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Cancer in Experimental Animals Exposed to Arsenic and Arsenic Compounds

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Abstract

Inorganic arsenic is a ubiquitous environmental contaminant that has long been considered a human carcinogen. Recent studies raise further concern about the metalloid as a major, naturally occurring carcinogen in the environment. However, during this same period it has proven difficult to provide experimental evidence of the carcinogenicity of inorganic arsenic in laboratory animals and, until recently, there was considered to be a lack of clear evidence for carcinogenicity of any arsenical in animals. More recent work with arsenical methylation metabolites and early life exposures to inorganic arsenic has now provided evidence of carcinogenicity in rodents. Given that tens of millions of people worldwide are exposed to potentially unhealthy levels of environmental arsenic, *in vivo* rodent models of arsenic carcinogenesis are a clear necessity for resolving critical issues, like mechanisms of action, target tissue specificity, and sensitive subpopulations, and in developing strategies to reduce cancers in exposed human populations. This work reviews the available rodent studies considered relevant to carcinogenic assessment of arsenicals, taking advantage of the most recent review by the International Agency for Research on Cancer (IARC) that has not yet appeared as a full monograph but has been summarized (IARC 2009). Many valid studies show that arsenic can interact with other carcinogens/agents to enhance oncogenesis, and help elucidate mechanisms, and these too are summarized in this review. Finally, this body of rodent work is discussed in light of its impact on mechanisms and in the context of the persistent argument that arsenic is not carcinogenic in animals.

Keywords

arsenicals; carcinogenesis; inhalation; mouse; oral exposure; rat; rodents; transplacental exposure

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Introduction

Millions of people worldwide are exposed to potentially harmful levels of arsenic in their drinking water (IARC 2004). Some of the first evidence that arsenic is a human carcinogen was a report by a British physician of skin cancers in people using medicinal arsenicals (Hutchinson 1888). Now there is considered to be *sufficient evidence* in humans for arsenic and inorganic arsenic compounds as lung, skin and urinary bladder carcinogens, and some evidence for carcinogenicity in kidney, liver and prostate (IARC 2009). Indeed, one of the first monographs from the IARC (IARC 1973), reported that inorganic arsenic exposure was consistently linked to human skin cancer. Arsenic can be carcinogenic in humans after inhalation or oral intake. However, there was considered to be a clear lack of convincing evidence in animal studies (IARC 1973). Since the 1973 monograph, the evaluation of arsenic and arsenic compounds in one form or another have been reported in two additional full-length monographs (IARC 1980, IARC 2004) and in the 1987 updating summarizations (IARC 1987). In the 1980 evaluation there was still considered *inadequate evidence* for the carcinogenicity of arsenic compounds in animals. The 1987 summarization evaluation considered there to be *limited evidence* for inorganic arsenicals and no adequate data available on organic arsenicals in animals. The ensuing 2004 monograph considered additional data to provide *sufficient evidence* of the carcinogenicity of the organo-arsenical, dimethylarsinic acid (DMA^V). DMA^V, a known biomethylation product in humans and rats, produced tumors in the urinary bladder (UB) of rats and lungs of mice (IARC 2004). However, studies on inorganic arsenicals as a group still were considered to provide *limited evidence* of carcinogenicity in animals (IARC 2004).

In 2009, IARC undertook the task of updating the status of known human inorganic carcinogens, including arsenic. A monograph on this evaluation has not yet been published, however, the results have been reported in summary form (IARC 2009). The IARC evaluates agents as *carcinogenic to humans* (Group 1), *probably carcinogenic to humans* (Group 2A), *possibly carcinogenic to humans* (Group 2B), not classifiable as to its carcinogenicity to humans (Group 3), and *probably not carcinogenic to humans* (Group 4) (IARC 2006). The 2009 IARC evaluation included various rodent studies that have emerged since the 2004 evaluation (IARC 2004) that provide evidence of complete carcinogenicity for inorganic arsenic or organoarsenical compounds, and add significantly to the prior database for carcinogenicity in animals. These studies with arsenicals have examined several different modes of exposure in animals ranging from, for example, oral exposure in drinking water or feed, to intravenous (i.v.) exposure. They also include life stage-based studies such as transplacental exposure. Work with arsenic and arsenic compounds has thus far primarily focused on inorganic salts of arsenite or arsenate and on DMA^V. This may be because the inorganic arsenicals (arsenite and arsenate) would be the primary forms of human environmental exposure, mostly via the drinking water, while DMA^V is one of the more common biomethylation products found in the body after arsenical exposure (IARC 2004).

As a human carcinogen, which despite many attempts over a very long period of time was inactive in rodents, arsenic was once thought of as a “paradoxical” carcinogen by many (e.g. Jager and Ostrosky-Wegman, 1997). In fairness, this paradoxical notion (Jager and Ostrosky-Wegman, 1997) was voiced long before many of the positive studies reviewed in the present paper were published (see IARC 2004 and IARC 2009 for summary). This perception of a lack of carcinogenic activity in rodents has persisted even in the face of accumulating evidence and, for instance, statements like “arsenic does not cause cancer in laboratory animals” have quite recently come out of leading workshops on risk assessment of environmental carcinogens (National Science Foundation [NSF], 2005). However, this notion must now be abandoned as, taken together, many studies have furthered our understanding of the role of arsenicals in carcinogenesis and provide unequivocal proof of

the carcinogenic capability of this metalloid in animals. To this end, this review discusses those studies that are considered relevant to the establishment of the carcinogenicity of arsenicals in rodents, with emphasis on those that impacted the 2009 IARC evaluation (IARC 2009). Obviously, our ultimate goal is to gain an enhanced understanding of the carcinogenicity of arsenicals in humans. The most recent IARC review (IARC 2009) considered arsenic and inorganic arsenic compounds to be Group 1 (human) carcinogenic agents. Epidemiological data show that human arsenic exposure via inhalation or drinking water causes lung, skin, and urinary bladder cancers (IARC 2009). There is more limited evidence that links human exposure from arsenic in the drinking water to cancers of the lung, liver, and prostate (IARC 2009). Corollary target sites in rodents were considered particularly important in this current review.

Differences in biokinetics between human and rodents to some researchers raise questions in regards to the relevance of inter-species comparisons of arsenic carcinogenicity based on *in vivo* studies. External doses are often labeled as relatively “high” in rodent studies compared to levels of exposure in the human environment (Waalkes et al., 2007). However, in order to make an appropriate comparison between species, one must take into account various critical factors in tissue dosimetry, as impacted by toxicokinetics, which varies considerably between species. In other words, it can not be assumed that the proportion of the dose delivered internally is the same from humans to rodents. In fact, although drinking water levels are between 100 and 200 times greater in some mouse carcinogenesis studies they result in similar blood arsenic levels as seen in humans exposed to much lower external doses (see Waalkes et al., 2007). However, human pharmacokinetic data are considered sparse or limited (El-Masri and Kenyon, 2008; Kenyon et al., 2008) and models as yet do not account for specific toxic effects.

Beyond studies that support actions as a complete carcinogen, arsenicals have been successfully administered in many studies after or during the application of known carcinogenic agents to enhance a carcinogenic response. A few studies have now emerged where arsenic, although inactive alone, acts to enhance carcinogenic response when given prior to other carcinogenic agents, even when a significant temporal gap exists between application of arsenic and the second agent. The precise meaning of such studies to the carcinogenicity of arsenic as a single agent is complex, but these studies can be useful in elucidation of mechanisms. In defining carcinogenic potential, however, this review focuses on the studies where the arsenicals in question, when given alone, results in tumor formation. Therefore, for the sake of clarity, a complete carcinogen is herein defined as a compound which, when given by itself, causes a statistically significant increase in tumor incidence in a given organ or tissue over natural background.

Oral Exposure and Arsenical Carcinogenesis

Naturally contaminated drinking water is the most common source of inorganic arsenic in humans (IARC 2004). Inorganic arsenic (arsenite plus arsenate) is the most common arsenic species in natural water with mono- and di-methylated species (MMA^{V} , DMA^{V} , MMA^{III} , DMA^{III}) also being found in minor amounts (IARC 2004). Worldwide, naturally elevated levels of arsenic in groundwater range in concentration from $< 0.5 \mu\text{g/L}$ to $7550 \mu\text{g/L}$ and significant exposures in humans have been identified in countries such as Bangladesh, India, Taiwan, China, Japan, Viet Nam, Australia, Mexico, Argentina, Chile, and the United States (IARC 2004). Clearly, oral exposure is one of the most important modes to investigate and establish models in rodents. Chronic oral exposure to arsenicals via the drinking water or feed is carcinogenic in rodents in several studies which are summarized in Table 1.

In genetically altered or naturally cancer sensitive mice, several studies indicate a carcinogenic potential for arsenicals (Cui et al. 2006; Hayashi et al. 1998; Kinoshita et al. 2007). For instance, oral sodium arsenate in the drinking water for 18 months enhanced lung tumor multiplicity and lung tumor size in male strain A/J mice (Cui et al. 2006), which are sensitive to pulmonary carcinogens. Similarly, chronic (≥ 50 weeks) exposure via drinking water in strain A/J mice to the methylated arsenical metabolite, DMA^V, increased both incidence and multiplicity of lung adenoma or carcinoma (Hayashi et al. 1998). Chronic oral DMA^V can also increase lung tumors in mutant *Ogg*^{-/-} mice, which are unable to repair key types of oxidative DNA damage, but not in the repair competent *Ogg*^{+/+} mice (Kinoshita et al. 2007), implicating some form of oxidant DNA damage as a possible means to tumor formation, although this was never shown directly (Kinoshita et al. 2007).

As discussed earlier, arsenic and inorganic arsenic compounds are considered to be human lung carcinogens by oral exposure (IARC 2004; 2009) and a primary route of inorganic arsenic exposure in humans is the drinking water (IARC 2004). This makes the findings of oral carcinogenic activity in the lung particularly important, even in genetically hypersensitive animals (Cui et al. 2006; Hayashi et al. 1998; Kinoshita et al. 2007). However, the methylated organo-arsenicals, such as DMA^V, would rarely occur in drinking water, although as biomethylation products of inorganic arsenic they would be commonly found as circulating or urinary metabolic products after inorganic arsenic exposure (IARC 2004).

Tumor end-point results have been generated using oral exposure to DMA^V in rats (Table 1). Oral DMA^V exposure in the drinking water for up to two years produced clear exposure-response relationships in male rats for induction of urinary bladder (UB) carcinoma and combined papilloma or carcinoma (first reported as a short communication in Wei et al., 1999; reported in full in Wei et al., 2002). The tumors induced were transitional cell carcinoma (TCC). The UB is a critical target of arsenic and arsenic compounds in humans and pathologically TCC is concordant with the tumor type most often found in arsenic exposed humans (IARC 2004; IARC 2009). Thus, these data are very important as they are the first to show the UB as a cancer target site of an arsenical alone in rodents (Wei et al., 1999; 2002). Furthermore, they duplicate the specific tumor found in humans (Wei et al., 1999; 2002), which greatly fortifies their human relevance.

When DMA^V is added to the feed it induces an exposure-response related increase in UB tumors in female rats but not males over two years (Table 1; Arnold et al., 2006). The malignant tumors were TCC. Although tumors primarily occurred in female rats, preneoplasia (urothelial cell hyperplasia; not shown) was clearly increased in male and female rats (Arnold et al., 2006). The reasons for this gender-based sensitivity are unknown. No tumors were induced at other sites in rats. The study by Arnold et al. (2006) is quite comprehensive and presents critical confirmatory evidence that oral DMA^V can induce UB cancer in rats. They also studied similar groups of male and female B6C3F1 mice treated with DMA^V (Arnold et al., 2006) but no UB tumors or tumors at other sites were induced under similar circumstances. The basis of the species-related difference in susceptibility to arsenical-induced UB carcinogenesis is undefined, but mice may be just insensitive. Oral exposure to another organo-arsenical biomethylation product, trimethylarsine, over two years in the drinking water induces liver adenoma in rats (Table 1; Shen et al. 2003).

Oral monomethylarsonic acid (MMA^V), an intermediate biomethylation production of inorganic arsenicals, when given to rats for up to two years did not induce tumor formation in a comprehensive dose-response study that included male and female rats and mice (Arnold et al., 2003).

Several recent studies using oral exposure to arsenicals give some indication of a carcinogenic response, but they have significant interpretation issues. One study appears to provide evidence of a positive but low carcinogenic response in the skin of K6/ODC mice given DMA^V or sodium arsenate orally (slight increase skin tumor incidence and multiplicity; Chen et al. 2000) but provides insufficient information to adequately interpret this finding (no descriptive statistics, lack of specific group sizes, statistical analysis technique undefined). Because the response is marginal and the reporting is limited such that an independent re-evaluation is impossible this study (Chen et al. 2000) is not interpretable. A dose-response study with sodium arsenate in the drinking water in male and female Sprague-Dawley rats using 0, 50, 100 and 200 mg/L NaAsO₂ in the drinking water produced a maximal response for combined benign and malignant renal tumors (100 and 200 mg/L, females, both 5/50) which was statistically insignificant compared to contemporaneous control (1/50; Soffritti et al., 2006). However, the authors point out that this rate of renal tumor formation with arsenic is high compared to their historical control (Soffritti et al., 2006) but do not give numbers for the historical control. Accordingly, these are difficult to interpret, but the role of inorganic arsenic in renal tumor development bears additional study because it is a suspected tumor site in humans (IARC 2009). A study apparently finding elevated total tumors in various organs in two strains of mice after chronic oral sodium arsenate exposure (Ng et al., 1999), although discussed in the 2004 IARC monograph (IARC 2004), unfortunately the investigators did not perform actual histopathological evaluation of the gross lesions. The IARC preamble is quite clear that the number of animals examined *histopathologically* is a major factor in judging study adequacy and no subsequent reporting of this work has appeared with appropriate histopathological data. It must therefore, unfortunately, be considered preliminary in nature. Quantitative macroscopic evaluation of tumors is of limited value in defining carcinogenic potential with few, very well-defined exceptions, like skin and perhaps lung, and then only if supported by histopathological evaluation of representative lesions at termination. In another study, DMA^V in the drinking water of p53+/- or C57BL/6J mice for 18 months increased the incidence of mice with tumors at any site (C57BL/6J) and the number of tumors at any site/mouse (both strains) but did not show a specific target site (Salim et al., 2003). Combining tumors from different organs raises a variety of issues particularly in the absence of a known discrete target site. Therefore, these data should be considered of limited value.

Overall, in acceptably designed and interpretable studies, oral exposure to inorganic arsenate produces lung tumors in mice (Cui et al. 2006), while oral exposure to DMA^V produces lung tumors in mice (two studies: Hayashi et al. 1998; Kinoshita et al. 2007), and UB tumors in rats (two studies: Wei et al., 2002; Arnold et al., 2006). In addition, oral exposure to trimethylarsine produces benign liver tumors in rats (Shen et al. 2003).

Intratracheal Instillations

Intratracheal instillations (ITs) of arsenicals have been utilized in several rodent studies to attempt to mimic inhalation exposure (Table 2). Repeated weekly ITs (15 times) of calcium arsenate, at a level that caused moderate early mortality (15%), induced lung adenomas in hamsters when observed over their lifespan (Pershagen and Björklund, 1985). In a similarly designed study, male hamsters receiving multiple weekly ITs of calcium arsenate at the experimental onset showed increased lung adenoma or combined lung adenoma and carcinoma formation during their lifetime (Yamamoto et al., 1987). In negative portions of these two studies, they also found that repeated ITs of arsenic trisulfide did not produce lung tumors and were highly toxic, producing a high level of dosing deaths (29–40%) making it difficult to draw any conclusions (Pershagen and Björklund, 1985; Yamamoto, 1987).

One IT study in rats used “Bordeaux mixture” that contained calcium arsenate but, beyond being a complex mixture, contained high levels copper (Invankovic et al. 1979), a redox active metal. Another study used arsenic trioxide in hamsters (Ishinishi et al., 1983). While these studies did produce a tumor response, the study on Bordeaux mixture did not account for the high levels of copper with any sort of control (Invankovic et al. 1979). Also, both of these studies showed a high percentage (~50%) of animal death during dosing (Ishinishi et al. 1983; Invankovic et al. 1979). The tumor response in the Ishinishi et al. (1983) study was, by accepted convention, statistically insignificant (i.e. $p > 0.05$). A study using IT instillation of gallium arsenide in hamsters has critical design flaws, including short duration, small group sizes, etc., and shows no indication of tumors (Ohyama et al., 1988). Therefore, the conclusions that can be drawn about the IT carcinogenicity of arsenic from these particular studies must be considered limited.

Overall, there are in two acceptably designed and interpretable IT studies in which with inorganic arsenic was carcinogenic in the lungs of hamsters (Pershagen and Björklund, 1985; Yamamoto, 1987).

Inhalation Exposure

The primary route of human exposure to arsenic in the workplace is through inhalation. A study by the US National Toxicology Program (NTP 2000) provided clear evidence of the carcinogenicity for gallium arsenide after inhalation in rodents. In female rats exposed via inhalation to several levels of gallium arsenide particulate for up to ~2 years, dose-related lung alveolar/bronchiolar tumors and adrenal medulla pheochromocytomas occurred (Table 4). There is some controversy as to exactly how rat pheochromocytomas may correspond to human tumors or predict human risk (Powers et al., 2008; Greim et al., 2009). In male rats, though treatment-related tumors were not observed, a dose-related increase in the incidence of atypical hyperplasia of the lung alveolar epithelium occurred. In the female rats, increases also occurred in leukemia at the highest dose. In a separate component of this study, mice exposed via inhalation to several doses of gallium arsenide particulate for ~2 years did not show treatment-related tumors, but both males and females showed exposure concentration-related increases in the incidence of lung epithelial alveolar hyperplasia. This is considered to be an acceptably designed and interpretable inhalation study providing evidence of the carcinogenicity of inhaled gallium arsenide (NTP 2000).

Perinatal Arsenical Exposures Leading to Cancer in Adulthood

Several studies have used exposure during the perinatal period in rodents, which is typically a period of high sensitivity to carcinogens, to investigate the carcinogenic potential of arsenic (Table 3). In one such study, pregnant mice were treated subcutaneously (s.c.) with arsenic trioxide on one day during the last third of gestation (gestation day [GD] 14, 15, 16 or 17) and then the offspring were treated s.c. on multiple days *post partum* (days 1, 2 and 3) with the arsenical (Rudnai and Borzsonyi, 1980,1981 [same study re-reported]). The offspring initially treated on day 15 of gestation developed an excess of lung tumors (63%) compared to control (18%) while the groups treated on GD 14, 16 or 17 failed to develop tumors. Interpretation of this study is complicated by many factors, including under-reporting of the experimental design, which includes the absence of key information such as number of dams used, and if male, female or pooled mice were used (Rudnai and Borzsonyi, 1980,1981). None-the-less, these data (Rudnai and Borzsonyi, 1980,1981) indicate a sensitivity of rodents towards arsenic carcinogenesis during the perinatal period.

A series of transplacental studies in mice have been performed with sodium arsenite (Waalkes et al., 2003; 2004; 2006a; 2006b). The first such study exposed pregnant mice orally to sodium arsenite alone (Waalkes et al., 2003) while subsequent studies followed this

prenatal arsenic exposure scenario with exposure of the offspring to tumor promoters (12-*O* – tetradecanoyl phorbol-13-acetate [TPA]) or estrogen-like compounds (diethylstilbestrol, tamoxifen) *post partum* to enhance tumor response (Waalkes et al., 2004; 2006a; 2006b). Here a discussion will be confined to the tumor response in groups from these studies that received arsenic alone, and the multiple chemical tumor promotion/interactions are discussed below (see Arsenic Enhances the Carcinogenic Effects of Known Carcinogens section).

In the initial transplacental study, pregnant C3H mice were exposed to different levels of sodium arsenite (0, 42.5 and 85 ppm) in the drinking water from GD 8 to 18, allowed to give birth, and, at weaning, groups of males or females were formed and observed for tumor formation (Table 3; Waalkes et al. 2003). No additional arsenic was given. The levels of arsenic exposure used in this and other transplacental studies (Waalkes et al., 2003;2004;2006a;2006b) did not alter dam weights, drinking water consumption, litter size, newborn weights, or weanling weights or give any other indication of overt toxicity during exposure (Waalkes et al. 2003). Over the next 90 weeks *post partum* female offspring exposed to arsenic *in utero* developed dose-related increases in lung adenocarcinoma, benign ovarian tumors and combined benign or malignant ovarian tumors. Female offspring also developed arsenic dose-related uterine and oviduct preneoplasias after fetal arsenic exposure. After *in utero* arsenic exposure, male offspring showed dose-related increases in incidence of liver adenoma, hepatocellular carcinoma, liver adenoma or carcinoma, and adrenal cortical adenoma. Additionally, arsenic exposed male offspring showed arsenic-induced, dose-related increases in liver tumor multiplicity (tumors/mouse) which was maximally over 5.6-fold over control (Waalkes et al. 2003).

A second study again looked at the carcinogenic effects in C3H mice of various doses of sodium arsenite (0, 42.5 and 85 ppm) in the drinking water of pregnant dams from GD 8 to 18, with or without subsequent exposure to the tumor-promoting phorbol ester, TPA. TPA was applied to the shaved back of the offspring after weaning from 4 to 25 weeks of age in order to potentially promote skin cancers initiated by prenatal arsenic exposure (Waalkes et al. 2004). Only arsenic exposure results are reported here. Over the next two years, no elevation in skin tumors occurred regardless of arsenic exposure (or additional TPA). However, with arsenic alone, female offspring showed an increased incidence of ovarian tumors (Table 3) and dose-related increases in uterine and oviduct preneoplasia incidence. Male offspring exposed to arsenic *in utero* showed dose-related increases in incidence of arsenic-induced liver adenoma, hepatocellular carcinoma, combined adenoma or carcinoma, and adrenal cortical adenoma. Male arsenic-treated mice also showed marked increases in liver tumor multiplicity. Arsenic before TPA did enhance tumor outcome in some cases (see below).

Pregnant CD1 mice received sodium arsenite (0 or 85 ppm) in the drinking water from GD 8 to 18, were allowed to give birth, and female (Waalkes et al., 2006a) or male (Waalkes et al. 2006b) offspring were treated with diethylstilbestrol (DES) or tamoxifen (TAM) s.c. on *post partum* days 1 to 5. In female offspring over the next 90 weeks, arsenic exposure alone increased the incidence of ovarian tumors, combined uterine adenoma or carcinoma, and adrenal cortical adenoma (Table 3). Arsenic alone also increased the incidence of oviduct hyperplasia as seen in C3H mice. In male offspring (Waalkes et al. 2006b), prenatal arsenic exposure alone increased liver adenoma, hepatocellular carcinoma, combined liver adenoma or carcinoma, lung adenocarcinoma, and adrenal cortical adenoma (Table 3). In these male mice, prenatal arsenic also induced renal cystic tubular hyperplasia, considered a preneoplastic lesion.

A complex study using sodium arsenate that included 20 s.c. injections of pregnant mice with or without an additional 20 weekly s.c. injections of the offspring proved impossible to interpret because 19/55 control mice were still surviving at the time the work was reported in 1971 (Osswald and Goerttler, 1971). No subsequent report of this study has become available which makes these data of limited value.

Overall, there are in four acceptably designed and interpretable perinatal studies in which inorganic arsenic was carcinogenic in three strains of mice (Rudnai and Borzsonyi, 1980, 1981 [same study re-reported in different languages]; Waalkes et al., 2003; 2004; 2006a; 2006b [2006a,b same study reporting sexes separately]). Studies provide evidence of prenatal arsenic dose-response relationships in adult tumor formation in two cases (Waalkes et al., 2003; 2004). Target sites for tumor production for perinatal arsenic exposure studies include the lung, liver, ovary and adrenal (three studies each), as well as the uterus (one study). It is now considered that there is evidence of carcinogenicity for arsenic and arsenic compounds in humans for both the lung (*sufficient*) and liver (*limited*), two repeated target sites of the perinatal studies with inorganic arsenic (Rudnai and Borzsonyi, 1980, 1981; Waalkes et al., 2003; 2004; 2006b).

Intravenous Exposure

Two studies in mice have used multiple i.v. injections of sodium arsenate to assess carcinogenic potential (Osswald and Goerttler, 1971; Waalkes et al., 2000). Although one study appeared to find increased incidence of hematopoietic tumors, about 35% of the control animals were alive at the time the work was reported, making interpretation of data problematic (Osswald and Goerttler, 1971). No subsequent complete reporting has appeared. The other study looked at male and female mice (Waalkes et al., 2000) using the doses of Osswald and Goerttler (1971) and found no evidence of elevated tumor formation with sodium arsenate. An elevated incidence of uterine hyperplasia did occur with repeated arsenate (Waalkes et al., 2000), but neither this study nor the study by Osswald and Goerttler (1971) are considered to add interpretable data to the issue of actions of arsenic as a complete carcinogen.

Pellet Implantation

A brief report in rats describes the surgical implantation of permeable pellets containing arsenic trioxide in fat-wax mixture into a pouch created in the stomach which produced benign and malignant tumors at the site of the implantation over 2 years (Katsnelson et al., 1986). However, benign tumors were also observed with implantation of pellets without arsenic (Katsnelson et al., 1986), indicating the pellet itself stimulated an oncogenic response, making interpretation of the role of arsenic in the results of this study difficult.

Summary of Complete Carcinogenic Effects; Arsenicals, Routes, Species and Sites

There is now a variety of acceptably designed and interpretable studies in which various arsenicals are complete carcinogens by several routes, at several sites and in multiple species. For inorganic arsenicals, oral exposure to sodium arsenate in the drinking water was carcinogenic in the lungs of mice in one study (Cui et al., 2006). Multiple intratracheal instillations of calcium arsenate in hamsters caused lung tumors in two separate experiments (Pershagen and Bjorkland, 1985; Yamamoto et al., 1987). Perinatal exposure to arsenic trioxide, specifically, maternal s.c. exposure on GD 15 plus early postnatal s.c. injections, produced lung tumors in adulthood in mice in one study (Rudnai and Borzsonyi 1980, 1981). Transplacental exposure in mice via maternal consumption of sodium arsenite in the

drinking water during gestation (GD 8–18) was carcinogenic in males and/or females in the liver, adrenal, and ovary in three separate studies (Waalkes et al. 2003; 2004; 2006a; 2006b), in the lungs two studies (Waalkes et al. 2003; 2006b), and the uterus in one study (Waalkes et al. 2006a). These transplacental studies involved two different mouse strains (C3H and CD1; Waalkes et al., 2003; 2004; 2006a; 2006b). This makes for a total of seven studies in which inorganic arsenicals, in one form or another, by several routes and often at multiple sites, are carcinogenic in rodents. Two of the target sites (lung, liver) are concordant with known or suspected human target sites of arsenic or inorganic arsenic compounds (IARC 2009).

Oral exposure to DMA^V in the drinking water produced UB tumors in male rats (Wei et al., 1999; 2002). While DMA^V in the feed produced UB tumors in female, but not male rats (Arnold et al., 2006). Oral exposure to DMA^V in the drinking water induced lung tumors in mice (Hayashi et al., 1998; Kinoshita et al., 2007). This makes four studies in which DMA^V alone causes cancer in rodents, two of which cause TCC in the UB, which is concordant with human data indicating that the UB is an important target site of arsenic carcinogenesis and TCC is common (IARC 2004; IARC 2009).

Oral exposure to trimethylarsine oxide in the drinking water also induced an elevated incidence of liver adenoma in rats in one study (Shen et al. 2003).

A comprehensive study showed inhalation of gallium arsenide is carcinogenic in female rats at multiple sites including the lungs and forms preneoplastic lung lesions in female and male rats and mice (NTP 2000).

Arsenic Enhances the Carcinogenic Effects of Known Carcinogens

An arsenical given prior to, concurrently with, or after other exposures can sometimes enhance carcinogenic outcome with or without showing clear carcinogenic activity alone. In this review, such studies were only considered if they had an arsenical alone group and did actual histopathological confirmation of tumors.

Designs where treatment with another carcinogen was followed by exposure to an arsenical are summarized in Table 5. In a short report, rats that underwent partial hepatectomy then were given diethylnitrosamine by injection and one week later oral sodium arsenite in the drinking water for ~24 weeks showed an increased renal tumor incidence while arsenic alone produced no renal tumors (Shirachi et al., 1983). A much more comprehensive study used rats “initiated” with a mixture of organic carcinogens [including diethylnitrosamine, *N*-methyl-*N*-nitrosourea, 1,2-dimethylhydrazine, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, and *N*-bis(2-hydroxypropyl)nitrosamine] followed by DMA^V in the drinking water (at four levels) for 24 weeks (Yamamoto et al., 1995). The combined carcinogen mix/arsenical-treated rats developed an increased incidence of UB tumors, kidney carcinoma, liver tumors, and thyroid tumors, none of which were seen with DMA^V alone. Additionally, DMA^V dose-related trends occurred for kidney, liver, and thyroid tumors with the DMA^V/carcinogen mix. These are important initial data (Yamamoto et al., 1995) as they indicate DMA^V can promote UB carcinogenesis, a human target site (IARC 2004; IARC 2009). In another study, rats were given *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in the drinking water as an initiator followed by four levels of DMA^V for up to 32 weeks. The combined organic carcinogen and arsenical treatment increased UB papilloma and carcinoma, while DMA^V alone was without effect (Wanibuchi et al., 1996). Yamanaka et al. (1996) initiated mice with 4-nitroquinoline 1-oxide (s.c.) then exposed them to DMA^V in the drinking water and found a marked increase in lung tumors.

Several other arsenical “promotion” style studies were considered flawed in regards to interpretation of carcinogenic potential. For example, in K6/ODC mice treated topically with 7,12-dimethylbenz[α]anthracene (DMBA) then DMA^V in a cream applied to the same skin the organo-arsenical appeared to double the skin tumor multiplicity compared to DMBA alone (Morikawa et al., 2000). This study was deemed insufficient because it had only two DMA^V alone control mice. In a small study, rats pretreated with *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine in the drinking water followed by DMA^V for 36 weeks showed an increase in UB hyperplasia. Effects were not seen with the nitrosamine or DMA^V alone controls (Li et al., 1998). Although a few tumors occurred, the response was not significant (Li et al., 1998), and the very small group sizes (8) make interpretation of these data limited.

In some cases, arsenic alone has no carcinogenic effect but enhances the carcinogenic response when given concurrently or before the other agent (Table 6). For instance, TPA alone produces epidermal papillomas in Tg.AC mice, which are engineered to be sensitive to skin carcinogenesis. Germolec et al. (1997;1998) orally administered sodium arsenite just before and concurrently with TPA and provided initial evidence that this co-treatment markedly enhances the epidermal carcinogenic response compared to TPA alone. Although these studies may lack statistical analysis and the specific information to independently perform such analyses, given the level of response, the repeatability, and the extensive database with other agents in this mouse model, these studies are noteworthy as the initial work that indicates inorganic arsenic has the potential to act in the skin to cause cancer in a complex fashion with other agents. The skin is an important target site of arsenic in humans (IARC 2004;2009), and this initial work (Germolec et al., 1997;1998) set the stage for various skin “co-carcinogenesis” studies (Rossman et al. 2001;Burns et al., 2004;Uddin et al., 2005).

Studies have since focused on combined treatments with oral sodium arsenite in the drinking water and multiple exposures to excess topical ultraviolet (UV) irradiation (85% UVB, <1% UVC, 4% UVA, remainder visible) in Crl:SK1-*hr*BR hairless mice (Rossman et al. 2001; Burns et al., 2004; Uddin et al., 2005). Arsenic treatment alone was consistently without carcinogenic effect in the skin (Rossman et al. 2001; Burns et al., 2004; Uddin et al., 2005). However, compared to UV treatment alone, which did cause some tumors, the arsenic plus UV co-treatment decreased time to first skin tumor, increased total number of tumors, increased the portion of tumors that were highly invasive squamous cell carcinoma (SCC), and increased skin tumor volume (Rossman et al. 2001). This enhancement of UV skin carcinogenesis occurred in an arsenic exposure concentration-related fashion (Burns et al., 2004) and could be blocked by antioxidants in the diet (Uddin et al., 2005). These data are important in that they show arsenic acts as a co-carcinogen producing a skin tumor of concordant type (SCC) as that seen in humans exposed to arsenic (IARC 2004). Given that both inorganic arsenic and UV irradiation can reasonably be expected to commonly occur in the human environment makes these findings increasingly relevant to human populations. In another skin study, mice were exposed to topical 9,10-dimethyl-1,2-benzanthracene and oral sodium arsenate in the drinking water concurrently for 2 weeks and then arsenic only until 25 weeks of age, representing a combined co-treatment and promotion design (Motiwale et al., 2005). Arsenic alone was without impact, yet increased the skin tumors/mouse and skin tumor size when combined with the organic carcinogen compared to the carcinogen alone (Motiwale et al., 2005).

Arsenic exposure has also been shown to enhance carcinogenic effects when given prior to additional treatment (Table 6). The impact of postnatal treatments with TPA (Waalkes et al., 2004), DES or TAM (Waalkes et al., 2006a,b) after maternal arsenic exposure were not discussed in the **Perinatal Exposure** section but because of the timing involved should be viewed as instances in which arsenic acts in advance of additional, subsequent treatments to

enhance carcinogenic outcome. For instance, prenatal sodium arsenite exposure via maternal drinking water (0, 42.5 and 85 ppm) when combined with postnatal topical TPA exposure (weeks 4–25 of age) increased liver tumor incidence (and multiplicity) in an arsenic dose-related fashion (female offspring) and lung adenomas (high dose arsenical, female offspring; low dose arsenical male offspring) compared to control. These effects were not seen with TPA or arsenic alone (Waalkes et al., 2004). TPA applied topically is easily absorbed and does have systemic promotion capability. Similar prenatal arsenic exposure via maternal drinking water (0 or 85 ppm) followed by postnatal DES increased uterine and vaginal carcinomas, UB hyperplasia, UB total proliferative lesions (incidence of hyperplasias plus tumors), and liver tumors in female offspring compared to control, effects not seen with DES or arsenic alone (Waalkes et al., 2006a). The increase seen with combined prenatal arsenic and postnatal DES in UB total proliferative lesions included three TCCs, which, although not statistically significant, are noteworthy because of their spontaneous rarity in mice, and because TCCs of the UB are a tumor commonly associated with arsenic exposure in humans (IARC 2004). In further results from this study, female offspring after prenatal arsenic exposure followed by postnatal TAM showed similar increases in both hyperplasias and total proliferative lesions in the UB (Waalkes et al., 2006a). In male offspring, prenatal arsenic exposure followed by postnatal DES increased liver tumor response (based on incidence in mice at risk and hepatocellular tumor multiplicity; see Waalkes et al., 2006b for graphics), UB hyperplasia, and UB total proliferative lesions, compared to control, effects not seen with DES or arsenic alone (Waalkes et al., 2006b). Prenatal arsenic exposure followed by postnatal TAM in the male offspring increased UB hyperplasia, and UB total tumors (Waalkes et al., 2006b). The postnatal promotion of prenatally initiated lesions induced by arsenic into UB tumors by TAM included a TCC (Waalkes et al., 2006b), indicating arsenic could put into place events *in utero* that could be acted upon later to cause UB cancer by other agents.

In another study in which arsenic was given prior to another agent, pregnant Tg.AC mice were treated from GD 8 to 18 with oral sodium arsenite in the drinking water (0, 42.5 or 85 ppm) and their offspring were topically exposed to TPA from 4 to 40 weeks of age. The Tg.AC mouse is genetically sensitive to skin cancer development. Arsenic alone had no effect but when combined with TPA it markedly increased the multiplicity of malignant epidermal cancers (SCC) in a dose-dependent fashion compared to TPA alone (Waalkes et al., 2008). Because arsenic is rapidly cleared from the body (IARC 2004) these data indicate that *in utero* arsenic is causing persistent/permanent changes in the skin that make it more sensitive to carcinogenic stimulus, and further data from this study shows an aberrant accumulation of cancer stem cells (CSCs) in the SCC arising from combined treatments (Waalkes et al., 2008). This indicates that arsenic exposure may be altering stem cell (SC) response to chemical carcinogens later in life and thereby altering sensitivity to carcinogenesis, at least in skin (Waalkes et al., 2008). Thus, arsenic exposure *in utero* in this case may be looked upon as a toxicological fetal basis of adult sensitivity to disease.

Summary of Studies Where Arsenicals Enhance the Effects of Other Carcinogens

There are now a variety of acceptably designed and interpretable studies in which various arsenicals can be given before, during, or after other agents and act to enhance end-point carcinogenic response. In studies where arsenic is given as a promoter of cancer (i.e. after initiation with a known carcinogen) oral exposure to either sodium arsenite (Sharachi et al., 1983) or DMA^V (Yamamoto et al., 1995) promoted renal tumors initiated by organic carcinogens in rats. Other studies showed oral exposure to DMA^V promoted UB tumors initiated by organic carcinogens in rats (Yamamoto et al., 1995; Wanibuchi et al., 1996). Liver and thyroid carcinogenesis in rats (Yamamoto et al., 1995) or lung carcinogenesis in

mice (Yamanaka et al., 1996) pre-initiated by organic carcinogens could all be promoted by subsequent oral DMA^V exposure.

Concurrent exposure to arsenicals and other chemical or physical skin carcinogens can enhance oncogenic outcome. Co-treatment with oral sodium arsenite enhanced chemical- or irradiation-induced epidermal cancer in five separate studies in mice (Germolec et al., 1997, 1998; Rossman et al. 2001; Burns et al., 2004; Uddin et al., 2005). One study shows that with topical exposure to an organic skin carcinogen, concurrent and subsequent treatment with oral sodium arsenate increases skin tumor response (Motiwalé et al., 2005).

Prior arsenic exposure can also impact the carcinogenic effects of subsequent exposures. One study each shows *in utero* exposure to sodium arsenite enhances skin SCCs (males and females; Waalkes et al., 2008), liver tumor (females; Waalkes et al., 2004) and lung adenoma (females and males; Waalkes et al., 2004) formation with subsequent TPA treatment in adulthood. One study shows fetal exposure to sodium arsenite enhances uterine carcinoma, vaginal carcinoma, UB proliferative lesions, and liver tumors with subsequent DES treatment in female offspring (Waalkes et al., 2006a,b [one study reporting different sexes]). One study shows *in utero* exposure to sodium arsenite enhances UB tumors with subsequent TAM treatment in male offspring (Waalkes et al., 2006b).

Discussion

Impact of Rodent Studies on Overall Evaluation

Arsenic is clearly an important environmental human carcinogen and millions of people world-wide are exposed to what are very likely un-healthy levels of the metalloid in the drinking water (IARC 2004). Mode of action is often difficult to define in humans, because one is frequently looking at past events and trying to piece together outcome, all overlaid by the complexities of human behavior. *In vivo* whole animal models of carcinogenesis provide important information on the carcinogenic potential and mechanisms of carcinogenesis in humans. The accumulated evidence that arsenic is carcinogenic in rodents is compelling, and it acts as a complete carcinogen in numerous studies (Tables 1–4). These rodent data clearly supported the very recent IARC classification of arsenic and inorganic arsenic compounds as carcinogenic to humans (IARC 2009). In addition, arsenicals have been shown to act in concert with other agents in rodents to enhance end-point cancer production (Tables 5 and 6). In light of all these data it would appear extremely difficult to defend previously held scientific positions that arsenic is not carcinogenic in animals, even though such opinions have been voiced in the very recent past (NSF 2005). The stance that arsenic is not carcinogenic in animals is no longer tenable or warranted.

Rodent to Human Target Site and Tumor Concordance

Together, the carcinogenesis studies in rodents show arsenicals can impact a range of tumor sites with human relevance, including *all* those for which there is considered sufficient evidence for arsenicals in humans, namely the UB, lung and skin (IARC 2009), and the majority for which there is now considered more limited evidence in humans (i.e. the liver and kidney; IARC 2009). Such tissue concordance between humans and rodents in target site is truly remarkable. In our review of the literature, we found seven acceptably designed and interpretable studies that used a total of four different inorganic arsenical compounds (sodium arsenate, arsenic trioxide, sodium arsenite and calcium arsenite) in a total of two species (mice and hamsters) that gave clear evidence of complete carcinogenic activity for inorganic arsenicals (Rudnai and Borzsonyi 1980; 1981; Pershagen and Björklund, 1985; Yamamoto, 1987; Cui et al., 2006; Waalkes et al. 2003; 2004; Waalkes et al. 2006a, 2006b). The target sites for arsenic alone in all these studies include one either of the lung or liver or

both, again noteworthy as known or possible target tissues of human arsenic carcinogenesis (IARC 2009). Oral DMA^V was also carcinogenic by itself in four separate studies, two in the rat UB (Wei et al., 1999; 2002; Arnold et al., 2006) and two in mouse lung (Hayashi et al., 1998; Kinoshita et al., 2007). DMA^V is a major biomethylation metabolite produced from inorganic arsenic in humans and rodents and is found in the urine (IARC 2004). The UB TCC produced in these rat studies (Wei et al., 1999; Wei et al., 2002; Arnold et al., 2006) are concordant with tumors commonly found in humans exposed to arsenic and produced by a biomethylation product that is found in the urine (IARC 2004). The human relevance of the evidence for carcinogenesis of arsenic in rodents is enhanced by both the target site and tumor type concordance with arsenic-exposed humans. This fact should be considered when evaluating the carcinogenic capability of arsenicals in laboratory animals.

Rodent Bioassay and Mechanism of UB Cancer

The IARC considers DMA^V a Group 2B carcinogen (possibly carcinogenic to humans; IARC 2009) on the basis of sufficient evidence of cancer in animals. DMA^V has been used as a pesticide but, more importantly it is the ultimate biomethylation product of inorganic arsenic in mammals. Some of the key studies in rodents are those that show UB cancer after oral exposure, specifically TCC (Wei et al., 1999; Wei et al., 2002; Arnold et al., 2006). A recent review by Cohen et al. (2007) discusses possible mechanisms by which DMA^V could induce UB cancer in the rat (Wei et al., 1999; Wei et al., 2002; Arnold et al., 2006). They propose protracted oral DMA^V exposure, when it reaches the urine, induces urothelial cytotoxicity, causing subsequent persistent regenerative proliferation, which ultimately leads to compensatory hyperplasia and eventually UB cancer (Cohen et al. 2007). It is thought that DMA^V may first require conversion to a reactive trivalent metabolite (possibly DMA^{III}) which then causes oxidative stress in the bladder cells (Cohen et al. 2007). This mechanism would be consistent with chronic exposure to arsenic where damage (cytotoxicity) and compensatory (regenerative) proliferation could eventually lead to cancer formation, but would appear specific to the UB. This makes it entirely possible that arsenicals have multiple mechanisms that might depend on target site. There is no reason that this proposed mechanism in the rat UB (Cohen et al. 2007) could not apply to humans. Although not discussed in the review, this mode of action could also be possible for other pentavalent forms of arsenic, such as MMA^V (which could be converted to MMA^{III}) (Cohen et al. 2007). The recent findings that maternal oral inorganic arsenic exposure *in utero* followed by postnatal DES or TAM results in UB hyperplasia or TCC in mice in adulthood would not seem to fit into this proliferative hyperplasia to tumor hypothesis, as all arsenic exposure would have ended by GD 18, long before any preneoplasia or tumor formation in adulthood (Waalkes et al, 2006a,b). Perhaps there is more than one mechanism by which arsenicals can influence UB tumor formation, and this needs further exploration.

Rodent Bioassay and Skin Cancer Mechanisms

Other tumor end-point rodent studies with arsenicals also provide mechanistic insight. In addition to the evidence of complete carcinogenesis, there is the fact that arsenicals, when given before, during or after a variety of chemical or physical agents, can enhance their carcinogenic outcome while having little or no oncogenic effect by themselves. Such studies can provide remarkable insight into mechanism, as they indicate arsenicals have the capacity to modify the carcinogenic outcome of other agents by modifying tissue responsiveness short of cancer causation. For instance, such factors may be particularly important for the skin, which is a known human target for arsenic carcinogenesis (IARC 2004; 2009). In the experimental studies, it appears arsenic cannot cause tumors by itself, but requires either prior or co-treatment with another chemical (Germolec et al., 1997; Germolec et al., 1998; Motiwale et al., 2005) or physical agent (UV; Rossman et al., 2001; Burns et al., 2004; Uddin et al., 2005) to produce skin cancer. There is also one instance in which *in utero*

arsenic exposure enhances skin SCC response when animals are exposed to TPA in adulthood (Waalkes et al., 2008). So for one major human target site (i.e. the skin; IARC 2004; 2009) the animal data indicate that arsenic is not a complete carcinogen, even in strains very sensitive to skin cancer, such as the Tg.AC mouse (Germolec et al., 1997; Germolec et al., 1998; Waalkes et al., 2008). This leads to the conclusion that the mechanism with inorganic arsenic may be quite different in skin than other organs, since arsenicals do show the ability to be complete carcinogens in some other tissues. Several studies convincingly point towards co-carcinogenic effects of arsenic and UV irradiation in skin cancer (Rossman et al., 2001; Burns et al., 2004; Uddin et al., 2005), which have important implications in human exposures. Thus, arsenic needs a co-carcinogen in the skin but is a complete carcinogen in other tissues and, therefore, has multiple carcinogenic mechanisms of action. Further, the recent results of the transplacental skin “initiation” study (Waalkes et al., 2008) provides a novel insight into the effects of arsenic exposure on skin cancer formation. This study shows a clear over-abundance of CSCs in the SCC produced after *in utero* arsenic exposure followed by TPA as compared to TPA alone (Waalkes et al., 2008). Coupled with *in vitro* data indicating arsenic stalls skin SC differentiation (Patterson and Rice, 2007; Patterson et al., 2005) and induces malignant transformation of normal SCs into CSCs (Tokar et al., 2009), these *in vivo* data showing CSC over-abundance in mouse skin cancers after arsenic (Waalkes et al., 2008) strongly suggest that arsenic had previously targeted and altered the skin SC phenotype to predispose it to skin carcinogenesis by other agents. This SC population may then remain quiescent until promoted by a subsequent exposure later in life, such as from TPA (Waalkes et al., 2008), or UV, fortifying the concept of a toxicological fetal basis of adult disease and the emerging notion that SCs play a pivotal role in the carcinogenic process (Thomas et al., 2006; Ailles and Weissman, 2007). Further investigations into the link between SCs and arsenic carcinogenesis are warranted.

Rodent Bioassays in Non-traditional Models

It has been recently stated that “arsenic is unusual among chemicals of concern in that it does not cause tumors in **common** laboratory animal models, although it clearly does so in humans” (NSF, 2005). Such “uncommon” animal models would include, presumably, exposure during the perinatal period (Rudnai and Borzsonyi 1980, 1981; Waalkes et al. 2003; Waalkes et al. 2004; Waalkes et al 2006a; Waalkes et al 2006b; Waalkes et al., 2008) or from tests using rodents that are hypersensitive to cancer development (Germolec et al., 1997; Germolec et al. 1998; Hayashi et al. 1998; Cui et al., 2006; Waalkes et al. 2008; Kinoshita et al. 2007). Nonetheless, data are interpreted by IARC as supportive evidence of complete carcinogenic potential of arsenicals in rodents (IARC 2004; IARC 2009). However, because such data have been labeled by other reviewers as not providing any evidence of carcinogenic potential because they are derived from “uncommon” or non-traditional rodent models (NSF, 2005), the concept of arsenic as a paradoxical carcinogen persists.

With regard to the use of rodents that are hypersensitive to cancer development, it is clear that for many years laboratory animals were considered refractory to arsenic carcinogenesis to the point that arsenic was considered a uniquely “paradoxical” carcinogen by many (e.g. Jager and Ostrosky-Wegman, 1997), being potently active in humans but totally inactive in test animals. It is considered by many that, for unknown reasons, rodents are insensitive to arsenic carcinogenesis compared to humans (e.g. NSF, 2005), a notion that we support even with the emergence of positive evidence of rodent carcinogenicity (Waalkes et al., 2007). But, as to testing, this lack of sensitivity in rodents alone fully justifies the use of animal strains that would be generally susceptible to carcinogenesis when designing animal tumor end-point assays. It seems, at best, counterintuitive to test an agent for which carcinogenic activity has proven difficult to show, in animals that are insensitive or normal sensitivity to

chemical carcinogenesis. The IARC preamble clearly states that “All known human carcinogens that have been studied adequately in experimental animals have produced positive results ...” (IARC 2004). Perhaps part of the adequate animal testing in the case of arsenicals is to use rodents that are generally sensitive to cancer because rodents may have an innate insensitivity to arsenic carcinogenesis compared to humans.

As to early life exposure, contrary to being unusual, it is well-established that perinatal exposures have led to cancer in humans, as with *in utero* exposure to DES and vaginal cancer in young adulthood as a prime example (Anderson et al., 2000). Furthermore, there is emerging evidence that perinatal arsenic exposure is carcinogenic in humans, where lung and liver cancers show remarkable increases in subjects exposed to arsenic in the drinking water during early childhood and/or *in utero* (Smith et al., 2006; Liaw et al., 2008). From the perspective of the rodent work, this strongly fortifies the relevance of these mouse perinatal carcinogenesis data with inorganic arsenicals (Rudnai and Borzsonyi 1980; 1981; Waalkes et al. 2003; Waalkes et al. 2004; Waalkes et al 2006a; Waalkes et al 2006b) in which the lung and liver are common tumor target sites. People who are exposed to inorganic arsenic in the environment, without intervening remediation, are frequently exposed for their entire life times, including during their fetal and early childhood life stages. It is clear that the mechanisms of arsenic carcinogenesis are complex and multi-factorial, and that within this context perinatal exposure could be difficult to model. However, based on accumulating data from both humans and rodents that transplacental and/or early life exposure to arsenic can be carcinogenic (Rudnai and Borzsonyi 1980; 1981; Waalkes et al. 2003; 2004; Smith et al., 2006; Waalkes et al 2006a; 2006b; Liaw et al., 2008), arsenic-induced perinatal events leading to cancer in animals should certainly be taken into account when assessing risk or modeling human arsenic carcinogenesis. Indeed, all environmental chemicals of concern for carcinogenesis should probably be tested for transplacental/early life carcinogenic potential, because it still remains typical that potential perinatal consequences that might help determine human cancer risk are largely ignored, as was pointed out two decades ago (Tomatis, 1989). The perinatal period is clearly a time of high sensitivity to chemical carcinogenesis (Anderson et al., 2000), and testing environmental carcinogens as if they were occupational carcinogens (i.e. during adulthood only) because a carcinogen bioassay during adulthood is a “common” model may grossly underestimate the impact of exposure during what could be one of the most critical life stages in accumulated cancer risk.

Future Directions

Rodent tumor end-point studies afford opportunities to define mechanisms that cannot be readily addressed in human epidemiological studies. Although the rodent studies discussed here have added greatly to our understanding of arsenic as a carcinogen much necessary information remains to be acquired. For instance, lacking are *in vivo* tumor end-point studies in rodents investigating the effects of the trivalent methylated arsenicals, such as methylarsonous acid (MMA^{III}). Recent evidence has led some to hypothesize that these trivalent arsenicals, particularly MMA^{III} , generated by metabolism of inorganic arsenicals may be the ultimate carcinogenic form of arsenic because of their higher reactivity and toxicity compared to the parental inorganic forms (e.g. Wang et al., 2007). This should be directly tested in whole animals and studies exploring the potential carcinogenic effects of MMA^{III} *in vivo* are clearly needed. In addition, humans in many situations clearly are exposed to arsenic throughout their entire lifetime, and not just during the fetal or adult stages of life. Therefore, studies in which the animals are exposed to arsenicals during their entire life times would most accurately model the effects of environmental arsenic exposure in humans. It remains a major gap that there is not one arsenic inhalation study in rodents, even though arsenic has been long accepted as a lung carcinogen in humans after inhalation (IARC 1980) and increased cancer risks are similar when people ingest or inhale arsenic

(Smith et al., 2009). Comparison of systemic versus inhaled mechanisms, knowledge of potential distant tumors after inhalation, etc., could all be of great value and explored if an animal model of arsenic inhalation lung carcinogenesis were developed. Such animal studies as these are the essential next step in defining the effects and mechanisms of arsenic carcinogenesis in humans and would further help to prevent and control cancers induced by this common environmental contaminant.

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Abbreviations

(DMBA)	7,12-dimethylbenz[α]anthracene
TPA	12- <i>O</i> -tetradecanoyl phorbol-13-acetate
(CSC)	cancer stem cell
DES	diethylstilbestrol
(DMA^V)	dimethylarsinic acid
IARC	International Agency for Research on Cancer
(MMA^{III})	methylarsonous acid
MMA^V	monomethylarsonic acid
SCC	squamous cell carcinoma
TCC	transitional cell carcinoma
TAM	tamoxifen
UV	ultraviolet irradiation
UGS	urogenital system

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Table 1

Carcinogenic Effects of Oral Arsenic and Arsenic Compounds in Rodents

Species, strain (sex) Duration	Dosing regimen, Number/group at start	Incidence of tumors	Reference
Mice, A/J (Male) 18 months	0, 1, 10, 100 ppm Arsenate Drinking water 30, 30, 30, 30	Tumor/lung [*] (all doses) Tumors > 4 mm/lung [*] (all doses)	Cui et al.(2006)
Mice, A/J (Male) 50 weeks	0, 50, 200, 400 ppm DMA ^V Drinking water 14, 14, 14, 14	Lung adenoma or adenocarcinoma: 2/14, 5/14, 7/14, 10/13 [*] Tumors/lung: 0.50, 1.07, 1.07, 1.36 [*]	Hayashi et al. (1998)
Mice, <i>Ogg1</i> ^{-/-} and <i>Ogg1</i> ^{+/+} (Male & Female) 72 weeks	0, 200 ppm DMA ^V Drinking water <i>Ogg1</i> ^{-/-} : 10, 10 <i>Ogg1</i> ^{+/+} : 12, 12	<i>Ogg1</i> ^{-/-} : Lung tumors: 0/10, 5/10 [*] Lung tumors/mouse: 0, 0.5 [*] <i>Ogg1</i> ^{+/+} : Lung tumors: 1/10, 0/10 Lung tumors/mouse: 0.1, 0	Kinoshita et al. (2007)
Rats, Fischer F344 (Male) 104 weeks	0, 12.5, 50, 200 ppm DMA ^V Drinking water 36, 36, 36, 36	Urinary bladder (UB) papilloma: 0/28, 0/33, 2/31, 2/31 UB carcinoma: 0/28, 0/33, 6/31 [*] , 12/31 [*] UB papilloma or carcinoma: 0/28, 0/33, 8/31 [*] , 12/31 [*]	Wei et al. (1999); Wei et al. (2002)
Rats, F344 (Female) 104 weeks	0, 2, 10, 40, 100 ppm DMA ^V In feed 60, 60, 60, 60, 60	UB carcinoma: 0/60, 0/59, 0/60, 0/59, 6/60 ([*]) Combined UB papilloma and carcinoma: 0/60, 0/59, 0/60, 0/59, 10/60 ([*] trend)	Arnold et al. (2006)
Rats, Fischer 344 (Male) 2 years	0, 50, 200 ppm trimethylarsine oxide in drinking water 42, 42, 45	Liver adenoma: 6/42, 10/42, 16/45 [*]	Shen et al. (2003)

*
= Significant difference.

Table 2**Intratracheal Instillation Carcinogenesis of Arsenic and Arsenic Compounds**

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumors	Reference
Hamsters, Syrian golden (Male)	0, ~3 mg As/kg Calcium arsenate	Lung adenoma: 0/26, 4/35*	Pershagen and Björklund (1985)
Lifespan (up to ~145 weeks)	1X/wk, 15 wks 29, 41		
Hamsters, Syrian golden (Male)	0, 0.25 mg As as Calcium arsenate	Lung adenoma: 0/22, 6/25 [*/]	Yamamoto et al (1987)
Lifespan (up to 121 weeks in control and 115 weeks in treated)	1X/wk, 15 wks 22, 30	Lung carcinoma: 0/22, 1/25 Lung adenoma or carcinoma: 0/22, 7/25 [*/]	

* = Significantly different.

[*/] = Statistical significance determined performed during review (Fisher exact test, one-way, $p < 0.05$)

Table 3

Carcinogenicity of Inhaled Gallium Arsenide in Rodents

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumors	Reference
Rat, Fisher 344 (F) 105 wks NTP (2000)	0, 0.01, 0.1, 1.0 mg/m ³ 6 hr/d, 5 d/ wk 50, 50, 50, 50	Lung adenoma: 0/50, 0/50, 2/50, 7/50 ^{*(<i>trend</i>)} Lung carcinoma: 0/50, 0/50, 2/50, 3/50 Lung adenoma/carcinoma: 0/50, 0/50, 4/50, 9/50 ^{*(<i>trend</i>)} Adrenal medulla: 4/50, 6/49, 6/50, 13/49 ^{*(<i>trend</i>)} Mononuclear-cell leukemia: 22/50, 21/50, 18/50, 33/50 ^{*(<i>trend</i>)}	NTP (2000)

* Significantly different.

(*trend*) = Statistically significant dose-related trend.

Table 4

Perinatal Carcinogenicity of Arsenic and Arsenic Compounds

Species, strain (sex) Duration	Dosing regimen, Animals/group at start	Incidence of tumors in offspring as adults	Reference
Mice, CFLP (Unspecified – sexes apparently pooled) 1 year	Maternal: 1.2 mg/kg arsenic trioxide bw s.c., 1X, GD 14, 15, 16, or 17 Offspring: 5 µg arsenic trioxide/ mouse s.c. <i>post partum</i> day 1, 2 and 3 (Controls untreated). Initial group sizes not specified	Lung tumors (control) 3/17, (day GD 14) 14/36, (GD 15) 12/19*, (GD 16) 3/20, (GD 17) 6/20	Rudnai and Borzsonyi (1980); Rudnai and Borzsonyi (1981) [the 1981 paper re-reports the 1980 work but in Hungarian]
Mice, C3H/HeNCR (Female) 90 <i>post partum</i> weeks	Maternal exposure: 0, 42.5, 85 ppm As as sodium arsenite in the drinking water GD 8–18	Ovarian tumors benign: 2/25, 4/23, 8/24*(*trend) Ovarian tumors benign or malignant: 2/25, 6/23, 9/24*(*trend) Lung carcinoma: 0/25, 1/23, 5/24*(*trend)	Waalkes et al. (2003)
Mice, C3H/HeNCR (Male) 74 <i>post partum</i> weeks	Offspring groups: 25, 25, 25 (males and females)	Liver adenoma: 9/24, 9/21, 20/23* Liver carcinoma: 2/24, 8/21, 14/23*(*trend) Liver adenoma or carcinoma: 10/24, 11/21, 20/23*(*trend) Adrenal cortical adenoma: 9/24, 14/21, 21/23*(*trend)	
Mice, C3H/HeNCR (Female) 104 <i>post partum</i> weeks	Maternal exposure: 0, 42.5, 85 ppm As as sodium arsenite in drinking water from GD 8–18	Ovarian tumors: 0/24, 5/23*, 4/21*	Waalkes et al. (2004)
Mice, C3H/HeNCR (Male) 104 <i>post partum</i> weeks	Offspring groups: 25, 25, 25 (males and females)	Liver adenoma: 10/24, 12/23, 19/21* Liver carcinoma: 3/24, 8/23, 10/21*(*trend) Liver adenoma or carcinoma: 12/24, 14/23, 19/21*(*trend) Adrenal cortical adenomas: 9/24, 15/23, 15/21*	
Mice, CD1 (Female) 90 <i>post partum</i> weeks	Maternal exposure: 0, 85 ppm As as sodium arsenite in the drinking water from GD 8–18 Offspring group sizes: 35, 35	Ovarian tumors: 0/33, 7/34* Uterine adenoma or carcinoma: 0/33, 5/34* Adrenal cortical adenoma: 1/33, 9/34*	Waalkes et al. (2006a)
Mice, CD1 (Male) 90 <i>post partum</i> weeks	Maternal exposure: 0, 85 ppm As in drinking water from GD 8–18 Offspring group sizes: 35, 35	Liver adenoma: 2/35, 8/35* Liver carcinoma: 0/35, 5/35** Liver adenoma or carcinoma: 2/35, 11/35* Lung adenocarcinoma: 2/35, 9/35* Adrenal cortical adenoma: 0/35, 13/35*	Waalkes et al. (2006b) [run contemporaneously with Waalkes 2006a]

GD = Gestation Day. Note: Lung tumor incidence data, although the same, shift one GD later between the Rudnai and Borzsonyi (1980) and (1981; in Hungarian) manuscripts. Direct communication with the primary author revealed this discrepancy is due to a difference in calling the first day of pregnancy day 1 of gestation in the 1981 version rather than day 0 as in the original 1980 work (Rudnai and Borzsonyi, 1980). Here the data from the primary paper are reported.

* = Significant.

(*trend) = Statistically significant dose-related trend.

Table 5

Studies Where Arsenicals Given After Other Agents Enhance Carcinogenesis

Species, strain (sex) Duration Reference	Arsenical Dosing regimen, Animals/group at start	Incidence of tumors	Reference
Rats, Wistar (M) 175 days	Sodium arsenite diethylnitrosamine (DEN), then 160 ppm As in drinking water Control 10, DEN 7, As 9, DEN + As 10	Renal tumors: 0/10, 1/7, 0/9, 7/10*	Shirachi et al. (1983)
Rat, F344/DuCrj (M) 30 weeks	DMA ^V Treatment with carcinogen mix then DMA ^V p.o. 6–30 20, 20, 20, 20, 20 (Mix alone, Mix + 50 ppm DMA, Mix + 100 ppm DMA ^V , Mix + 200 ppm DMA ^V , Mix + 400 ppm DMA)	UB total tumors: 2/20, 17/20*, 16/19*, 17/20*, 16/20* [mostly TCC] Kidney adenocarcinoma: 0/20, 0/20, 2/19, 1/20, 7/20*(*trend) Liver carcinoma: 0/20, 2/20, 0/19, 8/20*, 8/20* Thyroid gland tumors: 3/20, 2/20, 8/19, 6/20, 9/20*(*trend)	Yamamoto et al. (1995)
Rats, F344 (M) 36 Weeks	DMA ^V N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) in the drinking water 4 wks then 0 to 100 ppm DMA ^V for 32 wks 20, 20, 20, 20, 20 (BBN alone, BBN + 2 ppm DMA, BBN + 10 ppm DMA, BBN + 50 ppm DMA, BBN + 100 ppm DMA)	UB: Papilloma: 3/20, 2/20, 7/20, 11/19*, 13/20*, 17/20* Carcinoma: 1/20, 2/20, 3/20, 7/19*, 10/20*, 12/20*	Wanibuchi et al. (1996)
Mice, ddy (M) 25 weeks	DMA ^V 4-nitroquinoline (4NQO) s.c. then 200 or 400 ppm DMA ^V p.o. 25 wks 9, 13, 13 (4NQO, 4NQO + 200 ppm DMA, 4NQO + 400 ppm DMA)	Macroscopic lung tumors/mouse: 0.22, 3.92, 4.38	Yamanaka et al. (1996)

* = Significant.

(*trend) = Statistically significant dose-related trend. See individual papers for organic carcinogen treatment details. .p.o = per os.

Table 6

Studies Where Arsenicals Given With or Prior to Other Agents Enhance Carcinogenesis

Species, strain (sex) Duration	Arsenical Dosing regimen, Animals/group at start	Incidence of tumors or tumor multiplicity	Reference
Mice, Tg.AC homozygous (Female) 14 weeks	Sodium arsenite 0 or 0.02% As p.o. through study plus topical TPA wk 5 & 6 20 each (control, As, TPA, As + TPA)	Macroscopic skin papillomas/mouse: control or As none, TPA alone ~0.5/mouse, ~2.1/mouse As + TPA	Germolec et al. (1997)
Mice, Tg.AC homozygous (Female) 24 weeks	Sodium arsenite 0 or 0.02% As p.o. through study plus topical TPA wk 5 & 6 20 each (control, As alone, 1.25 TPA, 2.5 TPA, As + 1.25 TPA, As + 2.5 TPA)	Macroscopic skin papillomas/mouse: 0 in control, As alone, and 1.25 TPA alone; As + 1.25 TPA maximal ~5/mouse, 2.5 TPA ~3/mouse, in arsenic + 2.5 TPA ~7/mouse	Germolec et al. (1998)
Mouse, Crl: SKI-hrBR (hairless) 29 weeks	Sodium arsenite 10 mg/L sodium arsenite in drinking water through study plus dermal solar radiation (termed UVR) 3X/wk starting at 3 wks 5, 5, 15, 15 (control, As, UVR, As + UVR)	Skin tumors: Control and As alone 0/5, 0/5 Time to first occurrence: As + UVR* vrs. UVR Total all mice: UVR 53 vrs. As + UVR 127* Highly invasive squamous cell carcinoma: UVR 14/53 UVR vrs. As + UVR 64/127*	Rossman et al. (2001)
Mouse, SK1 (hairless) 29 weeks	Sodium arsenite Experiment 1: 0, 1.25, 2.50, 5.00, 10.0 mg/L sodium arsenite p.o plus UVR 3X/wk. Group sizes: 10 each Experiment 2: 10.0 mg/L sodium arsenite p.o. plus UVR 3X/wk. Group sizes: 10 each	Skin tumors: Control and arsenic 0/10, 0/10 Experiment 1: Skin tumors/mouse: 2.4* (UVR), 5.4* (1.25 As + UVR), 7.21* (2.5 As + UVR), 11.1* (5.0 As + UVR), 6.8* (10.0 As + UVR) Experiment 2: Skin tumors/mouse: 3.5 (UVR), 9.6 (As + UVR)* [vrs. UVR]	Burns et al. (2004)