose, postnatal growth retardation was reported, and at the low dose no effects were seen. A Russian study (Nadeenko *et al.,* 1978, available with English abstract and translated data tables) treated rats with .0025 mg/kg arsenic (form unspecified) by gavage for a 7 month period including gestation. On gd 20, a significantly increased number of embryonic deaths, including post-implantation death, was reported. Arsenic was also reported to cause morphological changes at the microscopic level in brain, brain ventricles, bladder and kidneys. Taken together, these studies suggest that *in utero* and postnatal mortality and growth retardation are associated with chronic oral exposures; malformation were not assessed in these studies.

Maternal toxicity

It is common for toxicants that are administered during pregnancy to affect both the mother and the fetus. In this case, developmental effects are still considered to represent developmental toxicity and should not be discounted according to current risk assessment guidance in this area (US EPA, 1990). However, the relationship between developmental and maternal toxicity needs to be considered. When effects occur at the same doses in

the adult and developing organism, it may indicate that both are sensitive to that dose level. It is also possible that the fetal effects are secondary to maternal effects. Information on the relationship between maternal and developmental toxicity endpoints in arsenic studies is presented below.

In studies using single i.p. or gavage doses (Table 3), maternal mortality was frequently either described or documented. The documentation was not complete enough to determine whether developmental toxicity occurred only at doses that also produced maternal mortality. Further, the small group sizes in many studies may have precluded detection of an increased maternal mortality rate. In a series of studies of sodium arsenite where maternal mortality was systematically reported, maternal mortality did not appear to covary systematically with developmental toxicity rate across routes, day of treatment and dose (Figure 4). Unfortunately, studies using minipump administration in hamsters, which provide the most detailed information on developmental toxicity, did not report indices of maternal toxicity, although this route is not likely to evoke an acute toxicity syndrome in the dam.

More detailed information concerning maternal toxicity (food intake, pregnancy weight gain, mortality, gross and histo pathology) is available from developmental toxicity studies conducted by gavage (WIL, 1988a, 1988b). These studies included a maternally toxic dose at which increased maternal mortality and reduced weight gain were seen in the dams. It is not possible to determine whether fetal toxicity occurred in the absence of maternal toxicity; it is possible to state that fetal toxicity occurred in the absence of severe maternal toxicity (>10% mortality).

A mouse multigeneration study also included information on both developmental and maternal toxicity (Hazelton, 1990). Both maternal and fetal mortality occurred at the high dose in this study in both generations. Effects on postnatal weight gain occurred at the mid dose in the absence of effects on maternal mortality, weights or weight gain in the F0 generation. A comparison of effects on maternal and fetal weights in these studies during lactation is presented in Figure 5. Fetal abnormalities (overall and for each type alone except for hemorrhage) were significantly increased relative to controls. Maternal weight was also affected. Combined Cu and As led to decreased fetal weight and an increase in overall abnormalities, and in skeletal retardation and ectrodactyly considered separately. When all three agents were administered together all endpoints of fetal toxicity, as well as maternal weight gain, were significantly affected relative to controls. In a related abstract (Hood *et al.,* 1979 decribed in Mason *et al.,* 1989), no teratogenic effects of CCA treated sawdust were identified in mice exposed via diet or rabbits exposed dermally.

The possible mediation of arsenic teratogenicity by maternal trauma has been investigated. Recently, data has been presented demonstrating that diverse toxicants, when administered to rats during pregnancy, can increase maternal hepatic metallothionein (MT), sequestering Zn in liver, and reducing embryonic zinc accrual (Taubeneck *et al.*, 1994). Induction of MT is a well documented component of the acute phase response, which can

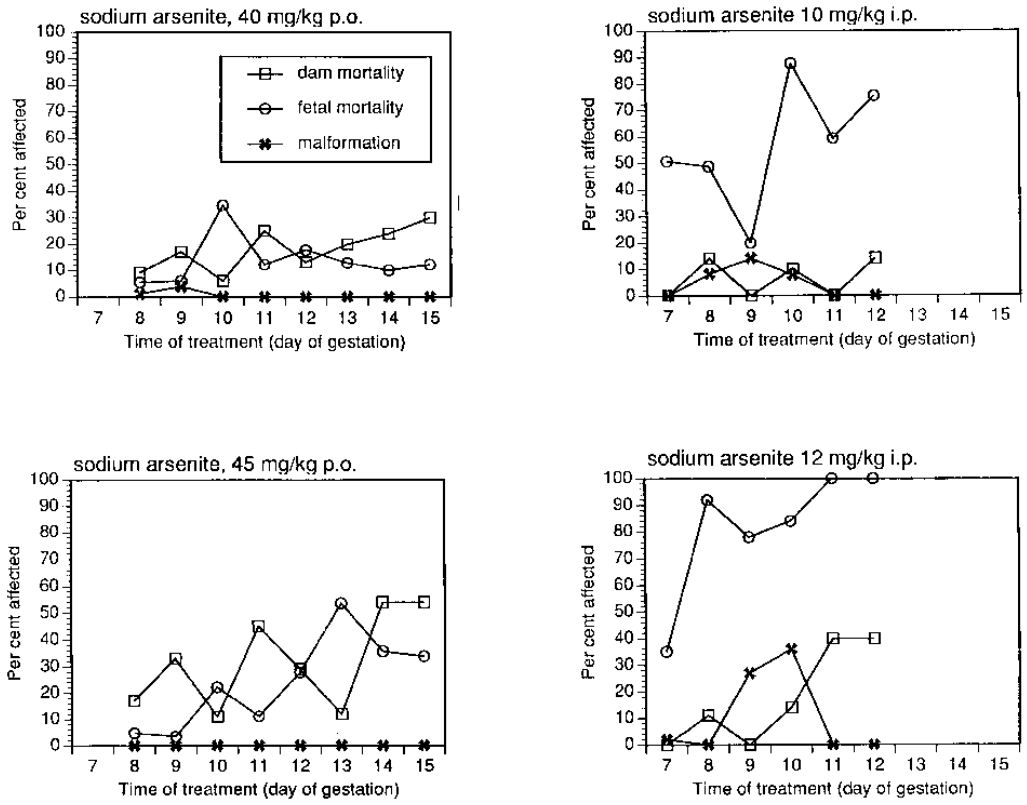

Figure 4. Comparison of maternal and developmental toxicity of arsenite administered to mice at different doses, by different routes, and on different gestation days. Data are from Hood (1972) and Baxley *et al.* (1981). Average group sizes (dams treated per time point and dose) were n=8.2 for i.p. administrations and the p.o. 45 mg/kg administration and n=20 for the 40 mg/kg p.o. administration. Malformations included gross and soft tissue malformations; skeletal malformations are not included. Fetal mortality included early and late resorptions and fetal death. Controls (gavage) had no maternal mortality, no malformations and 5.2% fetal mortality. Controls (injection) had no maternal mortality, no malformations and 1.78% fetal mortality.

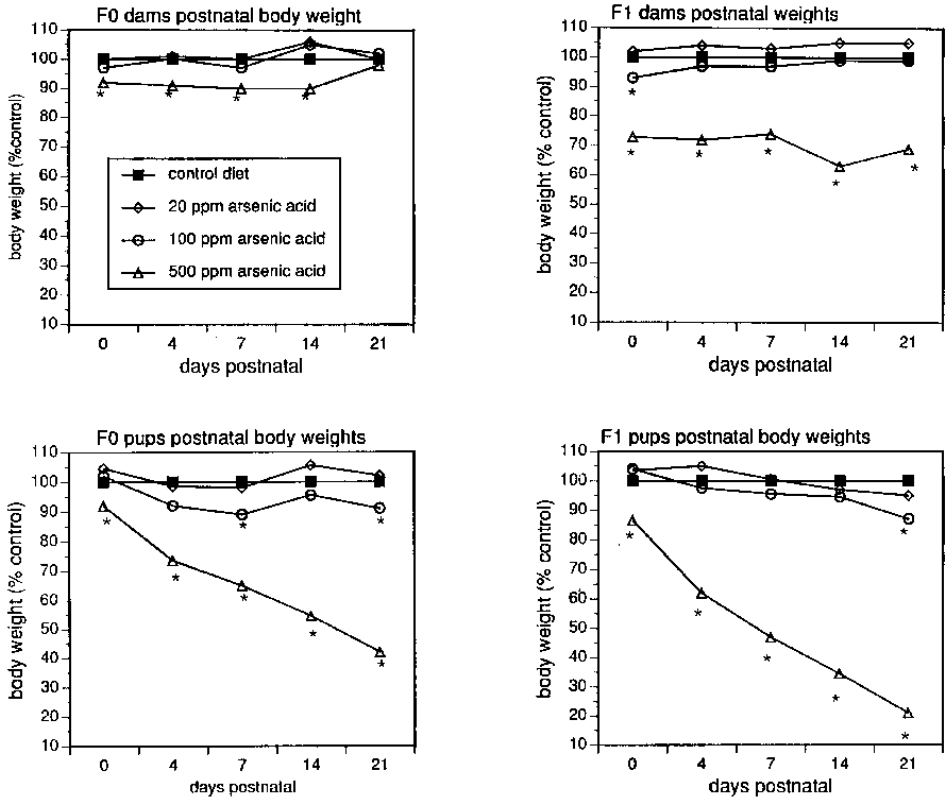

Figure 5. Comparison of maternal and offspring toxicity in terms of weights during the postnatal period. Data are from a multigeneration study in mice, with arsenic acid administered in feed (Hazelton, 1990). Weights are expressed as a percentage of the mean of the control group. For offspring, percentages were calculated separately for males and females and averaged. Both maternal and offspring mortality during lactation was seen at the high dose (F0 maternal 38%, offspring 53%; F1 maternal 88%, offspring 62%). No maternal of offspring mortality was observed at the mid dose. The number of litters represented at weaning for the control, 20, 100 and 500 ppm groups was 22, 23, 26 and 12 for the F0 generation and 26, 19, 25 and 2 for the F1 generation. * Statistically significant differences from controls as stated in the study report.

be triggered by inflammation or trauma. Because zinc deficiency is developmentally toxic, causing malformation as well as growth retardation and embryotoxicity, it has been proposed that this mechanism could contribute to developmental toxicity of agents that induce maternal hepatic MT. Arsenic has been investigated in this regard (Taubeneck *et al.,* 1994). Sodium arsenate was administered i.p. to rats at a dose (50 mg/kg) that was shown to produce complete embryolethality by the end of gestation (Beaudoin, 1974). This dose led to reduced maternal food intake and maternal and embryonic weight gain 18 h after injection. Induction of MT was demonstrated, but maternal hepatic MT levels were lower than those produced by an equivalent amount of food restriction. Embryo zinc content 18h after injection did not differ from *ad libitum* fed controls.

Protection from and potentiation of arsenic developmental toxicity

Chromated copper arsenate (CCA, 34% As) is a wood protection treatment. A study was conducted by Mason *et al.* (1989) to determine the combined developmental toxicity of the three components (chromium, copper, arsenate) in rats. Arsenic (sodium arsenate, 5 mg As/kg, i.p, gd 8-19) did not influence fetotoxicity (resorption, live fetuses), fetal weight, or maternal toxicity (maternal weight gain) but did significantly increase the incidence of fetal abnormalities as a group (retarded skeletal development, vertebral absence or delayed ossification, short ribs, ectrodactyly, subcutaneous hemorrhage) and of ectrodactyly considered separately. Chromium alone (sodium dichromate 2 mg Cr/kg, i.p.) led to reduced litter size, and copper alone (Cu sulfate, 2 mg Cu/kg, i.p.) increased fetal hemorrhage. Combined Cr and As led to decreased live fetuses per litter (compared to both controls and Cr alone), decreased fetal weight and increased resorptions.

In an earlier study, combinations of intravenously administered As, Cd and Se were studied in hamsters (Holmberg and Ferm, 1969). Se, which did not have a teratogenic action, had a protective effect against As and Cd teratogenicity. Also As was found to alleviate the developmental toxicity caused by Se deficiency. Muth *et al*. (1971) reported that As (1 ppm in diet as sodium arsenate) significantly reduced the occurrence of myopathy (as reflected in serum enzymes) in 6 wk old lambs whose dams were fed an Se deficient diet during pregnancy.

Two physical treatments, hyperthermia and restraint stress, potentiated teratogenicity caused by i.p. arsenic exposure. Hyperthermia increased the incidence of exencephaly and encephalocele in hamsters (Ferm and Kilham, 1977) while restraint stress increased the incidence of exencephaly and also decreased fetal weights relative to either treatment alone in mice (Rasco and Hood, 1994). Hyperthermia may have increased arsenic exposure through decreased kidney function (Hanlon and Ferm, 1986c). No specific mechanism was proposed for the restraint effect, although effects on maternal food and water consumption did not occur. These studies point out the ability of arsenic to interact with nonchemical environmental developmental toxicants.

The ability of chelators to prevent arsenic-induced developmental toxicity supports the hypothesis that arsenic is the active agent. Hood and Vedel-Macrander (1984)

determined that BAL (British anti-Lewisite, 2,3-dimercapto-1-propanol), the standard chelator used for acute arsenic poisoning, was not effective in preventing teratogenicity when administered before, with, or after sodium arsenite treatment (12 mg/kg i.p.). However, BAL was protective against sodium arsenate-induced teratogenesis when given 4 h before, concurrently with, or 4 h after i.p. injection of 40 mg/kg (Hood and Pike, 1972). Unfortunately, BAL itself is teratogenic at high doses, presumably due to chelation of essential trace metals. Domingo and colleagues have recently studied the ability of newer, less toxic dimercapto metal chelators to prevent the developmental toxicity of arsenic. DMSA (2,3-dimercaptosuccinic acid) alleviated the effects of arsenic (sodium arsenite, 12 mg/kg, i.p., gd 10) on embryo/fetal lethality and gross and skeletal abnormalities (Domingo *et al.*, 1991). The effect on structural abnormalities appeared to be dose dependent across 3 administered doses of DMSA, whereas all 3 doses prevented embryo/fetal lethality. Treatments given up to 1 h after arsenic administration were effective, whereas those delayed 4 or 12 h were not. A second agent, DMPS (sodium 2,3-dimercapto-1 propanesulfonic acid), was protective against arsenite induced developmental toxicity (embryo/fetal lethality, gross and skeletal malformations) when given s.c. at 0, 24, 48, and 72 h after i.p. injection of sodium arsenite (12 mg/kg on gd 9) (Domingo *et al*., 1992). Malformations were reduced at 2 of 3 administered doses, and embryo/fetal lethality was reduced at the highest dose. Maternal toxicity was also prevented at the highest dose. BAL, at doses that ameliorated some maternal toxicity, reduced the number of gross and skeletal abnormalities at the intermediate, but not the higher, dose of arsenic. Chelator studies help establish that arsenic is the active agent in inducing developmental toxicity, but do not help distinguish whether the primary effect is on maternal or fetal tissues.

Recently, folic acid supplements have been found to decrease the incidence of neural tube defects in some human populations. Ferm and Hanlon (1986) studied the effect of folic acid administered by minipump beginning on gd 6 on induction of neural tube defects in hamsters by arsenate (administered i.p.on gd 8). Folate was not found to be protective in this study.

3. Other relevant data

Maternal fetal distribution

Arsenic is readily distributed to the fetus after maternal administration. Maternal-fetal distribution of inorganic arsenic has been demonstrated in rats, mice, rabbits, hamsters, and monkeys (Gerber *et al*., 1982; Lindgren *et al.*, 1984; Hanlon and Ferm, 1986a,b; Hanlon and Ferm, 1987; Hood *et al.*, 1987,1988). Several of these studies include speciation and estimates of pharmacokinetic parameters. Animal studies suggest that the placenta selectively concentrates arsenic. Placenta arsenic concentrations exceeded those in maternal blood after 48 h exposure to arsenate via minipump on gd 6-7 (Hanlon and Ferm, 1987). Also, the concentration of arsenic in the placenta exceed that of the fetus after i.p. administration of sodium arsenate or sodium arsenite to mice on gd 18 (Hood *et al.,* 1987).

Limited data in humans also indicate that arsenic readily distributes from mother to fetus. In a comparison of maternal and cord blood in 101 births, mean maternal and cord blood arsenic levels were similar although the correlation was not significant (Kagey *et al.*, 1977). Arsenic accumulation in placenta has been identified in women receiving organic arsenicals therapeutically (Eastman, 1931). In comparing the maternal and fetal portions of the placenta after arsenophenamine treatment, the fetal tissues had higher arsenic concentrations than the maternal tissues. Elevated tissue arsenic was also found in the aborted fetus of a woman poisoned with arsenic (Bollinger *et al.*, 1992). Placental arsenic was 3 times higher (23 vs 7 μg/kg) in women living in a smelter vs control area in Bulgaria (Tabacova *et al.,* 1994). A 6 fold increase in placental arsenic was also reported in a rural area where arsenical pesticides were used vs urban areas (Thieme *et al.*, 1977, cited in Barlow and Sullivan, 1982). However, placental arsenic was similar in cities with high vs low air pollution, although maternal plasma arsenic was 2 fold higher in the polluted city (Kagey *et al.*, 1977).

Arsenic is apparently transferred to milk in cows, goats and humans (studies discussed in Vahter, 1983; Smith *et al.*, 1990; and Barlow and Sullivan, 1982), but no systematic studies of excretion into milk and uptake via nursing were identified.

Pathogenesis of embryonic defects

The pathogenesis of neural tube closure and renal agenesis defects were studied by examining embryos obtained at short time intervals after arsenic teratogenic treatments (Willhite, 1981; Morrissey and Mottet, 1983; Carpenter, 1987; Burk and Beaudoin, 1977). Willhite (1981) did not observe any changes from control in embryos 2 h after i.v. sodium arsenate administration to pregnant hamsters on gd 8. At 6 h after treatment, embryos appeared generally retarded and at 10 h after treatment, failure of elevation of neural folds in the cephalic region was noted. Morrissey and Mottet (1983) noted failure of elevation and apposition of cephalic neural folds in mouse embryos recovered 6-8 h after i.p. administration of sodium arsenate to the dam. Cytoplasmic inclusions were also observed in the neural folds of treated embryos. In studies of renal agenesis, pregnant rats injected with sodium arsenate on gd10, no differences from control embryos were observed 24 h after treatment, but retarded growth of the mesonephric duct was seen beginning at 48 h after treatment (Burk and Beaudoin, 1977).

Embryo culture

Embryo culture studies can help clarify whether arsenic can have direct effects on the conceptus. Arsenic developmental toxicity has been investigated *in vitro* using preimplantation mouse embryo culture and postimplantation mouse and rat embryo culture. In preimplantation mouse embryos, arsenite inhibited blastocyst formation, cell proliferation, and hatching in a dose dependent manner (Muller *et al.*, 1986). In postimplantation (gd 8) mouse embryos, growth retardation (yolk sac diameter, crown rump length, head length), developmental delay (somite numbers), malformation (neural tube closure failure, prosencephalon hypoplasia, hydropericardium, somite formation

alterations), and embryolethality were induced in a dose dependent manner by both sodium arsenate and sodium arsenite during a 48 hour incubation (Chaineau *et al.*, 1990).

Recently, Tabacova *et al.* (1996) studied the influence of different doses, durations and gestational ages on arsenite and arsenate induced developmental effects in two strains of mouse embryos. Data tables from this study are included in Appendix II. A consistent pattern of defects was found which included failure of neural tube closure, hypoplastic prosencephalon, hypoplastic otic and optic vesicles, abnormal somites, pharyngeal arch defects and malrotation. The earliest embryos studied (3 somite stage) were most sensitive to teratogenic actions. Treatment at this stage resulted in 100% open cranial neural tubes in arsenite and arsenate treated embryos as compared to 29% in controls. A one hour exposure was sufficient to induce developmental toxicity, and a 6 hour exposure was as effective as a 24 hour exposure in producing some malformations. The authors pointed out the similarity between their findings and those obtained *in vivo* and concluded that *in vivo* findings may be due to the direct effects of arsenic on the embryo.

In a study combining *in vivo* exposure with *in vitro* development (Beaudoin and Fisher, 1981), sodium arsenate (30 mg/kg/day) was administered intraperitoneally to rats either 4 or 24 hours prior to removal of embryos on gd 10 for a 24 hour incubation. The arsenate treated embryos demonstrated a marked reduction in closed neural tubes, in anterior limb buds, and in rotated embryonic axis relative to controls when dams were treated 24 hours prior to culture. A smaller percentage were affected when treated 4 hours prior to culture. Lower DNA, RNA and protein content of embryos was also seen after 24 h in culture (Fisher, 1982).

In general, embryo culture studies demonstrated a similar malformation syndrome as *in vivo* studies, including, prominently, neural tube closure defects, and a similar relative potency of arsenite and arsenate. Some malformations reported after *in vivo* administration such as axial skeletal defects and renal agenesis, would not be detectable in short term embryo culture experiments. However, micrognathia observed in term fetuses could originate in pharyngeal arch maldevelopment seen in cultured embryos, and somite abnormalities in cultured embryos can be seen as a precursor of rib and vertebral anomalies at term. Two malformations reported in cultured embryos, hydropericardium and abnormal tail flexion, have been previously reported at a low incidence with *in vivo* administrations of arsenate (Willhite, 1981; Hood *et al.*, 1978).

4. Integrative interpretation

The majority of information concerning arsenic developmental toxicity is from animal studies with single injection or gavage administrations during embryogenesis. Data from studies in mice, rats and hamsters demonstrate that arsenic can produce malformation,

intrauterine death, and growth retardation. The characteristic pattern of malformations described in injection studies included neural tube defects, renal and gonadal agenesis, eye defects and rib malformations. Developmental toxicity effects were dependent on the dose, route, species and the day of gestation when arsenic was administered. Arsenic readily crosses the placenta. A similar pattern of developmental toxicity has been reproduced in embryo culture. Studies using minipump administration examined dose-response relationships and identified a minimally teratogenic maternal blood concentration of arsenic in hamsters. Studies by the gavage route in mice reported intrauterine death, growth retardation and skeletal malformations. Other characteristic malformations (although not at statistically significant rates) were reported in one mouse study using gavage administration. Agents that chelate arsenic were found to protect against developmental toxicity.

Three studies are available which used chronic oral administration in feed or drinking water before conception and during gestation and lactation. Reduced litter sizes, intrauterine death and postnatal mortality, and growth retardation were among the effects reported in these studies. Malformations were not assessed. No studies were identified that used gestational exposure and measured offspring endpoints postnatally.

In the majority of studies using single administrations during embryogenesis, maternal toxicity was not reported, or reported only as maternal mortality. Studies using minipump administration did not provide data on maternal toxicity. In most studies, both maternal toxicity and developmental toxicity were recorded at the same doses, and in some studies developmental toxicity occurred at doses that did not influence the maternal toxicity endpoints recorded.

Although several human studies address the association between arsenic exposure and adverse reproductive outcomes, interpretation of most of these studies is complicated because study populations were exposed to multiple chemicals. Only one ecological study and three case studies were able to evaluate exposure to arsenic in the absence of other known toxic exposures. The ecological study found associations between living in an area with high drinking water arsenic content and spontaneous abortion and stillbirth. Other studies have examined populations exposed to arsenic via drinking water and smelter emissions. In case control studies of arsenic concentrations in drinking water, associations were found with a cardiac defect in one study and with spontaneous abortion in another. A series of studies in populations working at or living near a smelter found associations with spontaneous abortion, low birth weight and malformations..

Table 3
STUDIES CONTAINING DATA ON INORGANIC ARSENIC
DEVELOPMENTAL TOXICITY
page 1 of 10

Species	Treatments	Reported Effects	Reference
Single or multiple day administration during embryogenesis			
hamsters	sodium arsenate Single i.v. injection gd 8 0, 20 mg/kg (fetuses assessed on gd15)	No statistical analysis. 20 mg/kg - 45/216 no malformations; 5/216 renal agenesis; 56/ 216 an/exencephaly; 110/216 other malformations; amniotic fluid volume increased in fetuses with an/exencephaly. No information on maternal toxicity.	Ferm and Saxon 1971
hamsters (LVG)	sodium arsenate Single i.p. injection gd 8 0 or 20 mg/kg (fetuses assessed on gd 9, 10, 11, 12, 13, 14 or 15)	No statistical analysis 20 mg/kg- increased prenatal mortality, increased % malformed fetuses (>90%), especially neural tube defects.	Carpenter 1987
hamsters (Syrian)	sodium arsenate Single i.p. injection gd 8 40.1, 48.2, 64.2 μmol/kg (fetuses assessed on gd 13)	No control group data. The three doses produced 20, 38 and 95% neural tube defects. Folate infusion did not affect the incidence of neural tube defects. No information on maternal toxicity.	Ferm and Hanlon 1986
hamsters	sodium arsenate Single i.v. injection 0, 5, 20 or 40 mg/kg	Control-4.4% resorptions, 0% malformations. 5 mg/kg- 16.7% resorption or death. 20 mg/kg-35% resorptions, 48.6% malformations. 40 mg/kg- all fetuses resorbed.	Ferm and Carpenter 1968[3]
hamsters (LVG)	sodium arsenate Single i.p injection gd 8 10 mg/ kg (fetuses assessed on gd 13-15)	No control group data. 10 mg/kg- 12/242 resorptions, 4/226 encephalocele. Hyperthermia increased the incidence of neural tube defects and rib defects; embryo weights not reported. No information on maternal toxicity.	Ferm and Kilham 1977

Table 3
STUDIES CONTAINING DATA ON INORGANIC ARSENIC
DEVELOPMENTAL TOXICITY
page 2 of 10

hamsters (LVG)	sodium arsenite Single i.p. injection gd 9 or 10 2.5 mg/kg. gd 8, 11 or 12 5 mg/kg (fetuses assessed on gd 15)	2.5 mg/kg - No significant effects on number of litters, fetal weight, prenatal mortality or incidence of malformation. 5 mg/kg - no significant effects on number of litters or incidence of malformations. Significant depression of fetal weights on treatment days 11 and 12. Significant increase in prenatal mortality on gd 8 and 11. No information on maternal toxicity.	Hood and Harrison 1982
hamsters (LVG)	sodium arsenite Single oral dose gd 9 or 10 20 mg/kg gd 8, 11 or 12 25 mg/kg (fetuses assessed on gd 15)	Maternal mortality: 1/20 (5%) at 20 mg/kg and 6/36 (16.7%) at 25 mg/kg. Control: 1/51 (2%) maternal deaths 20 mg/kg - no significant effects on number of litters, fetal weight, prenatal mortality or malformations. 25 mg/kg - no significant effect on number of litters or malformations; significant increase in prenatal mortality on gd 8 and 12; significant decrease in fetal weight on gd 12.	Hood and Harrison 1982
hamsters (golden)	sodium arsenate, dibasic Single i.v. injection gd 8 [9 a.m.], (15, 17.5, 20 mg/kg), gd 8 [3 p.m.], (15, 7.5, 20 mg/kg) or gd 8 [9 p.m], (15m 20, 25 mg/kg) (fetuses assessed gd 15)	No statistical analysis. Increasing rates of resorption and malformation from 15 to 20 mg/kg. Rates varied from <10% to >90%; type of malformation and rates of resorption varied with time of day. No information on maternal toxicity.	Ferm *et al.* 1971
hamsters (golden)	sodium arsenate single i.v. injection gd 8 20 mg/kg, (fetuses assessed on gd 13, gross malformations only)	No control group data. No statistical analysis. 48% of the embryos were malformed and 84% were malformed and/or resorbed. Sodium selenite, injected simultaneously, reduced malformation rate. Embryo weights not reported. Dose described as “barely sublethal”.	Holmberg and Ferm 1969

Table 3
STUDIES CONTAINING DATA ON INORGANIC ARSENIC
DEVELOPMENTAL TOXICITY
page 3 of 10

hamsters (golden)	sodium arsenite Single i.v. injection gd 8 0, 2, 5, or 10 mg /kg (fetuses assessed on gd 14)	No statistical analysis. 2 mg/kg - Slight increase in prenatal mortality and % abnormal live fetuses. 5 mg/kg - "increased" prenatal mortality and "increased" % abnormal fetuses. 10 mg/kg - "Marked" increase in prenatal mortality and "increased" % abnormal live fetuses. "No overt maternal toxicity" reported.	Willhite 1981
hamsters (LVG)	sodium arsenate Subcutaneously implanted mini-pumps containing 0, 150, 175, 200, 225, or 250 mg /mL (approximately 0, 74, 86, 100, 110 and 130 μmol/kg-d) Pumps inserted on day 4, 5, 6 or 7 of gestation. (fetuses assessed on gd 13)	No statistical analysis. In all groups, fetal weight was depressed as length of exposure increased. As exposure period increase, the resorptions rate (embryonic or early fetal death) increased. "A strong relationship between increasing dose and resorptions rate was seen: 150 mg/mL group. 7% rose to 65% in 250 mg/mL group". Congenital malformations in live fetuses. No correlation to exposure duration but strong correlation to dose level: 0 mg/mL - <1%; 150 mg/ml - 10%; 175 mg/mL - 24%; 200 mg/mL - 39%; 225 mg/mL - 46%; and 250 mg/mL - 59%. No information on maternal toxicity.	Ferm and Hanlon 1985
mice (Swiss)	sodium arsenite Single i.p. injection gd 10 0, 12 mg/kg (fetuses assessed gd 18, gross and skeletal)	Full statistical analysis. Increased dead or resorbed fetuses/ litter. Decreased live fetuses/litter. Increased external abnormalities. Increased skeletal abnormalities. (arsenic effects were prevented by BAL). No information on maternal toxicity.	Domingo *et al.* 1991
mice (Swiss)	sodium arsenite Single i.p. injection gd 9 0, 12 mg/kg (fetuses assessed gd18, gross and skeletal)	Full statistical analysis. Increased % dead or resorbed. Decreased fetal weight. Increased gross malformations. Increased skeletal malformations. Maternal toxicity: 3/22 deaths; 6/22 hemorrhage; 7/22 complete resorption.	Domingo *et al.* 1992

Table 3
STUDIES CONTAINING DATA ON INORGANIC ARSENIC
DEVELOPMENTAL TOXICITY
page 4 of 10

mice (Swiss-Webster)	sodium arsenite Single i.p. injection gd 7, 8, 9, 10, 11, or 12. 0, 10, or 12 mg /kg (fetuses assessed on gd 15)	Statistical analysis used. 10 mg/kg - Significant increase in % resorbed or dead fetuses on all treatment days. Significant decrease in fetal weights on all treatment days except 12. Significant increase in incidence of malformations on treatment days 8, 9 and 10. 12 mg/kg - Significant increase in % resorbed or dead fetuses on all treatment days. On days 8, 9, 10, 11 and 12, the % of litters totally resorbed ranged from 75-100%. Significant decrease in fetal weights on all treatment days in which fetuses were produced. Significant increase in incidence of malformations on treatment days 9 and 10. Maternal mortality - 3/48 (6%) at 10 mg/kg and 10/51 (19.6%) at 12 mg/kg.	Hood 1972
mice (Swiss-Webster)	sodium arsenate, dibasic Single i.p. injection one day gd 6-12 0, 45 mg/kg (fetuses assessed gd 18, gross and skeletal)	Minimal statistical analysis (body weights only). Significant reduction in body weights after gd 6-11 injections. Increased resorption, particularly after gd 11, 12 injections. Variety of malformations with highest rates for rib malformations, exencephaly and hydrocephalus, eye defects, shortened jaw and protruding tongue. No maternal toxicity reported, dose described as “barely sublethal”.	Hood and Bishop 1972
mice (CD-1)	sodium arsenite Single i.p. injection gd 9 or 12 0, 12 mg /kg	No statistical analysis. Day 9-Increased prenatal mortality (50% vs. 4% in controls). Increased skeletal malformations. No significant effect on fetal weight. Day 12-Increased prenatal mortality (87% vs. 8% in controls). No significant effect on fetal weight or incidence of malformations. Maternal mortality: 18% at 12 mg/kg on gd 9.	Hood and Vedel-Macrander 1984

Table 3
STUDIES CONTAINING DATA ON INORGANIC ARSENIC
DEVELOPMENTAL TOXICITY
page 5 of 10

mice (Balb/c)	sodium arsenate Single i.p. injection gd 7, 8 a.m.; gd 8, 8 a.m. and 2 p.m.; gd 9, 7 a.m. 0, 15, 20, 45, 60, 75 mg/kg (doses used varied with time)	45 mg/kg gd 8, 8 a.m. significant decrease in fetal weight. 45 mg/kg gd 8, 2 p.m., significant increase in incidence of exencephaly (65%). No significant effects on implantation sites or % resorptions. Maternal toxicity: LD50 69.2 mg/kg for pregnant dams; 45 mg/kg "without apparent maternal effects".	Morrissey and Mottet 1983
mice (CD-1)	sodium a rsenate Single i.p. injection gd 9 20 mg/kg (fetuses assessed on gd 18, gross and skeletal)	Full statistical analysis. No effect on maternal weight gain, live fetuses, resorptions, fetal weight or incidence of exencephaly, rib defects or hematoma. (Restraint plus sodium arsenate decreased fetal weight, and increased incidence of malformations relative to controls)	Rasco and Hood 1994
mice (CD-1)	sodium arsenate gavage gd 1-17 0, 5 mg/kg, i.p. injections 0, 2 or 4 mg/kg (offspring evaluated through pnd 40)	No effect on birth weight, postnatal weight, physical development. 5 mg/kg - postnatal survival reduced. 5 mg/kg -increased errors in maze learning on day 1 of testing. No information on maternal toxicity.	Earnest and Hood 1981 (abstract)
mice (CD-1)	sodium arsenate Single i.p. injection gd 9 or 10 0, 40 mg /kg	No statistical analysis. Increase prenatal death. Decreased fetal weight. Increase in number of malformations. Maternal death - 28% at 40 mg/kg on day 9 and 9% on day 10.	Hood *et al*. 1977
mice	sodium arsenite Single oral dose gd 9 or 10 0, 120 mg/kg	No statistical analysis. Slight increase in prenatal death. Decreased fetal weight (day 10 only). Low incidence of malformations. Maternal mortality 18% on day 9; 11% on day 10.	Hood *et al.* 1977
mice (CD-1)	sodium arsenate Single i.p. injection one day gd 7-15 0, 40 mg /kg (fetuses assessed on gd 18)	Significant decrease in fetal weight on day 7, 9 and 10. Significant increase in % resorptions or fetal deaths on day 7, 8, 9, 10, 11, 13, 14, and 15. Significant increase % malformations on day 9 and 10. No maternal mortality reported.	Hood *et al.* 1978

Table 3
STUDIES CONTAINING DATA ON INORGANIC ARSENIC DEVELOPMENTAL TOXICITY
page 6 of 10

mice (CD-1)	sodium arsenate Single oral dose one day gd 7-15 0, 40, 50, 60, 80, 100, 120 mg/kg (fetuses assessed on gd 18)	Statistical analysis used. 100 mg/kg or less - No significant difference from controls. 120- mg/kg - Significant decrease in fetal weight on day 10, 11, and 15. Significant increase in incidence of resorptions or fetal deaths on day 11. Significant increase in skeletal malformations on day 9. No information on maternal toxicity.	Hood *et al.* 1978
mice (CFLP)	arsenic trioxide Inhalation exposure gd 9-12, 4 h/day 0.26, 2.9 and 28.5 mg/m^3 (fetuses assessed gd 18)	28.5 mg/m^3 significantly decreased average litter weight, increased number of growth retarded fetuses, number of fetuses with skeletal malformations, number of fetal liver cells with chromosomal damage. 0.26 and 2.9 mg/m^3- significantly decreased average weight of fetus. No information on maternal toxicity.	Nagymajtenyi *et al.* 1985
mice (CD-1)	sodium arsenite Single oral gavage, one day, gd 8-15 0, 20, 40, 45 mg/kg (fetuses assessed on day 18)	Statistical analysis used. 40 mg/kg - significant increases in dead or resorbed fetuses on gd 10 and 12. No significant effects on fetal body weight or gross malformations. 45 mg/kg - Significant increases in dead or resorbed fetuses on gd 10, 12, 13, 14, and 15. Non-significant increases in gross malformations onbgd 9. Significant depression of fetal body weight on gd 9, 10, 14, and 15. Maternal mortality: 19% at 40 mg/kg; 36% at 45 mg/kg.	Baxley *et al.* 1981
mice (ICR)	sodium arsenate Single gastric intubation gd 9, 10 or 11. 0, 10, 20, or 40 mg/kg (fetuses assessed on gd 18).	Statistical significance not reported. Low incidence of minor malformations noted in some groups, but not dose-dependent. 40 mg/kg - increased number of prenatal deaths and decrease in fetal weight. No information on maternal toxicity.	Matsumoto *et al*. 1973 (abstract)

Table 3
STUDIES CONTAINING DATA ON INORGANIC ARSENIC
DEVELOPMENTAL TOXICITY
page 7 of 10

mice (CD-1 ICR-BR)	75% arsenic acid (As^{+5}) gavage gd 6-15 3.96, 12.67, 25.34 mg As/kg/day	Statistical analysis used. 25.34 mg As/kg - Significantly decreased maternal weights and weight gain, increased resorptions, increased post implantation losses decreased viable fetuses decreased corpora lutea; 2/146 exencephaly; 1/146 omphalocele. 12.67 mg As/kg - Decreased maternal weight gain, gd 6-9, gd 15-18, 2/263 thoracogastroschisis, 1 total resorption 3.96 mg As/kg - Decreased maternal weight gain gd 6-9; 1/231 exencephaly with facial cleft, 1/231 micro/anophthalmia.	WIL 1988a
rabbits (New Zealand White)	75% arsenic acid (As^{+5}) gavage gd 6-18 0, 0.10, 0.40, 1.58 mg As/kg/d	1.58 mg As/kg - Maternal mortality, abortion and total litter resorptions. 0.40 and 0.10 mg As/kg, no effects.	WIL 1988b
rats (Sprague -Dawley	sodium arsenate Single i.p. injection gd 11.5, 2-3 p.m. 0, 50 mg/kg (fetuses assessed 18 h after treatment)	Full statistical analysis. 50 mg/kg - Reduced maternal food intake and body weight gain. Reduced embryo weight. Reduced embryo zinc uptake.	Taubeneck *et al.* 1994
rats (Wistar)	sodium arsenate Single i.p. injection gd 8 0, 5 mg As/kg (fetuses assessed on gd 19)	Full statistical analysis. No effect on maternal weight gain, implants/litter, live fetuses per litter, fetal weight, resorptions. 5 mg/kg - Significant increase in total abnormalities, and ectrodactyly: no effect on skeletal retardation. abnormal vertebrae, rib abnormalities, or subcutaneous or internal hemorrhage.	Mason *et al.* 1989

Table 3
STUDIES CONTAINING DATA ON INORGANIC ARSENIC
DEVELOPMENTAL TOXICITY
page 8 of 10

rats (Wistar)	sodium arsenate Single i.p. injection one day gd 7-12 0, 20, 30, 40 or 50 mg /kg (fetuses assessed on gd 20)	Statistical analysis fetal weights only. 20 mg/kg - Increased % of live fetuses with malformations on treatment day 9 and 10. 30 mg/kg - Increased resorptions on treatment day 8, 9 and 10. Increased % of live fetuses with malformations on treatment day 8, 9 and 10. Significant decrease in fetal weight on day treatment day 8 and 9. 40 mg/kg - Increased resorptions on treatment day 7, 8, 9, 10 and 11. Increased % of live fetuses with malformations on treatment day 8, 9 and 10 as well as some increases on day 11 and 12. Decrease in fetal weight on day treatment day 8, 9 and 11 (significant on day 11). 50 mg/kg - Lethal to all embryos on all treatment days. No information on maternal toxicity.	Beaudoin 1974
rats (Wistar)	sodium arsenate Single i.p. injection gd 9, 10, or 11 0, 30, 40 or 50 mg /kg (fetuses assessed on gd 20)	Statistical analysis for fetal weights only. 30 mg/kg - Significant decrease in fetal weight on day treatment day 8 and 9. -Increased % of live fetuses with malformations on treatment day 10. 40 mg/kg - Increased % resorbed or dead fetuses on treatment day 9. Increased % of live fetuses with malformations on treatment day 9 and 10. -Decrease in fetal weight on day treatment day 9 10 and 11 (significant on day 11) 50 mg/kg - Increased % resorbed or dead fetuses on treatment day 9, 10 and 11. - Increased % of live fetuses with malformations on treatment day 9 and 10. -Decrease in fetal weight on day treatment day 9 10 and 11 (significant on gd 11)	Burk and Beaudoin 1977

Table 3
STUDIES CONTAINING DATA ON INORGANIC ARSENIC
DEVELOPMENTAL TOXICITY
page 9 of 10

Chronic Studies			
mice (Balb/c)	unspecified form of arsenic Drinking water, gd 0 through 2 generations; only female breeders exposed 0, 0.05, 0.1, 0.5, 10.0, 50.0 mg As/L	No statistical analysis. No apparent effects on pregnancy length, litter size, dam or offspring hematocrit, dam or offspring tissue weights, immunoglobulin serum concentrations in F2 offspring, highest As dose. 50 mg As/L- possible effect on offspring survival and body weight at pnd 45 No maternal toxicity data reported; no fertility parameters reported.	Gershwin *et al.* 1987 (unpublished)
mice (CD)	unspecified arsenite salt Drinking water 3 generations 5 ppm (orig article) Hardin-Barlow *et al* estimates: 1 mg As/kg/d	Partial statistical analysis. No significant effect on materrnal deaths, number of litters, age at first litter, interval between litters, dead litters, young deaths, failures to breed or runts. Significantly increased number of small litters (2-5 pups).	Schroeder and Mitchener 1971
rats	unspecified arsenite salt Gavage 7 mo period including gestation 2.5 μg/kg/d	Significantly increased embryonic death; histological organ changes. No information on maternal toxicity.	Nadeenko *et al.* 1978 [partial translation & described in Hardin-Barlow, 1989]
rats	arsenic trioxide Inhalation 5 months 0.3, 1 or 3 μg/ m^3	1 and 3 μg/m^3- delayed ossification and statistically significant increase in preimplantation mortality. No maternal toxicity data mentioned in secondary source.	Kamkin 1982[3]
rats	arsenic trioxide Feed during gestation & lactation 0.5, 2.5, 5.0 mg As/kg/d	No significant differences between treated and control rats. No maternal toxicity data mentioned in secondary source.	Kojima 1974[3]

Table 3
STUDIES CONTAINING DATA ON INORGANIC ARSENIC
DEVELOPMENTAL TOXICITY
page 10 of 10

sheep	potassium arsenate Oral capsule 0.5 mg/kg/day for 45, 140 or 147 days during pregnancy, 0.75 mg/kg/day for 18 days during pregnancy, 1 ewe per exposure	0.75 mg/kg - dams' gums and tongue changed from pink to black, lamb was "very small, full term, hairy". 0.5 mg/kg - no maternal data, lambs described as normal.	James *et al.* 1966

[1] The abbreviation "i.p." refers to intraperitoneal, "i.v." refers to intravenous and "s.c." refers to subcutaneous. Some table entries from Cal/EPA/PETS, 1992.
[2] Unless otherwise noted, dose is given as "mg of administered agent per kg body weight". ("mg As/kg" refers to mg of arsenic per kg body weight.)
[3] Original not available. Information from Hardin-Barlow *et al.* 1989.

B. Female Reproductive Toxicity

Data are more limited for female reproductive toxicity than for developmental toxicity. The primary source of information is multigeneration studies in animals.

1. Human data

Recently, urinary arsenic, along with cadmium and lead, was determined in 50 women in a heavily industrialized region of Bulgaria (Tabacova *et al.,* 1994). No differences were found in urinary arsenic of subgroups who experienced pregnancy complications (toxemia, anemia, threatened abortion). A study of the association between arsenic in drinking water and spontaneous abortion and stillbirth (Aschengrau *et al.,* 1989; Borzsonyi *et al*., 1992), described above (section III.A.1) may be relevant to female reproductive toxicity. No other relevant studies were identified

2. Animal data

The primary source of data on reproductive toxicity is from multigeneration studies in mice and rats.

In an early two generation study, Morris *et al.* (1938) fed rats diets containing 26.8 or 215 mg arsenic (as As trioxide)/kg diet and mated both within groups and with controls. The authors reported no effects on fertility, fecundity, number of offspring produced or postnatal survival or weight gain. Another early 3 generation study administered arsenite (salt not specified) in drinking water (5 ppm, 1 mg/kg/day) to mice. Both males and females were apparently treated. Litter sizes were smaller in the arsenic-treated group than in controls in all 3 generations (Schroeder and Mitchener, 1971).

A more recent 2 generation study (Hazelton, 1990) in mice used arsenous acid administered in feed (0, 0.53, 2.65, 13.25 mg As /kg/day). Both males and females were treated. In the high dose group, litter size was lower than controls for both generations, with the difference (8.0 vs 10.96 pups) being significant in the F2 generation. The viability and weaning index were also significantly affected in the F0 dams (viability, high dose 90%, control 98%, $p \leq 0.01$: weaning, high dose 65%, controls, 94%, $p < 0.05$), and the weaning index was also significantly affected in the second generation (high dose 51%, control 99%, $p \leq 0.01$). These results appear to reflect primarily an effect on viability in the conceptus and pups. In terms of general toxicity, dam mortality and weight gain were also affected in the high dose group. F2 dams were about 30% smaller than controls from weaning, continuing through gestation and lactation; mortality was also higher than controls.

Remarkably, reproductive indices (mating, female fertility, gestation indices) in the F0 generation were not adversely affected by arsenic treatment. The high dose group female fertility index and pregnancy rate were equivalent to or better than controls in the F1

generation and the days-to-mate measure was significantly lower. This suggests that fertility was not specifically affected even at doses that produced systemic toxicity.

In a third study with both female and male exposures (Blanusa *et al.*, 1988), 3 generations of male and female rats were given drinking water containing 3 effluents from a coal gasification plant. Only the third effluent, which contained arsenic, led to reproductive toxicity in the form of lower body weights of offspring. No effects on fertility or litter size were reported. The effluents contained varying amounts of other essential and nonessential trace element contaminants. Arsenic body burdens were not determined.

In a chronic 2-year feeding study, reproductive organ weights and reproductive organ pathology were examined in female rats or dogs fed 5 doses of sodium arsenite or sodium arsenate in diet (Byron *et al.*, 1967). Effects on mortality and body weight were reported but there was no mention of reproductive organ effects.

3. Integrative interpretation

Multigeneration studies in rats and mice have provided data most relevant to female reproductive toxicity. Results of the available studies report dose-dependent effects on growth and viability of the conceptus and offspring but no effects on fertility. There are no human data concerning effects on fertility. However, associations between arsenic exposure and spontaneous abortion have been reported in 3 human studies.

C. Male Reproductive Toxicity

Data are available primarily from dominant lethal and multigeneration studies in animals.

1. Human data

In studies of pregnancy outcome at the Rönnskar smelter (see section on human developmental toxicity above), paternal effects on spontaneous abortion were investigated. When both mother and father worked at the smelter the abortion rate was higher than if only the mother was employed there (19.4 vs 13.5%) (Beckman, 1978). A higher abortion rate was found for parity >2 (n=117) but not for parity 1 or 2 (Nordstrom *et al.*, 1979a). No further information was located concerning the male reproductive toxicity of arsenic in humans.

2. Animal data

The findings of dominant lethal studies are outlined in Table 4. No dominant lethal effect of arsenic (sodium arsenite, 5 mg/kg i.p, single injection) was detected in mice based on implantations (Deknudt *et al.*, 1986). Similarly no sperm shape abnormalities were seen 35 days after treatment in this study. These authors cite a non-English report (Sram and Bencko, 1974) which also found no dominant lethal effect in mice after a

single oral administration of 250 mg/kg sodium arsenite or after chronic administration of 10 or 100 mg/L in drinking water over 4 generations. No dominant lethal effects were reported in mice with oral administration (0.25, 0.5 or 1 mg/kg arsenite) (Gencik *et al.*, 1977 In fact, these authors reported that the lowest dose resulted in lower total dominant lethality than controls. However, Sram (1976) found that sodium arsenite (10 mg/L administered in drinking water for 8 weeks) potentiated the dominant lethal effects of TEPA (tris aziridinyl phosphine oxide). The author attributed this effect to inhibition of DNA repair enzymes by arsenic.

A recent study found no effect of arsenic trioxide administered by intratracheal administration to rats on reproductive organ weights or sperm parameters (Omura *et al.*, 1996). In this study, similar administration of gallium arsenide and indium arsenide altered sperm counts, and gallium arsenide influenced sperm morphology.

Poma *et al.* (1981) did not find chromatid or chromosome aberrations in spermatogonia obtained from mice up to 48 h after i.p. arsenic trioxide injection. Labeled arsenic was not found to accumulate in the testes as was the case for Cd and Cr, but did accumulate in the lumen of the duct of the epididymis (Daniellson *et al.,* 1984).

In a chronic 2-year feeding study, sodium arsenite and sodium arsenate (five doses plus a control for each agent) were fed to rats and dogs of both sexes (Byron *et al.*, 1967 Reproductive organ weights and reproductive organ pathology were examined but no effects of arsenic were mentioned in the report. Effects on body weight and survival were found at the highest doses.

Testicular pathology was reported in mice given 50 mg As/L as arsenic trioxide in drinking water for 265 days (Bencko *et al.*, 1968 described in Barlow and Sullivan, 1982). In a study using inhalation administration of cesium arsenate (Silaev and Lemeshevskaya, 1980, described in Hardin-Barlow *et al.*, 1989) various male reproductive effects were reported (see Table 4), but the relative roles of cesium and arsenic are not known. These are the only reports mentioning positive findings concerning inorganic arsenic effects on male reproduction.

Multigeneration studies are described in Section III.B. The mouse multigeneration study of arsenic acid (Hazelton, 1990) included calculation of a male fertility index in both generations. There was no apparent effect on this index. Other multigeneration studies (Schroeder and Mitchener, 1971; Blanusa *et al*., 1988) did not report male fertility indexes. In the Morris *et al*. (1938) study, no effects on fertility were reported when males fed 26.8 or 215 mg As/kg diet were mated with control females.

3. Integrative interpretation

Studies in experimental animals have addressed dominant lethal effects, sperm and reproductive organ pathology, and fertility. No dominant lethal effects were reported in several studies of mice using injection, single oral administration or chronic oral administration. No chromosomal aberrations or abnormal sperm morphology was found after injection administration to mice. Chronic feeding studies in rats and dogs and studies using intratracheal administration in rats failed to find reproductive organ pathology. Male fertility indexes were not affected in a single available multigeneration study in mice. There are two studies published in non-English journals which were described in secondary sources as reporting testicular toxicity. One used arsenic trioxide administration in drinking water to mice and the other used cesium arsenate administration via inhalation to rats. In the latter study, cesium toxicity may have been involved.

One human study reported that rates of spontaneous abortion were higher when both father and mother worked at a smelter that processed high arsenic ore than when the mother alone was employed at the smelter. Arsenic exposure was not determined and there were no controls for confounding with other exposures.

Table 4
STUDIES OF INORGANIC ARSENIC MALE REPRODUCTIVE TOXICITY

Species	Route	Admin. agent	Dose	Reference
mice	i.p.	sodium arsenite	-5 mg/kg (no dominant lethal effects) - 2.5, 5.0, 7.5 mg/kg (no sperm abnormality effects)	Deknudt *et al.* 1986
mice	daily oral doses	sodium arsenite	-250 mg/kg, single dose -10, 100 mg As/L drinking water for 8 wks, 4th generation tested (no dominant lethal effects)	Sram and Bencko 1974 (described in Barlow and Sullivan 1982 and Cal/EPA/PETS, 1992)
mice	drinking water	arsenic trioxide	5, 50 mg As/L ("heavy deterioration of testicular germinal epithelium")	Bencko *et al.* 1968 (described in Cal/EPA/PETS, 1992)
rats	inhalation	cesium arsenate	0.33 , 4.96, mg/m^3 (0.33 mg/m^3, "intensified cell division of spermatogenic epithelium; 4.96 mg/ m^3 "changes in sperm cells, preimplantation death, reduced life of progeny")	Silaev and Lemeshevskaya 1980 (described in Hardin-Barlow *et al.,* 1989)
mice	oral	sodium arsenite	0.25, 0.5, 1.0 mg /kg/d (no dominant lethal effects)	Gencik *et al.* 1977 (described in Barlow and Sullivan 1982 and Cal/EPA/PETS, 1992)
rats	intra-tracheal installation	arsenic trioxide	1.3 mg/kg (no effect on testes weight, epididymal weight, sperm count, sperm morphology)	Omura *et al.* 1996
mice	oral (diet)	arsenic acid	0.53, 2.65, 13.25 mg As/kg/d (no effects on male fertility index)	Hazelton 1990

IV. Summary

A. Developmental Toxicity

There is a large literature concerning arsenic-induced malformations in animals (mice, rats and hamsters). A characteristic pattern of malformations was seen and intrauterine death and growth retardation were also reported when inorganic arsenic was administered by injection routes. With oral administration, intrauterine death, growth retardation and skeletal malformations were reported. When maternal toxicity was examined, it occurred at the same doses as developmental toxicity in many studies, but in others developmental toxicity occurred at a lower dose than the endpoints of maternal toxicity measured. Human studies are limited in number and design. They have reported primarily associations between arsenic exposure and spontaneous abortion and stillbirth.

B. Female Reproductive Toxicity

No effects on fertility or reproductive organs were reported in available animal studies. Effects on conceptus and offspring growth and viability were reported in multigeneration animal studies where both parents were exposed to arsenic. Human studies with limitations in design and exposure assessment have reported associations between arsenic exposure and spontaneous abortion and stillbirth.

C. Male Reproductive Toxicity

No dominant lethal effects, effects on sperm parameters or effects on fertility were reported in available studies in animals. Chronic studies did not report reproductive organ pathology. There are no studies of fertility, reproductive organ damage, or sperm parameters in humans.

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VI. Appendices

Appendix I. Information on arsenic exposure in human populations.

Tables are reproduced from secondary sources to provide general information.

ARSENIC LEVELS IN FOODS[a]
Reproduced from USPHS/ATSDR, 1987

Food Group	Arsenic Concentration (mg/kg)[b] Range of Mean Values	Maximum
Meats, eggs, and milk	0.01-0.03	0.5 (chicken)
Vegetables and fruits	0.01-0.03	0.3 (potato products)
Cereal, nuts, and sugar products	0.01-0.04	0.4 (rice)
Finfish and shellfish	0.07-1.47	19.1 (finfish)

[a]Adapted from EPA 1982b, after Jelinek and Corneliussen 1977.
[b]Arsenic levels are reported as concentrations of As_2O_3, but this does not imply that arsenic exists in this form in the food samples.

SUMMARY OF ESTIMATED LEVELS OF HUMAN EXPOSURE TO ARSENIC[a]
Reproduced from USPHS/ATSDR, 1987

Route	Probable Form	Exposure (μg/day) Typical	Maximum	Assumptions
		Ingestion		
Surface water (99.6% sources	Arsenate	5	200	*Typical:* Most levels of D.W. survey) in U.S. <10 μg/L; mean-2.5 μg/L; consumption of 2L/day *Maximum:* Maximum level in drinking water supply 100μg/L, consumption of 2 L/day
Groundwater sources	Arsenite from 25-100%; some arsenate in less reduced water	5	4,000	*Typical:* Small sample of average groundwater levels at 10 μg/L or less, consumption of 2 L/day; there are many incidences of higher groundwater levels *Maximum:* Maximum levels of 2000 μg/L in naturally contaminated supplies, consumption of 2 L/day
Food-total diet	All forms	21	190	*Typical:* FDA total diet study estimate *Maximum:* Total diet with seafood
Wine consumption	Arsenite predominately and arsenate	0.004	500	*Typical:* Wine containing 100 μg/L, consumption of 28 mL/day *Maximum:* Wine containing maximum levels of 500 μg/L, consumption of 1 L/day

Soil ingestion (children)	Arsenate or organic arsenicals	0.02	20	*Typical:* Soil containing 2.1 mg/kg consumption of 10 mg soil/day *Maximum:* Soil containing 2100 mg/kg, consumption of 10 mg soil/day
		Inhalation		
General atmosphere	Arsenic trioxide	0.06	0.6	*Typical:* Average ambient concentrations of 0.003 $\mu g/m^3$, respiratory flow of 20 m^3/day *Maximum:* Typical urban concentration in cities (containing smelters) of 0.03 $\mu g/m^3$
Cigarette smoking	Arsenic trioxide	-	90	Arsenic concentration of 12 µg/cigarette, 15% volatilized; consumption of 50 cigarettes/day

[a]Adapted from EPA 1982b

EXPOSURE TO INORGANIC AND ORGANIC ARSENIC COMPOUNDS IN THE GENERAL POPULATION

Reproduced from Vahter, 1994

Source	inorganic μg As/day	organic μg As/day
Air	0.05	--
Water	5-20	--
Food	<1-10	5-1000
Smoking	1-20	--

Appendix II. Tables of malformations from studies using administration of arsenate to mice via injection or gavage, or incubation medium (embryo culture).

Effects of Arsenate Administered by Injection on Fetal Development in Mice
Single intraperitoneal injections of 45 mg/kg on one of days 6-12 of gestation
Litters examined on Day 18
Reproduced from Hood and Bishop (1972)

Developmental Toxicity						
Day of Treatment*	No. of Litters	Total Implantations	Fetal Weights (gm ± SE)	% Resorbed or Dead	Litters Completely Resorbed	% Grossly Malformed
6	10	110	0.88 ± 0. 02[1]	50.96	3	1.69
7	8	95	0.67 ± 0.3[1]	36.84	0	33.87
8	11	135	0.87 ± 02[1]	55.56	2	35.94
9	8	95	0.61 ± 03[1]	60.00	1	63.04
10	10	107	0.79 ± 03[1]	51.40	3	26.42
11	10	120	0.94 ± . 03[1]	69.17	5	8.11
12	8	88	1.05 ± 0.01	4.20	0	1.04

[1]decreased fetal weights when compared with controls injected with distilled H_2O on one of gestation days 6-12($P < .05$).

Table of Gross Anomalies														
Gestation Day of Treatment														
	6		7		8		9		10		11		12	
Anomaly[1]	**N[2]**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Encephalocole	0/56	0	0/62	0	1/64	2	0/46	0	0.56	0	0/37	0	0/19	0
Exencephaly	0/56	0	2/62	3	20/64	31	25/46	54	0/56	0	0/37	0	0/19	0
Shortened jaws	0/56	0	3/62	5	8/64	12	21/46	46	1/56	2	0/37	0	0/19	0
Protruding tongue	0/56	0	3/62	5	17/64	27	20/46	43	0/56	0	0/37	0	0/19	0
Anophthalmia	0/56	0	1/62	2	7/64	11	4/46	9	0/56	0	0/37	0	0/19	0
Missing or displaced pinna	0/56	0	0/62	0	2/64	3	0/46	0	0/56	0	0/37	0	0/19	0
Agnathia	0/56	0	0/62	0	2/64	3	0/46	0	0/56	0	0/37	0	0/19	0
Exophthalmos	0/56	0	3/62	5	7/64	11	14/46	30	0/56	0	0/37	0	0/19	0
Open eye	3/56	5	12/62	19	9/64	14	9/46	20	1/56	2	0/37	0	0/19	0
Cleft palate and lip	0/56	0	1/62	2	1/64	2	0/46	0	0/56	0	0/37	0	0/19	0
Umbilical hernia	0/56	0	11/62	18	0/64	0	4/46	9	0/56	0	0/37	0	0/19	0
Eventration	0/56	0	1/62	2	0/64	0	1/46	2	0/56	0	0/37	0	0/19	0
Ectrodactyly	0/56	0	0/62	0	0/64	0	0/46	0	2/56	4	1/37	0	0/19	0
Micromelia	0/56	0	0/62	0	0/64	0	0/46	0	1/56	2	0/37	0	0/19	0
Twisted limb	0/56	0	0/62	0	0/64	0	1/46	2	1/56	2	0/37	0	0/19	0
Missing or short tail	0/56	0	0/62	0	0/64	0	3/46	7	9/56	16	1/37	3	0/19	0
Twisted tail	1/56	2	0/62	0	0/64	0	1/46	2	5/56	9	1/37	3	0/19	0
Dorsal hemorrhage	0/56	0	0/62	0	0/64	0	0/46	0	2/56	4	1/37	3	0/19	0
Hydrocephalus	0/13	0	1/16	6	3/16	19	4/12	33	0/14	0	0/10	0	0/4	0
Fusion of ribs	0/17	0	0/17	0	5/18	28	11/11	100	0.15	0	0/12	0	0/7	0
Forking of ribs	0/17	0	0/17	0	5/18	28	11/11	100	0/15	0	0/12	0	0/7	0
Fusion of vertebrae	0/17	0	0/17	0	0/18	0	1/11	9	11/15	73	0/12	0	0/7	0
Anomalous vertebrae	0/17	0	0/17	0	1/18	6	0/11	0	2/15	13	0/12	0	0/7	0

[1] Only the following anomalies were observed in control fetuses: one bent forelimb (1/405) and three dorsal hemorrhage (3/405).

[2] Values (N) represent the number of affected animals/total number of fetuses examined.

Effects of Arsenate Administered by Gavage on Fetal Development of Mice
Arsenic acid (75% arsenate) was administered to Swiss Webster mice on gd 6-15.
Data from WIL (1988a)

	arsenic acid (mg/kg/day)			
	0	10	32	64
implantation sites	328	246	289	265
% dead/resorbed	6.4	6.1	9.0	44.9
fetal weight (g)	1.30±.094	1.32±.108	1.23±.082	0.99±.183
(litters)	(25)	(20)	(24)	(22)
fetal anomalies (				
# embryos examined	307 (25)	231 (20)	263 (23)	146 (16)
(litters)				
carpal /tarsal flexure	2(2)	1(1)	1(1)	0(0)
thoraco-gastroschisis	0(0)	0(0)	2(2)	0(0)
omphalocele	0(0)	0(0)	0(0)	1(1)
exencephaly	0(0)	1(1)*	0(0)	2(1)
micro/anophthalmia	0(0)	1(1)	0(0)	0(0)
facial cleft	0(0)	1(1)*	0(0)	0(0)
cleft palate	1(1)	1(1)	1(1)	1(1)

* this fetus had exencephaly and a facial cleft

Effects of Arsenate Administered in vitro on Fetal Development of CD-1 Mice after 24-hr exposure at the 4-6 Somite Stage
Reproduced from Tabacova et al. (1996)

		Sodium arsenate (μm)			
Parameter	**Control**	**5**	**10**	**20**	**50**
		ICR strain			
Number of embryos examined	62	15	28	27	10
Somites (mean number)					
Initial	4.7	4.7	4.9	4.7	4.9
Final	23.0	23.0	22.3	18.5	
Embryolethality (% embryos)	0	0	0	3.7	100
Abnormal (% embryos)	4.8	20.0	60.7	100	
Specific abnormalities % embryos examined					
Cranial neural tube open	4.8	13.3	53.6	70.4	
Collapsed neural folds	0	0	17.9	55.6	
Prosencephalic hypoplasia	0	6.7	50.0	92.6	
Pharyngeal arch defects	0	0	14.3	77.8	
Optic vesicle hypoplasia	0	6.7	7.1	33.3	
Otic vesicle hyypoplasia	0	0	3.6	14.8	
Somite dysmorphology	0	0	0	14.8	
Malrotation	0	0	14.3	63.0	
Yolk sac defects	0	0	0	14.8	
		CDI strain			
Number of embryos examined	43	14	21	10	10
Somites (mean number)					
Initial	4.6	5.0	4.7	4.7	4.7
Final	23.3	23.8	22.1	21.2	
Embryolethality (% embryos)	0	0	0	10	100
Abnormal (% embryos)	7.0	7.1	61.9	90.0	
Specific abnormalities % embryos examined					
Cranial neural tube open	2.3	0	42.9	60.0	
Collapsed neural folds	0	0	19.0	60.0	
Prosencephalic hypoplasia	0	0	47.6	90.0	
Pharyngeal arch defects	0	7.1	28.6	60.0	
Optic vesicle hypoplasia	0	0	0	40.0	
Otic vesicle hypoplasia	0	0	0	0	
Somite dysmorphology	0	0	0	10.0	
Malrotation	7.0	0	9.5	50.0	
Yolk sac defects	0	0	0	20.0	

Appendix III. Ototoxicity

Ototoxicity as an endpoint of arsenic developmental toxicity

Two studies have reported hearing loss in children exposed to As. Parallel studies in adults are not available. It is unclear whether this effect is characteristic of developmental toxicity or would occur also in adults. Further, in the case of the Bencko et al. (1977) study, exposure might have occurred prenatally and/or postnatally. Thus, the status of these studies in developmental toxicity hazard identification is unclear. The following summary was provided by Tabacova (1986):

" In 1955 over 12,000 infants in Japan were accidentally poisoned with dry milk contaminated with inorganic As at a level of 15-24 mg As/kg (Hamamoto, 1955; Nakagawa and Ibuchi, 1970). Symptoms usually appeared after a few weeks of exposure and included fever, insomnia and anorexia. Anaemia, kidney and liver damage were found, and 130 deaths were reported. Disturbances of CNS functions were reported in the survivors 15 years after they had been exposed as infants to average daily doses of 3.5 mg As for about one month. The effects included severe hearing loss in 18% of 415 children examined in the follow-up study and electroencephalographic abnormalities (Yamashita *et al.,* 1972; Ohira and Aoyama, 1972). A number of pathological eye changes including a case of bilateral optic atrophy were found.

Moderate hearing losses apparently connected with inner ear damage were reported in children 10 years of age living near a coal-fired plant in Czechoslovakia which emitted large amount of As (Bencko *et al*., 1977)."