A Quantitative Assessment of Inorganic Arsenic in Apple Juice

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EXECUTIVE SUMMARY

The purpose of this assessment is to provide quantitative estimates of long-term cancer risk presented by inorganic arsenic in apple juice. Inorganic arsenic is the primary toxic form of arsenic and has been associated with both acute and chronic health effects. Sampling over the past several years by the U.S. Food and Drug Administration (FDA) and others has revealed the presence of inorganic arsenic in apple juice in varying concentrations. In response to these findings, FDA conducted this assessment to quantify risk at various concentrations of inorganic arsenic in apple juice that correspond to concentrations seen in the samples.

The assessment is intended to inform decision making by FDA on how best to manage the risk and protect the public health. FDA selected long-term cancer risk to assess because it is the outcome of greatest concern with lifetime exposure. Current FDA policy is based on evaluations of shorter term exposures and future guidance for individual products where exposure is expected to be temporary may continue to be based on noncancer endpoints.

Much of the data employed in the dose-response modeling in this assessment, and many of the assumptions that were made, were derived from a recent evaluation by the Joint Expert Committee on Food Additives and Contaminants (JECFA) of the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) (WHO, 2011). These include data from two long-term observational reports of a cohort in Taiwan that appeared to develop both urinary tract cancers (Chen et al., 2010a) and lung cancers (Chen et al., 2010b) as a result of drinking well water contaminated with arsenic. These are the most recently published studies that contain information applicable to dose-response modeling. The assessment calculated total cancer incidence by adding urinary tract and lung cancers together.

The exposure modeling used in this assessment included both the consumption of apple juice as a stand-alone commodity and as an ingredient in other juices. Consumption data were taken from food consumption surveys administered by the Centers for Disease Control and Prevention in its National Health and Nutrition Examination Survey (NHANES). A distribution of the various concentrations of total arsenic in apple juice was developed from the results of FDA sampling that occurred between 2008 and 2011, and a distribution of concentrations of inorganic arsenic alone was developed from FDA results collected in the last year.

The assessment modeled risk at the following concentrations of inorganic arsenic: 3 parts per billion (ppb), 5 ppb, 10 ppb, 20 ppb, and 50 ppb. A disease rate was calculated for each of these concentrations based on an assumption that all juice contained exactly that concentration. Additional disease rates were calculated for each of the above concentrations based on the average arsenic concentration in the FDA juice samples that were at or below the concentration in question, e.g., the average of all juice concentrations that did not exceed 3 ppb. This approach provides disease rates that are more realistic than those based on an assumption that all juice contains the same concentration of inorganic arsenic.

Disease rates were also calculated for:

(1) Childhood exposure alone, assuming that juice consumption essentially stops after six years of age. [0 to 6 is the age range that the Agency typically uses to characterize exposure of young children.];

(2) Early lifetime exposure (0-50 years) based on evidence of a latency period of 25 years between exposure and peak cancer rate elevation (Marshall et al., 2007); and

(3) Lifetime exposure with no emphasis on early exposure.

Modeling based on early lifetime exposure is likely to be the most relevant for a cancer risk assessment for arsenic. It also produced the highest calculated disease rates of the three approaches. Estimated cancer rates for the total US population attributable to apple juice consumption based on three different putative limits are as follows:

Limit (ppb)	Total Cancer Rate (per million)
3	2.5 (0.0, 6.8)
5	4.8 (0.0, 12.8)
10 and above	8.0 (0.0, 21.3)

Disease rates for 20 ppb and 50 ppb were estimated to be the same as the rate for 10 ppb because the data set used in the modeling (FDA 2011 survey of inorganic and organic arsenic in apple juice) did not contain any inorganic arsenic levels above 10 ppb. Older data indicate that inorganic arsenic concentrations above 10 ppb will occur infrequently, so risk at 20 ppb should be somewhat higher. No total arsenic concentrations above 50 ppb have been found in any FDA apple juice samples collected over the past 8 years.

The Scope and Purpose of the Present Document

Over the last few years, the FDA Chemical Hazard Assessment Team has used the Environmental Protection Agency (EPA) reference dose (RfD) to evaluate the hazards from chronic exposures from juices and other products that are less than lifetime. The RfD of 0.3 µg/kg body weight per day is based on observations of hyperpigmentation, keratosis and possible vascular complications with oral doses estimated to be 30 times higher (EPA, 1993). Exposures to arsenic that are below the RfD are generally considered to have negligible risks for effects other than cancer. The level of concern identified in 2008 for pear and apple juice using the RfD was 23 ppb, which was calculated using a daily intake of 13 g/kg/day. However, because arsenic is considered to be a human carcinogen (IARC, 2012), there is a basis for concern for cancer risks at lower levels of exposure.

The purpose of the present document is to provide a quantitative evaluation to be used in support of a guidance level for inorganic arsenic in apple juice. The present analysis is not prepared with the expectation that it will solely determine the guidance values recommended by the agency. In particular, it is anticipated that the guidance will depend, at least in part, on the feasibility of achieving a specified level. In addition, arsenic risk assessment is a very active field, and it is likely that the methodology used to characterize the dose-response relationships for the toxic effects of arsenic will evolve in the near future. FDA has been working closely with the EPA IRIS Program Arsenic Working group. We are following its progress and will carefully review

the recommendations that the National Academy of Sciences is expected to issue in draft form in early fall 2013. Similarly, EPA is closely following the activities of FDA surrounding the assessment of arsenic in food commodities. The current draft assessment is based upon the best science available today on the risk of arsenic in apple juice products. As with all FDA risk assessments, the agency will review new significant scientific findings as they become available.

Arsenic Toxicology

Since extensive reviews have recently been conducted elsewhere (ATSDR, 2007; EFSA, 2009; WHO, 2011; IARC, 2012), and for the sake of brevity, this document does not provide a comprehensive review of the literature on arsenic. Although key references are provided for some statements, complete documentation for the summary given below may be found in the full reviews listed above.

Introduction

Arsenic (As) is a naturally occurring element that is released from volcanoes and from erosion of mineral deposits containing arsenic. Arsenic exists in many chemical forms and valence states (-3, 0, +3 and +5). The forms fall broadly into two categories with public health relevance: inorganic and organic. Inorganic forms are the primary toxic forms of arsenic.

Human activities such as burning coal, oil, gasoline and wood, mining, and the use of arsenic compounds as medicinals, pesticides, herbicides and wood preservatives (primarily chromated copper arsenate (CCA)), also contribute to the arsenic environmental burden. Low concentrations can be found in air, water, soil and food. These concentrations can consist of inorganic forms, organic forms, or a combination of the two ("total" arsenic). The background soil content of arsenic varies widely, typically ranging from one to 40 parts per million (ppm) with an average of five ppm (ATSDR, 2007). Arsenic concentration in natural surface and groundwater is generally less than 10 parts per billion (ppb), but may exceed this level in contaminated areas or areas with high soil levels of arsenic (ATSDR, 2007). Drinking water in the U.S. contains, on average, 2 ppb total arsenic; although this calculation is based on including all sources of water, i.e., municipal water supplies as well as surface and ground water sources (ATSDR, 2007). For example, naturally occurring arsenic-contaminated groundwater has severely affected people in Bangladesh where they have been chronically exposed to elevated arsenic in drinking water from groundwater wells (ATSDR, 2007; EFSA, 2009; WHO, 2011).

The primary forms of arsenic found in drinking water are the inorganic forms (iAs), arsenite (+3) and arsenate (+5). Seawater typically contains 1.5 – 1.7 ppb total arsenic (EFSA, 2009). Ambient air arsenic background concentrations generally range from one to three nanograms per cubic meter (ng/m^3), but concentrations in an urban area may range up to 100 ng/m^3. Food arsenic concentrations usually range from 20 – 140 ppb (ATSDR, 2007). Higher total arsenic levels are found in seaweed, seafood, mushrooms, rice and rice products and some meats, and very high levels generally are encountered in seafood where the arsenic occurs in organic forms known as arsenobetaine and arsenocholine, which are considered to be of no toxicological concern (ATSDR, 2007). Two organic forms that are of toxicological concern, monomethylarsonic acid (MMA^{+5}) and dimethylarsinic acid (DMA^{+5}), can also be found in various types of finfish, crabs, and mollusks, but often at very low levels (EFSA, 2009). These

organic forms do not appear to be as toxic as inorganic forms of arsenic. Arsenosugars are the major species detected in seaweed and are also found to a lesser extent in marine mollusks. Small amounts of MMA^{+5} and DMA^{+5} are also found in rice, some vegetables and fruit juices (ATSDR, 2007; EFSA, 2009; WHO, 2011).

Absorption, Distribution, Metabolism and Excretion

Soluble inorganic As is highly bioavailable and is rapidly absorbed. It is cleared from blood in humans and in most animals except rats, in which As binds to red blood cells, thus delaying clearance. Once absorbed, inorganic As is metabolized by reduction from As^{+5} to As^{+3} in the blood and is taken up by cells in tissues, mainly the liver, followed by intracellular oxidative addition of methyl groups to form MMA^{+5} and DMA^{+5}. Alternative pathways include the production of methylated arsenical glutathione metabolites, a process that also occurs in the liver. Trivalent inorganic arsenic (arsenite) is taken up into cells more extensively than the pentavalent inorganic arsenic (arsenate). Arsenite is also a preferred substrate for arsenite methyltransferase over arsenate, thus is metabolized more extensively than arsenate. Arsenobetaine, the major form of arsenic in most seafood and fish, is not metabolized and is excreted intact in humans (ATSDR, 2007).

Two basic processes are involved in the metabolism of inorganic arsenic: 1) reduction/oxidation reactions that interconvert As^{+3} and As^{+5} and 2) methylation reactions that convert arsenite to monomethylarsonic acid and dimethylarsonic acid, although there is uncertainty as to the metabolic pathway (Sams II et al., 2007; Hayakawa et al., 2005). Methylation reactions facilitate the excretion of inorganic arsenic from the body as both MMA and DMA are more readily excreted in urine. Inorganic arsenic can also be excreted directly in the urine. In contrast, with the exception of arsenosugars, ingested organic arsenicals such as MMA, DMA and arsenobetaine, do not readily enter the cell, undergo limited metabolism, and are excreted unchanged in the urine (ATSDR, 2007). High variability of arsenic metabolism and toxicokinetics was reported among different species, population and individuals. Some species (marmoset monkey, guinea-pig, chimpanzee) have minimal or no arsenic methylation capability (Cui et al., 2008).

In humans, inorganic arsenic is extensively methylated and its metabolites are excreted primarily in the urine. Ingested inorganic As is excreted via the kidney within a few days as inorganic As^{+5} and As^{+3} and as the pentavalent methylated metabolites MMA^{+5} and DMA^{+5}, with lesser amounts of the trivalent methylated metabolites MMA^{+3} and DMA^{+3} and thioarsenical metabolites. Age, gender and smoking may contribute to the large individual variations in arsenic methylation in humans (EFSA, 2009; ATSDR, 2007). Similar urinary metabolic profiles were reported among family members (Chung et al., 2002). An increase in DMA excretion was observed in individuals with a specific allele on a gene coding for a form of glutathione S-transferase, suggesting its possible association with a genotype that protects against arsenic toxicity (Paiva et al., 2010). Other than genetic polymorphisms and wide differences in methyltransferase activities, nutritional status may also influence methylation capacity (ATSDR, 2007; EFSA, 2009). The presence and level of arsenic in urine is commonly used as a measure of recent exposure. Arsenic levels in hair and nails have been shown to provide reliable biomarkers for long-term chronic exposure to arsenic in humans (Marchiset-Ferlay et al., 2012; Lin et al., 1998; Karagas et al., 1996).

There are interspecies differences in the metabolism (methylation and excretion) of inorganic arsenic. Humans excrete the majority of ingested inorganic arsenic in urine within a few days (ATSDR, 2007). By measuring the relative amount of arsenic metabolites in urine, it has been shown that intracellular metabolism of inorganic arsenic involves extensive metabolism to DMA^{+5} and MMA^{+5} in most animal species including humans. According to a study of the U.S. population based on NHANES 2003-2004 data, DMA^{+5} is generally the most abundant methylated arsenical in urine, comprising an average of 45% of total arsenic in urine (Caldwell et al, 2009).

Based on urinary excretion data, ingested MMA and DMA are well absorbed (at least 75-85%) from the gastrointestinal (GI) tract in several species, including humans (ATSDR, 2007). We know of no studies on the distribution of orally ingested MMA or DMA in humans. Studies in other animals have shown that MMA and DMA are distributed to all tissues after acute oral doses. In mice, MMA rapidly distributes throughout the body with peak concentrations largest in the bladder and concentrations in kidneys and lungs, larger than that in the blood (ATSDR, 2007).

In contrast to ingested inorganic arsenic, which undergoes extensive intracellular metabolism to DMA, ingested organic arsenicals undergo limited intracellular metabolism with the exception of arsenosugars, which may undergo extensive metabolism. The available data suggest that the methylarsenates are not demethylated to inorganic As either in humans or in animals (ATSDR, 2007). In most species (except rats), MMA and DMA are mostly excreted in the urine unchanged. Human volunteers excreted in their urine 78% of a single oral dose of MMA^{+5} given in drinking water within 4 days (Buchet et al., 1981). Furthermore, 87% of the excreted dose was as the parent compound, MMA^{+5} and only 13% was converted to DMA^{+5}. Similarly, 75% of a single oral dose of DMA^{+5} was excreted in urine as the parent DMA^{+5} within 4 days with no evidence for further methylation or demethylation (Buchet et al., 1981). Only one study reports trimethylarsine oxide (TMAO, 4%) in human urine following exposure of one male individual to an extremely high oral dose of DMA^{+5} (Marafante et al., 1987). Similarly, small percentages of orally ingested DMA^{+5} are excreted as TMAO in urine of mice and hamsters (Marafante et al., 1987). In contrast, rats have high urinary TMAO levels after DMA^{V} exposure (Yoshida et al., 1998).

In vitro studies demonstrated differences in the uptake of DMA^{+5} and DMA^{+3} into red blood cells among animals (Shiobara et al., 2001). DMA^{+3} was taken up more efficiently than DMA^{+5} in the rat, hamster, mouse, and human, and DMA^{+3} was taken up most efficiently in the rat cells and least efficiently in the human cells. Because of much higher binding of DMA^{+3} to sulfhydryl groups of hemoglobin in the rat than in other species, greater intracellular retention of DMA^{+3} was also shown in the rat red blood cells. In addition, the oxidation efficiency of DMA^{+3} to DMA^{+5} was shown to be in the order of hamster > human > rat (Shiobara et al., 2001).

Toxic Effects of Arsenic

Biochemical mechanisms. Inorganic arsenic binds to the sulfhydryl groups of cellular proteins, inhibiting the pyruvate and succinate oxidative pathways. It also competes with phosphorus in the oxidative phosphorylation process. Although chronic exposure to inorganic arsenic in drinking water has been associated with cancers in humans, the exact molecular mechanisms are

not clear. Several modes of action (MOA) of inorganic arsenic in carcinogenesis have been proposed, including induction of oxidative stress; genotoxicity as induction of mutations and chromosomal aberrations; modulation of signal transduction and apoptosis (growth factors, cell proliferation, and promotion); and alterations in gene expressions via hyper- and hypomethylation of DNA (ATSDR, 2007).

In studies in rodent models from one laboratory, either DMA or arsenate and arsenite administered in the diet or drinking water to rats and mice induced cytotoxicity and necrosis of the urothelial superficial layer and hyperplasia in the urinary bladder of the animals. The authors postulate that arsenic-induced bladder cancer is a non-linear process, involving urothelial cytotoxicity and regenerative proliferation (Suzuki et al., 2008; Cohen et al., 2007; Arnold et al., 2013; Suzuki et al., 2010). Recent evidence suggests that arsenic activates "Hedgehog signaling," a signaling pathway that transmits information to cells for proper development; malfunctions of this pathway have been implicated in some cancers (Fei et al, 2010). This evidence also suggested that there is a strong positive correlation between arsenic exposure and high levels of "Hedgehog" activity in a cohort of bladder cancer patients

Another recent study evaluating gene expression changes in a small number of cultured human primary uroepithelial cells treated with mixtures of inorganic arsenic and its metabolites indicates changes in other key signaling pathways such as oxidative stress, protein folding, growth regulation, metallothionine regulation, DNA damage sensing, thioredoxin regulation, and immune response (Yager et al., 2013). Arsenic does not directly react with DNA, but it has been shown damage DNA through an indirect effect. (For more information on this indirect mode of action, see Nesnow et al, 2002.) Inorganic arsenic has been shown in both in vitro and in vivo studies to break chromosomes and cause extensive damage to DNA in a variety of human tissues. It is also probable that more than one of these mechanisms is involved in the carcinogenicity of inorganic arsenic.

Some of these proposed mechanisms may operate via non-linear dose-responses including a threshold for effects; however no consensus view has yet emerged.

Recently findings were published from the Health Effects of Arsenic Longitudinal Study (HEALS), a prospective cohort study of increased overall mortality and chronic-disease mortality associated with arsenic in drinking water in the Araihazar region of Bangladesh. The HEALS cohort includes concentrations at the low end of the dose-response curve and concentrations at the high end at which known health effects occur. The authors report a dose-related trend in mortality with exposure to increasing concentrations, with no apparent threshold. (Argos, et al 2010.) However, it should be noted that, while the study data appeared to support a dose related trend in mortality, the only statistically significant increase in mortality was recorded at levels above 150 ppb. Thus, as discussed in a paper by EPA scientists (Kitchin and Conolly, 2010), there are multiple possible mechanisms underlying the carcinogenic effects of inorganic arsenic. These include the genotoxicity and clastogenicity of organic and inorganic arsenicals that may warrant linear extrapolation as well as other mechanisms such as oxidative stress that may be expected to exhibit nonlinear characteristics. Because different biochemical mechanisms may operate in different organ systems or even the same organ at different life stages, current knowledge does not allow the dose-response relationship for arsenic to be characterized based on purely theoretical considerations.

Acute Exposure. Ingestion of large doses of arsenic can result in death (ATSDR, 2007). The oral lethal dose of arsenic trioxide is reported to be between 70 and 180 mg/day. The estimated minimum lethal dose in humans ranges from one to three milligrams per kilogram of body weight per day (mg As/kg bw/day). Poisoning may appear with daily doses of inorganic arsenic as low as a few milligrams for a short period of time, e.g. weeks. For example, over 200 adults were poisoned by contaminated soy sauce with an estimated daily exposure of three mg of arsenic for two to three weeks (ATSDR, 2007). Depending on dose and duration of exposure, adverse health effects caused by inorganic arsenic can occur in many organs. Symptoms of acute exposure to arsenic in drinking water at doses of 0.2 mg/kg bw/day or above usually occur within the first several hours. Essentially all cases of short-term high-dose exposure to inorganic arsenic show clinical signs of gastrointestinal effects.

Short-term exposure. Exposure to elevated arsenic for weeks to months in drinking water can result in gastrointestinal effects such as abdominal pain, vomiting, diarrhea, and muscular cramping; hematological effects such as anemia and leucopenia; and peripheral neuropathy such as numbness, burning or tingling sensation or pain in extremities. Metallic taste, garlic odor in breath and feces and salivation may also be present. (ATSDR, 2007).

Chronic exposure. With longer exposures, lower lethal doses of 0.014 to 0.065 mg/kg bw/day from drinking water were reported specifically contributing to Blackfoot Disease in an endemic area of Taiwan (ATSDR, 2007). Chronic exposure to arsenic in drinking water typically causes specific dermal effects. Diffuse or spotted hyperpigmentation followed by palmoplantar hyperkeratosis occurs after six months to three years of ingestion of high doses of arsenic (0.04 mg/kg bw/day) or five to 15 years of ingestion of low doses of arsenic (0.01 mg/kg bw/day or higher (EFSA, 2009). Chronic exposure to 0.02 mg/kg bw/day or higher has been shown to cause skin lesions and other health outcomes including peripheral vascular effects, cardiovascular effects, diabetes mellitus, peripheral neuropathy, diseases of the respiratory system, negative impacts on fetal and infant development (low birth weight) and cancers (skin and internal organs; ATSDR, 2007; EFSA, 2009; IARC, 2012).

Human Clinical Studies. A few studies in humans regarding oral toxicities including cancer of organic arsenicals have been published. Vomiting was reported to occur after ingesting 793 mg/kg arsenic as monosodium methanearsenate (MSMA) in a suicide attempt (ATSDR, 2007). Induced vomiting, abdominal pain, hyperactive bowel, and diarrhea resulted from ingesting 78 mg DMA/kg (as dimethyl arsenic acid and dimethyl arsenate) but hematological effects were not observed in all cases (ATSDR, 2007). In another suicidal case, ingesting 1714 mg MSMA/kg showed no adverse cardiovascular, renal, and respiratory effects after a chelation treatment (ATSDR, 2007).

Epidemiology Studies. The main adverse effects reported to be associated with long-term ingestion of inorganic arsenic in humans are cancer, skin lesions, developmental effects, cardiovascular disease, neurotoxicity and diabetes. Of these, the greatest strength of evidence for a causal association is for cancers of the skin, urinary tract and lung and for skin lesions (hyperkeratosis, hyperpigmentation and hypopigmentation) observed in studies in which

inorganic arsenic exposure was high due to inorganic arsenic in drinking water (e.g. ≥100 µg/l). The nutritional status of exposed populations has been observed to influence cancer risk. Thus, compromised nutrition (e.g. low protein intake) may be associated with significantly higher risk (EFSA, 2009).

Toxicity of Organic Species. There are no human studies of chronic exposure to DMA or MMA through ingestion; more research is needed to understand the mode of action of these methylated arsenicals. Studies of DMA^{+5} oral exposure in experimental animals have found effects on the urinary bladder, kidneys, thyroid and fetal development (EFSA, 2009). DMA^{+5} has been found to be carcinogenic for the urinary bladder of male and female rats (Wei et al., 2002; Arnold et al., 2006), but not in the urinary bladder of male and female mice fed in the diet up to 500 ppm (equivalent to 94 mg/kg/day; Arnold et al., 2006). While IARC (2012) concluded that there is "sufficient evidence" for carcinogenicity of DMA^{+5} in experimental animals, ATSDR (2007) concluded that differences in uptake, metabolism and elimination of DMA^{+5} in rats as compared with other species indicate that the rat is not a good animal model for humans for this chemical. In chronic studies, oral administration of MMA^{+5} to experimental animals has been shown to have effects on the gastrointestinal tract, kidney, thyroid and reproductive system, with the effect seen at the lowest doses being diarrhea (ATSDR, 2007). MMA^{+5} was not carcinogenic in two-year bioassays when fed to male rats at up to 200 mg/L in drinking water or to male and female mice or rats up to 400 mg/kg in the diet (Arnold et al., 2003; Shen et al., 2003). DMA^{+5} and MMA^{+5} were not mutagenic in the Ames test, but they can cause chromosomal aberrations and mutations at cytotoxic concentrations (EFSA, 2009). DMA^{+5} or MMA^{+5} did not produce any dose-related developmental toxicity effects in rats and rabbits following oral ingestion of levels below the threshold for maternal toxicity (Irvine et al., 2006; EFSA, 2009). DMA^{+5} and MMA^{+5} did not result in clinical signs of neurotoxicity or brain lesions in rats or mice after chronic dietary exposures (ATSDR, 2007). Unlike inorganic arsenic, DMA^{V} and MMA^{V} have not been found to cause cardiovascular effects (ATSDR, 2007).

Arsenic Toxicity in Children

The documented toxic effects of arsenic exposure are almost entirely associated with effects in adults. There is little to no evidence of toxic effects in the epidemiological literature of arsenic that occur specifically in children. However, exposure during childhood may still be relevant for effects that occur as a result of chronic exposure. There is evidence from several studies that increased cancer in adults may occur as a result of exposure during childhood (Tokar et al., 2011; IARC, 2012). Most of the epidemiology studies that have found increased rates of cancer in populations with higher exposures to arsenic involved exposure that began during childhood. In particular, an ecological study of a Chilean cohort that was exposed to elevated levels of arsenic over a 12 year period exhibited an increase in lung and bladder cancer that peaked 25 years after the elevated exposure had stopped (Marshall et al., 2007).

Although some initial pharmacokinetic studies indicated that children may metabolize arsenic at a slower rate than adults (ATSDR, 2007), other studies have found that the metabolic profile may be faster in children than adults. Lindberg et al. (2008) found a 30% variation between the exposure level of arsenic and gender and age of the test subjects. Furthermore, after adjustment of the dose for body weight, children may be expected to exhibit the same dose-response relationship for acute and short-term chronic effects as adults, and the temporal evidence from

episodic exposures (e.g., Marshall et al., 2007) indicates that exposures earlier in life are likely to be more important. Therefore, an exposure assessment that places greater weight on earlier exposures is warranted.

Data for Cancer Dose Response Assessment

Although FDA has used noncancer guidance values (e.g. the EPA RfD of 0.3 µg/kg/day) in the past to evaluate hazards from short term exposures to individual products, the present evaluation is intended to evaluate the hazard from exposure to arsenic in apple juice consumed as apple juice or when used as ingredients in juice blends or as a sweetener in other foods.

At low levels of inorganic arsenic exposure, regardless of the source, the strongest evidence of negative effects is for induced cancers of the lung and urinary tract, and therefore a dose response assessment was conducted for these adverse health outcomes. It is important to note that other endpoints may still be considered more important for exposures of a shorter duration.

For this cancer dose-response assessment, the data and modeling assumptions identified by JECFA were used (WHO, 2011). The studies that provided data for the modeling involved both relatively high concentrations of inorganic arsenic in well water (e.g. >300 µg/l) and relatively low concentrations (e.g. <100 µg/l) that allowed modeling of concentrations in ranges that are normally encountered in the rest of the world. A prospective cohort study in north-eastern Taiwan was selected as the pivotal study for urinary cancer (Chen et al., 2010a) and lung cancer (Chen et al., 2010b). Other studies considered by the committee included earlier studies with different cohorts in Taiwan where lung and bladder cancer were the primary endpoints (Wu et al., 1989; Chen and Wang, 1990), and studies of skin cancer and other dermal lesions in Bangladesh and China (Ahsan et al., 2006; Rahman et al., 2006; Xia et al., 2009). Some of the former studies have been used for cancer risk assessments by the U.S. EPA. While JECFA also modeled results of skin lesions from Bangladesh, the Taiwanese studies were considered to be best suited for dose-response modeling because demonstrable (i.e. statistically significant) changes in disease rates were observed at two levels of exposure and because lung and bladder cancer are more serious effects.

In total, the Taiwanese cohort began with 8,086 subjects aged 40 years and older that were recruited into the study, and had an average of 11.5 years of follow-up. Total arsenic concentrations in drinking water were available for 6,888 of these subjects. Studies that have speciated arsenic in drinking water in Taiwan have found it to be primarily inorganic arsenic (Lin et al., 1998; Chen et al., 1995). A significant dose-response trend of urinary/lung cancer risk was associated with increasing arsenic drinking-water concentration. An advantage of the prospective cohort study design is that the cohort is classified in relation to exposure before disease develops, thereby reducing the likelihood of exposure misclassification resulting from associating individuals with a well from which they do not have long-term exposure. Standardized incidence ratios can also be estimated from this study design, unlike the case-control design, which yields only odds ratio (OR) estimates.

In order to utilize the adjustment made for other variables (e.g. smoking) in the original studies of lung cancer (Chen et al., 2010b) and urinary tract cancer (Chen et al., 2010a), adjusted cases were calculated for each exposure group (i.e. other than the referent group) from relative risks.

This two-step process involved calculating case frequency by multiplying the rate in the referent group by the relative risk and then estimating the number of adjusted cases by multiplying the number of subjects by the case frequency. The resulting adjustment was small relative to the reported cases (see Tables 1 and 2).

The epidemiological study of urinary tract cancer (Chen et al., 2010a) showed a significantly increased trend risk as assessed by the risk ratio (RR) with increasing arsenic concentration in drinking water when adjusted for sex, age and smoking. For exposures above 100 µg/L, RRs were more than four and the differences were statistically significant, whereas the RRs were elevated but not significantly so for exposures below 100 µg/L. Table 1 provides the data used in this dose-response modeling for urinary tract cancer.

The dose-response assessment of lung cancer (Chen et al., 2010b) also found a significant increased trend ($P = 0.001$) of lung cancer risk associated with increasing drinking water arsenic concentration. As with the urinary RRs for urinary cancer, the increase in RR was non-significant below 100 µg/L, but a significant increase in RR was shown for exposures above 100 µg/L. Table 2 shows the data used in dose–response modeling for lung cancer.

While the primary goal of the JECFA evaluation was to generate a Benchmark Dose (BMD) estimate (roughly equivalent to a No-Observed Adverse Effect Level, or a dose with effects that are below the level of quantification in a particular study), a cancer risk assessment is typically intended to generate an estimate of risk after lifetime exposure. To accomplish this, cohort incidences were adjusted by an additional factor (i.e. a factor not used in the JECFA assessment) to account for the fact that the period of follow up was 11.5 years rather than for a complete lifetime. This value is based on average life expectancy in Taiwan relative to the period of observation, and assumes that the disease rate at ages of less than 40 is negligible, and that the ages of the members of the cohort are representative of the general population. Because of the uncertainties associated with these assumptions, an uncertainty range spanning from two to three was used as an adjustment factor, where the upper range of three corresponds to (75-40)/11.5.

Table 1. Association of Urinary Cancer in Relation to Person-years of Observation with Arsenic Exposure in North-eastern Taiwan

Inorganic arsenic in water		Inorganic arsenic total dietary exposure[b]		Cohort incidence[c]	RR	*N*	Adjusted cases[d]
Category range (µg/L)	Central estimate[a] (µg/l)	(µg/person per day)	(µg/kg bw per day)				
<10	5	90	1.6	0.0022	1	2288	5
10–49.9	30	165	3.0	0.0036	1.66	2093	8
50–99.9	75	300	5.5	0.0053	2.42	907	5
100–299.9	200	675	12.3	0.00905	4.13	909	8
≥300	450	1425	25.9	0.0170	7.8	691	12

[a] Point estimate of the range of inorganic arsenic in drinking water.

[b] Central estimate, assuming consumption of three liters of water per day, including that used in cooking, and 75 µg of inorganic arsenic in food per day and body weight of 55 kg.

[c] Referent group (<10 µg/l) is actual case rate per person; other rates are calculated from relative risks.

[d] Referent group is actual cases. Other case estimates are obtained by multiplying group size by incidence.

Source: Chen et al. (2010a).

Table 2. Association of Lung Cancer Cases in Relation to Total Population Studied with Arsenic Exposure in North-eastern Taiwan

Inorganic arsenic in water		Inorganic arsenic total dietary exposure[b]		Cohort incidence[c]	RR	*N*	Adjusted cases[d]
Category range (µg/L)	Central estimate[a] (µg/L)	(µg/person per day)	(µg/kg bw per day)				
<10	5	90	1.6	0.021	1	2288	48
10–49.9	30	165	3.0	0.023	1.1	2093	48
50–99.9	75	300	5.5	0.021	0.99	907	19
100–299.9	200	675	12.3	0.032	1.54	909	29
≥300	450	1425	25.9	0.047	2.25	691	33

[a] Point estimate of the range of inorganic arsenic in drinking water.

[b] Central estimate, assuming consumption of three litres of water per day, including that used in cooking, and 75 µg of inorganic arsenic in food per day and body weight of 55 kg.

[c] Referent group (<10 µg/l) is actual case rate per person; other rates are calculated from relative risks

[d] Referent group is actual cases. Other case estimates are obtained by multiplying group size by incidence.

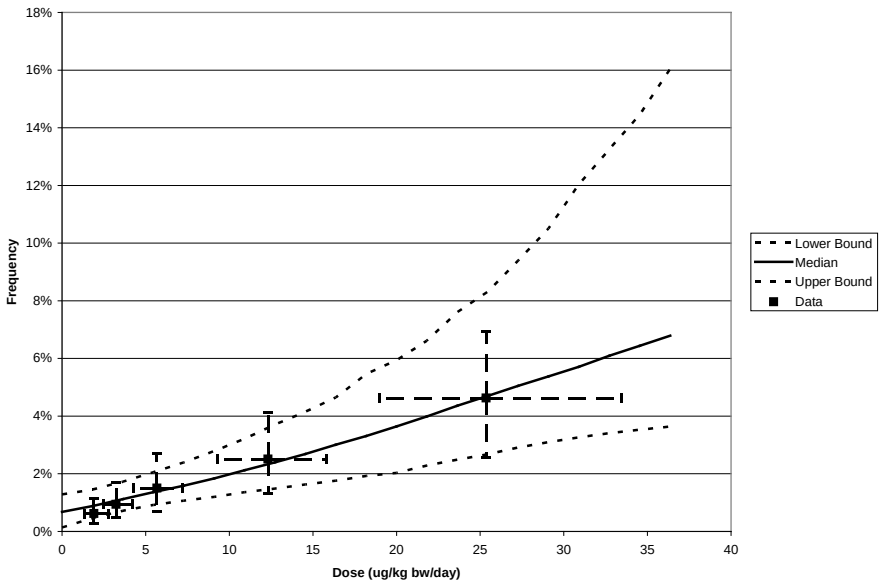
Source: Chen et al. (2010b).

Dose-Response Modeling

Because of the very large uncertainties associated with theoretical approaches to characterizing the dose-response relationship for arsenic-induced cancer, an approach that largely relies on empirical support is appropriate and necessary. Eight different candidate models were used to model the data; gamma, logistic, loglogistic, logprobit, probit, Weibull, plus two alternative versions of the log-logistic and log-probit that utilized background dose terms to model background effects (i.e. extra-risk rather than added-risk). While the first six were also used by JECFA for the BMD analysis, the latter two were not. Models were fit by least squares regression analysis using Microsoft Excel Solver.

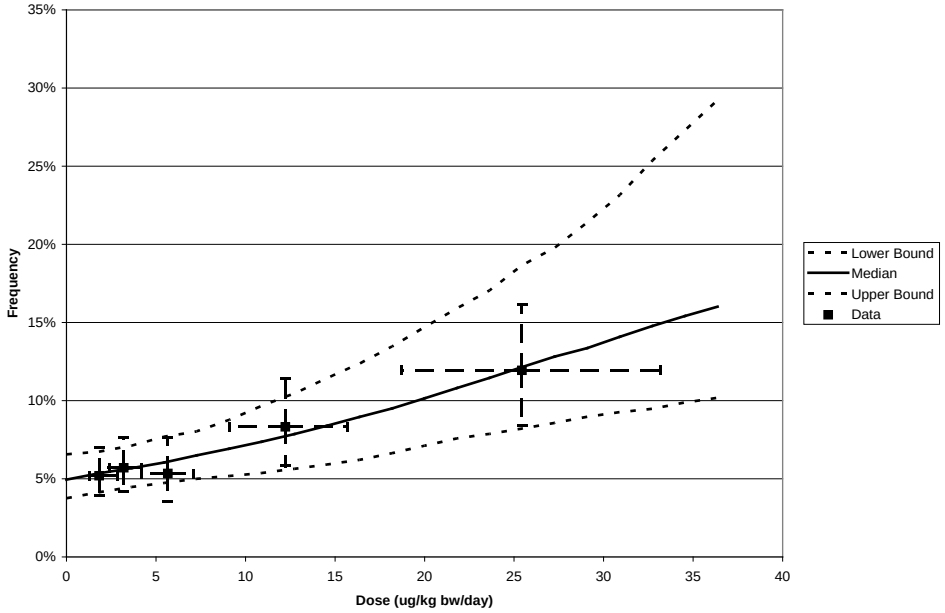
A 300-iteration bootstrap analysis was used to represent multiple uncertainties associated with the dose-response relationship. First, uncertainties associated with the dose in each group were represented by using a range of plausible values for both drinking water consumption and arsenic intake from food (see Tables 1 and 2). Second, a binomial distribution was used to represent uncertainties in the frequency of disease occurrence in the cohort and a rectangular distribution for the lifetime risk adjustment factor. Third, model uncertainty was represented by using only the best fitting model for each bootstrap iteration. The results of the bootstrap analysis are shown in Figures 1 and 2. Actual model usage for both lung and urinary tract cancer is listed in Table 3. While the loglogistic model is the predominant model in both cases, the alternative models for the two endpoints differ. Table 4 provides the frequency of cases values, from Figures 1 and 2, relative to five examples doses and shows the related number of cases on a per million basis (as reported in Table 5).

Figure 1: Urinary Tract Cancer, Best Model



Dose-response model for inorganic arsenic and urinary tract cancer, based on a prospective epidemiology study in northeastern Taiwan (Chen et al., 2010a). The confidence intervals (5^{th} and 95^{th} percentiles) reflect uncertainties arising from the dose-estimates (represented by the error bars) and the model used to represent the dose-response relationship.

Figure 2: Lung Cancer, Best Model



Dose-response model for inorganic arsenic and lung cancer, based on a prospective epidemiology study in northeastern Taiwan (Chen et al, 2010b). The confidence intervals (5^{th} and 95^{th} percentiles) reflect uncertainties arising from the dose-estimates (represented by the error bars) and the model used to represent the dose-response relationship.

Table 3. Best Model Usage from 300 Iteration Bootstrap Analysis

Model	Lung Cancer	Urinary Tract Cancer
Gamma	1 (0. 3%)	8 (2.7%)
Logistic	9 (3%)	28 (9.3%)
LogLogistic	232 (77.3%)	227 (75.7%)
LogProbit	43 (14.3%)	5 (1.7%)
Probit	6 (2%)	14 (4.7%)
Weibull	4 (1.3%)	10 (3.3%)
LogLogistic-ER	1 (0. 3%)	7 (2.3%)
LogProbit-ER	4 (1.3%)	1 (0.3%)

Each value represents the number of iterations each model used per 300 uncertainty iterations, with percentages representing a relative probability for each model given in parentheses.

Table 4. Cases Estimated for Lung and Bladder Cancer at Five Example Doses of Inorganic Arsenic

	Dose (µg/kg bw/day)				
Δ Disease Rate[1]	0.029[2]	0.3[3]	1	3[4]	10
Lung Cancer (%)	0.0 (0.0, 0.0)	0.0 (0.0, 0.1)	0.1 (0.0, 0.4)	0.5 (0.0, 1.4)	2.0 (0.2, 4.4)
Lung Cancer (per million)	30 (0, 123)	369 (0, 1292)	1284 (0, 4298)	4634 (7, 13594)	20242 (1763, 43882)
Bladder Cancer (%)	0.0 (0.0, 0.0)	0.0 (0.0, 0.1)	0.1 (0.0, 0.2)	0.4 (0.0, 0.7)	1.3 (0.2, 2.5)
Bladder Cancer (per million)	32 (0, 69)	338 (0, 726)	1143 (1, 2483)	3574 (43, 7441)	12968 (2256, 25100)

[1] - Change in frequency of disease over background rate
[2]- Dose from tap water at 1 ppb, 2L/day, 70 kg bw.
[3] - Corresponds to current EPA Reference Dose
[4] - Corresponds to 2010 JECFA BMDL

U.S. Environmental Protection Agency Cancer Dose-Response Models for Inorganic Arsenic

The U.S. Environmental Protection Agency (EPA) has produced several different risk estimates for inorganic arsenic over the last 25 years (EPA, 2007). The model developed for the EPA (2001) regulation of arsenic in drinking water is the last dose-response analysis to be finalized and used as the basis for a regulation. Like the dose-response analysis described above, these estimates are based on epidemiological studies conducted in Taiwan on populations exposed to arsenic from drinking water from highly contaminated wells. They are also largely focused on lung and bladder cancer.

However, there are several differences among dose-response characterizations performed for the EPA and FDA. In particular, the following underlying issues are all important:

- *The cohorts are different.* Our assessment is based on two reports published in 2010 (Chen et al., 2010 a,b) that describe the results of a 12 year prospective study, whereas the EPA assessments (EPA, 2001, 2007) are based on ecological studies published in 1988, 1989 and 1990 (Wu 1989; Chen et al., 1988; Chen and Wang, 1990). A common difficulty with using epidemiological data for dose-response modeling is that observed associations between dose and disease outcome may not be causally related. Furthermore, even when it can be firmly established that there is a causal relationship, the

relationship may not be completely causal. Since non-causal associations may vary between cohorts, some differences may be expected. As a further complication, causal relationships may involve interactions with other risk factors. For example, the development of lung cancer may in some individual cases be contingent on exposure to both smoking and arsenic, so that arsenic may have a stronger causal relationship on a population of smokers than in a population of nonsmokers. This issue may be addressed, at least in part, by pooling data or using a meta-analysis that integrates results from different cohorts. Since we do not have individual subject data from any of the studies, pooling and meta-analysis are beyond the scope of the current assessment. Such a modeling effort could potentially address complex interactions.

- *The study dose estimates are different.* Another generic problem with modeling data from epidemiological studies is that because the exposures are not controlled, the actual dose that each individual is exposed to must be estimated. Since arsenic was measured in well water, drinking water intake must be estimated in order to calculate actual arsenic intake. In addition, water used for food preparation will also be consumed, and arsenic may also be consumed from other parts of the diet. The estimates used in our analysis are similar those used in the EPA 2001 analysis.
- *The models are different.* The data sets used for dose response modeling typically have a dose range that includes measurable outcomes at high doses and low doses where effects are too small to be measured reliably. Different mathematical models used to represent dose-response relationships may describe the high dose results equally well yet yield different predictions at low doses where most exposures typically occur. Whereas our risk assessment used several different models to represent this source of uncertainty, EPA risk assessments for arsenic and other compounds typically employ a single model.
- *Lifetime Disease Rate Estimation.* Prospective epidemiological studies are most suited for dose-response analyses. However, the studies only follow each individual for a limited period of time, and therefore results are expressed on a person-year basis rather than per person over a lifetime. Because disease rates are often dependent on subject age, estimates of lifetime risk must adjust for the age to the population. We used a relatively simple method that assumes that the age distribution in the cohort is representative of the general population. Individual subject data allow age to be modeled as a cofactor, and the resulting model can then be used to model cumulative risk over a lifetime (EPA, 2007).
- *Uncertainty analysis.* Cancer risk assessments typically have a confidence interval that ostensibly portrays the range of plausible estimates of the risk. In addition to uncertainty arising from potential sampling error that is portrayed by most assessments, the current assessment employs a bootstrap analysis that also incorporates uncertainties arising from the dose estimates, model uncertainty, and lifetime risk calculations.

For purposes of comparison, Table 5 presents estimates for the risk for drinking water containing 1 ppb inorganic arsenic from both the model developed by the FDA and the model underlying EPA's 2001 regulation of arsenic in drinking water (EPA, 2000a). Since the estimates are based on assumed values for both water consumption and arsenic concentration, they are not estimates of actual risk. While the central estimates are nearly identical, the confidence intervals are much wider in our assessment. The greater width of the confidence intervals can be attributed to the

inclusion of uncertainties arising from dosimetry and dose-response model choice in our analysis.

Table 5. Comparison of Risk Estimates[1] for Inorganic Arsenic in Drinking Water at a Concentration of 1 ppb

Endpoint	FDA 2013[2]	EPA 2000a (Morales *et al*, 2000)[3]
Lung Cancer	30 (0, 123)	33 (29, 38)
Bladder Cancer	32 (0, 69)	32 (28, 37)
Total	66 (1, 165)	66 (59, 73)

[1]Estimates are cases per million people.
[2] Estimate based on analysis of Chen et al (2010a and 2001b) study (shown in Figures 1 and 2) with a water intake of 2 L per day, and a body weight of 70 kg.
[3]Estimate based on model number 1 from Table 8 in Morales et al (2000), with linear extrapolation from the ED01.

Consumption of Apple Juice

For the purpose of estimating lifetime exposure, currently available food consumption surveys (i.e. the National Health and Nutrition Examination Survey (NHANES)) that characterize food consumption for only two days are inadequate for estimating individual consumption or the variation in long-term consumption in a population. In particular, the surveys are conducted at a particular point of time during a lifetime and do not characterize consumption during childhood and adulthood for the same individual. Therefore, it is not possible to estimate lifetime consumption rates at specific population percentiles. However, since NHANES does characterize food consumption of the population at all ages, the population average is representative of average per capita exposure over a lifetime. In order to characterize risks for consumers with higher consumption of apple juice, consumption levels of three times the average were also considered.

While the focus of this assessment is on lifetime consumption and exposure, children are always a population of concern when considering juice consumption. Consequently, this assessment considered the impact of only children's exposure and separately estimated risk from consumption of apple juice by children aged six or less.

NHANES is a two non-consecutive day food survey that measures consumption of over 6,000 different foods and beverages. The survey participants provide a recall of the types of foods and beverages consumed over the two days. This assessment used data collected from 2007 to 2008. From this two non-consecutive day food survey, consumption estimates for apple juice as an ingredient in juices and juice products were derived for 19 juices and juice products found in this survey. In order to derive estimates of apple juice consumption when used as an ingredient, recipes from the United States Department of Agriculture's (USDA) Food and Nutrient Database for Dietary Studies 4.1 (FNDDS 4.1) along with the Food Commodity Intake Database (FCID)

developed jointly by EPA and the USDA's Agricultural Research Service based on the Continuing Survey of Food Intakes by Individuals (CSFII) 1994 – 96, 1998 were used to estimate the percentage of apple juice in a particular juice or juice product.

Shown in Table 6 are the 19 specific food codes that were used in this analysis. For the first seven food codes the USDA (2010) FNDDS 4.1 database was used because it provided a detailed recipe for these specific juices and juice products, but did not provide a recipe for the remaining 12 food codes. Therefore, the EPA (2000b) FCID database was used to provide the recipe amount that apple juice contributed to these juices and juice products.

Table 6. Food Codes and Recipe Files Used to Derive Apple Juice Consumption Estimates.

Food Codes	Food Name	Recipe %	Recipe Source
64100100	FRUIT JUICE, NFS (INCLUDE MIXED FRUIT JUICES)	0.36	USDA (2010)
64100110	FRUIT JUICE BLEND, 100% JUICE	0.4	USDA (2010)
64100200	FRUIT JUICE BLEND, WITH CRANBERRY, 100% JUICE	0.3	USDA (2010)
64101010	APPLE CIDER (INCLUDE CIDER, NFS)	1	USDA (2010)
64104010	APPLE JUICE	1	USDA (2010)
67202000	APPLE JUICE, BABY	1	USDA (2010)
67202010	APPLE JUICE, W/ CALCIUM, BABY	1	USDA (2010)
67203000	APPLE W/ OTHER FRUIT JUICE, BABY	0.8	EPA (2000b)
67203200	APPLE-BANANA JUICE, BABY	0.93	EPA (2000b)
67203400	APPLE-CHERRY JUICE, BABY	0.96	EPA (2000b)
67203450	APPLE-CRANBERRY JUICE, BABY	0.92	EPA (2000b)
67203500	APPLE-GRAPE JUICE, BABY	0.75	EPA (2000b)
67203600	APPLE-PEACH JUICE, BABY	0.67	EPA (2000b)
67203700	APPLE-PRUNE JUICE, BABY	0.78	EPA (2000b)
67204000	MIXED FRUIT JUICE, NOT CITRUS, BABY	0.86	EPA (2000b)
67204100	MIXED FRUIT JUICE, NOT CITRUS, W/ CALCIUM, BABY	0.3	EPA (2000b)
67211000	ORANGE-APPLE-BANANA JUICE, BABY	0.4	EPA (2000b)
67230000	APPLE-SWEETPOTATO-JUICE,BABY FOOD	0.73	EPA (2000b)
67250150	MIXED FRUIT JUICE W/ LOWFAT YOGURT, BABY FOOD	0.19	EPA (2000b)

Exposure estimates are reported for two age groups (zero to six years of age and zero to 100 years of age). The mean exposure estimates for these two groups were derived using the Crème (2011) software. These exposure estimates incorporate the statistical survey weights from the 2007 – 2008 NHANES survey. Since all of the dose-response models employed in the current assessment have a linear dose-response relationship at low doses, the per capita mean exposure may be used to estimate disease rates for the total population. Consumers with higher rates of consumption will have higher estimated risks, and an exposure corresponding to three times the per capita average was used to characterize the risk of frequent consumers of apple juice. The resulting apple juice consumption estimates are presented in Table 7.

Table 7. Apple Juice Consumption Estimates

	NHANES Average (g/kg-day)	3 x Average (g/kg-day)
Children aged 0-6	4.1	12.3
All Persons aged 0-50	0.83	2.5
All Persons	0.62	1.9

The per capita average is based on a two day survey. While the per capita average for chronic and lifetime exposure may be expected to be the same, it is not possible to estimate chronic exposure at particular population (e.g. 90th, 95th) percentiles. The value corresponding to an intake that is three times higher than the average is used for a sensitivity analysis for high-intake apple juice consumers.

Arsenic Concentrations in Apple Juice

FDA has been monitoring arsenic in apple juice since 2005 as part of the Toxic Elements Program (TEP). The TEP has tested 159 samples during that time with the majority of those coming in the last year. Many of the TEP samples were juice concentrates (107 of 159). These require calculation of equivalent concentrations of juice when reconstituted by the addition of water. In addition to the TEP, an apple juice survey (AJS) consisting of 94 samples was collected in October 2011. Unlike the TEP samples, these were all finished apple juice products collected at the retail level. Results are summarized in Tables 8 and 9. Since the arsenic concentrations in most of the samples were not speciated, total arsenic is reported. However, concentrations of organic arsenic species were below the level of quantitation in most of the samples that were speciated, indicating that most of the arsenic in the apple juice samples was inorganic arsenic.

Table 8. Total Arsenic Concentration Results by Survey and Year

Survey and Year[1]	Samples	As Concentration[2] Range (ppb)	As Concentration Average[3] (ppb)
TEP 2005	3	ND to 4	1.7
TEP 2006	2	ND to 7	3.8
TEP 2007	1	ND	0.5
TEP 2008	29	ND to 45	8.8
TEP 2009	16	ND to 25	7.8
TEP 2010	11	ND to 34	6.6
TEP 2011	97	ND to 29	2.7
All TEP[4]	153	ND to 45	4.7
AJS 2011	94	ND to 36	6.2
All 2011	191	ND to 36	4.4
All[4]	247	ND to 45	5.2

[1] –Toxic Elements Program (TEP 2005 to 2011) data are available at http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/Metals/ucm273328.htm . Apple Juice Survey (AJS 2011) data are available at http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/Metals/ucm283725.htm .

[2] - For samples where As was measured in concentrate, corresponding single strength values were calculated.

[3] - For the purpose of calculating average levels, levels below one or the level of quantitation were assumed to be 0.5.

[4] - Because of a change in analytical methodology in 2008, the six 2005 to 2007 samples were excluded.

Visit the FDA Arsenic in Apple Juice webpage for more information, http://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm280209.htm.

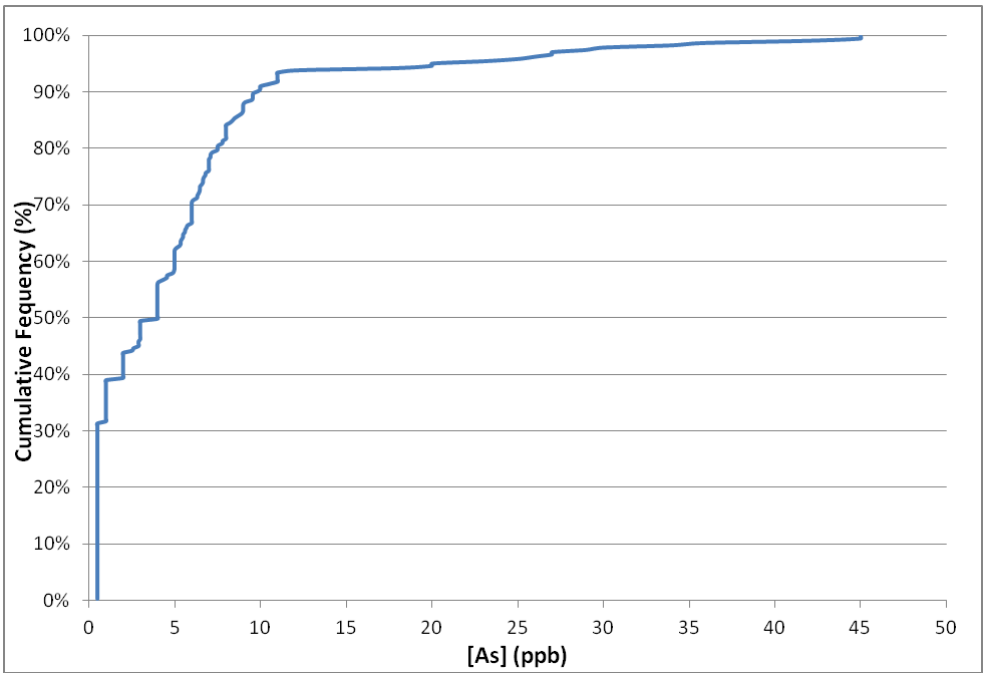
Exposure assessments for inorganic arsenic were estimated with several scenarios. First, exposure was calculated by assuming fixed levels for inorganic arsenic in apple juice. Specifically levels of three, five, 10, 20, and 50 ppb were evaluated. As a more realistic alternative, lifetime exposure was assumed to correspond to the average concentration in apple juice, and limits of three, five, 10, 20, and 50 ppb were evaluated by truncating empirical distributions at the specified limit. Two different empirical distributions were employed. The first used all 247 observations from both the Toxic Element Program and the Apple Juice Survey (see Figure 3) collected between 2008 and 2011. The second used results from the Apple Juice Survey only and used determinations of inorganic arsenic rather than total arsenic (see Figure 4).

Table 9. Comparison of Total Arsenic Determinations from Concentrate vs. Finished Product

Survey and Sample Type	Samples	As Concentration Range[1] (ppb)	As Concentration Average[2] (ppb)
TEP All Concentrate[3]	103	ND to 34	2.6
TEP 2008-2010 Juice	30	ND to 45	11.0
TEP 2011 Concentrate	77	ND to 8	1.8
TEP 2011 Juice	20	ND to 29	5.9
TEP All Juice[3]	50	ND to 45	9.0
AJS 2011 Juice	94	ND to 36	6.2
All 2011 Juice	114	ND to 36	6.1

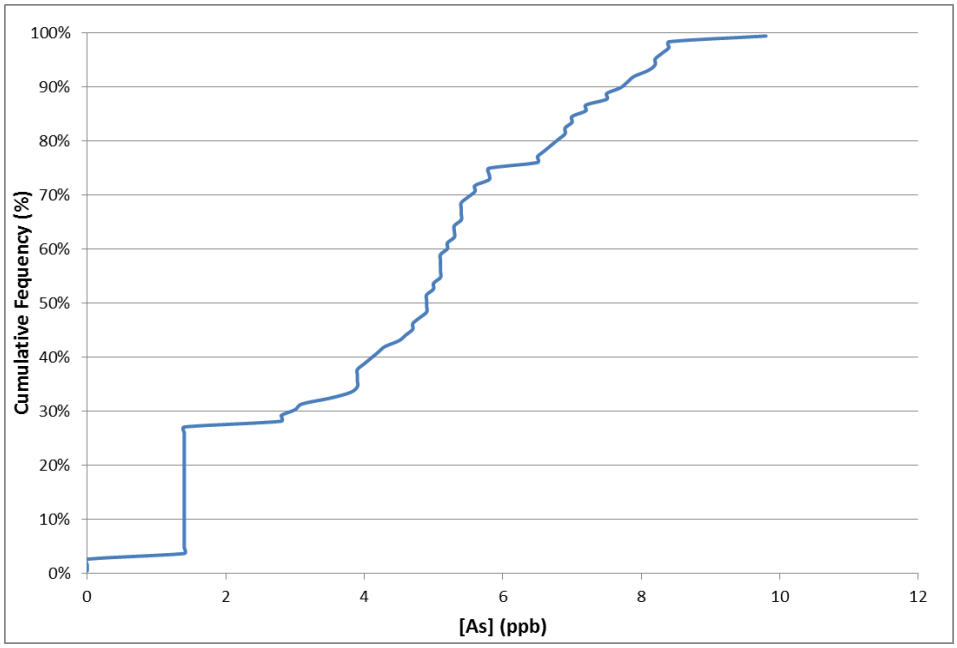
[1] - For samples where As was measured in concentrate, corresponding single strength values were calculated. This calculation assumes no additional arsenic is added when the juice is reconstituted by the manufacturer.
[2] - For the purpose of calculating average levels, levels below 1 ppb or the level of quantitation were assumed to be 0.5.
[3] - Because of a change in analytical methodology in 2008, the six samples from 2005 to 2007 were excluded.

Figure 3: Cumulative Frequency Distribution for All 2008-2011 Samples



Distribution of 247 samples collected from both the Toxic Elements Program Survey and the targeted Apple Juice survey between 2008 and 2011. Both finished juice product and single-strength juice concentrate values are included.

Figure 4: Cumulative Frequency Distribution for Inorganic As in 2011 Juice Samples Only



Distribution of 94 samples collected from the targeted Apple Juice survey in 2011. As concentrations reflect inorganic As only.

Visit the FDA Arsenic in Apple Juice webpage for more information, http://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm280209.htm.

Some of the differences between the two data sets may be attributed to the fact that the first data set characterizes a distribution for total arsenic in apple juice, rather than just inorganic arsenic. This impacts the distributions in two different ways. First, since the test for total arsenic is more sensitive, concentrations are reported at much lower levels. Second, while about 3% of the samples are above 10 in the total data set, there are no samples above 10 in the 2011 data set. As three of the samples were above 10 ppb for total arsenic, this difference can largely be attributed to the fact that inorganic arsenic was specifically measured. However, speciation does not change the fact that a higher percentage of the 2011 juice sample appears to have levels between three and zero ppb than would be expected on the basis of historical concentrations in juice concentrates. Average concentrations following the application of putative limits of three, five, 10, 20, and 50 ppb are given in Table 10.

Table 10. Average Concentrations of As in Apple Juice with Varying Limits

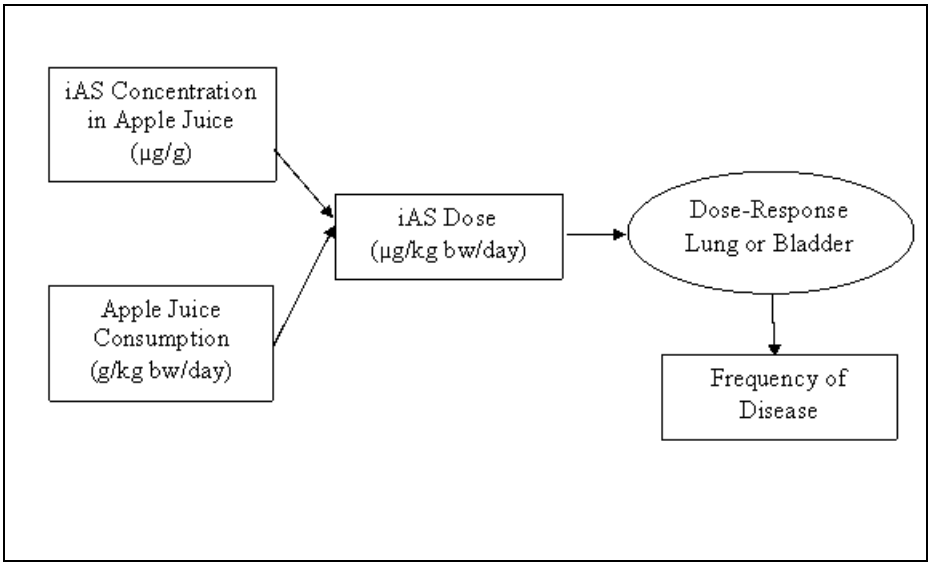
Limit	All 2008-2011 Total As Average[1] (ppb)	Juice 2011 iAs Only Average[2] (ppb)
3	1.0	1.4
5	1.7	2.7
10	3.4	4.4
20	3.9	4.4
50	5.2	4.4

[1] - For the purpose of calculating average levels, levels below 1 ppb or the level of quantitation were assumed to be 0.5 ppb.
[2]- For the purpose of calculating average levels, levels below 1 ppb or the level of quantitation were assumed to be 1.4 ppb.

Estimated Risks

The risk of cancer from exposure to inorganic arsenic in apple juice was determined by integrating the dose-response model and the exposure assessment using a Monte-Carlo simulation model with the structure shown in Figure 5. The exposure assessment provides an estimate of different doses based on consumption patterns (see Table 7) and levels of inorganic arsenic in apple juice (see Figures 3 and 4) for different populations (see Table 11). The dose-response model provides the relationship between the frequency of bladder and lung cancer relative to difference doses (see Figures 1 and 2). Tables 11-13 provide the estimated risk of cancer for different exposures (childhood, ages 0 to 50, and lifetime) and different concentration levels of inorganic arsenic in apple juice (3-50 ppb).

Figure 5: Diagram Showing Interrelationship of the Risk Assessment Model Components



Risks were estimated as cancer disease rates attributable to inorganic arsenic from apple juice consumption at each arsenic juice level for three different chronic exposure estimates:

- The first estimate is based on childhood exposure only (ages 0-6). The estimated average lifetime dose was prorated for a 50 year (see below) exposure period (i.e. 50/7, or a factor of about seven). That is, these estimates presume that there is no exposure to arsenic after the age of six.
- The second estimate is based on exposure from age zero through age 50, where 50 represents the average lifetime in Taiwan minus the 25 years required for increased cancer incidence to peak after a 12 year episode of increased exposure to arsenic in northern Chile. We believe that this exposure estimate is the most relevant for a cancer risk assessment for arsenic.
- Finally, an estimate for risk based on average lifetime exposure is presented, which represents the traditional method for estimating lifetime risks.

Results for total cancer incidence, where urinary tract and lung cancers were added together, are shown in Table 11. Because the risks estimated from lifetime exposure include arsenic intake at later ages as well, the estimated risks are higher than when exposure from children is considered alone.

Table 11. Total Lung and Urinary Tract Cancer Disease Rates Per Million From Exposure to Inorganic Arsenic in Apple Juice at Five Different Fixed Concentration Levels

iAs Level (ppb)	Average Consumption (per million)	3x Average Consumption (per million)
	Childhood Exposure Only	
3	3.8 (0.0, 10.2)	11.7 (0.0, 30.5)
5	6.4 (0.0, 16.9)	19.8 (0.1, 50.8)
10	13.1 (0.1, 33.9)	40.1 (0.4, 101.6)
20	26.4 (0.2, 67.7)	81.8 (1.2, 203.1)
50	67.8 (0.9, 169.3)	211.0 (5.6, 507.7)
	Exposure Ages 0-50	
3	5.4 (0.0, 14.4)	16.8 (0.1, 43.2)
5	9.1 (0.0, 24.0)	28.1 (0.2, 72.0)
10	18.7 (0.1, 48.0)	57.3 (0.7, 143.9)
20	37.8 (0.3, 96.0)	116.9 (2.2, 287.8)
50	96.6 (1.6, 239.9)	303.1 (10.1, 721.1)
	Lifetime Exposure	
3	4.0 (0.0, 10.8)	12.4 (0.1, 32.3)
5	6.8 (0.0, 17.9)	20.9 (0.1, 53.8)
10	13.9 (0.1, 35.8)	42.4 (0.4, 107.5)
20	28.0 (0.2, 71.7)	86.5 (1.3, 215.0)
50	71.9 (1.0, 179.2)	223.4 (6.2, 537.4)

All estimates are for combined case rates for both lung and urinary tract cancer per million persons with the 5th and 95th percentiles given as confidence intervals.

In Table 11, disease rates were calculated assuming that all apple juice consumed by an individual would be at the specified residue level over a lifetime. Tables 11 and 12 show estimated disease rates if apple juice is consumed at an average concentration over a lifetime at each arsenic limit. For Table 12, the average residue level is based on all 248 samples analyzed by the FDA between 2008 and 2011. Samples below the level of quantitation of approximately one ppb were assigned a value of 0.5 ppb. For Table 13, the average residue level is based on 94 samples analyzed by the FDA in the last year, where all samples were specifically analyzed for inorganic arsenic. Samples below the level of quantitation of approximately 2.8 ppb were assigned a value of 1.4 ppb. The impact of each limit was evaluated by assuming that juices in excess of the specified limit are eliminated from the food supply, so that the average concentration is reduced. If high and low concentrates were blended together to reach the limit, the average concentration would remain the same and the estimated risk would also be unchanged.

Table 12. Total Lung and Urinary Tract Cancer Disease Rates Per Million From Exposure to Total Arsenic in Apple Juice at Five Different Concentration Limits, All Sample Data 2008-2011

iAs Limit (ppb)	Average As[1] (ppb)	Average Consumption (per million)	3x Average Consumption (per million)
		Childhood Exposure Only	
3	1.0	1.3 (0.0, 3.4)	3.8 (0.0, 10.2)
5	1.7	2.1 (0.0, 5.8)	6.5 (0.0, 17.3)
10	3.4	4.3 (0.0, 11.5)	13.3 (0.1, 34.5)
20	3.9	4.9 (0.0, 13.2)	15.4 (0.1, 39.6)
50	5.2	6.6 (0.0, 17.6)	20.6 (0.1, 52.8)
		Exposure Ages 0-50	
3	1.0	1.8 (0.0, 4.8)	5.4 (0.0, 14.4)
5	1.7	3.0 (0.0, 8.2)	9.3 (0.0, 24.5)
10	3.4	6.1 (0.0, 16.3)	19.0 (0.1, 48.9)
20	3.9	7.1 (0.0, 18.7)	21.9 (0.1, 56.1)
50	5.2	9.5 (0.0, 24.9)	29.2 (0.2, 74.8)
		Lifetime Exposure	
3	1.0	1.3 (0.0, 3.6)	4.0 (0.0, 10.8)
5	1.7	2.3 (0.0, 6.1)	6.9 (0.0, 18.3)
10	3.4	4.6 (0.0, 12.2)	14.2 (0.1, 36.6)
20	3.9	5.2 (0.0, 14.0)	16.3 (0.1, 41.9)
50	5.2	7.0 (0.0, 18.6)	21.8 (0.1, 55.9)

All estimates are for combined case rates for both lung and urinary tract cancer per million persons with the 5th and 95th percentiles are given as confidence intervals.

[1]-Total arsenic. The risk estimates presume that all of the arsenic is inorganic. Since most but all of the arsenic is inorganic, these estimates are slightly conservative.

Table 13. Total Lung and Urinary Tract Cancer Disease Rates Per Million From Exposure to Inorganic Arsenic in Apple Juice at Three* Different Concentration Limits, 2011 Sample Data Only

iAs Limit	Average iAs[1]	Average Consumption	3x Average Consumption
(ppb)	(ppb)	(per million)	(per million)
		Childhood Exposure Only	
3	1.4	1.8 (0.0, 4.8)	5.4 (0.0, 14.3)
5	2.7	3.4 (0.0, 9.0)	10.4 (0.0, 27.1)
10 and above	4.4	5.6 (0.0, 15.0)	17.5 (0.1, 45.0)
		Exposure Ages 0-50	
3	1.4	2.5 (0.0, 6.8)	7.7 (0.0, 20.3)
5	2.7	4.8 (0.0, 12.8)	14.9 (0.1, 38.5)
10 and above	4.4	8.0 (0.0, 21.3)	24.9 (0.2, 63.8)
		Lifetime Exposure	
3	1.4	1.9 (0.0, 5.0)	5.7 (0.0, 15.1)
5	2.7	3.6 (0.0, 9.6)	11.0 (0.0, 28.7)
10 and above	4.4	6.0 (0.0, 15.9)	18.5 (0.1, 47.7)

*This table only displays risk at 3 ppb, 5 ppb, and 10 ppb since none of the samples in the subset of 94 samples taken during October 2011 had inorganic arsenic levels above 10 ppb. Consequently, risk at all arsenic concentrations above 10 ppb would be identical to risk at 10 ppb. All estimates are for combined case rates for both lung and urinary tract cancer per million persons with the 5th and 95th percentiles are given as confidence intervals.
[1]-Inorganic arsenic only.

While the risks based on average consumption are indicative of per capita disease rates, the risks will obviously be higher for those individuals who consume more apple juice. Because the modeled dose response relationship is approximately linear at low doses, the estimated risks at three times average consumption are roughly three times higher than at average consumption. It is not unlikely that a few individuals have a lifetime apple juice consumption rate that is 10 times the average, and those individuals may be expected to have roughly 10 times the risk. However, since available food consumption surveys are not designed to provide estimates of chronic lifetime exposure, it is not possible to estimate how many individuals have those levels of apple juice consumption.

Conclusions

Arsenic is a ubiquitous environmental elemental contaminant that arises from natural and anthropogenic sources. Arsenic is found in a number of substrate/media, including food and drinking water, and exists in a variety of forms/species. The forms of greatest toxicological and public health concern are the inorganic forms, tri- and pentavalent arsenic. Inorganic arsenic is the primary form found in water. Organic species of arsenic are the primary forms found in food, particularly in seafood, but inorganic forms can also be found in some foods, particularly rice and in some fruit juices. The inorganic forms can elicit a variety of toxicological effects,

including cancers in several organ systems. As a result, the primary health concern with long-term, lifetime exposure is the carcinogenic effects, particularly in the lung and urinary tract. The key hazard information for a dose-response assessment is the evidence from prospective human environmental epidemiology studies of the adverse effects of lifetime exposure to inorganic arsenic in well water.

The resulting risk estimates indicate that there are per capita urinary tract and lung cancer risks of approximately one in one hundred thousand (based on the modeled disease rates of 8.0 cases per million people at average levels of consumption, and noting the confidence intervals as reproduced in the table below). Persons with higher rates of apple juice consumption may be expected to have risks that are proportionally higher. The comparison of the estimates between lifetime exposure and childhood exposure indicate that a majority of the exposure is achieved during childhood, and therefore, most of the risk from apple juice occurs as a result of exposure during childhood. Because there is strong evidence of a 25 year latent period for the peak impact of arsenic on lung and bladder cancer (Marshall et al., 2007), we believe the estimates utilizing an exposure period of 0-50 years of age is the most appropriate for arsenic cancer risk. We also believe that the samples of finished juice product are more representative of apple juice currently sold in the United States than are estimated values based on juice concentrate. As a result, we conclude that the values presented in the table below are best suited for the purpose of evaluating potential guidance values for inorganic arsenic in apple juice:

Limit (ppb)	Total Cancer Rate (per million)
3	2.5 (0.0, 6.8)
5	4.8 (0.0, 12.8)
10 and above	8.0 (0.0, 21.3)

These values are reproduced from Table 12. They are presented here for emphasis.

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