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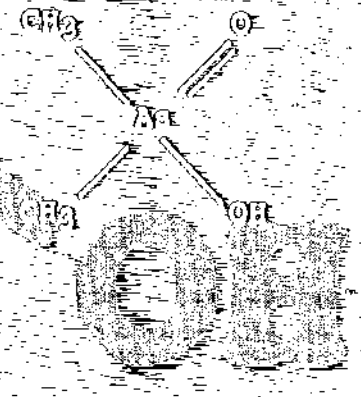
Cacodylic Acid: Agricultural Uses, Biological Effects, and Environmental Fate



VA Monograph
December 1983

Cacodylic Acid: Agricultural Uses, Biologic Effects, and Environmental Fate

Ronald D. Hood, Ph.D.

Department of Biology
The University of Alabama
Tuscaloosa, Alabama
and
R. D. Hood and Associates
Consulting Toxicologists
Northport, Alabama

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Preface

The intent of this monograph is to provide a single source of information on cacodylic acid (CA) and its sodium salt (NaCA). These two compounds were the active ingredients in Agent Blue, one of the group of herbicides used in Vietnam to defoliate hiding places, supply lines, and staging areas held by the opposing forces. Along with the phenoxy herbicides such as Agent Orange, Agent Blue was used experimentally in the early to mid-1960's and more extensively from 1965 to 1970. As with the phenoxy herbicides, these organic arsenicals had been widely used in agriculture and forestry for many years with no known risk to human health. But as with Agent Orange, albeit to a somewhat lesser extent, there has been an expression of concern on the part of some veterans of the Vietnam conflict that exposure to these chemicals might have a potential for adverse health effects. It is for this reason that the Veterans Administration has undertaken the preparation of this monograph. It is hoped that in addition to providing detailed information to Veterans Administration health care and research professionals, it will be of use to the general scientific community as a reference guide to virtually all of the available literature on CA and some material on related methylated arsenicals.

The first five chapters address in great detail the very complex and highly technical aspects of the chemical and physical properties as well as the production, agricultural uses, and environmental considerations of these compounds. It is, therefore, recommended that the reader whose primary interests are with biological aspects such as pharmacology, metabolism and toxicology start with chapters 6, 7, or 8. Chapter 9 provides a summary overview and discusses areas where additional research is needed to fill the gaps in our knowledge of these interesting compounds.

Virtually all the literature available through 1984 covering the many aspects of the methylated arsenicals has been included in this monograph. The chapters on agricultural uses and environmental sources and fate put into perspective the various ecologic and economic consequences of the use of these herbicides. The inclusion of an extensive review of the analytic methodology for methylated arsenicals should be of considerable benefit to future researchers interested in these compounds. Such information will also facilitate assessment of the accuracy of previous studies that relied on the analysis of these compounds in biologic or environmental samples. Related material on other methylated arsenicals is included to give a broader perspective of the role and likely effects of cacodylate.

The very extensive body of literature dealing with these organic arsenicals is widely scattered among traditional journals as well as variety of other sources not commonly indexed. For this reason, many documents were more difficult to locate or to obtain than were the more traditional journal articles. With the exception of one 1975 review done by the Midwest Research Institute for the U.S. Environmental Protection Agency, which itself is not widely available, there appears to have been no previous attempts to assemble all the literature on CA in one source document. Some of the more recent literature on CA has also been included in a review series done for the Veterans Administration, but the major emphasis of this effort has been on the phenoxy herbicides and dioxins.

It is hoped that this monograph will serve as a valuable reference source to a broad range of interested users, including health care providers, research scientists, and science writers and editors dedicated to the important role of interpreting highly complex and technical literature to the less scientifically oriented public.

Acknowledgements

I wish to acknowledge the assistance of those who helped me in preparing this monograph. I am particularly indebted to Lt. Col. Alvin L. Young, Ph.D., Senior Policy Analyst for Life Sciences, Office of Science and Technology, Executive Office of the President. Lt. Colonel Young sparked my interest in the project and provided both encouragement and logistic support while he was associated with the Agent Orange Projects Office of the Veterans Administration.

I am also grateful for assistance by Lt. Colonel Young's staff, especially Mr. Larry Stockmoe and Ms. Michel Bright, of the Agent Orange Projects Office. Invaluable aid was also provided by Edwin A. Woolson, Ph.D., of the U.S. Department of Agriculture's Pesticide Degradation Laboratory, Beltsville, Maryland. Dr. Woolson made available his extensive files of references on methylated arsenicals and was kind enough to criticize the chapter on analytic methods. Walter W. Melvin, M.D., Sc.D., of Colorado State University reviewed the entire manuscript. Once Lt. Colonel Young left the project, final publication was facilitated by Barclay M. Shepard, M.D., Director, Agent Orange Projects Office, and by his staff, Mr. Layne A. Drash, Mrs. Elaine M. Morrow, Publications Coordinator, and Mrs. Laverne L. Cooper.

I also express my special gratitude to my wife, Barbara, and to my daughter, Rebecca Ann, for their encouragement, patience, and understanding while I was preparing the manuscript.

CONTENTS

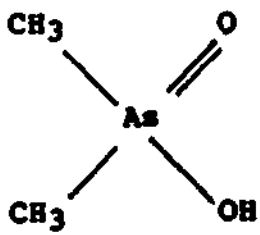
Chapter 1

Chemical and Physical Properties of Cacodylic Acid

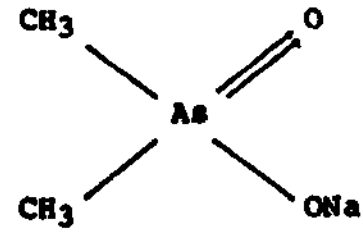
Physical properties

Cacodylic acid, $(CH_3)_2As(O)OH$, was perhaps first described by Bunsen (1843), who referred to it as "alkargene." It is an organic arsenical with a molecular weight of 137.99 and the empirical formula $C_2H_7AsO_2$ (MRI, 1975). It is also referred to as hydroxydimethylarsine oxide, dimethylarsinic acid, or CA. Sodium cacodylate or NaCA, $C_2H_6AsO_2Na$, has a molecular weight of 159.98 and is also known as sodium dimethylarsinate. The structural formulas of the acid and sodium salt are:

cacodylic acid



sodium cacodylate



The prefix "caco" was derived from the Greek *kakos* or "bad," and the Greek *kakodes* refers to an offensive odor (MRI, 1975). Although CA and its sodium salt are odorless, the radical $As(CH_3)$ or two such radicals united as $(CH_3)_2AsAs(CH_3)_2$ are known for their garlic-like scent.

CA and NaCA are both colorless solids, and CA forms triclinic crystals whose unit-cell dimensions have been described as: a = 6.53 nm, b = 6.82 nm, c = 6.61 nm, $\alpha = 77° 30'$, $\beta = 98° 45'$, $\gamma = 55° 9'$ (Trotter and Zobel, 1965). The crystal was said to resemble that of carboxylic acids, with centrosymmetrical hydrogen-bonded dimers, rather than the more complex hydrogen bonding associated with arsonic acids, i.e., $RAsO(OH)_2$. Smith et al. (1970b), however, list the dimensions as a = 8.34 nm, b = 6.82 nm, c = 10.16 nm, $\alpha = 59.5°$, $\beta = 89.3°$, $\gamma = 106.0°$. According to Trotter and Zobel (1965), the configuration around the arsenic atom is tetrahedral, and the bond angles range from 106° to 115°.

CA is hygroscopic (MRI, 1975), with a calculated density of 1.95 g/cc (Smith et al., 1970b), a melting point around 200°C (Dietz and Moore, 1978), and an enthalpy of fusion of 4.96 kcal/mol

(Smith et al., 1970a). The binding energy of the electrons in the 3*d* orbital of the arsenic of CA was found by Hulett and Carlson (1971) to be 44.7±0.7 eV, with a charge on the As atom of + 1.5. In addition, the Raman spectrum of CA has been published by Guha (1941), and a magnetic susceptibility of 49.12 was calculated by Prasad and Mulay (1951).

CA is quite soluble in water (66 g in 100 g at 25°C), according to Ansul (1971). Bailey and White (1965) state that it is relatively soluble in ethanol as well (28.5 g in 100 ml at 15°C). CA is also soluble in acetic acid, but not in ethyl ether or other common organic solvents (Windholz et al., 1976; Dietz and Moore, 1978).

Sodium cacodylate is deliquescent, with a solubility in water of 83 g/100 g at 22°C. A 4% solution of the salt has a pH of 7.7 to 8.0 (Masucci and Moffat, 1923), and NaCA has a melting point greater than 330°C (Dietz and Moore, 1978). NaCA can also form a trihydrate with a lower melting point (82°C to 86°C).

Synthesis and production methods

In 1958, the Ansul Company began the first commercial production of CA as an herbicide, although the compound had been known for well over a hundred years (Bunsen, 1843). Among the major commercial patents describing methods for the production of technical grade CA are those obtained by Moyerman and Ehman (1965) and Schanhals (1967), although general and specific reactions for production of CA and related arsenicals had been proposed much earlier (Baeyer, 1858; Dehn and McGrath, 1906; Quick and Adams, 1922; Guinot, 1923; Fioretti and Portelli, 1963). In addition, Challenger and Ellis (1935) had noted the production of CA by the oxidation of trimethylarsine and of dimethylalkylarsine.

The manufacturing process patented by Schanhals (1967) begins with the production of disodium methanearsonate (DSMA) from arsenic trioxide, sodium hydroxide, and methyl chloride in a two-step process:

$$(1)\ As_2O_3 + 6\ NaOH \xrightarrow{H_2O} 2\ As(ONa)_3 + 3\ H_2O$$

$$(2)\ As(ONa)_3 + CH_3Cl \xrightarrow{H_2O} CH_3AsO(ONa)_2 + NaCl$$

The DSMA is then reacted with sulfur dioxide to produce methylarsine oxide:

$$CH_3AsO(ONa)_2 + SO_2 \xrightarrow{H_2O} CH_3AsO + Na_2SO_4$$

Disodium methyl arsenite is then produced by use of sodium hydroxide:

$$CH_3AsO + 2\ NaOH \xrightarrow{H_2O} CH_3As(ONa)_2 + H_2O$$

This step is followed by production of sodium dimethyl arsinate when methyl chloride is again employed:

$$CH_3As(ONa)_2 + CH_3Cl \xrightarrow{H_2O} (CH_3)_2AsO(ONa) + NaCl$$

The final step in this sequence involves reaction with hydrochloric acid to yield CA:

$$(CH_3)_2AsO(ONa) + HCl \xrightarrow{H_2O} (CH_3)_2AsO(OH) + NaCl$$

Schanhals (1967) recommends an additional step to "reduce toxicity, odor, etc." of the reaction mixture. This step requires the addition of an oxidizing agent, preferably sodium hypochlorite, and results in reactions such as:

$$CH_3AsO + H_2O + NaOCl \longrightarrow CH_3AsO(OH)_2 + NaCl$$

$$As(ONa)_3 + NaOCl \longrightarrow OAs(ONa)_3 + NaCl$$

$$As(OH)_3 + NaOCl \longrightarrow OAs(OH)_3 + NaCl$$

$$As_2O_3 + 2NaOCl \longrightarrow As_2O_5 + 2NaCl$$

Sodium cacodylate can be readily obtained by the addition of sodium hydroxide to an aqueous solution of CA. Further details of the CA and NaCA production process, including the exact concentrations, temperatures, pressures, and other requirements can be found in the original patents.

Chemical properties

The compound is named as an acid, but "CA" has been found to be an amphoteric electrolyte (Zawidzki, 1903; Holmberg, 1910), although this finding was initially disputed by Hantzsch (1904). The pKa of CA has been estimated as 6.288 (Wauchope, 1976). CA has been widely used as a buffer, especially by electron microscopists (Weakley, 1977). It was first proposed for use in microscopy by Sabatini et al. (1963), but its use as a buffer (in the pH range 5.2 to 7.2) was initially suggested by Plumel (1948). That use has been questioned, however, by Banks et al. (1979), on the grounds that CA may interfere with the activity of enzymes that possess vulnerable SH groups. Also, reducing agents that possess SH groups are often used to stabilize enzymatic reactions and could react with CA in buffers (Jacobsen et al., 1972).

The acid strength of CA in water has been measured by a number of workers with varying results (MRI, 1975). For example, where

$$K_a = \frac{(Me_2AsO^-)(H_3O^+)}{(Me_2AsOOH)}$$

values for K_a have been given as 4.2×10^7 at 25°C (Zawidzki, 1903, as recalculated by MRI, 1975), 5.33×10^7 (Kilpatrick, 1949), or 7.1×10^7 (Jacobsen et al., 1972). Conversely, when K_b has been investigated, where

$$K_b = \frac{(Me_2AsOOH{\cdot}H^+)}{(Me_2AsOOH)(H_3O^+)}$$

results (at 25°C) such as 5.1×10^{13} (Holmberg, 1910) and 3.2×10^{13} (Kilpatrick, 1949) were among those obtained when the data were recalculated by MRI (1975).

The rate constants for the transfer of protons between a number of donor and acceptor molecules were determined by Ahrens and Maass (1968). For the reaction:

$$(CH_3)_2AsOOH + C_2H_3O_2 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (Ch_3)_2\ AsOO + C_2H_3O_2H$$

$$CA + \text{acetate ion} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{cacodylate ion} + \text{acetic acid}$$

k_1 was 6.2×10^7 l/mol/sec and k_2 was 1.7×10^9 l/mol/sec at an ionic strength of 1 M and a temperature of 20°C. Also, Clifford (1959) stated that on a relative scale of 0 to 4, the electronegativity of the cacodylate ion would have a rating of 3.15, and the electroreduction of CA has been studied by Elton and Geiger (1976, 1978).

Dehn and McGrath (1906) suggested that the arsenic in CA must be in the pentavalent state because of its resistance to oxidation by agents such as nitric acid and halogens. Jacobsen et al. (1972), however, state that the arsenic in CA is in the trivalent oxidation state.

Reactions

A variety of papers dealing with the chemistry of CA were published in the late nineteenth and early twentieth centuries, mainly in Europe. A number of these manuscripts were difficult to obtain or could not be translated in time to be included in this monograph, but anyone interested in these articles can find references for them in the bibliographies of the works that are included.

In 1843, Bunsen published a lengthy paper that mentioned his still earlier work on "cacodyl compounds." He discussed the lack of effect of sulfuric and oxalic acids, ferrous oxide, hydrogen gas, and other chemical agents on CA, while phosphoric acid was said to liberate cacodyl oxide when heated with a solution of CA. Acidic stannous chloride and metallic zinc were each said to reduce CA. Hydriodic and hydrobromic acids reacted with CA to yield alkyl halides, while hydrogen sulfide plus CA yielded cacodyl sulfide and free sulfur. CA was said to be stable in the presence of nitric acid and sulfuric acid-dichromate mixtures.

Bunsen (1843) also made the astonishing statement that CA was essentially nontoxic. Most of the relevant experimental evidence was attributed to a Professor Kurschner, however, who must have been an unreliable source or who used the wrong compound in his experiments (unless he was being misquoted by Bunsen).

Bunsen (1843) described a series of reactions between CA and other compounds. These included production of salts from silver oxide, carbonic acid silver oxide, nitric acid silver oxide, mercuric oxide, and copper chloride. Bunsen additionally synthesized cacodyl sulfide, cacodyl subsulfide, cacodylic lead oxide, and sulfocacodylates of gold, copper, bismuth, and lead, as well as other types of compounds. All of these syntheses were said to be for the purpose, as Bunsen put it, of learning more about "the true nature of the organic compound."

In 1906, Auger discussed the decomposition of CA when it was heated with sodium hydroxide, while Barthe and Minet (1909) described the reaction of CA with antimony trichloride, yielding antimony dichlorocacodylate plus hydrochloric acid. The formation of uranyl cacodylate by the treatment of sodium cacodylate with uranyl nitrate was presented by Isnard (1925).

In a more extensive study, Tiollais (1936a) described the syntheses of the calcium, strontium, and barium salts of cacodylate. He also published a detailed description of the crystals formed by these compounds (Tiollais, 1936b), and with a colleague later presented two related techniques for the formation of zinc cacodylate (Tiollais and Perdreau, 1937).

More recently, Feltham et al. (1967) found that dimethylarsinoiodide was generated when CA was reacted with potassium iodide, hydrochloric acid, and sulfur dioxide. They also reported the reduction of CA to dimethylarsine by use of zinc/hydrochloric acid, a method they attribute to Raiziss and Gavron (1923). This reduction is usually carried out by use of sodium borohydride in current methods for analysis of methylated arsenicals, based on the work of Braman and Foreback (1973).

Details of ester formation when arsinic and arsonilic acids react with mono- and dihydric alcohols have been published by Levskaya and Kolomiets (1967). For example, they prepared butyl dimethylarsinate from CA and butyl alcohol. The following year, Schmidbaur and Kammel (1968) gave a method for formation of aluminum, gallium, and indium derivatives of CA by reacting the arsenical with the trimethyl derivatives of these metals.

Reactions of CA with organic thiols were shown by Jacobsen et al. (1972), who used 2-mercaptoethanol, L-cysteine, L-glutathione, and dithiothreitol as the source of SH groups. This work was confirmed by Banks et al. (1979), who found that CA and the thio amino acid cysteine could readily combine in aqueous solution, giving S-dimethylarsinocysteine.

The previous discussion does not exhaust the literature on the chemistry of CA and related arsenicals. Additional material can be found in sources such as the 1975 review by the Midwest Research Institute and the 1970 book by Doak and Freedman. A summary of the types of reactions entered into by CA is shown in Figure 1.1.

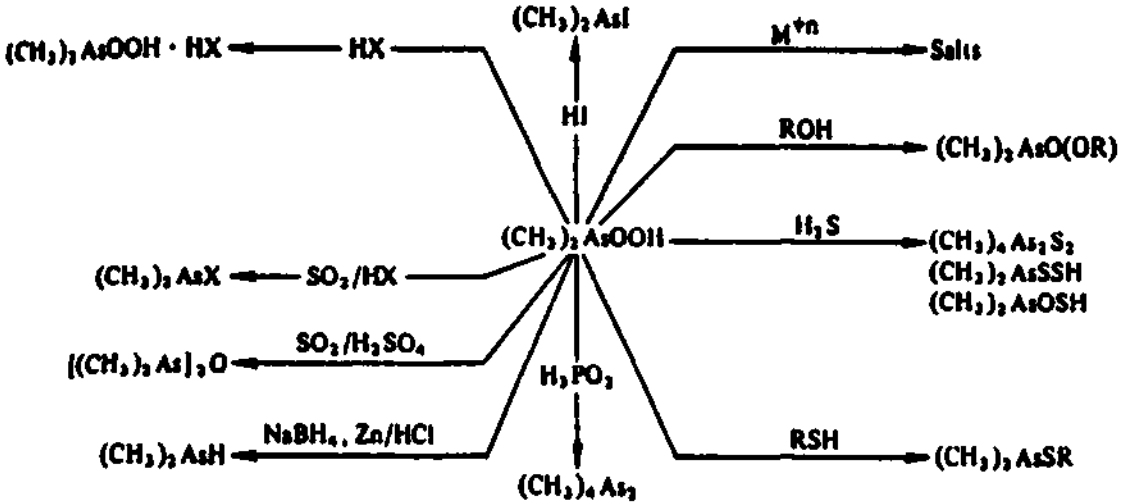


Figure 1.1 Reactions of cacodylic acid

Source: National Academy, 1977.

Chapter 2

Analysis of Methylated Arsenicals

Analytic methods for methylated arsenicals have greatly improved in recent years. Such improvements have come primarily from two techniques. One such method employs reduction of the arsenicals to volatile arsines by use of sodium borohydride (sodium tetrahydroborate, $NaBH_4$) followed by cold-trapping of the arsines and sequential evolution of arsine, methylarsine, and dimethylarsine. The arsines are then quantitated by means of atomic absorption spectrophotometry (AAS) or glow-discharge detection. Other recently developed procedures rely on either ion-exchange chromatography or high-pressure liquid chromatography (HPLC) for separation, followed by quantitation with atomic absorption spectrophotometry.

Because of the improved methodology now available, the following review will emphasize the most useful current methods. For an additional review of methodology in use by 1975, see Talmi and Bostick (1975b), and for a later review, see Lauwerys et al. (1979).

Original methodology

Among the earliest methods for determining methylated arsenicals, those used to test commercial products depended on demethylation followed by measurement of total (inorganic) arsenic by titration with iodine or other means (Zweig and Sherma, 1978). Until recently, analysis for methyl-arsenic pesticide residues in environmental or food samples also depended on total arsenic quantitation, but such methods could not be used to look for the specific arsenicals present. Because of such deficiencies, attempts were made to separate CA, methanearsonic acid (MAA), and inorganic arsenic, with some effort being made to further distinguish arsenite from arsenate.

Von Endt et al. (1968), for example, used thin-layer chromatography (TLC) to separate As(III), As(V), and monosodium methanearsonate (MSMA) residues extracted from soils. Later Sachs et al. (1971) employed paper chromatography to separate As(III), As(V), CA, and MAA in extracts from bean plants.

Colorimetric procedures

Peoples et al. (1971) quantitated inorganic arsenic and MAA in urine by a colorimetric procedure, while Lakso et al. (1973) used a similar colorimetric method to analyze for those arsenicals in Johnson grass and cottonseed. This colorimetric method was later improved by Lakso et al. (1979), who used it to analyze for arsenite, arsenate, CA, and MAA in both biologic and environmental samples. In their procedure, a two-step reduction of the arsenic species to their corresponding arsines (first suggested by Aggett and Aspell, 1976) was employed. In the first step, arsenite and CA were reduced to arsine and dimethylarsine with sodium borohydride at pH 4. In the second step, the pH was lowered to 1, and arsenate and MAA were reduced to arsine and methylarsine by further addition of $NaBH_4$. After each round of reduction, the evolved arsines were reacted with silver diethyldithiocarbamate (SDDC), whereupon they formed two differently

colored complexes. The relative amount of each complex could then be calculated by using simultaneous equations. The method was said to allow quantitation at a level of 1.0 μg of elemental arsenic for any of the four arsenic species.

Howard and Arbab-Zavar (1980) published a colorimetric approach to differentiate As(III) and As(V). They relied on generation of arsine from solutions of different pHs, with the assumption that only As(III) would be reduced at pH 6, while As(V) would form arsine at a lower pH. The separately generated arsines were then reacted with SDDC and quantitated by spectrophotometry. This method was said to suffer from interferences due to the presence of any CA or MAA in the samples tested, an interference that the authors could not overcome. Thus, it would only be accurate for defined samples known not to contain methylarsenicals.

Sandhu and Nelson (1978) investigated the abilities of a number of possible contaminant ions to alter the results of the SDDC colorimetric methods. They found that certain ions, particularly antimony and mercury, could interfere. At concentrations typically found in water, however, results were usually not significantly compromised. Following digestion with potassium permanganate and sulfuric and nitric acids, CA could be quantitated, but with only an 89% recovery.

Stringer and Attrep (1979) investigated methods of sample digestion before quantitation by colorimetry or by atomic absorption spectrophotometry. They found that two different digestion procedures were effective. These included heating with sulfuric acid and hydrogen peroxide or irradiation with ultraviolet light in the presence of small amounts of the above reagents. In each case, digested samples were treated with hydrochloric acid, potassium iodide, and stannous chloride, with the evolved arsine being trapped in an absorption tube containing SDDC.

When reagent standards were analyzed, percentage recoveries of arsenic following digestion with the acid-peroxide mixture were 99.9 ± 2.3, 103.5 ± 1.2, and 101.0 ± 5.2 for CA, MAA, and triphenylarsine oxide, respectively (Stringer and Attrep, 1979). Recoveries from settled raw sewage and sewage treatment plant effluent samples spiked with known amounts of the various arsenicals ranged from 89.1% to 104.4%. Results for spiked samples digested by UV light appeared to be only slightly more variable.

Ion-exchange chromatography

Ion-exchange chromatographic procedures have proven useful for separation of various arsenicals, including CA (Tagawa and Kojima, 1980). In 1975, Yamamoto published a method for separating arsenate, CA, and MAA in water and soil extracts by using a cation-exchange resin column (Dowex 50W-X8). The method worked well on standard solutions, but recoveries of pond sediment samples spiked with the three arsenicals varied from 98% to a low of 80%. Comparable results were reported by Wauchope and Yamamoto (1980), who stated that methanearsonate was less strongly bound to sediments than was inorganic arsenate.

Dietz and Perez (1976) reported using a similar column to purify CA and MAA. Elton and Geiger (1978) also separated CA and MAA by the ion-exchange method of Yamamoto (1975) and quantitated the arsenicals by differential pulse polarography. They then used their procedure to analyze commercial herbicide samples for the sodium salts of CA or MAA, with good results.

An ion-exchange separatory procedure was employed by Tam et al. (1978) to separate arsenicals in urine and plasma of dogs, with use of an x-ray spectrometer to quantitate arsenic compounds in fractions of the column eluate. In a further study, Tam et al. (1979b) confirmed the identities of inorganic arsenic and of CA in dog urine separated by the ion-exchange system employed in their earlier study. In both cases, a Bio-Rad AG 50X8 column was used. They used cellulose TLC sheets with a fluorescence indicator to identify the arsenic species.

In 1979, Iverson et al. employed a modification of the ion-exchange procedure of Yamamoto (1975) to separate inorganic arsenic, CA, and MAA. Detection and quantitation were by use of graphite furnace atomic absorption spectrophotometry (GFAAS). When dried river sediment samples were initially extracted sequentially with ammonium chloride, ammonium hydroxide, acid ammonium oxalate, and hydrochloric acid, recoveries were poor, averaging only 68% to 70% for CA and MAA. Samples not requiring such extraction could be analyzed more accurately.

The following year Henry and Thorpe (1980) reported the use of both cation- and anion-exchange resins (Dowex 50 X-X8 and AG 1-X8, respectively) to separate CA and MAA from inorganic arsenic. They then digested the isolated methylarsenic factions with hot perchloric acid, reduced the liberated arsenic (V) to arsenic (III) with SO_2 and used differential pulse polarography for quantitation of the resultant arsenite.

More recently, Maher (1981) examined arsenicals in marine organisms and sediments subjected to alkaline extraction followed by reacidification and separation on a cation-exchange column (Dowex 50 AG-X8). Reduction of arsenic (V) to arsenic (III) was necessary, along with reduction of both CA and MAA. Samples were then converted to arsines by use of a zinc reductor column and the arsines quantitated by GFAAS. The authors reported 92% to 97% recoveries of added arsenicals in the 5 to 10 μg range from spiked samples.

Conversely, Ricci et al. (1981) relied on an anion-exchange resin (*Dionex*) to separate the four arsenic species mentioned above, as well as *p*-aminophenyl arsonic acid. These workers used a heated quartz absorption cell in the light path of an atomic absorption spectrophotometer for quantitation of arsines generated by use of sodium borohydride. Detection limits of 10 ng/ml were claimed for each arsenic species.

Grabinski (1981) independently published a similar method. However, he used a separatory column containing both a cation- and an anion-exchange resin (Bio-Rad AG 50 X-X8 and AG 1-X8, respectively) and used the procedure to determine As(III), As(V), CA, and MAA. GFAAS was employed for quantitation, obviating the need for generation of arsines. Detection limits were claimed to be 10 ppb for each arsenical, along with recoveries of 97% to 104% from natural water samples spiked with the various species.

Recently, Persson and Irgum (1982) introduced a method for the specific purpose of determining CA present at sub-ppb levels in environmental samples. These authors preconcentrated CA from a relatively large sample volume on a strong cation-exchange resin (Dowex AG 50W-X8). They then eluted inorganic arsenic and MAA by using 0.1 M phosphoric acid, followed by 0.02 M ammonia. CA could then be eluted from the column with 1 M ammonia. Quantitation was accomplished by GFAAS.

The method was said to allow determination of CA in the presence of a 10^5-fold excess of inorganic arsenic. The detection limit for CA was given as 0.02 ng of arsenic per milliliter. It was necessary, however, to use the standard additions method to attain acceptable recoveries when CA spiked samples of natural seawater were analyzed. This was attributed to the ability of organic acids eluted with the CA to interfere with CA detection by GFAAS. A sample with a high organic acid content (urine) proved to be too difficult to analyze by Persson and Irgum's method.

Anion-exchange with a Dowex 1-X4 column conditioned with acetic acid was employed by Takamatsu et al. (1982) to separate As(III), As(V), CA, and MAA extracted from soils. In a procedure similar to that of Tagawa (1980), the soil samples were extracted with HCl, and the As(III) was extracted with benzene and back-extracted with H_2O. KI was then added to an additional portion of the original soil extract, and the remaining arsenicals (arsenite, CA, and MAA) extracted with benzene followed by H_2O. The Dowex column was eluted with acetic acid followed by HCl, and the arsenicals quantitated by GFAAS. Two additional arsenicals were detected in soils but were only tentatively identified.

Gas-liquid chromatography (GLC)

Among the earliest studies describing the use of GLC for determining an arsenical was that of Gudzinowicz and Driscoll (1963), who separated alkyl/aryl and perfluorinated compounds. Then, in 1968, Roy used the technique to separate monobrominated isomers of dimethylphenylarsine from dimethylphenyl-amine and -phosphine. Johnson et al. (1972) condensed MAA with ethylene glycol "by azeotropic removal of water from a benzene solution." The resultant chelate could be chromatographed on a variety of GLC columns. The minimal amount detected was 1 ng, and quantitation was linear up to 250 ng.

Not until the work of Soderquist et al. (1974) appeared was the GLC technique employed in an attempt to separate inorganic arsenic from methylated arsenicals. Their method was based on conversion of CA to iododimethylarsine, with quantitation by use of an electron-capture detector. Detection limits were said to be below 0.5 ppm in water or soil samples. Drawbacks of the method included the inability to achieve a practical separation of CA from other arsenicals and instability of the solutions involved.

Talmi and Bostick (1975a) applied GLC with a microwave emission spectrometric detector to analyze alkylarsenicals. These authors used sodium borohydride to produce arsines and GLC for their separation and quantitation. The microwave emission spectrometer was said to give excellent selectivity and sensitivity for arsenic. When cold-trapping of arsines was used before their introduction into the GLC, a sensitivity of 1 to 3 ng/L could be achieved.

According to Talmi and Bostick (1975a), molecular rearrangements could take place during the process of arsine generation, with production of small amounts of monomethylarsine from CA or of arsine, dimethylarsine, and trimethylarsine from MAA. These undesirable reactions could be minimized by rapid introduction of borohydride, elimination of dissolved oxygen in the samples, or lowering the pH of the samples to be reduced. The authors used their GLC method for determining CA and MAA in water and pesticide samples, but the determination of inorganic arsenic was not possible with their technique.

A different GLC procedure for analyzing methylated arsenicals was proposed by Daughtrey et al. (1975) and elaborated upon by Mushak et al. (1977). In their method, freeze-dried tissue samples (e.g., kidney, liver) were extracted with benzene and the arsenicals chelated with diethylammonium diethyldithiocarbamate. Determination of CA and MAA was achieved with a GLC equipped with an electron-capture detector, and recoveries of 98% to 100% achieved for MAA and CA, respectively. Again, inorganic arsenic recovery was poor (about 60%), and the method was recommended only for alkylarsenic. An additional drawback was the required periodic extensive silanization of the GLC column packing.

In the study of Henry and Thorpe (1978), trimethylsilyl derivatives of arsenate, arsenite, and CA were prepared and analyzed by GLC with a flame ionization detector. The reported detection limits were 1 ng for CA and 0.1 ng for As(III) or As(V). It was also suggested, but not shown, that MAA could be determined as a trimethylsilyl derivative.

Another GLC-based method for determining CA and MAA was that of Beckermann (1982), who tested samples of water, urine, and whole blood. The procedure was simple, involving only oxidation with hot acid, adjusting the pH of a rediluted sample with buffer, and derivatization with thioglycolic acid methylester (TGM). Arsenicals were extracted with cyclohexane and separated by use of a GLC with either a flame ionization or a thermionic-specific detector. The arsenic derivatives were stable for several days in solution, and interferences from other ions in the biologic samples were not a problem when EDTA and an excess of TGM were used.

Reproducibility was good, with a relative standard deviation of 4%, and a linear response was seen at concentrations of 5 to 200 ng of arsenic. Methylarsenic was determined at concentrations of 10 ng/ml in a 5 ml sample extracted with 0.5 ml of cyclohexane. Detection limits were said to be 0.2 ng for CA and 0.4 ng for MAA. Inorganic arsenic was not investigated, but since it forms a TGM derivative, such a method might yet be developed.

The most recent method involving GLC appears to be that of Odanaka et al. (1983). Arsines were generated with $NaBH_4$ and collected in *n*-heptane in a cold trap flushed with helium and cooled with dry ice and acetone. An aliquot of the arsine-containing *n*-heptane was then injected into a GC/mass spectrometer with a multiple ion detector. Separation was accomplished with a column packed with 5% PEG-20M on Chromosorb 101. The system could separate and detect arsine and methyl, dimethyl, and trimethyl arsines, although all four could not be determined simultaneously.

Quantitation was said to be linear between 0.2 and 2,000 ng of arsenic per ml, with absolute detection limits of 20 pg of arsenic for injected standards of methyl- or dimethylarsine and 30 μg of arsenic for arsine or trimethylarsine. When arsines were generated with $NaBH_4$ from 50 ml samples, collected in 3 ml heptane, and 5 μl aliquots injected in the GLC, relative detection limits were 0.2 ng of arsenic per ml for CA or DSMA and 0.4 ng of arsenic per ml for As(V) or trimethylarsine oxide. Reproducibility was also good, with relative standard deviations of 2% to 5% for the various arsenicals.

High-pressure liquid chromatography (HPLC)

HPLC, originally known as high-pressure liquid chromatography, has at times been referred to as high-performance liquid chromatography. Rapid development of HPLC methodology has led to its use in an increasing number of applications, including arsenic speciation.

Among the earliest studies employing HPLC to separate organometals was the work of Brinckman et al. (1977), who used GFAAS as the detection method. These authors described the technical aspects of HPLC separation of a variety of organometal species in some detail, but triphenylarsine was the only arsenical examined. Stockton and Irgolic (1979) interfaced a high-performance liquid chromatograph with a Zeeman effect graphite furnace atomic absorption spectrophotometer, but only reported on the separation of arsenobetaine, arsenocholine, and inorganic arsenic.

In an effort to achieve improved separation of arsenic species and to avoid the signal-quenching effect of previously used HPLC solvents, Woolson and Aharonson (1980) employed an anion-exchange column and an elution gradient of 100% water/methanol (80/20, v/v) to 100% 0.02 M $(NH_4)_2CO_3$/methanol (85/15, v/v). The HPLC was connected to a graphite furnace atomic absorption spectrophotometer via an automatic sampler. Separation and quantitation of As(III), As(V), CA, and MAA was accomplished, with detection limits of 0.25 ppm of arsenic in solution.

Calibration curves for all four arsenic species were linear from 0.1 to 2.0 ng for single furnace atomizations and from 5 to 200 ng for sequential, multiple atomizations of eluent from the HPLC. Quenching from solvents used and from extracts of soil samples was said to be minimal. Because the solvents used did not affect the spectrophotometric signal response, there was no advantage in attempting a correction for quenching by use of the "method of addition." Quantitation was also simplified, in that all four arsenicals yielded a virtually identical signal response, allowing use of internal standards or injection of only one standard into the atomic absorption instrument.

The report of Woolson and Aharonson (1980) was followed by another from the same laboratory, detailing procedures for extracting arsenicals from soil samples for analysis by the HPLC-GFAAS method (Iadevaia et al., 1980). The method for soil samples involved extraction with 2 M NH_4OH, clean-up on a carbon-Celite chromatographic column, and concentration by evaporation. Use of 8-quinolinol sulfate to complex and remove interfering ions significantly enhanced recovery of both CA and MAA. Samples containing 0.5 ppm CA or MAA were subjected to further isolation by TLC to remove excess salts.

The procedure of Iadevaia et al. (1980) resulted in recovery from spiked samples of over 90% of added CA and 80% of MAA. Recoveries of As(V) and As(III) were much lower, however, being only 56% and 64%, respectively. Additionally, inorganic arsenic could not be speciated according to valence, as all arsenite recovered was oxidized to arsenate by the soil extract.

Brinckman et al. (1980) published a study similar to those from Woolson's laboratory. They tested two HPLC-GFAA combinations for separation and quantitation of arsenite, arsenate, CA, and MAA, and determined that all four arsenicals could be separated on a reversed-phase column. Brinckman et al. achieved their best separatory results with a mobile-phase of water/

methanol (75/25) at pH 7.6, saturated with tetraheptylammonium nitrate (THAN). This mixture was followed with pure methanol, in order to elute arsenate, the last arsenic species coming off the column.

Brinckman et al. (1980) state that identical amounts of arsenic in the different arsenic species did not produce like responses from their GFAAS, with up to a twofold difference observed in response between CA and MAA. This finding differed from the results of Woolson and Aharonson (1980). Also, when soil samples containing inorganic or methylated arsenicals were extracted with 0.5 M NH_4F and 0.1 M NaOH, only a small portion of the arsenic recoverable by acid digestion of a like sample was solubilized. Thus, the soil extraction technique of Iadevaia et al. (1980) appears to be superior to that of Brinckman et al. (1980).

A recent paper by Morita et al. (1981) describes the use of HPLC for arsenic speciation, but differs from the foregoing studies in that arsenic detection was accomplished by using an inductively coupled argon plasma-atomic emission spectrometer (ICP). Separation of arsenicals was achieved with anion-exchange columns, and the most efficient employed Nagel-N-$(CH_3)_3$ column packing and use of 0.05 M phosphate buffer. A cation-exchange column was tried, but did not separate arsenate from arsenite. Relative sensitivities for five arsenic species, As(III), As(V), CA, MAA, and arsenobetaine, were almost identical, judging from relative peak areas. Detection limits were said to be 30, 30, 19, and 41 ng for As(III), As(V), CA, and MAA, respectively.

Peak heights for five arsenicals were said to be linearly related to the amount of arsenic present in the range of 50 to 1,000 ng. Results with the ICP detection system were also compared with data derived from atomic absorption and direct current plasma atomic emission spectrometry. The latter two methods were judged significantly less sensitive for arsenic quantitation of the HPLC samples tested. The HPLC-ICP method appeared to be useful for detecting arsenicals in samples of seaweed extract, as well as in prepared solutions of pure arsenicals, and gave a detection limit of 2.6 ng of arsenic.

Atomic absorption spectrophotometry (AAS) and glow-discharge detection

In 1973, Braman and Foreback published their pioneering work on the determination of environmental levels of methylated arsenicals. Their system depended on reduction of arsenicals to volatile arsines by the use of sodium borohydride ($NaBH_4$) at different pHs, and was used to quantitate CA, MAA, As(III), and As(V). The arsines were collected in a U-trap partially packed with glass beads and cooled with liquid nitrogen. When the trap was warmed, the arsines were carried from the trap in the order of their boiling points, and they could then be quantitated by atomic emission spectrometry, using a helium glow-discharge detector. Braman and Foreback reported detection limits of 0.5 ng for CA and MAA, and 0.05 ng for arsenate and arsenite.

The initial reduction at pH 4 to 5 resulted in arsine production from As(III). The sample was then acidified to pH 1 to 2 with oxalic acid. Cyanoborohydride was added to reduce any As(V) present to As(III). Addition of more sodium borohydride then resulted in production of arsine from the new As(III). Use of a different acidified sample aliquot was necessary to determine CA and MAA, however, as hydrogen cyanide produced in the previous procedure interfered with

detection of methylarsines. Braman (1977) later reviewed the utility of such methods in analysis of environmental levels of arsenicals.

A similar method was employed by Edmonds and Francesconi (1976) to analyze for arsenate, CA, and MAA in aqueous solutions, but quantitation was achieved with an atomic absorption spectrophotometer. Samples were acidified with hydrochloric acid. No sample splitting was required, but analysis times were lengthy (13 minutes) and detection limits were quite high (500 ng for inorganic and 1 μg for methylated arsenic).

An elaborate analytic scheme was published by Andreae (1977) for analysis of arsenate, arsenite, CA, MAA, and trimethylarsine oxide (TMAO). This method was also based on separation of cold-trapped arsines by sequential volatilization, and detection was accomplished by use of atomic absorption or GLC instrumentation. Again, $NaBH_4$ was used to convert As(III) to arsine in a sample buffered to ca pH 6. Tris-HCl buffer was used, as it neither resulted in interferences nor precipitated with seawater samples, as would the oxalate buffer used by Braman and Foreback (1973).

Following removal and quantitation of the arsine, HCl was used to bring the pH to ca 1; $NaBH_4$ was then added to convert As(V), CA, MAA, and TMAO to their respective arsines for quantitation by either AAS or GLC. With AAS, the detection limit for arsine was 0.05 ng of arsenic. When using GLC, detection limits of 0.4, 0.2, and 15 ng of arsenic for mono-, di-, and trimethylarsines, respectively, could be attained with an electron-capture detector. Due to nonlinearity, however, the useful range for quantitation was only 20 to 50 times the detection limit. A flame ionization detector was less sensitive for CA and MAA but had a much greater range for quantitation. Additionally, it was more sensitive than electron capture for trimethylarsine quantitation. Andreae advocated use of both detectors operated in parallel, connected to a column effluent splitter.

The advantages of the GLC detection system were ability to quantitate methylarsines over a broader range of concentrations and ability to identify peaks by use of the peak height ratio between the two detectors, as well as by their relative retention times. Although slower and more burdensome than quantitation by AAS, use of GLC was said to offer greater sensitivity (in the parts-per-trillion range often needed for analysis of environmental samples). It was also recommended that samples be acidified with hydrochloric acid to prevent loss of a portion of the methylated arsenicals on standing. In order to prevent loss of As(III) by oxidation, especially in acidified samples, storage below -15°C was suggested.

Also in 1977, Braman et al. published a more detailed report of their analytic method, with various added refinements of technique. For example, they had determined that by buffering their samples they could prevent premature reduction of As(V); this allowed production of arsine from As(III) only. If the pH was kept in the range of 3.5 to 4.0, the reduction of As(III) by $NaBH_4$ was faster than it was at higher pH values. Use of cyanoborohydride was replaced by repeated additions of $NaBH_4$ at pH 1 to 1.5. The repeated additions were required to overcome loss of borohydride due to its rapid hydrolysis.

It was said to be necessary to use a CO_2 absorber employing small beads of NaOH to avoid interference with detection of arsines. As before, the evolved arsines were precipitated in a liquid N_2 cold trap and evolved sequentially in the order of their respective boiling points. Helium was

used as the carrier gas, as N_2 could freeze in the cold trap. Again, quantitation was accomplished by passing the arsines through a direct current electrical discharge and using a recording scanning monochromator to detect arsenic atomic emission.

Detection limits for arsine, and mono-, di-, or trimethylarsines were near 1 ng (as arsenic), and responses were similar in arsenic content for each of the arsines. The method was said to be useful for arsenic speciation in fresh water and seawater, for air particulates and filtered air, as well as for urine and other biologic materials.

Crecelius (1978) used the same basic methodology as Braman et al. (1977), but suggested using an additional water vapor trap between the sample reaction chamber and the liquid N_2 arsine trap. This consisted of a U-tube immersed in an ice-water/salt bath. For samples such as wine or certain natural waters, it was said that removal of H_2S and CO_2 was helpful to prevent interference with arsenic quantitation due to high concentrations of these gases. Removal could be accomplished by using a lead acetate trap and an additional sodium hydroxide trap between the water vapor and arsine traps. Additionally, it was said that saturation of the system (except for the reaction chamber) with inorganic arsenic and with CA before running samples would give more consistent results.

Following the lead of Braman et al. (1977), Shaikh and Tallman (1978) employed hydride generation and cold-trapping of arsines to speciate arsenicals. They used GFAAS for arsenic determination and obtained good precision with inorganic arsenic and only slightly poorer results with CA or MAA. For the methylated arsenicals, response linearity was seen up to 600 ng. Detection limits were 10 ng of arsenic for CA and 15 ng of arsenic for MAA.

In a more recent publication, Feldman (1979) proposed a variety of further refinements of the method of Braman et al. (1977). According to the author, the technique was more rapid and convenient. Among the suggested changes were use of concentrated (at least 0.25 M) buffers to prevent increase in pH due to addition of the alkaline sodium borohydride. The best yields of mono- and dimethylarsine from MAA and from CA, respectively, were obtained by using oxalic acid. This procedure also avoided production of arsine from MAA and methylarsine from CA, a problem encountered when sulfuric acid was used.

Leakage of gas from the system was said to be reduced by adding $NaBH_4$ solution via a pump mechanism, rather than by the previous method of injection through a septum. Joints that required occasional disconnection were constructed from o-ring flanges, held with clamps in a further effort to prevent leaks. A rate-meter and a flow-controller were used to control gas flow rates accurately.

Water vapor aerosols created during the bubbling caused by generation of arsines and particularly during evolution of excess hydrogen gas in the borohydride reactions had to be removed to prevent clogging of the cold trap with ice. Feldman accomplished this by using two impingers containing phosphoric acid, a hygroscopic liquid. In addition, a trap containing NaOH beads was employed to remove CO_2 and other (unknown) impurities in the helium stream that caused interference with arsine quantitation by the direct current glow-discharge technique. Warming the trap with the airstream from a large fan was said to give more reproducible peak heights.

Improvements in design of the glow-discharge chamber were also described. Retention times for arsine and mono-, and dimethylarsines were said to be only around 5, 8, and 10 seconds, respectively, with good peak resolution. The detection limits given for Feldman's method were limited primarily by the amounts of arsenic and interfering elements present as contaminants in the reagents used, and were said to be approximately 0.1 ng of arsenic.

Another of the earlier studies was that of Fitchett et al. (1975), who used solvent extraction to separate arsenicals from water or urine samples. Such samples were mixed with hydrochloric acid and heated, and a concentrated potassium iodide solution was then added. After further mixing, arsenicals (as the iodides) were extracted into chloroform. The chloroform was then back-extracted with water to remove inorganic arsenic or with dilute sodium dichromate solution to sequester both organic and inorganic arsenic. Aliquots of the aqueous phase were subjected to analysis by GFAAS.

All the arsenicals were presumed to have been extracted into the aqueous phase regardless of the presence of dichromate; however, the oxidant was apparently necessary to reoxidize methylarsenic to prevent loss of the methylated arsenous (reduced) forms by volatilization during sample ashing and drying. According to the authors, recoveries of inorganic arsenic added to water or to urine were 87% and 93%, respectively, while similar recoveries for CA were approximately 99% and 88%.

In a subsequent publication from the same laboratory, Mushak et al. (1977) addressed the issue of arsenic speciation during analysis of mammalian tissue samples. Lyophilized tissue homogenates (liver, kidney, etc.) were treated with concentrated hydrochloric acid followed by deionized water. For other samples, KI solution was added with the HCl to reduce As(V) to As(III). Both types of samples were then extracted with benzene.

Inorganic and monomethylarsenic could be removed from the benzene layer into deionized water, or 2 N nitric acid could be used to extract inorganic, mono-, and dimethylated arsenic. The water extract was acidified with HCl followed by addition of KI, and the mixture further extracted with chloroform. Re-extraction of the chloroform with water was said to yield only inorganic arsenic. The various arsenicals could then be quantitated by GFAAS, either directly or through subtraction of values for single species from sums of known combinations.

The tissue samples analyzed by Mushak et al. (1977) were freeze-dried following homogenization in deionized water to prevent dilution of the acid used to free the bound arsenic. The precision of the method was not high initially, with relative standard deviations on the order of 8% to 12% for series of five samples, but use of large sample aliquots for introduction into the graphite furnace (30 to 40 μl vs 10 μl) was said to greatly improve analytic precision.

In a related approach, Yasui et al. (1978) reported a method for selective speciation of arsenicals from biologic samples. Their technique involved solubilization of arsenicals with 6 N HCl and extraction of As(III) into toluene. As(V) was extracted with toluene after reduction by KI. Organic arsenicals remained in the HCl solution, and they were then wet-ashed with an acid mixture (HN_3), H_2SO_4, and $HClO_4$) and quantitated by GFAAS.

Buchet et al. (1980) investigated various acidification and solvent extraction procedures for separating arsenic species before quantitation by GFAAS. They determined that only As(III)

would be extracted with toluene from samples acidified with concentrated HCl. When acidification was carried out with a concentrated $HCl/HClO_4/HBr$ mixture and followed by toluene extraction, both As(III) and As(V) were removed. If HCl plus KI was employed, CA and MAA, as well as total inorganic arsenic, could be extracted.

All of the above, plus arsanilic acid and arsenic complexed with organic molecules (e.g., 2,3-dimercaptosuccinic acid) could be extracted by wet-ashing the sample with a concentrated mixture of HNO_3, $HClO_4$, and H_2SO_4, followed by addition of HCl and KI and extraction with toluene. In each case, the toluene extract was back-extracted with a solution of $Co(NO_3)_2$ in dilute nitric acid, before quantitation by GFAAS. The results of the wet-ashing plus extraction procedure were said to be identical to those of dry-ashing and extraction with toluene after sample acidification and KI treatment.

When the method of Buchet et al. (1980) outlined above was applied to urine samples, rather than to aqueous laboratory solutions, problems arose. Arsenite alone or total arsenic could still be accurately ascertained, but only 94% of the MAA and 85% of the CA could be extracted from spiked samples in the presence of 0.005 M dichromate (as had been suggested by Fitchett et al., 1975).

Buchet et al. (1980) also determined that the complex organoarsenical present in the urine of individuals who had consumed certain seafoods (e.g., shrimp) was not extracted during any of their procedures as described above, with the exception of ashing. They employed a modification of the hydride generation method of Braman and Foreback (1973), and found that they could determine the CA, MAA, and total inorganic arsenic and its metabolites in man, even when the subjects had recently consumed seafood.

Among the most recent work involving hydride generation and cold-trapping was that of Howard and Arbab-Zavar (1981), who attempted further improvements in methodology and investigated possible chemical interferences. They used a unique apparatus for mixing the sample and reagents for arsine production.

Interconnected mixing coils derived from Technicon AutoAnalyzer® components were used to mix sample, sodium borohydride, and EDTA solutions. The complete mixture was then passed through a scrubber containing lead acetate that was designed to remove any hydrogen sulfide present. Further passage through a sodium hydroxide moisture trap occurred before the arsine-containing N_2 gas reached a liquid N_2 cold trap. The U-shaped trap contained glass beads silanized with trimethylchlorosilane. Detection was accomplished by using an AAS equipped with a tubular quartz atomizer heated by an air-acetylene flame.

Samples were digested with warm concentrated hydrochloric acid, in an attempt to avoid oxidation of As(III) to As(V). The method was claimed to allow determination of CA, MAA, arsenate, and arsenite. Detection limits were approximately 0.25 ng of arsenic for the mono- and dimethylated species and for arsenate, but the detection limit was higher for arsenite (0.62 ng of arsenic).

Howard and Arbab-Zavar (1981) stated that phosphoric acid absorbed arsines and could not be used in a moisture trap. This is in contradiction to the statement by Feldman (1979) to the effect that phosphoric acid was suitable for use in a moisture trap in the gas stream. Both agreed,

however, that sodium hydroxide did not remove arsines. Additionally, Howard and Arbar-Zavar contend that silanization of the cold trap is essential, although no other authors relied on such a procedure.

Pierce and Brown (1977) reported an elaborate study of inorganic interferences with arsenic analysis by various atomic absorption methods. They stated that interferences differed by method (e.g., hydride generation vs GFAAS).

Howard and Arbab-Zavar (1981) also tested a series of ions for their ability to interfere with arsenic analyses. The presence of certain of the tested ions did result in a decrease in the detected amount of one or more of the arsenic species quantitated. Most such interferences could be overcome by adding EDTA before sample reduction, with the exception of the effects of antimony, gold (III), nitrite or a large (5,000 x) excess of iron (III). All of these, except for the iron, could be removed by using a cation-exchange resin.

Peacock and Singh (1981) also tested various complexing agents for their ability to overcome interference from various elements and found thiourea to be superior to EDTA, dimethyl glyoxime, triphenylphosphine, trimethyl phosphite, cyanide, thiocyanate, and fluoride. In a similar attempt, pyridine-2-aldoxime was found to be a superior chelating agent for eliminating the suppressing effect of other metals on arsenic quantitation (Dornemann and Kleist, 1981).

According to Hinners (1980), the use of a cold-trapping step to separate arsine from mono- and dimethylarsine allows accurate quantitation of each type. It is common, however, for arsenic mixtures containing both methylated and inorganic arsenic to be analyzed for total arsenic content by direct hydride analysis using inorganic arsenic standards. Under such conditions, the methylarsines do not give as great a response as does the AsH_3 according to Hinners. This would result in an underestimation of the total arsenic present. This finding was echoed by Arbab-Zavar and Howard (1980). Also, Hinners stated that in methods purporting to differentiate As(III) from As(V) by reduction at two different pHs, a potentially significant contribution to the apparent amount of As(III) can be made by As(V) present in the sample. This was said to be the case even with well-buffered samples.

The findings of Hinners (1980) and of Arbab-Zavar and Howard (1980) regarding total arsenic analysis have been disputed by Norin and Vahter (1981). The latter authors state that when mixtures of inorganic arsenic, CA, and MAA were analyzed, peak area was independent of the relative proportions of the individual arsenicals. They proposed using direct hydride analysis of urine samples to monitor environmental arsenic exposure.

F. M. Tatum and R. D. Hood (unpublished), however, found that when a mixture of equal proportions of As(V), CA, and MAA was analyzed by hydride generation AAS, there was a 25% decrease in total peak area, compared with that resulting from an amount of As(V) alone equal to the arsenic in the combined total of the mixture. Further, E. A. Woolson (personal communication) stated that addition of ammonium or potassium iodide to reduce the samples prior to the addition of acid and $NaBH_4$ will eliminate such differences in peak area.

Elimination of methylarsenic in total arsenic determination

An early study of analysis of methylated arsenic was conducted by Hamme et al. (1970), who developed the method for use on soils from areas sprayed with a CA-based herbicide. Samples were extracted with HCl, H_2SO_4, and carbon black. Total arsenic was determined by atomic absorption spectrophotometry of the solution.

Recent papers have described methods for determining total arsenic in samples such as water and urine. These methods included procedures for decomposing organoarsenicals in order to allow quantitation by atomic absorption spectrophotometry. For example, a method for total arsenic was also described that involved wet-ashing lyophilized tissue homogenates in two steps, conversion to trivalent iodides, and extraction into chloroform of arsenicals chelated with diethyldithiocarbamate (Mushak et al., 1977). The arsenic was reextracted into dilute nitric acid, which broke the chloroform soluble arsenic-carbamate complex. Analysis again was by GFAAS, with relative standard deviations of around 3% to 7%.

An interesting method was proposed by Shaikh and Tallman (1977), who used a solution of SDDC in chloroform/ephedrine to trap arsine for analysis by GFAAS. Prior wet-ashing was said to be essential in order to quantitate methylated arsenicals accurately.

Cox (1980) suggested destroying organoarsenicals by wet digestion with hot nitric and perchloric acids, followed by hot sulfuric acid and then by HCl and KI. $NaBH_4$ was used to generate arsine from the sample, and quantitation was done by AAS with an electrically heated quartz absorption tube. The procedure was said to result in complete breakdown of CA to the inorganic state and to be superior to both the use of potassium permanganate followed by nitric and sulfuric acids as described by Sandhu and Nelson (1978) and to the acid persulfate method of Goulden and Brooksbank (1974). The latter authors stated, however, that for "relatively clean" water samples, they found no difference between the use of acidified potassium persulfate and the use of sulfuric-nitric acid digestion to break down organoarsenicals.

Arbab-Zavar and Howard (1980) acknowledged the necessity of decomposing any significant amounts of methylarsenic present before total arsenic determination by methods employing hydride generation. These authors recommended either fusion with NaOH or wet-ashing with a combination of nitric and sulfuric acids and hydrogen peroxide. The latter method was also suggested by Arafat and Glooschenko (1981).

Subramanian and Meranger (1982) used only nitric and perchloric acids for sample digestion, but reported recoveries of just 75% for arsenic from bovine liver standards certified by the National Bureau of Standards (NBS). Conversely, when Agemian and Thomson (1980) ashed NBS liver standard reference material with nitric acid, followed by sulfuric plus perchloric acids, recoveries were close to 90%. Nitric and perchloric acids were also used by Agemian and Bedek (1980) for wet-ashing soil and sediment samples, but were followed by hydrofluoric acid to release arsenicals from the silicate lattice.

A different approach to sample preparation before total arsenic analysis was used by Uthus et al. (1981), who dry-ashed plant and animal tissues. Samples were placed in glass tubes with cellulose and $Mg(NO_3)_2$ and dried with moderate heat. They were then placed in a muffle furnace, ashed at 600°C, and the residue was dissolved in 5 N HCl followed by deionized water. Arsine

was generated from the samples by using $NaBH_4$ in the presence of EDTA to reduce interference from other elements. The arsine was concentrated in a liquid N_2 cold trap before quantitation by GFAAS. This procedure was said to yield a sensitivity of 0.11 ng and detection limit of 0.14 ng, and to give values agreeing well with those given for NBS bovine liver, oyster tissue, pine needles, and orchard leaves.

Tam and Lacroix (1982) also dry-ashed samples, using $Mg(NO_3)_2$ plus MgO to aid the ashing process. Samples were redissolved in water followed by 6 N HCl, and the As(V) was reduced to As(III) with KI solution before quantitation by AAS. The method was said to be useful for biologic samples.

An elaborate scheme was published by Thiex (1980) for total arsenic determination in plant and animal tissues. Thiex's method involved sample digestion with hot nitric acid, followed by perchloric and sulfuric acids. Concentrated digests were rediluted with water and with solutions of iodide and sodium metabisulfite. Such treatment was said to digest the sample, break down organic arsenicals, and reduce As(V) to As(III). The As(III) was then extracted with diethylammonium diethyldithiocarbamate in chloroform and analyzed by GFAAS. Results from analysis of rat tissues containing ^{74}As were compared with those from quantitating the radioactivity present. They ranged from 77% to 134%, with a mean of 103%.

Miscellaneous analytic methods

Although little used in comparison with the previously described methods, other means of analysis for methylated arsenicals have been described in the literature. For example, Care (1968) used a solution of potassium bromate and nitric acid to decompose MAA, DSMA, and other organoarsenicals to inorganic arsenate, followed with quantitation by means of a titration procedure. Holak (1972) quantitated CA by nuclear magnetic resonance spectroscopy. Samples were mixed with succinic acid, which was used as an internal standard. To prevent broadening of the CA peak, ferric ions were precipated with KOH. Quantitation was done by integrating the single peak characteristic of CA.

In 1975, Edwards et al. described a technique involving the use of electrophoresis on cellulose thin-layer chromatography sheets. Samples were spotted on the sheet and electrophoresed in 0.05 M sodium citrate buffer. The sheets were then air-dried and exposed to ammonium sulfide, followed by Jungnickel's Reagent A. This caused arsenite to appear as a yellow and MAA as a light blue spot. The sheet was then exposed to UV light, resulting in the appearance of arsenate as a dark blue and CA as a pink spot. The method was said to be simple, inexpensive, sensitive, and rapid, but is useful only for qualitative analyses. It was applicable, however, to complex samples such as sewage sludge.

A more recently described method involved determining inorganic arsenic by differential pulse polarography (Henry et al., 1979). In this case, methylated arsenicals were discussed only as interferents. It was concluded that the presence of CA was not a problem, but that a high ratio of MAA to inorganic arsenic could result in inflated arsenic values due to MAA breakdown during sample preparation.

Chapter 3

Production and Use

Commercial formulations

Agent Blue was a clear, yellowish tan liquid (Young et al., 1978). It was water soluble but not soluble in organic solvents such as diesel fuel, and had a specific gravity of 1.32 at 25°C. One gallon (3.8 L) of the herbicide weighed 11 lb (5 kg) and contained roughly 3.1 lb (1.4 kg) of the active ingredient (as CA). According to A. L. Young (personal communication), Agent Blue was obtained from the Ansul Company, Marinette, Wisconsin, and was designated by the company as Phytar® 560-G. This formulation had slightly more surfactant and active ingredient than the commercial Phytar® 560. Also, early in the Vietnam War, 4,500 kg of another cacodylate-based formulation, Phytar® 138, were used. The formulation for Phytar® 560-G was given by Young et al. (1978) as:

Sodium cacodylate	26.4%
Cacodylic acid	4.7%
Sodium chloride	5.5%
Surfactant	3.4%
Antifoam agent	0.5%
Water	59.5%

Although Ansul no longer manufactures cacodylate-based herbicides, they are currently produced in the United States by Vineland Chemical Company, Vineland, New Jersey, and by Vertac Chemical Company, Memphis, Tennessee. Cumberland Chemical Company, Houston, Texas, has also registered with the U.S. Environmental Protection Agency (EPA) as a potential producer and may be in production by the time this monograph appears.

In addition to Phytar®, formulations containing CA or NaCA have been marketed under trade names such as Phytar® 160, Ansar® 138, 160, and 560, Silvisar® 510, Rad-E-Cate®, Rad-E-Cate® 25 and 35, Broadside®, Chex-Mate®, Bolls-Eye®, Clean-Boll®, Check-Mate®, Hi-Yield®, and Super K-Gro® Liquid Fence and Grass Edger.

According to the major manufacturing patents (Moyerman and Ehman, 1965; Schanhals, 1967), commercial cacodylate-based formulations may contain impurities, such as MAA, arsenic acid, or sodium sulfate, in addition to sodium chloride. Formulations based on the commercial processes are said to be free of arsenite and to lack the typical garlic odor of cacodyl, arsines, and their oxides. If the commercial products are stored in containers lined with metals, such as zinc or iron, however, reducing conditions could result in the formation of small amounts of cacodyl oxide, causing a garlic odor.

Federally approved uses

Pesticides based on CA or NaCA have been registered for a number of uses. These registered uses are listed below (MRI, 1975, pp. 98-100):

1. General weed control in noncrop areas such as drainage ditchbanks; rights-of-way; along sidewalks, driveways, and fences; along railways, highways, and other roads; around buildings, ornamentals, lumber yards, grain elevators, parking lots, etc. Rate: 2.5 to 5.0 lb acid equivalent per 100 gal of water, applied at a volume sufficient to cover the unwanted vegetation to just short of runoff. Reapply as required (no limitations).

2. Weed control as a "directed application" in nonbearing citrus orchards (orange, grapefruit, tangerine, lemon, and lime orchards), to be applied in interspaces between and around the bases of trees. Rate: 3.75 to 5.0 lb acid equivalent per acre, to be mixed with water at the rate of 2.5 to 5.0 lb acid equivalent in 50 to 100 gal, and applied as a full-coverage spray to just short of runoff. Application should be repeated as required if regrowth occurs, but no more than three applications per year are permitted. This use is not permitted in Florida.

3. Lawn renovation by application of CA to lawn mowed to about 1 in height, preferably on a warm, sunny day. Rate: About 8.5 lb acid equivalent per acre, equal to 3 oz of acid equivalent per 1,000 sq ft, in 4 gal of water. This rate corresponds to 10 fluid ounces of a CA formulation containing 2.5 lb acid equivalent per gallon in 4 gal of water per 1,000 sq ft. If green areas remain, reapply after five days. When top growth is all brown, dead vegetation should be removed, and the lawn may be promptly reestablished because the phytotoxic properties of CA are quickly inactivated on contact with soil.

4. Defoliation of irrigated and dryland cotton by aerial or ground application, to be applied when 50% or more of the cotton bolls are open, and 7 to 10 days before anticipated picking. Rate: On dryland cotton, about 0.8 to 1.0 lb acid equivalent per acre. In airplane applications, 5 to 10 gal of water should be used, and 15 to 25 gal of water per acre for ground applications.

5. For general postemergent weed control in noncrop areas, a combination product is registered and recommended that contains MSMA and CA at the ratio of 2.4 parts MSMA and 1 part CA. This combination offers quick burn-down of vegetation in conjunction with the systemic effect necessary to control certain deep-rooted perennial grasses and weeds. Weeds against which this combination product is registered and recommended include puncture vine, wild mustard, wild oats, chickweed, sandburn, common ragweed, pigweed, crabgrass, lambs-quarter, common plantain, prostrate spurge, giant foxtail, and yellow foxtail. It provides top-kill of certain perennial grasses such as Johnson grass, dallisgrass and nutsedge. Rate: 4.25 to 8.5 lb of combined active ingredients in 40 to 100 gal of water per acre. Reapply as required.

6. Crown kill of undesirable trees, including both conifers and hardwoods, through spaced-cut injection methods. Rate: About 1 ml of a formulation containing 50% CA per cut per 2 in of tree diameter at breast height (dbh) for trees below 8 in dbh; 1 ml of 50% formulation per cut per 1 in dbh for trees 8 in dbh and larger. Rate: Recommended rates vary somewhat depending upon whether conifers or hardwoods are to be killed, and whether the treatment is made during the growing or the dormant season.

7. Bark beetle control is used by professional foresters and entomologists only. CA can also be used to control Southern pine beetle, spruce beetle, Engelmann spruce beetle, mountain pine beetle, round-headed pine beetle, Arizona 5-spined beetle, pine engraver beetle, and the California 5-spined beetle. Suggested uses include preflight treatment (trap tree technique), preharvest treatment (elimination of logging debris as brood material), precutting treatment (in areas to be disturbed as, for instance, in trail building), and postflight treatment (lethal trap technique). Rate: For best results, a complete, trough-like frill has to be made around the entire tree within 18 in of the ground. One milliliter of a 50% CA formulation per inch of tree circumference is to be applied evenly in the frill. Preflight treatment (for spruce beetle only) may be made in October, with treated trees to be felled two to four weeks after treatment. Preharvest and precutting treatments involve treating 4 weeks before cutting the tree. Postflight treatments should be made within two to three weeks after the tree is attacked.

Production volume

Accurate data on the amounts of CA-based pesticides, silvicides, and desiccants produced commercially are not readily available. For example, the annual reports of the United States Tariff Commission do not list such products separately but include them in the category "Pesticides and Related Products, Acyclic." The Midwest Research Institute (MRI, 1975) estimated from an independent survey of trade sources that domestic production in 1973 and 1974 was between 600,000 and 800,000 kg (660 and 880 tons) annually, as the acid equivalent. It also speculated that CA was not being imported into the United States and that exports of the arsenical were low, probably not more than 10% of domestic production.

Estimated use by user and function

The major categories of domestic use for cacodylate-based agricultural products have been estimated for the year 1973 (MRI, 1975), and the results are shown in Table 3.1. The data are based on two different assumptions regarding total use, Case A (1.4 million lb or 635,000 kg) and Case B (1.6 million lb or 726,000 kg), which are near the estimated upper and lower values for total use (1.3 million lb or 590,000 kg to 1.7 million lb or 771,000 kg). According to the MRI estimate (1975), the major uses of CA were for weed control and cotton desiccation. Other uses were quite limited. It was also stated that use on cotton was increasing.

Table 3.1—Estimated uses of cacodylic acid in the United States by major functions and areas of use, 1973[a]

Function	Estimated share of total use	Estimated use, lb of AE[b] Case A[c] (lb)	Case A[c] (kg)	Case B[d] (lb)	Case B[d] (kg)	Primary area of use
Nonselective weed control[e]	52%	725,000	329,000	830,000	376,000	Western and southern states
Cotton defoliation	42%	585,000	265,000	670,000	304,000	Cotton states
Forest management	1%	20,000	9,000	20,000	9,000	Northwest
All other uses[f]	5%	70,000	32,000	80,000	36,000	All areas
Total U.S. use	100%	1,400,000	635,000	1,600,000	726,000	

[a] Adopted from MRI (1975).
[b] AE = acid equivalent.
[c] Case A: Assuming total U.S. use = 635,000 kg AE.
[d] Case B: Assuming total U.S. use = 726,000 kg AE.
[e] Including directed application in nonbearing citrus orchards.
[f] Including lawn renovation, and all other (including nonregistered uses).

Source: RvR Consultants, Shawnee Mission, Kansas.

Detailed records of domestic CA use are apparently kept only in California (MRI, 1975). Such information is available in quarterly and annual California Department of Agriculture pesticide use reports. In 1973, for example, the reported use of CA was 69,553 kg (153,364 lb) (MRI, 1975). Of that amount, 40,574 kg (89,466 lb) were used on cotton, 1,075 kg (2,370 lb) on other crops, 8,354 kg (18,421 lb) on highways and roads, and 19,549 kg (43,106 lb) for "other uses." The "other use" category included governmental agencies; park departments; school districts; reclamation, irrigation, flood control, water resource, and vector control districts; the University of California; and uses in industrial, residential, turf and other nonagricultural areas.

It is likely that the values given above are useful as an indication of the patterns of CA usage, rather than as a highly accurate estimate of total use. This is because CA was not classified as a "restricted or injurious material," by the California State Department of Agriculture (MRI, 1975) and thus it was not subject to strict reporting and use guidelines.

Chapter 4

Agricultural and Related Uses

Effects on plants

The herbicidal effects of methylated arsenicals such as CA have been known for more than 30 years (Schwerdle, 1954), and these chemicals have been widely used for much of that period (Hiltbold, 1974). In fact, 82 products containing CA or NaCA have been registered for use as pesticides according to the EPA's Office of Pesticide Programs (OPP, 1981).

Methanearsonates are more commonly used when a degree of selectivity in killing is desired, while CA and its sodium salt are considered relatively nonselective (Stevens, 1966). For example, CA is more than six times as phytotoxic as DSMA to rice seedlings (Ehman, 1965). Thus, it is rarely used on crops, unless a desiccant effect is sought. CA is also used when turfgrass killing is needed before reestablishing desired species, and it has been used as a silvicide as well (Rumberg et al., 1960).

Both the mono- and dimethylated forms are also less toxic to animals and to man than are the inorganic arsenicals that had long been in use as pesticides. Indeed, a specific CA formulation, Phytar® 560, was promoted by Stevens (1966) as having the following characteristics: "(1) low toxicity, (2) no residual soil toxicity, (3) complete water solubility, (4) non-volatility, (5) broad spectrum of response."

Grass and weed control. Because of their nonselective herbicidal activity, mixtures of cacodylate and sodium cacodylate are currently used to eliminate weeds and grasses around trees and shrubs, as a chemical "edger" along driveways, sidewalks, and fences, and to eradicate vegetation from rights of way, drainage ditches, and other noncrop areas. Mixtures of CA, NaCA, and MSMA are also used for general control of broadleaf weeds and grasses and top-kill of perennial grasses.

According to the patent issued to Schwerdle (1954), both CA and MSMA are active against crabgrass. While CA is the more potent herbicide, MSMA is selective to a greater degree and generally affects crabgrass more than it affects desirable lawn grasses. CA also acts somewhat more quickly (in three to five days) against crabgrass than does MSMA (two to seven days). When the two arsenicals are used together, a synergistic effect can be seen with regard to selective killing of crabgrass. CA was also said to be too toxic to lawn grasses for routine use early in the growing season.

In another early report, CA at relatively high treatment levels was said to be effective against both ryegrass and crabgrass (Skogley and Ahlgren, 1955). CA was also said to eliminate weeds such as curly dock and mullein.

It can generally be said that even with the monomethylated arsenical herbicides, application rate is important in determining whether weed species will be killed without undue harm to turf

grasses (Rumburg et al., 1960). Both arsenicals also generally require repeated applications at intervals of 14 to 21 days on perennial weeds (National Research Council, 1968).

In addition, elevated ambient temperature at the time of application was found by Rumburg et al. (1960) to enhance the phytotoxicity of DSMA, but not that of CA or sodium arsenite. Stevens (1966), however, stated that warm temperatures cause CA to act more rapidly, although its effects under such conditions may not be as long-lasting. Stevens also reported that longer, more intense periods of sunlight appear to hasten the phytotoxic action of CA and to increase its permanence. He further stated that since CA is poorly translocated in vegetation, thorough coverage of the treated plants is essential, and due to the water solubility of CA-containing formulations such as Phytar® 560, rain, overhead irrigation, or even heavy dews will reduce the herbicidal effect. This is particularly true since concentrated CA solutions are much more effective than dilute ones.

Although CA has been cited as having a greater toxic effect than that of monomethylated arsenicals on grasses, there are also reports to the contrary. For example, when Sckerl et al. (1966) sprayed Bermuda grass infested with Johnson grass with DSMA or MSMA, killing of the Johnsongrass was nearly complete. There was no obvious harm to the Bermuda grass. When CA was used, only minor damage resulted to either grass. Application rates were 3.4 kg/ha four times per season. Even in the DSMA- and MSMA-treated plots, however, Johnsongrass regrowth was seen the following year.

Arnold and Aitken (1973) reported later that although CA with or without MSMA initially damaged Bahia grass in a pecan orchard, it was not as effective in preventing regrowth as were other herbicides tested, such as Dalapon® (2,2-dichloropropionic acid). According to Stevens (1966), such results are to be expected in that several applications of CA are needed to kill deep-rooted perennial grasses completely.

A study of the effects of CA use on a large test area at Eglin Air Force Base in northwest Florida was performed by Oliver et al. (1966). They reported that CA applied to a "sandhill plant community" at a rate of 2.25 kg/ha had no lasting effect, although some plant species were defoliated or exhibited dead or brown leaves. A 6.8 kg/ha application had a greater effect, while 34 kg/ha appeared to have killed all pine trees in the area and caused a 75% defoliation of the oaks. The highest application rate had a significant effect on all of the area's dominant vegetation. Considerable rainfall in the area during the study may have diminished the effect of the lower CA applications.

A less well-drained (hammock) community was tested by spraying individual plants (Oliver et al., 1966). The 2.25 kg/ha rate, applied before heavy rains, was ineffective, while the 34 kg/ha rate applied before a dry period caused defoliation or complete browning of the leaves of all plants tested.

A grassland area, covered with broomsedge, wiregrass, palmettos, and yucca was sprayed at four different application rates and evaluated at four weeks. At the low rate, 2.25 kg/ha, recovery was nearly complete. At 6.8 kg/ha, only the lower leaves of exposed plants were still brown. Following 17 kg/ha, wiregrass, broomsedges, and oaks were alive, but recovery was modest following the initial 75% to 90% kill. At the highest application rate, 34 kg/ha, there was a

complete initial kill of vegetation, and only wiregrass appeared to be making a significant recovery.

Another study of the effects of an herbicide on an entire plant community was conducted in a fescue grass meadow by Malone (1972). Beginning in mid-June, he compared heat stress caused by covering large areas with polyethylene sheeting for three days with the effects of single or multiple sprayings with Phytar® 560. Application rates were 3.4, 10.1, or 30.2 kg/ha for the single treatments, and 10.1 kg/ha applied three times for the repeated treatment. Results indicated that heat stress and single CA applications had similar effects. Recovery of the plant community was rapid, and pretreatment diversity and biomass were largely regained in one growing season.

The repeated herbicide application inhibited fescue growth, decreased species diversity and biomass, and resulted in more rapid decomposition of litter. Malone (1972) attributed the recovery following single sprayings to the fact that CA is a nonselective contact herbicide that is rapidly inactivated and has only limited ability to kill perennial grasses such as fescue. Repeated applications of such materials were said to stress plant communities severely, however, altering the community's structure and reducing both litter and biomass.

In a later study of a salt marsh ecosystem, Edwards and Davis (1975) found that repeated treatments at up to 10,000 ppm of CA failed to alter the density of smooth cordgrass, although dry weight was decreased. Thirty applications of 1,000 ppm of CA inhibited production of flowering shoots and increased the proportion of dead and dying tissue on live shoots, as did a single application of 90,000 ppm.

Use of cacodylic acid in cotton defoliation. Commercial preparations containing NaCA and CA are currently in use as cotton defoliants to aid in mechanically harvesting the cotton bolls. The defoliant is typically applied a week to 10 days before harvest, when more than half of the bolls have opened. Spraying can be done by aircraft or by ground equipment capable of thorough coverage of the cotton leaves.

Chemical defoliation has been said to reduce the decay of the lower bolls and to inhibit the deterioration of both cotton seed and fiber (Burhan and Gleadle, 1973). According to Kurdikeri and Koraddi (1975), using a sodium cacodylate preparation known as Chemaid® resulted in an increased cotton yield. Ray (1977) also found that using Chemaid® on cotton was advantageous.

In 1978, Iruthayaraj et al. compared paraquat and Chemaid® on seed cotton. They obtained a higher yield of cotton when using Chemaid®, although paraquat caused a greater degree of defoliation. They attributed this to the fact that the NaCA preparation caused leaf abscission without killing the plant, thus allowing further boll development. On the other hand, paraquat desiccated the bolls to the extent that they shriveled and sometimes dropped from the plants. Combinations of Chemaid® with paraquat were not superior to the arsenical alone in affecting yield. The previous results were contrary to those of Singh and Kumar (1978), as they reported no significant differences between an NaCA preparation (Bolls-Eye®) and either paraquat or ethrel on seed cotton yields. Use of any of the defoliants increased the yield of late-maturing cotton varieties but not those of early-maturing strains. No treatment affected the properties of the cotton fibers or germination of the seed.

Silvicidal effects. According to Smith (1966), CA was first proposed as an agent for killing unwanted trees by J. L. Harrison-Smith in 1963. A similar suggestion was made by Day (1965), based on results with Ansar® 138 and Ansar® 160 on hardwoods, although secondary sprouting was said to be a problem. In the same year, Smith (1965) stated that according to preliminary results, single injections of a 30% CA formulation (Ansar® 160) were highly effective in controlling a number of species. These included quaking aspen, red maple, paper birch, red oak, jack pine, and red pine treated in Wisconsin. All trees were given 1.0 to 2.0 ml of Ansar® 160 per inch of tree "diameter at breast height" (dbh) during August and evaluated within a few months.

Smith (1965) speculated that there would be a considerable degree of difference in susceptibility among species, based on the degree of spirality of their water-conducting elements. He suggested that trees with greater spiraling of xylem elements would conduct the herbicide to a higher proportion of the tree's crown. Smith (1966) later theorized that crown kills from CA are due to desiccation of meristematic tissues of buds and twigs and the abscission layer of the petals.

Peevy (1969) reported excellent rates of top growth killing (96% to 100%) with an herbicide containing 50% CA (Silvisar® 510) injected into dormant blackjack oak during January. Injections were given at a level of 2 or 4 ml per tree in 4- to 9-in trees, with injection spacings of 3, 5, or 7 in. Top growth killing rates for hickory under the same conditions were poor (14% to 41%), and when blackjack oaks were treated in June, top growth killing occurred at only 54% to 76% rates for trees given one or two injections, respectively.

Better results were obtained with mixtures of Silvisar® 510 and either GC-7887® (hexafluoroacetone trihydrate), paraquat (1,-1'-dimethyl-4,4'-bipyridylium dichloride), or Banvel-D® (2-methoxy-3,6-dichlorobenzoic acid), especially when given in two injections. Nevertheless, another agent, picloram, either alone or mixed with other herbicides, was said to be superior to any other herbicide tested. It had the ability to kill with only a single injection and killed a wider range of species.

February application of Ansar® 160 to 4-in dbh loblolly pines was evaluated by Wiant and Walker (1969). They found that 0.5 or 2 ml per tree applied in frills or single bore holes appeared initially to give good kill rates, but when evaluation was done after two years, kill rates were found to be low (only 10% to 35%). Results with similarly treated 3-in dbh white oak, post oak, southern red oak, blackgum, sweetgum, and hickory trees (given 1 or 2 ml per inch dbh) ranged from 17% to 58% of a given species killed with no sprouting after two years. Best results were obtained with frill application, regardless of dose.

In a second trial, involving only American hornbeam, sweetgum, water oak, willow oak, and southern red oak, kill rates were significantly better, particularly for frill application, although frilling alone gave relatively high kill rates (35% and 59% for winter and summer treatment, respectively). CA treatment in frills was said to have killed 85% to 100% of trees, although lack of sprouting may have been due to flooding of the bottomland treatment sites used in that trial.

More recently, Heilman (1975) reported equivalent results from using chemical thinning with CA (Silvisar® 510) or MSMA (Glowon®), and from saw thinning of young Douglas fir.

Studies have been published evaluating the use of a silvicide injector known as a Hypo-Hatchet™ with a variety of herbicides and herbicide mixtures on a number of hardwood species.

According to Holt and Voeller (1972), neither CA, MSMA, nor a mixture of the two proved to be as satisfactory as certain other herbicides in killing trees and particularly in preventing sprouting. These authors reported similar results the following year (Voeller and Holt, 1973).

Ahrens (1977) performed a study to determine the likelihood of tree damage from exposure to herbicides when spraying was used to control weeds in orchards. He found that trees without basal sprouts were unaffected, but those with basal sprouts suffered damage to their crowns.

Effects on plants from cacodylate residues in soil. Because of concern over the possibility of a buildup of phytotoxic levels of arsenic in soils or the appearance of residues resulting from use of arsenical pesticides in crops, studies have been done to measure such effects on cultivated plants. Among the earliest reports of such research was that of Ehman (1965), who stated that grass could be planted on the same day that CA had been applied at a rate of 22.5 kg/ha for lawn renovation with no harm to the subsequently emerging grass seedlings.

Land treated with 5.8 kg of CA per hectare was seeded with alfalfa and ryegrass after three days, and no arsenic residues were found in hay from two cuttings (Ehman, 1965). When peanuts, sorghum, cotton, and soybeans were planted in soil sprayed with 42.5 kg/ha, however, the peanuts required replanting a month later. With the exception of the soybeans, development of the plants was adversely affected and they contained arsenic residues. When CA was used to control weeds in orchards, no arsenic was found in nuts or fruit at harvest. Also, even after application of as much as 89.5 kg/ha, no arsenic was found in grapefruit.

The cacodylate-based herbicide Phytar® 560 was tested over a period of two years by application in aqueous solution directly to the soil (Raab, 1970; Raab and Klosterboer, 1970). In the first year, plots of sandy clay loam soil near Weslaco, Texas, were treated with CA applications of approximately 2.8, 8.5, 28.3, or 85 kg/ha or left untreated. The plots were planted with corn, grain sorghum, cotton, soybeans, sugar beets, or wheat on the day the herbicide was applied.

Yields of these crops were unaffected at any treatment level. In corn, arsenic was significantly increased in the leaves and stalks or in the grain at the high treatment level, but sorghum exhibited no increase in arsenic content. Increased arsenic levels were found in cottonseed, soybeans, and wheat only from the high-dose plots. In the case of sugar beets, no increased residues were found.

In the second year, only the 2.8 and 8.5 kg/ha applications were repeated, but all previously used plots were planted and harvested. In the case of corn, increased arsenic residues were found in leaves and stalks from the plots treated in the previous year at the highest application rate (85 kg/ha); similar results were seen for wheat grain. In the case of soybeans, beans from all except the low-dose plot were found to contain significantly increased arsenic levels (0.32 to 0.70 ppm of arsenic). Sugar beets harvested from the plots previously given the two highest applications of CA also had increased arsenic levels. Again, no effects were seen on yields of any crop, a result reported for two additional years by Raab and Sandberg (1972).

In another long-term trial, Woolson and Isensee (1981) applied CA (as Phytar® 560), MSMA (as Ansar® 529 H.C.), and sodium arsenite directly to a silt loam soil annually for a period of seven years. Treatment levels were 0, 1, 2, and 10 times the recommended application rates of

11.2, 5.6, and 9.0 kg/ha for CA, MSMA, and sodium arsenite, respectively. The high doses were skipped in 1975, and that of CA decreased in 1974 only, while the 10 × dose of arsenite was skipped in 1972.

Soybeans and radishes were planted annually on each treated plot. Soybean yields from plots treated with the high level of CA or of arsenite were decreased in four of the seven years, while MSMA was associated with reduced yields in only two years. CA at the lower application rates did not reduce soybean yields. In the case of radishes, 10 × CA- or arsenite-treated soil was associated with reduced yields in five of the seven years. The same was true of MSMA for two of the seven years. Generally, only the highest application of CA had an effect on radish production, although in two of the last three years, even the low rate appeared to be deleterious.

The results of Woolson and Isensee (1981) were obtained under rather severe conditions compared to typical agricultural practices because arsenical herbicides are typically applied primarily to foliage and not before planting, thus allowing time for leaching, binding to soil, microbial metabolism, and volatilization.

The long-term effects of MSMA have also been studied. Hiltbold et al. (1974) applied the herbicide to three soil types by annual spraying at rates of up to 40 kg/ha before planting cotton over a six-year period. No effects were seen on yield, and only traces of arsenic (less than 0.2 ppm) were found in cotton seed by the end of the test.

More recently, Horowitz (1977) mixed CA, MSMA, and DSMA at levels of 250 to 1,000 ppm with sandy loam soil and planted the treated soils with seeds of cotton, peanuts, corn, sorghum, tomato, or watermelon. Seedlings grew only in the soils treated with the lowest level of MSMA or DSMA, and none survived in the CA-treated soil. When the experiment was repeated with 10, 50, or 100 ppm concentrations, 50 ppm of CA suppressed development of all plant species tested.

Talbert et al. (1977) compared CA, MSMA, and calcium arsenate added to the soil at 1.1, 3.4, or 10.1 kg of arsenic per hectare on two rice cultivars. They found that the two higher applications of CA reduced growth of the rice plants by mid-season, and both organic arsenicals reduced grain yield. The inorganic arsenical was significantly less injurious to rice.

In a trial comparing soil types, phytotoxicity was said to be higher on sandy loam than on clay, but the data were not clearly consistent with such a statement, particularly in the case of DSMA. Horowitz (1977) also used sorghum in a bioassay for persistence of methylated arsenicals in clay soil. He made repeated plantings in soil treated with various amounts of CA, MSMA, and DSMA, and weighed the plants two weeks after each seeding. By one month after the soil treatment, no effects were seen in plants from soil treated with up to 10 ppm of CA, 150 ppm of MSMA, or 200 ppm of DSMA.

Miscellaneous effects on plants. According to Stevens (1966), CA can cause defoliation of susceptible plants by a selective effect on the leaf abscission layer. This effect can occur at treatment levels considerably below those needed for tissue desiccation. Affected plants often grow new leaves rapidly, however, so they are not greatly harmed. Burt and Muzik (1971) reported a similar effect in CA-treated petiole explants of beans and privet, but not in coleus.

Stevens (1966) also mentioned that CA given to plants early in their growth had adverse effects on later flowering and seed production, with possible sterility of gametes. Taylorson (1966) found CA-induced malformation of the inflorescences and decreased seed production in a variety of grasses. The severity of the effect increased if the plants were treated in later growth stages.

Subsequently, Powell and Taylorson (1967) treated pearl millet with CA sprayed at application rates of 46 or 184 g/ha. Treatment at five or six weeks after planting resulted in delayed flowering and reduced the height of the plants, particularly at the higher dose and later spraying time, and chromosome pairing was inhibited. The inflorescences were grossly malformed, especially after the high-dose treatment, and the female parts were more affected than were the male components. Seed production was affected more by the early, high rate CA application.

CA was found to be ineffective in causing sterilization of the male gametes of the sugar beet, however, when applied as a foliar spray at 100 or 200 ppm at various developmental stages (Hecker et al., 1972). At the 200 ppm dose, CA was highly phytotoxic.

Sachs and Michael (1971) compared equimolar amounts of CA, MSMA, sodium arsenite, and sodium arsenate applied to the foliage of various plants (soybeans, morning glories, radishes, oats, and rice). They found that CA was generally more toxic than the other arsenicals when sprayed on the foliage. When translocation of the arsenicals was measured in beans, the methylated forms were found to be transported more readily than were the inorganic salts, and they caused greater inhibition of stem elongation. Nevertheless, when the arsenicals were applied to the bean root systems, the order of their toxicity was arsenite > arsenate > MSMA = CA.

With regard to the total amount absorbed, the inorganic arsenicals were absorbed much more readily than were the organic forms, but CA was far more rapidly transferred to the shoot tips. Even though the arsenicals were all absorbed somewhat more readily from the bean roots than from the shoots, only the inorganic forms were more phytotoxic when given via the root system. When Sachs and Michael (1971) looked for evidence of metabolism of CA or MSMA by the bean plants, they found none.

Effects on insects

Use as a pesticide. CA has been investigated for use in controlling a number of destructive bark beetles. The technique employed consisted of using trap trees, either "trees of merchantable size felled to attract infestations" (Nagel et al., 1957), killed with silvicide (Chansler and Pierce, 1966), killed with silvicide and later felled (Buffam, 1971), or baited with beetle pheromones (Copony and Morris, 1972). When trap trees were treated with CA, the silvicide was typically either injected into the tree or poured into frills cut with an ax. If the treatment was successful, developing beetles were killed before they could mature and infest living trees in the area. In many cases, adults were killed as well.

Although the trap-tree method had been used previously with other pesticides, the first test with CA was done by Chansler and Pierce (1966). They found that injecting CA formulations (Ansar® 160 or Silvisar® 510) significantly decreased survival of the young of a number of *Dendroctonus* beetles in ponderosa pine, Douglas fir, and Englemann spruce. Treatment was accomplished between the initial attack on the tree and the hatching of the beetle's eggs.

McGhehey and Nagel (1967) reported similar results with Silvisar® 510 on *Pseudohylesinus* species in western hemlock, as did Newton and Holt (1971) with CA, MSMA, and a mixture of the two on mountain pine beetles and pine engraver beetles in ponderosa pine. Buffam and Yasinski (1971), Buffam (1971), Buffam et al. (1973), Frye and Wygant (1971), and Frye et al. (1977) found heavy mortality among spruce beetles on Engelmann spruce treated with Silvisar® 510.

Hostetler and Brewer (1976) found no effect on European elm bark beetles when American elms were frilled and treated with Silvisar® 510, even when the inner phloem contained as much as 1,700 ppm of CA. Rexrode (1974), however, reported that pressure injection of CA into American elms resulted in almost complete control of European elm bark beetles, and O'Callaghan et al. (1980) stated that trap trees killed with CA or MSMA (Silvisar® 510 or 550) and baited with the pheromone "multilure" were highly effective in eliminating survival of beetle broods in elms.

According to Rexrode and Lockyer (1974), CA levels of 900 to 1,000 ppm in diets were lethal when fed to both European elm bark beetles and to oak bark beetles. Pressure injection of CA into oak trees was also found to decrease bark beetle infestation (Rexrode, 1977).

The time of the year when trees were treated was considered in some studies. For example, Stelzer (1970) stated that CA application in August was more effective in killing Arizona 5-spined beetles on ponderosa pines than was treatment in any of six other months, and similar results were seen by Stevens et al. (1974). Buffam and Flake (1971) stated that frilling with a hatchet rather than with a power saw resulted in a higher level of mortality in round headed pine beetles and their offspring in CA-treated ponderosa pines.

A further refinement was attempted by Copony and Morris (1972), who baited loblolly pines with Frontalure®, a mixture of frontalin (a pine beetle pheromone) and a tree-produced beetle attractant known as alpha-pinene. Their target was the southern pine beetle, a species reported by Ollieu (1969) to be susceptible to CA. Copony and Morris reported that Frontalure® application increased the effectiveness of beetle control when Silvisar® 510 poisoned trap trees were placed adjacent to naturally infested areas.

Factors influencing the effectiveness of this beetle-trapping system were investigated by Coulson et al. (1973a and 1973b), and were said to include original infestation size, brood development stage, tree size, numbers of baited CA-treated trees, and time of the year. According to Coulson et al. (1973b and 1975), however, southern pine beetle attack density was not increased by Frontalure® application, in comparison with the use of CA alone.

The mechanism by which CA kills bark beetles is currently only speculative. Bark beetle mortality has been related to rapid drying of treated trees (Stelzer, 1970), but Frye and Wygant (1971) stated that moisture content was probably not a factor. In fact, Williamson (1970) was reported by Coulson et al. (1975) to have said that increased moisture content of the inner bark may be a factor adverse to bark beetle development.

Newton and Holt (1971) proposed that beetle mortality following arsenical treatment may be due to arsines generated from CA or from MSMA by the action of fungi or bacteria attacking the dead trap trees, but did not produce any direct evidence that this indeed occurred. Whatever the

mechanism, it involves an indirect effect by modification of the beetle's microhabitat or else there are considerable species differences in resistance to CA. Some species (e.g., the striped ambrosia beetle) were unaffected by treatments killing most spruce beetles, while other species were intermediate in response (Frye and Wygant, 1971).

It is also of interest that when CA, CA plus NaCA (Phytar® 560), arsenic trioxide, or arsenic pentoxide were given in drinking water to meadow katydids, their longevity was reduced by 90%. The decrease occurred within 14 days at arsenic levels greater than 5 μg/g (dry weight) from any of the arsenicals according to Watson et al. (1976). Their data suggest similar toxicity of Phytar® 560 and the inorganic forms at equivalent arsenic contents. Total arsenic uptake in the katydids at LD_{50} doses was also found to be similar between Phytar® 560 and As_2O_3, and both were lower than the incorporated amounts of pure CA or As_2O_5.

Although Watson et al. (1976) took their data to suggest that the arsenic from the methylated arsenicals in Phytar® 560 was as toxic as that from arsenic trioxide, an alternative explanation would be that other ingredients in the herbicide formulation (e.g., the wetting agent) added toxicity of their own or exacerbated the effects of the arsenicals. This appears likely, in that CA was less toxic in the pure form than in the herbicide formulation.

Effects on beneficial insects. The potential toxicity of methylated arsenicals to honeybees has also been investigated. Moffett et al. (1972) sprayed MSMA and CA on cages containing bees and found that both arsenicals resulted in high mortalities within a few days after treatment. Morton et al. (1972) and Morton and Moffett (1972) also reported toxicity of methylated arsenicals (CA, MSMA, DSMA, and MAA) to honeybees. In this case, the pesticides were fed ad libitum to the bees in a 60% sucrose solution. CA killed half the bees given the 10 ppm dose in 4.1 days, while 100 and 1,000 ppm solutions resulted in 50% mortality in 2.6 and 2.1 days, respectively. The other arsenicals were less effective than CA at the low dose, but similar to CA in toxicity at the high dose. CA and MAA were technical grade and the MSMA and DSMA used were the commercial formulations, Ansar® 170 and Ansar® 184.

In a further study, Atkins et al. (1973) determined that dusting honeybees with CA at a level of 157 μg per bee would result in 5.6% mortality within 48 hours. Similar treatment with 24 μg of MSMA caused 6.2% lethality, while 218 μg of DSMA per bee was followed by 9.8% deaths. Accordingly, the authors labeled these arsenicals as being "relatively nontoxic to honeybees," as they caused no deaths at doses of up to 11 μg per bee.

The foregoing results with honeybees suggest that the mode of exposure to methylated arsenicals is an important factor in determining the degree of toxicity. It is likely that the absorbed dose from dusting with dry powder was considerably less than that obtained from either spraying or ingestion of the pesticide.

Microbial methylation and demethylation of arsenic, and arsenic cycling

Increasing evidence has shown that a number of heavy metals (e.g., Hg, Sn, Pt, Au, Tl) and metalloids (e.g., As, Se, Te, S) can be methylated in the environment (Ridley et al., 1977). The evidence for arsenic methylation was reviewed in 1978 by McBride et al., and proposed mechanisms of methylation were discussed by Challenger in 1978. Early work by Challenger et al. (1933) demonstrated that a mold (*Penicillium brevicaule*, now *Scopulariopsis brevicaulis*) could transform arsenious acid, MSMA, or CA. Similar results were reported by Challenger (1947) with *Penicillia* and *Aspergilli*, by Cox and Alexander (1973a, 1973b), and by Cullen et al. (1979a, 1979b) with *Penicillia* and other fungi grown on media containing CA. The end product was trimethylarsine, $As(CH_3)_3$, a highly toxic gas.

It is not known whether higher forms also elaborate trimethylarsine, although it has been said that the characteristic garlic-like odor of this gas has been noticed following administration of CA or its sodium salt. Puntoni (1917) attributed the garlic odor he detected after oral CA treatments to the effect of the intestinal microflora. He claimed to detect a similar odor following the culture of such organisms on a CA-containing medium, but Challenger and Higginbottom (1935) were unable to obtain similar results using media containing CA or other arsenicals.

More recently, Cullen et al. (1979a and b) found that cell-free homogenates from the fungus *Candida humicola* produced MAA and CA from arsenate and elaborated CA and trimethylarsine oxide from MAA. MAA and trimethylarsine oxide resulted when CA was used as the substrate. When a marine yeast, *Rhodotonula rubra*, was grown in culture with arsenate, the result was generation of arsenite, MAA, CA, and methylarsines (Vidal and Vidal, 1980).

It has also been found that certain bacteria (e.g., *Methanobacterium*) can transform inorganic arsenate to dimethylarsine (McBride and Wolfe, 1971). Under anaerobic conditions, arsenate is reduced to arsenite and then methylated to methylarsonic acid. Further methylation and reduction result first in CA and ultimately in dimethylarsine.

Under aerobic conditions, several bacterial species could transform arsenate to methyl-, dimethyl-, and trimethylarsines (Wong et al., 1977; Shariatpanahi et al., 1981). It is also interesting to note that pure cultures of *Escherichia coli*, bacteria typically found in the gut of animals, were capable of methylating both arsenite and arsenate to MAA and CA. This organism could also methylate MAA to CA, but not to TMA.

The marine bacterium *Serratia marinorubra* could reduce arsenate and methylate it to MAA under both aerobic and anaerobic conditions, but failed to produce detectable amounts of CA or of arsines (Vidal and Vidal, 1980).

Sanders and Windom (1980) reported that certain marine algae could take up inorganic arsenic, particularly arsenate, from seawater and convert it in part to CA without subsequently

producing arsines, while Andreae and Klumpp (1979) found that such algae took up arsenate and released both MAA and CA. Such algae also formed other organic arsenicals that remained within their cells. These findings suggest that metabolism by phytoplankton provides the source of much of the CA found in the marine environment (Andreae and Klumpp, 1979; Sanders and Windom, 1980).

Microorganisms can degrade as well as methylate organic arsenicals. For example, Dickens and Hiltbold (1967) and Von Endt et al. (1968) reported microbial decomposition of MAA in soils and found that pure cultures of actinomycetes and bacteria isolated from soil could degrade MSMA. Woolson and Kearney (1973) added CA to soils and detected degradation to arsenate of 41% of the added CA by 24 weeks. No such degradation was seen when soil conditions were anaerobic.

Schuth et al. (1974) also observed degradation of CA to arsenate, as well as to arsenite, and they found $^{14}CO_2$ following microbial attack on ^{14}C-labeled CA in a model ecosystem. Braman (1975) collected arsines evolved over areas planted in lawn grass and found both di- and trimethylarsines after treatment of the soil with CA. Only trimethylarsine was observed following treatment with MAA or with As(III).

According to Cheng and Focht (1979), however, CA and MAA added to soils were reduced to their respective methylarsines, with no further methylation. An additional fraction was said to be demethylated before reduction. Similar results were obtained from pure bacterial cultures as well. Cheng and Focht (1979) further criticized the reported microbial production of trimethylarsine as presented by Cox and Alexander (1973), Braman (1975), and Woolson (1977b), basing their critique on methodological grounds.

Microbial alteration of methylated arsenicals has also been examined in aquatic environments. For example, demethylation of CA and its subsequent oxidation to arsenate was accomplished by mixed aerobic bacterial cultures isolated from an estuary (Sanders, 1979). Andreae (1979) obtained similar results with stored seawater samples, while identical samples that had been sterilized were stable over the same time periods. Holm et al. (1980) modeled the demethylation of CA and MAA to inorganic arsenic in spiked anaerobic river bottom sediments. The demethylation process was attributed to microbial activity, since sterilized control samples remained unchanged. Sanders and Windom (1980), however, found no metabolism of CA added to cultures of marine algae.

The primary proposed environmental transformations of arsenicals are shown in Figure 5.1 (from Woolson, 1977a). This figure depicts possible methylation, demethylation, oxidation, and reduction reactions.

Data on environmental arsenic oxidation, reduction, methylation, and demethylation have led to speculation regarding "arsenic cycling" in both aquatic and terrestrial environments (Sandberg and Allen, 1975; Woolson, 1977a; Sanders, 1980). For example, Sandberg and Allen (1975) presented a detailed model of arsenic cycling in agricultural environments. They proposed arsenic inputs from use of MSMA- and CA-based herbicides, with minor additions from arsenic in irrigation water and fertilizers and from oxidized arsines. Arsenicals from industrial and municipal wastes were mentioned but were not quantitatively estimated. Arsenic transfer

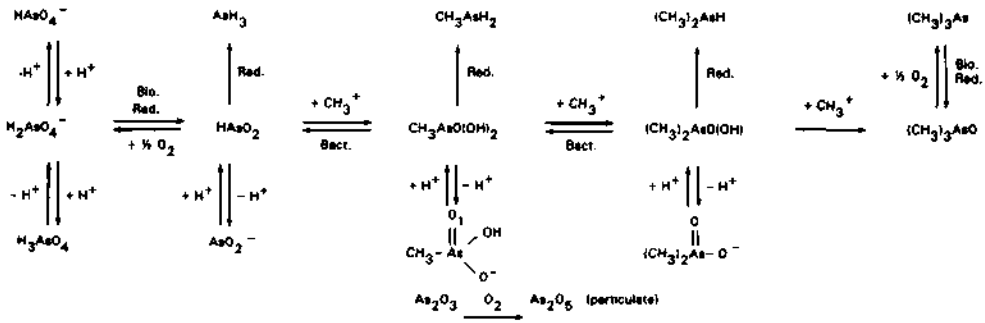


Figure 5.1. Conversion pathway for biotic metabolism of arsenic.

Source: E. A. Woolson, Fate of arsenicals in different environmental substrates, *Environmental Health Perspectives* 19:77 (1977).

mechanisms were said to be oxidation and reduction, uptake and translocation in plants, leaching, soil erosion, and harvesting of plant material. The proposed cycle is diagrammed in Figure 5.2.

Sandberg and Allen (1975) concluded that arsenic does not build up in the water, air, or plant material of an agricultural ecosystem due to its mobility in the environment. Arsenicals were said to accumulate in soil, but redistribution generally prevented buildup of hazardous amounts.

According to Woolson and Kearney (1973), arsenic persistence is a function of soil type, with arsenic being lost more rapidly under anaerobic conditions. Arsenic loss was presumably due to formation and volatilization of arsine, methylarsines, and other organoarsenicals.

Indeed, Woolson (1977b) reported finding alkylarsines generated from arsenical-spiked soil and supported the view that loss of arsenic to the atmosphere is a significant factor in natural soil arsenic cycles. Woolson (1977c) estimated the mean half-life of CA, MSMA, and arsenate added to certain agricultural soils as 5.2, 5.6, and 6.0 years, respectively.

Ferguson and Gavis (1972) presented detailed models of arsenic cycling in natural waters, including oxidation, reduction, and methylation steps (Figure 5.3). Woolson (1977a) subsequently published detailed models for arsenic cycles in a variety of environments. He suggested, for example, that while alkylarsines can be transported in air in the gaseous form, CA, MAA, or trimethylarsine oxide may be transported while adsorbed to airborne particles.

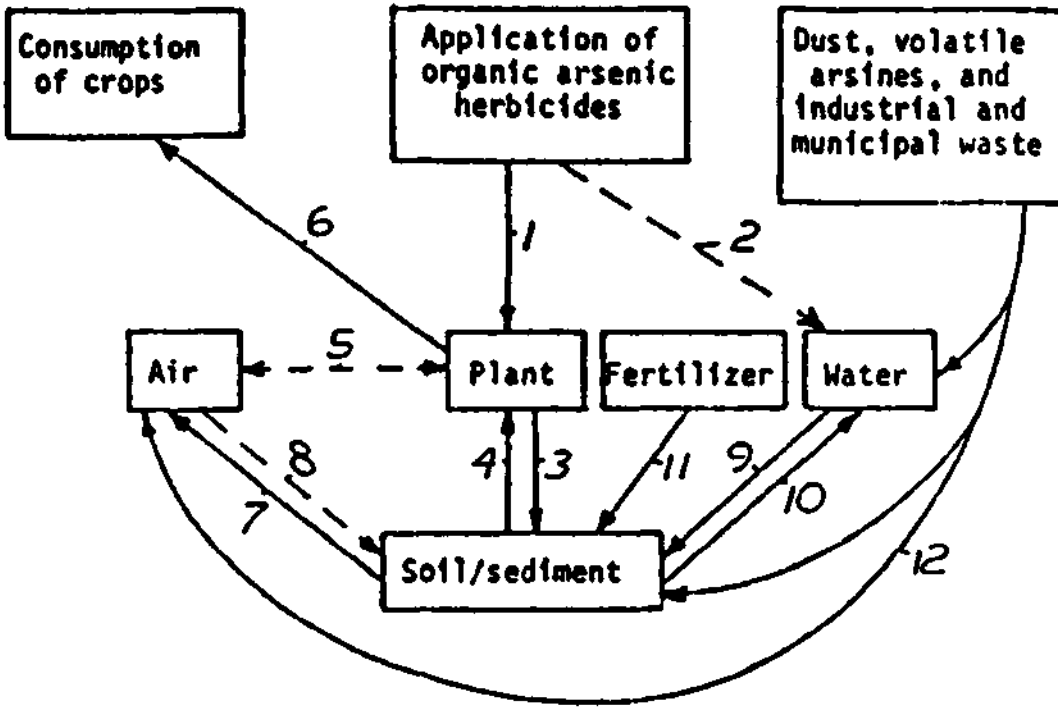


Figure 5.2. A proposed model for the arsenic cycle in an agronomic ecosystem.

Source: G. R. Sandberg and I. K. Allen, A proposed arsenic cycle in an agronomic ecosystem. In: E. A. Woolson (ed.), *Arsenical Pesticides*, ACS Symposium Series 7:124-47, Washington, American Chemical Society, 1975.

Environmental generation of di- and trimethylarsines was confirmed by Johnson and Braman (1975a), when they sampled air over a variety of areas. They also found arsines in air inside a house and in a plant store, as well as in a number of greenhouses containing plants. Relatively high levels of trimethylarsine were seen in some air samples from three of five greenhouses, suggesting possible production of alkylarsines by higher plants or by microorganisms associated with them.

In water, methylated arsenic could be transported in solution or in living organisms. Woolson (1977a) maintains that the complex nature of exchanges of arsenicals between the various portions of the environment, as well as between living organisms and their surroundings, makes precise quantitation of transport difficult. Woolson's postulated relationships between arsenicals and environmental compartments are shown in Figure 5.4.

A remaining question about arsenic transformations and movements concerns the insoluble fraction, which may or may not enter into biologically catalyzed reactions. Also, although there were few experimental data at the time from which to calculate environmental transformation rates for the various arsenicals, the interconnections of the necessary reactions have been suggested by Woolson (1977a), as portrayed in Figure 5.5. It can be seen from Woolson's

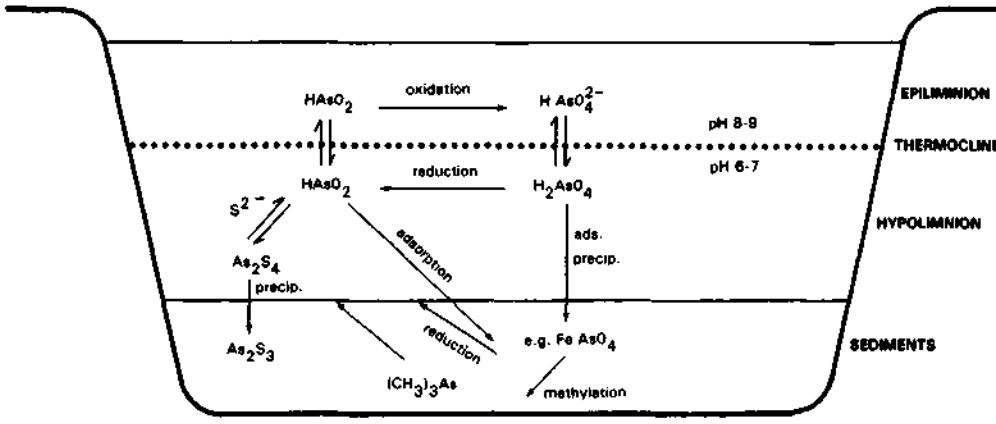


Figure 5.3. Arsenic reactions in a stratified lake [taken from Ferguson and Gavis (*11*)].

Source: E. A. Woolson, Fate of arsenicals in different environmental substrates, *Environmental Health Perspectives* 19:74 (1977).

equation that perturbation of the system would occur whenever additional amounts of an arsenical are introduced, until a new equilibrium state was attained.

More recently, Burau (1981) addressed the issue of the kinetics of the transformation of arsenicals in soils, at least under aerobic conditions. He trapped arsines on cellulose filter discs impregnated with an acidified solution of $Hg(NO_3)_2$ and air-dried before use. The air samples were obtained from over a calcareous soil (Temple clay loam) taken from an orchard and mixed with sodium arsenate, MSMA, or CA at rates of 0, 15, or 120 mg of arsenic per kg of air-dried soil.

Amounts of arsenic from air above the untreated and arsenate-treated soils were below the detection limits of the GFAAS employed. Arsines were generated from 3 to 9.4 times more rapidly from CA-treated soil, with arsine evolution being proportional to treatment application rate. Thus, it appeared that arsenic loss to the atmosphere could be calculated as a first order function. The arsine evolution rate was rather variable over time. There was no obvious pattern to the changes observed, but they may have been influenced by varying environmental temperatures or by interactions of microbial populations.

Burau (1981) attempted to calculate the annual amount of arsenic lost from soil treated with CA or MSMA and obtained values ranging from 0.03% to 1.6%. Such amounts are considerably lower than those reported by others, such as Sandberg and Allen, 1975; Braman, 1975; Woolson,

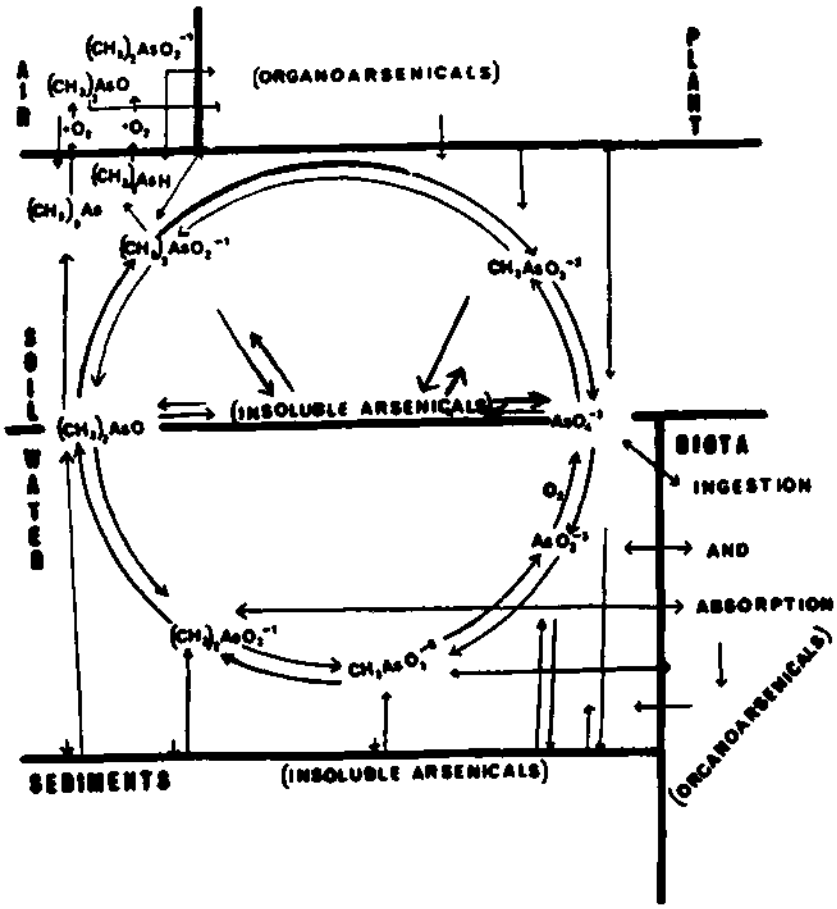


Figure 5.4. Cyclical nature of arsenic metabolism in different environmental compartments.

Source: E. A. Woolson, Fate of arsenicals in different environmental substrates, *Environmental Health Perspectives* 19:77 (1977).

1977b; and Woolson et al., 1982. Although Burau postulated a number of possible reasons for this discrepancy, data were not available to allow a choice among them.

In a recent study, Matapeake silt loam soil was mixed with hay, sewage sludge, or cow manure at rates equivalent to 50 or 100 T/ha, with various moisture levels (Woolson et al., 1982). The soil mixtures were incubated in a closed system designed for sampling the degradation of arsenicals. ^{14}C-labeled CA was added to the samples at a level of 10 ppm by weight as arsenic.

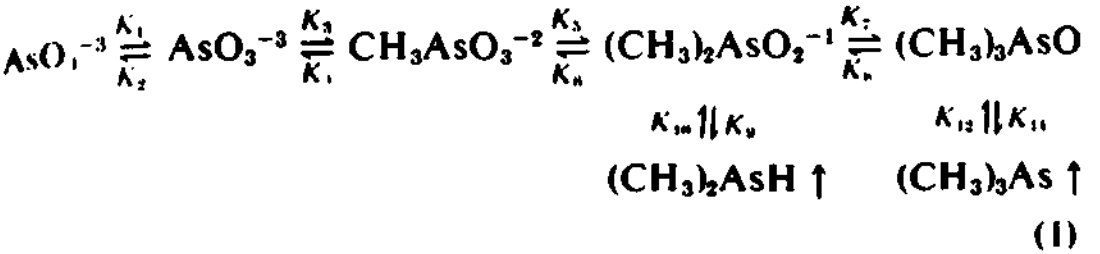


Figure 5.5. Transformations of arsenic in the environment.

Source: E. A. Woolson, Fate of arsenicals in different environmental substrates, *Environmental Health Perspectives* 19:79 (1977).

Eighty percent of the added CA was degraded within two months in moist, aerobic samples, with production of alkylarsines, arsenate, MAA, and CO_2. In untreated soils, CA was degraded more rapidly than in the soils with added organic matter, and the high level of sewage sludge actually inhibited CA alteration to the same extent as did soil sterilization with mercuric chloride. The half-life of CA in amended soils (excluding samples given the high-level sludge treatment) was 31 days, and that of untreated soils was only 20 days.

Flooding of the soil samples in the study by Woolson et al. (1982) with sufficient water to produce anaerobic conditions caused a reduction in the rate of CA degradation and resulted in higher levels of MAA when soils were sampled at 60 days. More of the degraded CA molecule was trapped as $^{14}CO_2$ under aerobic conditions, and thus presumably more ^{14}C-methane was released from the flooded (anaerobic) samples.

Woolson and his coworkers also added sodium arsenite, CA (as Phytar® 560), and MSMA (as Ansar® 529) to field plots. Alteration of the added CA resulted in a buildup of MAA in the soil, while MAA was degraded to both arsenate and CA. In soil treated with arsenite, small amounts of MAA and CA were detected.

According to Woolson et al. (1982), their results suggest that aerobic metabolism can degrade CA to MAA and then to arsenate. When they applied high levels of CA or MAA to field plots (10 × the recommended application rate annually for seven years), both CA and MAA persisted in the soil for more than a year after the last application. Half-lives of CA and MAA in the field plots were 20 and 22 days, respectively, which agreed with the laboratory results for CA.

Arsenic cycling in a marine system has also been described in some detail. Sanders (1980) investigated a near-shore environment which was characterized by receiving its major supply of arsenic (primarily as arsenate) via intruding water from the Gulf Stream.

Sanders' proposal for arsenic cycling in such an environment is shown in Figure 5.6, with his estimates of transfer rates. Such rates would of course vary considerably in other marine environments, depending on factors such as phytoplankton levels and seasonality, arsenic inputs, and water flow. Arsenic methylation by phytoplankton and subsequent degradation by bacteria are obviously important factors in this model.

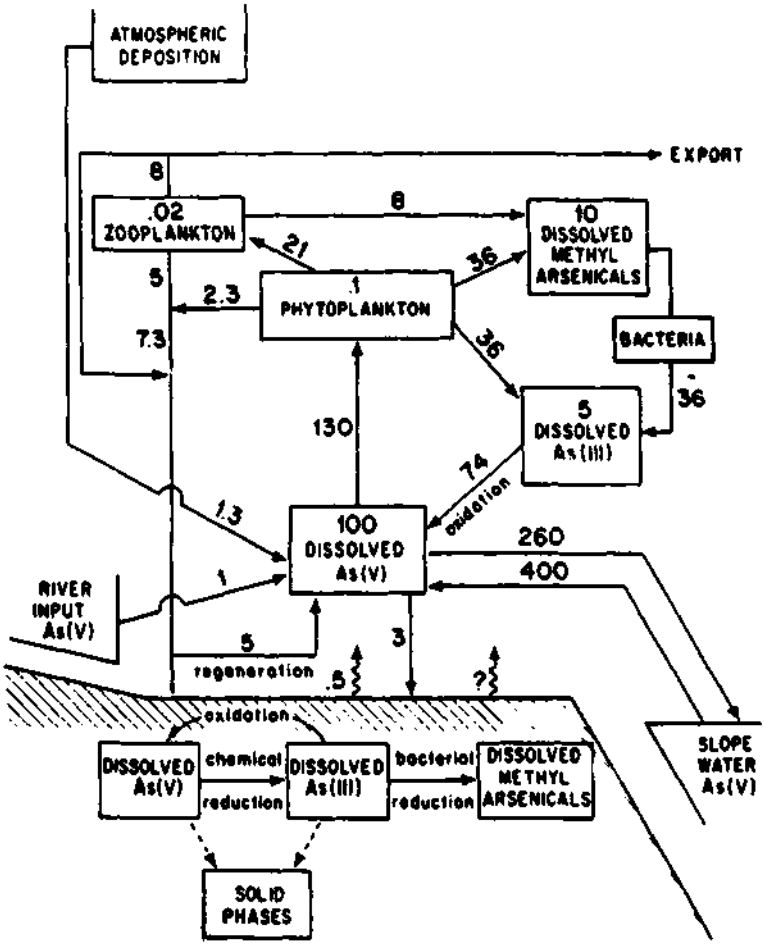


Figure 5.6. The cycling of arsenic in the Georgia Bight. Arsenic reservoirs are in 10^4 kg, transfer rates are 10^4 kg/yr.

Source: James G. Sanders, Arsenic cycling in marine systems, *Marine Environmental Research* 3:263 (1980).

Methylated arsenicals in fresh and sea waters and in sediments

Insufficient data are available on distribution of arsenicals in the aquatic environment (Andreae, 1978), and such information regarding methylated arsenicals has often been gathered merely as a secondary aspect of methodologic studies of arsenic analysis (e.g., Braman and Foreback, 1973; Braman et al., 1977; Grabinski, 1981).

An early study that addressed the issue of possible contamination of natural waters with methylated arsenicals was limited to measuring total arsenic for lack of an available technique to measure low levels of CA at that time (Lehn et al., 1970). The results showed that the levels of arsenic present in streams near a very heavily sprayed test area were below the detection limits for the atomic absorption spectrophotometer (0.05 ppm). This was the case even though rainfall in the test area tended to be high, and runoff was abundant due to the sloping topography.

These analytic data of Lehn et al. (1970) were supported by data on fish species diversity which showed no adverse effect. The fact that detectable levels of arsenic were not found in the stream water samples was attributed to retention by soil in the sprayed area or to dilution by stream flow. Further, when Salman et al. (1972) analyzed for arsenic in water from irrigation ditches whose banks and bottoms had been sprayed with MSMA (5.8 kg/ha), arsenic concentrations decreased to no more than 0.06 ppm within 10 minutes after water was released in each ditch.

More recently, Norris et al. (1983) found no significant increases in arsenic levels of a number of streams located in forests that were commercially thinned with MSMA or CA. These western coniferous forests were sampled during silvicide application, storms, and spring runoff.

Additional data on content of methylated arsenicals in fresh waters were generally collected in a less systematic way. The findings of several workers are presented in Tables 5.1 and 5.2, primarily as indications of the range of amounts of methylated and inorganic arsenicals that are likely to be encountered in random sampling. It must be kept in mind that the waters sampled were primarily in only a few states of the United States, and may not be representative of other environments.

The data presented show that concentrations of cacodylate in natural waters ranged from less than 0.01 up to 1.00 ppb (as arsenic), but amounts in most cases were no greater than 0.32 ppb. Amounts of MAA and inorganic arsenite were also generally quite low, while arsenate values were often above 1 ppb in both fresh and salt waters.

One sample of well water tested by Braman and Foreback (1973) was found to contain 0.20 and 0.11 ppb of CA and MAA, respectively. No other values were found in the literature for amounts of methylated arsenic in drinking water, which suggests little current interest in the subject.

Table 5.1 — Content of arsenicals found in fresh waters (in ppb as As)

Source	As(III)	As(V)	MAA	CA	Reference
Hillsborough River (FL)a	<0.02	0.25	<0.02	<0.02	Braman & Foreback (1973)
Withlacoochee River (FL)	<0.02	0.16	0.06	0.30	
Well Water (FL)	<0.02	0.27	0.11	0.20	
Pond, Withlacoochee Forest (FL)	<0.02	0.32	0.12	0.62	
Research Pond, Univ. of South Florida	0.79	0.96	0.05	0.15	
Lake Echols (FL)	2.74	0.41	0.11	0.32	
Lake Magdalene (FL)	0.89	0.49	0.22	0.15	
Golf Course Pond (FL)	6.4[b]		7.40	trace	Braman et al. (1977)
Lake Carroll (FL)	2[b]		0.18	0.25	
Sacramento River (CA)	0.04	1.08	0.02	<0.01	Andreae (1977)
Owens River (CA)	0.09	42.5	0.06	0.22	
Colorado River (AZ)	0.11	1.95	0.06	0.05	
Colorado River, slough (CA)	0.09	2.25	0.13	0.31	
Saddleback Lake (CA)	0.05	0.02	<0.01	0.01	
Rainwater (La Jolla, CA)					
10 September 1976	<0.01	0.18	<0.01	0.02	
11 September 1976	<0.01	0.09	<0.01	<0.01	
Colorado River (CA)	0.12	3.05	0.09	0.01	Andreae (1978)
Alamo River (CA)	0.02	6.90	0.56	0.01	
Kaweah River (CA)	0.11	2.45	0.01	0.20	
New River (CA)	0.03	11.2	0.01	0.02	
Owens River (CA)	0.09	42.5	0.06	0.01	
Tamiami Canal (FL)	0.47	0.08	0.01	0.05	
Truckee River, Lake Tahoe Outlet (CA)	0.48	0.79	0.01	0.03	
Truckee River, Truckee (CA)	1.30	<0.01	0.01	0.02	
Coot Bay Pond (FL)	0.81	1.27	0.01	0.32	
Donner Lake (CA)	0.06	0.07	0.01	<0.01	
Paurotis Pond (FL)	0.04	0.03	0.01	0.01	
Saddlebag Lake (CA)	0.05	0.02	0.01	0.01	
Squaw Lake, surface (CA)	0.87	2.76	0.02	0.05	
Squaw Lake, at 2m depth (CA)	0.53	2.96	0.02	0.11	
Senator Wash Reservoir (CA)	0.58	2.47	0.01	0.01	
Beaulieu River (England)	0.10	1.02	0.06	0.23	Howard & Arbab-Zavar (1981)
Restronguet Creek (England)	1.60	19.40	<0.20	<0.20	

[a]State or country where located.

[b]Total inorganic [As(III) + As(V)].

Table 5.2 — Content of arsenicals found in seawater (in ppb as As)

Source	As(III)	As(V)	MAA	CA	Reference
Bay, Causeway (FL)[a]	0.12	1.45	<0.02	0.20	Braman & Foreback (1973)
Tidal Flat (FL)	0.62	1.29	0.08	0.29	
McKay Bay (FL)	0.06	0.35	0.07	1.00	
Scripps Pier CA Andreae (1977)					
5 November 1976	0.02	1.75	0.02	0.12	
11 November 1976	0.03	1.70	0.02	0.12	
San Diego Trough (CA)					
surface	0.02	1.49	0.01	0.21	
25 m depth	0.02	1.32	<0.01	0.14	
50 m depth	0.02	1.67	<0.01	<0.01	
75 m depth	0.02	1.52	<0.01	<0.01	
100 m depth	0.06	1.59	<0.01	<0.01	
Southern California Bight (CA)					Andreae (1978)
1–3 m depth	0.01–0.87	0.16–1.34	<0.01–0.02	0.11–0.26	
7–10 m depth	0.06–0.68	0.81–1.21	<0.01–0.03	0.17–0.24	
13–17 m depth	0.03–0.60	1.13–1.27	<0.01–0.02	0.11–0.20	
19–26 m depth	0.01–0.06	1.17–1.33	<0.01–0.02	0.10–0.16	
30–35 m depth	0.01–0.04	1.20–1.45	0.01	0.10–0.17	
40–45 m depth	0.03	1.19–1.34	<0.01–0.01	0.03–0.13	
50–55 m depth	0.03–0.42	1.35–1.42	<0.01–0.01	0.01–0.06	
62 m depth	0.11	1.45	0.01	0.01	

[a]State where located.

The most extensive research on arsenic distribution and speciation in natural waters has been done by Andreae (1977, 1979), who analyzed a variety of fresh and salt waters for CA, MAA, arsenite, arsenate, mono-, di-, and trimethylarsines, and trimethylarsine oxide. Of these arsenic species, only the first four were said to be present in detectable amounts (Andreae, 1977).

According to Andreae, CA and MAA in seawater are primarily found in the upper waters where they are produced by biologic methylation, with CA the more abundant of the two. Andreae speculated that the productivity of phytoplankton in freshwater is positively correlated with the concentration of methylated arsenicals. No methylated arsenicals were identified in water separated from marine sediments, due either to immediate irreversible binding following arsenical formation or to lack of methylating activity in the sediments (Andreae, 1979).

Methylated arsenicals in aquatic organisms

Organisms living in many aquatic environments are continually bathed in a mixture of various arsenicals which they absorb to some degree from the fluid medium. Aquatic animals may also consume considerable amounts of arsenicals in their diets, whether animal or vegetable. It is thus of interest to determine how such creatures manage to deal with environmental arsenicals and whether such compounds are biomagnified by the aquatic food chain.

The occurrence of arsenicals in aquatic organisms has been known for some time. In 1926, Chapman wrote of the still earlier work of others who described arsenic occurring naturally in both plants and animals, including the finding of relatively high levels in seaweed.

These observations were extended by Chapman himself, who found arsenicals in a number of marine organisms and remarked on the relatively high levels of a complex arsenical in shellfish and crustaceans. He also theorized that if the arsenic in marine organisms was not playing some role in cellular metabolism, it might be present in a form produced as a detoxification mechanism, a view still held today (Chapman, 1926).

Among the first to address the issue of CA and MAA uptake by aquatic plants and animals were Isensee et al. (1973). Using model ecosystems, they measured uptake of ^{14}C-labeled CA by mosquito fish (*Gambusia affinis*), snails (*Physa*), daphnia (*Daphnia magna*), and algae (*Oedogonium cardiacum*).

After 11.5 ppb of CA was added to the water, 92% remained after one day, and by 32 days, 58% was left in solution. When assayed at 32 days, the alga was found to contain most of the bioaccumulated CA (95%), although it accounted for only 74% of the total biomass. The snail was the second most efficient accumulator of CA (4%), followed by the daphnia (0.64%). The fish accumulated the least (0.09%), but were exposed in the system for only three days.

When snails exposed to CA for 32 days were subsequently placed in untreated water for an additional 16 days, they lost from 50% to 75% of their accumulated CA. Whether a portion of the ^{14}C-labeled CA counted in the snails initially was contained in ingested algae was not clear. Such a factor could clearly influence the results obtained, with regard to both the apparent initial CA retention and the magnitude of CA loss following termination of exposure.

The contribution of CA from consumption of algae by snails was apparently not a major factor, however, as snails in CA-treated water accumulated much more of the arsenical than did individuals in uncontaminated water that were fed CA-containing algae. The same was true of fish in CA solution versus those only fed treated daphnia.

Similar results were obtained in concurrent experiments with dimethylarsine oxide. Such data indicate that a variety of aquatic organisms could absorb methylated arsenicals from their environments. Such absorption, rather than accumulation from dietary sources, may be the major source of uptake, and the potential for biomagnification of CA in food chains does not appear great.

A more elaborate system was used by Schuth et al. (1974), who mixed ^{14}C-labeled CA solutions with clay loam, silt loam, and sandy loam soils. They placed the soils in freshwater aquaria to which a variety of organisms were added after one week. These included algae, snails, and daphnia, as in the previous study, plus duckweed, catfish, and crayfish. Water sampling occurred over 60 days.

In tanks containing clay or silt loam, there was an increase in CA in the water during the first 40 days, followed by a decline. In the sandy loam tank, no major decrease was observed. All organisms were harvested at 22 days after their addition, replaced after nine days with a like assortment, and the replacements harvested after an additional 20 days.

Again, CA did not appear to have been greatly biomagnified in the food chain. In fact, the bioaccumulation ratios (^{14}C concentration in tissues/^{14}C concentration in water) were lowest at the top of the experimental food chain. Uptake of ^{14}C tended to be greater than that of arsenic in

the first group of organisms harvested, with the exception of the crayfish, and was greater still in the second group.

Such results suggest that CA was broken down by microorganisms in the aquatic environment and that much of the ^{14}C accumulated in the biomass was not taken up as part of an intact CA molecule. This contention was further supported by the failure of chromatography and autoradiography of extracts from algae and snails to show intact ^{14}C-CA and by detection of a garlic-like odor above the tanks (presumably from arsines). MSMA has also been found to be taken up from water by crayfish and was concentrated by a factor of 2.7 to 3.8 over 56 days (Abdelghani et al., 1976).

Howard and Arbab-Zavar (1981) reported finding mono- and dimethylarsines at levels of 0.03 to 0.29 μg/g for the former and 0.27 to 1.74 μg/g for the latter compound in tissue from mollusks obtained from an estuarine environment. Algae samples contained 0.05 and 2.79 μg/g, respectively. Andreae (1978) also reported finding both forms in marine algae, but at considerably lower levels (0.36 to 5.36 ng/g for the mono- and 1.12 and 10.8 ng/g for the dimethylated species).

Whether the arsenicals in the two studies were taken up from the environment or were synthesized from inorganic arsenic by the organisms was not investigated. It is also not certain whether the simple methylated species were initially present as such in the samples or whether they were released upon hydrolysis of more complex organic arsenicals. The latter view is supported by the findings of Woolson et al. (1976) that neither MSMA nor CA was extracted from algae, daphnia, *Gambusia* minnows, or crayfish exposed to inorganic arsenate.

Methylated arsenicals in soils

Methylated arsenicals, particularly CA and MAA, enter soils from a variety of sources, including microbial methylation of inorganic arsenic. Of greatest interest, however, are the inputs from human application of methylated arsenicals, such as CA, DSMA, MSMA, MAA, and MAMA. These agents, whether used as herbicides or defoliants, are typically sprayed onto plant surfaces. They reach the soil if they miss the vegetation during application or when washed off by rainfall or sprinkler irrigation. Additional arsenicals enter the soil by exudation from the roots (Wells et al., 1973) or when killed plant material decays (Hiltbold, 1975).

Arsenic is widely distributed in agricultural soils, but typical pesticide monitoring programs have not employed tests capable of distinguishing methylated arsenicals from inorganic forms (e.g., Stevens et al., 1970; Wiersma et al., 1971 and 1972). Nevertheless, specific attempts have been made to investigate the behavior of methylated arsenicals in soils.

Johnson and Hiltbold (1969) measured arsenic distribution after application of MSMA, DSMA, and MAMA over four years to sandy loam areas planted in Bermuda grass. They found that arsenic content decreased at soil depths below 5 cm, with little difference in arsenic residues remaining after application of any of the three herbicides. Most of the arsenic applied at the lowest rate (2.23 kg/ha) remained in the upper 30 cm of the soil profile, but the percentage of recovery in this upper layer declined with increasing application rates. When the soil was

fractionated into sand, silt, and clay, it was found that around 90% of the arsenic recovered was in the clay fraction.

Hiltbold et al. (1974) found that no arsenic from MSMA had migrated to depths greater than 15 cm in two soil types, and arsenic found slightly deeper in a third soil was attributed to soil mixing during plowing of the area. Ehman (1965) also found greater retention of arsenic in clay soil than in sandy soil following application of CA or DSMA to the soil surface.

Such findings were substantiated by Dickens and Hiltbold (1967), who tested soils, minerals, and soil fractions for their ability to adsorb DSMA. They also reported greater adsorption of the arsenical on clays and on soils with a high clay content. When pure clays were tested, kaolinite adsorbed significantly more arsenical than did vermiculite or montmorillonite. Limonite (a hydrous iron oxide) strongly adsorbed DSMA.

Differences in DSMA binding ability based on clay content were also reflected in soil leaching experiments, but varying the pH from 5.5 to 6.5 had no effect. The latter result is not surprising, since in the pH range between 5.0 and 7.0 the univalent ion of methanearsonate would greatly predominate, leaving little cause for variability of results (Hiltbold, 1975).

Enhanced adsorption of methanearsonate was also reported to be related to soil clay content by Hiltbold et al. (1974) and by Horowitz (1977). The latter author tested CA as well, and found that it leached more readily than did the monomethyl form.

Similar results were seen when a variety of Mississippi Delta soils were tested for their ability to adsorb arsenicals (Wauchope, 1974). Binding ability was positively associated with clay content. Added arsenate and DSMA were bound in similar amounts, about 17% greater than for CA.

After MSMA had been sprayed over a six-year period on silt loam, fine sandy loam, and loamy sand planted to cotton, 57%, 67%, and 39% of the applied arsenic was recoverable from the soil (Hiltbold et al., 1974). Johnson and Hiltbold (1969) applied MSMA, DSMA, or MAMA to Bermuda grass sod during four growing seasons and could recover from 50% to 100% of the arsenic. The lowest recoveries were associated with the highest application rate (8.95 kg/ha).

In another long-term study, CA and MSMA were applied annually over a six-year period to the surface of bare Hidalgo sandy clay loam just before cotton, corn, or grain sorghum planting (Sandberg et al., 1973). Rates of application were 2.2 and 6.7 or 2.8 and 8.4 kg/ha for MSMA and CA, respectively.

After six years, soil analyses indicated increased values for total arsenic in the upper 15 cm of soil at all treatment levels. At the 15 to 31 cm depth, only the higher application rates of either herbicide resulted in arsenical buildup, while no increase was seen in the 31 to 46 cm soil profile. Residue levels varied greatly among samples, making it difficult to model the arsenic loss trend accurately.

Robinson (1975) treated plots of fine sandy loam with MSMA annually for five years at levels of 4.4 to 288 kg/ha. No significant increases in arsenic levels were detected colorimetrically in the

upper 10 cm of soil at the end of the first year. After two years, increases in soil arsenic levels were seen in plots treated at levels of 72 kg/ha and above.

Similar results were seen for the remaining five years, except for modest decreases for arsenic in the plots treated at the two highest levels (144 and 288 kg/ha). After five years, roughly 50% of the total arsenic applied had left the upper 10 cm of soil. Robinson speculated that arsenic accumulation did not occur at the lower application levels because annual dissipation losses were sufficient to prevent a buildup.

After the second and fifth treatment years, additional samples were taken to a depth of 90 cm in the 9, 144, and 288 kg/ha plots (Robinson, 1975). Analysis of these samples revealed increased arsenic at depths of 0 to 15 and 15 to 30 cm only for the plots treated at the two highest doses, and no increases were seen at greater depths. Bioassays with rice plants confirmed the analytic results, in that only soils treated at levels of 72 kg/ha or more were toxic.

Wilkinson and Hardcastle (1969) saw an increase in arsenic levels in the upper 2.5 cm of soil after six applications of MSMA (2.24 kg/ha) to a cotton field in a single growing season, but soil type was not specified. Similar results were obtained more recently by Woolson and Isensee (1981), who applied CA or MSMA annually for seven years to silt loam soil at 1, 2, or 10 times the recommended maximal rates (17.9 and 89.6 kg/ha, respectively). The soil was tilled to a 15 cm depth before herbicide treatment and to a 7 cm depth immediately after application.

According to colorimetric analysis, arsenic levels slowly increased following all levels of either arsenical. Nevertheless, only half of the arsenic applied at the highest application rate was still present at the last analysis. Total arsenic losses were estimated at approximately 15% of the amount applied per year, which should be sufficient to prevent accumulation to phytotoxic levels when the herbicides are applied at recommended rates. Most of the arsenic residue was found in the upper 15 cm of the test soil.

In a recent study of arsenic transfer in a forest environment, MSMA was applied to frills made in conifers in northeastern Washington, and the surrounding soils were sampled and analyzed for arsenic (Norris et al., 1983). The data presented indicate no significant elevation in soil arsenic near treated ponderosa pines or western larch trees. Arsenic increased in soils near treated Douglas firs, but the increase was statistically significant only in the closest sample (0.5 crown radius).

Reactions of arsenicals within soils are important factors determining their availability for transformation and their relative persistence in different soil types. As was discussed above, the relative proportion of clay in a given soil is apparently the major factor determining arsenical binding ability. Woolson (1977a) diagrammed the relationships of arsenicals to the soil environment, as shown in Figure 5.7.

Methylated arsenicals in terrestrial organisms

Arsenical pesticides such as CA enter plants primarily through absorption from sprayed foliage. Absorption from the soil is generally only a minor source of uptake. The amount and

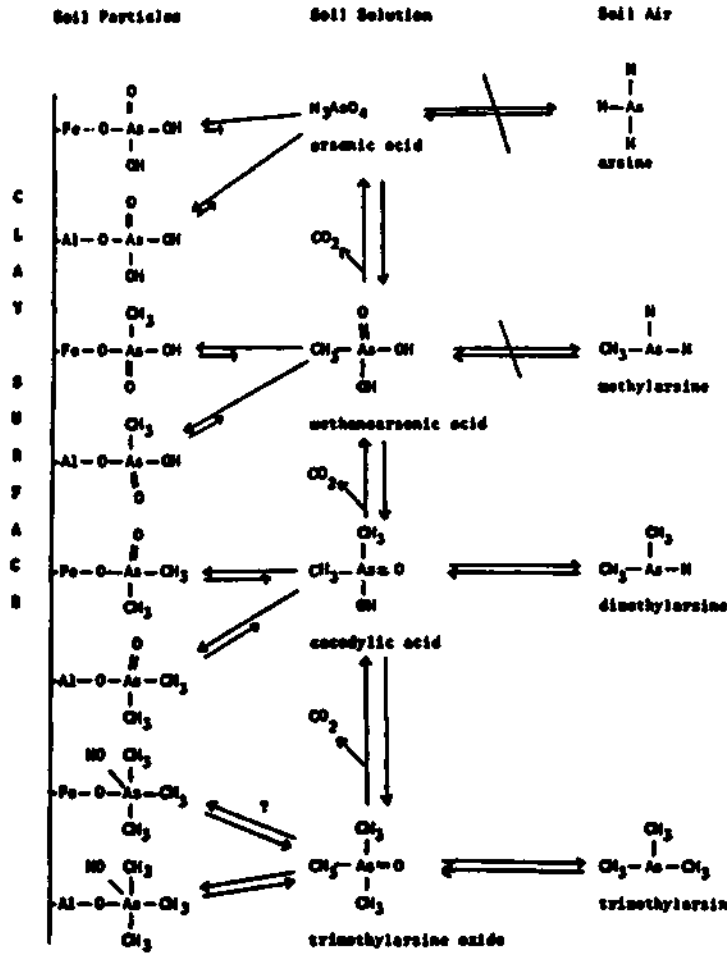


Figure 5.7. Dissolution and reactions of arsenicals within the soil environment

Source: E. A. Woolson, Fate of arsenicals in different environmental substrates, *Environmental Health Perspectives* 19:78 (1977).

speed of absorption of sprayed material depends on such factors as plant type and density, spraying patterns and conditions, and the particular arsenical involved.

Studies of methylated arsenic in terrestrial plants have typically involved determinations of uptake after application of mono- or dimethylated herbicides. Among these was the work of Wilkinson and Hardcastle (1969), who used neutron activation to determine total arsenic in leaves of cotton plants sprayed with MSMA. The plants were treated from one to six times with a dose of 2.24 kg/ha, applied so that only the lower leaves were directly sprayed.

Arsenic was found in greater amounts in both upper and lower leaves with increasing numbers of applications, but a dramatic increase in arsenic in directly sprayed leaves after the last two or three applications appears to have been related to the fact that the plant surfaces were not cleaned by rainfall before the later sampling times. Background arsenic levels were 0.05 and 0.17 μg/g in the younger and older cotton leaves, respectively, rising to 0.23 and 0.90 μg/g following three sprayings, and to 4.8 to 10.3 and 30 to 41 μg/g after five to six MSMA sprayings.

Cotton seed from soils sprayed with MSMA at levels of up to 40 kg/ha before planting were found to contain only slight amounts of arsenic (0.2 μg/g) by Hiltbold et al. (1974), although seed from cotton plants grown on soil sprayed four times annually in four prior years with MSMA, DSMA, or MAMA (2.23 to 8.95 kg/ha 4 × per year) had arsenic contents of 9.07, 7.43, or 3.68 μg/g, respectively, according to Johnson and Hiltbold (1969).

Johnson and Hiltbold (1969) also assayed seeds from corn and soybeans, as well as top growth from sorghum, oats, crimson clover, and hairy vetch grown on similarly treated soil. Arsenic content tended to be dose-related and ranged from 1.35 μg/g for clover in low-dose soil to 3.95 μg/g in the first cutting of high-dose sorghum. Arsenic uptake varied by herbicide, with the greatest uptake following application of MSMA. Further, significant arsenic levels were not found in tea leaves or brewed tea from plants previously treated with MSMA (Anon, 1973, as quoted in Ray, 1975).

Uptake of organoarsenicals by plants is relatively rapid (Rumberg et al., 1960). For example, Mason et al. (1979) measured 50% uptake of MSMA by Johnson grass within six hours of application. Later absorption was greatly slowed, apparently because of the initial damage to the plant. Keely and Thullen (1971a) noted control of yellow nutsedge exposed for only 5 or 15 minutes to MSMA or DSMA, respectively. Rapid effect was not universal, however, as they found 24- to 48-hour exposures necessary for control of purple nutsedge.

Recently, Pyles and Woolson (1982) identified trace amounts of MAA in vegetables grown on silt loam soil treated only with inorganic arsenate (100 ppm of arsenic, as arsenic acid). Included were tops of lettuce, and swiss chard, as well as the flesh (parenchyma) and peel of potatoes. Additional arsenic appeared to be present in a more complex organic form, apparently a nonpolar lipid.

Amounts of MAA in broccoli, beets, cabbage, corn, tomatoes, and green beans, if present, were below detection limits. Detectable amounts of MAA were not found in the soil, which suggests that the amounts determined in the plant materials were synthesized within the plant rather than by soil microorganisms.

In addition to uptake and retention of methylarsenic, plants have been said to exude such compounds from their roots. In 1973, Wells and Anastasia reported that by three days, bean plants and green ash seedlings had released 12.8% and 2.7%, respectively, of the ^{14}C-cacodylate applied to their leaves. These values had risen to 19.1% and 9.6% after nine days.

Wells and Anastasia (1973) also found that increased CA application enhanced the exudation rate unless the dose used was high enough to damage the plant, in which case exudation was greatly inhibited. It thus appears that woody plants may release a greater proportion of a low dose of CA than would herbaceous types, although such findings are in need of confirmation. At

effective herbicidal use levels, such CA release would be minor, and most loss of arsenicals would result from the decay of killed plants.

A number of studies on the translocation of methylated arsenicals have been carried out using the purple or yellow nutsedge as test plants. For example, Holt et al. (1967) found that arsenic was transferred to other shoots on the same tuber when only one shoot was treated with amine methylarsonate. If shoots from one tuber were treated, arsenic was translocated laterally through rhizomes to other connected tubers, particularly to the terminal tuber of a connected chain.

Similar results were reported by Duble et al. (1968), who used DSMA. They found that little ^{14}C-labeled DSMA was degraded in the nutsedge plant. A small amount of what may have been $^{14}CO_2$ was respired, but the authors speculated that the radioactivity measured may have been from volatilization of ^{14}C-DSMA.

Translocated DSMA was concentrated in actively growing tissues, such as shoots, roots, rhizomes, and terminal tubers, but 86% remained in the treated shoot. DSMA applied to young leaves was translocated to other leaves more readily than if it had been applied to older leaves. Movement to developing rhizomes and other shoots was not influenced by leaf age nor was DSMA movement within a single leaf blade.

More recently, when Keeley and Thullen (1970) applied DSMA or MSMA to the foliage of yellow nutsedge (3.4 kg/ha), they found significantly more arsenic in tubers from treated plants (4 to 33 ppm) than in those from controls (1 ppm). A higher level of arsenic was located in small tubers (23 to 33 ppm) than in larger ones (4 to 12 ppm). The same authors later found that both DSMA and MSMA could kill yellow nutsedge more readily than they killed the purple variety, and this differential effect was correlated with a greater uptake of both herbicides by the yellow nutsedge (Keeley and Thullen, 1971a).

When plant extracts were chromatographed, no apparent metabolites of either DSMA or MSMA were found. MSMA tended to penetrate the nutsedge leaves more readily than did DSMA, a finding similar to results with the cotyledons from cotton seedlings (Keeley and Thullen, 1971b).

Working with Johnson grass, Sckerl and Frans (1969) found rapid uptake of MAA from a nutrient solution through the roots, with translocation throughout the plant in four hours. When the arsenical was applied to the leaves or stems of either Johnson grass or cotton, both acropetal and basipetal movement occurred, suggesting translocation via both xylem and phloem.

Addition of DSMA to a nutrient solution was followed by rapid uptake in coastal Bermuda grass, but uptake was much more meager after soil application, indicating binding by soil components (Duble et al., 1969). When foliar DSMA was applied, translocation again was seen to have occurred both acropetally and basipetally. Within five days, roughly one-fourth of the DSMA applied to the Bermuda grass foliage was translocated to the rhizomes and roots.

Arsenical translocation in both xylem and phloem was also reported for bean plants (Anastasia and Hurtt, 1970). Stem girdling by steam application decreased movement of root-absorbed CA into the plant tops and reduced its herbicidal effectiveness, particularly at low treatment levels.

Treatments ranged from 2.5×10^{-4} to 7.5×10^{-4} M CA in the nutrient solution. Levels of CA in the roots were unaffected by girdling.

In a recent study, cotton plants were grown with a nutrient solution containing up to 40 μg of arsenic per ml as CA (Marcus-Wyner and Rains, 1982). The herbicide was transferred through the entire plant, in contrast to similarly applied arsenic trioxide (to 8 μg of arsenic per ml) which was not readily translocated, even though it was taken into the roots. Nevertheless, the inorganic arsenical was considerably more toxic to the cotton plants.

When translocation of organic arsenicals added at levels of 25 μg of arsenic per ml was compared with that of arsenic trioxide at 5 μg of arsenic per ml, roots of 42-day-old cotton plants took up MSMA, DSMA, and As_2O_3 more readily than they absorbed CA in the first week after treatment. During the second week, their total uptakes (expressed as a percentage of the first week's value) were 80%, 79%, and 232% for CA, DSMA, and MSMA, respectively.

No data were obtained for As_2O_3, as the roots of those plants treated with the inorganic arsenical had been killed. More arsenical was found in the shoots of CA-treated plants than in those of plants treated with MSMA or DSMA, but the arsenic content of the plant tops had not increased during the second week after treatment.

In further experiments, Marcus-Wyner and Rains (1982) applied CA to the foliage of 49-day-old cotton plants growing in a greenhouse. Application rates were one and two times the recommended rate (1.41 kg/ha), and plants were harvested and sampled at one, four, or six weeks after treatment. Such treatments resulted in little translocation of CA from the leaves, which tended to abscise.

The reproductive parts had been covered during spraying, so that CA uptake by translocation alone could be measured. Seed from plants sprayed with 2.81 kg/ha contained 3.31 μg of arsenic per g at six weeks. In a similar experiment with plants sprayed at 69 days and harvested in one week, the seeds and fiber combined contained up to 5.3 μg of arsenic per g.

When field grown cotton was sprayed with Bolls-Eye® at a CA level of 0.71 kg/ha at 105 days after planting, the roots contained only 1 μg of arsenic per g after 11 or 27 days. Litter around the cotton plants (mainly dead leaves and abscised reproductive parts) was relatively much higher in arsenic (32 to 53 μg/g), as were the upper bracts (20 to 22 μg/g). Seeds from the same plants were much lower in arsenic, containing only 0.5 to 0.7 μg/g.

Such results support the concept that CA is not rapidly taken up from soils when it is incorporated in organic material, and that CA sprayed on mature cotton is not transferred to the seeds in large amounts. Further, in all the foregoing experiments of Marcus-Wyner and Rains (1982), it must be kept in mind that although the arsenical was determined and reported as "arsenic," the authors were almost certainly measuring CA rather than inorganic arsenic.

In their effort to determine the degree to which use of organoarsenical herbicides in Western forests contributed to levels of environmental arsenicals, Norris et al. (1983) found no statistically significant increases in arsenic levels of herbaceous plants or of browse materials (terminal 10-cm branch tips) near MSMA-treated ponderosa pines. Some increases were seen around Douglas fir, and in one case near western larch trees.

Another attempt to investigate possible environmental pollution with organic arsenicals was the work of Ehman and Birdsall (1963). They applied 5.8 or 17 kg of Ansar® 138 (65% CA) to "pasture grass" by spraying, and after five days sampled and analyzed the grass for arsenic.

Untreated grass contained 0.3 ppm of arsenic, while the low-and high-dose samples contained 134 and 197 ppm of arsenic, respectively. Portions were then burned, and when the ash was analyzed, the untreated, low-, and high-level samples contained 2, 190, and 392 ppm of arsenic, respectively. These results suggest that approximately 81% and 51% of the arsenic (probably still as CA) was given off in the smoke from the 5.8 and 17 kg/ha treated grass samples, respectively. This would amount to roughly 3 mg of arsenic in the smoke from 30 g of burned grass hay in both cases.

Additional data on arsenical residues in crops can be found in the reports of Raab (1970), Raab and Klosterboer (1970), and the Ansul Company (undated) discussed in the chapter on Agricultural and Related Uses.

It appears that only a few studies have dealt with the absorption of methylated arsenicals from their environment by terrestrial animals other than man. Among them was the work of Maycumber (1974). He collected hair from 37 cattle before they were let out for summer grazing near the end of June, about one year after trees on the grazing area had been thinned with silvicides containing CA (Silvisar® 510) or MSMA (Glowon®). In mid-October a second hair sample was obtained from the same animals. In a second trial, hair was taken from 28 cattle late in May before the animals were released to graze. They were resampled in early September.

Silvicide application with MSMA (Silvisar® 550) took place while the cattle were grazing the area to be thinned. In both cases there was an increase in arsenic in the hair, according to the results of neutron activation analysis. There is a problem inherent in interpreting Maycumber's data, however, as no samples were taken from cattle grazing untreated areas to provide a concurrent control baseline for arsenic levels. Analysis of soft tissue from two cattle and a deer taken from the thinned area found no arsenic detectable by wet-ashing followed by colorimetric determination.

Maycumber was followed by Norris (1977) in a study of cattle from a similar area partly thinned with MSMA in previous years, with a portion thinned during the grazing period. Hair from a dozen cattle was sampled in mid-June and again in mid-October and compared with hair from 12 cattle grazing untreated areas.

A significant increase in arsenic content was seen between the initial and later samples taken from the cattle grazed on untreated areas, but not in those from the cattle on treated areas. Such results were said to indicate that natural levels of arsenic in the plants consumed in the test forest areas were higher than those consumed during the previous time. It was therefore assumed that treatment of a portion of the trees in the area did not cause an increase in arsenic levels detectable by changes in hair.

Another confounding factor in the Norris study was the presence of apparently higher initial levels of arsenic in the hair of the test cattle than were seen in the control animals. Regardless of such considerations, the arsenic levels measured in the hair samples of cattle from both studies (Maycumber, 1974; Norris et al., 1977) grazing on areas where arsenicals had been used were low

(0.69 to 0.74 ppm) and fail to indicate any potential hazard. It should be kept in mind, however, that hair analysis is subject to many potentially confounding factors which could result in erroneous conclusions.

In a study of arsenic levels in wildlife collected from forest areas thinned with CA or MSMA, residues were not found in birds or in the single deer tested (Schroedel, 1974). A variety of small mammals (voles, shrews, mice, and chipmunks) collected within the first month after treatment contained arsenic residues of up to 9.8 ppm, but most were less than 5 ppm.

Animals collected later than 30 days after silvicide application generally failed to contain tissue arsenic residues as high as 0.5 ppm. A single ground squirrel contained 17 to 30 ppm of arsenic in various tissues, but arsenic levels in other ground squirrels were similar to those in the other small mammals.

An exception to the foregoing was the finding of a number of freshly dead snowshoe hares that had apparently died from eating materials heavily contaminated with MSMA by thinning crews emptying and washing their spray equipment and washing their hands. High arsenic levels were found in plant and soil samples from such areas. Arsenic in the livers of most of the dead hares ranged from 140 to 460 ppm, and the arsenic levels in their stomachs ranged from 130 to 530 ppm, suggesting arsenical poisoning. Hares trapped in untreated areas did not contain detectable arsenic levels.

Evans (1974) found that the snowshoe hare was much more sensitive to MSMA than were other species reported on in the literature, lending credence to the idea that MSMA was the cause of death in the report of Schroedel (1974). Such data also reinforce the concept of the need for cleanup of pesticide application equipment in ways that do not significantly increase environmental contamination.

Other studies that have addressed the issue of environmental arsenic uptake have looked solely at total arsenic. According to the 1977 report of Sharma and Shupe, small mammals (primarily rock squirrels) from an arid environment contained 1 to 9 ppm of arsenic in their livers. It is likely that at least a portion of the arsenic found was in CA or MAA. Nevertheless, what fraction of any such methylated arsenicals would be endogenous and what, if any, was absorbed as such also remains to be determined.

Elfving et al. (1979) reported finding total body burdens of arsenic in adult meadow voles, pine voles, and white-footed mice ranging from 2.6 to 28 μg when the animals were trapped from orchards that had been sprayed for a number of years with inorganic arsenic. Soil arsenic content ranged from 31 to 94 ppm (dry weight). Arsenic contents of control animals from environments lower in arsenic (2.4 ppm in soil) ranged from below detection limits (limits not specified) to 0.8 μg per animal.

Chapter 6

Pharmacology of Methylated Arsenicals

Cacodylate absorption and distribution

Current knowledge of the fate of methylated arsenic in humans and other animals comes largely from studies of the metabolism of inorganic arsenic, but a few studies have dealt with the absorption of exogenously supplied CA. Among the first was the report of Peoples (1968) that discussed CA excretion in the milk of Holstein cows fed a ration providing a daily intake of 24.5 mg of CA. The lactating cows were given the treated diet for 60 days, and periodic sampling of their milk failed to reveal CA. The detection limit of the analysis used was 0.25 ppm.

Urine samples contained 75% to 85% of the ingested dose. When the cows were slaughtered at the end of the test period, only low levels of arsenic were found in most tissues, with liver containing the highest levels (0.4 to 0.5 ppm). Only two cows were used in the CA study, but similar results were seen with two cows given arsenic acid. Peoples later fed methanearsonic acid at levels up to 75 mg per cow per day to groups of three dairy cows for nine weeks and found no significant increases in the arsenic content of their milk (Peoples, 1968).

Hwang and Schanker (1973) found that a number of organic arsenicals, including CA, were absorbed by rats after direct administration into the small intestine. The absorption rate constants for three arsenicals ranked in the same order as did their chloroform-to-water partition coefficients, with a rate constant for CA of 0.207 and an absorption half-time of 201 minutes. Absorption appeared to occur by a nonsaturable process, as it was proportional to concentration over a 100-fold range (1 to 100 mM).

In a more extensive study, Stevens et al. (1977d) administered ^{14}C- or ^{74}As-labeled CA by the oral, intravenous, or intratracheal routes to rats. CA absorption following oral dosing was much slower ($t_{1/2}$ = 248 minutes) than absorption from the lung ($t_{1/2}$ = 2.2 minutes). Maximal plasma levels of CA were seen 5 to 10 minutes after intratracheal dosing, and the plasma clearance curve obtained was similar to that found after intravenous administration (Figure 6.1).

Oral dosing did not result in peak plasma CA levels until one hour, but the concentrations reached in whole blood were higher than those achieved following treatment by the other routes, and CA retention remained greater through 105 days. In general, the relative distributions of CA by one hour in a variety of organs (lung, spleen, liver, kidney, brain, whole blood) were similar after high (33 μg) or low (3.47 μg) doses of cacodylate. By 105 days posttreatment, with the exception of whole blood values, only a few differences were observed in tissue levels of CA following treatment by the three routes.

When female rats treated intravenously with CA were compared with males, no sex-related differences were seen in tissue distribution. When pregnant rats were treated (*apparently intravenously*) on gestation day 21, levels of CA in fetal blood and liver were similar to those of the mother. CA amounts in fetal brain were lower and those in kidney higher than levels for maternal tissues.

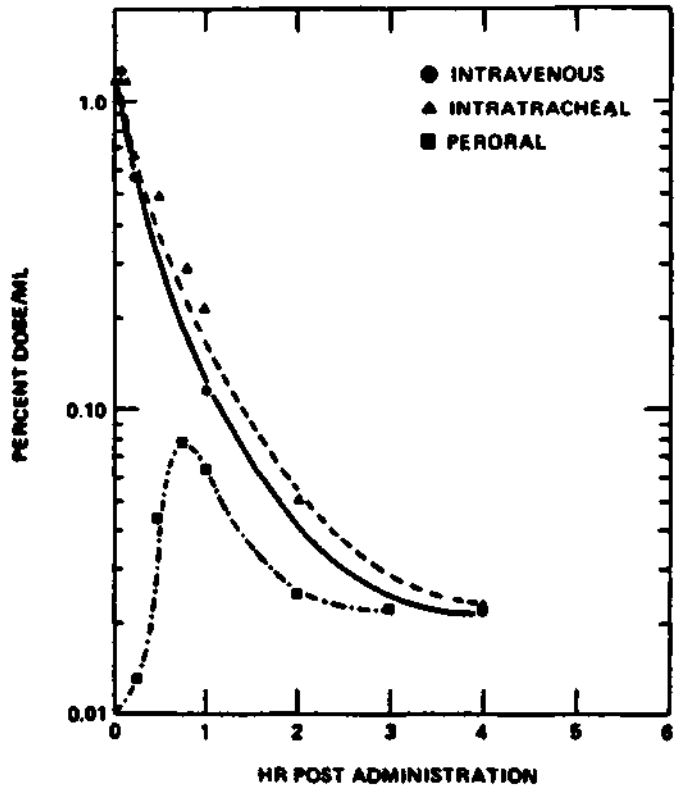


Figure 6.1. Comparison of plasma curves after (●) intravenous, (▲) intratracheal, and (■) peroral administration of 33 μg of ^{14}C-cacodylic acid and 3.5, 13.8 and 6.9 μg of ^{74}As-cacodylic acid given to male sherman rats.

Source: Stevens et al., Disposition of ^{14}C and/or ^{74}As-cacodylic acid in rats after intravenous, intratracheal, or peroral administration, *Environmental Health Perspectives* 19:151 (1977).

The administration of five daily oral doses (totaling approximately 80 μg) by Stevens et al. (1977d) resulted in blood CA levels four times as great as those resulting from a single oral dose of 40 μg, but at 105 days the percentage of dose remaining in the blood was less following the multiple dosing than that for a single dose given by any of the three routes tested. No clear explanation for such results was evident.

Stevens et al. (1977d) also analyzed the plasma CA data following intravenous dosing and computed a three-exponential equation with half-times of 0.014, 0.217, and 3.42 hours. The apparent volume of distribution was 15.3 ml. Such data on distribution of arsenicals are difficult to use for extrapolating results, however, as the rat appears to handle arsenicals differently from humans and other test species (Odanaka et al., 1980).

Unfortunately, other studies involving exogenous cacodylate also employed the rat as animal models. Siewicki (1981) fed purified diets containing two levels of witch flounder, a fish naturally high in arsenic, or a combination of a low arsenic fish (winter flounder) and CA. The diets were fed for a total of 42 days.

CA-fed rats retained significantly more arsenic in their livers, and particularly in their spleens and erythrocytes, than did the animals fed only fish arsenic. In fact, erythrocyte arsenic was two orders of magnitude higher in the spleens of the CA-fed rats on both the high- and low-dose diets.

The sum of dietary arsenic was similar in fish arsenic and in the CA-supplemented diets (approximately 16 and 29 ppm for the two levels), except that 5 ppm of the arsenic in the CA-supplemented diet was supplied by the winter flounder. Although the endogenous arsenical in the flounders was not specified, it was most likely to have been arsenobetaine (Oladimeji et al., 1979; Edmonds and Francesconi, 1981b; Luten et al., 1982).

In a previous study, Woods and Fowler (1978) had compared the relative amounts of arsenic retained in the testes and livers of rats orally dosed with various arsenicals. They found that the order of retention was fish arsenic < arsenic trioxide < CA < sodium arsenate.

Lindgren et al. (1982) investigated CA distribution in adult male mice and found that ^{74}As-labeled CA tended to concentrate in certain organs. It appears that the thyroid and lens of the eye were involved, but the full data were not included.

In order to obtain information about uptake and retention of methylated arsenicals in pregnant mice and their offspring, Hood et al. (1983c) gave intraperitoneal injections of either 600 mg/kg of NaCA or 800 mg/kg of disodium methanearsonate to mice on day 12 of gestation and killed individuals at intervals after treatment. Samples of fetuses and of maternal liver, erythrocytes, serum, and urine were analyzed for total arsenic.

Results indicated rapid uptake in fetuses, liver, and both blood fractions, with peak values in the 15-minute samples. Arsenic levels also declined rapidly, however, especially in the blood. Both serum and erythrocyte levels were generally below 0.1 μg per milliliter or per gram by 6 to 12 hours after treatment. By 12 hours, arsenic in fetal tissues had declined from a level of 30 to 1.5 μg/g in the case of cacodylate and from 37 to 2.5 μg/g in the case of methanearsonate.

Whether a mammal can effectively metabolize CA is controversial. The older literature speaks of a garlic-like odor on the breaths of humans and animals treated with CA (e.g., Heffter, 1901; Simon, 1932; Goodman and Gilman, 1941; Ehman, 1973a; Wagner and Weswig, 1974), presumably from alteration of a portion of the arsenical to its corresponding arsine, or to cacodyl or cacodyl oxide, but these observations await confirmation with modern analytic techniques.

Indeed, Stevens et al. (1977b) failed to find evidence of CA metabolism by liver homogenate fractions in vitro, and Stevens et al. (1977d) found little evidence of metabolism of CA in vivo. Odanaka et al. (1983) did, however, recently report finding trimethylated arsenic (which might have been derived from methylation of CA) in the urine of rats, mice, and hamsters following oral dosing with inorganic arsenate. They obtained like results with human urine from an untreated individual.

The older literature often contained statements to the effect that CA was broken down to inorganic arsenic in the tissues (e.g., Goodman and Gilman, 1941; Sollmann, 1948). Such a belief may have been based on the symptoms of CA toxicity, rather than on analytic data. Dawes and Jackson (1907) stated that arsenate could be detected in the urine of human patients and in urine, feces, and tissues of dogs chronically injected intramuscularly with CA. The inorganic arsenic was not found until some two to six weeks after treatment was begun, and the reliability of the methodology used is not clear.

It has also been stated that gastric acid would liberate inorganic arsenic from orally administered CA, resulting in increased toxicity (Goodman and Gilman, 1941; Blacow, 1972), but current knowledge of the resistance of CA to hydrolysis with hydrochloric acid suggests that cacodylate breakdown in the stomach is unlikely.

BAL (British anti-lewisite or 2,3-dimercaptopropanol) has been used clinically as a chelator in attempts to decrease the toxicity of inorganic arsenic and to promote its excretion (Levine, 1970). According to Hashimoto (1953), BAL failed to enhance CA excretion in rabbits or to affect its concentration in the blood within 24 hours. Based on Hashimoto's results, Ehman (1973a) suggested that BAL would probably not be useful in treating cases of CA poisoning.

Metabolism and excretion of inorganic arsenicals

Braman and Foreback (1973) were the first to discover methylated arsenic metabolites in human urine. They found both cacodylic acid and methylarsonic acid, and suggested that methylation served to detoxify inorganic arsenic before excretion. These results have been confirmed by a number of other workers (eg., Smith et al., 1977; Crecelius, 1977; Tam et al., 1979a).

One or both methylated arsenic metabolites have also been found in most other mammalian species examined, including the cow (Lakso and Peoples, 1975), dog (Lakso and Peoples, 1975; Tam et al., 1978, 1979b; Charbonneau et al., 1979), rat (Odanaka et al., 1978a, 1978b, 1980; Yamauchi et al., 1980; Shirachi et al., 1981; Vahter, 1981; Rowland and Davies, 1982; Marafante et al., 1982), mouse (Odanaka et al., 1980; Vahter, 1981), hamster (Odanaka et al., 1980; Charbonneau et al., 1980), rabbit (Odanaka et al., 1980; Bertolero et al., 1981; Marafante et al., 1982), and cat (Odanaka et al., 1980).

According to Tam (quoted in Pomroy et al., 1980), a monkey of unspecified species excreted CA as its arsenic metabolite, but Vahter et al. (1982) reported that a male marmoset excreted only inorganic arsenic after being given arsenite by intraperitoneal injection.

Further studies focused on the fate of inorganic arsenate and arsenite following their administration to mice late in gestation (day 18). Hood et al. (1983d and 1983e) employed both the oral (by gavage) and intraperitoneal treatment routes. The doses employed (in mg/kg) for As(V) were 20 and 40 for mice treated intraperitoneally and orally, respectively. As(III)-treated mice received 8 mg/kg intraperitoneally or 25 mg/kg orally. Pregnant females were sampled at intervals up to 24 hours after dosing. In addition to total arsenic, both methylated arsenic metabolites (cacodylate and methanearsonate) were quantitated in the fetuses.

Total fetal arsenic levels peaked at two and six hours following intraperitoneal and oral arsenate treatment, respectively, while in the case of arsenite, the highest fetal levels were seen at 24 hours. In both arsenate- and arsenite-treated mice, animals given intraperitoneal injections had higher peak levels of arsenic, even though the dose administered by injection was considerably smaller. Total arsenic absorption as measured by comparing the areas under the uptake curves was similar following oral and intraperitoneal administration, however.

According to the results of both studies (Hood et al., 1983d and 1983e), a significant amount of arsenic was methylated in all cases. After both maternal arsenate and arsenite administration, mono- and dimethylated arsenic were present in the fetuses in similar amounts through the first hour, after which the dimethylated form greatly predominated.

At 24 hours after treatment, the relative proportion of dimethylarsenic to the monomethylated form was greater in animals given arsenate (97% to 98%) than in those given arsenite (77% to 81%), although the proportion of total fetal arsenic remaining in the inorganic form was similar (26% vs 21% for intraperitoneal and 14% vs 14% for oral arsenite and arsenate, respectively).

In addition to their decreased toxicity in comparison with inorganic arsenic, methylated arsenicals are also excreted more rapidly, especially MAA (Buchet et al., 1981a). This more rapid elimination may be due to a lower affinity of methylated arsenic for tissue constituents (Marafante and Vahter, 1982).

When one arsenic metabolite has been found to predominate greatly, as is the case in the dog (Tam et al., 1978), mouse, rabbit, cat (Odanaka et al., 1980), and hamster (Charbonneau et al., 1980), it is invariably the dimethyl form. In humans, however, although cacodylate is the major metabolite, it accounts for only two-thirds to three-fourths of the methylated products found in urine, the remainder being MAA. This relationship holds true regardless of whether the exposure was through inhalation or by ingestion.

Additionally, it has been found that 20% to 30% of the inorganic arsenic humans absorb is excreted without methylation (Smith et al., 1977; Crecelius, 1977; Tam et al., 1979a; Buchet et al., 1981a), and Vahter (1981) and Lerman and Clarkson (1984) both found arsenite to be methylated more readily than arsenate.

According to Crecelius (1977), a human subject was fed wine containing 50 μg of As(III) and 13 μg of As(V) per liter. Arsenic of both valence states was largely excreted within 20 to 40 hours, in the case of arsenate and arsenite, respectively (Figure 6.2). Methylated arsenic concentrations in the urine peaked around 40 hours and decreased thereafter.

Later, when Buchet et al. (1980) gave oral arsenite doses (500 μg of arsenic) to three male volunteers, 46% was excreted in the urine by the end of four days, with the proportion of methylated metabolites rising rapidly by 8 to 12 hours after dosing. From the second to fourth days, CA accounted for more than 60% of the excreted arsenic. Yamauchi and Yamamura (1979a) also reported similar results for one male subject given arsenic trioxide.

When individuals ingested considerably larger arsenite doses (500 to 1,000 mg or more) accidentally and so developed arsenic poisoning, a considerable degree of methylation occurred, particularly by six to nine days after arsenic ingestion (Buchet et al., 1980; Mahieu et al., 1981).

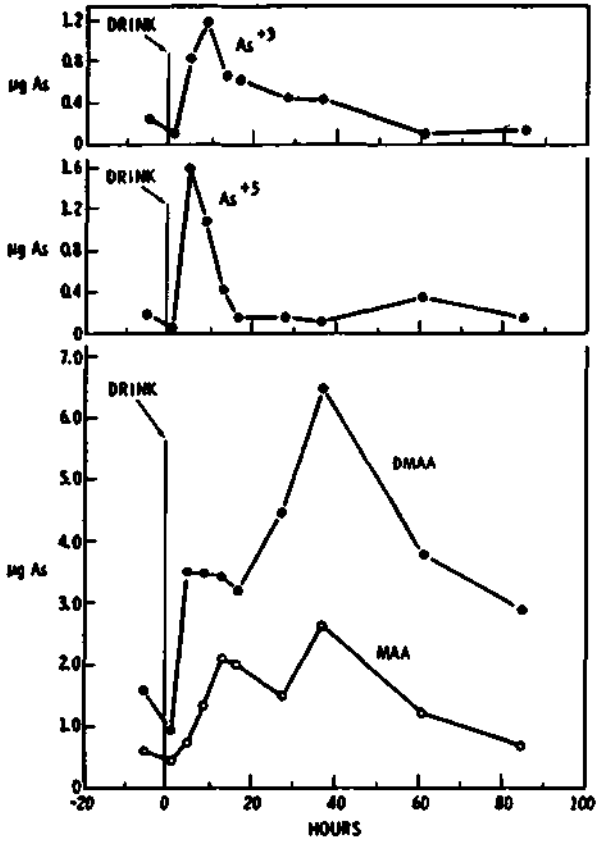


Figure 6.2. Amount of arsenic compounds in urine with time after ingestion of wine containing 50 μg As^{+3} and 13 μg As^{+5}. The amounts of methylarsonic (MAA) acid and dimethylarsinic (DMAA) acid are given in μg as arsenic.

Source: Eric A. Crecelius, Changes in the chemical speciation of arsenic following ingestion by man, *Environmental Health Perspectives* 19:149 (1977).

Whether the timing of the onset of high levels of methylation was influenced by the arsenic dose or by the BAL chelation therapy these patients were receiving could not be determined, however.

It was additionally suggested that the quantities of methylated metabolites versus inorganic arsenic in the urine might be used to time the duration of chelation therapy (Mahieu et al., 1981).

Tam et al. (1979a) followed a group of six men who ingested ^{74}As-labeled arsenate (as arsenic acid) and reported the results shown in Figure 6.3. Although 50% of the radioactivity in the urine

was found in inorganic arsenic on the first day, this value decreased to less than 20% by day 2, while the percentage of MAA remained relatively constant, and that of CA increased to 70% by day 5.

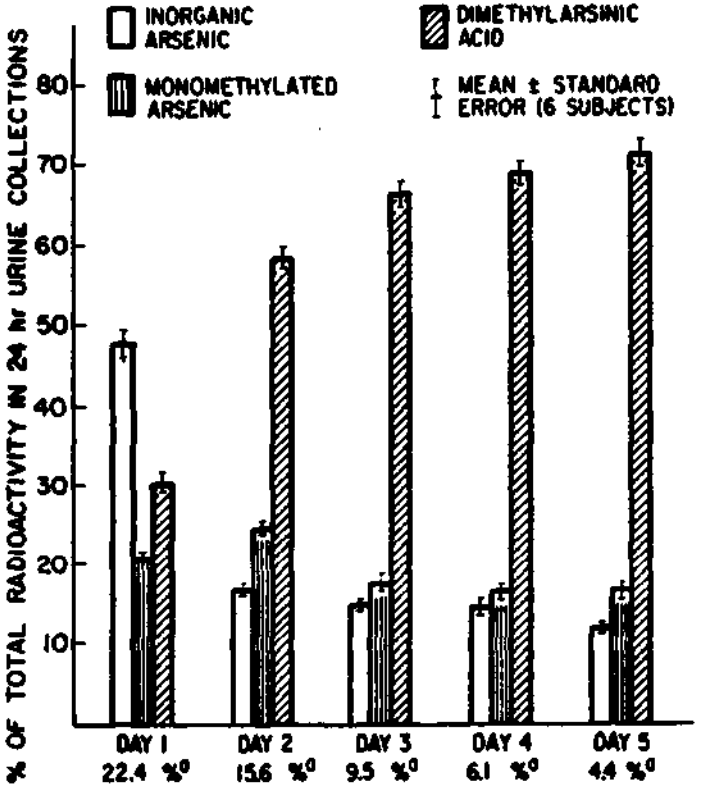


Figure 6.3. Percentage of total radioactivity present as various forms of arsenic in human urine following a single oral dose of inorganic arsenic (^{74}As). (a) Percentage of ingested dose excreted in urine.

Source: G. K. H. Tam et al., Metabolism of inorganic arsenic (^{74}As) in humans following oral ingestion, *Toxicology and Applied Pharmacology* 50:320 (1979).

During the first five days after treatment, 58% of the total dose had been recovered from the urine, with 51.07 ± 2.6% as the dimethyl metabolite and 20.6 ± 1.5% in the monomethylated form. The degree of methylation was quite constant between individuals. In a second report on what appeared to be the same subjects (Pomroy et al., 1980), it was further stated that no ^{74}As was found excreted via the sweat of one individual.

The above findings differed somewhat from those of Yamauchi and Yamamura (1979b), who fed a seaweed extract containing 2.88 ppm of arsenic, of which 86% was As(V), 7% As(III), and 7% CA, to three test subjects. They stated that after excretion of only 36% of the ingested arsenic, urinary arsenic levels had returned to the pretreatment baseline. CA was the predominate metabolite, as expected, but the authors reported an apparent reduction of As(V) to As(III) before excretion in the unmetabolized inorganic fraction.

Initial suspicions that CA and MAA found in urine were derived from methylated arsenicals found in plants (Peoples et al., 1971; Lakso et al., 1973)—or in the case of cattle, from the action of rumen bacteria—were laid to rest by Lakso and Peoples (1975). They fed sodium arsenate or potassium arsenite to dogs and detected methylated metabolites in the urine.

When a monomethylated arsenical, ferric methanearsonate [$(CH_3AsO_3)_3Fe_2$], was administered orally to rats, it too was methylated to CA (Odanaka et al., 1978 a and b). Rat microflora have also been shown to methylate inorganic arsenic (Rowland and Davies, 1981), but not in amounts sufficient to account for the amount produced in that animal (Rowland and Davies, 1982).

Further proof that inorganic arsenic is a primary source of excreted methylated arsenicals was obtained by Tam et al. (1978, 1979a, 1979b), Charbonneau et al. (1979, 1980), Bertolero et al. (1981), and Marafante et al. (1982). They administered ^{74}As to a variety of mammals, including humans, and subsequently isolated ^{74}As-labeled methylated metabolites. These findings are evidence that the methylated arsenicals isolated from urine are not of dietary origin. Trace amounts of MAA (generally less than 0.01 ppm) were found in some vegetables, however, by Pyles and Woolson (1982).

Although considerable research has addressed the issue of microbial methylation of arsenic, relatively little is known of the methylation process as it takes place in mammals. Tam et al. (1978) proposed that the cacodylate they observed in dogs 30 minutes after intravenous injection of arsenic acid was derived from metabolism by the liver, but direct evidence that mammalian liver could methylate inorganic arsenic was not forthcoming until the report of Shirachi et al. in 1981. According to their findings, methylating activity resulting in formation of monomethylated arsenic was seen in nuclear, mitochondrial, microsomal, and 100,000 $\times$ gravity supernatant fractions from the livers of male Wistar strain rats. Dimethylated arsenic was found primarily in the supernatant fraction.

Shirachi et al. (1981) made the assumption that the inorganic arsenate added to their liver fractions was methylated in two steps, first to mono- and then to dimethyl-arsenic. Because the relative distribution of the two methylating activities differed, it appeared that two enzymes were involved. It was also suggested that the liver homogenate contains an endogenous inhibitor of arsenic methylation and that it particularly affects the reaction resulting in the dimethylated metabolite.

Shirachi et al. (1981) used very high amounts of inorganic arsenic. Their method for qualitative analysis is not the most accurate available, and others have been unable to duplicate their results. For example, Rowland and Davies (1982) were unable to obtain *in vitro* methylation of either valence state by rat liver homogenates or isolated hepatocytes. F. M. Tatum and R. D. Hood (unpublished results) also found no methylation when they tried to duplicate the results of

Shirachi et al. (1981) with rat or mouse liver or kidney homogenate, either whole or fractionated. Nevertheless, Tatum and Hood also found that isolated rat primary hepatocytes plus two cell lines derived from rat hepatomas could methylate arsenate, while cells from three additional lines failed to do so. Increasing levels of arsenate in the culture medium blocked further methylation of the monomethylated metabolite, and resulted in its accumulation in the cells and culture medium.

Lerman and Clarkson (1984) have reported metabolism of both arsenic valence forms by rat hepatocytes, as well as by liver and kidney slices. Both liver and kidney were said to methylate arsenite more readily than they did arsenate. Kidney was much more efficient at methylating arsenate than was liver, and the authors suggested that kidney may be more efficient at reducing arsenate, a necessary step in arsenate methylation.

Rowland and Davies (1982), had found that when arsenate was given by injection into a loop of small intestine isolated by ligation, it was largely reduced by the time it reached the portal blood. Similar results were seen after injection via the jugular vein, however, suggesting that such reduction may be due to reducing agents in the blood or other tissues.

In 1982, Marafante et al. compared the distribution and metabolism of inorganic arsenite in the rat and rabbit. Their results confirmed the evidence of others that rats handle arsenic atypically. After intraperitoneal injection, rats were found to accumulate arsenic in the blood, mainly in the bound form in association with intracellular proteins of the erythrocytes. This was not the case with rabbits, where arsenic largely remained in a diffusible state.

The same was also true of other tissues from both species, such as kidney and liver. Such differences were also reflected in the clearance of arsenic from the tissues, with retention being greater in the rat. Retention of arsenicals in the rat erythrocyte has been attributed to reduction by glutathione reductase and subsequent reaction with the SH groups of hemoglobin.

According to Marafante et al. (1982), methylation of the administered arsenite occurred rapidly in both species, with CA being the predominant metabolite. Arsenic was retained to a considerably greater degree in the rat by 16 to 48 hours after treatment. In fact, even untreated rats have been found by Marafante et al. (1982), Knowles and Benson (1983), and R. D. Hood (unpublished results) to contain arsenic accumulated from the low levels found in commercial laboratory animal diets.

In an attempt to influence in vivo arsenic methylation in man, Buchet et al. (1981a) fed methionine, choline, inositol, and vitamin B_{12} for eight days to five men, and left three others untreated. All were given 500 μg of As(III), which was administered on the fourth day of treatment to the men given methyl donors. The percentages of inorganic, mono-, and dimethylated arsenic excreted over four days following arsenic ingestion were not significantly altered by the additional treatment. That result was attributed to the low arsenic dose that was unlikely to have tested the capacity of the test subjects.

Buchet et al. (1981a) gave 500 μg of arsenic as CA or MAA orally to four men each and collected their urine for four days. Seventy-five percent of the CA and 78% of the MAA were eliminated via the urine within the four-day period. This is in contrast to the 45% to 49% elimination of the ingested dose from men given inorganic arsenite. Half of the total amount

excreted was lost within 4, 11, or 28 hours in the case of MAA, CA, and inorganic arsenic, respectively.

When the urinary arsenicals were speciated after MAA ingestion, an increasing proportion of the remaining MAA was found to have been methylated and excreted as CA by 8 to 12 hours. When CA was administered initially, however, the proportions of inorganic arsenic, MAA, and CA in the urine remained relatively constant, with no suggestion of possible demethylation of the CA. Buchet et al. (1981a) also found that arsenite doses of up to 1,000 μg of arsenic per day for five days did not saturate the methylating capacity of their male subjects (Buchet et al., 1981b).

Although urinary excretion of methylated arsenicals is more commonly measured, there is evidence that the rat excretes a large portion of ingested CA (27% to 37%) via the feces by 24 hours (Stevens et al., 1977d). Somewhat less CA was excreted in the urine (20% to 29%), and the remaining portion was retained.

When dosing was by the intravenous or intratracheal routes, the urinary excretion route was primary, accounting for 43% to 80% of the dose administered versus 0.2% to 14% found in the feces. Evidence of biliary excretion of CA was also presented, which may account for much of the fecal CA measured after parenteral dosing. Whether this occurs in humans is as yet unknown.

Chapter 7

Animal Toxicology

In the absence of epidemiological or clinical data, toxicologic testing with laboratory animals is the only currently reliable method of evaluating in advance the possibility that a chemical will have adverse effects in man. Because of the likelihood of exposure of humans, domestic animals, and both terrestrial and aquatic wildlife to methylated arsenicals, a variety of tests have been carried out on these compounds.

A number of the tests were performed with rats, however, and some questions arise as to the applicability of such test results to man and other animals, since the rat is an atypical species in terms of the pharmacokinetics of arsenicals, including CA.

Toxicologic testing of methylated arsenicals has been done using the oral, inhalation, and dermal exposure routes in attempts to mimic the most commonly expected exposures of man and animals. Addition to their aqueous environment has also been used in the case of aquatic organisms.

Most of the test results available were obtained for use in Federal registration of methylated arsenicals as pesticides. Currently, pesticide use and registration are monitored by the U.S. Environmental Protection Agency (EPA) under the regulatory provisions of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). Much of the available information on CA was gathered by the Ansul Company of Marinette, Wisconsin, formerly a major producer of methylated arsenical pesticides.

Between 1963 and 1976, a number of the toxicologic studies with CA were performed for Ansul by Industrial Bio-Test Laboratories (IBT), Inc., Northbrook, Illinois, and by the Wisconsin Alumni Research Foundation Laboratories, Madison, Wisconsin. Additional studies, especially those involving inhalation toxicology, were done by EPA personnel.

In 1976 a number of the toxicologic tests performed by IBT were questioned by the Food and Drug Administration because of alleged irregularities in their conduct and in the reporting of their results (Marshall, 1983b). This led to a recent mail fraud conviction of members of IBT's management (Marshall, 1983c). Such circumstances suggest that the IBT data on CA (e.g., IBT 1965, 1973 a, b, c, d and 1976 a, b) may be unreliable and should thus not be considered definitive, particularly in view of the consistently negative pathologic findings in the IBT reports.

Tests involving methylated arsenicals include those for acute, subacute, and chronic toxicity, for teratogenicity, for mutagenicity, and for carcinogenicity. Data derived in each of these categories and by the various dosing routes employed will be covered in turn in the following pages.

Acute toxicity

Oral exposure. A number of studies have been reported in which CA was administered by gavage, as can be seen in Table 7.1. This method is often used in order to avoid inaccuracies of dosing inherent in administering chemicals in the diet or in drinking water.

Table 7.1. Results of acute toxicity testing with cacodylic acid, sodium cacodylate, and commercial products based on these arsenicals

Treatment		*Test Subject*		LD_{50}	*Reference*
Agent	**Route**	**Species**	**Sex**	**(mg/kg)**	
Cacodylic Acid	p.o.	mouse	M	>184	Lebedeff (1956)
		rat	M + F	830	Weed Society of America (1967)
			?	700	Fairchild et al. (1977)
	s.c.	dog	?	1,000	Windholz et al. (1976)
	i.p.	mouse	M	520	Stevens et al. (1979)
			F	600	
		rat	M	720	
			F	520	
	i.v.		F	470	
Sodium Cacodylate	p.o.	rat	?	1,250	Frost (1967)
			?	600	Ansul Company (1971)
			?	1,000	Arena (1974)
			?	2,600	Fairchild et al. (1977)
Ansar® 160 (29.3% CA)	p.o.	bovine[1]	?	1,230[2]	Ansul Company (1965)
Ansar® 560 (22.7% NaCA and 3.9% CA)		rat	M + F	1,700[2] 2,600[2]	IBT (1965)
Phytar® 560[3]	dermal	rabbit	M + F	>2,000[2]	
Phytar® 138 (65.6% CA)	p.o.	rat	?	1,312[4]	Stevens et al. (1977a)

[1]Holstein dairy calves.
[2]Total formulation.
[3]Same formulation as Ansar® 560 above.
[4]As mg/kg CA.

Even the gavage method is not free of problems, however, because handling the animals stresses them and there is the possibility of introducing the test agent into the trachea, although this can be avoided by a skillful operator and is usually obvious if it occurs. The rate of absorption and the toxicity seen often differ from those produced by incorporation in the diet, and even the volume of diluent can profoundly influence the end result (Gralla, 1981).

Early data compiled by Lebedeff (1956) suggested an LD_{50} greater than 184 mg/kg for CA in male mice, while the Weed Society of America (1967) and Fairchild et al. (1977) published values around 700-800 mg/kg for the oral LD_{50} of rats. IBT (1965) reported an oral LD_{50} value in the rat for Ansar® 560 (22.7% NaCA and 3.9% CA) of 2.6 g/kg, with 95% confidence limits of 2.1 to 3.2 g/kg. These results were for four male and four female rats at each of four dose levels (from 1.4 to 4.6 g/kg). Generalized inactivity, slight loss of appetite, and mild diarrhea were observed in the intoxicated rats, with mild ataxia at the high dose. As was typical of the IBT data, no "significant gross pathologic alterations" were observed at necropsy.

In another IBT study, orally administered CA-based herbicides were tested in 181-kg Holstein calves, resulting in an estimated LD_{50} of 1.23 g/kg for formulated Ansar® 160 and 1.7 g/kg for Ansar® 560 (Ansul Company, 1965). The calves were observed for seven days, but all those that died succumbed in 36 to 72 hours, with symptoms of being "listless," "drawn," and having copious salivation, nasal mucus, and diarrhea. Dying animals became progressively weaker before death. Symptoms persisted for only four to five days in animals that recovered. Necropsies were apparently not performed.

Stevens et al. (1977a) mentioned a value of 1.31 g/kg for the CA in Phytar® 138 when it was given orally to rats at the EPA's Health Effects Research Laboratory in Research Triangle Park, North Carolina, but no details were given.

Additionally, data on the acute oral toxicity of MSMA are available. For example, the above study of CA in dairy calves (Ansul Company, 1965) included replicates treated with MSMA-based herbicides (Values for MSMA-based products not shown in Table 7-1). The resulting LD_{50} for Ansar® 170 was 200 mg/kg and for Ansar® 529 was 250 mg/kg. Such values indicate that the oral toxicity of MSMA herbicides in cattle is much greater than that of those containing CA as the active ingredient, although the symptoms observed were essentially the same in both cases.

Another test employed rats fasted for 16 hours and gavaged with MSMA (as Ansar® 170) (IBT, 1964). The calculated acute LD_{50} was 1.8 g/kg, with a 95% C.I. of 1.5 to 2.2 g/kg. These values were equivalent to 0.93 and 0.77 to 1.13 g/kg, respectively, for MSMA alone. The test rats were observed for two weeks. Survivors were seen to have severe weight loss at doses of 1.4 g/kg and above, but again, no "significant gross pathologic alterations" were reported at necropsy of the animals dying due to the treatment. The symptoms of MSMA intoxication in rats were the same as those reported for CA (IBT, 1965), and these results also support the premise that MSMA is more toxic to mammals than is CA.

A further study with Silvisar® 550 in snowshoe hares yielded an LD_{50} of 173 mg/kg (95% CI = 97 to 306) as MSMA and indicated a greater sensitivity in this species (Evans, 1974).

Inhalation exposure. An aerosol of CA as Phytar® 560 was tested in two male and five female rats at a concentration of approximately 16.01 mg per liter of air for four hours (IBT, 1976a). No mortality or other signs of toxicity were seen during the subsequent 14-hour observation period, and necropsy were said to have revealed no gross pathologic changes. Similar negative results were seen with MSMA (Ansar® 529 H.C.) at 8.12 mg per liter of air and with DSMA (as Ansar® DSMA liquid) at 9.43 mg per liter of air. Only the inhalation data derived from commercial products containing CA are summarized in Table 7.2.

Table 7.2. Results of inhalation toxicity testing of commercial products containing cacodylic acid

Treatment Agent	Duration	*Test Subject* Species	Sex	LC_{50} (mg/m³)	*Reference*
Phytar® 560 (aerosol) (22.7% NaCA and 3.9% CA)	4	rat	M + F	>16,000[a]	IBT (1976a)
Phytar® 138 (aerosol) (65.6% CA)	6		M + F	>146[b]	Stevens et al. (1976)
	2		M	>6,900[b]	Stevens et al. (1979)
	2		F	3,900[b]	
	2	mouse	M	>6,400[b]	
	2		F	>6,400[b]	
	2		M + F	>165	Stevens et al. (1977a)
	6		M + F	>83	

[a]Given as mg of the total formulation.
[b]Given as mg of CA.

A series of reports from the EPA's Health Effects Research Laboratory outlined an extensive toxicologic study with technical-grade CA as Phytar® 138, with DSMA as Ansar® 170 or Ansar® 8100, and with purified CA in rats and mice (Stevens and Farmer, 1977; DiPasquale and Stevens, 1977; Stevens et al., 1977a and 1977b; Stevens et al., 1979). According to these reports, aerosols of Phytar® 138 and Ansar® 170 in water and of Phytar® 138 and Ansar® 8100 as dust were used in the inhalation studies.

When rats and mice were exposed for as long as six hours to the aqueous aerosols of Phytar® 138 or Ansar® 170 at mean concentrations up to 146 and 69 mg/m³, respectively, no mortality was seen by 14 days after treatment (Stevens et al., 1977a). Although mild eye and nasal irritation was observed, no overt respiratory distress was noted, nor was there gross lung or liver pathology.

Lungs and livers of rats treated with Phytar® 138 for 6 hours at the highest dose were subjected to histopathologic examination at 115 days following treatment. Perivascular lymphocytes, nodular foci of lymphocytes, histiocytes, and neutrophils, intraalveolar foamy histiocytes, congestion and edema, and focal hemorrhage were found. It was stated, however, that similar findings had been observed in untreated rats. The data presented do not clearly show if concurrent controls were used in the rat inhalation studies, but comparison of treated animals with historical controls only would be a less than ideal procedure.

Mice exposed to Phytar® 138 for six hours at 83 mg/m³ were killed at 275 days after exposure and the lungs, livers, kidneys, spleens, hearts, and pancreases examined histopathologically. No significant findings were reported, but one female had a pulmonary adenoma.

A single male mouse was found to have a poorly differentiated osteosarcoma at 162 days after exposure for six hours to 58 mg/m³ of MSMA in Ansar® 170 (Stevens et al., 1977a). The remaining mice given the same treatment were killed 300 days after exposure and given a gross pathologic examination. Two treated males and one female were seen to have small lung nodules, while one female had a fatty liver. One control male had peripheral liver nodules. Nodules and tumors are not uncommon in mice of such age, and in view of the small numbers of animals involved, such observations are not particularly meaningful.

When the test aerosols were used at exposure levels of up to 6.9 g of CA per liter for two hours, labored respiration, rhinorrhea, porphyrin encrustation of the eyes, and diarrhea were observed in the test rats (Stevens et al., 1977a). At the highest dose, rats had erythematous lesions of the feet and ears and "reddish tinged fur." A single male mouse died 20 days after treatment, while one male rat died at 25 days and nine female rats succumbed within two weeks.

The animals that died were not necropsied, but those surviving were necropsied at 158 days following exposure. The one surviving female rat had hydronephrosis and kidney stones, while five of eight males had kidney anomalies. The LC_{50} value was calculated to be 4.33 g/m^3 (95% CI = 3.79 to 5.37 g/m^3). Based on this value and assuming essentially 100% absorption, the authors computed an LD_{50} value of 346 mg/kg by inhalation.

Rats exposed to a lower dose of CA in Phytar® 138 (mean concentration 2.6 g/m^3) were necropsied at 110 days and exhibited a number of histopathologic changes, with all 20 animals having focal loss of staining in the kidney tubular epithelium. It was stated, however, that a similar condition had been observed previously in untreated rats. Mice exposed to DSMA (in Ansar® 8100) and killed at 110 days showed no gross effects, but histopathologic results were not available at the time of the report.

The levels of arsenical-containing dust used in the Stevens et al. (1977a) study were considerably higher than would be expected to be encountered in the work environment. Based on their data, the authors concluded that CA and DSMA should be considered slightly to moderately toxic by inhalation. They found it hard to evaluate the inhalation toxicity of MSMA properly, since it was difficult to generate a high density aerosol with this viscous, hygroscopic liquid.

In an effort to carry out a more detailed investigation of the effects of organoarsenical pesticide inhalation, DiPasquale and Stevens (1977) evaluated respiratory irritation by whole-body plethysmography. Their study was done according to methodology developed by Kane et al. (1979). Male mice were exposed for five minutes to dusts from purified CA at concentrations of 175 to 2,080 mg/m^3 and from Ansar® 8100 at DSMA concentrations of 204 to 2,667 mg/m^3.

Even at the highest concentration used, pure CA decreased the respiratory rate of test mice by only 36% and CA in Phytar® 138 at 1,760 mg/m3 decreased respiratory rate by only 23%. The calculated RD_{50} (concentration resulting in a 50% decrease in respiration rate) for the DSMA in Ansar® 8100 was 1,398 mg/m^3 while MSMA at 1,490 mg/m^3 (in Ansar® 170) was associated with only a 1.7% change in respiration.

Such data suggest that brief exposures to extremely high levels of methylated arsenical pesticides have a relatively low potential for lung irritation of the type that results in changes in breathing rates, although they were somewhat more irritating than diatomaceous earth used as a control. Further, the data were derived from only one animal model.

Stevens et al. (1977b) proceeded to evaluate the effects of methylated arsenical pesticides on pulmonary and hepatic microsomal metabolism in rats and mice. Their results were said to indicate only transient effects rather then significant toxicity.

Stevens and Farmer (1977) further investigated inhalation effects when they measured the levels of glutathione and vitamin E in mice exposed for two hours to CA (purified or in Phytar® 138) or to MSMA (in Ansar® 170) aerosols in water at mean concentrations of 165 or 71 mg/m^3, respectively.

Both arsenical exposures were followed by reductions in lung and liver glutathione in males at three hours after treatment. By 24 hours, glutathione was actually increased in lungs and livers of CA-treated animals of both sexes, but not in those given MSMA. Similar, but not identical, results were seen in rats, and MSMA appeared to reduce lung glutathione levels in males at 24 hours.

When CA (334 mg/m^3 in Phytar® 138) was given to rats as a dust, liver glutathione levels increased by the end of the two-hour exposure in both sexes and 48 hours later in males. Glutathione levels in lung decreased by the end of exposure and had returned to normal by 48 hours.

When 10 additional rats of each sex were exposed to 6,935 mg/m^3 of CA (in Phytar® 138) for two hours and their orbital sinus blood was sampled serially for 48 hours, changes in glutathione levels occurred. The most notable change was seen in females, with glutathione values declining to 70% of control levels by 48 hours, a finding not observed in male rats. Additionally, nine of the females and only one of the males subsequently died, apparently as a result of the treatment.

Stevens and Farmer (1977) later looked at the effect of CA inhalation (4,647 mg/m^3 for one hour), injection of diethylmaleate (a glutathione-depleting agent), or a combination of the two in groups of four female rats. Both CA and diethylmaleate reduced glutathione levels in the rats, as did the combined treatment. One rat given CA and two rats given the combination died before the 48-hour sampling. The combined treatment appeared to have an additive effect on both glutathione levels and toxicity, but the numbers of animals were too low to assess such a likelihood properly.

In the above report, results of assays of plasma, lung, and liver for vitamin E following exposure of rats to CA at 3,341 mg/m^3 (in Phytar® 138) for two hours were presented. The results indicated changes in levels of the various forms of vitamin E, with treatment-related decreases of hepatic tocopherol acetate and increases in plasma alpha-tocopherol in both sexes at 48 hours after exposure.

Tocopherol quinone was reduced by 74% in the lungs of females immediately following treatment, but by 48 hours the depletion was only 50%. The authors suggested that lung depletion of tocopherol quinone (the active form) resulted in mobilization of hepatic tocopherol acetate stores, and that alpha-tocopherol was then released into the blood stream to resupply the depleted lung tissues.

Other exposure routes. A portion of the data described above was summarized by Stevens et al. in 1979. They also presented additional data on LD_{50} values which were included in Table 7.1. According to these data, the median lethal doses of CA given intraperitoneally were similar in rats and mice, while female rats and male mice were more sensitive than the opposite sexes of their species.

These data are somewhat at odds with those of Rogers et al. (1981), who reported rats to be considerably more sensitive than mice to chronic oral administration of CA, but this discrepancy could be due to the known tendency of rats to accumulate arsenicals.

Toxicity symptoms seen after intraperitoneal or intravenous administration of CA by Stevens et al. (1979) were said to be similar to those observed following exposure by inhalation. Death tended to occur within four days after exposure. The animals developed rough fur, breathed with difficulty, became unable to right themselves, assumed the fetal position, lost body temperature, and became rigid. They exhibited a small, red thymus, bright red lungs, darkening of the liver, spleen, and adrenals, impaction of the caecum, irritation of the stomach lining, and blood and mucus-like material in the intestine. Such findings are at variance with the negative pathologic reports for rats treated orally with CA (IBT, 1965).

The dermal route has also been used to test for CA toxicity. Young albino rabbits were treated over approximately 30% of their skin surface with undiluted Phytar® 560 liquid at a dose level of 2,000 mg/kg (IBT, 1976b). Two rabbits of each sex were treated (one each with abraded and intact skin) and none died within two weeks following a 24-hour exposure. Weights of the animals were unaffected, and no signs of toxicity were reported other than those associated with mild skin irritation.

The above results were based on an inadequate number of test animals for reliability, but although no details were given, Phytar® 560 was assigned by the authors "in accordance with FIFRA" to category III (3.6/8.0) for primary skin irritation and category III (8.7/110.0) for eye irritation in the rabbit.

In another dermal study, undiluted Ansar® 529 (MSMA) was applied to two rabbits at each of three doses (2, 4, or 6 ml/kg) for 24 hours and the animals observed for two weeks (Nees, 1969). All rabbits given the two highest doses died within two to four days after exposure. The two low-dose animals both survived the test. No histopathologic data were given on any of the test rabbits.

In an early study with rabbits, Simon (1932) treated the animals intravenously with NaCA at various injection rates and concluded that rapid injection with 1,200 mg/kg would cause immediate death. Similar treatment with DSMA yielded a value of 1,240 mg/kg (Simon, 1933). The minimum NaCA dose resulting in death within several hours was 400 mg/kg for intravenous and 500 mg/kg for subcutaneous injection (Simon, 1932), and values for like treatments with DSMA were 660 and 730 mg/kg, respectively (Simon, 1933). A number of gross pathologic findings were noted upon autopsy and described in detail in both of Simon's studies.

In an additional report (Edgewood Arsenal, undated), results of treatment of male mice with DSMA were as follows: no effect at 388 mg/kg, given orally; subcutaneous minimum lethal dose (MLD) = 3,350 mg/kg; intravenous MLD > 316 mg/kg. DSMA in rabbits gave an "immediate" intravenous MLD of 1,240 mg/kg and a "late" MLD of 660 mg/kg. Results with MAA in male mice indicated an oral LD_{50} of 185 mg/kg. A dose of 100 mg/kg given orally to rats had no lethal effect.

Windholz et al. (1976) list an LD_{50} value of 1.0 g/kg for CA given subcutaneously to dogs, but give no reference for the original data.

Effects in lower organisms. In 1966, Butler reported no effect on pink shrimp or longnose killifish exposed to aqueous CA concentrations of 26 ppm for 48 hours. Eastern oysters were said to be unaffected at 1 ppm.

In a study of pesticide-induced lethality in small bluegill sunfish, Hughes (1969) found LC_{50} values of 90 ppm for the surfactant-containing CA formulation Phytar® 560 at 24 to 48 hours: at 72 and 96 hours the LC_{50} was 80 ppm. A nonsurfactant formulation containing 6% more CA (Phytar® 160) yielded test LC_{50} values of 2,500, 1,500, 750, and 750 ppm at 24, 48, 72, and 96 hours, respectively. Thus the addition of surfactant made the herbicide much more toxic.

It could not be determined from the foregoing data if the enhanced effect was due primarily to the surfactant or to an interactive effect of arsenical plus surfactant. According to a later report, however, the enhanced toxicity was equivalent to that seen with the surfactant alone (Ansul Company, 1971). Test results with Ansar® 529 gave LC_{50} values of 400 ppm at 24 hours and 300 ppm at the later times.

Results of tests involving bluegill sunfish were also reported by Johnson and Finley (1980), who found a 96-hour LC_{50} of 17 mg/L (95% CI = 15 to 19) for Phytar® 560. Also, Abdelghani et al. (1980) reported the following values for MSMA in sailfin mollies: 48 hours, LC_{50} = 1,600 ppm; 96 hours, LC_{50} = 1,300 ppm (as arsenic).

Judd (1977) reported lethal effects for MSMA as Ansar® 529 H.C. when spadefoot toads were exposed to 1,000 to 100,000 ppm aqueous solutions of the herbicide. Juvenile toads were more susceptible than adults, with all toads dying at the highest MSMA concentration within two hours.

Crustaceans have also been exposed to arsenicals in a variety of toxicity tests. Sanders (1970) reported that neither Phytar® 560 nor Ansar® 529 at 100 ppm had a lethal effect on scud within 96 hours, ranking the arsenicals among the least toxic to this organism of an extensive series of herbicides tested.

An LC_{50} value of 28 ppm (95% CI = 14 to 58) was reported for Phytar® 560 in glass shrimp by Johnson and Finley (1980), while a 48-hour LC_{50} value of 7.13 (49.4 to 103.0) ppm, with a no-observed-effect level of 56 ppm, was reported for pure CA with brine shrimp (Bionomics, 1974).

Subacute and chronic toxicity

Effects on laboratory and domestic animals. Information on the effects of longer term exposure of domestic mammals or poultry is relatively sparse in comparison with acute toxicity data.

The Ansul Company (1971) stated that data available from 90-day feeding trials showed no effect in rats or dogs from diets containing up to 30 ppm of either CA or MAA. Dairy calves given cottonseed meal containing 4,700 ppm of CA or 4,000 ppm of MAA were said to have become anorexic in three to six days. After seven days, the calves were placed back on a normal diet and resumed feeding. In another report that appeared to refer to the above data on dogs,

however, weight gain in the high-dose females was said to have been inhibited (Carcinogen Assessment Group, undated).

In a more detailed report, Palmer (1972) presented the results of 10-day oral toxicity tests with CA, MSMA, and DSMA in cattle, sheep, and chickens. These rather complex data are presented here in tabular form taken from the original publication (Tables 7.3-5).

Table 7.3. Results of multiple oral dosing of cattle, sheep, and chickens with hydroxydimethylarsine oxide acid[1]

Animal and dosage received (mg/kg)	Doses (Number)	Means of dosing	Results and remarks[2]
Cattle:			
5	10	Capsule	NIE
10	10	"	"
10	10	Drench	Irritation effect after 2, 5-percent weight loss[3]
25	10	"	NIE
25	10	Capsule	"
25	10	"	"
25	10	Drench	Poisoned after 8 and survived, 5-percent weight loss
50	7	"	Poisoned after 1 and died 4 days after last dose
Sheep:			
10	10	"	NIE
25	10	"	"
25	10	Capsule	"
25	10	"	"
25	10	"	"
25	10	"	14-percent weight loss
50	10	"	Poisoned after 3 and survived, 21-percent weight loss
50	10	"	Poisoned after 2 and survived, 22-percent weight loss
Chickens:[4]			
50	10	Pipette	55-percent weight gain
100	10	"	63-percent weight gain
100	10	"	42-percent weight gain
175	10	"	33-percent weight gain
250	10	"	36-percent weight gain
500	10	"	13-percent weight gain
Controls			53-percent weight gain

[1]Phytar® 560, 26.5 percent emulsifiable concentrate of hydroxydimethylarsine oxide (cacodylic) acid with 12.7 percent total arsenic. The Ansul Co., Marinette, Wis.
[2]NIE indicates no ill effects apparent.
[3]Affected by chemical reaction of the formulation on the pharyngeal mucosa, resulting in mandibular area enlargement accompanied by partial anorexia and dyspnea.
[4]Average results of 5 treated chickens.
Source: Palmer (1972)

Table 7.4. Results of multiple oral dosing by capsule of cattle, sheep and chickens with monosodium methanearsonate[1]

Animal and dosage received (mg/kg)	Doses (Number)	Results and remarks[2]
Cattle:		
5	10	NIE
5	10	"
10	5	Poisoned after 2 and died
25	7	"
50	4	"
Sheep:		
25	10	NIE
25	10	"
50	10	"
50	6	Poisoned after 4 and died
50	7	Poisoned after 3 and survived, 18-percent weight loss
100	2	Poisoned and died
Chickens:[3]		
25	10	62-percent weight gain
50	10	60-percent weight gain
100	10	57-percent weight gain
250	10	53-percent weight gain
Controls		64-percent weight gain

[1]Ansar® 170, 51.3 percent emulsifiable concentrate of MSMA, The Ansul Co., Marinette, Wis.
[2]NIE indicates no ill effects apparent.
[3]Average results of 5 treated chickens.
Source: Palmer (1972)

Table 7.5. Results of multiple oral dosing by capsule of cattle, sheep and chickens with disodium methanearsonate[1]

Animal and dosage received (mg/kg)	Doses (Number)	Results and remarks[2]
Cattle:		
10	10	NIE
10	10	"
25	6	Poisoned after 2 and died 3 days after last dose
Sheep:		
10	10	NIE
10	10	"
25	6	Poisoned after 5 and died
25	5	Poisoned and survived
Chickens:[3]		
25	10	67-percent weight gain
50	10	62-percent weight gain
100	10	55-percent weight gain
250	10	64-percent weight gain
375	10	47-percent weight gain
500	10	40-percent weight gain
Controls		59-percent weight gain

[1]Ansar® 184, 63 percent wettable powder of DSMA, The Ansul Co., Marinette, Wis.
[2]NIE indicates no ill effects apparent.
[3]Average results of 5 treated chickens.
Source: Palmer (1972)

According to Table 7.3, 10 daily CA doses of 25 mg/kg (in Phytar® 560) may have been a marginally toxic level for "mixed breed" yearling cattle, while 50 mg/kg for seven days was lethal to the individual treated at that dose. In the case of sheep (ewes and wethers), a 50 mg/kg/d dose was clearly toxic, but 25 mg/kg/d was again a borderline dose. When two groups of five 6-week old chickens were given 100 mg/kg/d doses for 10 days, reduced weight gain was seen in one group but not in the other. Doses of 175 to 500 mg/kg/d were clearly toxic but not lethal.

Poisoned animals generally had diarrhea and were anorexic. The yearling that was poisoned exhibited hemorrhaging of the abomasum and intestines, distended blood vessels, enlargement of the kidneys, spleen, and liver, and inflamed bladder mucosa.

In the case of MSMA (given in Ansar® 170, Table 7.4), yearling cattle were poisoned at doses of from 10 to 50 mg/kg/d, and died after four to seven doses. Two sheep became intoxicated at 50 mg/kg/d, and one died after six doses, but another survived all 10 doses with no obvious ill effects. The one sheep given 100 mg/kg/d died after two doses. Weight gain in chickens was slightly reduced at 100 to 250 mg/kg/d.

Cattle affected by MSMA became anorexic and exhibited hematuria, diarrhea, and listlessness (Palmer, 1972). When necropsied, hemorrhage of the lining of the rumen, abomasum, and intestines was apparent, and the blood vessels were distended. The liver and kidneys tended to be congested, the spleen was enlarged, and excessive fluid was seen in the abdominal cavity, but in two individuals the liver appeared abnormal.

When DSMA was administered (in Ansar® 184, Table 7.5), the one yearling given 25 mg/kg/d died three days after receiving six doses. One of two sheep given 25 mg/kg/d died after six doses. The other was given only five doses and survived. Chickens were intoxicated at 375 to 500 mg/kg/d.

The intoxicated yearling and sheep had symptoms similar to those given MSMA had no hematuria. When necropsied, they were found to have mucosal hemorrhage of the abomasum and intestines. The mesenteric lymph nodes were edematous and the kidneys swollen, with petechiae of the cortical surface. The sheep also had abdominal fluid accumulation and a distended pericardium, and the yearling had pathologic changes of the adrenals.

The foregoing results of Palmer (1972) suggest that chronic oral exposure to methylated arsenicals in herbicide formulations can be hazardous at moderate dose levels in ruminants. Both MSMA and DSMA appear to be somewhat more toxic on a milligram per kilogram basis than CA, a finding similar to results from acute toxicity tests. It was also apparent that chickens were considerably more resistant to all three arsenicals than were the cattle or sheep. Symptoms of arsenical intoxication tended to be similar in both cattle and sheep and among the arsenicals as well.

Dickinson (1975) also treated cattle with CA in a commercial herbicide formulation (Silvisar® 510). A cacodylate dose of 10 mg/kg/d was given to one heifer and four steers for three weeks, followed by three additional weeks at 20 mg/kg/d.

All animals survived the first dosage period, with persistent diarrhea the only symptom of toxicity. After five weeks the heifer exhibited anorexia and weight loss, its treatment was terminated, and the animal eventually recovered. Two of the steers died by the sixth test week and were necropsied. The remaining two were killed since they were in a moribund condition (asthenic and completely anorexic) and were also necropsied.

Pathologic changes included enteritis and renal and hepatic degeneration, with renal tubular degeneration suggested as having been the cause of actual or impending death. Tissue arsenic levels (by neutron activation) were said not to be elevated to levels expected in cases of arsenic poisoning, including those seen in the author's previous study with MSMA (Dickinson, 1972).

Results in cattle similar to those seen with CA were reported for MSMA in herbicide formulations (Glowon® or Ansar® 170 H.C.) by Dickinson (1972). When steers or heifers were given MSMA levels of 10 mg/kg/d, four or five treated individuals died or were killed when they became moribund after 10 or fewer doses. The one survivor apparently recovered from the intoxication after several days.

Symptoms observed included anorexia, diarrhea, weight loss, lethargy, and asthenia. On necropsy, the animals often exhibited hemorrhaging of the gastrointestinal tract and kidneys. The

tissues from two steers were examined microscopically, and one was seen to have had focal liver necrosis. Both had marked renal tubular degeneration, and nephrosis was suggested as the probable cause of death or morbidity.

More recently, when Siewicki (1981) fed rats for 42 days with purified diets containing either 21 or 42 ppm of CA plus added fish meal, no effects were seen on feed intake or weight gain, spleen or liver weight, hematocrit, hemoglobin, or urinary aminolevulinic acid or coproporphyrin. This was true even though CA-fed rats retained considerable amounts of the arsenical in their livers (54 ppm, as arsenic), spleens (292 ppm), and erythrocytes (1,430 ppm).

Effects on wildlife. As early as 1970, a report was prepared dealing with effects of CA on wildlife (Lehn et al., 1970). Six sampling stations on three freshwater streams on Eglin Air Force Base in Florida were monitored for changes in fish species diversity. A 2.6-km^2 test area through which the streams ran was sprayed between July 1 and August 21, 1969, with Agent Blue (4.7% CA plus 26.4% NaCA) totaling 10,309 kg in 7,892 liters of solution. Spraying took place on seven different dates, and sampling was done from the preceeding April until the following December.

One sampling station had a decrease in one fish species (*Notropis hypselopterus*). A variety of benthic organisms were also sampled, and none showed a change in relative population density, lending support to the author's conclusion that the single instance of variability in fish diversity was probably not due to herbicide toxicity.

In a study conducted the following year, a survey of animal life on and around the spray test area at Eglin was conducted (Pate et al., 1972). Mammals, birds, reptiles, amphibians, and fishes were observed in the field or were collected. The authors concluded that the study was too brief and qualitative to be definitive regarding the possible impact of herbicides on the test area. That area not only received applications of Agents Blue and White during the sampling period but also had been previously sprayed with Agent Orange.

Nevertheless, Pate et al. (1972) reported 10 vertebrate species seen only on the sprayed area and 35 species living both on and off the test area. They concluded that most of the differences between the species found on versus off the test area were probably due to known habitat preferences. They also stated that species diversity in the test area was relatively great, even though the area had often been sprayed with high levels of several herbicides. It would appear that the damage to vegetation in the sprayed area would also have an effect on selection of the habitat area by animals, but this was not mentioned.

More recently, Spehar et al. (1980) assayed the toxicity of NaCA, DSMA, As(III) (as arsenic trioxide), and As(V) (as arsenic pentoxide) in a 28-day laboratory flow-through test. Immature rainbow trout and a variety of invertebrates, including snails, a stonefly, and an amphipod were subjected to nominal concentrations of 100 or 1,000 ppm (as arsenic) of each arsenical.

As expected, As(III) was the most toxic arsenical tested; it significantly reduced amphipod survival after one week at the higher concentration. Neither arsenite nor any of the other arsenicals significantly affected survival of the other test species at any concentration. Data on the amphipods were not analyzed after 14 days due to unexpected deaths in the control groups. Survival and production of offspring by *Daphnia* were also not affected by a two-week exposure to any arsenical.

In other laboratory tests, 10- to 15-day old mallard ducklings and bobwhite quail were fed diets containing 5,000 ppm of technical-grade (34.8%) NaCA (IBT, 1973d and 1973c). A group of 10 individuals of each species was given the test diet for five days, observed for three additional days on control diets, killed, and subjected to gross pathologic examination.

No unusual behaviors or other signs of intoxication were reported in either species, and necropsy findings were said to have been negative. Both body weights and food consumption were also reported as within normal limits. A test on mallard ducklings with technical-grade MSMA (51.3%) at 5,000 ppm was reported as giving identical results to the trial with CA (IBT, 1973b).

When bobwhite quail were subjected to MSMA in their diet, the results differed (IBT, 1973a). Several MSMA doses from 312.5 to 5,000 ppm were tested on the quail. Two of 10 quail died at 1,250, none at 2,500, and 7 of 10 at 5,000 ppm, yielding an estimated dietary LC_{50} of 3,300 ppm (95% CI = 1,941 to 5,610 ppm). Decreased food consumption and weight gain were seen in the high-dose groups, but it was claimed that no outward signs of intoxication were noticed and no gross pathologic changes were reported at necropsy.

Again, the data support the assumption that methanearsonates are generally more toxic than cacodylate, although there are significant species differences in sensitivity as well. As mentioned previously, the consistently negative pathologic findings in the IBT data differ from the findings of others upon necropsy of arsenical-treated test animals (e.g., Palmer, 1972; Dickinson, 1972 and 1975; Stevens et al., 1979).

In a relatively rigorous field test with MSMA (as Ansar® 529), Edwards and Davis (1975) subjected an area of salt marsh to a single spraying of the vegetation with 90,000 ppm, 30 applications of up to 10,000 ppm, or 30 floodings with 100 ppm given twice a day for five consecutive days in each of three consecutive months. Although the populations of periwinkles declined by 68% after the high-dose multiple sprayings, no other treatment had any effect on their numbers. It was presumed that they were ingesting the arsenical while feeding on the sprayed vegetation. When rib mussels were placed in the flooded areas, 6 of 16 individuals died in treated areas versus none in control areas.

Teratogenicity and reproductive toxicity

Few studies have been published that deal with the developmental effects of exposure to CA or to methanearsonate. The first such report appears to have been a brief study by Ancel and Lallemand (1941), who exposed chick embryos to a variety of agents including NaCA. Exposure of 25-somite embryos to a dose of 2 mg per egg resulted in a low level of abnormalities involving the tail bud. Ancel, (1945) later reported similar results for both NaCA and DSMA. When earlier embryos were exposed to 1 mg of DSMA, spina bifida could be produced (Ancel, 1946-47).

In 1949, Landauer injected chicken eggs with 2.5 mg of NaCA before incubation and observed increased mortality during incubation, with a significant incidence of acaudal or brachycaudal embryos. Additional eggs were given NaCA plus nicotinamide, which had no effect on survival but did decrease the incidence of malformed embryos. When pyruvic acid was administered instead of nicotinamide, a protective effect was observed with regard to both malformations and survival.

In the only study employing an amphibian, Rostand (1950) treated larvae of the russet frog just as the rear limb buds were forming. No limb or other gross malformations were observed, but an unspecified portion of the individuals treated for three weeks with 100 ppm of NaCA were found to be unable to flex their femorotibial joints once limbs were developed.

The initial study of the effects of CA in pregnant mammals was that of Chernoff and Rogers (1975), who treated both mice and rats with CA contaminated with methanearsonic acid (1.7%), inorganic arsenic (0.9%), unidentified organic compounds (2%), and NaCl (5%). Treatments were given daily by gastric intubation during midgestation (days 7 to 16) at dose levels of 200 to 600 and 40 to 100 mg/kg/d for mice and rats, respectively.

CA treatment of mice caused dose-related maternal toxicity, with some mortality at the high dose. Further dose-related effects were seen in the fetuses, including reductions of both weight and skeletal ossification. Cleft palates were also noted, but only at the higher doses, where maternal toxicity was also observed.

In rats, maternal deaths were associated with CA doses of 75 to 100 mg/kg/d, and dose-related fetotoxic effects similar to those seen in mice were reported. No cleft palates were seen in rat fetuses, but they did exhibit an increase in incidence of aberrant palatine rugae (mismatched in apposition at the midline ridge). Micrognathia and apparently hypoplastic lungs were also reported in the offspring of treated rats, but neither effect was seen in a later study employing pure CA (Rogers et al., 1981). Although these initial results may have been due to the methylated arsenic, the possibility of effects from the inorganic arsenic present must also be considered, particularly in the high-dose animals, so a more definitive study was performed.

That pure CA could have adverse effects on developing offspring was confirmed by Rogers et al. (1981), who treated pregnant mice or rats on days 7 to 16 of gestation. Mice were given doses of 200 to 600 mg/kg/d by gavage, while rats received 7.5 to 60 mg/kg/d. Maternal toxicity, as evidenced by decreased weight gain, was seen even at the low dose in mice, and increased maternal mortality occurred at the high dose. In rats, maternal weight gain was reduced at dose levels of 40 mg/kg/d or higher, while maternal survival was greatly decreased at the high dose.

Fetal survival in mice decreased significantly only at the highest dose. Both the 400 and 600 mg/kg/d treatments were associated with a significant incidence of cleft palate, as well as retardation of both fetal growth and skeletal ossification. A reduction in the occurrence of supernumerary ribs was also observed.

In the rat, treatment at the two highest dose levels elevated the incidence of prenatal deaths, while doses of 40 mg/kg or higher resulted in fetal stunting, retardation of skeletal ossification, and a lower incidence of supernumerary ribs. In addition, at all doses higher than 15 mg/kg, significant numbers of fetuses were seen that had palatine rugae mismatched in apposition at the midline of the palate.

The results of Rogers et al. (1981) tended to confirm those of their earlier study (Chernoff and Rogers, 1975), with the exception that the occurrence of lung hypoplasia and micrognathia in the rat was not duplicated. Developmental defects were seen in the rat at doses that did not appear to cause maternal toxicity, while this was not the case with mice.

Results similar to those of Chernoff and Rogers (1975) were reported the following year (WARF Institute, 1976). An initial range-finding trial indicated that fetal and maternal deaths occurred when pregnant rats were dosed with CA by gavage at 50 to 60 mg/kg/d from gestation days 6 through 13. Four dose levels (0, 25, 35, and 45 mg/kg/d) were then administered to groups of 20 pregnant females each. Although CA treatment decreased maternal weight gain, fetal weights on gestation day 20 did not differ between treatment groups.

The only significant findings in the offspring were dose-related increases in irregular palatine rugae and retarded skeletal growth. Additional groups of 10 females each were given the same treatments as those in the teratology study but were allowed to deliver their litters and care for them for one week. No significant differences were observed in the number of offspring produced, their growth, survival, or gross morphology.

In a study involving pregnant hamsters, Wilhite (1981) administered CA and methylarsonic acid at dose levels of 20, 50, and 100 mg/kg. The arsenicals were given intravenously on gestation day 8. No dose-related increase in prenatal deaths was seen, although there were a few gross and skeletal malformations, especially in the CA-treated litters at the two higher doses. No fetal weight data were reported and the small numbers of litters observed (five per test group) make interpretation of the data difficult.

Single high doses of CA were also employed by Kavlock and Chernoff (1980). In a brief report, they stated that doses estimated to be the LD_{10} and LD_{40} were given to pregnant mice (exact dose and administration route were not specified) on gestation day 8. Such treatments resulted in findings of toxicity to the conceptus, including prenatal mortality and supernumerary lumbar ribs. Several types of malformations (encephalocele, exencephaly, cleft face, and fused ribs) were also observed.

The remaining reports concerned with methylated arsenicals in pregnant mammals consist of a series of studies from the same laboratory.

For example, Hood et al. (1983a) treated mice on one of gestation days 8 to 15 with either 800 or 1,200 mg/kg of NaCA or with 1,200 or 1,500 mg/kg of DSMA. When given on days 8, 9, or 10, the high doses of both arsenicals, which were close to the maternal minimum lethal doses, were associated with increased incidences of malformed fetal skeletons. This is the period when the developing mouse skeleton is most susceptible to adverse influences. A few gross malformations were associated with both arsenicals in fetuses from day 8- or 9-treated litters, but no visceral defects were observed.

In cacodylate-exposed litters, treatment late in gestation decreased survival of the fetuses, but fetal weight was generally unaffected. In the case of DSMA-treated litters, prenatal survival decreased following treatment on most gestation days, and fetal weights tended to be slightly reduced.

In a similar study involving hamsters (Hood et al., 1983b), pregnant females were given 500 mg/kg of DSMA or 900 to 1,000 mg/kg of NaCA by intraperitoneal injection on one of gestation days 8 to 12. Acute exposure to the high dose of NaCA was found to cause 75% to 100% prenatal mortality, depending on the day treatment was given. At 900 mg/kg, cacodylate

treatment resulted in increased fetal wastage and generally was associated with stunting as well. Gross and skeletal defects and some edematous fetuses were also seen.

The DSMA treatment given was less harmful to the fetus, although in some cases it was also associated with a degree of fetotoxicity. Only a few malformations were seen, and their incidence was not significantly different from that of controls. The frequency of maternal deaths was similar for both treatments and all dose levels, however, indicating that the results were obtained under conditions toxic to the mother.

An additional article dealing with the effects of exposure of developing mammals to methylated arsenicals dealt with CA toxicity to preimplantation embryos. In an attempt to develop a short-term test procedure for screening potentially embryotoxic or teratogenic compounds, Williams et al. (1979) obtained early cleavage stage embryos from mice and rabbits and maintained them in embryo culture for 24 hours. The embryos were treated with CA at levels in the culture medium ranging from 100 ppb to 10,000 ppm and cultured for three additional hours in the presence of tritiated leucine. They were then examined for signs of abnormal development or death and their incorporation of ^{3}H-leucine into new protein was determined.

According to the results of Williams et al. (1979), CA concentrations of 1,000 ppm or higher altered cleavage rates. Most mouse embryos treated with 10,000 ppm failed to cleave and there was an apparent adverse effect on protein synthesis at doses greater than 100 ppm. Such findings do not appear to indicate a significant potential for toxicity, though, as the same authors found 10,000 ppm of sodium chloride to have an even greater delaying effect on cleavage. In the case of rabbit embryos treated with cacodylate or NaCl, the cleavage results were quite similar to those obtained for mouse embryos, while the leucine incorporation data were said to be "impossible to interpret."

The foregoing data on prenatal effects of cacodylate treatment suggest that maternally toxic doses are generally required before significant adverse effects on the conceptus are seen. The exception to such a generality is found in the data on chronically treated rats (e.g., Rogers et al., 1981) but, as has been pointed out elsewhere herein, the rat handles arsenic atypically. Preliminary evidence (Tatum and Hood, 1983, and unpublished data) indicates that at comparable maternal doses of CA (50 mg/kg, given intraperitoneally) and comparable developmental stages (day 12 for mice, day 14 for rats), both rat and mouse fetuses contained similar CA levels at three hours after treatment (1.14 vs 1.02 μg/g), as well as at 12 hours, (0.39 vs 0.32 μg/g, respectively). Much higher levels of CA were retained in the maternal blood of the rat at 3 and 12 hours (38 and 58 μg/ml) than in that of the mouse (0.88 and 0.03 μg/ml). Such results suggest that the greater sensitivity of the rat conceptus to CA is due to an inherent susceptibility or that it is related to longer maternal CA retention.

Information on reproductive success in higher animals or in human beings exposed to CA appears to be lacking in the open literature. One report, however, covered the results of feeding NaCA to male rats for three weeks (Nees, 1960). Dose levels were said to be 0%, 20%, and 40% of an estimated LD_{50} (approximately 900 mg/kg), although Rogers et al. (1981) later stated that only 60 mg/kg/d of oral CA (by gavage) in pregnant rats resulted in 67% maternal mortality after just 10 days of treatment. Thus, the dosing information given for the Nees study may have been incorrect unless feeding the sodium salt of CA in the diet of male rats is far less toxic than gavage of pregnant females with the free acid.

At any rate, treatment decreased body weight and food consumption, indicating dose-related toxicity other than that manifested by death. Histopathologic examination of the testes of five males from each dose group revealed indications of beginning atrophy of seminiferous tubules, as well as a reduction in spermatogenesis in all males exposed to the high dose. The significance of these data is questionable, however, as the dose was quite high and produced overt toxicity in the animals. As mentioned elsewhere, results of studies of arsenical effects in the rat may not be useful for extrapolation to other species.

MSMA was also involved in two reports on reproductive effects. In the work of Exon et al. (1974), rabbits were fed 50 ppm of MSMA in their diet for 12 weeks before as well as throughout pregnancy. The rabbits were allowed to give birth, and offspring were analyzed for arsenic content at 1 or 20 days of age. No arsenic was found in the liver, kidney, or stomach contents of any of the offspring of treated females. Detection limits for arsenic were said to be 0.15 ppm. No mention was made of any treatment-related reproductive difficulties.

Lopez and Judd (1979), however, reported alteration in nest-building behavior of white-footed mice exposed to 477 ppm of MSMA (in Ansar® HC) in their drinking water for two weeks. Such a behavioral effect could influence reproductive success in this species.

Mutagenicity

Mutagens have been defined as agents that can cause alterations (mutations) in the DNA of either somatic or germ cells. A number of reports have appeared regarding the mutagenic potential of methylated arsenicals (Table 7.6).

Among the reports involving methylated arsenicals was part of a microbial mutagenicity screening study of over 100 herbicides (Anderson et al., 1972). Both CA and MSMA were found to give negative results when evaluated with histidine-requiring mutants of *Salmonella typhimurium*. A similar test with *S. typhimurium* plus the addition of a liver microsomal fraction was also negative for both CA and MSMA, as well as for arsenic trioxide (Moore, 1976).

Simmon et al. (1977) also reported no effect with *S.* typhimurium and with the *trp*-deficient strain *Escherichia coli* WP2, both with and without a microsomal fraction. Similar results were found by Felkner (1980) with various mutant strains of *Bacillus subtilis*, according to the EPA's Office of Pesticide Programs (1981).

Although results of mutagenicity tests of CA in bacterial systems have proven uniformly negative, a variety of tests involving eukaryotes have yielded some positive findings. For example, tests involving yeast (*Saccharomyces cerevisiae*) strains heterozygous for various mutant genes can provide data on mitotic crossing over, gene conversion, and reverse mutation.

Using such systems, Mortelmans et al. (1980) found CA to be strongly mutagenic in all three *Saccharomyces* systems, both with and without an added microsomal fraction. Similar positive results with mitotic crossing over were also seen by Simmon et al. (1977).

Table 7.6. Experimental evidence regarding mutagenicity of cacodylic acid (CA).[1]

Test and System		Condition and Dosage of CA		Results	Reference
GENE MUTATIONS IN VITRO					
I. Point Mutations					
S. typhimurium	*his*$^{+}$[2]	−MA[3]	1–1000 μg/plate	−	Simmon *et al.*, 1977
S. typhimurium	*his*$^{+}$	+MA	1–1000 μg/plate	−	Simmon *et al.*, 1977
E. coli WP2	*trp*$^{+}$	−MA	1–1000 μg/plate	−	Simmon *et al.*, 1977
E. coli WP2	*trp*$^{+}$	+MA	1–1000 μg/plate	−	Simmon *et al.*, 1977
S. typhimurium	*his*$^{+}$	−MA	1–5 μl/plate	−	Anderson *et al.*, 1972
Mouse lymphoma	L5178Y tk^{+}/$^{-}$	+MA	1–1000 μg/ml	+	Jotz & Mitchell, 1980
Mouse lymphoma	L5178Y tk^{+}/$^{-}$	−MA	1–1000 μg/ml	+	Jotz & Mitchell, 1980
S. typhimurium		+MA	0.1, 1 and 10 μg/plate	−	Moore, 1976
B. subtilis	Rec Assay	+MA	50–300 mg/plate	−	Felkner, 1980
B. subtilis	Rec Assay	−MA	50–300 mg/plate	−	Felkner, 1980
B. subtilis	*met*$^{+}$ *his*$^{+}$	+MA	50–300 mg/plate	−	Felkner, 1980
B. subtilis	met^{+} his^{+}	−MA	50–300 mg/plate	−	Felkner, 1980
II. Gene Conversion					
S. cerevisiae	D7 *trp*	+MA	0.5–5% and 0.5–2%	+	Mortelmans *et al.*, 1980
S. cerevisiae	D7 *trp*	−MA	0.5–5% and 0.5–2%	+	Mortelmans *et al.*, 1980
III. Reverse Mutation					
S. cerevisiae	D7 *ilu*	+MA	0.5–5% and 0.5–2%	+	Mortelmans *et al.*, 1980
S. cerevisiae	D7 *ilu*	−MA	0.5–5% and 0.5–2%	+	Mortelmans *et al.*, 1980
IV. Mitotic Crossing Over					
S. cerevisiae	D7 Adenine $^{+}$/$^{-}$	+MA	0.5–5% and 0.5–2%	+	Mortelmans *et al.*, 1980
S. cerevisiae	D7 Adenine $^{+}$/$^{-}$	−MA	0.5–5% and 0.5–2%	+	Mortelmans *et al.*, 1980
S. cerevisiae	D3	+MA	5%	+	Simmon *et al.*, 1977
S. cerevisiae	D3	−MA	5%	+	Simmon *et al.*, 1977

Table 7.6. Continued

Test and System	Condition and Dosage of CA	Results	Reference
HERITABLE IN VIVO EFFECTS			
I. Sex–Linked Recessive			
Drosophila melanogaster	Oral administration, 3, 500, and 1000 ppm aliquots to different groups	±	Valencia, 1977
II. Dominant Lethal			
Swiss–Webster mice	200 mg/kg, intraperioneal injection	–	Hodge, 1977
DNA EFFECTS			
I. DNA Damage			
E. coli BR513 β-galactosidase induction	25 μl (spot test and tube test)	–	Hume, 1981
II. DNA Repair			
E. coli strains W311C and p 3478	1 mg/disc	–	Simmon *et al.*, 1977
B. subtilis strains H17 and M45	1 mg/disc	–	Simmon *et al.*, 1977
III. Unscheduled DNA Synthesis			
Human diploid fibroblasts – WI 38 cells	+MA 10^{-5}–10^{-3}M	–	Simmon *et al.*, 1977
Human diploid fibroblasts – WI 38 cells	–MA 10^{-7}–10^{-3}M	–	Simmon *et al.*, 1977

[1]Adapted from Office of Pesticide Programs (U.S. EPA) (1981).

[2]Mutant genes or specific strains or assays that allow detection of further mutagenic effect.

[3]+MA = with metabolic activation; –MA = without metabolic activation.

Using a mammalian cell system capable of detecting point mutations (mouse lymphoma L5178Y cells heterozygous for thymidine kinase), Jotz and Mitchell (1980) obtained positive results that were apparently dose-related and indicative of a relatively weak mutagen. The mutagenic effect seen with the lymphoma cells occurred only at cytotoxic doses. Again, the positive nature of the data obtained was unaffected by the presence or absence of a microsomal fraction derived from activated liver from rats pretreated with an enzyme inducer. Such findings would be expected, however, as there is no evidence that microsomal enzymes alter CA in significant amounts. In the study of Jotz and Mitchell (1980), the presence of the liver-derived fraction appeared to decrease the cytotoxicity of CA, though not its mutagenicity, but this effect was probably not enzyme mediated.

Valencia (1977) screened CA, MSMA, and DSMA with a sex-linked recessive lethal test in *Drosophila*. Male flies were exposed to the herbicides for 72 hours by feeding. This allowed not only oral intake but also contact with the test material, as the *Drosophila* walked on glass fiber filter discs lying in solutions of the herbicides dissolved in a 1% glucose solution. The results with all of the herbicides were negative, but in the case of CA they were not entirely clearcut, and CA given at 1,000 ppm resulted in sterility of a number of the treated males.

In a dominant lethal test involving Swiss-Webster mice, Hodge (1977) observed only negative results after a single intraperitoneal injection of CA. Hodge's results may not be very useful, however, in that he gave only a single treatment using only one dose level, and the treatment route did not mimic expected human exposure.

In tests for DNA damage, CA was not seen to have adverse effects (Hume, 1981; Simmon et al., 1977). The tests used included assaying for DNA damage (measured by β-galactosidase induction in *E. coli* strain BR513), DNA repair (using *E. coli* strains W311C and p 3478, as well as *B. subtilis* strains H17 and M45), and unscheduled DNA synthesis in human diploid fibroblasts (WI 38 cells).

Further studies of effects related to mutation and chromosome damage were conducted by a number of investigators. Tests for sister chromatid exchange in cultured Chinese hamster ovary cells were negative in the presence or absence of a liver microsomal fraction (Evans and Mitchell, 1980), but a test for production of micronucleated polychromatophilic erythrocyte stem cells in marrow (a test for clastogenic activity) was positive (Kirkhart, 1980). In the latter study, mice were treated intraperitoneally and the highest doses (where the clastogenic effect was clearcut) were acutely toxic, resulting in a number of deaths among the test animals. No positive control was tested in this study for comparison purposes and to validate technique.

Additionally, older studies evaluated the capacity of CA to inhibit mitosis. Dustin (1929, 1934) mentioned that NaCA was one of a number of toxic substances that could increase the percentage of cells seen in mitosis. Dustin and Piton (1929) suggested that CA affected the thymus, lymph nodes, and crypts of the small intestine, but not the testes. The dividing cells affected by CA exhibited chromosomal condensation and were swollen with clear cytoplasm, although mitosis proceeded as far as telophase.

Dustin and Gregoire (1933a and b) gave CA at a level of 1,500 mg/kg by subcutaneous injection to mice bearing Crocker sarcoma cells. The observed number of dividing tumor cells increased 10-fold by 24 hours, as the cells were arrested in mitosis, but most of the affected cells

appeared eventually to complete mitosis on succeeding days. Cellular abnormalities seen included large, often abnormal mitotic figures along with both enlarged and dwarfed cells.

When Ludford (1936) repeated the preceding experiment, he observed similar effects. The dose used was lethal to several of the mice, but others "recovered completely." Ludford also treated a tumor-bearing mouse and one day later transplanted the tumor into 10 additional mice. The treated tumors grew as well in the host mice as did an untreated tumor similarly transplanted into 10 additional mice. Other tumor-bearing mice were treated and left intact. Their tumors also grew at a normal rate, suggesting that once the CA is eliminated, toxic effects do not persist.

Ludford (1936) also exposed cultured tissue from a carcinoma to NaCA (5,000 ppm for five hours) and noted mitosis arrested in metaphase. The cells generally died within 24 hours, as might be expected from the high concentration used. At 500 ppm, NaCA was less toxic but was still said to affect mitosis, although oddly enough, outgrowth from the culture was said to be unaffected. No effect was seen on mitosis or growth at 50 ppm.

Ludford (1936) and later King and Ludford (1950) suggested that CA acted as a spindle poison similar in this regard to colchicine. This contention was supported by Gregoire and Lison (1934), who treated crayfish and found blocked mitoses in the lymphatic tissue. Chromosomes were dispersed in the cytoplasm, with no evidence of a spindle apparatus.

More recently, Ansano (1953) confirmed the colchicine-like effect of CA on dividing cells when he injected the arsenical intraperitoneally in mice carrying transplanted tumor cells. Since subcutaneous CA injection had no such effect, the results seen with intraperitoneal injection were apparently due to a direct CA effect on ascites cells in the abdominal cavity. Further, CA did not inhibit tumor growth as measured by the survival time of the treated animals.

CA apparently has no mutagenic capability when tested in bacterial systems. The foregoing studies on mutagenicity and related effects of CA demonstrate, however, that it is capable of causing mutations and chromosomal effects in eukaryotes, at least at relatively high doses. Still, the evidence presented to date does not appear to be a cause for alarm, since human exposures cannot be expected to be high enough to result in appreciable mutagenic effects.

Carcinogenicity

The available literature does not adequately address the carcinogenic potential of CA and other methylated arsenicals. Such compounds have received little attention in this regard, although they are widely used as pesticides and defoliants and are known metabolites of inorganic arsenic, which is widely believed to be a carcinogen (Fishbein, 1976). Indeed, Hartwell et al. (1946) tested NaCA for *antitumor* activity and found it to be positive.

In a preliminary report, Innes et al. (1969) noted that a number of pesticides and other chemical agents have been tested for carcinogenicity in mice. CA dissolved in water was given by oral intubation at a maximally tolerated dose (46.4 mg/kg/d) to 18 animals of each sex from two different strains. The CA treatment was given daily from the 7th to the 28th days of age. According to the EPA's Office of Pesticide Programs (OPP, 1981), the animals were then fed 121 ppm of CA in their diet for 18 months. The animals were killed at around 18 months, necropsied,

and subjected to a histopathologic examination (although the brain was apparently not examined), with negative results in the CA-treated mice.

More recently, in a brief report to the Ansul Company, Moore (1976) stated that two in vitro tests with CA, MSMA, and arsenic trioxide yielded only negative results. The tests involved transformation of embryonic cells from Fisher rats or cloned cells from BALB/3T3 mice. CA and MSMA were both tested at concentrations up to 10 μg/ml (10 ppm) and arsenite was tested at levels up to 100 μg/ml (100 ppm).

Although these data are negative, the animal studies were done with small numbers and at only one dose. It is difficult to assess how meaningful they are with regard to the risk of human carcinogenesis. This can also be said because the majority of the numerous studies done in an effort to induce tumors with inorganic arsenic in laboratory animals have yielded only negative or equivocal results (Axelson, 1980).

Nevertheless, human epidemiologic data have been widely viewed as implicating inorganic arsenic as a carcinogen (IARC Working Group, 1980), although concurrent exposure to other chemical agents often confounded these studies. Thus, if inorganic arsenic is indeed carcinogenic and if methylated arsenicals such as CA were to act by the same mechanism, it might be difficult to detect such an effect by using typical animal screening systems.

It has also been suggested that inorganic arsenic is not a primary carcinogen but acts by enhancing the carcinogenicity of primary carcinogens (c.f. Hadler and Cook, 1979; Leonard and Lauwerys, 1980). This concept could explain much of the conflicting human and animal data, but to determine if CA might play such a role would require specifically designed tests.

Another consideration is that regardless of the role of inorganic arsenic in carcinogenesis, it might be that one or both of its methylated metabolites is the active form in the cell. Nevertheless, as there is no evidence in the literature that CA or other methylated arsenicals are involved in carcinogenesis, it would be purely unsupported speculation to propose such a role at this time.

Toxicity to microorganisms

In addition to tests of CA involving higher animals, microorganisms have also been subjected to toxicologic examination. The rationales for these tests have ranged from concern for such organisms in the environment (where CA-based pesticides may be applied) to the use of bacteria as initial screening organisms for toxicity testing of chemicals.

In an early study of the effects of CA on flora and fauna at Eglin Air Force Base in Florida, Oliver et al. (1966) found reduction of algal productivity by CA concentrations equivalent to those expected from a spray rate of 2 kg/ha over a 30.5 cm water depth.

Macklin and Witkamp (1973) later investigated the effects of defoliation of tulip poplars with CA on the decomposition of the consequent leaf litter. High CA application rates (2.2 g/5 cm dbh) resulted in decreased evolution of CO_2 from forest floor litter. At a lower application rate (0.7 g/5 cm dbh), fungal mycelial growth was inhibited, but loss of weight from the leaf litter and the rates of litter breakdown and CO_2 evolution were unaffected at any application rate.

In 1974, Bollen et al. tested the toxicity of CA and MSMA to pure cultures of a number of common molds and bacteria found in forest and agricultural soils. They found that the organisms tested were relatively resistant to the two herbicides.

Both CA and MSMA in concentrations containing 1,000 mg of arsenic per liter only slightly affected two of the test bacteria in nutrient broth, and a concentration of 10,000 mg of arsenic per liter significantly decreased—but did not completely prevent—growth of all bacteria tested (molds were not tested in broth). When all test organisms were cultured on agar, saturation of paper discs with herbicide solutions of up to 10,000 mg of arsenic per liter did not prevent growth up to the edge of the disc.

In additional tests, Bollen et al. (1974) subjected three forest soils to a depth of 15 cm (along with overlying forest floor material) to CA and MSMA solutions of from 0 to 1,000 ppm arsenic. As concentrations of both herbicides increased above 10 ppm of arsenic, the evolution of CO_2 from forest floor detritus declined slightly. MSMA had the opposite effect on soils, as it increased CO_2 release, while CA had no effect.

In a later report, Bollen et al. (1977) evaluated ammonification of added peptone nitrogen and nitrification of the resultant ammonia (aerobic oxidation by autotrophic bacteria) in the same soil and forest floor samples used in their previous study. They found that increasing levels of CA appeared to stimulate ammonification. The effect was not statistically significant, however, although MSMA significantly increased ammonification in both substrates.

Nitrification was low in all cases, apparently because soil from coniferous forests is typically low in nitrifying potential. Both herbicides caused decreases in nitrification, but the absolute change induced was so low that the authors considered it of little practical significance. This assessment appears correct since only very small effects were seen at the lower doses, and the higher doses would be unlikely to occur unless herbicide were spilled in an area.

Three additional bacterial species were isolated from soil by Anderson and Abdelghani (1980) and subjected to MSMA, sodium arsenate, or sodium arsenite. The LC_{50} for MSMA was found to be 27 mg/ml, while those for arsenate and arsenite were 1.3 and 0.27 mg/ml, respectively. As expected, the organic arsenical was much less toxic than the inorganic forms. It was also found that the organisms tested could adapt to the presence of MSMA, so that by 96 hours of incubation the LC_{50} had increased to 220 mg/ml.

Further, Moura Nunes et al. (1980) noted growth of a *Penicillium* mold on tissue stored in 0.1 M HCl-NaCA buffer, a situation probably made possible by adaptation of the fungus over time.

In a field study, Malone (1971) determined that the numbers of soil bacteria increased following a single application of a high level (30.3 kg/ha) of a NaCA-based herbicide (Phytar® 560) to control vegetation in a fescue grass meadow. Areas receiving an application of 10.1 kg/ha were unchanged. Repeated monthly spraying at the low dose resulted in a transient increase in bacterial densitity. Numbers of soil fungi were initially reduced, but recovered to control levels in the low-dose plots and to even greater numbers in the high-dose areas and following repeat applications.

Laboratory tests produced similar results. At soil concentrations of 10 or 1,000 ppm, increases in numbers of soil bacteria were found. Herbicide concentrations of 5 ppm or above were also associated with reduced numbers of fungi, followed by recovery at the two highest concentrations (10 and 1,000 ppm). Additionally, soil respiration rates were briefly elevated by 2 ppm or higher doses. Whether the results seen were attributable to the arsenical, to other ingredients of the herbicide formulation such as the detergent, or to both was not determined.

Mechanisms of cacodylate toxicity

Although much has been written about the effects of CA in humans and lower animals, relatively little attention has been paid to the mechanism(s) by which this arsenical exerts its effects. The early literature speculated that CA effects were due to liberation of inorganic arsenic, either in the gut (if CA was taken orally) or in the tissues (Dawes and Jackson, 1907; Goodman and Gilman, 1941; Sollmann, 1948). Nevertheless, there is no recent evidence to support such contentions (OPP, 1981).

Da Costa (1972) exposed cultures of several fungi to growth-inhibiting concentrations of NaCA, or to inorganic arsenate or arsenite. Adding phosphate to the culture media abolished the growth inhibition due to arsenate and reduced the toxic effect of arsenite, but had no effect on the toxicity of cacodylate. Such results indicate that dimethylated arsenic has a different mechanism of toxicity (at least to fungi) than does arsenate. It was not clear, however, why arsenite acted similarly to arsenate in this study, unless a significant amount of the As(III) oxidized to As(V) under the experimental conditions.

A similar study was conducted with yeast by Furakawa et al. (1979), who compared growth inhibition due to sodium methanearsonate with that caused by sodium arsenate. In this case, they found that addition of phosphate abolished the toxic effects of both arsenicals.

According to the results of the foregoing mechanistic studies (Da Costa, 1972, and Furakawa et al., 1979), MAA and CA may have different mechanisms of toxicity, since the toxic response to MAA was influenced by phosphate while the effect of CA was unchanged. Nevertheless, it would be helpful to have confirmation of such results by trials involving both compounds in the same test organisms.

That methylated arsenicals may inhibit enzymes has been suggested by Knowles and Benson (1983). For example, they propose that unchanged methanearsonate, with an oxidation state of +3, has an effect on the malic enzyme (responsible for catalyzing the use of maleate in the CO_2-utilizing system of C_4-plants). Malic enzyme was inhibited, however, by SH reagents such as $MeAs(OH)_2$ (methanearsonous acid) with a +1 oxidation state.

According to Knowles and Benson, methanearsonic acid could serve as an oxidant in the Hill reaction of chloroplasts, presumably by oxidizing the enzyme thioredoxin reductase, and become an enzyme-inhibitory SH reagent, i.e., methanearsonous acid. They also concluded that when arsenic is in either the +1 or -1 oxidation state, an attached hydroxyl group is free to exchange with an SH group on another molecule.

More direct evidence of enzyme inhibitory ability comes from the work of Yates (1969). He found that a nonspecific adenine nucleotide deaminase isolated from a bacterium was competitively inhibited by 134 mM CA, as well as by agents such as Hg^{2+} that are known to bind SH groups.

More recently, Stevens et al. (1977b) published the results of studies of rat inhalation exposure to aerosols of CA (in Phytar® 138) or MSMA (in Ansar® 170 in water or Phytar® 138 as a dust). They found inhibitory effects on pulmonary and hepatic microsomal benzphetamine n-demethylase in some cases and stimulatory effects in others. The response seen was dependent on the sex of the exposed rats and the time after exposure.

Similarly mixed results were seen in mice exposed to the aqueous aerosols. Reductions were also seen in the levels—but not the specific activities—of cytochrome P-450 in treated rats. Stevens et al. (1977b) speculated that the likely site where CA acted on the mixed function oxidase system was the terminal oxidase, i.e., cytochrome P-450.

In vitro studies by Stevens et al. (1977b) failed to show an effect of CA (purified or in Phytar® 138) on benzphetamine n-demethylase activity from isolated mouse hepatic microsomes, although CA weakly bound to cytochrome P-450. Further, when ^{14}C-labeled CA, DSMA, or MSMA were incubated with subfractions from lung or liver homogenates, no clear evidence of metabolism of any arsenical was seen.

Colilla et al. (1974) found effects of cacodylate on the multifunctional enzyme Glc-6-P phosphohydrolase (also called glucose-6-phosphatase, EC 3.1.3.9). CA at low concentrations (ionic strength = 0.1) increased the phosphotransferase activity slightly and the phosphohydrolase activity markedly. At higher concentrations these activities were inhibited, however.

The effects of arsenicals in rats are influenced by their unusual affinity for the rat erythrocyte. For example, Stevens et al. (1977c) found that CA given either intravenously or intratracheally soon became associated with the rats' red cells. A similar amount was found regardless of dose for doses between 150 and 400 mg/kg. The CA was apparently bound intact, as results were similar for both ^{14}C- and ^{74}As-labeled CA.

The half-life of CA in rat erythrocytes (90 days) was approximately the same as that of the erythrocytes themselves (95 days), indicating irreversible CA binding to the rat hemoglobin. When species comparisons were made, rat erythrocytes were found to bind CA much more readily than did those of the rabbit or guinea pig, with human red blood cells binding the least of all.

Stevens et al. (1977c) attributed the disparity in CA binding by hemoglobin to a possibly greater number of binding sites per erythrocyte in the rat, while Knowles and Benson (1983) speculated that the enhanced affinity of arsenicals for rat red cells was due to higher levels of reduced glutathione reductase. They suggested that the enzyme acts on the arsenical, allowing it to bind reactive SH groups, of which each hemoglobin tetramer has two. Rat erythrocytes apparently do not contain more glutathione reductase than do those of other species, but more of the rat enzyme is in the reduced form.

The ability of CA to react with SH groups had been noted previously by Jacobsen et al. (1972), who found that the arsenical could react with biologically important molecules, such as cysteine and glutathione. They and Banks et al. (1979) also speculated that CA could react with SH groups of proteins and possibly interfere with SH-requiring enzymes.

Chapter 8

Human Exposure and Toxicology

Relatively little information is available in the recent literature with regard to typical human exposure to CA and other methylated arsenicals or the health effects of such exposure. The scarcity of literature encompasses both environmental exposures and those derived from occupational uses, such as herbicide manufacturing and agricultural applications.

Sources of human exposure

Sources of exposure to CA include ingestion via the diet and inhalation or dermal absorption by pesticide applicators and bystanders in the vicinity of pesticide application. The Office of Pesticide Programs (OPP) of the U.S. Environmental Protection Agency included a number of estimates of potential exposure to CA in its *Cacodylic Acid: Decision Document*, published in 1981. The following discussion is based in part on information in that document.

Dietary exposure. The EPA has proposed estimated environmental concentrations (EECs) for CA based on worst-case estimates of pesticide use (OPP, 1981). With application rates of 5.6 kg/ha, EECs were estimated in parts per million as 1,200 for short grasses, 530 for long grasses, 600 for dense foliage, 30 for seeds and grain, and 1.15 for pond water.

Based on the above premises and their estimates for CA toxicity, the EPA estimated that when used properly, CA should offer a relatively low risk of harm to wildlife. Since wildlife is assumed to live in contact with as well as consume sprayed vegetation, thus receiving a higher dose of CA than would most humans, the risk for man may also be low.

The maximum permissible levels of arsenical residues in human foods and certain livestock feeds in the United States are set and monitored by the Food and Drug Administration. Although an acceptable daily CA intake value has not been published, tolerances were established for CA (*expressed as arsenic trioxide*) in 1972 (37 *Federal Register* 739, January 18, 1972) and include 28 ppm for cottonseed, 1.4 ppm in bovine kidney and liver, and 0.7 ppm in bovine meat, fat, and meat products. The purpose of the tolerance for cottonseed is that cattle may be fed cottonseed meal or hulls and thus absorb CA from their diet.

Periodic efforts are made to sample the U.S. food supply for a variety of pesticides to ensure that typical diets do not contain unsafe levels of these toxic substances. Studies published in the mid-1960s indicated the presence of relatively low levels of pesticides in samples of foods after processing for consumption (Duggan et al., 1966).

Levels of toxic substances associated with raw foods tend to be higher than those in the same items when they are prepared for eating, so that studies of unprocessed foods would be misleading as indicators of actual pesticide intake (Duggan and Weatherwax, 1967).

Both the frequency of occurrence of arsenic and the levels found in food items have consistently been low, generally being within or only a little above the levels expected from the natural environmental background (Corneliussen, 1972; Duggan and Corneliussen, 1972).

Since only total arsenic is measured, it is not possible to distinguish inorganic from methylated arsenic. Thus, it is not known what proportion of dietary arsenic may have been derived from CA or methanearsonate. If methods were used to distinguish organic arsenicals, it would still be impossible to determine directly if they were derived from herbicide use.

In any event, the presence of organic forms should decrease any perceived ill effect from dietary arsenic. Fortunately, arsenic residues have been low enough that they do not seem to present a significant hazard, even if they are totally present as inorganic arsenic.

The EPA has calculated a series of estimates of daily dietary intake of CA based on worst-case premises (OPP, 1981). Its estimates were based on assumptions about average values for the entire population at risk (60 kg weights for exposed individuals and a 1.5 kg total daily food intake) and maximum dietary levels of CA derived from cattle and cottonseed.

Such estimates give a value of 0.0014 mg/kg/d. If this value is adjusted for the proportion of the cotton crop believed to be actually treated with CA (2%), the total intake estimate becomes 0.000028 mg/kg/d. Actual CA intake would also vary according to the proportion of treated cottonseed products fed to the cattle consumed in a given area of the country.

The EPA also projected a worst-case estimate for the CA obtained from consumption of a single serving. Values for bovine muscle, fat, and byproducts together, and for beef kidney or liver were 1.33 and 2.66 μg per kilogram of serving, respectively. Although such estimates are not based on measured dietary values, if tolerance levels are not exceeded in the diet, they do represent true worst cases and suggest little likelihood of toxicity from this source of CA.

Dermal and respiratory exposure. In calculating expected exposures for pesticide applicators, the OPP (1981) started with the assumption that the methods used would include ground application, involving applicators using either hand-operated or boom-type, tractor-mounted sprayers, and aerial application, with possible exposure of pilots, mixer-loaders, flaggers, and occasional bystanders. Since exposure of such individuals would commonly involve both dermal and inhalation routes they will be discussed together, as they were in the EPA document. In addition, the data have been summarized in Tables 8.1-3.

Table 8.1. Summary of dermal and respiratory exposure, per use site/application method[1]

Use Pattern	Dermal Exposure (mg/hr)	Respiratory Exposure (mg/hr)
Aerial application,		
Dry land cotton		
Pilot	0.40	0.03
Flagger	22	0.005
Irrigated cotton		
Pilot	0.47	0.03
Flagger	26	0.006
Mixer/loader (dry land and irrigated)		
Open system	–	0.0055
Closed system	–	0.0013
Ground boom application		
Dry land cotton	42	0.09
Irrigated cotton	50	0.11
Non–bearing citrus orchards	59	0.24
Hand boom application,		
Non–bearing citrus orchards	71	0.24
Ditchbanks and rights-of-way	35.5	0.05
Knapsack or hand sprayer		
Ditchbanks, rights-of-way, and home uses	60	1.9
Hand-held sprinkler and dry formulations	Negligible	Negligible

[1]Adapted from OPP (U.S. EPA) (1981).

Table 8.2. Estimates of exposure to CA from non-dietary sources (mg/kg/day) [1, 2, 3]

Use Pattern	Dermal	Respiratory	Total
Aerial application			
Dry land cotton			
Pilot	0.046	0.003	0.049
Flagger	2.5	0.006	2.506
Irrigated cotton			
Pilot	0.054	0.003	0.057
Flagger	2.97	0.007	2.977
Mixer/loader (dry land and irrigated)			
Open system	–	0.006	0.006
Closed system	–	0.001	0.001
Ground boom application			
Dry land cotton	4.8	0.01	4.81
Irrigated cotton	5.7	0.013	5.713
Non-bearing citrus	6.7	0.027	6.727
Ditchbanks and rights-of-way	4.1	0.006	4.106
Hand boom spray application			
Non-bearing citrus	8.1	0.027	8.37
Knapsack or hand sprayer			
Ditchbanks, rights-of-way, and home uses	6.9	0.217	7.117
Hand-held sprinkler and dry formulations	Negligible	Negligible	Negligible

[1]Assumes 70 kg body weight for workers involved in application practices.
[2]The following exposure estimates have been calculated for bystanders to aerial applications of cacodylic acid on cotton, assuming an exposure duration of one hour and a body weight of 70 kg. Potential exposure to bystanders is dermal rather than respiratory: *dry land cotton*: 35.9 ng/kg at 83 feet (25 meters), 18.3 ng/kg at 149 feet (45 meters), 8.3 ng/kg at 314 feet (96 meters), 3.9 ng/kg at 644 feet (196 meters), and 2.0 ng/kg at 1304 feet (397 meters); *irrigated cotton*: 43.0 ng/kg at 83 feet, 22.0 ng/kg at 149 feet, 10.0 ng/kg at 314 feet, 4.6 ng/kg at 655 feet, and 2.4 ng/kg at 1304 feet.
[3]Adapted from OPP (U.S. EPA) (1981).

Table 8.3. Worst-case non-dietary risk from exposure to cacodylic acid: estimated margins of safety for fetotoxic effects[1]

Use Pattern	Dermal[2]	Respiratory	Total
Aerial Application[3]			
Dry land cotton			
Pilot	326	5,000	306–30,600
Flagger	6	25,000	6–600
Irrigated cotton			
Pilot	278	5,000	263–26,300
Flagger	6	2,142	5–500
Mixer/loader (dry land and irrigated)			
Open system	–	25,000	25,000–2,500,000
Closed system	–	150,000	150,000–15,000,000
Ground boom application			
Dry land cotton	3.1	1,500	3.1–3,100
Irrigated cotton	2.6	1,154	2.6–2,600
Non-bearing citrus	2.2	556	2.2–2,200
Ditchbanks and rights-of-way	3.7	2,500	3.7–3,700
Hand boom spray application			
Non-bearing citrus	1.9	556	1.8–1,800
Knapsack or hand sprayer			
Ditchbanks, rights-of-way, and home uses	2.2	69	2.1–2,100
Hand-held sprinkler and dry formulations	Negligible exposure	Negligible exposure	Negligible exposure

[1]Adapted from OPP (U.S. EPA) (1981).

[2]In the absence of data on the rate of dermal absorption, 100% absorption is assumed, a worst-case assumption. If 10% absorption is assumed, margins of safety for dermal exposure would be multiplied by 10; if 1% absorption is assumed, margins would be multiplied by 100; if 0.1% absorption is assumed margins would be multiplied by 1000.

[3]The following worst-case margins of safety (100% dermal absorption assumed) have been calculated for bystanders exposed for 1 hr as a result of aerial applications to cotton: *dry land cotton*: 418 at 83 feet (25 meters), 820 at 149 feet (45 meters), 1,807 at 314 feet (96 meters), 3,846 at 644 feet (196 meters), and 7,500 at 1,304 feet (397 meters); *irrigated cotton*: 349 at 83 feet, 682 at 149 feet, 1,500 at 314 feet, 3,261 at 644 feet, and 5250 at 1,304 feet.

The EPA estimates for exposure of pilots involved in aerial cotton spraying are based on application rates of 1.12 kg/ha for dryland cotton and 1.34 kg/ha for irrigated cotton. They yield respective dermal exposure values of 0.4 and 0.47 mg/h for the two application rates. Values for exposure via inhalation were projected to be around 0.03 mg/h in both cases.

The above values for application rates were also used in estimating exposure to flaggers. For mixer-loaders, the EPA assumed that any CA in respirable air was derived from the physical mixing process, such as splashing, rather than from an actual equilibrium between "aqueous and vapor phases of the compound."

Further assumptions included an inhalation rate of 1 m^3/h, complete retention of inhaled CA (obviously a worst-case projection for retention), and either an open or a closed mixing system. Although a degree of dermal exposure of mixer-loaders to CA is also probable, no estimates were attempted. Each drop of a 28.4% concentrate was said to contain approximately 19 mg of CA, but the typical amount of skin contamination to be expected was unknown.

Values calculated by the EPA (OPP, 1981) for exposure due to use of boom sprayers were derived by extrapolation from the data of Wolfe et al. (1961) obtained from tests with parathion, endrin, or herbicide (DNOSBP) spray applications. The exposure projections for cotton spraying were based on the use of maximum spray solution concentrations of 0.95% and 0.79% CA for irrigated and dryland fields, respectively.

All projections for human exposure from spraying of nonbearing citrus orchards were based on maximum amounts of CA equivalent to a 1.18% solution, while exposure values for applicators spraying ditch banks and rights of way were calculated for use of 0.59% solutions.

EPA exposure estimates for applicators of CA solutions on ditch banks, rights of way, and on lawns and weedy areas around homes by use of knapsack or hand-held sprayers were based on 0.59% CA concentrations (OPP, 1981). Exposure of users of CA solutions applied from a hand-held sprinkler were considered minimal since the preparations are premixed and relatively dilute.

Dry CA-based herbicides contain 6% cacodylate and are used for spot applications. Contact with significant amounts of CA when using such a preparation was also considered minimal, unless an applicator tried to unclog a spreader without first putting on impermeable gloves.

In the experience of the Threshold Limit Values (TLV) Committee of the American Conference of Governmental Industrial Hygienists (Anonymous, 1980), it has not been thought necessary to identify the organoarsenicals specifically. The TLV-TWA (time-weighted average) for arsenic (CAS 7440-38-2) and its soluble compounds reported as arsenic, has a TWA of 0.2 mg/m^3.

In the production of arsenic trioxide, the exposure has been assigned to the category A2. This categorization refers to compounds that are industrial substances suspected to have human carcinogenic potential. Note that this value is only for arsenic trioxide production and does not apply to industrial exposures to other arsenicals or their soluble salts. These guidelines are current for 1983-84. A further explanation for the rationale used to arrive at these values is contained in the TLV, ACGIH Document for 1980 (Anonymous, 1980).

Report XIX of the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area of the Federal Republic of Germany (Commission, 1983) does not contain a maximum allowable concentration (MAC), either in parts per million or milligrams per cubic meter. References are made to arsenic trioxide, arsenic pentoxide, arsenous acid, arsenic acid, and their salts.

In 1983, arsenic and its compounds, with the exception of arsine, was on the Technische Richtkonzentrationen (TRK) List at 0.2 mg/m^3. TRK values are assigned only for hazardous working materials (particularly suspected carcinogens) for which a MAC value confirmed by toxicologic or industrial medical experience cannot be established at the present time. Arsenic was assigned to this group because of the question of cancer and mutagenesis, but the German commission does not list values for organic arsenicals.

A National Institute of Occupational Safety and Health document (NIOSH, 1978) lists recommended exposure limits for inorganic arsenic but does not list such limits for organic arsenicals. Similarly, an Occupational Safety and Health Administration document (OSHA, 1981) lists permissible exposure levels only for inorganic arsenicals.

Health-related effects of human exposure

Occupational exposure is the most likely source of significant health effects of exposure to methylated arsenicals. Peoples et al. (1979) recorded physicians' reports of patients with job-related accidental exposures to organoarsenical pesticides in California from 1975 through 1977. During the three-year period, 4,326 cases of illness presumed due to occupational exposure to pesticides were noted.

Thirty-four cases — less than 1% of the total — were believed to be related to organoarsenical exposure. Of these, two-thirds were associated with cacodylate or sodium cacodylate and the rest with MSMA. The symptoms noted included vomiting, diarrhea, and abdominal pain (9 cases), eye irritation (13 cases), and contact dermatitis or allergic rash (12 cases). The eye and skin problems were said to be mild and to respond well to treatment. No long-term illness was reported, even in cases of pesticide ingestion.

The most serious case occurred when a man operating a spray rig was accidentally doused with MSMA because of a faulty hose connection. Part of the spray entered his mouth, whereupon he spat out the spray material and rinsed his mouth with water. During the evening he was hospitalized with a number of symptoms, including stomach cramps, paralysis, numbness, irregular breathing, and syncope. He was released after five days, when he had no further apparent symptoms.

Peoples et al. (1979) concluded that both CA and MSMA were irritants and could cause a mild, transient, easily treatable conjunctivitis when the eye was exposed. Dermal contact, especially if the herbicide was left on the skin for a time, typically resulted in contact dermatitis. At times there was a short-lived allergic response. Ingestion could result in nausea, colic, vomiting, and diarrhea, and only rarely in other symptoms, but exposure did not appear to result in long-term toxic effects.

In all cases examined, ill effects were due to careless or improper work practices or faulty equipment, resulting in direct contact with the arsenical. None of the reported cases was the result of approved application practices with proper safety devices and intact spraying equipment.

In a more extensive search that included both work-related and other exposures, the EPA examined the files of OPP's Pesticide Incident Monitoring System for 1966 to July 1980 (EPA,

1980). Of 37,291 incident reports on file, 70 involved CA, invariably combined with the sodium or (in two cases) the triethanolamine salt of CA. In several cases there was exposure to other pesticides as well. In 49 of the reports there was evidence of a likely effect on humans, involving 61 individuals.

The great majority of the reports involved skin rash, contact dermatitis, or eye irritation. Nausea or abdominal cramping occurred in a few instances. Occasional findings included symptoms such as diarrhea, dizziness, lung irritation, drowsiness or stupor, vomiting, paresthesia, fever, loss of appetite, or ataxia. The more serious and infrequent symptoms were generally associated with particularly significant exposures, such as accidental ingestion of apparently rather large amounts of pesticide. In none of the cases listed, however, was there any indication of long-term or persistent effects.

Two other studies involved forest workers applying CA or MSMA in tree thinning (Tarrant and Allard, 1972; Wagner and Weswig, 1974). In the first of these studies, application methods included "hack and squirt," injection hatchet, and injector tool used by one or two men each in three six-man crews (see Table 8.4 for details).

Table 8.4. Thinning procedures assigned to men in each crew

Crewman	Chemical Used	Application Method
1	None	None
2	Cacodylic acid	Injection hatchet
3	MSMA	Injection hatchet
4	Cacodylic acid	Hack and squirt
5	MSMA	Hack and squirt
6	MSMA	Injector tool

Source: Tarrant and Allard, 1972.

One man per crew did not apply herbicide and served as a control. Each crewman was given fresh clothing daily, including two pairs of cotton gloves, and was supplied with goggles or wrap-around sunglasses. The men were instructed to wear the gloves when applying silvicides and to change them at midday or if they became contaminated. Washing facilities were provided, and the men were told to wash their hands before eating or smoking.

The study was carried out over a nine-week period, and urine samples were collected each Monday morning and Friday afternoon (Tarrant and Allard, 1972). Urines were analyzed for total arsenic and corrected for differences in urine concentration by using values for osmolality, since 24-hour samples were not obtained. According to the results of the arsenic analyses, applicators had significantly higher urinary arsenic values than did control workers, and such values were higher on Fridays than on Mondays.

Differences associated with mode of application were not statistically significant, although some may have been so if a larger sample of workmen had been available. Unusually elevated individual values (up to 2.5 ppm) could ordinarily be associated with accidental exposures or

laxity in safety measures. There was no apparent buildup of arsenic levels over the nine-week test period.

During the 136 man-weeks of exposure in the Tarrant and Allard (1972) study, the applicators' physical complaints (with the number of men complaining of each in parentheses) were: headache (14), abdominal cramping (6), asthenia (5), nausea (4), and skin rash (1). During 27 man-weeks, controls reported nausea (1) and asthenia (1). Whether the apparent overrepresentation of headache among applicators was due to the arsenicals or to the fact that the men knew they were involved in a health study of arsenicals could not be determined.

A significantly larger, longer-term study, preferably with some applicators applying a sham silvicide, would be needed to determine if there were real health effects associated with exposure to CA or to MSMA. Nevertheless, the data do suggest that exposures of the workers to arsenicals in the study were too high and that better protective measures would have been useful.

That worker exposure to arsenical silvicides could be minimized was borne out by the results of Wagner and Weswig in 1974. In their study, five workers were given plastic face masks and plastic-lined cotton gloves for use while applying CA by the "hack and squirt" method. The test was carried out for two months, with 24-hour urine samples obtained for the last workday of each week (late Thursday afternoon through late Friday afternoon). Blood samples were also taken following the end of work on Fridays. Test samples were analyzed for total arsenic in comparison with monthly samples from five unexposed workers.

Blood arsenic values increased for the first four weeks of the study and unexpectedly declined thereafter, while urinary arsenic levels exhibited no comparable decrease during the exposure period. Assuming the analytic data were accurate, such results are puzzling. If the decreased blood levels were due to increased care by the workmen in avoiding exposure to CA, urinary arsenic levels should have shown a comparable decline.

The lowered blood values might have been related to an increase in ability to clear methylated arsenicals from the blood, but no such mechanism is currently known. It also seems unlikely that such increased clearance would require a four-week exposure in order to develop. Increased environmental temperatures were associated with the initial decine in blood arsenic, suggesting that additional amounts of CA might have been eliminated in perspiration, but when temperatures returned to more normal values arsenic levels remained low. At any rate, such results suggest that urinary arsenic values may be better than blood levels as a long-term measure of CA exposure.

None of the workers in the Wagner and Weswig (1974) study lost time from work, although one did complain of mild nausea, aching, and diarrhea over two days. Although such symptoms were not uncommon in the general population and can have a variety of causes, it is of interest to note that nausea and diarrhea were common complaints of others exposed to CA (e.g., Peoples et al., 1979).

All of the workers using arsenicals reported a strong garlic-like odor within two days of CA application in the treated forest areas. The odor was apparently that of arsine generated by soil micro-organisms (Braman, 1975; Cheng and Focht, 1979). Because of the potentially hazardous nature of arsines, blood cell counts were done on two of the five exposed men. The counts

revealed no signs of hemolytic anemia, however, indicating that any arsine exposures had not been sufficient to lead to signs of overt toxicity.

At no time did the workers studied by Wagner and Weswig (1974) have urinary arsenic levels in excess of 0.2 ppm of arsenic. This is in contrast to the results of Tarrant and Allard (1972), who frequently found values of 0.3 ppm or more. Wagner and Weswig attributed the difference primarily to superior education and supervision of the workers and additional protective devices and clothing. The methods of urine sample collection differed as well.

A more serious case of poisoning with a methylated arsenical occurred when a man was exposed to an MSMA spray from aerial spraying of cotton for approximately 1.5 h/d for five consecutive days (Hessl and Berman, 1982). He did not try to avoid the spray, and his head, neck, hands, and feet were exposed. The worker developed a severe peripheral neuropathy similar to those seen in cases of poisoning with other arsenicals. His symptoms also included anemia, leukopenia, and dyserythropoiesis.

The patient was treated with BAL followed by D-penicillamine, but a year after the poisoning incident his neuropathy had not decreased and he had flexion contractures of the hands as well as palmar keratoses. The significance of this case is difficult to assess, though, as the patient had also been exposed to other pesticides, including 2-sec-butyl-4,6-dinitrophenol and the isopropylamine salt of glyphosphate.

According to Stevens (1966), the much more moderate exposures to methylated arsenicals encountered by production workers did not result in notable toxic effects. Nevertheless, Weakley (1977) published a paper on the potential for toxic effects from the use of cacodylate-based buffers, as in electron microscopy. She appears not to have seen much of the literature on the toxicity of CA and to have been advised by sources who did not differentiate the toxicity of methylated arsenicals from that of the inorganic forms. Consequently, Weakley suggested that the relatively slight CA exposure likely to be encountered in the laboratory might be a significant danger. Judging from the available literature, however, this seems an unnecessary fear.

Early clinical trials with cacodylic acid

Around the turn of the century a number of arsenicals were tested as potential drugs for the treatment of a variety of human diseases. The results of such studies have been summarized in various pharmacology texts.

Sollmann (1926), for example, mentions that an overdose of CA could produce toxicity. It was often administered subcutaneously or intramuscularly because of the prevalent belief that orally administered CA would be demethylated and so made more toxic. The recommended dose was 0.025 to 0.15 g/d taken orally, injected subcutaneously, or given in an enema.

For syphilis, CA had been recommended at a dose of 0.13 to 0.26 g given once intramuscularly, but clinicians such as Cole (1916) found CA to be ineffective even at doses toxic to the patient. It was also pointed out in Sollmann's (1926) discussion that CA had been found to cause venous fibrosis when given intravenously and to result in nephritis in some individuals. In a

later edition of his book, Sollmann (1948) presented virtually the same material on CA but added the caveat that "Cacodylate is not effective in chemotherapy."

In 1941, Goodman and Gilman's *Pharmacological Basis of Therapeutics* concluded that certain organic arsenicals such as cacodylate "liberate inorganic arsenic in the body," being "converted to cacodylic oxide and then to inorganic arsenic." It was also stated that "After oral ingestion, inorganic arsenic is likely to be freed rapidly by the action of the acid gastric juice and serious poisoning can result from ingesting an amount that would be perfectly safe to inject." They give a typical oral dose as 60 mg and a subcutaneous dose as 200 mg.

As previously mentioned, only a slight amount of CA is apparently altered in the body (which would nevertheless be sufficient to account for the garlic-like odor observed), and CA is highly resistant to the type of acid found in the stomach. The same apparent misinformation was being repeated 31 years later, however, in *Martindale, The Extra Pharmacopoeia* (Blacow, 1972), where it was also mentioned that NaCA was still listed in the pharmacopoeias of Austria, Brazil, Chile, Italy, Mexico, the Netherlands, Portugal, Romania, Spain, and Switzerland.

It was further stated that injections of iron cacodylate (ferric dimethylarsinate) and strychnine cacodylate had previously been advocated as stimulants for individuals in weakened conditions. It is of interest to note that sodium methanearsonate was also prescribed as a medication to be used orally or by injection, at a dose of no more than 200 mg/d. The usual dose was 30 to 120 mg. That arsenical has been sold under the trade names Arrhenal, Arsozon, and Disomear. It was said to have properties similar to those of CA, but that its use did not result in the offensive odor of garlic.

According to a variety of sources (e.g., Dawes and Jackson, 1907; Simon, 1932), the French physician Gautier first used CA as a drug before 1900. It was initially believed to be a superior substitute for inorganic arsenicals, and was particularly popular in Europe. In 1903, however, a report by Fraser suggested that CA remained unchanged in the body. Since it had been believed that CA produced useful pharmacologic effects primarily through liberation of inorganic arsenic in the tissues, this resulted in a temporary lull in its use in therapy.

The paper of Dawes and Jackson (1907), in which the authors stated that a portion of the CA given chronically did liberate arsenic after varying lengths of administration, led to renewed interest in CA. They also reported that some 76% of patients they had treated with NaCA were cured or at least improved. The conditions treated included lack of appetite, anemia, eczema, and amenorrhea, psoriasis, chronic headache, chorea, and "neuroses."

Treatment by intramuscular injection was even said to have resulted in euphoria, causing some patients to request further injections after the treated symptoms were relieved! Of course, it is impossible to assess the actual efficacy of such treatments, since no controls were used and none of the treatments was done "blind." Also in 1907, de Biehler presented a detailed account of the effects of CA administration in humans and animals, but a translation was not available for inclusion in this monograph.

By 1916, NaCA was still being used in the treatment of syphilis, even though its effectiveness had been called into doubt by various workers such as Nichols (1911), who found it to be ineffective against spirochetes.

Cole (1916) treated 120 syphilitic patients with NaCA given at an initial intravenous dose of 250 mg, followed by 450 to 500 mg every three to five days, for a total of five to six doses. None of these treatments was successful, and in two cases treatment resulted in apparent kidney damage. Cole also mentioned that a nonprescription medication marketed as "Venarsen" consisted largely of NaCA, and its ineffectiveness was confirmed by Brayton (1917).

According to Simon (1932), NaCA was initially used primarily to treat skin diseases, "some blood diseases," and tuberculosis, but it eventually lost favor except for treating anemia and as a tonic. Simon also stated that the arsenical was claimed in several reports to alleviate "incurable spastic nerves" when given intravenously in high doses.

The clinical studies discussed above are only a sample of those in the early literature. Many of the potential references were incomplete or in obscure sources. Additionally, most of the early clinical studies were published in French, German, Italian, or Russian and would require translation. Nevertheless, it should be clear from the studies that were included that CA was one of the potential medications that were initially tested for a variety of conditions and eventually found ineffective, finally considered too toxic at the doses employed, or replaced by superior drugs.

The symptoms of mild intoxication with arsenicals such as MAA (and presumably, CA) have been said to include colicky abdominal pain, a burning sensation in the throat, and a salty taste (Weed Society of America, 1967). More acute poisoning might result in "headache, vomiting, diarrhea, dizziness, stupor, convulsions, general paralysis, and death." A garlic-like odor may also be noted. According to Ehman (1973b), a patient intoxicated with an organic arsenical would be likely to have urinary arsenic values above approximately 0.6 ppm.

Margin of safety estimates for fetotoxicity

Using the limited data available from modern studies of the toxicity of CA, the EPA has attempted to estimate margins of safety (MOS) with regard to certain risks (OPP, 1981). In order to compute such values, estimates for CA uptake expressed in milligrams per hour (Table 8.1) must be converted to milligrams of CA per kilogram of body weight per day (mg/kg/d).

Values calculated for individuals in various use categories are given in Table 8.2. These estimated exposure values are based on assumptions of an eight-hour work day and a 70 kg body weight. MOS values were then calculated by dividing the daily exposure estimates by the no-observed-effect levels (NOELs) derived from the toxicity data.

The EPA estimate of the NOEL for fetotoxicity (OPP, 1981) is based on the observation of irregular palatine rugae in rats (Rogers et al., 1981). The EPA document states that this estimate is only a tentative value, especially since the rats were exposed orally, while pesticide applicators would be exposed dermally and by inhalation.

Other caveats might be added: the rat may not be a good model for CA effects (Marafante et al., 1982). Further, the body weight estimate of 70 kg is based on an average man, and for fetotoxicity the exposed parent would be a woman. Although females in late stages of pregnancy

would more nearly approximate males in total body weight, throughout much of gestation this would not be the case.

The worst-case estimates for occupational exposures are shown in Table 8.3. They were based on assumptions of 100% absorption of both dermal and inhaled CA and thus are likely to overstate significantly the actual probability of fetotoxicity.

When the EPA computed margins of safety associated with dietary exposure to CA, all values were greater than 5,000 (OPP, 1981). This was true even if it was assumed that 100% of the cotton crop was treated with CA. These high MOS values would be seen for either continued dietary consumption or single servings.

The calculated MOS data were taken to mean that risks to the fetus from dietary exposure to CA were negligible (OPP, 1981). It was also stated, however, that if dermal absorption rates were high, some occupational groups such as pilots and mixer-loaders would be at risk. In addition, it was suggested that respiratory exposure might be hazardous in certain situations (knapsack or hand sprayer use on ditch banks, rights of way, and home use) unless suitable respirators or masks were worn. Since such conclusions were based on somewhat improbable worst-case scenarios, however, it seems likely that there is only slight, if any, actual risk to individuals in any of the listed occupational groups.

The EPA has also published recommended maximum concentrations for organic arsenicals in streams in areas where such compounds are used as silvicides (EPA, 1977). The agency recognized that there are invariably natural background levels of arsenic in forest environments. Such levels would be present in addition to those from anthropogenic sources.

Based on the assumption of man as the critical species and a no-observed-effect level of 0.12 mg/l, criteria were set in terms of 24-hour mean concentrations in parts per million. Arsenic concentration maxima for organic arsenical silvicides were 0.1 ppm in all streams with flows of 10 ft^3/s or less, 0.05 ppm for larger streams and rivers whose water was classed as potable, and 0.1 ppm for all others. If natural background levels already exceeded the suggested maxima, an increase of up to 10% for no more than one year was considered acceptable.

These criteria were based originally on those for inorganic arsenite. The assumption was made that the silvicides would be pentavalent compounds, but no mention was made of the lesser toxicity of the methylated arsenicals actually involved.

Chapter 9

Overview: Summary, Conclusions, and Research Needs

The literature dealing with cacodylic acid (CA) is extensive. This can easily be seen from the bibliography of more than 400 references assembled for this monograph. Even so, there is still much to be learned about this interesting compound, particularly with regard to its human health effects. In the following pages an attempt will be made to highlight briefly the information contained in the previous chapters and to mention some specific needs for future investigation.

Chemical and physical properties

Cacodylic acid, $(CH_3)_2As(O)OH$, and its sodium salt have long been known to chemists. Their physical properties are generally known, but there is no universal agreement on some, such as the solubility and crystal structure of CA. Several production methods have been developed and some have been patented. CA is actually an amphoteric electrolyte, with a pk_a generally estimated near 6.3, making CA practical as a buffer for certain uses.

Beginning with the early work of Bunsen, numerous reactions of CA have been described. Of recent interest is the manner in which CA and MAA (methanearsonic acid) react with organic compounds containing thiol groups. How such reactions might affect biologically active molecules is of obvious significance and must be further resolved.

Analytical methods

Until recently, methods for analyzing methylated arsenicals were relatively crude or inefficient, and trace levels found in environmental samples could not be readily quantitated. There are now highly sensitive techniques that allow such analyses. Determination of methylated arsenicals can be accomplished by acid hydrolysis of samples, followed by separation on an ion-exchange resin or by high-pressure liquid chromatography.

Another common method employs the generation of arsines by use of a reducing agent and trapping of these volatile compounds in a liquid nitrogen cold trap. When the trap is allowed to warm, the arsines volatilize sequentially, and arsine and methyl- and dimethyl-arsines separate. After separation, quantitation is typically accomplished by atomic absorption spectrophotometry.

Production and uses

Commercial formulations containing CA or NaCA (sodium cacodylate), including Agent Blue (Phytar® 560-G), are commonly clear, yellowish brown liquids. Agent Blue contained 26.4% NaCA and 4.7% CA, along with a surfactant, an antifoam agent, sodium chloride (a byproduct of the production process), and water. It was produced by the Ansul Company, but today only

the Vineland Chemical and Vertac Chemical Companies are commercial producers of CA-based agricultural products.

Federally approved uses of these formulations include weed control in noncrop areas, weed control in nonbearing citrus orchards, lawn renovation, cotton defoliation, postemergent weed control in noncrop areas, crown kill of undesirable trees, and bark beetle control. Production volume is difficult to estimate accurately, but probably amounts to several hundred thousand kilograms annually in the United States.

Agricultural and related uses

CA, methanearsonic acid, and their sodium salts have been widely used in a variety of situations for a number of years. Among the most extensive uses of CA is the killing of weeds and broadleaf grasses. It has been used as a preemergence herbicide on crops and for killing vegetation indiscriminately, as on roadsides and ditch banks. CA is used as a defoliant and desiccant on cotton to aid in boll ripening as well as in removing leaves, both of which facilitate mechanical harvesting. This remains a current major use. CA has also been used as a silvicide, primarily to kill trees during commercial thinning in the northwestern United States.

CA has been investigated for use against a variety of beetles, primarily those infesting conifers, and its toxicity to beneficial insects, primarily honeybees, has also been examined. It was found to decrease brood survival of bark beetles, but was among the least toxic to bees of a large series of tested pesticides.

Environmental sources and fate

Inorganic arsenic can be metabolized to CA or MAA by microorganisms. Such organisms can also demethylate CA to arsines and to inorganic arsenic in both terrestrial and aquatic environments, forming a basis for so-called "arsenic cycling." Arsenic present in or added to the environment is oxidized, reduced, methylated, or demethylated, according to the conditions. The various arsenicals thus produced may then volatilize, leach, or remain bound to soils, particularly the clay fraction.

Due to such cycling, added arsenical pesticides do not generally build up to high levels in the soil. They tend to reach an equilibrium at which the amount lost by leaching and by volatilization as arsines is equal to the amount added by man.

Levels of methylated arsenicals in the aquatic environment are usually quite low. Values for CA range from 0.01 to 1.00 ppb (as arsenic), but have generally been below 0.3 ppb in samples analyzed to date.

Since aquatic organisms are continually bathed in water containing dissolved arsenicals, they have evolved means for dealing with arsenic toxicity, generally by incorporating arsenicals into more complex molecules. Algae seem to accumulate CA more extensively than do aquatic animals, but unlike methylmercury, CA does not appear to be biomagnified in food chains.

Whether used as herbicides or defoliants, methylated arsenicals are sprayed on plant surfaces. They can reach the soil if they miss the vegetation during application or if they are washed off the plants. Additional amounts enter soil by exudation from the roots or when killed plant material decays. When studies have been made of fields treated with CA for several years, little accumulation was usually detected except in cases where very high levels were applied, and there generally appeared to be no harmful effects on subsequent crops.

Arsenical pesticides such as CA enter plants primarily through absorption from the sprayed foliage, with uptake from the soil generally being only minor. Initial uptake is rapid but slows greatly as the plant becomes damaged, and locally applied arsenicals can be translocated through various parts of the plant.

Few attempts have been made to look at the uptake of methylated arsenicals from the environment by animals. Residues generally appeared quite low in such cases, with the exception of those found in certain snowshoe hares that were believed to have been poisoned by consumption of plants heavily contaminated with monosodium methanearsonate by tree thinning crews who carelessly emptied and washed their application equipment. These animals appear to be especially sensitive to methylated arsenic, but the source of that sensitivity remains in need of investigation.

Pharmacology

Unfortunately, although the rat is now recognized as an inappropriate model for research with arsenicals, since it retains such compounds much longer than do other species, much of the literature on CA is based on rat studies. At any rate, CA in rats has been found to be absorbed rather rapidly ($t_{1/2}$ oral = 248 minutes and by inhalation = 2.2 minutes). CA levels also decline rapidly after exposure, except of course in the rat.

CA and MAA are the metabolites of inorganic arsenic in man, with CA predominating. Twenty percent to 30% of inorganic arsenic is excreted without methylation. Most other mammalian species studied have been found to produce one or both methylated metabolites, with CA the more abundant.

When either CA or MAA was administered orally to humans, 75% or more was eliminated in the urine within four days. When MAA was administered, a significant proportion was methylated to CA, but when CA was given, it remained unchanged. CA loss in feces was not measured in these studies, but this was the excretion route for one-third of the ingested CA in rats.

Such evidence indicates that CA is the predominant arsenic metabolite in mammals. It is presumably produced as an attempt to partially detoxify inorganic arsenic, and it is readily excreted. The question remains as to whether a small portion of absorbed CA is metabolized to volatile compounds. Indeed, a garlic-like odor has been described following human exposure to CA.

Animal toxicology

Many tests for CA toxicity have been done with rats. In addition, a number of the toxicity studies were done by Industrial Bio-Test, a contract laboratory whose results have been questioned because of alleged irregularities. It is thus difficult to be confident of the validity of some of the available test results or their applicability to man.

CA and NaCA have been given orally to rats, resulting in a range of LD_{50} values from 600 to 2,600 mg/kg. Such results, along with data from other administration routes in a number of species, suggest that CA has only a moderate degree of toxicity. In comparison, for example, the oral LD_{50} for aspirin in the mouse and rat has been reported to be 800 and 1,000 mg/kg, respectively. Also, when CA was applied dermally to rabbits, the lethal dose appeared to be between 4 and 6 g/kg, a level indicative of low toxicity. Inhalation test results also suggest only a relatively low acute toxicity for CA.

Symptoms of oral CA poisoning generally include inactivity, loss of appetite, and diarrhea. In the case of inhalation toxicity, labored breathing, nasal discharge, and eye irritation have also been seen (in rats). Brief (five-minute) exposures of mice to CA dust by inhalation at concentrations up to 2 g/m^3 had only a moderate effect on respiration.

When animals that died from CA poisoning were necropsied, irritation of the gastrointestinal tract lining and bleeding were frequent findings.

When CA was added to water for LC_{50} tests on aquatic organisms, its toxicity was generally found to be relatively low. In fact, when surfactant-containing CA-based herbicide formulations were tested, the toxicity of the mixture appeared to be limited by that of the surfactant rather than by that of the arsenical.

There are relatively few data on longer-term exposure to CA. The major studies have employed livestock and poultry. In one such study, all test animals were given 10 daily oral doses. Intake of at least 25 mg/kg/d produced effects in cattle and sheep, while chickens were resistant and tolerated doses of around 100 mg/kg/d.

Poisoned animals had the symptoms previously listed for acute toxicity, along with damage to other organs such as kidneys, liver, spleen, and bladder. Monosodium methanearsonate (MSMA) and disodium methanearsonate (DSMA) were tested with similar results, but as was also the case with acute toxicity, the monomethylated arsenicals seemed more toxic than did CA.

In another test, CA (in Silvisar® 510) was given to cattle for three weeks at 10 mg/kg/d and then for five to six weeks at 20 mg/kg/d. It was lethal at this dose, and renal failure due to tubular degeneration was suggested as the cause of death.

When fish species diversity was examined following repeated spraying with Agent Blue (seven sprayings between July 1 and August 21, with a total of 7,892 liters containing 10,309 kg of herbicide), only one species at one of six sampling stations appeared to have decreased in numbers. A later study found a variety of vertebrate wildlife still in the test area.

In a laboratory test, NaCA levels of up to 1,000 ppm as arsenic in the water for 28 days had no effect on survival of immature rainbow trout or a variety of invertebrates. When 10- to 15-day-old mallard ducklings or bobwhite quail were fed diets containing 1,740 ppm of NaCA for five days, no ill effects were reported.

A few studies of CA's potential for causing birth defects in animals have been published. When pregnant rats and mice were given high oral doses of CA from days 7 to 16 of gestation, dose-related effects were seen in the fetuses, including weight reduction and delayed skeletal ossification. Cleft palates in mice and irregular palatine rugae in rats were the only morphologic anomalies. Effective doses were 400 to 600 mg/kg in mice and 40 to 60 mg/kg in rats.

In subsequent studies, mice given 1,200 mg/kg of NaCA intraperitoneally (close to the maternal lethal dose) once during midgestation showed increased rates of skeletal malformation but few grossly observable defects. In a similar study with hamsters, doses of 900 to 1,000 mg/kg resulted in increased incidences of gross and skeletal malformations. The dose levels employed in these injection studies were maternally toxic and resulted in some maternal deaths, however, and lower doses were not associated with obvious effects on the offspring.

There appear to be no reports in the literature on the reproductive success of humans or animals exposed to CA. In particular, there are no data on what effect, if any, paternal CA exposure would have on reproductive success or on subsequently sired offspring. Such data might be helpful in understanding whether effects might be expected from exposure of men who served in South Vietnam.

When tests for mutagenicity were done with CA, results of microbial screens were invariably negative. Some tests with eukaryotes were positive, however, including results with yeast and with mouse lymphoma cells, although the latter results were positive only at cytotoxic doses. Results of a sex-linked recessive lethal test with *Drosophila* were ambiguous, and high doses tended to result in sterility of test males.

CA was not found to cause DNA damage in bacteria or in cultured fibroblasts. Further studies of chromosome damage and related effects were negative for sister chromatid exchange. They were positive for clastogenic response in mice, but only at highly toxic doses. Older studies found that CA could inhibit mitosis, with a colchicine-like effect in mice. Such results suggest that if CA is a mutagen, it is probably effective only at high doses. Further studies with more adequate controls in mammalian systems would be useful.

CA has also been tested for carcinogenicity, with negative results. The *in vivo* tests were inadequate as to numbers and protocols, and further testing would be warranted.

It should be kept in mind, however, that animal carcinogenicity tests with inorganic arsenicals have generally yielded negative results, although human data suggest that some arsenicals are carcinogenic. Negative animal test results with an organic arsenical may thus not be entirely reliable. Perhaps the tools of molecular biology will eventually resolve such problems. Until that time, the animal data must suffice except in cases where sufficient human epidemiologic results are available.

CA has been tested for toxicity to microorganisms, both in the laboratory and in the field. At high levels it has some adverse effects on bacteria and fungi, but many such organisms appear able to adapt over time to the presence of CA.

Unfortunately, little is known about the mechanism(s) by which CA exerts toxic effects. Enzyme inhibition is a mechanism suggested by several authors. The general assumption is that SH-dependent enzymes are particularly vulnerable. In addition, it is now well recognized that the rat erythrocyte has an unusual affinity for CA, with the arsenical apparently being irreversibly bound to rat hemoglobin. Such binding has been attributed to high levels of reduced glutathione reductase in the rat red cell, with the enzyme acting on CA to enhance its ability to bind to the SH groups of the hemoglobin tetramer, but more evidence is needed.

Human exposure and toxicology

Relatively little recent literature is available. Although arsenic levels in the human diet are regularly monitored, no attempt has been made to distinguish methylated from inorganic arsenic.

Occupational exposure is the most likely source of contact with CA. In a study of physicians' reports of job-related accidental exposure to pesticides in California from 1975 through 1977, a total of 4,326 cases of illness was recorded. Thirty-four were believed due to methylated arsenicals; two-thirds were attributed to CA and one-third to MSMA.

The symptoms noted included nausea, vomiting, diarrhea, and abdominal pain (9 cases), eye irritation (13 cases), and contact dermatitis or allergic rash (12 cases). The eye and skin problems were said to be mild and to respond well to treatment. No long-term illness was reported, even in cases of pesticide ingestion.

Both CA and MSMA were irritants and could cause a mild, transient, easily treatable conjunctivitis when the eye was exposed. Dermal contact, especially if the herbicide was left on the skin for a time, typically resulted in contact dermatitis. At times there was a short-lived allergic response. Ingestion could result in nausea, colic, vomiting, and diarrhea, but only rarely in other symptoms. Again, exposure did not appear to result in long-term toxic effects.

In all cases examined, ill effects were due to careless or improper work practices or faulty equipment which resulted in direct contact with the arsenical. None of the reported cases was the result of approved application practices with proper safety devices and intact spraying equipment.

In a more recent case, a worker who was repeatedly sprayed over five days with MSMA did develop an apparently permanent severe neuropathy along with palmar keratoses. He had also been exposed to two other pesticides, however, which made the etiology in this case less than clearcut.

Two other studies involved forest workers applying CA or MSMA in tree thinning. Some increases in urinary arsenic were found, especially when safety precautions had been lax, but no obvious health effects were reported that could be definitely attributed to CA exposure.

The EPA's Office of Pesticide Programs has estimated potential exposure to CA, based on a number of scenarios including dietary intake and various work exposures. The FDA has established maximum permissible levels of CA in human foods and certain livestock feeds. Dietary levels are likely to be well below these suggested guidelines, judging from actual dietary samples. According to the EPA's worst-case scenarios, CA toxicity from dietary exposure should not be a significant concern as long as the FDA tolerance levels are not greatly exceeded.

According to scenarios for occupational exposure using toxicity data derived from rats and assuming 100% absorption from both dermal contact and inhalation, some exposures of pregnant women (e.g., pilots and mixer-loaders) might result in a risk of harm to the conceptus; some respiratory exposures were also suggested as possibly hazardous unless appropriate safety precautions were taken. Since these conclusions were based on rather unlikely worst-case premises, however, there is probably little cause for alarm. This is especially likely when the data base was derived from rat studies.

There are also several references in the literature from the early 1900s that describe the use of CA as a drug for the treatment of a variety of illnesses. It was eventually discarded for such use due to ineffectiveness and toxic side effects at the high doses employed. Adverse effects included venous fibrosis at the injection site when CA was given intravenously and sometimes severe nephritis.

Suggested doses were 60 mg daily given orally or 200 mg by subcutaneous injection. Intravenous injections of 500 mg every three to four days for four to six doses were said to have caused blood and albumin in the urine. Such results from the older clinical literature indicate that although CA was apparently not highly toxic, it did have significant adverse effects when given chronically at relatively high dose levels.

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A: Acre.

AAS: Atomic absorption spectrophotometry (*q.v.*).

Abscission layer: A layer that forms between two plant parts before their separation, as between leaf and stem before loss of the leaf.

Acaudal: Lacking a tail (posterior region).

Acid equivalent: The amount of a salt (e.g., NaCA) equal to a given amount of the free acid form of the same compound (e.g., CA).

Acropetal: In plants, moving upward from the base toward the apex.

Actinomycetes: Organisms of the genus *Actinomyces*, a type of fungus.

Acute toxicity: Injury resulting from a single exposure to a toxin or from multiple exposures within a 24-hour period.

Adenine nucleotide deaminase: An enzyme that removes an amino (NH_2) group from the nucleotide adenine.

Adenoma: A benign, i.e., noncancerous, tumor or overgrowth arising from a gland.

Adsorption: The attachment of one substance to the surface of another.

Aerobic: In the presence of oxygen.

Aerosol: A solution of a drug or other chemical finely atomized or dispersed in air.

Agent Blue: Phytar® 560-G, an arsenical herbicide (used in South Vietnam) containing 26.4% NaCA and 4.7% CA.

Agent Orange: An herbicide (used in South Vietnam) containing 2,4-D and 2,4,5-T.

Agent White: An herbicide (used in South Vietnam) containing the triisopropanolamine salts of 2,4-D and picloram.

Alkylarsine: Methyl-, dimethyl-, or trimethylarsine or related compounds.

Amenorrhea: Absence or abnormal discontinuation of the menses.

Aminolevulinic acid: $COOH(CH_2)_3CONH_2$, a molecule involved in porphyrin biosynthesis.

Ammonification: Ammonia formation by bacterial action on proteins.

Amphoteric electrolyte: A substance that dissociates into both hydrogen (H^+) and hydroxyl (OH^-) ions.

Anaerobic: Without oxygen.

Anemia: The presence in the circulating blood of a lower than normal number of red blood cells and/or a smaller than normal concentration of hemoglobin.

Anion: A negatively charged ion.

Anorexic: Having no appetite.

Ansar® 138: An arsenical herbicide containing 65.6% CA.

Ansar® 160: An arsenical herbicide containing 29.3% CA.

Ansar® 170: An arsenical herbicide containing 51.3% MSMA.

Ansar® 170 H.C.: An arsenical herbicide containing 59% MSMA.

Ansar® 184: An arsenical herbicide containing 63% DSMA.

Ansar® 529 H.C.: An arsenical herbicide containing MSMA.

Ansar® 560: An arsenical herbicide containing 22.73% NaCA and 3.88% CA.

Ansar® 8100: An arsenical herbicide containing 81.4% DSMA.

Arsenate: Arsenic in the pentavalent state; As^{5+} or As(V).

Arsenic cycling: Addition of arsenic to and removal of arsenic from the environment and the factors that influence the rates of these processes.

Arsenic pentoxide: Arsenic acid anhydride; As_2O_5.

Arsenic trioxide: Arsenous acid; As_2O_3.

Arsenite: Arsenic in the trivalent state; As^{3+} or As(III).

Arsenobetaine: $(CH_3)_3As^+CH_2COO^-$ an organic arsenical produced as an arsenic metabolite by certain fishes and invertebrates.

Arsine: AsH_3, a volatile, toxic arsenical.

As: Arsenic as an element not combined with any other element, or as one part of a chemical compound, as in As_2O_3.

As(III): Arsenite (trivalent arsenic).

As(V): Arsenate (pentavalent arsenic).

^{74}As-CA: Cacodylic acid labeled with a radioactive isotope of arsenic to aid in detecting the compound.

Ascites: Excessive fluid in the abdominal cavity.

Asthenic: Lacking or having lost strength and energy, weak.

Ataxia: Decreased coordination of muscular movement.

Atomic absorption spectrophotometry: An analytic technique based on absorption of light at specific wave lengths by atoms.

Autoradiography: A technique using photographic emulsion to visualize the location of radioactive materials in experimental samples.

Autotrophic: Pertaining to organisms that can produce organic nutrients from CO_2 and inorganic salts; self-nourishing.

BAL: British anti-lewisite or 2,3-dimercaptopropanol, a chelating agent capable of binding arsenic.

Basipetal: In plants, moving from the apex downward.

Benthic: On the bottom or at the greatest depths of a body of water.

Benzphetamine *n*-demethylase: An enzyme that demethylates (removes a methyl group from) certain aromatic hydrocarbons.

Biliary: In or pertaining to the bile.

Bioaccumulation: Selective absorption and retention of chemicals from their environment by living organisms.

Biomagnification: An increase in the concentration of a substance in organisms higher in a food chain, due to retention in the tissues.

Biomass: The mass (weight) of living organisms within an area or environment.

"Blind": In drug efficacy research, a technique in which test drugs or experimental modifications are coded so that neither those administering them, the experimental subjects, nor those evaluating the results know which test subjects received a given treatment or remained untreated as controls until all data have been analyzed.

Brachycaudal: Having a shortened posterior.

Buffer: A substance capable of decreasing the magnitude of change in hydrogen ion concentration (pH) that would otherwise result from addition of a given amount of an acid or a base to a solution.

CA: Cacodylic acid (*q.v.*).

Cacodyl: Tetramethyldiarsine, $(CH_3)_2As\text{-}As(CH_3)_2$, an arsenical with a strong garlic-like odor.

Cacodylic acid: $(CH_3)_2As(O)OH$, an organic arsenical containing two methyl (CH_3) groups. Also known as hydroxydimethylarsine oxide, dimethylarsinic acid, or CA.

^{14}C-Cacodylic acid: CA labeled with a radioactive carbon isotope to aid in detecting the compound.

Calcareous: Pertaining to or containing lime (calcium oxide).

Carcinogen: An agent capable of causing the formation of a malignant tumor (cancer).

Carcinogenicity: Ability to cause malignant tumors (cancers).

Cation: A positively charged ion.

Chelate: To bind firmly and sequester a metallic ion.

Chemaid®: A defoliant containing NaCA.

Chemotherapy: Therapy based on the use of chemicals (drugs).

Chloroplast: Structures found in the cells of green plants. They contain chlorophyll and function in photosynthesis.

Chorea: A nervous disorder with involuntary, irregular, persistent movements of the extremities, trunk and face.

Chromatography: A method of separating and analyzing chemical mixtures based on their preferential adsorption onto solid or liquid adsorbent substances.

Chronic toxicity: Toxic effects attributed to long-term exposure to an injurious agent, generally over a substantial portion of the test subject's life span.

CI: Confidence interval. See confidence limits.

Clastogenic: An agent capable of causing breaks in chromosomes.

Clearance: Removal from the blood.

Cleavage: Division of the fertilized egg into increasing numbers of smaller cells early in embryonic development.

Cloned cells: Cells grown in culture, all of which derive from a common original cell and share the same genetic makeup.

cm: Centimeter, a metric unit of measure equal to one-hundredth of a meter, 2.54 cm equaling 1.0 inch.

Colchicine: A plant-derived chemical (alkaloid) capable of arresting cell division.

Cold-trapping: Causing materials carried in a gas to freeze and precipitate out so they will be available for analysis.

Conceptus: The products of conception; everything that results from the development of a fertilized egg.

Confidence limits: In statistics, the limits of an interval that has a specified probability (generally 95% or 99%) of containing the true population mean.

Conjunctivitis: Inflammation of the conjunctiva, the delicate membrane lining the eyelids, and the whites of the eyes.

Coproporphyrin: A porphyrin compound formed in the intestine from bilirubin, a pigment derived from hemoglobin.

Cotyledon: The seed leaf of a plant embryo.

Cysteine: A sulfhydryl-containing amino acid.

Cytochrome P-450: Hemoproteins present in liver and other tissues which aid in the processes of drug metabolism.

Cytoplasm: The protoplasm of a cell, exclusive of the nucleus.

Cytotoxic: Toxic to cells.

dbh or DBH: Diameter (of a tree trunk) at breast height.

Defoliant: An agent capable of causing leaf shedding by plants.

Defoliation: Process of causing leaf shedding by plants.

Deliquescent: Tending to become liquid by absorbing moisture from the air.

Demethylation: Removal of a methyl (CH_3) group from a molecule.

Dermal: On or associated with the skin.

Dermatitis: Inflammation of the skin.

Desiccation: Drying, as of plant parts.

Detection limit: The concentration of an element in dilution that can be detected with 95% certainty.

Detoxification: The process of making a toxic substance less harmful.

Differential pulse polarography: An analytic technique for determining the composition of dilute electrolyte solutions.

Diluent: Liquid used to dissolve a chemical administered in drug or other chemical testing.

Dimethyl: Containing two methyl (CH_3) groups.

Dimethylarsine: $(CH_3)_2AsH$, a volatile organic arsenical.

Disodium methanearsonate: An arsenical herbicide, the disodium salt of methanearsonic acid; $CH_3AsNa_2O_3$.

Diversity: The number of species in an ecosystem and the relative abundance of the individuals in those species.

DNA: Deoxyribonucleic acid, the basic chemical of which genes are constructed.

Dominant lethal: A gene that can cause an organism to die when present in the heterozygous condition (one copy).

D-penicillamine: A chelating agent sometimes used to treat poisoning with arsenicals.

DSMA: Disodium methanearsonate (*q.v.*).

Dyserythropoiesis: Abnormal production of red blood cells.

Ecosystem: The entire set of interacting organisms and their interactions with one another and with their environment.

Eczema: An inflammatory skin disease.

Edema: Abnormal accumulation of fluid in tissues; edema can cause swelling felt and seen immediately beneath the skin and in deeper structures.

Edematous: Exhibiting edema.

EDTA: Ethylenediaminotetraacetate, a chelating agent used to bind cations.

EEC: Estimated environmental concentration.

Embryo culture: Maintaining a developing embryo in an artificial environment (outside the mother's uterus).

Encephalocele: Hernia of the brain through a congenital defect (opening) in the skull.

Enteritis: Inflammation of the intestine.

Enthalpy of fusion: The negative of the heat required to melt a substance.

Enzyme inducer: An agent capable of stimulating the formation of enzymes, particularly chemical-metabolizing enzymes.

EPA: U.S. Environmental Protection Agency.

Erythematous: Having reddened skin due to congestion of the capillaries.

Erythrocyte: Red blood cell.

Estuarine: Formed or deposited in an estuary (the wide mouth of a river where the tide meets the river's current).

Eukaryote: Organisms whose cells have true nuclei that divide by mitosis (e.g., "higher organisms," as opposed to viruses, bacteria, and the like but including unicellular yeasts).

Euphoria: A sense of well-being. In psychiatry, an abnormal or exaggerated sense of well-being.

Exencephaly: A birth defect characterized by the absence of all or part of the roof of the skull, leaving the brain exposed.

Exogenous: Coming from outside an organism, not made internally.

Exudation: Escape of fluid, cells, and cellular debris from blood vessels, usually as a result of inflammation.

Fauna: Animal life.

Fecal: In or pertaining to feces.

Fetal: Pertaining to a fetus.

Fetus: The later stages of a developing mammal; in humans, from the third month until birth.

Fetotoxicity: Toxic effects to the fetus, such as growth stunting, delayed maturation, or decreased viability.

Flexion contracture: Fixation of one or more joints in the bent position due to changes in the muscles or the tissues around the joints.

Flora: Plant life.

Foliar spray: Spray applied to plant leaves or foliage.

Frill: A series of shallow cuts (generally made with an ax) girdling the trunk of a tree.

g: Gram; equal to 15.4 grains.

Gamete: Male or female reproductive cell.

Gas-liquid chromatography: A form of chromatography in which volatile samples carried in a gaseous moving phase are separated by use of a liquid adsorbent.

Gastric: Pertaining to the stomach.

Gastric intubation: Administration by stomach tube; gavage.

Gavage: Introduction into the stomach by way of a tube passed down the throat (esophagus).

Gestation: Pregnancy.

GFAAS: Graphite furnace atomic absorption spectrophotometry (*see* Atomic absorption spectrophotometry).

GLC: Gas-liquid chromatography (*q.v.*).

Glc-6-P phosphohydrolase: An enzyme that removes the phosphate (PO4) group from glucose-6-phosphate; glucose-6-phosphatase.

Glowon®: An arsenical herbicide containing 44.9% MSMA.

Glucose: A simple sugar; blood sugar.

Glutathione reductase: An enzyme that acts to reduce (add hydrogen to) glutathione.

Graphite furnace atomic absorption spectrophotometry: GFAAS; use of a furnace constructed of graphite to atomize samples for quantitation by atomic absorption spectrophotometry.

Gross malformation: An externally visible birth defect.

ha: Hectare (10,000 m^2, or about 2.47 A).

Half-life: The time required for one-half of the amount of a chemical present to be removed, destroyed, or changed to another form.

HCl: Hydrogen chloride, often used as an abbreviation for the aqueous solution hydrochloric acid.

Hematocrit: The volume percentage of red blood cells in whole blood.

Hematuria: Presence of blood in the urine.

Hemoglobin: The oxygen-carrying protein of red blood cells.

Hemolytic anemia: Anemia (*q.v.*) caused by blood cell destruction (or hemolysis) and the resultant liberation of hemoglobin.

Hepatic: Pertaining to the liver.

Hepatocyte: A type of liver cell.

Herbicide: A chemical that injures or kills plants.

Heterozygous: Possessing different alleles (genes at the same locus) for a given character.

High-performance liquid chromatography: *High-pressure liquid chromatography (q.v.).*

High-pressure liquid chromatography: A form of chromatography employing liquid adsorbents in a pressurized system for high resolution analysis of chemical mixtures.

Hill reaction: The part of photosynthesis that involves evolution of oxygen.

Histiocyte: A large phagocytic interstitial cell forming part of the reticuloendothelial system.

Histopathologic: Pertaining to the process of or the results of an examination of tissues with microscopy for evidence of injury, disease, or other abnormality.

HPLC: *High-pressure liquid chromatography (q.v.).*

Hydrolysis: The splitting of a molecule by addition of a hydroxyl group and a hydrogen ion derived from water.

Hydronephrosis: Distention of the kidney's internal cavity with urine as a result of ureteral obstruction.

Hygroscopic: Able to attract or absorb moisture from the air.

Hypoplastic: Incompletely developed.

ICP: Inductively coupled plasma-atomic emission spectrometry (*q.v.*).

Inductively coupled plasma-atomic emission spectrometry (ICP): An analytic technique for determining the concentration of elements.

Inflorescence: The structure or arrangement of flowers.

Inorganic: Chemical compounds in which carbon is not the principle element (with the exception of cyanates, cyanides, and carbonates).

Intoxicated: Exhibiting effects of toxicity; poisoned.

Intraalveolar: Within the alveoli or tiny air sacs of the lungs where gases exchange between the blood and the air.

Intramuscular: Within a muscle.

Intraperitoneal: Within the peritoneal (abdominal) cavity.

Intratracheal: Within or through the trachea.

Intravenous: Within a vein.

In vivo: In a living organism.

Ion-exchange chromatography: A form of chromatography in which the adsorbents used are ion-exchange resins.

i.p.: Intraperitoneally.

i.v.: Intravenously.

kg: Kilogram, a metric unit of weight equal to 1,000 grams, or about 2.2 pounds.

Kinetics: Turnover or rate of change per unit of time.

Lactating: Producing milk.

LC_{50}: Median lethal concentration; the concentration of a toxin in their environment calculated as capable of killing 50% of the animals exposed to it under the same test conditions.

LD_{50}: Median lethal dose; the dose of a toxic agent calculated as capable of killing 50% of the animals exposed to it under the same test conditions.

Leaching: Removal from the soil by solution in rain or irrigation water.

^{3}H-Leucine: Tritiated leucine (*q.v.*).

Leukopenia: Reduction in the number of leukocytes (white blood cells).

Litter: Accumulation of fallen leaves on the forest floor forming the surface layer, usually slightly decomposed.

Lymphocyte: A variety of white blood cell arising in the lymphatic tissue.

MAA: Methanearsonic acid (*q.v.*).

Malformation: An anatomic defect, especially one severe enough to impair an individual's ability to function normally.

MAMA: Monoammonium methanearsonate (*q.v.*).

Meristematic tissues: Tissues associated with the growing and differentiating tips of plant stems.

Metabolism: The chemical processes carried on in living organisms.

Metabolite: Any compound produced by metabolism.

Metaphase: The second of the four phases of mitosis (cell division).

Methanearsonic acid: $CH_3AsO(OH)_2$, a monomethylated metabolite of arsenic.

Methyl donor: A compound capable of transferring its methyl (CH_3) group to another compound.

Methylarsine: CH_3AsH_2, a volatile organic arsenical.

Methylated arsenical: An organic arsenic compound containing one or more methyl (CH_3) groups.

Methylation: Addition of a methyl (CH_3) group to a molecule.

mg: Milligram, a metric unit of weight equal to one-thousandth of a gram (10^{-3} g).

mg: Microgram, a metric unit of weight equal to one-millionth of a gram (10^{-6} g).

Microflora: Microscopic plant life in a particular environment, e.g., the intestinal bacteria.

Micrognathia: Abnormal smallness of the jaw.

Microhabitat: An individual's immediately surrounding environment.

Microsomal: Pertaining to microsomes.

Microsome: A fragment of the endoplasmic reticulum, a system of intracellular membranes.

Mitosis: Cell division.

Mitotic crossing over: Exchange of genetic material between homologous chromosomes during mitosis.

Mixed-function oxidase system: An enzyme system that metabolizes drugs and other chemicals, generally to less harmful or more easily excreted molecules.

MLD: Minimum lethal dose.

mM: Millimolar, one thousandth of a gram molecular weight (mole).

Monoammonium methanearsonate: Ammonium salt of methanearsonic acid.

Monomethyl: Containing one methyl (CH_3) group.

Monosodium methanearsonate: The monosodium salt of methanearsonic acid, CH_4AsNaO_3, an arsenical herbicide.

Moribund: Dying.

MOS: Margin of safety.

MRI: Midwest Research Institute, Kansas City, MO.

MSMA: Monosodium methanearsonate (*q.v.*).

Mutagenic: Capable of causing mutations.

Mutagenicity: Ability to cause mutation.

Mutation: A change in the DNA potentially capable of being transmitted to offspring (or daughter cells in the case of a somatic cell's DNA).

Mycelial: Pertaining to the vegetative body of those fungi that are composed of masses of filaments called hyphae.

N_2: Nitrogen in the molecular form.

NaCA: Sodium cacodylate, the sodium salt of cacodylic acid.

Necropsy: Examination of a dead animal or person to determine the cause of death and any pathological changes; autopsy, postmortem.

Necrosis: Death of cells or tissues, in contrast to death of the entire organism.

Nephritis: Inflammation of the kidney.

Nephrosis: Any disease of the kidney, but particularly those involving degeneration of the renal tubules (nephrons).

Neuropathy: Disease or disorder of the nervous system, usually of the peripheral nervous system.

Neuroses: Mental disorders not due to structural changes (and without the disruption of thought or loss of contact with reality found in the more severe disorders, i.e., psychoses).

Neutrophil: A type of white blood cell that is stainable by neutral dyes.

ng: Nanogram, a metric unit of weight equal to one-billionth of a gram (10^{-9} g).

Nicotinamide: A "B" vitamin, the amide of nicotinic acid.

Nitrification: Bacterial oxidation of ammonia to nitrite and nitrate in the soil.

NMR: Nuclear magnetic resonance.

Nodular foci: Lumps of cells observed at the beginnings of a morbid (disease) process.

Nodule: A small solid swelling or clump of cells distinct from the surrounding tissue.

NOEL: No-observed-effect level.

OPP: Office of Pesticide Programs (of the U.S. Environmental Protection Agency).

Orbital sinus: A venous cavity associated with the space (orbit) containing the eyeball.

Organic: In chemistry, compounds composed of carbon combined with hydrogen with or without oxygen, nitrogen, or other elements.

Osmolality: A property of solutions dependent on the concentration of dissolved particles per unit of solvent; solutions tend to become more dilute and so increase in volume.

Ossification: Formation of bone, generally by mineralization of some other tissue.

Osteosarcoma: A malignant tumor (cancer) derived from undifferentiated connective tissue cells originating from bone.

Oxidation: A chemical reaction that increases the oxygen content of a compound or in which a compound or radical loses electrons.

Palatine rugae: The transverse ridges extending outward on both sides of the midline (raphe) of the palate.

Palmar keratosis: Thickening of the outer horny layer (stratum corneum) of the skin of the palms.

Paraquat: An herbicide with the chemical formula 1,1'-dimethyl-4,4'-bipyridylium dichloride.

Parenteral: Administered by a route other than through the digestive system, usually by injection.

Paresthesia: An abnormal sensation, such as burning or prickling, without any stimulus to produce it.

Partition coefficient: The ratio of the concentration of a dissolved material (solute) contained in one solvent compared to that contained in another solvent when the two solvents are in contact and they have reached equilibrium.

Pathologic: Indicative of or caused by a diseased state.

Pathology: The study of the structural and functional changes that cause or are caused by disease.

Pentavalent: Having a chemical valence of 5.

Peptone nitrogen: Nitrogen derived from hydrolyzed protein.

Perivascular: Situated around a blood vessel.

Pesticide: A chemical used to kill plant or animal pests.

Petechiae: Small, unraised, round purplish-red spots caused by intradermal or subcutaneous hemorrhage.

pg: Picogram, a metric unit of weight equal to one-trillionth of a gram (10^{-12} g).

pH: An expression of the hydrogen ion (H^+) concentration of a solution; a measure of acidity or alkalinity.

Pharmacokinetics: Study of drug metabolism and action emphasizing the timing of absorption, duration of effect, distribution within the organism, metabolism, and excretion of the drug.

Pheromones: Chemicals used for communication among individuals of the same species.

Phloem: The part of the vascular bundle of plants consisting of sieve tubes and their parenchymatous tissue.

Phytar® 138: Ansar® 138 (*q.v.*).

Phytar® 160: Ansar® 160 (*q.v.*).

Phytar® 560: Ansar® 560 (*q.v.*).

Phytar® 560-G: The herbicide used in South Vietnam as Agent Blue, containing 26.4% NaCA and 4.7% CA.

Phytoplankton: Microscopic aquatic plant life.

Phytotoxic: Toxic (harmful) to plants.

Picloram: 4-amino-3,5,6-trichloropicolinic acid, a major component of the herbicide Agent White.

Plasma: The fluid portion of the blood.

Plethysmography: The process of recording changes in the size of a body part caused by the circulation of blood through the part.

p.o.: Per os; administered by mouth.

Point mutation: Mutation of a single gene due to change in its DNA at a single location.

Porphyrin: Any of a group of iron- or magnesium-free cyclic tetrapyrrole derivatives which are the basis of respiratory pigments; chemicals in living matter capable of combining with iron or magnesium to form a component of molecules such as hemoglobin or chlorophyll.

ppb: Parts per billion, one part in 1 billion parts.

Preimplantation: Before implantation of a developing embryo in the lining of the mother's uterus (womb).

Primary carcinogen: A direct-acting carcinogen (cancer-causing agent).

Psoriasis: A chronic, recurring skin disease that characteristically causes patches covered with silvery scales.

Pulmonary: Pertaining to the lungs.

Pyruvic acid: 2-oxopropanoic acid, an intermediate compound in sugar metabolism.

Rate constant: A numerical constant in a rate-of-reaction equation for a chemical reaction.

Recessive lethal: A gene that causes the death of an organism when it is present in the homozygous condition (as two copies), but not in the heterozygous condition (one copy).

Reduced glutathione: A tripeptide composed of glutamic acid, cysteine, and aminoacetic acid. It is present in red blood cells and protects them from the oxidant and hemolytic effects of certain drugs or other chemicals.

Renal: Pertaining to the kidney.

Respiratory exposure: Exposure by inhalation.

Reverse mutation: Reversion of a mutant gene to the original form.

Rhinorrhea: Free discharge of a thin nasal mucus; "a runny nose."

Rhizome: The subterranean root stock of a plant.

Sarcoma: A malignant tumor (cancer) derived from the connective tissue.

s.c.: Subcutaneously.

SDDC: Silver diethyldithiocarbamate (*q.v.*).

Sensitivity: In atomic absorption spectrophotometry, the concentration of an element in solution that will cause 1% absorption at the wave length of light used for analysis.

Sex-linked: The genetic traits due to genes located on the female sex (X) chromosome.

SH-group: Sulfhydryl group; sulfur plus hydrogen.

Silver diethyldithiocarbamate: A compound used in colorimetric analyses of arsenic compounds; abbreviated SDDC.

Silvicide: A chemical agent that injures or kills trees or brush.

Silvisar® 510: A silvicide containing 46% CA and 8.3% of the triethanolamine salt of CA.

Sister chromatid exchange: Exchange of genetic material between the two daughter strands of a duplicated chromosome.

Sodium arsenate: Na_2HAsO_4; a toxic pentavalent inorganic arsenical.

Sodium arsenite: $NaAsO_2$; a toxic trivalent inorganic arsenical.

Sodium borohydride: Sodium tetrahydroborate or $NaBH_4$, a reducing agent used to convert arsenic compounds to arsines by transferring hydrogen to them.

Speciation (of arsenic): Determination of the particular arsenic compounds present.

Spermatogenesis: Production of spermatozoa.

Spina bifida: A class of birth defects characterized by an abnormal opening in the bony casing of the spinal cord.

Spindle poison: A chemical that can inhibit cell division by damaging the spindle apparatus (microtubules) that guides the separating chromosomes.

Spirochete: A microorganism of the type that causes syphilis.

Subacute toxicity: Injury resulting from exposure to a toxin over a moderate period of time (e.g., 90 days for rodents).

Subcutaneous: Beneath the skin.

Supernumerary ribs: Extra ribs present in addition to the usual number for an animal species.

Surfactant: A surface-active agent such as a detergent.

Syncope: Fainting.

T: Ton.

Tail bud: The extension of the posterior portion of an early embryo in the region of the primitive streak and node.

Telophase: The last of the four major phases of mitosis (cell division).

Teratogenic: Capable of causing birth defects, either anatomic or functional.

Teratogenicity: The ability to cause birth defects.

Teratology: The study of birth defects.

Tetramer: A molecule composed of four subunits.

TGM: Thioglycolic acid methylester, a chemical agent used to form volatile derivatives for analysis by GLC.

TLC: Thin-layer chromatography, a technique used for separating and identifying compounds.

TMAO: Trimethylarsine oxide (*q.v.*).

alpha-Tocopherol: Vitamin E.

Tocopherol acetate: Acetate derivative of vitamin E.

Tocopherol quinone: Quinone derivative of vitamin E.

Tolerance level: The maximum amount of a harmful chemical allowed in human foods or livestock feeds under Federal regulation.

Toxicologic: Pertaining to the study of poisonous (toxic) substances.

Translocation: Transfer of materials from one part of a plant to another.

Trap trees: Trees treated so as to attract and kill harmful tree-infesting insects, such as bark beetles.

Trimethylarsine: $As(CH_3)_3$, a volatile arsenic compound.

Trimethylarsine oxide: $(CH_3)_3AsO$, a volatile organic arsenical.

Tritiated leucine: An amino acid labeled with a radioactive hydrogen isotope to aid in its detection.

Trivalent: Having a chemical valence of 3.

trp⁻: Bacterial strains that cannot grow without tryptophan in their culture medium.

Tubular epithelium (kidney): The cells lining the kidney tubules.

Unscheduled DNA synthesis: Synthesis used to repair damage to the DNA rather than to replicate chromosomes.

UV: Ultraviolet.

Venous fibrosis: Formation of fibrous tissue associated with a vein.

Visceral defect: Birth defect (malformation or malfunction) of an internal organ (viscera), e.g., heart or intestine.

Xylem: The woody portion of the vascular bundle of plants; contrasted with phloem.

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