

2 Arsenic

Arsenic (As) is a metalloid that occurs naturally in water and soil. It is also released from a number of human activities including mining, petroleum and natural gas extraction, wood preservation, and burning coal.⁹⁻¹⁶

Although As is rare in nature as a pure element, both inorganic and organic forms of As are commonly found in a number of different oxidation states, of which two (+3 and +5) occur in soil, water, and vegetation. Inorganic forms of As^{III} (e.g. the arsenite ion) may be found under reducing conditions; however, As^V (e.g. the arsenate ion) predominates in surface waters containing considerable dissolved oxygen.¹⁷ Organic arsenical compounds have also been used as herbicides, insecticides, and drugs.

Arsenic was one of the first drugs to be used successfully against the syphilis organism in humans. The organic arsenical roxarsone (4-hydroxy-3-nitrobenzenearsonic acid) is still used to control coccidia in poultry and swine. More recently, arsenic trioxide (As₂O₃) has been suggested for treatment of promyelocytic leukemia and multiple myeloma.¹⁸

Most of what was known about the toxicity of As prior to the 1980s was based upon the direct cytotoxic effects of As.¹⁹ More recently, however, chronic consumption of low-level As-contaminated drinking water has become associated with a variety of chronic maladies in human beings, including cardiovascular disease, “black-foot disease,” diabetes mellitus, spontaneous abortion, and, especially, cancer.^{9,20-22} Conversely, some reports^{23,24} suggest a hormetic (beneficial) effect of As at very low doses. So far the majority of the evidence for chronic effects in humans consists of epidemiological studies, but mechanistic, toxicologic explanations are appearing in the literature. The latter are important because they suggest these effects (and therefore dosages) are not likely relevant to our species of interest (see below for details).

The nomenclature of the arsenicals is often bewildering. In this text, the following conventions are used: the trivalent, methylated metabolites (mono)methylarsonous acid, and dimethylarsonous acid will be abbreviated as MMA^{III} and DMA^{III}, the pentavalent metabolites (mono)methylarsonic acid, and dimethylarsinic acid as MMA^V and DMA^V, and the inorganic ions such as arsenite and arsenate as iAs^{III} and iAs^V.

Essentiality

Although the physiological function(s) of As remains unknown, experiments with As deprivation in many species suggest As may be an essential element.²⁵ For example, rats maintained on a diet containing 30 ppb As exhibited decreased growth rate, rough hair coats, and decreased hematocrit levels when compared to controls supplemented with 4.5 ppm As.²⁶ Lactating goats consuming a diet containing less than 10 ppb As had decreased growth rates, decreased milk production, lower birth weights, and a higher incidence of mortality.²⁷ Female miniature pigs on a low As diet produced smaller piglets, and only 62% of pigs were reported to give birth when compared to pigs supplemented with 350 ppb As.²⁷ The results of experimentally induced deprivation in both *in vivo* and *in vitro* experiments suggest As plays a role in the methylation of both proteins and genetic molecules.²⁵ Although this and other, similar research is scientifically interesting, As deficiency has never been demonstrated in nature, probably because the apparent nutritional requirements are considerably lower than common background concentrations.

Metabolism

It is commonly accepted that, with the possible exception of the trivalent methylated metabolites (MMA^{III} and DMA^{III}), the inorganic forms of As are considerably more toxic in mammals than organic arsenicals.^{20,25,28-30} Since the inorganic forms are also by far the most common contaminants of water under field conditions and because MMA^{III} and DMA^{III} are too unstable to persist for any length of time under natural conditions, this review will focus upon exposure to the inorganic forms of As^{III} and As^V. Arsenate is absorbed from the gut by a two-stage process. First, it is concentrated in mucosal cells then, as binding sites become filled, it moves down the resulting concentration gradient into the portal circulation.²⁵ The absorption mechanism of iAs^{III} is less completely understood, but it is commonly accepted that iAs^{III} is even more readily absorbed than iAs^V, probably because of its greater water solubility.¹⁷ Once absorbed, As is transported to various tissues via blood. Distribu-

tion between the erythrocytic and plasma components of blood depends upon the dose of As given, the species of animal, and the valence of administered As. In general plasma concentrations increase relative to red cell concentrations as the dose increases, and trivalent As has a higher affinity for erythrocytes than As^V, resulting in slower clearance. Humans, rats, and mice have higher erythrocyte binding than other domestic mammals, also resulting in slower elimination.^{17,27}

The metabolism and excretion of As varies significantly between species and between genotypes within the human species.^{27,31-33} These species-specific differences in metabolism are important in the pathogenesis of As intoxication in any given species.^{17,27} Generally speaking, inorganic As is methylated *in vivo* via a series of sequential reduction and oxidative-methylation reactions. Inorganic As^V is reduced in a linked reaction with oxidation of reduced glutathione (GSH) to iAs^{III}, which is then methylated to MMA^V by reaction with S-adenosylmethionine. Monomethylarsonic acid (MMA^V) is, in turn, reduced and methylated to DMA^V and so forth.²² These metabolites are more readily excreted than inorganic As. In many, but not all, mammalian species, this process proceeds to DMA^V or the trimethyl arsenic metabolite trimethylarsine oxide (TMAO); however, in human beings, significant amounts of MMA^{III} and DMA^{III} apparently escape methylation and react with critical tissue components. The rate of methylation and physiologic site of metabolism are thus important determinants of the rate of elimination and the potential for chronic effects at very low doses.^{22,29}

As interacts with many other dietary factors, resulting in either increased or decreased toxicity. As has been known to minimize the toxicity of Se for many years.³⁴⁻³⁷ When As is fed with Se, the excretion of both elements in feces is enhanced.^{37,38} Simultaneous administration of Zn lessened the toxicity of As-spiked drinking water, possibly by induction of metallothionein, a metal binding protein.^{22,39} Folate can affect As methylation and both folate deficiency and excess As induce fetal malformations in rats, thus a dietary deficiency of folate potentiates As toxicity.^{22,40}

Toxicity

The acute toxicity of inorganic As has been attributed to the generation of reactive oxygen species (ROS) and oxidative stress^{18,21,39}, to the denaturation of critical protein moieties, and to interfering with phosphate metabo-

lism.^{17,41} In virtually all models, iAs^{III} is reported to be several fold more toxic than iAs^V.^{28,30,41,42} Trivalent iAs^{III} exerts its toxic effects by binding with specific functional groups (thiols and vicinyl sulfhydryls) on enzymes, receptor proteins, etc.^{10,17,27,43} For example, mitochondrial respiration is blocked by the reaction between As^{III} and the dihydrolipoic acid cofactor required for substrate oxidation.⁴¹ As cited by Thomas et al.⁴⁴, in 1966 Webb listed more than 100 enzymes that were inhibited by As^{III}. Pentavalent As^V, because of its chemical similarity with the phosphate ion, displaces phosphate from certain biochemical reactions, e.g. oxidative phosphorylation, resulting in depletion of ATP^{10,41,43-45}, but it may also be converted to As^{III} *in vivo* (i.e. in tissues) and in the rumen.⁴⁵ The clinical signs associated with acute toxicity in virtually all species include diarrhea, vomiting, abdominal pain, weakness, staggering gait, myocardial degeneration, and, in some cases, death.^{17,25,30,43,45}

Most acute As toxicity results from accidental exposure as a result of improper handling, use, and/or storage of arsenical compounds.^{13,15,45} Cattle began staggering and showing signs of abdominal pain, diarrhea, anorexia, and recumbency shortly after ingesting forage that had been sprayed with sodium arsenite (NaAsO₂). Analysis of the grass performed several days later revealed it contained 2 ppm As, but the author noted concentrations were probably higher immediately after spraying.⁴⁶ Cows and calves became recumbent and exhibited a rapid weak pulse, intermittent clonic convulsions, and paddling movements after drinking fluid from a dipping vat containing 200 mg As/L.⁴⁷ A herd of approximately 275 cattle allowed to graze a road right of way recently sprayed with sodium arsenate (Na₂HAsO₄) sickened within hours, and 80 died within four days. Samples of grass taken at the onset contained 10,500 ppm As.¹⁴ Selby et al. reported a similar scenario in Missouri in which the toxic vegetation contained 440 ppm As.⁴⁸ Cattle that consumed water containing 6-20 mg As/L and silage contaminated with 140 ppm As became progressively weaker, emaciated, and recumbent, had decreased milk production, and eventually died after several days.⁴⁹ Nine adult cattle developed acute hemorrhagic diarrhea, and two eventually died after consuming a dairy premix containing 5.50 ppm As; however, the As concentration of G.I. contents suggests the exposure may have been considerably higher.⁵⁰ Heifers became weak, recumbent, dysenteric, and died after ingesting vegetation containing approximately 2,000 ppm As from herbicide contamination.⁵¹ Approximately 12 hours after consuming pellets containing 27,000

ppm As and 20 ppm metaldehyde, cattle developed ataxia, profuse diarrhea, and muscle fasciculations.⁵² The authors suggested that most of the toxic effects were due to As because the predominate clinical signs did not fit metaldehyde.

The toxicity of the herbicide, lead arsenate (PbHAsO_4), is thought to be due to the As content rather than Pb.⁵³⁻⁵⁵ After licking bags containing PbHAsO_4 , cattle exhibited rapid pulse and respiration, oral mucosal erosions, diarrhea, and decreased milk production. Arsenic toxicity was confirmed when stomach contents were found to have 175 ppm As.⁵³ Yearling cattle exhibited severe colic, diarrhea, and death after consuming an undetermined amount of a powder containing 39% Pb and 10% As. Rumen contents from the animals contained 478-531 ppm As.⁵⁶ Five calves began showing signs of lethargy, ataxia, anorexia, decreased heart rates, and diarrhea after consuming powder containing 700,000 ppm As_2O_3 . Four of the animals eventually died.⁵⁷ Cattle receiving 67 g As_2O_3 and calves receiving 17 g As_2O_3 as a topically applied medication became depressed and exhibited bloody diarrhea and a staggering gait soon after treatment. By 20 hours post treatment, 94 of 101 animals were dead.⁵⁸ Depression, ataxia, weakness, recumbency, diarrhea, abdominal pain, and tachycardia developed in 15 cattle that ingested ash from As-preserved wood. The ash was found to contain 780 ppm As.⁵⁹ After 260 heifers were moved to a new pasture containing an abandoned cattle dip, they became restless and belligerent and began showing signs of profuse salivation and watery diarrhea. (Watery diarrhea is extremely dilute and may even be clear; it's usually a result of runaway secretory processes in the bowel.) More than 50% of the herd exhibited convulsions and became comatose, and 67 eventually died. The soil around the dip contained 10-150 ppm As and 50-500 ppm toxaphene. The authors suggested that, because tissue As concentrations were not diagnostically significant, the combination of As and toxaphene caused the die-off.⁶⁰ Experimentally, four of five cattle died within 10 days of being dosed with 10 mg As^{V} /kg BW/day as monosodium methanearsonate.⁶¹

More than 650 of 1,000 sheep developed diarrhea and died after consuming vegetation that had been sprayed with a PbHAsO_4 solution containing 0.58 % As.⁶² The condition was reproduced by dosing lambs with 12 mg As/kg BW.⁶² Lambs showed signs of depression, abdominal pain, salivation, and diarrhea after ingesting forage containing 62-95 ppm As due to contamination with a cotton defoliant; 172 of 923 lambs died.⁶³ Six doe

white-tailed deer were found dead after eating soil and vegetation contaminated by aerial spraying of an arsenical herbicide at the rate of 1.6 lbs As per acre. Later analysis of a combined soil and vegetation sample yielded 2.4 ppm As, and water samples yielded 0.36-0.48 mg As/L.⁶⁴ The application rate, however, calculates to a forage concentration of approximately 368 ppm or a dosage of approximately 11 mg As/kg BW in an animal consuming 3 % BW daily. The latter numbers are more consistent with reported tissue concentrations (18-19 ppm As) in the dead deer than the soil and water analysis.

Arsenic poisoning has also been reported in monogastrics. Nine thoroughbred racehorses died after showing signs of extreme distress, weakness, colic, rapid, weak pulse, hyperemic mucous membranes, and watery diarrhea. It was discovered that roughly 8 oz. of arsenical rat poison had spilled into their corn bin. *Post mortem* chemical analysis discovered significant amounts of As in the stomach and liver of two horses.⁶⁵ Working backward from the numbers presented, we estimate the horses received a dose of between 1-10 mg As/kg BW. Gastrointestinal cramps, vomiting, diarrhea, ECG changes, and liver disruption developed in a 27-year-old woman after she ingested 9,000 mg As_2O_3 .⁶⁶ Furr and Buck⁶⁷ poisoned cats with a commercial ant bait. Doses of As (as Na_2HAsO_4) greater than 8 mg/kg BW were lethal; the threshold of toxic signs was 2 mg/kg BW, and the no-effect level was 1.5 mg/kg BW.

In swine, arsanilic acid has been frequently used as a growth promotant and as a treatment for swine dysentery. In several cases, excessive doses or prolonged treatment periods have resulted in a chronic syndrome characterized by apparent blindness due to degeneration of the optic nerve and optic chiasma.⁶⁸⁻⁷⁴ The toxic mechanism and toxicity of this class of arsenical drugs are quite distinct from and less than inorganic As and will therefore not be considered further.

At present, despite convincing epidemiologic evidence that very low concentrations of As in drinking water can cause chronic disease, especially cancer, in human beings, there are no animal models that reliably duplicate these particular toxic effects without resorting to relatively high doses and/or pharmacologic and genetic manipulation to render them more sensitive.^{19,33} The current belief, derived from *in vitro* studies and specialized laboratory animal models, is that small amounts of DMA^{III} and MMA^{III} escape the methylation process in people and, over prolonged periods, cause cellular damage that results

in diseases such as cancer.^{10,20,23,32,33,41,44} Dimethylarsinic acid (DMA^V) and monomethylarsonic acid (MMA^V) are the main urinary metabolites of As excreted in most mammals; however, the trivalent As metabolites, dimethylarsinous acid (DMA^{III}), and monomethylarsonous acid (MMA^{III}) have been discovered in fresh urine of As-poisoned human patients.²⁰ The glutathione conjugate of DMA^{III} was actually more toxic to cells *in vitro* than inorganic As²⁰, and DMA^{III} causes single-strand breaks in DNA *in vitro*.^{20,75} These processes are apparently limited to human beings and specialized laboratory models; thus, based upon known differences in metabolism, this class of disease and dosages does not seem relevant for livestock and big game animals.

Developmental studies of orally administered MMA^V and DMA^V (the metabolites of inorganic As in most non-human mammals) in rats and rabbits determined the threshold of fetal damage was similar to that for maternal toxicity or about 36 and 48 mg/kg BW, respectively.⁷⁶ Administration of As by gavage to pregnant rats and mice did not produce morphologically evident teratogenesis at non-maternally toxic dosages, although there was some evidence of behavioral changes in pups born to dams consuming drinking water with slightly less As^{III} than the lowest maternally toxic dose.²² Conversely, Domingo⁷⁷ reported that As^{III}, via the oral route of exposure, was much less teratogenic in several species. This indicates that dietary limits safe for a dam should also provide adequate protection for her fetus.

There are very few reports of chronic toxicity in non-rodent animals. Due to the rapid excretion of As in cattle, sheep, dogs, etc., these species are able to clear less-than-acutely-toxic doses of As before they can cause much of a problem.^{25,78} The reports of chronic toxicity we discovered involved dosages similar to those reported for acute or subacute poisoning. Female beagle dogs fed 4 to 8 mg NaAsO₂ (2.3-4.6 mg As)/kg BW per day for 183 days exhibited decreased weight gains due to decreased feed consumption and slightly elevated liver enzymes.⁷⁹ Beagle dogs were fed varying concentrations of As as either As^{III} or As^V for two years. At 50 ppm and less, there were no measurable effects. At 125 ppm dietary As, the dogs lost weight and several died with lesions of inanition.⁸⁰ Three of four sheep given 88 mg PbHAsO₄/kg BW once per month died within 24 hours of the seventh dose. The other sheep in the study, given 22 and 44 mg PbHAsO₄ (4.9 and 9.7 mg As/kg BW), survived 11 doses with no clinical signs.⁵⁴ One of two lambs dosed with 1.5 mg As/kg BW/day as PbHAsO₄ died after 35 days; the other

survived until the study was stopped at 94 days.⁶² Sheep fed a mean daily dose of 1.4 mg As/kg (As species unspecified) for three weeks remained in good condition for the duration of the study.⁸¹ Bucy et al.⁸² fed potassium arsenite to feedlot lambs at doses as high as 3.26 mg As/kg BW/day for eight weeks without adverse effects. In a later study 1.75 mg/kg BW/day was not toxic, but higher doses caused feed refusal and clinical signs of toxicity.⁸³ Peoples⁷⁸ added arsenic acid to dairy rations at 1.25 ppm, a dose of approximately 0.48 mg As/kg BW/day for seven weeks with no effects. Virtually all of the ingested As was eliminated as quickly as it was eaten.

It has been proposed⁸⁴ that elk in the Madison-Firehole watershed of Yellowstone National Park have shorter lifespans because of naturally elevated As in water and feedstuffs. Exposure was estimated to be “greater than 1.25 mg/kg BW/day” based upon tissue concentrations and extrapolation from bovine studies and between “0.01 - 6.2 mg/kg BW/day” based upon forage and water analysis. The difference in longevity also may be due to other environmental differences between the Madison-Firehole area and the control site.⁸⁴ Forsberg et al.⁸⁵ demonstrated slight but measurable inhibition of normal rumen fermentation *in vitro* with As concentrations as low as 5 mg/L of rumen fluid, but they did not provide any data as to how this concentration related to dietary intake. Assuming for the moment that the rumen fluid concentration is equivalent to the combined concentrations in feed and water, the concentration would be equivalent to a dose of roughly 1 mg As/kg BW.

Summary

Our recommendations are based upon the toxicity of inorganic As^{III}, specifically the arsenite ion. Routine water quality analysis available to livestock producers does not distinguish between As species, and, although the less toxic pentavalent forms of As are more likely to occur in surface waters, trivalent As is seen frequently enough in specialized surveys to justify the assumption.^{86,87} It is suggested that ruminant animals are less susceptible to As than monogastrics.^{25,88} With the exception of laboratory rodents, however, we were not able to confirm this to be the case, thus we have assumed horses are equally sensitive to As as ruminants.

Chronic poisoning of the type (cancer, “blackfoot disease,” etc.) that prompted lowering the human drinking water standard from 0.05 to 0.01 mg As/L does not apparently occur in other animal species, as demonstrated

by the ongoing search for an appropriate animal model to study the human condition. The mechanism(s) putatively involved in the pathogenesis of chronic damage in people, i.e. chemical attack by methylated As^{III} metabolites on cellular macromolecules, do not appear to be relevant in livestock and wildlife. In domestic livestock, as opposed to people, most As is excreted via urine as DMA^{III}.³³ This, together with the shorter observed half-life in these species, suggests that relatively little trivalent As escapes methylation and excretion to cause cancer. Chronic poisoning in livestock species involves mechanisms similar to acute poisoning and requires dosages very similar to acute poisoning.

Given the accumulating evidence that As is a human carcinogen, the question of residues arises. Can food animals consuming As from water accumulate dangerous amounts of As in edible tissues without themselves showing signs of toxicity? The literature to date suggests cattle, sheep, etc. eliminate As too quickly for this to be a concern, and a study completed in 2007 by the University of Minnesota^{89,90} failed to find any evidence of As accumulation in milk or edible tissues from dairy cattle watered from As-contaminated (140 µg/L) wells.

The threshold toxic dose in domestic ruminants appears to be between 1-2 mg/kg BW. This dose is in general agreement with the NRC²⁵, which recommended 30-50 ppm dietary As as a maximum tolerated dose and with other reviews.^{88,91-93} It is quite distinct from the EU recommendation of 2 ppm dietary As, for which we have not been able to discover any justification. Sufficient quantitative data was not found to estimate a similar threshold for horses, but this dose is similar to that reported in another monogastric species (dogs)⁷⁹, and previous reviews suggest horses are similar to cattle in sensitivity and/or less frequently affected than cattle under similar conditions.^{93,94} Therefore, it *seems* reasonable that limits safe for cattle should be adequate for horses. The very limited data in wild ruminants suggest they are similar to cattle in sensitivity. Therefore, our recommendations are based upon dosage data from cattle and sheep. Assuming negligible As in feedstuffs, 5 mg As/L in drinking water will provide the minimum toxic dose of 1 mg As/kg BW to grazing animals in warm weather. Obviously, if animals are receiving any As from forage or medications, less will be required to achieve a toxic dose. Although we were not able to find any significant studies of As in Wyoming forages, limited data from our laboratories suggest natural background concentrations seldom exceed a few ppm, except in areas contaminated by geothermal runoff.

Assuming a NOAEL of 0.5 mg/kg BW/day and allowing for these small forage concentrations, we recommend drinking water for livestock and wildlife not exceed 1 mg As/L.