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INORGANIC COMPOUNDS

Tumors and proliferative lesions in adult offspring after maternal exposure to methylarsonous acid during gestation in CD1 mice

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Abstract Developmental exposure to inorganic arsenic is carcinogenic in humans and mice, and adult offspring of mice exposed to inorganic arsenic can develop tumors of the lung, liver, adrenal, uterus, and ovary. It has been suggested that methylarsonous acid (MMA3+), a product of the biological methylation of inorganic arsenic, could be a key carcinogenic species. Thus, pregnant CD1 mice were provided drinking water containing MMA3+ at 0 (control), 12.5, or 25 parts per million (ppm) from gestational days 8 to 18. Tumors were assessed in groups of male or female (initial $n = 25$) offspring up to 2 years of age. In utero treatment had no effect on survival or body weights. Female offspring exhibited increases in total epithelial uterine tumors (control 0%; 12.5 ppm 26%; 25 ppm 30%), oviduct hyperplasia (control 4%; 12.5 ppm 35%; 25 ppm 43%), adrenal cortical adenoma at 25 ppm (control 0%; 12.5 ppm 9%; 25 ppm 26%), and total epithelial ovarian tumors (control 0%; 12.5 ppm 39%; 25 ppm 26%). Male offspring showed dose-related increases in hepatocellular carcinoma (control 0%; 12.5 ppm 12%; 25 ppm 22%), adrenal adenoma (control 0%; 12.5 ppm 28%; 25 ppm 17%), and lung adenocarcinoma (control 17%; 12.5 ppm 44%). Male offspring had unusual testicular lesions, including two rete testis carcinomas, two adenomas, and three interstitial cell tumors. Overall, maternal consumption of MMA3+ during pregnancy in CD1 mice produced some similar proliferative lesions as gestationally applied inorganic arsenic in the offspring during adulthood.

Keywords Methylated arsenical · Carcinogenesis · Mice · Transplacental exposure

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Introduction

Inorganic arsenic is carcinogenic in humans and rodents (IARC 2004, 2011; NTP 2011; Tokar et al. 2010a, 2011). In humans, chronic exposure to inorganic arsenic is definitively linked to increased incidences of the cancers in lung, skin, and urinary bladder and may be associated with increased tumor incidences in liver, kidney, and prostate (IARC 2004, 2011). Because arsenic-contaminated drinking water is often the major source of inorganic arsenic in human populations, there is potential for both pre- and postnatal exposure to this carcinogen (IARC 2004, 2011; NTP 2011).

Recent epidemiological studies indicate that either fetal arsenic exposure via the mother or direct early-life exposure from ingestion of contaminated drinking water is associated with increased risk of liver, lung, and kidney cancer in later

life (Liaw et al. 2008; Smith et al. 2006; Yuan et al. 2010). Similarly, consumption of powdered milk heavily contaminated with inorganic arsenic during infancy has been linked to occurrence of skin, liver, pancreatic, and hematopoietic cancers nearly five decades after a brief period of exposure (Yorifuji et al. 2010, 2011). In mice, inorganic arsenic is an effective transplacental carcinogen. Maternal exposure to inorganic arsenic during pregnancy produces tumors in the liver, lung, ovary, uterus, and adrenal of adult offspring long after arsenic exposure has ended (Tokar et al. 2010a, 2011; Waalkes et al. 2007). Fetal arsenic exposure also predisposes mice to cancers induced or promoted by other agents in later life, like cancers of the skin (Tokar et al. 2010a, 2011; Waalkes et al. 2007, 2008). Thus, data in humans and rodents indicate that arsenic exposure during development initiates molecular events that can be stimulated much later during adulthood to become cancers.

Typically, inorganic arsenicals are enzymatically methylated in a series of reactions catalyzed by arsenic (3) methyltransferase (As3MT) that use S-adenosyl-methionine (SAM) as the methyl donor (Thomas et al. 2007; Thomas 2007). The intermediates and products produced during the formation of mono-, di-, and trimethylated metabolites of inorganic arsenic are thought to account for some of the adverse effects that have been associated with chronic exposure to inorganic arsenic. For example, dimethylarsinic acid (DMA5+) is a rodent urinary bladder carcinogen (Cohen et al. 2007; Tokar et al. 2010a), and the bladder is a well-defined human target site of arsenic (IARC 2004, 2011; NTP 2011). A monomethylated arsenical, monomethylarsonous acid (MMA3+), is one of the most toxic forms of arsenic biomethylation products and may be an ultimate carcinogen (Hirano et al. 2004; Kitchin 2001). However, there is no direct evidence for MMA3+ carcinogenicity in humans. Exposure to MMA3+ will cause DNA damage (Tennant and Kligerman 2011; Wnek et al. 2011) and malignant transformation of arsenic target tissue-relevant human cells in vitro, such as uroepithelial cells (Eblin et al. 2008). In one attempt to show MMA3+ carcinogenicity in rodents, adult K6/ODC transgenic mice, which are sensitive to chemically induced skin papilloma formation, were exposed to MMA3+ at doses up to 150 ppm in drinking water, but this treatment had no effect on skin tumor response (Chen et al. 2008).

The transplacental model for arsenic carcinogenesis is apparently more sensitive than adult exposure, which is consistent with this model being a sensitive time for carcinogenesis in general (Anderson et al. 2000; Waalkes et al. 2007). Thus, in the present study, we used MMA3+ in a mouse transplacental model system, which has consistently shown positive results with maternal inorganic arsenic during pregnancy and tumors in the offspring in adulthood (Tokar et al. 2010a, 2011; Waalkes et al. 2007).

Materials and methods

Animals and treatment

Methyloxoarsine synthesized by Dr. William R. Cullen, Department of Chemistry, University of British Columbia, was used. On dissolution in water, this produces methylarsonous acid (MMA3+). We evaluated the stability of MMA3+ in aqueous solution using the pH-selective method for hydride generation/cryotrapping/atomic absorption spectrometry (Devesa et al. 2006). Immediately after dissolution of methyloxoarsine in deionized water at a target arsenic concentration of 25 ppm, MMA3+ accounted for >99.6% of the methylarsenic present in these samples. After 48 h at room temperature, about 1.5% of the methylarsenic in these solutions was present as methylarsonic acid (MMA5+). Thus, MMA3+ was only slowly oxidized to MMA5+ at the maximum concentration of MMA3+ in drinking water used in the present study.

For this study, drinking water containing MMA3+ was made fresh and replaced every 2 days. The facility was provided with color-coded flasks containing quantities of the MMA3+-spiking solution that, upon addition of deionized water, yielded drinking water containing 12.5 or 25 ppm of arsenic as MMA3+. Pregnant mice were first exposed to MMA3+ on gestational day 8, and freshly prepared MMA3+-containing drinking water was provided on gestational days 10, 12, 14, and 16. Treatment lasted through day 18 of gestation. Controls received unaltered water.

CD1 mice were obtained from the Charles River Laboratory (Raleigh, NC). Pregnant CD1 mice were obtained by placing 2–3 females in one cage overnight with a male. A vaginal plug was considered to indicate pregnancy, which was confirmed by palpation on gestation day 8. Primigravid females were randomly divided into 3 groups of 10/treatment level and provided water ad libitum from days 8 to 18 of gestation. After birth, litters were culled to 8 or less. Mice were weaned at 4 weeks postpartum, offspring were randomly grouped ($n = 25$) according to maternal exposure, and the offspring were observed for a total of 104 weeks (including pre-weaning). Litters of origin were not followed in this study, and although litter effects could not be assessed, the use of 10 litters/group would have minimized any such impact (maximum 2–3 animals per litter going to any particular group). Animal care was provided in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources 1996). The animals were treated humanely and with regard for alleviation of suffering. Mice were housed under conditions of controlled temperature, humidity, and light cycle. A basal diet (5L79; Ralston Purina, St. Louis, MO) and acidified water were provided ad libitum.

Dam body weights were recorded between days 8 and 18 of gestation. Water consumption of the dams was recorded between gestation days 11 and 12 and between 15 and 16. Litter size was recorded on the day of birth and was not impacted by treatment. Neonatal weights were recorded at birth (time zero), then weekly until weaning, and every 5 weeks thereafter. Clinical signs were checked daily, and offspring mice were killed when significant clinical signs developed or at 104 weeks of age.

The NCI/Frederick animal facility, where the biopsy portion of the present study was conducted, and its animal program are accredited by the American Association for Accreditation of Laboratory Animal Care.

Tumor assessment

The animal portion of this study was designed and performed entirely within the National Cancer Institute, and it does not reflect the standard procedures, depth, or extent of a typical National Toxicology Program tumor endpoint study. A complete necropsy was performed on all moribund animals, animals found dead, or on mice at terminal killing. The ovaries/testes, oviduct, uterus, cervix, vagina, urinary bladder, kidneys, liver, lung, adrenal, spleen, gall bladder, thyroid, thymus, a sample of skin, and any grossly abnormal tissues were fixed in 10% neutral buffered formalin, paraffin-embedded, sectioned at 5 μm, and stained with hematoxylin and eosin. Pathological assessment was performed without knowledge of treatment group. For hepatic tumors, no hepatocellular carcinomas were detected that arose within a hepatocellular adenoma.

Data analysis

Data are expressed as incidence for tumors or proliferative lesions (number of mice with lesion/number of mice available for evaluation). Data are expressed as mean ± SEM for body weights and mean survival. Tumor incidence comes from 23 to 25 mice per treatment group originally derived from 25 mice per gender from groups made at random from 10 litters/treatment group (2–3 per group from each litter) at weaning. The decrease in some groups represents animals found dead and considered too autolytic for appropriate evaluation. In cases where more than one separate tumor occurred in a single tissue, it is counted as a case of the highest-grade tumor and the lower-grade tumor is noted as co-occurring in the tables. This would apply to lung and liver where separate adenomas and carcinomas in the same tissue were observed. Incidence data were compared by one-sided Fischer's exact test or two-sided chi-squared test for trend. Body weights, mean survival, and average tumor per mouse data were compared by two-sided Dunnett's multiple-comparison test after ANOVA.

Table 1 Survival and body weight in CD1 mouse born to mothers exposed to MMA3+ during pregnancy

Group	Mice remaining			Body weight	Average
	50[a]	75[a]	100[a]	Week 104 (*n*)	Survival[a]
Males					
Control (0 ppm)	25	20	7	54.7 ± 5.0 (7)	87.8 ± 2.9
12.5 ppm	25	18	10	52.6 ± 3.0 (9)	88.8 ± 3.3
25 ppm	22	18	7	51.7 ± 2.2 (6)	83.0 ± 4.3
Females					
Control (0 ppm)	23	21	8	45.0 ± 4.1 (7)	90.1 ± 3.9
12.5 ppm	24	21	5	44.6 ± 5.6 (5)	88.3 ± 3.6
25 ppm	24	16	3	47.4 ± 4.9 (3)	81.2 ± 3.2

See "Materials and methods" for treatment details. The maternal level of MMA3+ (in ppm) in the drinking water is given and was provided to the pregnant female between gestation days 8 and 18. Controls received 0 ppm MMA3+. The initial *n* in all offspring groups was 25. Body weight and survival data given as mean ± SEM of the indicated *n* or of *n* = 25 for average survival. There were no significant differences between groups in mice remaining, body weights at 104 weeks, or average survival

[a] Weeks of age of offspring

A $p < 0.05$ was considered significant in all cases. For trend tests, exact t values are given.

Results

Pregnant CD1 mice were given drinking water with 0 (control), 12.5, or 25 ppm MMA3+ from gestation days 8 to 18 and then allowed to give birth. At weaning, dose-appropriate groups of male and female offspring were formed and tumors were assessed in these offspring groups over a total of 104 weeks (see "Materials and methods"). Other than what would have been intentionally acquired during gestation, at no time were offspring treated with additional arsenic of any form.

The dosage levels of MMA3+ used did not affect survival of either male or female mice based on numbers of animals remaining at a given time point or average survival in weeks since birth (Table 1). Body weights of test animals were not altered by treatments during the study (week 104 shown as an example; Table 1). Maternal body weight, water consumption, neonatal birth weights, and litter size were similarly unaltered by the inclusion of MMA3+ in the drinking water of pregnant dams (not shown). Thus, dosage levels of MMA3+ in drinking water were well tolerated by the mothers and offspring.

In male mice, hepatocellular carcinoma incidence was significantly ($p < 0.05$) increased at the highest dose of MMA3+ (Table 2) and showed a significant dose-related

Table 2 Liver, adrenal, and lung tumors in male CD1 offspring born to mothers exposed to MMA3+ during pregnancy

Treatment group (*n*)	Liver			Adrenal	Lung		
	AD	CA	Total	AD	AD	CA	Total
Control (24)	2 (2)	0	2	0	6 (6)	4	10
12.5 ppm (25)	2 (2)	3	5	7*	3 (1)	11*	12
25 ppm (23)	4 (1)	5*	6	4*	2 (0)	9	9

See "Materials and methods" for treatment details. The maternal level of MMA3+ (in ppm) in the drinking MMA3+ water is given and was provided to the pregnant female between gestation days 8 and 18. *AD* Adenoma, *CA* carcinoma; *n* the number of mice available for analysis. Data are the number of mice with a given tumor. The number in parentheses under AD represents those mice that did not also have a higher-grade tumor (i.e., carcinoma). Total tumor represents number of cases of animals with single or multiple tumors of any grade. Liver tumors were hepatocellular. Lung carcinomas were adenocarcinomas. An asterisk (*) indicates a significant difference ($p < 0.05$) from control by one-sided Fisher's exact test

Table 3 Testicular tumors and proliferative lesions in male CD1 offspring born to mothers exposed to MMA3+ during pregnancy

Treatment group (*n*)	Interstitial cell		Rete testis			
	AD	HYP	AD	CA	Total	HYP
Control (24)	0	0	0	0	0	0
12.5 ppm (25)	3	1	1	0	1	5*
25 ppm (23)	0	1	1	2	3	1

See "Materials and methods" for treatment details. The maternal level of MMA3+ (in ppm) in the drinking water is given and was provided to the pregnant female between gestation days 8 and 18. *AD* Adenoma, *CA* carcinoma, *HYP* hyperplasia; *n* the number of mice available for analysis. Data are the number of mice with a given tumor or hyperplastic lesion. No animals that had AD also had a higher grade tumor (i.e., carcinoma). Total tumors represent number of cases of animals with single or multiple tumors of any grade. Hyperplasias were only counted if they occurred in the absence of tumor. The asterisk (*) indicates a significant difference ($p < 0.05$) from control by one-sided Fisher's exact test

trend across all doses ($p = 0.018$). Liver adenoma and total liver tumor incidence were not significantly increased at any dose and showed no dose-related trends. Adrenal cortical adenoma incidence (control 0%; 12.5 ppm 28%; 25 ppm 17%) significantly increased ($p < 0.05$) at both MMA3 + doses, but this response was not dose related. Lung carcinoma incidence increased in males at the low dose, but adenomas and total lung tumors were not increased. No classification of lung tumors was altered in the high-dose group and no dose–response relationship was found for lung tumors.

Unusual testicular lesions in mice exposed to MMA3+ in utero (Table 3) are noteworthy because of their relative rarity in CD1 mice (Newbold et al. 2000). For instance, there were three interstitial cell adenomas in the 12.5-ppm MMA3+ group (not significant) and one interstitial hyperplasia in both the low-dose and high-dose groups compared with no similar lesions in control mice. Various rete testis proliferative lesions also occurred in MMA3+-treated male mice, including four tumors (two carcinomas and two adenomas) and six cases of hyperplasias in the two MMA3+-treated groups compared to no rete testis tumors or hyperplasias in control mice. Rete testis hyperplasias were concentrated in the low-dose group (5/6 total cases) and were significantly ($p < 0.05$) increased in this group compared to control. Most (75%) of the rete testis tumors occurred in the high-dose MMA3+ group, potentially indicating a shift in stage of lesion, as hyperplasia incidence appeared to decrease in this group.

The female offspring of mothers consuming drinking water with MMA3+ during gestation showed various epithelial lesions in the reproductive tract (Table 4). Uterine adenomas increased ($p < 0.05$) at the higher MMA3+ dose, and total tumors were increased at both MMA3+ doses. Uterine adenomas ($p = 0.006$) and total epithelial uterine tumors ($p = 0.008$) increased in a clear dose-related fashion. Uterine carcinomas were also not uncommon (5 cases/13 total tumors in MMA3+ females), while no uterine tumors of any grade occurred in control animals. Ovarian adenomas (primarily cystadenoma) and total epithelial ovarian tumors significantly ($p < 0.05$) increased at both doses, although not in a dose-related fashion. A few ovarian carcinomas occurred (3/15 total tumors) with MMA3+ treatment, while no ovarian tumors occurred in control animals. Oviduct hyperplasias were significantly ($p < 0.05$) increased in a dose-related fashion to a maximum of 43% compared to 4% in control mice. Two oviduct adenomas occurred in mice exposed to MMA3+, one at each level of exposure.

Maternal consumption of MMA3+ during gestation had no effect on liver or lung tumors in female offspring (Table 5). In female offspring, adrenal cortical adenomas were significantly ($p < 0.05$) increased at the highest maternal MMA3+ dose and showed a significant ($p = 0.006$) dose–response relationship.

Incidental tumors not related to treatment in male or female offspring of mothers consuming MMA3+ during pregnancy are shown in Table 6. Lymphomas were common in all groups, particularly in females. Various mesenchymal uterine tumors occurred only in MMA3+ groups, including primarily leiomyomas, although leiomyomas did not occur to a level of statistical significance in any MMA3+ treatment group. If all mesenchymal uterine tumors were pooled (i.e., leiomyoma, leiomyosarcoma, and stromal cell sarcoma), a total of 6 occurred out of 23 mice in the 12.5-ppm dosage group, which was statistically significant from control (0/23). Pooling leiomyoma and leiomyosarcoma in the 12.5-ppm group (4/23) was not significantly different from control. Pooling mesenchymal

Table 4 Tumors and proliferative lesions of the reproductive tract in female CD1 offspring born to mothers exposed to MMA3+ during pregnancy

Treatment group (*n*)	Uterus				Ovary			Oviduct	
	AD	CA	Total[a]	HYP	AD	CA	Total[a]	HYP	AD
Control (23)	0	0	0	2	0	0	0	1	0
12.5 ppm (23)	2	4	6*	4	7*	2	9*	8*	1
25 ppm (23)	6*	1	7*	6	5*	1	6*	10*	1

See "Materials and methods" for treatment details. The maternal level of MMA3+ (in ppm) in the drinking water is given and was provided to the pregnant female between gestation days 8 and 18. *AD* Adenoma, *CA* carcinoma, *HYP* hyperplasia; *n* the number of mice available for analysis. Data are the number of mice with a given tumor or hyperplastic lesion. Only epithelial tumors are included. No animals had both adenoma and carcinoma in either uterus or ovary. Total tumors represent number of cases of tumors of any grade. Hyperplasias were only counted if they occurred in the absence of tumor. An asterisk (*) indicates a significant difference ($p < 0.05$) from control by one-sided Fisher's exact test

[a] Total epithelial tumors

Table 5 Liver, adrenal, and lung tumors in female CD1 offspring born to mothers exposed to MMA3+ during pregnancy

Treatment group	*n*	Liver			Adrenal	Lung		
		AD	CA	Total	AD	AD	CA	Total
Control	24	1	0	1	0	9	0	9
12.5 ppm	25	0	0	0	2	5 (4)	1	5
25 ppm	23	2	0	2	6*	4 (2)	3	5

See "Materials and methods" for treatment details. The maternal level of MMA3+(in ppm) in the drinking water is given and was provided to the pregnant female between gestation days 8 and 18. Data given as number of mice with the given tumors. *AD* Adenoma, *CA* carcinoma; *n* the number of mice available for analysis. Data are the number of mice with a given tumor. The number in parentheses under lung AD represents those mice that did not also have a higher-grade tumor (i.e., carcinoma). Total tumors represent number of cases of animals with single or multiple tumors of any grade. Hyperplasias were only counted if they occurred in the absence of tumor. The asterisk (*) indicates a significant difference ($p < 0.05$) from control by one-sided Fisher's exact test

uterine tumors in the 25-ppm dosage group did not lead to a statistically significant occurrence. Three renal adenomas occurred in MMA3+-treated males (1 in the 12.5-ppm group and 2 in the 25-ppm group) but not to statistical excess.

Discussion

Because MMA3+ is one of the most toxic products of inorganic arsenic biomethylation and may be an important carcinogenic species (Hirano et al. 2004; Kitchin 2001), we postulated that treatment with MMA3+ during sensitive life stages, such as the in utero life period (Anderson et al. 2000; Waalkes et al. 2007), might result in elevated rates of tumor formation later in life. Therefore, this study investigated the effects of maternal consumption of MMA3+ in the drinking water during gestation (from gestation days 8 to 18) on tumor formation in the offspring during adulthood. The design of this study mirrored that used in our prior transplacental studies that evaluated the effects of maternal exposure during pregnancy to oral inorganic arsenic, specifically sodium arsenite (Tokar et al. 2010a, 2011; Waalkes et al. 2007). Inorganic arsenic is the precursor for As3mt-catalyzed formation of MMA3+ (Thomas et al. 2007; Thomas 2007), and maternal oral intake of inorganic arsenic results in accumulation of mono- and dimethylated arsenicals in the mouse fetus (Devesa et al. 2006). Direct comparisons of the potency of inorganic arsenic and MMA3+ as transplacental carcinogens are difficult because the concentrations of arsenicals in maternal and fetal tissues were not determined. Placental transfer of inorganic and methylated arsenicals has been reported in rodents (Hood et al. 1987; Jin et al. 2006; Xi et al. 2010), although underlying dosimetric relationships are unclear. Similarly, the ontogeny of arsenic methylation in fetal tissue has not been well characterized. Additional work will be needed to elucidate the developmental factors that control the uptake and fate of inorganic arsenic and its methylated metabolites in the fetus.

In males, the incidence of hepatocellular carcinoma was significantly increased, and these malignancies occurred in a strong MMA3+ dose-related fashion to a maximum of 22%. Hepatocellular carcinomas occurred in the absence of an increase in total liver tumors or increased liver adenomas, which might tend to diminish confidence in the standalone finding of stimulated liver carcinoma formation. However, earlier work assessing liver tumors in adult male CD1 mice born to mothers consuming inorganic arsenic (85 ppm) via the drinking water during gestation found that the hepatocellular carcinoma response, although significant compared to control rate (0%), is actually less (14%) than the maximal hepatocellular carcinoma response in the present study. Nevertheless, both liver adenomas and total hepatocellular liver tumors increased in adult male CD1 offspring of mothers exposed to inorganic arsenic during pregnancy (Waalkes et al. 2006a). In addition, in two transplacental exposure studies using inorganic arsenic in the drinking water of pregnant C3H mice, male offspring mice also showed increased hepatocellular carcinomas, again with increases in total hepatocellular tumors (Waalkes et al. 2003, 2004a). Thus, the finding of dose-related hepatocellular carcinomas in the present work is consistent with a repeated finding of the same malignancy in male offspring mice of multiple strains, including the one used in the present study after maternal oral consumption of inorganic

Table 6 Incidental tumors unrelated to treatment in male or female CD1 offspring born to mothers exposed to MMA3+ during pregnancy

Treatment group	*n*	
Males		
Control	24	2 lymphomas, 2 liver hemangiosarcomas, 1 liver hemangioma
12.5 ppm	25	2 renal adenomas, 2 liver hemangiosarcomas, 1 liver hemangioma, 1 lymphoma, 1 leukemia, 1 epididymal histiocytic sarcoma
25 ppm	23	3 lymphomas, 2 renal adenomas, 2 liver hemangiosarcomas, 1 seminal vesicle adenocarcinoma, 1 gall bladder adenoma, 1 kidney lipoma, 1 subcutaneous fibrosarcoma, 1 epididymal histiocytic sarcoma
Females		
Control	23	7 lymphomas, 1 mammary adenocarcinoma
12.5 ppm	23	7 lymphomas, 3 uterine leiomyomas, 2 uterine stromal cell sarcomas, 2 mammary adenocarcinomas, 1 leiomyosarcoma
25 ppm	23	6 lymphomas, 2 uterine leiomyomas, 1 cervical hemangioma

The maternal level of MMA3+ (in ppm) in the drinking water is given and was provided to the pregnant female between gestation days 8 and 18. Controls received 0 ppm. Tumors were assessed in offspring over 2 years. *n* the number of mice available for analysis

arsenic during gestation (Waalkes et al. 2003, 2004a, 2006a). Female offspring did not show increased liver tumor response after maternal MMA3+ exposure in the present study but are typically less sensitive to hepatocarcinogens, including transplacental inorganic arsenic (Tokar et al. 2011; Waalkes et al. 2007).

In the present study, adrenal cortical adenomas were induced by maternal consumption of MMA3+ in both adult male and female offspring. In female offspring, induction of adrenal cortical adenomas was clearly related to MMA3+ dose; in male offspring, the response peaked at the lower dose for unknown reasons. In earlier studies, maternal consumption of inorganic arsenic during pregnancy very consistently induced adrenal cortical adenomas in the offspring of CD1, C3H, and Tg.AC mice (Tokar et al. 2010a; Waalkes et al. 2003, 2004a, 2006a, b). In fact, in Tg.AC mice, adrenal cortical adenomas occurred as early as 40 weeks after birth (Tokar et al. 2010a). Thus, maternal MMA3+ or inorganic arsenic consumption in the drinking water during pregnancy consistently induced benign adrenal tumors in the adult offspring.

Maternal consumption of MMA3+ induced tumors and proliferative lesions in the reproductive tract of adult female offspring, including the uterus, ovary, and oviduct. After maternal gestational MMA3+ exposure, uterine adenomas and dose-related total epithelial uterine tumors occurred in adult offspring along with ovarian adenomas and total epithelial ovarian tumors. There was some evidence of MMA3+ being associated with uterine mesenchymal tumors, but this was not dose-related. Oviduct hyperplasias were fairly common in MMA3+-treated mice and occurred in a dose-related fashion. Uterine and ovarian tumors or hyperplasias, and oviduct hyperplasias have been repeatedly observed in adult female offspring after maternal inorganic arsenic consumption during gestation in mice (Waalkes et al. 2003, 2004a, 2006b) including in CD1 mice (Waalkes et al. 2006b). Thus, maternal exposure to the parent compound (inorganic arsenic) leads to similar proliferative lesions in the reproductive tract of the female offspring of mothers consuming MMA3+ during pregnancy in CD1.

Increased lung adenocarcinomas occurred in male mice born to mothers consuming the low dose of MMA3 + during pregnancy in the absence of an increase in adenomas or total tumors. No similar response was seen in female offspring. Another study using maternal inorganic arsenic consumption (85 ppm in the drinking water) during pregnancy found induction of lung adenocarcinomas in adult male CD1 mouse offspring (Waalkes et al. 2006a). Lung lesions apparently initiated by prenatal maternal exposure to inorganic arsenic can be promoted to tumors by other agents given during adulthood in C3H mice (Waalkes et al. 2004a). However, the significance of the lung response in the present study must be tempered by the absence of a dose response or the finding of an effect on total tumors of the lung.

Testicular tumors that occurred in adult male offspring born to mothers consuming MMA3+ during pregnancy are noteworthy because they are relatively rare (Newbold et al. 2000). Proliferative lesions of the rete testis including two adenomas and two carcinomas in MMA3+ groups (4 tumors/48 treated mice; 8.3%) along with six cases of rete testis hyperplasias (12.5%) occurred in these mice. Rete testis tumors are considered rare in CD1 mice, while rete testis hyperplasia occurs at a spontaneous rate of approximately 6% (Newbold et al. 2000), which would have been one case in our control group (compared to an actual incidence of zero). In our earlier work with male CD1 mice born to mothers consuming inorganic arsenic during pregnancy, we did not observe any comparable rete testis lesions. Male offspring born to mothers consuming

MMA3+ during pregnancy also showed testicular interstitial cell adenomas (12% in the 12.5 MMA3+ group) and two cases of testicular interstitial cell hyperplasias (one in each treatment group). Spontaneous testicular interstitial cell adenomas occur at a low rate in CD1 mice (approximately 2%; Newbold et al. 2000). It is of interest that perinatal exposure of CD1 mice to diethylstilbestrol can also induce rete testis or interstitial cell proliferative lesions including tumors (Newbold et al. 1985, 1987). In this regard, diethylstilbestrol is also able to promote lesions initiated by prenatal exposure to inorganic arsenic into tumors in various tissues, including several sites within the female reproductive tract (Waalkes et al. 2006b) and in the male liver (Waalkes et al. 2006a) although this was not observed in the testes. Prenatal inorganic arsenic exposure activates estrogen receptors in various tissues (Shen et al. 2007; Waalkes et al. 2004b, 2006a, b); however, the role of estrogen receptor activation in the pathogenesis of cancer resulting from early-life inorganic arsenic or MMA3+ exposure is not fully understood.

This study was not designed to define the levels or form of arsenical that crossed the placenta or, perhaps more importantly, reached the target tissues in the fetus. In fact, the amount of a maternal dose of MMA3+ that will reach the fetus in this study is not known. Exposure of pregnant rodents to inorganic arsenic will result in the accumulation of monomethylated arsenicals in the fetus (Devesa et al. 2006), some of which has been attributed to the placental transfer of methylated compound, the valence of which is not clear (Jin et al. 2006; Xi et al. 2010). But there is the distinct possibility that MMA3+ could be modified in the maternal or fetal systems. However, how much MMA3+ actually reaches critical target cells in the fetus is an open question, which is a key data gap in actually assigning efficacy that is not available at this point. It is indeed possible that the active carcinogenic compound could be a monomethylated arsenical, a dimethylated derivative, or, perhaps, an inorganic arsenical generated by reverse reactions, and the role of arsenic methylation in carcinogenesis cannot be defined by this current study. Further study is required to define the role of methylated species in arsenic carcinogenesis.

Overall, maternal consumption of drinking water containing MMA3+ during pregnancy in CD1 mice was associated with liver hepatocellular carcinoma (males), uterine adenoma, ovarian adenoma, and adrenal adenoma (both sexes) in adult offspring in the present work. Many of these lesions have been detected in prior work with transplacental exposure to inorganic arsenic (Tokar et al. 2010a, b; Waalkes et al. 2003, 2004a, 2006a, b), which could be considered the parent compound to MMA3+. Maternal MMA3+ consumption during pregnancy was also associated with relatively rare proliferative lesions of the rete testis, including tumors.

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