

**Arsenic Bioavailability from Florida Soils:  
Uncertainty Evaluation of the University of Florida/  
Florida Department of Environmental Protection Study**

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## **A. INTRODUCTION**

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For nearly eighteen months, the Methodology Focus Group (MFG) has considered in-depth whether there is an appropriate technical basis to revise the assumed bioavailability factor for arsenic in soil used in calculating the direct-contact soil CTLs. This topic has actually been on the MFG agenda consistently since 1999, however, it has been the primary focus of four full-day meetings since August, 2001 with 20-30 stakeholders participating in each meeting. A research team from The Center for Environmental and Human Toxicology at the University of Florida headed by Professor Stephen Roberts was commissioned by the FDEP to complete a soil arsenic bioavailability study (the UF/FDEP study). Professor Roberts is a well regarded research scientist who has chaired national advisory panels, including for U.S. EPA, had numerous research articles accepted by the top scientific journals in the field, and has interacted with the other principal American research teams in the field of soil arsenic bioavailability for a number of years. He also currently serves on the Committee on the Bioavailability of Contaminants in Soils and Sediments of the National Research Council. Professor Roberts' team undertook to measure soil arsenic oral bioavailability from samples of Florida soils following their ingestion by cebus monkeys.

The objective of the UF/FDEP study was to develop data on relative arsenic oral bioavailability from soils for sites that are specifically relevant to Florida. Soil samples were collected from sites within Florida and were intended to represent a variety of types of arsenic contamination sources and typical varieties of Florida soils. Soil samples were collected from five locations as follows: two from pesticide sites; one from a power company site; one from a cattle dip site; and one from a chromated copper arsenate (CCA) wood treatment site.

During the August 1, 2001 MFG meeting, one issue discussed at length was whether the uncertainties associated with the UF/FDEP study could be qualified or quantified adequately to have confidence in the study's appropriateness for use in determining a state-wide, conservative, default bioavailability adjustment factor for arsenic in soils. Subsequent meetings of the MFG were conducted in Tampa on October 9, 2001, in Tallahassee on April 2, 2002 and in Tallahassee in December, 2002 (with the full Soils Forum) to consider additional issues and to discuss drafts of this Uncertainty Evaluation. This January, 2003 Uncertainty Evaluation discusses the primary areas of uncertainty under consideration by the MFG in anticipation of potential recommendations to the Contaminated Soils Forum and the FDEP.

## **B. PRIMARY SOIL TYPES IN FLORIDA**

Each of the soils that were fed to the monkeys during the UF/FDEP study was examined subsequently by a registered professional geologist to determine soil type and basic characteristics. The aliquots that were examined had been

screened with a 2 mm screen and sieved with a 250 micron sieve. This screening process was conducted before analysis for arsenic content and before dosing the monkeys. It is not expected that this screening process impacted appreciably the chemical or geologic characterization of the soils. It can be said that, in all likelihood, sieving to 250 microns helped to provide a more bioavailable, and “realistic” soil sample from an exposure perspective. The smaller, more uniform size particles more closely mimic the conditions of exposure that might be expected for adults or children who come in contact with soil (i.e., soil adhered to skin surface or airborne particles that may be inhaled and subsequently ingested).

## **1. Florida Soil Orders**

One way to approach the evaluation of whether the soils used in the UF/FDEP bioavailability study adequately typify a range of Florida soils is to compare the study samples with the major soil types present in Florida. Soils in Florida are represented by seven Orders (Natural Resources Conservation Service [NRCS] Soil Classification System, see Attachment A). Two Orders occur to a minor extent (Mollisols and Inceptisols), one occurs to a moderate extent (Alfisols), and four occur extensively (Histosols, Spodosols, Ultisols, and Entisols, see Attachment A). These Orders are further subdivided into five subcategories, resulting in dozens of different soil “types”. It is unrealistic to consider representation at this latter level. If the Orders that are present in Florida to a minor or moderate extent are eliminated, four Orders can be considered to represent the vast majority of Florida soils. One of these orders, Histosols, is a highly organic soil representative of peat and muck (swamp) areas. Areas with Histosols are unlikely to be developed for commercial or residential purposes and thus do not present a high potential for human exposure. The three remaining soil Orders are Spodosols, Ultisols, and Entisols.

Three of the five study samples were classified by FDEP as Ultisols, one as an Entisol, and the fifth was identified as fill material, probably dredged from offshore, and, therefore, is not classifiable with respect to soil type beyond observations about its origin. In this comparison, then, two of the three soil Orders likely to characterize a majority of areas with potential human exposure are represented by the study samples. Of the three most relevant Orders, the Order not represented, Spodosols, are commonly found in cool moist environments under coniferous forest vegetation. It should be noted that, although not classifiable in the typical manner, the fill material site may represent a considerable number of sites that may reside on comparable dredged/filled areas throughout the state, particularly in coastal locations.

The problem with trying to fit the study samples into representative soil Orders in Florida is that the NRCS system is a generic classification that does not define soil types based solely on mineralogy, but rather soil forming factors such as climate and parent material. In general, environments that share comparable soil forming factors produce similar types of soils. Two of the three major, relevant soil Orders in Florida are represented by the study samples.

## 2. Soils Used in the Bioavailability Study

Perhaps a more useful evaluation of the soil types used in the bioavailability study is to compare the lithologic/soil description with a qualitative evaluation of the typical surficial soils encountered in Florida. Additionally, soil mineralogy may be a key element in assessing the potential for a soil to contain arsenic at concentrations that pose a human health risk.

The raw soil samples were sieved to remove material coarser than 250 microns (0.25 millimeters), which corresponds to material coarser than fine grained sand. The sieving was conducted for ease of digestion and uniformity of size. Material excluded by the sieving consisted of organics (roots, sticks etc.) and predominantly quartz, with other components including mainly calcium carbonate (either calcite or aragonite) and, to a lesser extent, phosphatic grains. Calcium carbonate may comprise greater than 30% of a given sample. Phosphate usually comprises a very small fraction (<2%) of a sand sample. Quartz and calcium carbonate are not likely to contain appreciable quantities of arsenic as impurities in crystal lattice. While phosphatic grains may contain arsenic, phosphate generally comprises a very coarse fraction of a sand sample.

The five soils used in the bioavailability study were examined and described using standard lithologic descriptions and the Unified Soil Classification System (USCS). The descriptions are included in Attachment B. One of the study soils is described by FDEP as fill material, probably from offshore dredging, and is eliminated as a naturally occurring soil type.

Three of the remaining four samples are sands with varying amounts of finer grained material composed of silt, clay, and organic material. The fine-grained material may be in part composed of carbonate, as evidenced by reaction with dilute hydrochloric acid. The fourth sample is a fine-grained soil, predominantly silt and clay sized material, with abundant carbonate.

In a general sense, the four samples described above can be favorably compared to the types of natural surficial soils one encounters around the State. Sandy soils predominate across the state, especially in the upper few feet, and can vary from nearly pure quartz sands to sandy soils with varying amount of finer grained material. In Florida, it is generally accepted that sandy soils outnumber other soil types present in the upper foot of soil. While a clay-mineral rich soil was not represented in the study samples, that type of soil is not common in the upper foot, but more typically found at some depth. Clay minerals are a specific group of minerals characterized by small particle size and the ability to adsorb substantial amounts of water and ions on the surface and between layers in the particles. Clay size refers only to particles less than 2 to 4 microns in size, and may be composed of a variety of minerals and organic material as well as clay minerals. The Pesticide #1 and Pesticide #2 samples are both fine-grained and appear to contain quite a bit of clay-minerals. These samples provide a reasonable example of more clay-rich soils that also may be encountered in Florida.

### 3. Conclusion

*The UF/FDEP study does not include representation from all soil types in Florida, and in fact it would be impractical for any study to do so. The extent to which this represents a limitation in capturing the range of bioavailabilities possible in Florida soils is unclear because the role of the characteristics of soil, as the receiving medium for arsenic contamination, in determining arsenic bioavailability is poorly understood. To the extent to which soil type is important, a qualitative analysis of the soils used in the UF/FDEP study finds that they are typical of soils at the majority of sites where human health risks from arsenic contamination is an issue.*

## C. SITE TYPES, ARSENIC FORM AND AGING/WEATHERING

### 1. Discussion

Arsenic introduced into soils from anthropogenic activities initially may be present in a variety of inorganic and organic forms. Although arsenic is stable in four different oxidation states (-III, 0, +III and +V), non-mineralized forms of As(+III) and As(+V) are often the most prevalent forms at contaminated sites. Typically, inorganic arsenic contamination results from the application or disposal of arsenic trioxide (arsenous acid) or heavy metal arsenates and arsenites (e.g., lead arsenate, lead arsenite, chromated copper arsenate) that are associated with pesticides, herbicides and wood preservatives, as well as arsenic oxides and mixed metal oxides and sulfides from fly ash and from smelter operations. Organic arsenicals originate from derivatives of arsenic acid and arsonic acid, and include the herbicides cacodylic acid (dimethylarsinic acid), and the methylarsonic acid (MAA) derivatives, such as monosodium methanearsonate (MSMA) and disodium methanearsonate (DSMA). Two pesticide sites, a wood treatment site, a power company site and a cattle dip vat site were included in the UF/FDEP study in order to encompass and be representative of the typical anthropogenic arsenic release sources in the state. Other types of sites in Florida that may report arsenic impacts in soil include: golf courses, agricultural properties, railroads, sulfuric acid production, phosphorus mining/processing sites, steel mills/auto shredders, hide tanning and glass manufacturing plants. Site type may dictate the form of arsenic present, but the form of arsenic, as discussed further subsequently, is arguably not easily distinguished toxicologically and is not treated differently from a regulatory perspective.

The mobility and availability of arsenic at any particular site is related to its speciation and oxidation state as well as the site mineralogy. Organic arsenicals, while relatively immobile in soil, decompose, degrade and form volatile species at rates that are strongly dependent on factors such as soil sorption characteristics, soil Eh (redox potential) and pH, the intensity of UV irradiation

and the amount of microbial activity. Transformation processes of organic arsenicals include oxidation, reduction and demethylation or methylation reactions that can yield inorganic forms of arsenic and various volatile forms such as trimethylarsine. Significant amounts of cacodylic acid may be lost from anaerobic soil through volatilization, although demethylation processes tend to predominate. Soil microbes also can mediate the conversion of inorganic arsenic to methylated arsenicals. Microbial degradation or methylation of arsenic appears limited mainly to the soluble arsenic fraction. For reference, a typical soil half-life quoted for MSMA and DSMA is 180 days (OSU Extension and USDA ARS Pesticide Properties Databases), although considerable variability exists, with degradation proceeding slower in anaerobic soils than in aerobic soils. Cacodylic acid has a quoted half-life of 50 days (OSU Extension Pesticide Properties Database). While site-specific conditions may cause degradation rates to vary by more than an order of magnitude from nominal published rates, organic arsenicals are believed to ultimately degrade to arsenate through demethylation processes.

The binding of arsenic to soils is controlled by the soil Eh, pH, mineralogy and biogenic material. Both non-volatile organic and inorganic arsenic species adsorb most strongly to particles containing hydrous oxides of iron, followed closely by adsorption to aluminum oxides and, to a lesser extent, carbonate materials. Adsorption to manganese oxides or to particles containing organic carbon appears limited in many situations. In general, the presence of clay material (in acidic soils) or iron and aluminum oxides dominates the adsorption of arsenic, in spite of variations in organic matter, texture and cation exchange capacity. Once adsorbed, the development of a non-labile soil arsenic fraction has been observed, probably strongly chemisorbed within the matrix of iron and aluminum oxides. Precipitation reactions that deposit metal arsenates are another mechanism that contributes to limit arsenic mobility. The speciation of inorganic arsenic is influenced by the soil Eh/pH. Low Eh/pH conditions favor the more toxic arsenite species ( $\text{H}_3\text{AsO}_3$ ), whereas high Eh/pH result in the formation of arsenate ( $\text{H}_3\text{AsO}_4$ ). Because arsenite is adsorbed less strongly by soils than arsenate, flooding, acidification or other factors leading to low Eh/pH conditions can lead to increased arsenic mobility.

Analytical techniques have been developed for the speciation of arsenic, although there exists the potential to perturb the oxidation state(s) during sample collection and processing. Total arsenic is determined by digesting soil samples in strong mineral acids that may include the dissolution of silicates with hydrofluoric acid. Various extraction procedures have also been developed to examine inorganic fractions in soils. Generally, leaching procedures not utilizing concentrated mineral acids leach only a fraction of the total arsenic in a soil; however, sequential extraction procedures can be used to gain information about the manner in which arsenic is bound to soil. A variety of methods have been devised to measure organic arsenicals; non-oxidative leaching procedures are necessary to avoid transforming alkylated species. The aging of arsenic contaminated soils has been observed to diminish the recovery of organic and inorganic arsenic when utilizing mild leaching procedures.



Considerable differences between organic and inorganic arsenic forms, as well as between the different valence states of inorganic arsenicals may be observed in the environment. However, insufficient evidence is available to support a robust toxicological distinction between the forms, as evidenced by earlier University of Florida studies (see Attachments C and D; CEHT, 1998a; CEHT, 1998b) and the Toxicological Profile for Arsenic from the Agency for Toxic Substances and Disease Registry (ATSDR, 2000). This primarily is true because of the interconversion between organic and inorganic forms and between arsenate and arsenite valences.

## **2. Conclusion**

*Arsenic can exist in a variety of forms in soils. The form of arsenic in soil could conceivably influence its bioavailability, and also its toxicity, but there is little definitive information on this topic. The U.S. EPA and FDEP make the simplifying, and probably conservative, assumption that all arsenic in soil is present as inorganic arsenic (arsenate or arsenite) and estimate risks based on total arsenic content. The UF/FDEP study was conducted in a manner consistent with this approach, assessing arsenic bioavailability from soils in terms of total arsenic.*

## **D. STUDY DESIGN ELEMENTS**

### **1. Study Dose and its Relevance to Human Exposure**

Arsenic doses that were administered to the *Cebus apella* monkeys in the UF/FDEP study ranged from 0.3 to 1.0 mg As/kg body weight. This range is considerably higher than the typical American human dietary intake (approximately 0.0005 to 0.001 mg As/kg body weight; ATSDR, 2000), and the adult Provisional Tolerable Daily Intake of 0.0021 mg As/kg body weight (ATSDR, 2000). Other studies of the relative oral bioavailability of arsenic from soils also have used arsenic doses higher than typical human exposures in order to permit reliable measurement of arsenic excretion. If arsenic oral bioavailability is a function of dose, these studies may over- or underestimate arsenic bioavailability from soils at typical exposure levels. Data from the UF/FDEP bioavailability study in monkeys, as well as reports in the literature on bioavailability of arsenic in swine, have found no evidence of an effect of arsenic dose on absorption within the dose ranges assessed in the studies. However, arsenic bioavailability over a full range of potentially relevant doses has not been explored in detail.

### **2. Number of Samples/Monkeys**

There is no standard or convention regarding the number of animals that would be required to develop a valid measurement of relative oral bioavailability of

arsenic (or other environmental contaminants) from soils. For a site in Montana, the U.S. EPA relied upon relative arsenic bioavailability as measured in three (3) cynomolgus monkeys (Freeman et al., 1995). More recently, U.S. EPA Region 8 has measured arsenic relative bioavailability from soils in swine (Casteel et al., 1997; Casteel et al., 2001). This study design used three (3) or four (4) animals per dose group. The UF/FDEP study measuring arsenic bioavailability from Florida soils (Roberts et al., 2002) used five (5) monkeys per dose group. Among studies in the arsenic oral bioavailability literature, only one used more animals per dose group - six (6) per group in a study of arsenic absorption from bog soil in dogs (Goren et al., 1993). Thus, to the extent that uncertainty in bioavailability measurements is inversely related to treatment group size, the UF/FDEP study is equal to or better in this respect than all others except one that are noted in the literature.

### **3. Control Scheme**

The design of the UF/FDEP study is such that each animal serves as its own control (i.e., a “repeated measures study”). The design of a number of other studies in the literature (notably the swine studies conducted for U.S. EPA Region 8) is based upon comparison of absorption of arsenic from soils in one group of animals with the absorption from water in a separate group of animals. Only the UF/FDEP monkey studies (and others of comparable design) provide bioavailability measurements for individual animals, and consequently offer better information about variability in bioavailability.

### **4. One-Time Dose Versus Chronic Dose**

Studies using a single dose are the norm for absorption/metabolism/excretion studies that do not necessarily have an overt toxic effect as their endpoint. A single dose is desirable so that the pharmacokinetic behavior of that dose can be traced in detailed fashion. Continued dosing over time may better mimic human exposure conditions, and may impact blood levels and toxicity, but the potential impact on assessment of bioavailability is unclear. If arsenic bioavailability from the gut was controlled by an active transport process rather than by diffusion, it is possible that chronic dosing could alter bioavailability by increasing or decreasing arsenic transport. This is purely speculative, however, and an arsenic transporter in the gut has not been identified. Nor is there reason to expect, based on observations with other inorganics, that chronic doses will alter the relative bioavailability of arsenic from soils. Finally, the UF/FDEP study offers empirical evidence that repeated doses does not change bioavailability. While the bioavailability measurements for individual soils are based on a single arsenic dose, each animal subject received several arsenic doses over the course of the study (termed “episodic repeated dosing”). There was no evidence for a trend of either increased or decreased arsenic bioavailability over time in the experimental animals.

## 5. Fed Versus Fasted

In the majority of oral bioavailability studies, including the UF/FDEP study, the animals were dosed in a fasted condition. While this tends to remove one source of potential variability of results and the confounding effects of background metal (e.g., arsenic) levels in food, or the potential complicating effects of food in the gastrointestinal tract, it does not mimic typical human patterns. In general, the presence of food in the stomach intuitively would tend to diminish bioavailability, but this phenomenon has not been studied for arsenic in soils. As one potential comparison, studies of the bioavailability of lead from soils in humans found that the presence of food in the stomach markedly decreased lead bioavailability (approx. 10-fold; Maddaloni et al., 1998). If a similar effect occurs for arsenic in soils, then studies on fasted animals are likely to provide an upper bound estimate of bioavailability in comparison to realistic general circumstances.

## 6. Monkey vs. Swine vs. Rabbit

Data on the relative bioavailability of arsenic from soils are available using rabbit, swine and monkey models. All three models rely principally on measurement of the excretion of arsenic in urine and feces to assess its absorption. Given this method of measuring bioavailability, coprophagia (fecal ingestion behavior) by the rabbit is an important limitation in using this species. Only one report in the literature has used the rabbit to measure arsenic bioavailability from soil, using a soil sample from an area affected by atmospheric deposition from a smelter (Freeman et al., 1993).

Several studies developed on arsenic oral bioavailability from soils have used young swine. These data initially were developed as part of a study on the bioavailability of lead from soil. Immature swine were selected so as to address the well recognized influence of age on the absorption of lead. Since there was interest also in assessing the oral bioavailability of arsenic, the same animal model was used as a matter of convenience. Relative bioavailability measurements on 14 soil samples using the swine model have been presented in a U.S. EPA report (Casteel et al., 1997). These samples were from smelter wastes (i.e., tailings), smelter slag, and mining wastes. Low mass recovery after the arsenic dose was noted in that study, which casts doubt on the accuracy of the bioavailability measurements. Referring to this problem, a subsequent review article on oral bioavailability of inorganics (Ruby et al., 1999) stated, "This difficulty with the swine model causes considerable uncertainty regarding the accuracy of the RAFs derived from this model." [Note: "RAFs" refers to relative absorption factors, the terminology used in the Ruby et al. (1999) paper to designate relative oral bioavailability.] Shortly thereafter, the swine model was used to measure arsenic bioavailability from five soil samples and three iron slag samples taken from a site in Oklahoma (Rodriguez et al., 1999). Mass balance data were not reported, so it is unclear whether the mass balance problem with the swine model was corrected for this study. Finally, arsenic bioavailability was

measured using the swine model for five soil samples from a site with arsenic contamination from use of an arsenic trioxide containing herbicide (Casteel et al., 2001). The mass balance problem with the model was apparently resolved for that later study, as mass balance values were in the expected range. The mass balance difficulties associated with most of the swine data are not an inherent problem associated with the swine model per se, but nevertheless affect the reliability of most of the data produced thus far with this model.

Both Freeman et al. (1995) and the UF/FDEP study (Roberts et al., 2002) used monkeys to measure arsenic bioavailability from soils. The physiological similarities between monkeys and humans are well recognized, and the Cebus monkey has been used in preclinical studies related to drug development, including studies of absorption (Roberts et al., 2002). The UF/FDEP study explicitly addressed the predictive value of the monkey model in assessing arsenic absorption. Data on the urinary and fecal excretion of arsenic administered orally and intravenously were compared with literature reports on studies in human volunteers and found to be in excellent agreement. The choice of a monkey model, coupled with the efforts made to confirm that arsenic absorption and excretion in the model mimics that in humans, were viewed by the MFG as important features of the UF/FDEP study in reducing uncertainty in extrapolation of results to humans.

## **7. Conclusion**

*The choices of animal model and experimental approach for the UF/FDEP study are sound and have advantages over the models and designs used in a number of other arsenic bioavailability studies. Some aspects of the study do not duplicate expected environmental exposures (e.g., the size of the arsenic dose, the feeding state of the subjects, etc.), which introduces some uncertainty in the extrapolation of results to humans. However, the MFG found no reason to predict that any of these differences would lead to an underestimation of bioavailability in humans, and one factor (the use of fasted animals) would probably tend to overestimate bioavailability relative to typical human environmental exposures.*

## **E. FIFRA SAP ISSUES**

### **1. Discussion**

In September of 2001, the U.S. EPA Office of Pesticide Programs submitted to the FIFRA Scientific Advisory Panel (SAP) a background document proposing, in part, a recommended relative bioavailability of arsenic when ingested in soil (see relevant excerpt in Attachment E). The background document proposed 25% as a general value to define relative oral bioavailability, based primarily on the results of the UF/FDEP study. In late October, 2001, the FIFRA SAP held an open meeting to consider several issues related to childhood exposure to CCA-

treated wood playground structures and CCA-contaminated soil. The report from that meeting, and the associated transcript, were made available on December 12, 2001 (excerpted in part in Attachment E of this report). With the Panel's primary emphasis on CCA-treated wood, one of the major concerns was the lack of bioavailability studies on soils specifically related to CCA-treated wood applications. One of the soils in the UF/FDEP study was from a "wood treatment facility", and exhibited a mean relative bioavailability of approximately 17% for the five monkeys, making the generic 25% recommendation apparently conservative even with respect to the site-specific CCA site data. 6q

The panel noted that none of the samples tested in the UF/FDEP study was from a playground soil, which was the subject of the risk assessment under review. They pointed out that arsenic from CCA in soil from a wood treatment facility (as tested in the UF/FDEP study) might have different bioavailability from arsenic in soil from a CCA wood playground structure. The panel was divided between those who felt an interim value of 25% was reasonable for application to arsenic from CCA in playground soils and those that felt 50% might be more appropriate. Consistent with the panel's recommendation that the Agency use a probabilistic approach for its risk assessment, another element of the panel recommended that a range of values be utilized, with some suggesting 25 to 50%, and one panel member suggesting an extreme range of values from near zero to 98%,. The overriding recommendation was that additional research was needed, focusing specifically on soils from CCA-treated wood applications with appropriate animal models and doses that simulate the exposure of children playing on or near structures with CCA contamination.

The above-mentioned documents, as well as the complete transcripts for the October, 2001 meeting are available via the Internet at [www.epa.gov/scipoly/sap/2001/](http://www.epa.gov/scipoly/sap/2001/).

## **2. Conclusion**

*While the FIFRA SAP concluded that an insufficient basis is available to recommend a particular value for relative bioavailability of arsenic in soil from a nationwide perspective, it is important to recognize that their charge was narrowly targeted at soils that have been contaminated as a result of CCA-treated wood applications.*

## **F. ASSESSMENT OF OTHER STUDIES**

### **1. Discussion**

As part of this evaluation of the UF/FDEP study, the MFG considered the potential role of other studies in characterizing the bioavailability of arsenic from Florida soils. A number of studies have been published, as summarized in Ruby

et al., 1999. None of these studies used samples of arsenic-contaminated soils from Florida, and in fact nearly all of the soil samples in these studies were from types of contaminated sites not found in Florida (e.g., mining and smelter sites). This was regarded as a severe limitation in providing information relevant to Florida contaminated sites.

Most of the arsenic bioavailability measurements in the literature were conducted for U.S. EPA Region 8 using the swine model (Casteel et al., 1997; Casteel et al., 2001). As discussed above, all but the most recent of these studies failed to demonstrate a reasonable mass balance. Although it has been reported informally that this problem has been solved, and that the corrected values were not substantially different from the originally reported bioavailabilities, no formal report confirming this could be found. Consequently, the MFG considered these data to be useful only in a qualitative sense to demonstrate reduced bioavailability of arsenic in soils.

The MFG expressed strong preference for studies published in the peer reviewed literature. Only two such studies were identified — one involving measurement of arsenic from a smelter in the rabbit (Freeman et al., 1993) and the other measuring bioavailability of a soil and a dust sample from a mining area in monkeys (Freeman et al., 1995). As discussed above, data from the rabbit were considered unreliable due to coprophagia by the animal subjects. The monkey study by Freeman et al. used the same basic study design as the UF/FDEP study, and although a different monkey species was used, this model probably has the same predictive value for humans as the one used in the UF/FDEP study. The soil sample examined in this study — from a mining site — has questionable relevance to Florida sites, but it is worth noting that the measured value (about 20% relative bioavailability, on average), was within the range of values observed for soils in the UF/FDEP study.

## 2. Conclusion

*A number of studies measuring arsenic bioavailability have been conducted, but none is as well suited for characterizing bioavailability from Florida soils as the UF/FDEP study. In fact, the many limitations of the other studies reduce their value for this objective to qualitative terms only, in the judgement of the MFG. The contribution of these studies is primarily to demonstrate the consistency with which it is shown that absorption of arsenic from soils is substantially diminished.*

## G. SUMMARY

The MFG found the UF/FDEP study to be well designed and utilized an animal model with minimal uncertainty with regard to relevance to humans. Some aspects of the UF/FDEP study [and in fact all studies of arsenic bioavailability from soils] do not duplicate exactly conditions of likely environmental exposure,

resulting in some uncertainty in the extrapolation to humans. However, the MFG found no reason to predict that any of these factors would necessarily result in an underprediction of bioavailability, and one of the factors (the use of fasted animals) in fact probably leads to an overestimation of typical arsenic bioavailability from soils. The study used actual contaminated soils rather than soil spikes, which is important in view of evidence that “aging” of arsenic in soils affects its bioavailability. The use of actual contaminated soil samples helped insure that the observations from the study were realistic and relevant.

Bioavailability was measured in soils from five sites, representing an array of different types of arsenic contaminated sites in Florida. Some differences in bioavailability among the five samples were noted, but overall the means fell within a range of 10 to 25% relative bioavailability. The MFG discussed the ability of these five samples to represent the range of bioavailabilities in soils at various sites in Florida. Although uncertainty regarding this was acknowledged, they noted that the soil types of the samples were typical of soils found at most sites in Florida, and were representative of the most common types of arsenic contamination problems in Florida (namely, sites contaminated with arsenical pesticides, cattle dip sites, wood treatment facilities, and electrical substations). As such, the MFG considered the range of bioavailabilities observed in this study to be representative of most, if not all, sites in Florida.

The MFG was aware that the EPA SAP evaluated data from the UF/FDEP study for the purpose of assigning a default bioavailability value for assessing risks from arsenic in soils nationwide from CCA-treated wood playground structures. The SAP did not reach a consensus on the use of the UF/FDEP study for this particular purpose, or on a recommendation for a national default arsenic bioavailability from soils for playgrounds with CCA-treated structures. Given that the UF/FDEP study was not designed to support the EPA’s CCA risk assessment, reservations regarding its use for that purpose expressed by some members of the SAP were considered understandable. However, the MFG did not think that this diminished the value of the UF/FDEP study in achieving its primary objective, which was to characterize bioavailability of arsenic from contaminated soils in Florida.

In attempting to understand the range of bioavailabilities that might be encountered in soils from Florida sites, the MFG considered the results of other studies of arsenic bioavailability from soils. All were considered to be of secondary value (i.e., qualitative use only) for this purpose. This assessment was made based on a variety of reasons, including: 1) none of the studies used soils from Florida, and nearly all used soils from types of contaminated sites not found in Florida; 2) few were published in the peer reviewed scientific literature; 3) most of the observations were made in studies in which reasonable mass balance was not demonstrated; and 4) one study used an animal model with poor reliability in measuring oral bioavailability relevant to humans (the rabbit). The MFG considered the principal value of these studies, for the purpose at hand, was in demonstrating that arsenic absorption from soils is substantially diminished.

## H. CONCLUSIONS

In the UF/FDEP study, relative bioavailability measurements were obtained from five different soil samples (5 soil samples, each with bioavailability measured in five (5) different animals, for a total of 25 observations). The mean bioavailability results for each of the five sites in the UF/FDEP study ranged from a low of 10.7% to a high of 24.7%. The relationships between soil sample, animal, and bioavailability were examined through an analysis of variance, with “soil sample” and “animal” as main effects. The soil samples with the highest and lowest relative bioavailabilities were significantly different from each other ( $p < 0.05$ ); however, no other significant differences were observed among the five soils. Some variability in arsenic bioavailability was observed among animal subjects for each soil, which is to be expected. The average coefficient of variation was about 39%. Although some animals appeared to have somewhat greater bioavailability of arsenic than others, differences were not significant. The overall log-transformed mean for all 25 results was 16.8% and the overall 95% Upper Confidence Limit (UCL) of the transformed mean was 19.3%. Log transformation was performed because the data do not exhibit a normal distribution (U.S. EPA, 1992).

The data from the UF/FDEP study can be interpreted in a number of ways to identify upper bound and central tendency estimates of bioavailability. The following sections provide examples.

### 1. Potential Upper Bound Adjustment Factors

Several approaches are available for selecting an appropriate upper bound value in the range of approximately 25 to 32% to acknowledge arsenic bioavailability in soils. The 95<sup>th</sup> percentile value of the overall relative oral bioavailability results of the UF/FDEP monkey study (28.2%) represents a reasonable upper bound for adjustment of the oral component of the default SCTL. In addition, arithmetic mean values were calculated for each of the five soils that were used in the UF/FDEP study. The maximum of these values (24.7%) is a conservative, upper bound case for any particular soil type/site type combination in Florida. It should be noted that 25% bioavailability is the 85<sup>th</sup> percentile value for all of the relative bioavailability results from the UF/FDEP study, illustrating the upper bound nature of that value.

The maximum overall result of the UF/FDEP monkey study (maximum value for any soil sample; 32.4%) represents the absolute upper bound of the study. However, this is not judged to be an appropriately representative value for use in adjusting the Florida SCTL, based on historical precedent for the selection of other risk assessment assumptions. Only under highly specific, rare circumstances is the maximum value for a particular parameter used in environmental characterization, exposure assessment and risk assessment.



## 2. Potential Central Tendency Adjustment Factors

Central tendency values typically are presented as an estimation of the mid-range of a population distribution. One conservative and protective estimate of the central tendency is the 95% Upper Confidence Limit (UCL) of the mean. A relative oral bioavailability of 20% falls between the 95% UCL of the mean for all the results in the UF/FDEP study (19.3%;) and the overall mean of the Freeman et al. (1995) monkey study (20.4%; however, insufficient information and number of samples were available to calculate an individual 95% UCL for that later study).

The overall mean of the UF/FDEP study (16.8%) represents the 50<sup>th</sup> percentile value and, is the definitive central tendency value for adjustment of the oral component of the default SCTL, based on the UF/FDEP study. In addition, this value is in the range of the average values that have been demonstrated in other arsenic bioavailability study results.

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## **J. ATTACHMENTS**

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- Attachment A Soil Classifications
- Attachment B Descriptions of Soils Used in the Bioavailability Study
- Attachment C Risk Assessment of Organic versus Inorganic Arsenic
- Attachment D The Toxicity of Arsenite and Arsenate
- Attachment E Internet Access for FIFRA SAP Documentation

## ATTACHMENT A

### Soil Classification

Numerous soil classification systems are in use worldwide. The commonly used classification system in the U.S. is that of The Natural Resources Conservation Service (a branch of the U.S. Department of Agriculture). The major divisions in this classification system from general to specific, are: orders, suborders, great groups, subgroups, families, and series. At its lowest level of organization, the U.S. system of soil classification recognizes approximately 15,000 different soil series. The most general category of the NRCS soil Classification System recognizes eleven distinct soil orders: **Oxisols, Aridisols, Millisols, Alfisols, Ultisols, Spodosols, Entisols, Inceptisols, Vertisols, Histosols, and Andisols.**

#### Major Soil Orders in Florida

Seven soil orders represent the soils of Florida (Myers & Ewel, 1990):

1. Histosols – soils with a very high content of organic matter in the dark upper layer of the profile. Histosols form in places where organic matter is slow to decompose and thus accumulates over time. They are often found in bogs and swamps, and are often mined for peat. *These occur extensively in Florida.*
2. Spodosols – commonly found in cool moist environments under coniferous forest vegetation. Surface litter composed of pine needles breaks down in the presence of water to form a weak organic acid. Acidic soil water removes base ions in solution to create an acidic soil. Easily dissolved materials are leached from surface layers leaving behind the most resistant material like quartz creating an ashy-gray, near-surface layer. Layers at depth are stained with iron and aluminum oxides. *These occur extensively in Florida.*
3. Ultisols – highly weathered soils, they are often red/yellow in color reflecting the oxidation of iron and aluminum. Found in the moister portions of the humid subtropical climate, they have an illuvial clay layer which distinguishes them from oxisols, which do not. *These occur extensively in Florida.*
4. Entisols – immature soils lacking horizons because their parent material has only recently accumulated. Entisols also form where the parent material is quartz sand, in which horizons do not easily form. *Occur extensively in Florida, especially south Florida rocklands and sandhills.*
5. Alfisols – soils developed under forest covers and common in humid subtropical climates. These soils are well developed and contain a subsurface layer of clay called an argillic horizon. Very productive for agriculture. *These occur to a moderate extent in Florida.*

6. Mollisols – among the most fertile soils. Well known for their dark brown to black organic rich surface layers. *These occur to a minor extent in Florida.*
7. Inceptisols - soils just starting to show horizon development because the soil is quite young. *These occur to a minor extent in Florida.*

## ATTACHMENT B

### Description of Soil Samples Used in Bioavailability Study

Five soil samples were used in the bioavailability study:

<u>Designation</u>	<u>Location</u>
Power Co. #1	Holmes County
Cattle dip vat #1	Santa Rosa County
CCA #3	Alachua County
Pesticide #1	Monroe County
Pesticide #2	Dade County

The descriptions below are a combination of descriptive information supplied by the Florida Department of Environmental Protection (FDEP) and observations made by the author. Soil Order, when given, was supplied by FDEP. Descriptions by the author follow the Unified Soil Classification System (USCS).

Power Co. #1 – Yellowish-orange, fine- to very fine-grained quartz sand. Trace organics, trace to little silt and clay, USCS classification SP. Iron oxide/clay coatings on grains, very slightly plastic when moistened. Brief, mild effervescence in dilute (10%) hydrochloric acid (HCl). Soil Order – Ultisol.

Pesticide #1 – Brownish-gray, very fine-grained silt and clay. USCS classification ML. Described by FDEP as “limerock and silt fill, likely obtained from offshore dredging”. Strongly effervesces in dilute HCl.

CCA #3 – Brownish-gray, fine- to very fine-grained quartz sand. Trace organics, little silt and clay, USCS classification SP. Very slightly plastic when moistened. Mild effervescence in dilute HCl. Soil Order – Ultisol (loamy, siliceous).

Pesticide #2 – Orangish-brown clay and silt. Trace to little sand. USCS classification CL to ML. Forms ribbons when moistened. Vigorously effervesces in dilute HCl. Soil Order – Entisol.

Cattle Vat #1 Dip – Yellowish–orange, fine- to medium-grained quartz sand. Trace silt and clay. USCS classification SP. Slight plasticity when moistened. No reaction with dilute HCl. Soil Order – Ultisol.

## ATTACHMENT C

### Risk Assessment of Organic versus Inorganic Arsenic

0-01376

CEHT/TR-98-01

**TECHNICAL REPORT:**  
**RISK ASSESSMENT OF ORGANIC VERSUS INORGANIC ARSENIC**

Prepared for the  
Division of Waste Management  
Florida Department of Environmental Protection

L. E. Tonner-Navarro, Ph.D.  
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June 16, 1998

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## SUMMARY

Industrial, commercial, and agricultural activities involving arsenic compounds have resulted in numerous sites in Florida with elevated arsenic concentrations in soils. Chronic exposure to these soils is a potential health concern due to the toxic effects of arsenic, including carcinogenicity. Acceptable soil concentrations based on standard exposure assumptions and an incremental excess cancer risk of  $10^{-6}$  are only modestly above natural background concentrations for most Florida soils. As a result, even small elevations in arsenic concentrations above background can exceed cancer risk goals.

Arsenic exists in both organic and inorganic forms. Current regulatory practice does not distinguish between these forms, i.e., arsenic present in soils or other media is measured as total arsenic, and the risk of cancer or other health effects is based on this total arsenic concentration. A literature survey was conducted to determine whether a separate assessment of risk for inorganic and organic arsenicals could be justified. It was proposed that if such a distinction was defensible and, as acute toxicity studies suggest, organic arsenicals are less potently toxic, higher cleanup goals for organic arsenicals could be warranted. This would conceivably be important for sites where arsenic was introduced primarily in organic form, such as arsenic herbicide use on agricultural lands, golf courses, etc.

The literature survey addressed two issues: 1) What is the stability of organic arsenicals in the environment? Do they remain as organic arsenicals or are they converted over time to inorganic forms? and 2) Is there sufficient information with which to conclude that organic arsenicals are not carcinogenic? With regard to the fate of arsenicals in the environment, existing data indicate that there is interconversion of organic and inorganic forms, with a net conversion to inorganic arsenic. Some arsenic is lost through volatilization, but the rates are very slow. This means that whether applied in inorganic or organic form, the ultimate product of arsenic application is inorganic arsenic, and the arsenic is persistent. As a result, even if organic and inorganic arsenic were toxicologically distinct, consideration of risks in the future would have to assume that all of the arsenic was present in inorganic form.

Review of the toxicological literature suggests that at least some organic arsenicals may be carcinogenic. Dimethylarsinic acid (DMA) in particular has been studied and found to produce DNA damage as well as tumor promotion in the bladder, lungs, kidney, liver and other internal organs of laboratory animals. Long term administration of DMA has been found to result in increased bladder cancer in rats and fibrosarcomas in mice. Much less information relative to potential carcinogenicity is available for other organic arsenicals. It is possible that some may be potentially carcinogenic (like DMA) and others are not, but there is presently insufficient data to make this distinction. Clearly, a generalization that organic arsenicals are not potentially carcinogenic is inaccurate.

Information on the environmental fate and toxicity of organic arsenicals, though limited, do not support the development of separate risk-based acceptable soil concentrations for organic and inorganic forms of arsenic. In order for separate (higher) goals to be justified, organic arsenicals would have to be relatively stable in the environment and non-carcinogenic (or at least much less potently carcinogenic). Existing data do not adequately support either of these points, suggesting that arsenic should continue to be regulated on a total arsenic basis.

## INTRODUCTION

Arsenic is a metalloid present naturally in soils. Industrial, commercial, and agricultural activities have introduced additional arsenic into the environment, resulting in elevated soil arsenic concentrations at numerous sites in Florida. These sites are a potential health concern due to the well-established toxic effects of arsenic.

Although arsenic is capable of producing a variety of adverse health effects, the effect currently of greatest concern from chronic, low level exposure, such as from soils, is carcinogenicity. Ingestion of arsenic in drinking water has been associated with increased risk of cancer of the skin, bladder, lung, liver, kidney, and prostate (Chen et al., 1988, 1992; Chen and Wang, 1990) and the U.S. EPA has classified arsenic as a Group A carcinogen ("known to produce cancer in humans"). The U.S. EPA has used data from a large study of skin cancer in Taiwanese (Tseng et al., 1968; Tseng, 1977) to derive an oral cancer slope factor for arsenic, and this value is used in risk assessment to estimate cancer risks from arsenic ingestion from environmental media in general (i.e., water, soils, and sediments). The slope factor has also been used to develop risk-based criteria for arsenic in soils, such as U.S. EPA Soil Screening Levels (SSLs), U.S. EPA Region III Risk-Based Concentrations, and U.S. EPA Region IX Preliminary Remediation Goals, as well as Soil Cleanup Target Levels (SCTLs) for arsenic used by FDEP in recent rules (Chapter 62-770 and Chapter 62-785, F.A.C.).

Arsenic exists in both inorganic and organic forms. While the exposures upon which the arsenic oral slope factor are based were to inorganic arsenic (in drinking water), the U.S. EPA and other regulatory agencies currently make no distinction between inorganic and organic arsenic when assessing arsenic cancer risk. Concentrations in soils are measured as total arsenic, and this total (inorganic plus organic arsenic) concentration is used with an appropriate exposure model and the arsenic oral slope factor to calculate a cancer risk. Similarly, no distinction is currently made between inorganic and organic forms when comparing arsenic concentrations at a site with risk-based criteria or cleanup levels.

Using standard default assumptions regarding exposure, soil arsenic concentrations corresponding to a  $10^{-6}$  incremental excess cancer risk are calculated to be 0.8 ppm for residential land use and 3.7 ppm for industrial/commercial use (Tonner-Navarro and Roberts, 1997). These values are only modestly above natural background levels for most

of Florida soils, and as a result, relatively little arsenic contamination is needed to exceed these risk-based target concentrations. The extent of arsenic contamination problems in Florida, both in terms of numbers of sites and potential cleanup costs to achieve target risk levels, has led understandably to a close examination of acceptable arsenic concentrations for soils and their derivation.

One issue raised in discussions by the Arsenic Task Force is whether organic arsenicals should be evaluated differently from inorganic arsenicals. There is reason to believe that some arsenic contaminated sites in Florida were created by the introduction of predominantly organic arsenicals (e.g., golf courses and agricultural areas where methylated arsenicals were used as herbicides). The acute toxicity of organic arsenicals is significantly less than inorganic forms of arsenic leading to the impression that organic arsenicals are generally less toxic. If this were true, then perhaps higher arsenic soil concentrations could be justified for sites with primarily organic arsenical contamination. Because regulation of arsenic must consider its potential carcinogenicity, and carcinogenicity becomes the "risk driver" for arsenic when acceptable risk is defined as  $10^{-6}$ , the central question then is whether organic arsenicals should be considered carcinogens — and if so, at what potency? When contemplating whether to address inorganic and organic arsenicals separately, another issue that must be addressed is the stability of these forms in the environment. Risk-based evaluations must consider not only current site conditions, but conditions that can be anticipated in the future. If there is a transformation between organic and inorganic arsenicals under common environmental conditions, this could complicate risk assessment and management, particularly if there is a net conversion from one form to another.

In order to address these issues, a literature review was conducted for information pertaining to the potential carcinogenicity of organic arsenicals and to the fate of arsenicals in the environment. The information obtained from this literature review is summarized below.

## ARSENIC FATE IN THE ENVIRONMENT

Although inorganic insecticides such as lead arsenate, calcium arsenate, and copper arsenate are infrequently used today, historic use has resulted in the accumulation of inorganic arsenic in the soil of agricultural lands, groves, and orchards. The current use of

organic arsenical herbicides, such as arsenic acid, dimethylarsinic acid (DMA) (cacodylic acid), and monomethylarsonic acid (MMA) and its salts, monosodium methanearsonic acid (MSMA) and disodium methanearsonic acid (DSMA), continues to contribute arsenic accumulation in the environment (Ferguson and Gavis, 1972; Woolson, 1977). Following application, a large percentage of arsenicals are deposited in the soil and undergo a variety of interconversions and heterogeneous reactions producing various arsenic species. Different processes affecting the fate of arsenic ultimately impact the final concentration of arsenic in the soil, as well as surface water, groundwater, and air. These processes are part of the arsenic transport cycle. Typically, the cycling of arsenic between inorganic and organic states includes the adsorption and desorption to soils and sediments, as well as microbial interactions. While it is known that transformations and transport processes affect arsenic concentrations in soil, quantifying the degree of transport of the arsenical species within an ecosystem is difficult due to the complexity of the multimedia (sediment/soil-water-biota-air) exchanges of arsenic (Woolson, 1977).

**Inorganic arsenic.** Unlike organic arsenicals, the fate and transport of inorganic arsenicals in the environment is generally well understood and can be briefly summarized. In soils, inorganic arsenic can exist in mineral forms, as a complex with organic matter, or may bind to various soil cations. It can also be assimilated by plants, although the typical concentrations of arsenic in most major food items are below 0.1  $\mu\text{g}/\text{kg}$ . (Nriagu and Azcue, 1990). Generally, arsenic tends to be adsorbed by inorganic constituents found in soil matter by forming insoluble salts with soil cations (i.e. iron, aluminum, and calcium) which immobilize the arsenic in the soil matrix (Nriagu and Azcue, 1990). This immobilization process is more likely to occur in clayey soils and in soils with a high organic matter content, although arsenic may also be stable in sandy soils. The low volatility of arsenic, combined with the very low solubility of arsenic-soil cation complexes, means that arsenic has a low potential for leaching and can be very persistent in soil. As a result, arsenic introduced into the environment may accumulate in soil layers near the surface. The average residence of arsenic in soil is 2,400 years (Peters et al., 1996).

Soil phosphorus content can also affect the mobility of inorganic arsenic. Phosphorus and arsenic display similar chemistry in soil, and if the soil has a high phosphate ion content, there will be competition for binding sites by both arsenic and phosphate ions. In this case, arsenic may display slightly greater mobility and less stability in the soil. Inorganic arsenic in soil may also undergo redox reactions, which are

dependent on the iron level and pH of the soil. At high redox potential and iron levels, arsenic is usually found in the less toxic pentavalent arsenate form. Arsenate is the most prevalent form of arsenic found in soil, except when soils are extremely wet and the redox potential is very low. In these cases, more of the trivalent arsenite species will be present in soil. Arsenite may also be biooxidized by soil microorganisms to arsenate; this reaction is not dependent upon redox potential. The forms of arsenic produced by these redox reactions, i.e. arsenopyrite, are very stable in soil and do not account for loss of arsenic into other areas of the environment (Peters et al., 1996).

Although a major portion of inorganic arsenic in soil is adsorbed and rendered immobile by organic matter, the ultimate fate of arsenic in soil depends on several other factors as well. Arsenic in the top layers of soil can reach surface water via runoff. Initial deposition of arsenic on the soil surface may result in wind blowing the contaminated soil particles away from the site. It can be transferred to plants, or microbially decomposed to yield volatile arsine gases (Anderson and Abdelghani, 1985). The production of arsine gases from inorganic arsenic occurs as a result of both fungal and bacterial methylation processes. Gosio first discovered the process of fungal methylation in 1893. Since then, the formation of volatile arsenic species by fungi has been further verified (Challenger, 1933; Cox and Alexander, 1973). The methylation of arsenic by bacteria has also been extensively documented (Tamaki and Frankenberger, 1992). Arsenic as arsenate can also be reduced and methylated in soil to organoarsenicals, but this process is slow. Typically, the formation of inorganic arsenic from organoarsenicals and the production of arsine gases are the predominate reactions of arsenic in soil (Woolson, 1977).

**Organic arsenic.** Studies on fate and transport processes involving organic arsenicals are limited, most likely as a result of limitations in analytical methodology necessary for distinguishing among forms of organic and inorganic arsenic. Nevertheless, it has been found that organoarsenical herbicides, like inorganic arsenic, can be adsorbed to clay surfaces by interacting with iron and aluminum oxides, can leach from sandy soils, and are subject to metabolism (Woolson, 1977). The degree of interaction depends on soil type. In general, the net result of arsenic fixation by soil cations in combination with absorption to clay leads to a significant reduction in the mobility of organoarsenicals in the soil. Although data indicate that organic arsenicals can leach from sandy soils because they bind to soil matrices less strongly than inorganic arsenic (Woolson and Kearney, 1973), most studies agree that regardless of form, arsenic tends to remain in surface soils (Woolson, 1989; Bellet, 1992; and Coody and White, 1993). It should be noted, however, that DMA

and MSMA have the potential to be significantly mobile in soils with low organic/clay content. For example, if MSMA or DMA application is followed by heavy rain the arsenic can migrate to groundwater, especially when the depth to the groundwater table is limited (Personal communication with Dr. Woolson, January 7, 1998). Dickens and Hiltbold (1967), also suggest that DSMA association with soil is related to the clay content of the soil. As the percent clay decreases, leaching increases. Although leaching of organic arsenicals as a function of clay content is a valid concern regarding their use and application, evidence which suggests that organoarsenical herbicides can be metabolized by soil microorganisms is of greater curiosity (Hood, 1985).

Soil microbial species play a major role in the transformation and movement of organic arsenicals. Like inorganic species, organic arsenicals may also be modified by bacteria and fungi under both aerobic and anaerobic conditions to form volatile arsenic compounds. In studies conducted by Woolson (1977) using Mattapeake silt loam and radiolabeled DMA, MSMA, and arsenate (arsenic<sup>+5</sup>), it was concluded that the rate of formation of arsine gases in soil was dependent on the degree of methylation of the starting material and the level of aerobic activity. After 160 days, 18%, 12.5%, and 1% of the activity of DMA, MSMA, and arsenic<sup>+5</sup> was captured, respectively, indicating that organic arsenicals are more rapidly converted to arsine gases than inorganic arsenicals. As one might expect, reduction of methylated arsenicals is a direct process as compared to methylation followed by reduction required for the conversion of inorganic arsenicals to volatile arsenicals. In sewage, it was determined that low pH and anaerobic conditions facilitated the production of trimethylarsine (TA) gas from DMA, MMA, and arsenate by fungi, with production of detectable levels at pH 4 - 5 within 30 days and no production at pH 6 - 7 (Cox and Alexander, 1973). It has also been proposed that reduction to arsine gases from the corresponding arsenical with a like number of methyl groups, not methylation to trimethylarsine, is the primary mechanism for gaseous loss of arsenicals from soils (Chang and Focht, 1979). Using three soil types, Hanford sandy loam, Altamont clayey loam, and Domino silty loam, it was determined that arsine was produced in all three soils from all substrates (arsenic<sup>+3</sup>, arsenic<sup>+5</sup>, MMA and DMA), whereas methylarsine and dimethylarsine were produced only from MMA and DMA, respectively. In addition, soils treated with MMA and DMA produced arsine, suggesting that methylated arsenicals can also be demethylated and then reduced to arsine (Chang and Focht, 1979). Studies indicate that different mechanisms may be at work, i.e. methylation followed by reduction or demethylation followed by reduction, due to the variations in microorganisms. Nevertheless, arsine gases are formed as a by-product of organic arsenic in the soil

environment and are released into the atmosphere. Of the amount of arsenic lost from soil each year, release as arsine gases accounts for about 12 - 15% (Peters et al., 1996).

While volatilization in the form of arsine gases represents a mechanism for loss of organoarsenicals from soils, its impact on soil arsenic concentrations is probably minimal. Sandberg and Allen (1975) estimated the net loss of arsenic through volatilization following application of DMA (0.052 - 0.10 %/day) and MMA (0.055 - 0.070 %/day). When these compounds are applied at the maximum recommended rates, Sandberg and Allen predicted an increase in total soil arsenic residues at a rate of 2.6 to 3.3 ppm arsenic/ha/year for DMA and 1.5 to 1.9 ppm arsenic/ha/year for MMA, despite volatilization. Other factors that may influence rates of volatilization include amount of viable microbial populations in the soil and the iron/clay content of the soil. If these values are low, then the rates of volatilization will be reduced. As such, suspended arsenic application does not ensure dissipation of total arsenic residues over time (Personal communication with Dr. Woolson, January 7, 1998).

In addition to the formation of volatile arsenical gases from organic precursors, research has shown that methylated arsenicals, namely DMA, MMA, MSMA, and DSMA, are also subject to complete demethylation by a variety of soil microorganisms (Von Endt, et al., 1968; Abdelghani, et al., 1977; Woolson, 1977; Shariatpanahi, et al., 1981; and WHO, 1992.). In all studies it was found that the ultimate product of demethylation was inorganic arsenate and CO<sub>2</sub>. Von Endt et al. (1968) determined that bacteria had the greatest ability to demethylate DSMA, demethylating approximately 20% of 10 ppm DSMA within 3 days and up to 10% of MSMA in 60 days. Soil actinomycetes followed, with 10% metabolized in 7 days, and fungi were the slowest with only 3% metabolized in 11 days. Demethylation of MSMA was shown to peak at a rate of 29%/month (Abdelghani, et al., 1977). In moist aerobic soils, it was shown that 80% of 10 ppm DMA was degraded to arsenate and small amounts of MMA within 60 days, and under anaerobic conditions degradation was much slower. In field studies, MMA and DMA were still detected 1.5 years following application, but the major form was arsenate (Woolson et al., 1982). Based on this information it has been determined that the approximate half-life for field applied DMA or MMA is 20 - 22 days.

In summary, whether applied in inorganic or organic forms, the ultimate product of arsenic application is inorganic arsenic, namely arsenate, although under some conditions arsenite may be formed (Woolson, 1989). Two forms of oxidation are responsible for the

transformation of arsenicals: one destroys the carbon-arsenic bond and is associated with microbial activity and the other, a change in oxidation state, which may or may not be affected by microbial activity (Woolson, 1983). In the inorganic state, arsenate can react with iron and aluminum oxides to form insoluble complexes. In some situations, prior to metabolism of the organoarsenicals, the soluble forms such as DMA and MSMA can leach into groundwater or run-off into nearby surface water bodies. Also, the organic arsenicals and their demethylated by-products may be converted to volatile alkylarsines, although losses of arsenic from soil via this pathway are minor.

## POTENTIAL CARCINOGENICITY OF ORGANIC ARSENICALS

Organic arsenicals are of interest not only because of their introduction into the environment as pesticides and herbicides, but also because of their role in the disposition of arsenic within the body. Inorganic arsenic is methylated in the liver sequentially to MMA and DMA. In humans approximately 40-60% of an inorganic arsenic dose is excreted as DMA, 20-25% as inorganic arsenic, and 15-25% as MMA (ATSDR, 1993). Because methylated forms of arsenic (and other organoarsenicals) are less acutely toxic than inorganic arsenic, the methylation of arsenic has traditionally been regarded as a detoxification pathway. Recent research has suggested that this may not be the case, at least with respect to carcinogenicity.

Several researchers have shown that organic arsenicals are capable of inducing DNA damage. In both *in vivo* and *in vitro* studies, DMA has been demonstrated to cause DNA single strand breaks (Yamanaka and Okada, 1994; Kato, et al., 1994; Tezuka et al., 1993), heterochromatin clumping (Yamanaka and Okada, 1994; Nakano et al., 1992), protein cross-linking (Yamanaka et al., 1995; Kato, et al., 1994; Yamanaka, et al., 1993), adduct formation (Rin, et al., 1995), and tetraploidy (Endo, et al., 1992), several of which can lead to morphological alterations in cell structure and function. DMA induced Hsp 72 accumulation in the nucleus may also be indicative of the nucleus-specific damaging action of dimethylarsenic (Kato et al., 1997). In most cases, the DNA damage was attributed to the dimethylarsinic peroxy radical. Based on these observations, it has been proposed that DMA might be the carcinogenic form of arsenic (Brown et al., 1997; Endo et al., 1992).

The carcinogenicity of DMA has recently been studied in animals. In rat models, DMA has been shown to promote cancer in the bladder, lungs, kidney, liver, and other



internal organs (Yamamoto, et al., 1995 and 1997). This carcinogenic effect was seen in the bladder of rats at experimental concentrations as low as 50 ppm (the lowest dose used in this study). This effect at low concentrations, coupled with evidence that most of the absorbed DMA was excreted unchanged in the urine, may indicate that DMA is a bladder carcinogen or a potent promoter. Further evidence is provided by data that identify DMA as a promoter of bladder and liver cancer in rats (Wanibuchi, et al., 1996; Wanibuchi et al., 1997) and a promoter and progressor of lung tumorigenesis in mice (Yamanaka, et al., 1996). The bladder cancer studies revealed that there is a relatively low dose threshold for the promoting activity. Concentrations as low as 10 ppm DMA resulted in increased tumors, and one of the postulated mechanisms involved is increased cell proliferation of the bladder epithelium. The increased cell proliferation may be indicative of genotoxic damage. In a recent study (Okada, 1997), mice fed DMA for 50 weeks without previous initiation had an increased incidence of lung tumors. This suggests that, in addition to its promotion and progression activity, DMA may act as a complete carcinogen as well.

The USEPA's Health Effects Division Carcinogenicity Peer Review Committee has reviewed the carcinogenic potential of DMA (USEPA, 1993). This group recommended that DMA be classified as a probable human carcinogen (Group B2) based on increased bladder tumors in rats (Study No. PAL/010/CAC, 1989) and increased fibrosarcomas in female mice (Study No. PAL/014/CAC, 1990). Rats were fed DMA in concentrations from 0 to 100 ppm for 104 weeks, and had increased urinary bladder papillomas and carcinomas. Male rats had increased neoplasms at several doses, including the lowest (2 ppm), while female rats had increased neoplasms only at the highest dose. Non-neoplastic effects were also observed in treated rats, such as degeneration of the urinary bladder epithelium, nephropathy, and histological changes in the kidney and thyroid. In a study in which mice were fed DMA at concentrations from 0 to 500 ppm for 104 weeks, female mice at the highest dose had an increased incidence of multiple organ fibrosarcomas. In addition, degeneration of the urinary bladder epithelium, as well as glomerulonephropathy and nephrocalcinosis, was seen in the treated mice. The USEPA has not yet acted on the Peer Review Committee recommendation, and DMA remains listed as a Group D carcinogen on Integrated Risk Information System (IRIS).

Arguably, the animal evidence for carcinogenicity is stronger for DMA than for inorganic arsenic. In fact, it has been suggested that the carcinogenic effects of inorganic arsenic may be due to the organic metabolites:

“Based on the results of this and other studies, the acute lethality-based theory that methylation of inorganic arsenic is a detoxification pathway should be reconsidered. For carcinogenesis, methylation of inorganic arsenic may be a toxification pathway and DMA may be the active metabolite” (Brown et al., 1997).

Epidemiological studies of individuals exposed to organic arsenicals such as DMA would be helpful in assessing their carcinogenicity, but unfortunately such studies do not exist — the only data with which to evaluate carcinogenic effects of organic arsenicals are from animals and, at least in the case of DMA, these data are positive.

The observations with DMA appear to preclude a generalized assumption that organoarsenicals are not carcinogenic. The extent to which DMA is representative of other organoarsenicals in terms of carcinogenicity is unclear, however. It is possible that some organic forms are carcinogenic while others are not, but [with the exception of DMA] data relevant to assessing potential carcinogenicity of organoarsenicals are extremely limited. For the most part, information is simply not available with which to identify with confidence the organoarsenicals that do, and do not, have carcinogenic potential.

## CONCLUSIONS

Arsenic introduced into the environment is persistent and, regardless whether added in an organic or inorganic form, the ultimate product will be inorganic arsenic. This has important implications from a risk management perspective. Because arsenic in the environment is persistent, potential health impacts of arsenic contamination well into the future must be considered. Even if organic and inorganic forms of arsenic were toxicologically distinct and could be measured separately at a site, this would be relevant only for risks associated with exposure at the present. Net conversion of organoarsenicals to inorganic forms over weeks or months would change organic and inorganic arsenic concentrations, altering the attendant risks. Ultimately, the risk at the site would be based on all of the arsenic contamination as inorganic arsenic. For a site contaminated wholly or in part with organic arsenicals, any reduction in risk from considering organic forms to be less toxic would therefore apply only to the present, and not to the future.

Organic arsenicals are clearly less acutely toxic than their inorganic counterparts. However, with respect to the toxic effect of most interest currently from chronic arsenic exposure — carcinogenicity — there is some doubt whether organic forms of arsenic can in fact be considered less toxic. One organic arsenical, DMA, has been shown to be genotoxic, a tumor promoter, and positive in a cancer bioassay. Animal data for carcinogenicity of DMA are stronger than for inorganic arsenic, and there is even speculation that metabolism of inorganic arsenic to DMA in the body may be responsible for inorganic arsenic carcinogenicity in humans. Under the circumstances, it would be hard to argue that DMA in the environment should not be regulated as a carcinogen. For other organic arsenicals, there is much less information relevant to an assessment of potential carcinogenicity. It is certainly possible that there are non-carcinogenic organic forms of arsenic in the environment, but little information is available with which to clearly identify them as such.

In order to support a decision to regulate organic and inorganic arsenicals separately, there would need to be evidence of stability of these forms in the environment and a sound basis to distinguish between them toxicologically. A review of the literature and current state of knowledge unfortunately does not support either of these assumptions. Ongoing research may lead to new understanding of arsenic toxicology and fate in the environment, and it is possible that information will emerge that will permit a better, more refined estimate of risks posed by arsenic exposure. At this point in time, however, it would appear that the most prudent regulatory approach would be to continue to regulate arsenic contamination in soil without distinguishing between inorganic and organic forms, i.e., in terms of total arsenic.

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## **ATTACHMENT D**

### **The Toxicity of Arsenite and Arsenate**



## **FACT SHEET**

### **The Toxicity of Arsenite and Arsenate**

Prepared for the  
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November 6, 1998

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## **Background**

Arsenic, a metallic element found naturally in the environment in ores and soil, may exist in both organic and inorganic forms. Inorganic arsenic, whether naturally occurring or introduced anthropogenically, usually exists as either arsenate [ $\text{As}^{5+}$ ] (fully oxidized) or arsenite [ $\text{As}^{3+}$ ] (partially reduced). While analytical methods are available to distinguish between the organic and inorganic forms (Odanaka et al. 1983; Comber and Howard, 1989) and between the two valence states (Butler 1988; Mok et al. 1988; Rabano et al., 1989), they generally have not been approved for regulatory purposes. Concentrations of arsenic detected in environmental media are generally reported as total arsenic (e.g., without regard to speciation) (EPA 1983 1986a,b), except in Florida marine surface waters where arsenic is regulated as  $\text{As}^{3+}$  (Chapter 62-302 F.A.C.). The predominant form of inorganic arsenic in aerobic soil is arsenate. Arsenite predominates where there is low redox potential or where the soil is extremely wet (Sadiq, 1997; Peters et al., 1996). The purpose of this document is to review the available information on the relative toxicity of these two valence states of arsenic to determine if there is a toxicological basis for developing separate risk-based acceptable soil concentrations for arsenate and arsenite.

## **Acute Toxicity of Arsenate and Arsenite**

Arsenate and arsenite are thought to elicit acute toxicity via different mechanisms – arsenate by mimicking phosphate and interfering with ATP production in the mitochondria, and arsenite by binding to and inactivating sulfhydryl-containing enzymes (Lantz et al., 1994). Arsenite is generally considered more acutely toxic than arsenate (Naqvi et al., 1994). Reported arsenite  $\text{LD}_{50}$  values in rats vary from 15 mg/kg to 110 mg/kg, while a rat  $\text{LD}_{50}$  for arsenate has been reported as 110 mg/kg (ATSDR, 1993).

## **Chronic Toxicity of Arsenate and Arsenite**

Inorganic arsenic is associated with excess skin, lung, liver, bladder, and kidney cancers in humans following chronic exposure (Chen et al., 1988, 1990, 1992; IARC, 1980). Both arsenate and arsenite are genotoxic, capable of inducing chromosome aberrations and sister chromatid exchange in rodent and human cells. In this regard,

arsenite is approximately an order of magnitude more potent than arsenate (Wan, et al., 1982; Jacobson-Kram and Montalbano, 1985; Kochhar et al., 1996; Moore et al., 1997). Both forms of inorganic arsenic compromise pulmonary alveolar macrophage function at non-cytotoxic concentrations, with arsenite more potent than arsenate (Lantz et al., 1994). Both forms of inorganic arsenic produce tumors following intratracheal instillation to the lungs of hamsters (Yamamoto et al., 1987).

### **Metabolism of Arsenate and Arsenite**

Studies of arsenic metabolism in humans have primarily relied on the characterization of arsenic metabolites in the feces and urine following intake of various forms of arsenic. These studies suggest that two processes are involved: 1) reduction/oxidation reactions that interconvert arsenate and arsenite, and 2) methylation reactions that convert arsenite to monomethyl arsonic acid (MMA) and dimethyl arsinic acid (DMA). These methylation reactions are generally considered to be a protective mechanism against the acute toxicity of inorganic arsenic (ATSDR, 1992). Analysis of urine from humans exposed to either arsenates or arsenites reveals increased levels of DMA, MMA, arsenate, and arsenite, with the principal metabolite being DMA (Buchet et al., 1981a,b; Lovell and Farmer, 1985; Vahter et al. 1986). These illustrate that once absorbed, both arsenate or arsenite can be converted to the other valence state, confounding the toxicological distinction of the two species.

### **Conclusions**

In order to support the development of separate risk-based acceptable soil concentrations for arsenate and arsenite, there would have to be a sound basis to distinguish between them toxicologically. While the literature generally shows that arsenites are somewhat more toxic than arsenates, the Agency for Toxic Substances and Disease Registry (ATSDR) recommends the consideration of “the arsenates and arsenites as approximately equitoxic” (ATSDR, 1992). This recommendation is based on several factors, including the fact that in most cases, the differences in the relative potencies between these two chemical species are within the bounds of uncertainty of the no observed adverse effect level (NOAEL) and the lowest observed adverse effect level

(LOAEL). Additionally, the different forms of arsenic may be interconverted in the body and in the environment. Thus, the available scientific information does not support a decision to consider the two valence states of inorganic arsenic separately for regulatory purposes.

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## ATTACHMENT E

### Excerpted Material from the October 23-25, 2001 FIFRA SAP Meeting

The FIFRA SAP October 23 through 25, 2001 meeting materials may be found at the following internet address:

[www.epa.gov/scipoly/sap/2001/](http://www.epa.gov/scipoly/sap/2001/)

Scroll down to October 23-25, 2001 – CCA treated wood. The list of available documents includes the final report of the panel, *CCA Treated Wood Final Report*, as well as the transcripts of the meeting and the background document from which the questions/tasks were drawn, *Hazard Identification and Toxicology Endpoint Selection for Inorganic Arsenic and Inorganic Chromium*.



**SAP Report No. 2001-12**

**FIFRA Scientific Advisory Panel Meeting,  
October 23- 25, 2001, held at the Sheraton Crystal City  
Hotel, Arlington, Virginia**

**A Set of Scientific Issues Being Considered by the  
Environmental Protection Agency Regarding:**

**Preliminary Evaluation of the Non-dietary Hazard and  
Exposure to Children from Contact with Chromated Copper  
Arsenate (CCA)-treated Wood Playground Structures and  
CCA-contaminated Soil.**

## CHARGE

### **Issue: Short- and Intermediate-term Endpoint Selection for Inorganic Arsenic**

For inorganic arsenic, the data of Franzblau et al (1989) and Mizuta et al (1956) using a LOAEL value of 0.05 mg/kg/day is proposed for selection of short-term and intermediate-term incidental oral endpoints as well as short-term and intermediate-term dermal endpoints. An acceptable Margin of Exposure value of 100 is also proposed. The acceptable Margin of Exposure value includes a 10x factor for intraspecies variation as well as a 10x factor for use of a LOAEL value and the severity of the effects observed in the Mizuta study.

**Question 1: Please comment on the Agency's selection of the 0.05 mg/kg/day LOAEL value for use in assessing risks to the general population as well as children from short-term and intermediate-term incidental oral and dermal exposures, and the appropriateness of the use of a 10x factor for severity of the toxic effects observed in the Mizuta study. Please provide an explanation and scientific justification for your conclusions as to whether the presented data are adequate or whether other data should be considered for selection of this endpoint.**

### **Issue: Relative Bioavailability of Inorganic Arsenic**

The bioavailability of inorganic arsenic is dependent on the matrix in which it exists. For purposes of this discussion, the relative bioavailability of inorganic arsenic after ingestion of arsenic-contaminated soil is defined as the percentage of arsenic absorbed into the body from soil compared to that of arsenic administered in drinking water. Arsenic in drinking water is in a water-soluble form, and bioavailability by this route is high (i.e. 95-100%). Arsenic in soil, however, has reduced bioavailability due to existence in a water-insoluble form or its interaction with other soil constituents that impair absorption.

The available data on urinary and fecal recovery of arsenic after an intravenous dose of sodium arsenate in experimental animals compared to recovery after administration of sodium arsenate to experimental animals in soil was examined. Based on these data, a value of 25% bioavailability was selected for arsenic from soil ingestion. This value is based upon the data of Roberts et al. (2001) and Freeman et al. (1995) using non-human primates. These data were felt to best represent relative bioavailability of inorganic arsenic in soil based on the use of non-human primates and the physiological similarity in the pattern of metabolism with humans, and the use of CCA-contaminated soil in the study for estimation of bioavailability.

existing levels of arsenic in drinking water was cited as evidence for this. The US EPA has estimated that there are in excess of 1200 public drinking water systems in the United States that deliver drinking water with arsenic concentrations in excess of 20 ug/L (US EPA, 2001). Since the level of water consumption by some 3 year olds is 60 ml/kg (90<sup>th</sup> percentile estimate) (NRC, 2001), there appear to be many communities in the United States where young children have already been consuming >25 micrograms day. There are no reliable reports in the medical literature documenting or suggesting that adverse health effects from arsenic have occurred in these children. Several health surveys conducted in U.S. communities where the arsenic concentration in drinking water was several hundred micrograms per liter have also not detected adverse non-cancer effects (Harrington et al., 1978; Kreiss et al., 1983; Southwick et al., 1983). It was pointed out that both the Agency for Toxic Substances and Disease Registry (ATSDR) and U.S. EPA Region 8 have established health criteria for short- and intermediate-term exposure to arsenic of 0.005 mg/kg-day or higher, which is equivalent to an MOE of 10 or less [from a LOAEL of 0.05 mg/kg-day]. Finally, it was noted by one Panel member that clinical studies on children exposed to arsenic in drinking water associated the increased severity of observed multisystemic adverse effects in children compared to adults to a higher dose rate in children, and not to intrinsically increased susceptibility (Zaldivar, 1977; Zaldivar and Gullier, 1977; Zaldivar and Ghai, 1980).

Some Panel members cautioned that exposures above the MOE do not necessarily mean that health effects will occur and that the Agency should use the MOE in a screening level capacity only. That is, firm conclusions on the presence or absence of health effects should not be drawn solely on the basis of doses calculated to exceed the MOE.

**Question 2: Please comment on the choice of this data set and value chosen for representation of the relative bioavailability of inorganic arsenic from ingestion of arsenic-contaminated soil. Please discuss the strengths and weaknesses of the selected data and also provide an explanation as to whether this 25% value is appropriate for estimation of bioavailability in children.**

#### Recommendation

Panel members expressed a diversity of opinions regarding the designation of 25% as a value for the estimated relative bioavailability of inorganic arsenic from ingestion of arsenic-contaminated soil. Several members of the Panel felt that EPA should consider alternatives to a fixed value of 25% for the relative bioavailability of arsenic in soil in the vicinity of CCA contamination, while others felt that 25% was a reasonable interim value. Many members suggested an interim value of 50%. Several Panel members recommended that a range of values be considered: for some the suggested range was 25 to 50%, while another member suggested consideration of the full range of bioavailability for arsenic in soil reported in the literature (near zero to 98%).

In addition to oral absorption of arsenic from soil, consideration should be given to absorption of arsenic from nonsoil substances (such as wood chips or other buffer material) that might be subject to incidental ingestion.

Research is needed to obtain data on the relative bioavailability of arsenic from numerous sites that encompass the broad range of soil types and arsenic contamination specifically resulting from CCA-treated wood applications. These studies should be conducted in appropriate animal models preferably at doses that simulate the anticipated level of exposure of children playing on or around structures or sites subject to CCA contamination.

### Discussion

There is general scientific consensus that a number of physical, chemical, and biological factors may impact the extent of gastrointestinal absorption of a substance present in ingested soil relative to absorption of the same substance ingested in solution. For arsenic, as with several other metals, solubility of the form of arsenic present in soil is a key factor, such that increased solubility or extractability of the metal from soil to an aqueous solution is positively correlated with increased absorption. Chemical and physical factors influencing the solubility or extractability of arsenic from the soil include 1) the molecular form of the arsenic species; 2) the nature of its chemical and/or physical interaction with the constituents of the soil matrix (e.g., chemical bonding, sorption, complexation, rinding, or encapsulation); and 3) the size, porosity, compaction, and surface area of the arsenic-containing soil particulates or agglomerations. Biological factors may also influence the absorption of an ingested metal present in soil, including 1) the species-specific metabolism of the metal, including metabolism by microflora within the gastrointestinal tract (Hall et al., 1997), 2) the physical condition of the animal at the time of ingestion (e.g., the effect of drugs, physical stress, toxins, nutritional perturbations, or disease states on the animal's physiology), 3) the presence of other ingested material (food, drugs, or other substances) in the intestinal tract, and 4) in some cases the age and/or developmental stage of the animal. The dose regimen that characterizes the ingestion of the metal and the soil matrix may also exert influence on the absorption, in terms of either absolute amounts or the percent of the dose administered. For example, data on absorption of lead from soils (Kierski, 1992; Mushak, 1998) suggest that bolus administration of a large mass of metal and/or metal-containing soil matrix may be associated with a lesser degree of gastrointestinal absorption, in terms of percent of total ingested amount, than might result from administration of the same mass in smaller, divided doses.

Members of the Panel expressed concern that the findings of Roberts et al. (2001) and Freeman et al. (1995) have not provided a sufficient basis to establish a relative bioavailability of 25% for arsenic present in soil as a consequence of CCA related release or contamination. The single, high dose, bolus administration of arsenate and arsenic-containing soils used in the studies by Roberts et al. (2001) and Freeman et al. (1995) does not reasonably simulate the relatively low dose, repeated ingestion of arsenic-containing soil that would be anticipated with hand-to-mouth behavior of a child playing in the vicinity of a CCA application. The arsenic concentration of the test soils (ranging from 101 to 743 mg/kg) appears high relative to those measured in the vicinity of CCA-treated structures in children's playgrounds in several recent investigations. The experimental design used by the investigators resulted in these soils being introduced into the monkey test subjects in single high mass boluses. For example, in the case of soil obtained from a "wood treatment site", it may be calculated that the soil-associated arsenic dose of 0.3 mg As/kg body weight was achieved by administering a 3 kg monkey a single oral dose of 9000 mg of soil. In like manner, in Freeman et al. (1995), the monkeys (which weighed between 2 to 3 kg)

were given single, oral doses of 3000 to 4500 mg of soil containing 410 ppm arsenic. Enhanced confidence in the generalizability of the relative bioavailability values from such studies might be obtained from experimental designs that utilize multiple, smaller soil doses spanning a range of relevant arsenic concentrations.

There is uncertainty regarding the extent to which the test soils used in the studies by Roberts et al. (2001) and Freeman et al. (1995) reflect arsenic speciation and chemical and physical characteristics of the soil matrix in the vicinity of CCA contamination at a playground. Although a soil sample from the investigation by Roberts et al (2001) was identified as coming from a “wood treatment site,” this sample was not characterized further. The arsenic in that soil may have resulted in part from direct spillage of raw CCA product onto the soil, rather than leaching of arsenic from a weathered piece of CCA-treated wood.

The animal model used in the studies by Roberts et al. (2001) and Freeman et al. (1995) were the *Cebus apella* monkey and the cynomolgus monkey, respectively. Intravenous dosing with sodium arsenate suggested that these nonhuman primates were similar to humans with respect to excreting absorbed arsenic almost entirely through the urine (<5% of the recovered dose occurred in the feces). Also, the extent of excretion of an oral dose of sodium arsenate in urine and feces was quite similar between these monkeys and humans. At this point in time, the Panel is not aware of information regarding the biomethylation patterns of arsenic species in these nonhuman primates. This is an issue of some concern for some Panel members because other nonhuman primates, such as the marmoset monkey, do not biomethylate arsenic and exhibit prolonged retention of some arsenic species in vivo. These Panel members thought that this could potentially result in an underestimation of relative bioavailability if a significant proportion of the arsenic specie(s) present in the test soils was retained in the body for a longer period of time *relative* to the reference material, sodium arsenate in solution. Underestimation could also result if arsenic present in the test soil underwent greater relative biliary excretion compared to sodium arsenate. Other Panel members acknowledged these possibilities but expressed the opinion that these factors were not likely to significantly affect the findings.

At the present time, little is known regarding differential absorption and metabolism of arsenic in juvenile versus adult animals. Some Panel members expressed concern that the developmental age of the animal model might be a potentially significant variable, since it is known that infants and even older children as well as very young animals, sometimes have the potential for increased uptake of contaminants. Although the swine models have utilized juvenile pigs, the current monkey bioavailability data were obtained with adult animals. To the extent that the nutritional or dietary status of children and experimental test animals may affect the uptake of other substances, the absorption of arsenic (particularly arsenate) in the face of phosphorous deficiency is of potential concern for these Panel members. They noted, for example, that arsenate uptake by cells has been shown to be increased in low phosphate media (Huang and Lee, 1996) and suggested the need for further research on the impact of nutritional and developmental factors on bioavailability determinations. Other Panel members pointed out that the absorption of arsenite and arsenate, in absolute terms, is already extensive in adult animals and humans. As a result, the potential for greater absorption in children is limited, and consequently they did not think that use of arsenic bioavailability values from adult animals was

a significant concern (the Panel members thought that arsenic bioavailability values from adult animals were applicable to immature subjects).

As discussed in more detail elsewhere in this report, the interactive effect of metal combinations may influence arsenic absorption, biotransformation, and excretion. For example, when administered together with selenite, some inorganic arsenic compounds undergo increased biliary excretion (Levander, 1977; Gailer et al., 2000), a factor that may potentially serve to underestimate relative bioavailability in models that examine relative urinary excretion as a marker of relative bioavailability.

Panel members noted several other studies that have investigated the oral bioavailability of arsenic in soils. Widely divergent results for relative bioavailability have been reported, a finding that is not unexpected given the variability in soil-associated arsenic compounds, soil matrices, animal models, and experimental design. For example, Casteel et al. (2001), under the auspices of U.S. EPA Region VIII, recently examined the relative bioavailability of arsenic in soils from the VBI70 superfund site in Denver, CO. Using a swine model that investigated six soil specimens spanning a range of arsenic concentrations, the mean relative bioavailability was 31%, with a 95% upper confidence limit of 42%. This latter value (42%) has been utilized in risk calculations contained in the site's baseline risk assessment (US EPA, 2001). Other relative bioavailability studies have been noted or reviewed in the Inorganic Arsenic Report of the Hazard Identification Assessment Review Committee (HIARC, 8/21/2001), and a recent publication by Ruby et al. (1999). Results for relative bioavailability have ranged from near zero to 50%, with the exception of two soils from Aspen, CO, that yielded much higher results, albeit with extremely wide confidence intervals ( $62\% \pm 55$ ,  $98\% \pm 86$ ; Casteel et al., 1997; Ruby et al., 1999).

**Question 3: Please comment on the selection of the value of 6.4% for dermal absorption of inorganic arsenic and whether or not this value will be appropriate for use in all scenarios involving dermal exposure to arsenic from CCA-treated wood, including children's dermal contact with wood surface residues and contaminated soils.**

#### Recommendations

The Panel recommends that EPA use a value less than 6.4%, probably in the range 2-3%, for dermal absorption of inorganic arsenic. The Agency should consider using a figure for absorption rate (e.g., percent exposure absorbed per hour) rather than a value for percent absorption.

Research, using arsenic in more appropriate chemical form (that it is present in dislodgeable CCA residues and in soil beneath CCA-treated sites) and in a relevant matrix, should be carried out to improve estimates of dermal absorption.

FIFRA Scientific Advisory Panel Background Document

**Hazard Identification and Toxicology Endpoint Selection for  
Inorganic Arsenic and Inorganic Chromium**

September 25, 2001

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Antimicrobials Division

Recently, methylated trivalent forms of arsenic have been shown to nick and/or completely degrade  $\phi$ X174 DNA in vitro (Mass et al., 2001), while sodium arsenite, arsenate, and the pentavalent methylated forms of arsenic were without effect. In the single-cell gel assay (COMET assay) using human lymphocytes, inorganic arsenite and arsenate produced concentration-dependent linear increases in DNA damage, but the methylated trivalent forms of arsenic were observed to be 54-77 times more potent in this assay than the non-methylated forms. DNA damage occurred in the absence of metabolic activation in both assays.

### **Metabolism and Bioavailability**

Metabolism of inorganic arsenic first proceeds through non-enzymatic reduction of arsenate to arsenite, which can then undergo enzymatic methylation to the products monomethylarsinic acid and dimethylarsinic acid. These products are then reduced to the monomethylarsinous acid and dimethylarsinous acid products. The major site of methylation appears to be liver, where the methylation reaction is mediated by methyltransferase enzymes using S-adenylmethionine as a cosubstrate. The products of inorganic arsenic metabolism in urine have been identified as As(+3), As(+5), monomethylarsinous acid, and dimethylarsinous acid. Urinary products appear similar among species studied (ATSDR, 2000a), but the relative proportions of these products vary greatly.

The bioavailability of absorbed inorganic arsenic is dependent on the matrix in which it is exposed to. Arsenic in drinking water is in a water-soluble form, and it is generally assumed that its absorption from the gastrointestinal tract is nearly complete. Arsenic in soils, however, may be incompletely absorbed because they may be present in water-insoluble forms or interact with other constituents in the soil. The relative bioavailability of arsenic after it is been exposed (water versus soil) was defined as the percentage of arsenic absorbed into the body of a soil-dosed animal compared to that of animal receiving a single dose of arsenic in aqueous solution. This is a route specific issue. The Agency has considered several data sets in determination of the relative bioavailability of inorganic arsenic (soil vs. water), which are summarized below.

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### **Relative Bioavailability-Oral Route**

#### **Roberts et al. 2001**

The relative bioavailability of arsenic from selected soil samples was measured in a primate model. Sodium arsenate was administered to five male *Cebus apella* monkeys by the intravenous and oral routes, and urine and feces were collected over a four-day period. Pharmacokinetic behavior of arsenic and the fractions of dose excreted in urine and feces were consistent with previous observations in humans. Soil samples from four waste sites in Florida (one from an electrical substation, one from a wood preservative treatment (CCA) site, one from a pesticide application site, and one from a cattle dip vat site) were dried and sieved. Soil doses were prepared from these samples and administered orally to the monkeys. Relative bioavailability was assessed based on urinary excretion of arsenic following the soil dose compared with excretion following an oral dose of arsenic in



solution. Relatively consistent bioavailability measurements were obtained among monkeys given the same soil sample. Differences in bioavailability were observed for different sites, with relative bioavailability ranging from  $10.7 \pm 14.9\%$  (mean $\pm$ SD) to  $24.7 \pm 3.2\%$  for the four soil samples.

### **Freeman et al. 1993**

The relative bioavailability of arsenic from soil samples from Anaconda, Montana was measured. After a fasting period of approximately 16 hours, prepubescent male and female SPF New Zealand White rabbits (5/sex/group) were given a single oral (capsule) administration of soil (3900ppm As) at three dose levels (0.2, 0.5, and 1.0 g of soil/kg, corresponding to 0.78, 1.95 and 3.9 mg As/kg, respectively). Control groups included untreated controls, and an intravenous sodium arsenate group (1.95 mg As/kg). The relative bioavailability of arsenic in the soil was approximately 37 - 56 % (based on the As concentration in the excreted urine).

### **Freeman et al. 1995**

Oral absorption of arsenic in a group of three female Cynomolgus monkeys from a soluble salt, soil, and household dust was compared with absorption of an intravenous dose of sodium arsenate (Freeman et al. 1995). Mean absolute percentage bioavailability based on urine arsenic excretion was reported at  $67.6 \pm 2.6\%$  (gavage),  $19.2 \pm 1.5\%$  (oral dust), and  $13.8 \pm 3.3\%$  (oral soil). Mean absolute percentage bioavailability based on blood arsenic levels was reported at  $91.3 \pm 12.4\%$  (gavage),  $9.8 \pm 4.3\%$  (oral dust), and  $10.9 \pm 5.2\%$  (oral soil). The relative bioavailabilities of arsenic in the dust and soil were approximately 28.4% and 20.4% respectively (based on urine).

### **Groen et al. 1993**

Arsenic was administered as an intravenous solution ( $\text{As}_2\text{O}_5$ ) or orally as As in soil to groups of six beagle dogs, and urine was collected in 24-hour fractions for 120 hours. After 120 hours,  $88\% \pm 16\%$  of the dose administered intravenously was excreted in the urine, compared to only  $7.0 \pm 1.5\%$  excreted in the urine after oral soil administration. The calculated bioavailability of inorganic As from urinary excretion was  $8.3 \pm 2.0\%$ .

### **USEPA Region 10, 1996**

The relative bioavailability of arsenic and lead in soil or slag from the Ruston/North Tacoma Superfund Site has been studied in immature swine that received one single oral dose of soil or sodium arsenate (EPA, 1996). Following a 12 hour overnight fast, each animal was given a single administration of the appropriate test material. Solutions of sodium arsenate and lead acetate were administered separately and not mixed together prior to administration. The group receiving environmental media received a single oral administration of one of four quantities of soils at 25, 60, 100 or 150 mg soil/kg of body weight (BW) (0.04, 0.10, 0.16, or 0.24 mg As/kg BW and 0.03, 0.08, 0.14, or 0.20 mg pb / kg BW). Control groups include intravenous or gavage doses of solution arsenic, untreated controls (received aqueous vehicle only), and an intravenous sodium arsenate

group (1.95 mg As/kg). Because several urine samples were lost during sampling procedure, urinary arsenic excretion was not used as a biomarker in estimating bioavailability. Based on the blood level of arsenic, the relative bioavailability of arsenic (soil versus water) in the soil was 78% (56 - 111%).

### **USEPA Region 8, 1997**

The bioavailability of arsenic in soil has been studied in juvenile swine that received daily oral doses of soil or sodium arsenate (in food or by gavage) for 15 days (EPA 1997). The soils were obtained from various mining and smelting sites and contained, in addition to arsenic at concentrations of 100-300  $\mu\text{g/g}$ , lead at concentrations of 3,000-14,000  $\mu\text{g/g}$ . The arsenic doses ranged from 1 to 65.4  $\mu\text{g/kg/day}$ . The fraction of the arsenic dose excreted in urine was measured on days 7 and 14 and the relative bioavailability of the soil-borne arsenic was estimated as the ratio of urinary excretion fractions, soil arsenic:sodium arsenate. The mean relative bioavailability of soil-borne arsenic ranged from 0 to 98% in soils from seven different sites (mean $\pm$ SD, 45%  $\pm$ 32). Estimates for relative bioavailability of arsenic in samples of smelter slag and mine tailings ranged from 7 to 51% (mean $\pm$ SD, 35%  $\pm$ 27).

By carefully comparing data on the urinary and fecal recovery of arsenic in both experimental animals after an oral intravenous dose of sodium arsenate and in humans, the data of Roberts et al. (2001) using the monkey was considered an appropriate study model in evaluating the relative bioavailability of arsenic due to the similarity of monkeys to humans and the similarity in g.i. absorption characteristics. The Roberts et al. study also employed a variety of soil types including soil from a CCA-contaminated site. Therefore, based on the study results of Roberts et al. (2001) a relative bioavailability of 25% was chosen to represent oral bioavailability.

### **Relative Bioavailability - Dermal Route**

Wester et al. (1993) studied the dermal absorption of arsenic from both water and soil with Rhesus monkeys. The results of this study showed that in vivo percutaneous absorption of the low dose of arsenic in water was  $6.4 \pm 3.9\%$  (n=3); while  $2.0 \pm 1.2\%$  (n=4) was absorbed from the high dose. Percutaneous absorption of arsenic from soil was  $4.5 \pm 3.2\%$  (n=4) from the low dose and  $3.2 \pm 1.9\%$  (n=4) from the high dose. The dermal absorption of arsenic from water was not statistically different from the absorption from soil. Therefore, the relative bioavailability of arsenic by the dermal route (water versus soil) is 100%.

For inorganic arsenic, studies by the oral route in commonly used experimental animal species have not revealed a carcinogenic response. However, human data reveal a clear carcinogenic response. In epidemiological studies by Tseng, 1968, and Tseng, 1977, where chronic oral exposure to arsenic contained in food and water occurred, symptomatology consisted of hyperpigmentation, keratosis and possible vascular complications [Blackfoot disease] at the LOAEL of 0.17 mg/L of water, equivalent of 0.014 mg/kg/day. The NOAEL was calculated to be 0.009 mg/L of water equivalent to 0.0008 mg/kg/day. Several follow-up studies of the