2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO ARSENIC IN THE UNITED STATES

Arsenic is widely distributed in the Earth's crust, which contains ~3.4 ppm arsenic. In nature, arsenic is mostly found in minerals and only to a small extent in its elemental form. Arsenic is mainly obtained as a byproduct of the smelting of copper, lead, cobalt, and gold ores. Arsenic trioxide is the primary form in which arsenic is marketed and consumed. There has been no domestic production of arsenic since 1985. In 2003, the world's largest producer of arsenic compounds was China, followed by Chile and Peru.

In 2003, the United States was the world's largest consumer of arsenic. Production of wood preservatives, primarily copper chromated arsenate (CCA), CrO_3•CuO•As_2O_5, accounted for >90% of domestic consumption of arsenic trioxide. In response to consumer concerns, U.S. manufacturers of arsenical wood preservative began a voluntary transition from CCA to other wood preservatives for certain residential wood products. This phase-out was completed on December 31, 2003; wood treated prior to this date could still be used and CCA-treated wood products continue to be used in industrial applications.

Other uses for arsenic compounds include the production of agricultural chemicals, as an alloying element in ammunition and solders, as an anti-friction additive to metals used for bearings, and to strengthen lead-acid storage battery grids. High-purity arsenic (99.9999%) is used by the electronics industry for gallium-arsenide semiconductors for telecommunications, solar cells, and space research. Various organic arsenicals are still used in the United States as herbicides and as antimicrobial additives for animal and poultry feed. However, the use of inorganic arsenic compounds in agriculture has virtually disappeared beginning around the 1960s. Arsenic trioxide and arsenic acid were used as a decolorizer and fining agent in the production of bottle glass and other glassware. Arsenic compounds also have a long history of use in medicine, and have shown a re-emergence of late with the recent introduction of arsenic trioxide treatment for acute promyelocytic leukemia.

The principal route of exposure to arsenic for the general population is likely to be the oral route, primarily in the food and in the drinking water. Dietary exposures to total arsenic were highly variable, with a mean of 50.6 μg/day (range of 1.01–1,081 μg/day) for females and 58.5 μg/day (range of 0.21–1,276 μg/day) for males. U.S. dietary intake of inorganic arsenic has been estimated to range from 1 to 20 μg/day, with grains and produce expected to be significant contributors to dietary inorganic arsenic

intake. Drinking water generally contains an average of 2 μg/L of arsenic, although 12% of water supplies from surface water sources in the North Central region of the country and 12% of supplies from groundwater sources in the Western region have levels exceeding 20 μg/L. Arsenic is also widely distributed in surface water, groundwater, and finished drinking water in the United States. Surveys of arsenic concentrations in rivers and lakes indicate that most values are below 10 μg/L, although individual samples may range up to 3,400 μg/L. Arsenic released to the land at hazardous waste sites is likely to be relatively immobile due to a high capacity for soil binding, particularly to iron and manganese oxides. Exposure to arsenic from other pathways is generally small, but may be significant for areas with high levels of arsenic contamination or in occupational settings. For a more complete discussion of possible exposures to arsenic, see Chapter 6 of the profile.

2.2 SUMMARY OF HEALTH EFFECTS

Arsenic is a potent toxicant that may exist in several oxidation states and in a number of inorganic and organic forms. Most cases of arsenic-induced toxicity in humans are due to exposure to inorganic arsenic, and there is an extensive database on the human health effects of the common arsenic oxides and oxyacids. Although there may be some differences in the potency of different chemical forms (e.g., arsenites tend to be somewhat more toxic than arsenates), these differences are usually minor. An exception would be arsine, which is highly toxic. However, because arsine and its methyl derivatives are gases or volatile liquids and are unlikely to be present at levels of concern at hazardous waste sites, health effect data for these compounds are not discussed in this document. Humans may be exposed to organic arsenicals (mainly methyl and phenyl derivatives of arsenic acid) that are used in agriculture and to organic arsenicals found in fish and shellfish (arsenobetaine and arsenocholine). Although the toxicity of organic arsenicals has not been as extensively investigated as inorganic arsenicals, there are sufficient animal data to evaluate the toxicity of methyl arsenates (e.g., monomethylarsonic acid [MMA] and dimethylarsinic acid [DMA]) and roxarsone. The so-called “fish arsenic” compounds (e.g., arsenobetaine) are not thought to be toxic and health effects data are not discussed in this document.

It is generally accepted that the arsenic-carbon bond is quite strong and most mammalian species do not have the capacity to break this bond; thus, inorganic arsenic is not formed during the metabolism of organic arsenicals. In most species, including humans, ingested (or exogenous) MMA(V) and DMA(V) undergo limited metabolism, do not readily enter the cell, and are primarily excreted unchanged in the urine. This is in contrast to inorganic arsenic, which undergoes sequential reduction and methylation reactions leading to the formation of MMA and DMA. Inorganic As(V) is readily reduced to inorganic

As(III), which is taken up by the cell. Within the cell (primarily in the liver), As(III) is methylated to form MMA(V), which is reduced to MMA(III); MMA(III) subsequently undergoes oxidative methylations to form DMA(V). DMA(V) is the primary excretion product in humans. Because inorganic and organic arsenicals exhibit distinct toxicokinetic characteristics, the health effects and MRLs are considered separately.

Inorganic Arsenicals. Exposures of humans near hazardous waste sites could involve inhalation of arsenic dusts in air, ingestion of arsenic in water, food, or soil, or dermal contact with contaminated soil or water. Increased risk of lung cancer, respiratory irritation, nausea, skin effects, and neurological effects have been reported following inhalation exposure. There are only a few quantitative data on noncancer effects in humans exposed to inorganic arsenic by the inhalation route. Animal data similarly identify effects on the respiratory system as the primary noncancer effect of inhaled inorganic arsenic compounds, although only a few studies are available. Only limited data on the effects of inhaled organic arsenic compounds in humans or animals are available; these studies are generally limited to high-dose, short-term exposures, which result in frank effects.

Relatively little information is available on effects due to direct dermal contact with inorganic arsenicals, but several studies indicate that the chief effect is local irritation and dermatitis, with little risk of other adverse effects.

The database for the oral toxicity of inorganic arsenic is extensive, containing a large number of studies of orally-exposed human populations. These studies have identified effects on virtually every organ or tissue evaluated, although some end points appear to be more sensitive than others. The available data from humans identify the skin as the most sensitive noncancer target following long-term oral arsenic exposure. Typical dermal effects include hyperkeratinization of the skin (especially on the palms and soles), formation of multiple hyperkeratinized corns or warts, and hyperpigmentation of the skin with interspersed spots of hypopigmentation. Oral exposure data from studies in humans indicate that these lesions typically begin to manifest at exposure levels of about 0.002–0.02 mg As/kg/day, but one study suggests that lesions may appear at even lower levels. At these exposure levels, peripheral vascular effects are also commonly noted, including cyanosis, gangrene, and, in Taiwanese populations, the condition known as “Blackfoot Disease.” Other reported cardiovascular effects of oral exposure to inorganic arsenic include increased incidences of high blood pressure and circulatory problems. The use of intravenous arsenic trioxide as therapy for acute promyelocytic leukemia has raised further concerns about the cardiovascular effects of arsenic, including alterations in cardiac QT interval and the

development of torsades de pointes. Decrements in lung function, assessed by spirometry, have been reported in subjects exposed to approximately 0.008–0.04 mg As/kg/day in the drinking water who exhibited skin lesions.

In addition to dermal, cardiovascular, and respiratory effects, oral exposure to inorganic arsenic may result in effects on other organ systems. Nausea, vomiting, and diarrhea are very common symptoms in humans following oral exposure to inorganic arsenicals, both after acute high-dose exposure and after repeated exposure to lower doses; these effects are likely due to a direct irritation of the gastrointestinal mucosa. Acute, high-dose exposure can lead to encephalopathy, with clinical signs such as confusion, hallucinations, impaired memory, and emotional lability, while long-term exposure to lower levels can lead to the development of peripheral neuropathy characterized by a numbness in the hands and feet that may progress to a painful "pins and needles" sensation. Recent studies also have reported neurobehavioral alterations in arsenic-exposed children.

Chronic exposure of humans to inorganic arsenic in the drinking water has been associated with excess incidence of miscarriages, stillbirths, preterm births, and infants with low birth weights. Animal data suggest that arsenic may cause changes to reproductive organs of both sexes, including decreased organ weight and increased inflammation of reproductive tissues, although these changes may be secondary effects. However, these changes do not result in a significant impact on reproductive ability. Animal studies of oral inorganic arsenic exposure have reported developmental effects, but generally only at concentrations that also resulted in maternal toxicity.

Arsenic is a known human carcinogen by both the inhalation and oral exposure routes. By the inhalation route, the primary tumor types are respiratory system cancers, although a few reports have noted increased incidence of tumors at other sites, including the liver, skin, and digestive tract. In humans exposed chronically by the oral route, skin tumors are the most common type of cancer. In addition to skin cancer, there are a number of case reports and epidemiological studies that indicate that ingestion of arsenic also increases the risk of internal tumors (mainly of bladder and lung, and to a lesser extent, liver, kidney, and prostate).

The Department of Health and Human Services (DHHS) has concluded that inorganic arsenic is known to be a human carcinogen. The International Agency for Research on Cancer (IARC) cites sufficient evidence of a relationship between exposure to arsenic and human cancer. The IARC classification of arsenic is Group 1. The EPA has determined that inorganic arsenic is a human carcinogen by the

inhalation and oral routes, and has assigned it the cancer classification, Group A. EPA has calculated an oral cancer slope factor of 1.5 $(mg/kg/day)^{-1}$ and a drinking water unit risk of $5x10^{-5}$ $(\mu g/L)^{-1}$ for inorganic arsenic based on human dose-response data. The inhalation unit risk for cancer is calculated to be 0.0043 $(\mu g/m^3)^{-1}$. The unit risk is the upper-bound excess lifetime cancer risk estimated to result from continuous exposure to an agent at a concentration of 1 µg/L in water or 1 $\mu g/m^3$ in air. EPA is currently revising the assessment for inorganic arsenic; a more detailed discussion of the uncertainties associated with human cancer risk levels for arsenic is presented in Section 3.2.2.7.

The following sections discuss significant effects resulting from exposure to inorganic arsenic in greater detail: dermal, cardiovascular, respiratory, gastrointestinal, neurological, and cancer. Additional information on these effects and on other effects is discussed in Section 3.2.

Dermal Effects. The most characteristic effect of long-term oral exposure to inorganic arsenic compounds is the development of skin lesions; these lesions are often used as diagnostic criteria for arsenicosis. The three lesions most often associated with chronic arsenicosis are hyperkeratinization of the skin (especially on the palms and soles), formation of multiple hyperkeratinized corns or warts, and hyperpigmentation of the skin with interspersed spots of hypopigmentation. Numerous studies of long-term, low-level exposure to inorganic arsenic in humans have reported the presence of these lesions. In general, they begin to manifest at chronic exposure levels >0.02 mg As/kg/day. Chronic oral studies of lower exposure levels, ranging from 0.0004 to 0.01 mg As/kg/day, have generally not reported dermal effects. However, in a study with detailed exposure assessment, all confirmed cases of skin lesions ingested water containing >100 µg/L arsenic (approximately 0.0037 mg As/kg/day) and the lowest known peak arsenic concentration ingested by a case was 0.115 µg/L (approximately 0.0043 mg As/kg/day). Another large study reported increased incidence of skin lesions associated with estimated doses of 0.0012 mg As/kg/day (0.023 mg As/L drinking water). The mechanism(s) by which inorganic arsenic causes dermal effects is not well-understood. Elucidating the mechanism of dermal effects has been particularly difficult because the dermal effects common in humans have not been seen in studies in animals.

Dermal effects have also been reported following inhalation exposures to inorganic arsenic, although they are not as diagnostic as for oral exposure. Several studies of arsenic-exposed workers have reported the development of dermatitis; exposure levels required to produce this condition are not well-established. Altered dermal pigmentation and hyperkeratosis have also been reported in studies of humans exposed to inorganic arsenic by inhalation, although exposure levels have varied considerably. Direct dermal contact

with inorganic arsenicals may cause irritation and contact dermatitis. Usually, the effects are mild (erythema and swelling), but may progress to papules, vesicles, or necrotic lesions in extreme cases; these conditions tend to heal without treatment if exposure ceases.

Cardiovascular Effects. A large number of studies in humans have reported cardiovascular effects following oral exposure to inorganic arsenic compounds. The cardiac effects of arsenic exposure are numerous, and include altered myocardial depolarization (prolonged QT interval, nonspecific ST segment changes), cardiac arrhythmias, and ischemic heart disease. These effects have been seen after acute and long-term exposure to inorganic arsenic in the environment, as well as side effects from intravenous therapy with arsenic trioxide for acute promyelocytic leukemia. Exposure levels for environmental exposures have not been well characterized, but intravenous doses for arsenic trioxide therapy are generally on the order of 0.15 mg As/kg/day.

Chronic exposure to inorganic arsenic has also been shown to lead to effects on the vascular system. The most dramatic of these effects is "Blackfoot Disease," a disease characterized by a progressive loss of circulation in the hands and feet, leading ultimately to necrosis and gangrene. Blackfoot Disease is endemic in an area of Taiwan where average drinking water levels of arsenic range from 0.17 to 0.80 ppm, corresponding to doses of about 0.014–0.065 mg As/kg/day. The results of a another study suggested that individuals with a lower capacity to methylate inorganic arsenic to DMA have a higher risk of developing peripheral vascular disease in the Blackfoot Disease-hyperendemic area in Taiwan. Arsenic exposure in Taiwan has also been associated with an increased incidence of cerebrovascular and microvascular diseases and ischemic heart disease. While Blackfoot Disease itself has not been reported outside of Taiwan, other vascular effects are common in areas with high arsenic exposures, and include such severe effects as increases in the incidences of Raynaud's disease and of cyanosis of fingers and toes as well as hypertension, thickening and vascular occlusion of blood vessels, and other unspecified cardiovascular conditions. However, while the majority of human studies have reported cardiovascular effects following exposure to inorganic arsenic, some have found no such effects.

Changes in cardiac rhythm and in some vascular end points have also been reported in animal studies of inorganic arsenicals, but generally only at higher exposure levels and not to the degree of severity seen in humans.

Respiratory Effects. While case reports and small cohort studies have routinely reported an increase in respiratory symptoms of humans exposed occupationally to inorganic arsenic, dose-response data for

these symptoms are generally lacking. The only study that evaluated respiratory effects (changes in chest x-ray or respiratory performance) and reported an exposure estimate did not report significant changes at an exposure level of 0.613 mg As/m^3. Exposed workers often report irritation of the mucous membranes of the nose and throat, which may lead to laryngitis, bronchitis, or rhinitis. Increased mortality due to respiratory disease has been reported in some cohort mortality studies of arsenic-exposed workers, but no conclusive evidence of an association of these diseases with arsenic exposure has been presented. It is not known whether respiratory effects following inhaled inorganic arsenic compounds are due to a direct effect of arsenic on respiratory tissues, general effects of foreign material in the lungs, or an effect of arsenic on the pulmonary vasculature. Similar responses, including rales, labored breathing, and respiratory hyperplasia, have been noted in animal studies of inhaled or instilled inorganic arsenic compounds.

Respiratory effects have also been reported following oral exposure of humans to inorganic arsenic. Acute oral exposure to ≥8 mg As/kg may result in serious respiratory effects, including respiratory distress, hemorrhagic bronchitis, and pulmonary edema; however, it is not clear whether these are primary effects or are the result of damage to the pulmonary vascular system. In general, respiratory effects have not been widely associated with long-term oral exposure to low arsenic doses. However, some studies have reported minor respiratory symptoms, such as cough, sputum, rhinorrhea, and sore throat, in people with repeated oral exposure to 0.03–0.05 mg As/kg/day. More serious respiratory effects, such as bronchitis and sequelae (bronchiectasis, bronchopneumonia) have been observed in patients chronically exposed to arsenic and at autopsy in some chronic poisoning cases. There are few animal data reporting respiratory effects of oral exposure to inorganic arsenic, and those studies generally found effects only at very high dose levels.

Gastrointestinal Effects. Both short-term and chronic oral exposures to inorganic arsenicals have been reported to result in irritant effects on gastrointestinal tissues. Numerous studies of acute, high-dose exposure to inorganic arsenicals have reported nausea, vomiting, diarrhea, and abdominal pain, although specific dose levels associated with the onset of these symptoms have not been identified. Chronic oral exposure to 0.01 mg As/kg/day generally results in similar reported symptoms. For both acute and chronic exposures, the gastrointestinal effects generally diminish or resolve with cessation of exposure. Similar gastrointestinal effects have been reported after occupational exposures to inorganic arsenicals, although it is not known if these effects were due to absorption of arsenic from the respiratory tract or from mucociliary clearance resulting in eventual oral exposure.

Neurological Effects. A common effect following both oral and inhalation exposure to inorganic is the development of peripheral neuropathy. Following occupational exposure to inorganic arsenic in pesticide plants or smelters, exposed workers have shown increased incidence of neurological changes, including altered nerve conduction velocities. One study reported that these effects were seen after 28 years of exposure to 0.31 mg As/m^3. In another study, signs and symptoms of sensory and motor polyneuropathy on both upper and lower extremities were reported in workers at a power station in Slovakia. The average length of exposure was 22.3 years (standard deviation [SD] ±8.4 years) and the average arsenic exposure in inhaled air ranged from 4.6 to 142.7 μg/m^3.

Following high-dose (>2 mg As/kg/day) acute oral exposures to inorganic arsenicals in humans, reported effects include headache, lethargy, mental confusion, hallucination, seizures, and coma. Following longer-term exposure to 0.03–0.1 mg As/kg/day, peripheral neuropathy, characterized initially by numbness of the hands and feet and a "pins and needles" sensation and progressing to muscle weakness, wrist-drop and/or ankle-drop, diminished sensitivity, and altered reflex action. Histological features of the neuropathy include a dying-back axonopathy and demyelination. Following removal from exposure, the neuropathy is only partially reversible and what recovery does occur is generally slow. Reports of neurological effects at lower arsenic levels (0.004–0.006 mg As/kg/day) have been inconsistent, with some human studies reporting fatigue, headache, depression, dizziness, insomnia, nightmare, and numbness while others reported no neurological effects. Some studies also have reported that exposure to arsenic may be associated with intellectual deficits in children. Neurological effects have also been reported in oral studies of arsenic toxicity in animals, although these were generally performed at higher doses (0.4–26.6 mg As/kg/day) than has been reported in exposed human populations. The mechanism(s) of arsenic-induced neurological changes has not been determined.

Cancer. There is clear evidence from studies in humans that exposure to inorganic arsenic by either the inhalation or oral routes increases the risk of cancer. Numerous studies of copper smelters or miners exposed to arsenic trioxide have reported an increased risk of lung cancer. Increased incidence of lung cancer has also been observed at chemical plants where exposure was primarily to arsenate. Other studies suggest that residents living near smelters or arsenical chemical plants may have increased risk of lung cancer, although the reported increases are small and are not clearly detectable in all cases. In general, studies reporting long-term exposure to 0.07 mg As/m^3 or greater have shown an increased incidence of lung cancer, while at lower exposure levels, the association has been less clear or not present.

There is convincing evidence from a large number of epidemiological studies and case reports that ingestion of inorganic arsenic increases the risk of developing skin cancer. The most common tumors seen are squamous cell carcinomas, which may develop from the hyperkeratotic warts or corns commonly seen as a dermal effect of oral inorganic arsenic exposure. Early studies of populations within the United States did not suggest an increased risk of cancer from oral inorganic arsenic exposure. Later studies have found suggestive evidence that the possibility of arsenic-induced skin cancers cannot be discounted based on an association between toenail arsenic levels and incidence of skin cancer.

There is increasing evidence that long-term exposure to arsenic can result in the development of bladder cancer, with transitional cell cancers being the most prevalent. While studies have noted statistical dose-response trends in arsenic-induced bladder cancers, reliable quantitative assessments of dose-response relationships have not been presented. Several studies have also shown that chronic oral exposure to arsenic results in the development of respiratory tumors, making lung cancer an established cause of death from exposure to arsenic in drinking water. Exposure levels in studies evaluating respiratory and bladder cancers have been comparable to those in studies evaluating skin tumors. Studies of U.S. populations have not identified an increased risk of bladder or respiratory tumors following oral exposure to inorganic arsenic.

Animal studies of both inhalation and oral exposure to inorganic arsenicals have not resulted in increased incidence of cancer formation in adult animals. However, a series of studies have shown that inorganic arsenic can induce cancer in the offspring from mice exposed to arsenic during gestation (transplacental carcinogen) and acts as a co-carcinogen with UV light and polycyclic aromatic hydrocarbons (PAHs).

Organic Arsenicals. Humans may be exposed to organic arsenicals via inhalation of dusts, ingestion of organic arsenic in water, food, soil, or dermal contact with contaminated soil, water or plants following pesticide application. There are limited data on the toxicity of organic arsenicals following inhalation exposure in humans and animals and these data do not allow for identification of critical effects. Keratosis was observed in workers exposed to 0.065 mg/m^3 arsanilic acid (i.e., 4-aminophenyl arsenic acid); no alterations in gastrointestinal symptoms or hematological alterations were observed. In animals, very high concentrations (>3,000 mg/m^3) of DMA results in respiratory distress, diarrhea, and erythematous lesions on the feet and ears. No adverse effects were observed in rats exposed to DMA concentrations as high as 100 mg DMA/m^3 for 95 days.

Similarly, the available dermal toxicity data do not allow for identification of critical effects. Contact dermatitis was observed in workers applying DMA (and its sodium salt) and mild dermal irritation was observed in a Draize test in rabbits (adverse effect level not reported). Intermediate duration (21 days) exposure studies in rabbits did not result in systemic toxicity or skin irritation following 5 day/week exposure to 1,000 mg/kg/day MMA or DMA.

The preponderance of toxicity data for organic arsenicals involves oral exposure. Human data are limited to three case reports of individuals intentionally ingesting pesticides containing organic arsenicals. Gastrointestinal irritation (vomiting, nausea, and diarrhea) were consistently reported in these cases. Animal data has primarily focused on the toxicity of MMA, DMA, and roxarsone; these data suggest that the targets of toxicity may differ between the compounds.

MMA. The gastrointestinal tract appears to be the most sensitive target of toxicity for MMA. Diarrhea and tissue damage in the large intestine have been reported in several animal species following dietary, gavage, and capsule exposure. For diarrhea, both the time of onset and incidence appear to be dose-related. In rats, diarrhea was observed in 100% of females exposed to 98.5 mg MMA/kg/day, 55% of females exposed to 33.9 mg MMA/kg/day, and 5.1% of females exposed to 3.9 mg MMA/kg/day. The increased incidence of diarrhea was observed after 3 weeks of exposure to 98.5 mg MMA/kg/day, 4 weeks at 33.9 mg MMA/kg/day, and 18 months at 3.9 mg MMA/kg/day. Histological damage consisting of squamous metaplasia of the epithelial columnar absorptive cells in the cecum, colon, and rectum was observed in rats and mice chronically exposed to 72.4 or 67.1 mg MMA/kg/day, respectively. Hemorrhagic, necrotic, ulcerated, or perforated mucosa were also observed in the large intestine of rats exposed to 67.1 mg MMA/kg/day for 2 years. In rats, the damage to the large intestine resulted in intestinal contents leaking into the abdominal cavity and the development of peritonitis. The available data provide suggestive evidence that there may be some species differences in the sensitivity to gastrointestinal damage; however, some of these differences may be due to the route of administration. The lowest adverse effect levels, regardless of duration of exposure, for gastrointestinal effects in rats, mice, rabbits, and dogs are 25.7, 67.1, 12, and 2 mg MMA/kg/day, respectively; the no adverse effect levels in rats and mice (NOAELs were not identified in rabbits and dogs) were 3.0 and 24.9 mg MMA/kg/day. However, the rabbit and dog studies involved bolus administration (gavage and capsule administration), which may have increased sensitivity; the rat and mouse studies involved dietary exposure.

The kidney also appears to be a sensitive target in rats and mice chronically exposed to MMA. An increase in the severity of progressive glomerulonephropathy was observed in female rats exposed to 33.9 mg MMA/kg/day for 2 years and an increase in the incidence of progressive glomerulonephropathy was observed in male mice exposed to 6.0 mg MMA/kg/day for 2 years. Other adverse effects that have been observed in animals exposed to MMA include hypertrophy of the thyroid follicular cells in rats exposed to 33.9 mg MMA/kg/day in the diet for 2 years, reproductive toxicity, and developmental toxicity. Decreases in pregnancy rate and male fertility index were observed in F_0 and F_1 rats exposed to 76 mg MMA/kg/day for 14 weeks prior to mating and during the mating period; the findings were not significantly different than control values but were considered treatment-related because they were outside the range found in historical controls. This study also reported a decrease in pup survival in the F_1 and F_2 offspring of rats exposed to 76 mg MMA/kg/day; as with the reproductive effects, the incidence was not statistically different from controls but was considered biologically significant because survival in the MMA pups was outside the range found in historical controls. Another study reported impaired fetal growth (decreases in fetal weights and incomplete ossification) and minor skeletal defects (an increase in the number of fetuses with supernumerary thoracic ribs and eight lumbar vertebrae) in rat and rabbit fetuses exposed to 500 or 12mg MMA/kg/day, respectively; maternal toxicity was also observed at these dose levels and the effects may be secondary to maternal stress rather than a direct effect on the developing organisms. A 2-year bioassay did not result in significant increases in the incidence of neoplastic lesions in rats and mice exposed to doses as high as 72.4 and 67.1 mg MMA/kg/day, respectively.

DMA. The most sensitive targets of DMA toxicity in rats are the urinary bladder and kidneys. In the bladder, the effects progress from cytotoxicity to cellular necrosis to regenerative proliferation and hyperplasia. At dietary doses of 11 mg DMA/kg/day, cytotoxicity is observed as early as 6 hours after exposure initiation and cellular proliferation (as evident by increased BrdU labeling) was observed after 2 weeks of exposure. After 10 weeks of exposure, necrosis and hyperplasia were also observed. The lowest adverse effect levels for urinary bladder effects following intermediate or chronic duration exposure were 5 mg DMA/kg/day for evidence of regenerative proliferation and 3.1 mg DMA/kg/day for vacuolar degeneration of urothelium and hyperplasia. Vacuolization of the superficial cells of the urothelium was observed in mice exposed to 7.8 mg DMA/kg/day and higher for 2 years. However, unlike the vacuolar degeneration observed in rats, the vacuolization observed in mice was not associated with cytotoxicity, necrosis, inflammation, or hyperplasia.

Kidney damage characterized by increased urinary calcium levels, calcification, nephrocalcinosis, and necrosis of the renal papillae have been observed in rats following intermediate- or chronic-duration exposure. Increases in urine calcium levels and corticomedullary junction calcification were observed in rats exposed to 5 or 10 mg DMA/kg/day for 10 weeks and cortical degeneration and necrosis were observed in rats exposed to 57 mg DMA/kg/day for 4 weeks. Chronic-duration exposure to 3.1 mg DMA/kg/day resulted in an increased incidence of nephrocalcinosis and necrosis of the renal papillae in rats; these lesions are typical in aged rats, although DMA exposure appeared to exacerbate them. An exacerbation of age-related kidney lesion (progressive glomerulonephropathy and nephrocalcinosis) has also been observed in male mice exposed to 37 or 94 mg DMA/kg/day, respectively, for 2 years. A consistent finding in intermediate and chronic rat studies is an increase in urine volume, which corresponds to an increase in water consumption; the toxicological significance of this finding is not known. The observed decreases in electrolyte levels and specific gravity are likely due to the higher urine volume.

Although gastrointestinal effects have been observed in animals exposed to DMA, it does not appear to be as sensitive a target compared to MMA. Diarrhea has been observed in rats exposed to a lethal dose of 190 mg DMA/kg/day for 4 weeks and in dogs administered via 16 mg DMA/kg/day. No gastrointestinal effects were observed in rats or mice chronically exposed to 7.8 or 94 mg DMA/kg/day.

Other adverse effects that have been observed in animals exposed to organic arsenicals include hypertrophy of thyroid follicular cells in rats exposed to 4.0 mg DMA/kg/day in the diet for 13 weeks and 7.8 mg DMA/kg/day in the diet for 2 years and developmental effects in rats and mice. Decreases in fetal growth and delays in ossification have been observed in rat fetuses exposed to ≥36 mg DMA/kg/day; these alterations typically occur at doses associated with decreases in maternal weight gain. Other developmental effects that have been reported include an increase in the incidences of irregular palatine rugae in rats exposed to 30 mg DMA/kg/day, diaphragmatic hernia in rats exposed to 36 mg DMA/kg/day, and cleft palate in mice exposed to 400 mg DMA/kg/day. No developmental effects were observed in rabbits exposed to 12 mg DMA/kg/day.

The available data provide strong evidence that DMA is carcinogenic in rats. A 2-year exposure to DMA resulted in significant increases in the incidence of neoplastic urinary bladder tumors in rats exposed to 7.8 mg DMA/kg/day in the diet or 3.4 mg DMA/kg/day in drinking water. No increases in neoplastic tumors were observed in mice exposed to doses as high as 94 mg DMA/kg/day for 2 years; however, a

50-week exposure to 10.4 mg DMA/kg/day did result in an increased incidence of lung tumors in A/J mice.

The available data for DMA suggest that there are species differences in terms of the critical effects and sensitivity. In rats, the urinary bladder and kidneys are the most sensitive targets with effects occurring at 5 mg DMA/kg/day following intermediate-duration exposure and 3.1 mg DMA/kg/day following chronic-duration exposure. Although the urinary bladder and kidneys are also sensitive targets in mice with LOAELs of 7.8 and 37 mg DMA/kg/day, respectively, following chronic exposure, the effects are not associated with cytotoxicity or elevated urine calcium levels. In dogs, the most sensitive effect is gastrointestinal tract irritation (diarrhea), which occurs at 16 mg DMA/kg/day.

There is concern that the rat may not be a good model to predict the human risk associated with organic arsenic exposure due to the unique toxicokinetic properties of DMA in rats. In humans and most animal species, DMA is rapidly eliminated from the body; >90% of the dose is excreted 2–3 days after dosing. In contrast, DMA is slowly eliminated in rats. One study estimated that 45% of an initial oral DMA dose was eliminated with a half-time of 13 hours; the remaining 55% of the dose DMA dose had an elimination half-time of 50 days. In rats, DMA has a strong affinity for hemoglobin resulting in an accumulation of DMA in erythrocytes. Species differences in DMA metabolism have also been found. In particular, DMA undergoes further methylation to trimethylarsine oxide (TMAO) in rats. In most animal species, almost the entire oral DMA dose is excreted in the urine unchanged; however, in rats, about half of the dose is excreted in the urine as DMA and the other half as TMAO. During the metabolism of DMA to TMAO, DMA(III) is formed as a metabolic intermediate. The formation of this highly reactive intermediate and the excretion of small amounts of DMA(III) in urine may damage the urinary bladder.

There are limited data on the mode of action of DMA for most end points. Recently, there has been considerable research on the mode of action for the development of neoplastic urinary bladder tumors in rats. Although the mechanisms have not been fully elucidated, it has been proposed that the mode of action involves cytotoxicity leading to necrosis and subsequent regeneration of the urinary bladder urothelium. There is strong evidence to suggest that DMA(III) is the causative agent for the urothelial cytotoxicity. The strongest evidence comes from the finding that urinary concentrations of DMA(III) measured in rats exhibiting urothelial cytotoxicity are equivalent to DMA(III) concentrations that are cytotoxic to urothelial cells *in vitro*. Urothelial cytotoxicity, regenerative urothelial proliferation, and urothelial tumors have not been detected in other animal species. Other animal species, including

humans, only metabolize a small percentage of ingested DMA to TMAO; thus, much lower levels of DMA(III) are produced, suggesting that rats may be very sensitive to toxicity of DMA and therefore are not an appropriate model for human risk assessment.

Roxarsone. The available data on the toxicity of roxarsone suggest that following bolus administration, the gastrointestinal tract, kidney, and nervous system are sensitive end points of roxarsone toxicity. Vomiting and gastrointestinal hemorrhage were observed in dogs receiving a single capsulized dose of 50 mg/kg roxarone; no gastrointestinal effects were observed in rats or mice administered 4 or 42 mg/kg/day roxarsone for 2 years. Kidney effects included increases in kidney weight, minimal tubular epithelial cell degeneration, and focal mineralization in rats exposed to 32 mg roxarsone/kg/day for 13 weeks; no kidney effects were observed at 16 mg/kg/day or in mice exposed to doses as high as 136 mg/kg/day for 13 weeks or 43 mg/kg/day for 2 years. Hyperexcitability, ataxia, and/or trembling were observed in rats exposed to 20 mg/kg/day for 13 weeks or 64 mg/kg/day for 13 weeks. A 14-day study in rats reported slight inactivity in rats exposed to 32 mg/kg/day, but this was not observed in longer-term studies. Neurological effects were observed in mice exposed to doses as high as 136 mg/kg/day for 13 weeks or 43 mg/kg/day for 2 years, although a slight decrease in activity at 42 mg/kg/day was reported in a 14 day study. Pigs appear to be especially sensitive to the neurotoxicity of roxarsone. Muscle tremors have been observed at doses of ≥6.3 mg roxarsone/kg/day and myelin degeneration in the spinal cord was noted at 6.3 mg/kg/day. Both the clinical signs of neuropathy and the myelin degeneration followed a time-related pattern. Mild lethargy and ataxia were observed 7 days after exposure initiation, exercise-induced muscle tremors and clonic seizures were observed at day 11, paraparesis was observed at day 22, and paraplegia was observed at day 33. At day 11, equivocal lesions were observed in the cervical spinal cord, and the severity of these lesions increased with time; myelin degeneration was observed in the peripheral nerves and optic nerve starting at day 32 (2 days after exposure termination). Equivocal evidence of carcinogenicity (a slight increase in the incidence of pancreatic tumors) was found in male rats chronically exposed to roxarsone; no increases in neoplastic tumors were observed in female rats or male and female mice.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for arsenic. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive

health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990i), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inorganic Arsenicals

Inhalation MRLs. No inhalation MRLs were derived for inorganic arsenic. Human data suggest that dermal or respiratory effects may be the most prevalent (Lagerkvist et al. 1986; Mohamed 1998; Perry et al. 1948); respiratory or immunological effects appeared to be the most common following inhalation exposure to inorganic arsenic in animals (Aranyi et al. 1985; Holson et al. 1999). Adequate human studies evaluating dose-response relationships for noncancer end points were not located for inorganic arsenic, and animal data on the health effects of inorganic arsenic following inhalation exposure are limited to studies that did not evaluate a suitable range of health effects. Lacking suitable studies upon which to base the MRLs, no inhalation MRLs were derived for inorganic arsenic.

Oral MRLs

- An MRL of 0.005 mg As/kg/day has been derived for acute-duration (14 days or less) oral exposure to inorganic arsenic.

Mizuta et al. (1956) summarized findings from 220 poisoning cases associated with an episode of arsenic contamination of soy sauce in Japan. The soy sauce was contaminated with approximately 0.1 mg As/mL, probably as calcium arsenate. Arsenic intake in the cases was estimated by the researchers to be 3 mg/day (0.05 mg/kg/day, assuming 55 kg average body weight for this Asian population). The duration of exposure was 2–3 weeks in most cases. The primary symptoms were edema of the face, and gastrointestinal and upper respiratory symptoms initially, followed by skin lesions and neuropathy in

some patients. Other effects included mild anemia and leukopenia, mild degenerative liver lesions and hepatic dysfunction, abnormal electrocardiogram, and ocular lesions. For derivation of the acute oral MRL, facial edema and gastrointestinal symptoms (nausea, vomiting, diarrhea), which were characteristic of the initial poisoning and then subsided, were considered to be the critical effects. The MRL of 0.005 mg As/kg/day was calculated by applying an uncertainty factor of 10 (10 for use of a lowest-observed-adverse-effect level (LOAEL) and 1 for human variability) to the LOAEL of 0.05 mg As/kg/day (see Appendix A for MRL worksheets).

An intermediate-duration oral MRL for inorganic arsenic was not derived due to inadequacy of the database. The lowest LOAEL identified in a limited number of intermediate-duration human studies available was 0.05 mg As/kg/day in a study by Mizuta et al. (1956) (summarized above). While this study was considered appropriate to derive an acute-duration oral MRL for inorganic arsenic, there is considerable uncertainty regarding what the effects and severity might be beyond the relatively short 2–3 weeks of exposure that most subjects experienced. There are numerous studies in animals dosed for intermediate durations, but as indicated in Section 3.5.3, animals are not appropriate models for effects of inorganic arsenic in humans.

- An MRL of 0.0003 mg As/kg/day has been derived for chronic-duration (365 days or more) oral exposure to inorganic arsenic.

Tseng et al. (1968) and Tseng (1977) investigated the incidence of Blackfoot Disease and dermal lesions (hyperkeratosis and hyperpigmentation) in a large number of poor farmers (both male and female) exposed to high levels of arsenic in well water in Taiwan. A control group consisting of 17,000 people, including one group in which arsenic exposure was “undetermined” and which included those villages where arsenic-contaminated wells were no longer used or the level could not be classified, and a control population of 7,500 people who consumed water from wells almost free of arsenic (0.001–0.017 ppm) was also examined. The authors stated that the incidence of dermal lesions increased with dose, but individual doses were not provided. However, incidence data were provided based on stratification of the exposed population into low (<300 µg/L), medium (300–600 µg/L), or high (>600 µg/L) exposure levels. Doses were calculated from group mean arsenic concentrations in well water, assuming the intake parameters described by IRIS (IRIS 2007). Accordingly, the control, low-, medium-, and high-exposure levels correspond to doses of 0.0008, 0.014, 0.038, and 0.065 mg As/kg/day, respectively. The no-observed-adverse-effect level (NOAEL) identified by Tseng (1977) (0.0008 mg As/kg/day) was limited by the fact that the majority of the population was <20 years of age and the incidence of skin lesions increased as a function of age, and because the estimates of water intake and dietary arsenic intake are

highly uncertain. Schoof et al. (1998) estimated that dietary intakes of arsenic from rice and yams may have been 15–211 µg/day (mean=61 µg/day), based on arsenic analyses of foods collected in Taiwan in 1993–1995. Use of the 50 µg/day estimate would result in an approximate doubling of the NOAEL (0.0016 mg/kg/day) (see Appendix A for MRL worksheets). The MRL was derived by applying an uncertainty factor of 3 (for human variability) to the NOAEL of 0.0008 mg/kg/day.

The MRL is supported by a large number of well-conducted epidemiological studies that identify reliable NOAELs and LOAELs for dermal effects. EPA (1981b) identified a NOAEL of 0.006–0.007 mg As/kg/day for dermal lesions in several small populations in Utah. Harrington et al. (1978) identified a NOAEL of 0.003 mg As/kg/day for dermal effects in a small population in Alaska. Guha Mazumder et al. (1988) identified a NOAEL of 0.009 mg As/kg/day and a LOAEL of 0.006 mg As/kg/day for pigmentation changes and hyperkeratosis in a small population in India. Haque et al. (2003) identified a LOAEL of 0.002 mg As/kg/day for hyperpigmentation and hyperkeratosis in a case-control study in India. Cebrián et al. (1983) identified a NOAEL of 0.0004 mg As/kg/day and a LOAEL of 0.022 mg As/kg/day in two regions in Mexico. Borgoño and Greiber (1972) and Zaldívar (1974) identified a LOAEL of 0.02 mg As/kg/day for abnormal skin pigmentation in patients in Chile, and Borgoño et al. (1980) identified a LOAEL of 0.01 mg As/kg/day for the same effect in school children in Chile. Valentine et al. (1985) reported a NOAEL of 0.02 mg As/kg/day for dermal effects in several small populations in California. Collectively, these studies indicate that the threshold dose for hyper-pigmentation and hyperkeratosis is approximately 0.002 mg As/kg/day. While many of these studies also identified effects on other end points at these exposure levels, including effects on gastrointestinal (Borgoño and Greiber 1972; Cebrián et al. 1983; Guha Mazumder et al. 1988; Zaldívar 1974), cardiovascular (Tseng et al. 1995, 1996), hepatic (Hernández-Zavala et al. 1998), and neurological end points (Guha Mazumder et al. 1988; Lianfang and Jianzhong 1994; Tsai et al. 2003), the overall database for dermal effects is considerably stronger than for effects on other end points.

Organic Arsenicals

Inhalation MRLs. No inhalation MRLs were derived for organic arsenic. Human data are limited to an occupational exposure study of workers exposed to 0.065 mg/m^3 ansanilic acid (Watrous and McCaughey 1945). The exposed workers more frequently complained of keratosis than nonexposed workers. A limited number of animal studies have examined the toxicity of organic arsenicals following inhalation exposure. Respiratory distress and diarrhea were observed in rats and mice exposed to high concentrations of MMA and DMA (Stevens et al. 1979); at lower concentrations (1,540–3,150 mg DMA/m^3), respiratory irritation, as evidenced by a decrease in respiration rate, was observed in animals

exposed to MMA or DMA (Stevens et al. 1979). The acute-duration studies do not clearly identify the most sensitive targets of inorganic arsenical toxicity; the available studies are of limited scope and none included a comprehensive histological examination.

One study examined the toxicity of DMA in rats following intermediate-duration exposure. This study (Whitman 1994) found an increase in intracytoplasmic eosinophilic globules in the nasal turbinates of rats exposed to 34 or 100 mg/m^3 DMA 6 hours/day, 5 days/week for 67–68 exposures; no other adverse effects were observed in this comprehensive study. As discussed in greater detail in the oral MRL section, the toxicokinetic properties of DMA in rats differ from other species and rats do not appear to be a good model for human exposure. The half-time of DMA in the body is much longer in rats compared to other species, including humans, and DMA is more extensively methylated in rats. In the absence of data to determine whether the observed effect is due to a direct interaction of DMA, derivation of an intermediate-duration MRL using rat data is not recommended at this time.

No studies examined the chronic toxicity of organic arsenicals precluding the derivation of a chronic-duration inhalation MRL.

Oral MRLs

MMA. A limited number of animal studies have examined the acute oral toxicity of MMA. These studies consisted of LD_{50} studies in rats (Gur and Nyska 1990), mice (Kaise et al. 1989), and rabbits (Jaghabir et al. 1988) and developmental toxicity studies in rats (Irvine et al. 2006) and rabbits (Irvine et al. 2006); all studies administered MMA via gavage. Adverse effects reported in the LD_{50} studies included diarrhea in rats at 2,030 mg monosodium methane arsonate (MSMA)/kg (Gur and Nyska 1990), mice at 2,200 mg MMA/kg (Kaise et al. 1989) and rabbits at 60 mg MSMA/kg (Jaghabir et al. 1988) and respiratory arrest in mice at 1,800 mg MMA/kg/day (Kaise et al. 1989). These doses were at or near the LD_{50} levels of 2,449 mg MSMA/kg, 1,800 mg MMA/kg, 100 mg MSMA/kg for the rats, mice, and rabbits, respectively. In the developmental toxicity studies (Irvine et al. 2006), maternal effects included decreases in maternal body weight gain in rats (17% less than controls) and rabbits (70% less than controls) receiving gavage doses of 100 and 12 mg MMA/kg/day, respectively, and loose feces/diarrhea in rabbit does administered 12 mg MMA/kg/day. The NOAELs for maternal effects were 10 and 7 mg MMA/kg/day in the rats and rabbits, respectively. Minor developmental effects (decreased fetal weight, incomplete ossification, and supernumerary ribs) were also observed at the maternally toxic doses in the rats and rabbits (Irvine et al. 2006); these effects were probably secondary to the maternal stress. These

data, coupled with the results of longer-term studies (Arnold et al. 2003; Waner and Nyska 1988), suggest that the gastrointestinal tract is a sensitive target of MMA toxicity. The rabbit developmental toxicity study (Irvine et al. 2006) identified the lowest LOAEL (12 mg MMA/kg/day) for gastrointestinal irritation. However, this study is not suitable for the derivation of an acute-duration oral MRL for MMA because the MMA was administered via bolus doses. It is likely that the observed gastrointestinal effect is a concentration-dependent effect; thus, at a given dose level, effects are more likely to occur following bolus administration. A marked decrease in body weight gain was also observed at this dose level.

- An MRL of 0.1 mg MMA/kg/day has been derived for intermediate-duration (15–364 days) oral exposure to MMA.

Three studies have examined the intermediate-duration toxicity of MMA; two of these are chronic-duration studies reporting diarrhea and decreases in body weight gain after MMA exposure for <1 year. Diarrhea was observed in rats exposed to 30.2 mg MMA/kg/day in the diet (Arnold et al. 2003) and in dogs exposed via a capsule to 2 mg MMA/kg/day (Waner and Nyska 1988). Decreases in body weight were observed at the next highest doses, 106.9 mg MMA/kg/day in rats and 8 mg MMA/kg/day in dogs. In the rat study (Arnold et al. 2003), diarrhea was observed in 16.7 and 40% of the males and females, respectively, exposed to 30.2/35.9 mg MMA/kg/day during the first 52 weeks of the study; diarrhea first occurred after 4 weeks of exposure. At the highest dose level (106.9 mg MMA/kg/day), diarrhea was observed in all exposed male and female rats. In dogs, the increased incidence of diarrhea first occurred during weeks 25–28; at the highest dose tested in the study (35 mg MMA/kg/day), vomiting was also observed. A NOAEL of 3.5 mg MMA/kg/day was identified in the rat study; a NOAEL was not identified in the dog study. The remaining study in the intermediate-duration database is a 2-generation study that reported reproductive (decreased pregnancy rate and male fertility index in F_0 and F_1 generations) and developmental (decreased pup survival in F_1 and F_2 generation) effects in rats exposed to 76 mg MMA/kg/day in the diet (Schroeder 1994). The lowest LOAEL identified in the intermediate-duration database is 2 mg MMA/kg/day for diarrhea in dogs (Waner and Nyska 1988). Although dogs appear to be more sensitive to the gastrointestinal effects of MMA, a direct comparison of the two studies is not possible due to the difference in the routes of exposure. It is possible that the bolus administration of MMA, in the form of a capsule, resulted in increased sensitivity of the dogs. Because the most likely route of exposure for humans would be ingestion and the critical effect appears to be irritation of the gastrointestinal tract, studies involving bolus administration (gavage or capsule) were not considered for derivation of oral MRLs. The Arnold et al. (2003) and Schroeder (1994) studies were considered as the basis for an intermediate-duration MRL. Of these two studies, Arnold et al. (2003) identified the lowest

LOAEL, 30.2 mg MMA/kg/day, for gastrointestinal effects and was selected as the principal study for the intermediate-duration oral MRL.

Arnold et al. (2003) exposed groups of 60 male and 60 female Fischer 344 rats to 0, 50, 400, or 1,300 ppm MMA in the diet for 104 weeks. Using the average doses for weeks 1–50 reported in an unpublished version of this study (Crown et al. 1990), doses of 0, 3.5, 30.2, and 106.9 mg MMA/kg/day and 0, 4.2, 35.9, and 123.3 mg MMA/kg/day were calculated for males and females, respectively. Body weights, food consumption, and water intake were monitored regularly. Blood was taken at 3, 6, and 12 months for clinical chemistry measurements, and urine samples were collected at the same interval. Mortality was increased in high-dose males and females during the first 52 weeks of the study. Body weights were decreased in the mid- and high-dose groups of both sexes; however, at 51 weeks, only the body weight for the high-dose males was <10% of the control weight (14.5%). Food and water consumption was increased in the mid- and high-dose groups. Diarrhea was observed in 100% of the high-dose males and females and in 16.7 and 40% of the mid-dose males and females during the first 52 weeks of exposure. Diarrhea first occurred after 3 weeks of exposure to the high dose and 4 weeks of exposure to the mid-dose group; the severity of the diarrhea was dose-related. The gastrointestinal system was the primary target in animals dying early; numerous macroscopic and histological alterations were observed.

A benchmark dose (BMD) analysis of the incidence data for diarrhea was conducted; details of this analysis are presented in Appendix A. Using the female incidence data, a BMD (BMD_{10}) of 16.17 mg MMA/kg/day, which corresponds to a 10% increase in the incidence of diarrhea, was calculated; the 95% lower confidence limit on the BMD ($BMDL_{10}$) was 12.38 mg MMA/kg/day. The female incidence data were selected over the male data because the females may be more sensitive than the males. Thus, the intermediate-duration oral MRL of 0.1 mg MMA/kg/day is based on the $BMDL_{10}$ of 12.38 mg MMA/kg/day in female rats and an uncertainty factor of 100 (10 to account for animal to human extrapolation and 10 for human variability).

- An MRL of 0.01 mg MMA/kg/day has been derived for chronic-duration (365 days or longer) oral exposure to MMA.

The available data on the chronic toxicity of MMA in animals (no human data are available) suggest that the gastrointestinal tract and the kidney are the most sensitive targets. Diarrhea has been observed in rats and mice exposed to MMA in the diet for 2 years (Arnold et al. 2003). The NOAEL and LOAEL values for diarrhea are 3.0 and 25.7 mg MMA/kg/day in rats, respectively, and 24.9 and 67.1 mg MMA/kg/day

in mice, respectively. At 72.4 mg MMA/kg/day, necrotic, ulcerated, or perforated mucosa and metaplasia were observed in the cecum, colon, and rectum of rats. Squamous metaplasia was also observed in the cecum, colon, and rectum of mice exposed to 67.1 mg MMA/kg/day. Diarrhea was observed in dogs exposed via capsule to 2 mg MMA/kg/day for 52 weeks (Waner and Nyska 1988). The bolus administration used in the dog study probably increased the dog's sensitivity to MMA. In both the rats and mice, chronic administration of MMA resulted in an exacerbation of chronic progressive nephropathy. In female rats, significant increases in the severity of chronic progressive nephropathy were observed at 33.9 and 98.5 mg MMA/kg/day; the NOAEL was 3.9 mg MMA/kg/day (Arnold et al. 2003). In male mice, there was an increased incidence of slight progressive nephropathy at doses ≥6.0 mg MMA/kg/day; the NOAEL was 1.2 mg MMA/kg/day (Arnold et al. 2003; incidence data reported in Gur et al. 1991). Nephrocalcinosis was also observed in male mice exposed to ≥24.9 mg MMA/kg/day (Arnold et al. 2003). Other effects that have been observed following chronic exposure MMA include decreased weight gain in male and female rats exposed to 25.7/33.9 mg MMA/kg/day and higher (Arnold et al. 2003) and hypertrophy of the thyroid follicular epithelium in female rats exposed to ≥33.9 mg MMA/kg/day (Arnold et al. 2003). A variety of other lesions including peritonitis, pancreatitis, inflammation of the ureter, uterus, prostate, testes, epididymis, and seminal vesicles, hydronephrosis, pyelonephritis, and cortical tubular cystic dilation were also observed in rats; however, these alterations were probably secondary to the ulceration and perforation of the large intestine, which resulted in leaking of gastrointestinal contents into the abdominal cavity. Hyperplasia of the urinary bladder was also observed in rats exposed to 2.1 mg MMA/kg/day as MMA in drinking water for 2 years (Shen et al. 2003). Although hyperplasia of the urinary bladder is commonly observed in rats exposed to DMA, it was not observed in the Arnold et al. (2003) study at doses as high as 72.4 mg MMA/kg/day; thus, the significance of the results of the Shen et al. (2003) study is not known.

The lowest reliable LOAEL identified in the chronic oral MMA database was 6.0 mg MMA/kg/day for an increased incidence of progressive glomerulonephropathy in mice (Arnold et al. 2003). Although the investigators noted that the kidney lesions were consistent with the normal spectrum of spontaneous renal lesions and that there was no difference in character or severity of lesions between groups, ATSDR considers the dose-related increase in glomerulonephropathy to be treatment-related.

In the Arnold et al. (2003) study (incidence data reported in Gur et al. 1991), groups of 52 male and 52 female $B6C3F_1$ mice were exposed to 0, 10, 50, 200, or 400 ppm of MMA in the diet for 104 weeks. The average doses reported in Gur et al. (1991) were 0, 1.2, 6.0, 24.9, and 67.1 mg MMA/kg/day for males and 0, 1.4, 7.0, 31.2, and 101 mg MMA/kg/day for females. Body weights, food consumption, and

water intake were monitored regularly. Blood was taken at 3, 6, 12, 18, and 24 months for white cell counts. At sacrifice, complete necropsies were performed, including histological examination of at least 13 organs. No treatment-related increases in mortality were observed. Significant decreases in body weights were observed in males and females exposed to 67.1 or 101 mg MMA/kg/day, respectively; at week 104, the males and females weighed 17 and 23%, respectively, less than controls. Food consumption was increased in females exposed to 101 mg MMA/kg/day, and water consumption was increased in 67.1 mg MMA/kg/day males and 31.2 and 101 mg MMA/kg/day females. Loose and mucoid feces were noted in mice exposed to 67.1/101 mg MMA/kg/day. No changes were seen in white cell counts of either sex. Small decreases in the weights of heart, spleen, kidney, and liver weights were observed in some animals, but the decreases were not statistically significant. Squamous metaplasia of the cecum, colon, and rectum was observed at 67.1/101 mg MMA/kg/day. The incidence of metaplasia in the cecum, colon, and rectum were 29/49, 14/49, and 39/49 in males and 38/52, 17/52, and 42/52 in females; metaplasia was not observed in other groups of male or female mice. An increased incidence of progressive glomerulonephropathy (incidence of 25/52, 27/52, 38/52, 39/52, and 46/52 in the 0, 1.2, 6.0, 24.9, and 67.1 mg MMA/kg/day males, respectively) was observed in males; the incidence was significantly higher (Fisher Exact Test) than controls at ≥6.0 mg MMA/kg/day. Significant increases in the incidence of nephrocalcinosis was observed in the males at 24.9 and 67.1 mg MMA/kg/day (Fisher Exact Test) (incidences of 25/52, 30/52, 30/52, 45/52, and 45/51 in males and 0/52, 1/52, 1/52, 2/52, and 5/52 in females). A reduction in the incidence of cortical focal hyperplasia in the adrenal gland of male mice exposed to 67.1 mg MMA/kg/day was possibly related to MMA exposure; the toxicological significance of this effect is not known. Thus, this study identifies a NOAEL of 1.2 mg MMA/kg/day and a LOAEL of 6.0 mg MMA/kg/day for progressive glomerulonephropathy in male mice.

As described in greater detail in Appendix A, BMD was applied to the incidence data for progressive glomerulonephropathy in male mice using all available dichotomous models in EPA's Benchmark Dose Software (version 1.4.1) to calculate predicted doses associated with a 10% extra risk. As assessed by the Akaike's Information Criteria (AIC), the log-logistic model provided the best fit to the data. The predicted BMD_{10} and $BMDL_{10}$ are 2.09 and 1.09 mg MMA/kg/day. The $BMDL_{10}$ was selected as the point of departure and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive a chronic-duration oral MRL of 0.01 mg MMA/kg/day.

DMA. As discussed in greater detail in Section 2.2, urinary bladder effects characterized by cytotoxicity and regenerative proliferation and hyperplase have been observed in rats, but not in other species. The LOAELs for these effects are lower than the LOAELs for sensitive effects in other species. Additionally,

rats have a much greater capacity than other species to metabolize ingested DMA to form DMA(III) (a reactive intermediate) and TMAO (Cohen et al. 2006; Marafante et al. 1987b; Yoshida et al. 1998). It is likely that DMA(III) is the causative agent for the urothelial cytoxicity observed in rats (Cohen et al. 2006). Thus, rats were not considered a suitable model for humans and these data were not considered for derivation of MRLs for DMA.

There are limited data to assess the acute toxicity of DMA in species other than rats. Diarrhea, increased startle reflex, and ataxia were observed in mice exposed to a lethal gavage dose of 1,757 mg DMA/kg (Kaise et al. 1989); vomiting and diarrhea were also observed during the second week of a 52-week study in dogs exposed via capsule to 16 mg DMA/kg/day (Zomber et al. 1989). The remaining studies in the acute database are developmental toxicity studies in mice and rabbits. Rabbits appear to be more sensitive than mice to maternal and developmental effects. Gavage exposure to 48 mg DMA/kg/day on gestational days 7–19 resulted in maternal weight loss and abortion in approximately 75% of the does; no adverse effects were observed at 12 mg DMA/kg/day (Irvine et al. 2006). In mice, decreases in maternal body weight gain were observed at gavage doses of 200 mg DMA/kg/day on gestational days 7–16 (Rogers et al. 1981), decreases in fetal body weight, delays in ossification, and increased incidence of cleft palate were observed at 400 mg DMA/kg/day on gestational days 7–16 (Rogers et al. 1981) and fetal deaths, decreases in growth, and increased incidence of malformations were observed in mice administered 1,600 mg DMA/kg on gestational day 8 (Kavlock et al. 1985). The acute-duration database for DMA was not considered adequate for derivation of an oral MRL. The database is lacking a comprehensive toxicity study, which would be useful in establishing the critical target of toxicity. In a chronic-duration study in mice (Arnold et al. 2006), vacuolization was observed in the urinary bladder at ≥7.8 mg DMA/kg/day; it is not known if these effects would also be observed after acute-duration exposure. Thus, it is not known if systemic effects would occur at lower doses than the maternal developmental effects observed in rabbits exposed to 48 mg DMA/kg/day (Irvine et al. 2006); an acute-duration oral MRL for DMA is not recommended at this time.

Excluding rat studies, the database on the toxicity of DMA following intermediate-duration oral exposure is limited to a chronic study of dogs exposed to DMA via capsule 6 days/week for 52 weeks (Zomber et al. 1989). Diarrhea and vomiting were observed at 16 and 40 mg DMA/kg/day starting after the first week of exposure. A slight decrease in erythrocyte levels and increase in total leukocyte levels were observed in males exposed to 40 mg DMA/kg/day for 51 weeks. This dog study was not selected as the basis of an MRL because it is likely that bolus administration of DMA would increase sensitivity to the gastrointestinal effects.

- An MRL of 0.02 mg DMA/kg/day has been derived for chronic-duration (365 days or longer) oral exposure to DMA.

Two studies investigated the chronic-duration toxicity of DMA in a species other than rats. In dogs, diarrhea and vomiting were observed after 52 weeks of exposure to 16 or 40 mg As/kg/day (Zomber et al. 1989); no histological alterations were observed. In mice exposed to DMA in the diet for 2 years, vacuolization of the urothelium in the urinary bladder was observed at ≥7.8 mg DMA/kg/day and progressive glomerulonephropathy was observed at ≥37 mg DMA/kg/day (Arnold et al. 2006). As noted in Section 2.2, the vacuolization was not associated with cytotoxicity or proliferation. Because the bladder effects in mice occurred at the lowest adverse effect level for the database, it was selected as the critical effect and Arnold et al. (2006) was selected as the principal study.

In the Arnold et al. (2006) study, groups of 56 male and 56 female B6C3F_1 mice were exposed to 0, 8, 40, 200, or 500 ppm DMA in the diet for 2 years; the results of this study were also reported in an unpublished paper (Gur et al. 1989b) submitted to EPA under Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The investigators reported dietary doses of approximately 0, 1.3, 7.8, 37, and 94 mg DMA/kg/day. The following parameters were used to assess toxicity: clinical observations, body weight, food consumption, water consumption, differential leukocyte levels measured at 12, 18, and 24 months in mice in the control and 94 mg DMA/kg/day groups, organ weights (brain, kidneys, liver, and testes), and histopathological examination of major tissues and organs. No deaths or treatment-related clinical signs were observed. Decreases in body weight gain were observed in the male mice exposed to 94 mg DMA/kg/day; the difference was <10% and not considered adverse. An increase in water consumption was observed in males exposed to 94 mg DMA/kg/day during weeks 60–96. In the female mice exposed to 51 mg As/kg/day, a statistically significant decrease in lymphocytes and an increase in monocytes were observed at 24 months. Treatment related nonneoplastic alterations were observed in the urinary bladder and kidneys. In the urinary bladder, increases in the vacuolization of the superficial cells of the urothelium were observed in males exposed to 37 or 94 mg DMA/kg/day (0/44, 1/50, 0/50, 36/45, 48/48) and in females exposed to 7.8, 37, and 94 mg DMA/kg/day (1/45, 1/48, 26/43, 47/47, 43/43); incidence data reported in Gur et al. (1989b). An increased incidence of progressive glomerulonephropathy was observed in males at 37 mg DMA/kg/day (16/44, 22/50, 17/50, 34/45, 30/50) and an increased incidence of nephrocalcinosis was also observed in male mice at 94 mg DMA/kg/day (30/44, 25/50, 27/50, 29/50, 45/50). Neoplastic alterations were limited to an increased incidence of fibrosarcoma of the skin in females exposed to 94 mg DMA/kg/day (the incidence of 3/56, 0/55, 1/56,

1/56, and 6/56 in the 0, 1.3, 7.8, 37, and 94 mg DMA/kg/day groups, respectively); however, it was concluded that this lesion was not related to DMA exposure.

As described in detail in Appendix A, BMD analysis was applied to the incidence data for vacuolization of the urothelium in the urinary bladder of female mice using all available dichotomous models in EPA's Benchmark Dose Software (version 1.4.1) to calculate predicted doses associated with a 10% extra risk. As assessed by the AIC, the multi-stage model provided the best fit to the data. The predicted BMD_{10} and $BMDL_{10}$ are 2.68 and 1.80 mg DMA/kg/day. The $BMDL_{10}$ was selected as the point of departure and divided by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability) to derive a chronic-duration oral MRL of 0.02 mg DMA/kg/day.

Roxarsone. A series of three National Toxicology Program (NTP) studies in rats and mice (NTP 1989b) and a study in dogs (Kerr et al. 1963) have examined the acute toxicity of roxarsone; adverse effects have also been reported within the first 2 weeks of a longer-term study in pigs (Rice et al. 1985; Kennedy et al. 1986). A single exposure study reported diarrhea and ataxia in rats and mice exposed to doses that exceeded the LD_{50} (NTP 1989b). In another study, no alterations in hematological parameters (only end point assessed) were found after 10 or 9 days of dietary exposure in rats and mice, respectively (NTP 1989b). In a 14-day study (NTP 1989b), a decrease in body weight gain and slight inactivity were observed in rats exposed to 32 mg roxarsone/kg/day and slight inactivity was observed in mice exposed to 42 mg roxarsone/kg/day; a decrease in body weight gain was also observed in mice exposed to 168 mg roxarsone/kg/day. The dog study was considered inadequate because a small number (n=3) of animals were tested and no control group was used. In a 30-day dietary exposure study in pigs (Rice et al. 1985; Kennedy et al. 1986), mild lethargy and ataxia were observed from day 7 forward and exercise-induced muscle tremors and clonic seizures were observed from day 11 forward in pigs exposed to 6.3 mg roxarsone/kg/day; equivocal evidence of myelin degeneration was also observed in pigs sacrificed after 11 days of exposure. These data clearly identify pigs as the most sensitive species following acute-duration oral exposure; in the absence of data to the contrary, it is assumed that pigs are a good model to predict the toxic potential of roxarsone in humans. Because the lowest dose tested in pigs was a serious LOAEL for neurotoxicity and a NOAEL for this effect was not identified, an acute-duration oral MRL cannot be derived for roxarsone.

As with the acute-duration database, pigs appear to be the most sensitive species; neurotoxicity has been observed at ≥6.3 mg roxarsone/kg/day. In a study reported by Rice et al. (1985) and Kennedy et al. (1986), exercise-induced muscle tremors and clonic convulsions were observed in pigs during the early

part of the study; when the pigs returned to a recumbent position, the seizures and tremors stopped. Paraparesis, evidenced by reluctance to rise and the pigs dragging their hindquarters on the ground, was observed at day 22; paraplegia was observed 2 days after exposure termination. In addition to these clinical signs of neuropathy, histological alterations consisting of myelin degeneration was observed in the spinal cord, peripheral nerves, and optic nerve. The lesions were first detected in the spinal cord on day 15 and in the peripheral nerves and optic nerve 2 days after exposure termination. The Rice et al. (1985) and Kennedy et al. (1986) studies did not identify a NOAEL. Muscle tremors were also observed in pigs exposed to 10 mg roxarsone/kg/day for 28 days (Edmonds and Baker 1986). This study was not designed to assess neurotoxicity and did not include histological examination of the spinal cord or nerves. Trembling, ataxia, and hyperexcitability were also observed in rats exposed to 64 mg roxarsone/kg/day for 13 weeks (NTP 1989b). Other effects that have been observed include tubular degeneration and focal regenerative hyperplasia in the kidney and decreased body weight in rats exposed to 32 mg roxarsone/kg/day for 13 weeks (NTP 1989b) and decreased body weight in mice at 136 mg roxarsone/kg/day for 13 weeks (NTP 1989b). The lowest identified adverse effect level is 6.3 mg roxarsone/kg/day for serious neurological effects in pigs (Kennedy et al. 1986; Rice et al. 1985) and is not suitable for the derivation of an intermediate-duration oral MRL.

The chronic toxicity of roxarsone has been examined in rats (NTP 1989b; Prier et al. 1963), mice (NTP 1989b; Prier et al. 1963), and dogs (Prier et al. 1963) in 2-year dietary exposure studies. None of these studies reported adverse effects at the highest doses tested; the highest NOAELs for each species are 10, 43, and 5 mg roxarsone/kg/day for rats, mice, and dogs, respectively. The results from shorter duration studies suggest that pigs are more sensitive to the neurotoxic effects of roxarsone than rats, mice, or dogs. Because no chronic duration pig studies were identified and deriving an MRL using a potentially less sensitive species may not be protective of human health, a chronic-duration oral MRL is not recommended at this time.