

# Toxic Metals and Antioxidants: Part II. The Role of Antioxidants in Arsenic and Cadmium Toxicity

Lyn Patrick, ND

## Abstract

Exposure to toxic metals has become an increasingly recognized source of illness worldwide. Both cadmium and arsenic are ubiquitous in the environment, and exposure through food and water as well as occupational sources can contribute to a well-defined spectrum of disease. The symptom picture of arsenic toxicity is characterized by dermal lesions, anemia, and an increased risk for cardiovascular disease, diabetes, and liver damage. Cadmium has a significant effect on renal function, and as a result alters bone metabolism, leading to osteoporosis and osteomalacia. Cadmium-induced genotoxicity also increases risk for several cancers. The mechanisms of arsenic- and cadmium-induced damage include the production of free radicals that alter mitochondrial activity and genetic information. The metabolism and excretion of these heavy metals depend on the presence of antioxidants and thiols that aid arsenic methylation and both arsenic and cadmium metallothionein-binding. S-adenosyl-methionine, lipoic acid, glutathione, selenium, zinc, N-acetylcysteine (NAC), methionine, cysteine, alpha-tocopherol, and ascorbic acid have specific roles in the mitigation of heavy metal toxicity. Several antioxidants including NAC, zinc, methionine, and cysteine, when used in conjunction with standard chelating agents, can improve the mobilization and excretion of arsenic and cadmium.

(*Altern Med Rev* 2003;8(2):106-128)

## Introduction

Heavy metals are found in increasingly hazardous concentrations in air, food, and water. The Agency for Toxic Substances and Disease Registry (ATSDR) lists arsenic and cadmium among the top seven of the 275 most hazardous substances in the environment. This listing is based on the toxicity of the substance and the potential for exposure from air, water, or soil contamination at any one of the 1,560 National Priorities List Cleanup or "Superfund" sites.<sup>1</sup> Arsenic and cadmium, in addition to mercury and lead, have been identified as the most probable causes of heavy metal-related disease observed in primary care medicine.<sup>2</sup>

As the prevalence of heavy metal exposure is increasingly recognized and identified in individuals seen in private practice clinics, the need for effective prevention and treatment will increase. In this article, the clinically relevant aspects of arsenic and cadmium exposure are reviewed, as are current exposure data. The role of antioxidants in mitigating the damage of heavy metal toxicity and assisting the process of chelation has been explored in *in vitro* and animal studies; however, clinical trials in humans have been limited. The relationship of oxidant stress to the toxic effects of arsenic and cadmium is summarized, in addition to the potential role of antioxidants as adjunctive treatment in heavy metal exposure.

---

Lyn Patrick, ND – 1984 graduate, Bastyr University; associate editor, *Alternative Medicine Review*; private practice, Tucson, Arizona, 1984-2002.  
Correspondence address: 21415 Hwy 140, Hesperus, CO 81326  
Email: lpatrick@frontier.net

## Arsenic

### *Sources of Exposure and Symptoms of Toxicity*

It is estimated that several million people worldwide suffer the effects of chronic arsenic exposure resulting from environmental release of arsenic.<sup>3</sup> Arsenic was identified as a hazardous waste at 1,014 of the 1,598 National Priorities List “Superfund” sites in the United States in 2000.<sup>4</sup> The National Institute of Occupational Safety and Health (NIOSH) estimates that 55,000 workers in the United States were exposed to high levels of arsenic in the early 1980s.<sup>5</sup> These estimates are considered low because they exclude mining and agriculture, two occupational sources of arsenic exposure. Groundwater contamination provides the majority of worldwide arsenic exposure. In the United States, groundwater concentrations exceed the U.S. Environmental Protection Agency (EPA) limit of 50 ppb.<sup>6</sup> Levels as high as 166 ppb have been measured in public water systems in Utah<sup>7</sup> and a private well in Nevada was found to contain 1,312 ppb.<sup>8</sup> Levels of 20 ppb or more are found in the water supply of at least 725,000 people in the United States.<sup>9</sup>

Inorganic arsenic, the form found in soil, water, and crops, is classified by the EPA as a Group A human carcinogen, meaning that sufficient knowledge exists to substantiate a causal relationship between human exposure and cancer occurrence.<sup>10</sup> The current EPA water standard, known as the Maximum Contaminant Level (MCL) of 50 ppb (50 µg/L), has been criticized by the scientific community, specifically the National Academy of Sciences. Based on their 1999 Risk Estimates, the lifetime risk of contracting bladder or lung cancer from arsenic at a 50-ppb concentration is 1 in 100. Even at the EPA-proposed MCL of 10 ppb, rejected by the federal government in 2001, the lifetime risk is still 1 in 500. This risk estimate is significantly higher than the EPA's current acceptable cancer-risk definition for water contaminants: 1 in 10,000 risk of fatal cancer.<sup>10</sup> Inorganic arsenic is also found in environmental tobacco smoke and arsenic-treated wood, used in over 90 percent of the outdoor wooden structures in the United States.

Of growing concern is the presence of high levels of heavy metals in industrial waste processed for use as fertilizer. The presence of high levels of arsenic in agricultural fertilizer (which can be legally sold as organic fertilizer) has been shown to exceed EPA limits for arsenic in biosolids.<sup>11</sup> A minimal risk level (MRL) of 0.8 µg of arsenic/kg/day (approximately 5.6 µg for an adult) has been established for chronic arsenic exposure as a result of studies showing that approximately twice that dose resulted in hyperpigmentation and keratosis, both symptoms of chronic arsenic exposure-induced skin damage.<sup>12</sup>

High levels in soil used for agricultural purposes in Denmark are considered over 20 µg per gram.<sup>13</sup> Soils in Butte, Montana, collected near mine tailings (a source of zinc sulfate for fertilizer), contained as much as 13,800 µg arsenic per gram of soil, and soil collected from a previous hazardous waste site in Jersey City, New Jersey, contained 1,120 µg per gram.<sup>14</sup> Adults in Denmark, eating 376 grams (about 4 servings) of vegetables per day grown in soil containing 30 µg of arsenic per gram of soil, consumed 5.3 µg of arsenic daily.<sup>13</sup> Adults living near point sources of arsenic exposure may have a constant daily total inorganic arsenic intake as high as 12 µg/kg body weight/day.<sup>15</sup> Acute symptoms of arsenic poisoning – nausea, diarrhea, abdominal cramping, hyperesthesia in extremities, abnormal patellar reflexes, and abnormal electrocardiograms – have been estimated to occur at levels of exposure equal to 50 µg/kg body weight/day.<sup>16</sup>

Arsenic exposure has been linked to cardiovascular disease and diabetes. In epidemiological studies in Bangladesh, where arsenic toxicity is endemic as a result of tube-well contamination, arsenic has been linked to the prevalence of hypertension.<sup>17</sup> Exposure to 20 ppb or more in drinking water has been associated with increased mortality from cardiovascular diseases.<sup>18</sup> Cumulative arsenic exposure has also been positively associated with the incidence of type 2 diabetes in Taiwan, another area where arsenic contamination of water is common.<sup>19</sup>

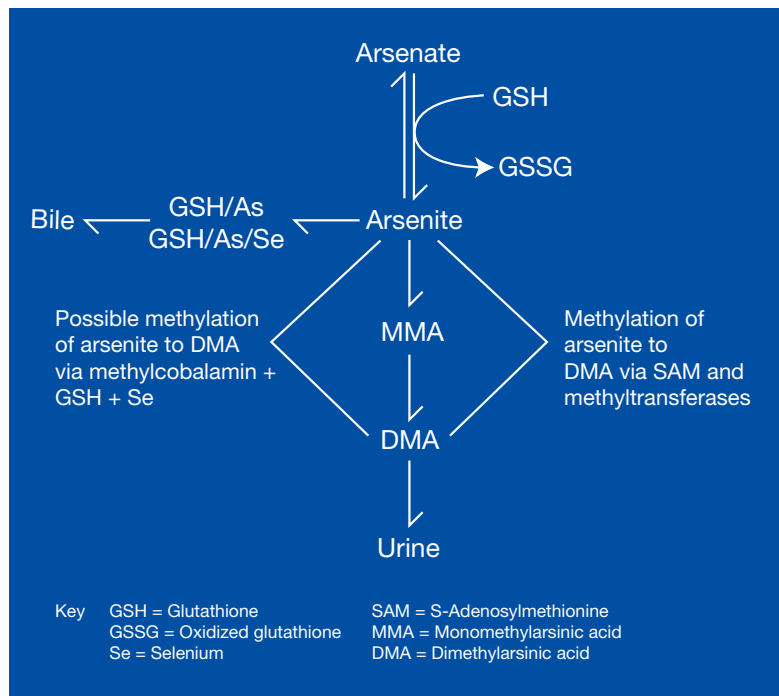
Chronic exposure is associated with anemia, peripheral neuropathy, liver and kidney damage, and irritation of the skin and mucous membranes. Peripheral vascular disease has also been seen in chronically exposed individuals.<sup>20</sup> Chronic inhalation of inorganic arsenic has been shown to be strongly associated with the risk of human lung cancer.<sup>2</sup> Because inorganic arsenic binds to sulfhydryl proteins, specifically keratin, deposits are left in skin, hair, and nails. Exposure to inorganic arsenic has been linked to arsenical keratoses, squamous cell carcinoma *in situ* of the skin, and basal cell carcinoma. Arsenic exposure has also been linked to hepatocellular carcinoma, angiosarcoma, cirrhosis, and hepatoportal sclerosis.<sup>21</sup> While animal studies have shown inorganic arsenic to be fetotoxic and teratogenic, few studies have looked at arsenic toxicity in pregnant females. Ingested arsenic can cross the placenta and result in cord blood concentrations that resemble maternal blood concentrations.<sup>22</sup> Arsenic, however, has not been detected in measurable amounts in the breast milk of arsenic-exposed women.<sup>23</sup>

Chronic arsenic intoxication may present as diffuse symptoms: headache, fatigue, confusion, polyneuritis with distal weakness, exfoliative dermatitis, hyperkeratosis (especially on the soles of the feet), hyperpigmentation, and Mees' lines (transverse white striae of the fingernails). Anemia, leucopenia, slight proteinuria, and liver enzyme abnormalities may also develop.<sup>2</sup>

### Arsenic Methylation and Detoxification

Arsenic exists in both inorganic and organic states. The organic forms that accumulate in fish and shellfish, arsenobetaine and arsenocholine, have been found to be essentially nontoxic.<sup>24</sup> The inorganic forms, airborne arsenic trioxide and arsenate/arsenite (found in soil, water, and food), are the forms of concern to human health. Arsenic is well absorbed, 40-60 percent if

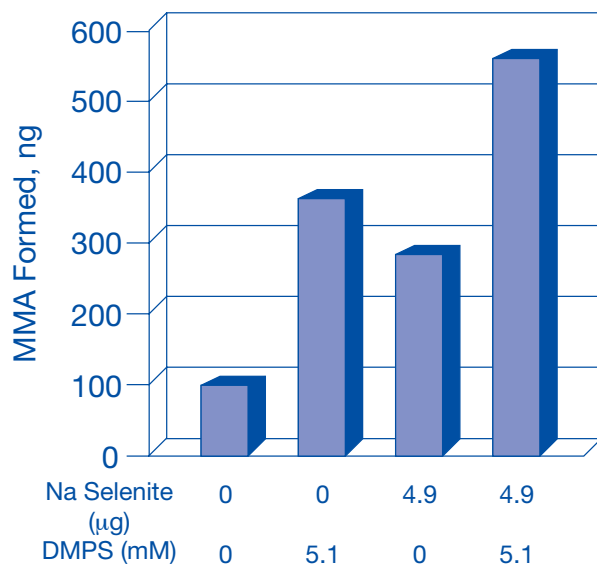
Figure 1. Proposed Detoxification Pathway of Inorganic Arsenic



inhaled<sup>25</sup> and approximately 95 percent if ingested.<sup>26</sup> Arsenic is distributed and stored in all tissues of the body and is metabolized for elimination by two sequential processes (Figure 1). The first are oxidation/reduction reactions that interconvert arsenate to arsenite. Glutathione has been shown to form complexes with arsenic and mediate the reduction of arsenate to arsenite. These glutathione complexes can be eliminated in the bile and a positive correlation has been found between glutathione and arsenic levels in bile.<sup>27</sup> Selenium is also able to complex with glutathione and arsenic to form a compound that is also excreted through the bile. Binding to unidentified proteins is another possible mechanism for arsenic detoxification. These proteins appear to be primary, along with glutathione and possibly selenium, in the removal of arsenic.<sup>28</sup>

The second step, methylation, which occurs mainly in the liver, requires s-adenosylmethionine (S-AdoMet) and possibly other methyl donors (choline, cysteine, glutathione, reduced lipoic acid) to produce monomethylarsinic

**Figure 2. DMPS and/or Sodium Selenite-Stimulated Nonenzymatic Methylation of Arsenite by Methylcobalamin**



Adapted from: Zakharyan RA, Aposhian HV. Arsenite methylation by methylvitamin B12 and glutathione does not require an enzyme. *Toxicol Appl Pharmacol* 1999;154:287-291.

acid (MMA) and dimethylarsinic acid (DMA).<sup>29</sup> Several studies have shown that SAME is actually essential for the methylation of arsenic and low methionine intakes can inhibit arsenic methylation in animals.<sup>30</sup> Both MMA and DMA have been found in human urine and are considered end-products of arsenic metabolism. Because DMA is cleared from cells more rapidly than MMA or inorganic arsenic, and methylation reduces the amount of arsenic retained in tissues by increasing the water solubility of arsenite, methylation is considered by some researchers to be a detoxification mechanism.<sup>31</sup> Other researchers disagree because MMA may be the most toxic intracellular form of arsenic due to its ability to induce enzyme inhibition, oxidative stress, and DNA damage.<sup>32</sup> Therefore, methylation may simply be a way of biotransforming arsenic rather than detoxifying it.<sup>28</sup>

Dermal and pulmonary tissues are unable to convert MMA to DMA as efficiently as other tissues, and both are the sites of specific arsenic-induced cancers. DMA is not a benign metabolite either, and has been shown to produce free radicals that may contribute to the mechanisms of arsenic-related cancers.<sup>33</sup> MMA and DMA have also been shown to complex with glutathione and other sulfhydryl proteins, resulting in sulfhydryl-related enzyme inhibition and cellular damage.<sup>31</sup>

The methylation of arsenic is a topic of significant debate and interest in the toxicology field because the ability to methylate and eliminate arsenic is influenced by nutrition, gender, lifestyle, and individual genetic polymorphisms.<sup>34</sup> There appears to be significant individual variation in the ability to methylate arsenic. Malnourished individuals exposed to high levels of arsenic are less able to methylate it and are more at risk for arsenic toxicity symptoms and diseases than well-nourished individuals.<sup>35</sup> In a study of an arsenic-exposed population, smoking more than 10 cigarettes daily had a stronger inhibitory effect on the ability to completely methylate arsenic than gender, age, or ethnicity.<sup>36</sup> However, these factors only accounted for 20 percent of the variation in methylation capacity. The amount of exposure appears to be the most important factor affecting arsenic methylation; the higher the chronic exposure the lower the individual's ability to methylate MMA to DMA. The ability to transform MMA to DMA is significant; for example, the dermal signs of arsenic exposure (including skin cancer) are related to a buildup in the body of MMA.<sup>31</sup>

### ***Selenium and Arsenic Toxicity***

The presence of selenium also affects arsenic toxicity. Animal research has established a bidirectional effect of selenium and arsenic with each metal preventing a toxic effect of the other.<sup>37</sup> As mentioned, selenium is believed to bind to arsenic to form an insoluble complex in the liver.<sup>28</sup> Animal studies with injected sodium selenite (0.5 mg/kg) increased the excretion of arsenite-selenium compounds in the bile and reduced hepatic

arsenite concentrations.<sup>38</sup> Rats given selenium-sufficient diets (0.2 ppm) and toxic doses of arsenic were able to eliminate arsenate, arsenite, and DMA more quickly than rats on a selenium-deficient diet (0.02 ppm).<sup>39</sup> The methylation of arsenic can also occur *in vitro* in the presence of methylcobalamin (methylated vitamin B12) and glutathione.<sup>40</sup> This methylation reaction was increased with the addition of selenium (in the form of sodium selenite) or the chelating agent 2,3-dimercaptopropane sulfonate (DMPS). When both DMPS and selenium were used, the amount of MMA produced from inorganic arsenic was approximately doubled (Figure 2). The authors of the paper suggest the vitamin B12, selenium, and methionine (the essential component of SAME) content of the diets of those exposed to inorganic arsenic be carefully considered as factors that assist in the elimination of arsenic.

### *Oxidative Stress in Arsenic Toxicity*

The exact cellular mechanisms of arsenic's carcinogenicity are not completely understood; however, it is believed to be a co-carcinogen and tumor promoter rather than a tumor initiator.<sup>31</sup> The lack of direct evidence is, at least in part, because there are no animal models for arsenic-induced carcinogenesis. Arsenic may be the only agent that has been determined to be a definite human carcinogen even though there is not enough evidence to prove it is a carcinogen in animals. Therefore, it is both possible and disconcerting that humans are actually more sensitive to the toxic effects of arsenic than experimental animals.

The four main areas of research on the cellular mechanisms of arsenic toxicity are: (1) mutation inductions and chromosomal aberrations; (2) altered signal transduction, cell-cycle control, cellular differentiation and apoptosis; (3) direct damage from oxidative stress; and (4) alterations in gene expression.<sup>41</sup> None of these mechanisms are exclusive, and oxidative stress has been shown to influence all of them, directly or indirectly.<sup>33</sup>

Arsenic-induced oxidative stress has been shown to cause DNA damage through the production of superoxide and hydrogen peroxide radicals.<sup>42</sup> This particular form of genotoxicity has

been linked to arsenic-related skin cancers. Oxidant-induced damage was found significantly more frequently in biopsies of individuals with known arsenic exposure and measurable arsenic in skin biopsies than in those with squamous-cell carcinoma who had no known arsenic exposure and no measurable arsenic in skin biopsies (78 percent versus 9 percent, respectively).<sup>43</sup> *In vitro* studies have found superoxide dismutase, catalase, dimethyl sulfoxide (DMSO), glutathione, N-acetylcysteine (NAC), and vitamin E can effectively block DNA mutations, prevent the production of high levels of superoxide, and protect fibroblasts from arsenic-induced chromosomal damage.<sup>44</sup> These studies indicate oxidative damage-related mechanisms are involved in arsenic genotoxicity.

Arsenic also has a direct toxic effect on cellular respiration in mitochondria.<sup>45</sup> This toxic effect on cellular respiration occurs because arsenic binds to lipoic acid in the mitochondria and inhibits pyruvate dehydrogenase. The resulting uncoupling of mitochondrial oxidative phosphorylation leads to increased production of hydrogen peroxide. The resulting oxidative damage may play an important role in altering gene-expression patterns, another mechanism for arsenic-induced carcinogenesis.<sup>46</sup> The uncoupling of oxidative phosphorylation, decrease in cellular respiration, and resulting increase in free radical production also lead to hepatotoxicity and porphyrinuria. These symptoms of arsenic toxicity are seen more commonly with acute exposure but also occur with low-dose chronic exposure.<sup>47</sup>

Evidence of oxidative stress has also been measured in humans with arsenic exposure. Studies in those with very high arsenic exposure from groundwater contamination (a mean of 410 µg/L or 400 ppb) had serum lipid peroxide levels significantly higher (24 percent) than a control group whose drinking water had much less arsenic (20 µg/L).<sup>48</sup> The high exposure group also had a 57-percent reduction in whole blood glutathione levels compared to the lower exposure group. On the whole, individual glutathione levels were inversely related to both whole blood inorganic arsenic concentrations and the presence of methylated forms of arsenic (MMA and DMA).

**Table 1. Effects of N-acetylcysteine, meso 2,3-Dimercaptosuccinic Acid and their Combination**

Group	GSH (nmol/mg protein)	GSSG (nmol/mg protein)	GSH/GSSG Ratio
I. Control	42.3 ± 1.0	1.40 ± 0.07	30
II. Arsenic	21.6 ± 0.9	4.45 ± 0.34	5
III. Arsenic + NAC	30.1 ± 2.5	2.97 ± 0.21	10
IV. Arsenic + DMSA	29.2 ± 3.2	3.30 ± 0.10	9
V. Arsenic + NAC + DMSA	37.2 ± 1.0	2.17 ± 0.21	17

NAC, N-acetylcysteine; DMSA, meso 2,3-dimercaptosuccinic acid; GSH, glutathione; GSSG, oxidized GSH

Adapted from: Flora SJ. Arsenic-induced oxidative stress and its reversibility following combined administration of N-acetylcysteine and meso 2,3-dimercaptosuccinic acid in rats. *Clin Exp Pharmacol Physiol* 1999;26:865-869.

Another human study of northeastern Taiwan residents confirmed these findings.<sup>49</sup> The coast of northeastern Taiwan is an area of endemic arsenic toxicity where well water concentrations vary from 0 to over 3,000 µg/L (3,000 ppb). Arsenic whole blood concentrations in individuals from this area with high arsenic exposure have been positively associated with plasma oxidant levels and negatively correlated with plasma antioxidant capacity. A correlation was also found between lower levels of plasma antioxidants and a lowered ability to methylate inorganic arsenic. Taiwan residents living in arsenic-hyperendemic areas diagnosed with arsenic-related ischemic heart disease had significant decreases in serum alpha- and beta carotene.<sup>50</sup>

### ***Arsenic, Antioxidants, and Chelating Agents***

Both dimercaptosuccinic acid (DMSA)<sup>51</sup> and DMPS<sup>52</sup> can be used as chelating agents in arsenic toxicity. Studies looking at the effects of antioxidants used in conjunction with chelating agents have investigated their role as potential aids to chelators. A study evaluating chronic arsenic

intoxication (100 ppm in water for 12 weeks) in rats evaluated the ability of NAC and a chelating agent, DMSA, to preserve hepatic and brain glutathione levels and to normalize erythrocyte enzyme levels.<sup>53</sup> Dosages of therapeutic agents were given orally to approximate those used in human treatment: NAC and DMSA each at a dose of 1 mmol/kg for five days. The combination treatment significantly elevated reduced glutathione levels in the liver and decreased levels of oxidized glutathione (Table 1). The simultaneous use of both compounds was significantly stronger than either individually. NAC treatment alone decreased levels of hepatic malondialdehyde (the result of arsenic-induced oxidant activity), while the effect of DMSA by itself was insignificant. Effects in the brain were less apparent, with neither treatment alone or in combination able to affect a significant shift in the reduced glutathione/oxidized glutathione ratio. Brain levels of malondialdehyde were significantly reduced, however. The ability of arsenic to alter heme synthesis was also evaluated. Arsenic toxicity is known to interrupt hemoglobin synthesis and changes in the erythrocyte enzyme delta aminolevulinic acid dehydratase (ALAD) levels were measured to reflect this.

Blood ALAD activity was reduced 62 percent by arsenic exposure. DMSA alone or with NAC was able to restore ALAD levels to those of controls (not exposed to arsenic); only the combination of both was able to restore RBC glutathione levels. The level of acute arsenic exposure in this study (100,000 ppb) was significantly higher than levels of chronic human exposure, even in hyperendemic areas of Taiwan and West Bengal where tube well contamination reaches 1,500-3,000 ppb.<sup>54,55</sup>

Arsenic has been detected in human placental tissue and human fetal tissue. Neonatal brain tissue has shown significant oxidative damage when exposed to arsenic, at levels as low as 50 ppb.<sup>56</sup> Four groups of female rats were fed arsenic-contaminated water for the length of gestation at concentrations of 300 ppb. The study compared arsenic alone to arsenic plus vitamin E, arsenic plus vitamin C, and arsenic with the chelating agent DMSA added for two days at the end of gestation. When vitamin C and vitamin E were added, significant improvements occurred in levels of lipid peroxidation and glutathione content (Table 2). The levels of antioxidants used were small – vitamin C at 2.5 mg/kg/day and vitamin E at 148µg/kg/day (0.148 IU/kg/day). Vitamin E at that level, however, was able to almost completely restore glutathione levels in brain tissue and increase catalase levels 100-percent higher than in the control group. Because arsenic-induced neuronal damage has been shown to be directly related to lipid peroxidation,<sup>55</sup> the role of antioxidants in protecting both adult and fetal nervous tissue is of increasing importance.

### Cadmium

Cadmium is considered one of the most toxic substances in the environment due to its wide range of organ toxicity and long elimination half-life of 10-30 years.<sup>57</sup> Cadmium was identified as a contaminant at 776 of the 1,467 EPA National Priorities List sites in 1998.<sup>58</sup> It has been estimated that at least 512,000 U.S. employees each year work in an environment that potentially exposes them to cadmium.<sup>59</sup> Cadmium-contaminated topsoil, however, is considered the most likely mechanism for the greatest human exposure through uptake into edible plants and tobacco.<sup>60</sup> The EPA estimates approximately 3.4 billion pounds of sewage sludge are transferred to soil annually in the United States, estimated to contain up to 1,000 µg/g cadmium.<sup>61</sup> Fertilizer raw materials are also contaminated with cadmium; 1.3 million pounds of cadmium-contaminated zinc sulphate containing up to 215,000 ppm cadmium entered the United States in November 1999. It is not known how much of this product has been sold and applied to agricultural lands.<sup>62</sup> Fertilizers

*Table 2. Effects of Vitamins C and E and DMSA as Protective Agents*

	Lipid peroxidation (ΔOD/mg protein)	GSH content (µg/mg protein)
Arsenic	131 ± 7	77 ± 9
Arsenic + vitamin C	81 ± 5 *	82 ± 11 ***
Arsenic + vitamin E	64 ± 11 *	98 ± 8 **
Arsenic + DMSA	38 ± 14 *	88 ± 12 ***

\* value lower than control  
 \*\* value same as control  
 \*\*\* p < 0.05 compared to arsenic only

Adapted from: Chattopadhyay S, Bhaumik S, Purkayastha M, et al. Apoptosis and necrosis in developing brain cells due to arsenic toxicity and protection with antioxidants. *Toxicol Lett* 2002;136:65-76.

continue to be contaminated with cadmium as a result of the recycling of industrial waste sold as zinc sulphate or other raw materials for agricultural and home use fertilizers. Assays of commonly sold dry fertilizer and soil amendment in Washington State in 1998 revealed concentrations of cadmium as high as 160 mg/kg dry weight.<sup>63</sup>

### *Cadmium Exposure*

The uptake of cadmium from the soil through produce results in elevated concentrations in vegetables, fruits, and grains, with the highest levels in leafy greens and potatoes. High levels are also found in shellfish (up to 30 mg/kg) and organ meats.<sup>64</sup> The current federal minimal risk level (MRL) for cadmium – a level at which chronic exposure in humans is not likely to cause cancer or adverse health effects – is 0.2 µg/kg/day (14.0 µg for the average adult). The average American diet in 1986 provided 0.4 µg/kg/day of cadmium.<sup>65</sup> The overall range of dietary cadmium in Swedish diets in 1994-1996 was 2.0-175 µg/day and is estimated to be increasing at a rate of two percent per year.<sup>57</sup> The World Health Organization has shown that dietary cadmium exposure has a very wide range: inhabitants of worldwide nonpolluted areas have a daily dietary intake of approximately 40-100 µg, while inhabitants of polluted areas may obtain 200 µg or more as an average daily intake.<sup>57</sup>

### *Cadmium Absorption*

Between 10-50 percent of cadmium fumes are absorbed through the respiratory tract and approximately five percent of oral cadmium is absorbed through the digestive tract. Smokers absorb 1-2 µg cadmium per pack of cigarettes, approximately doubling the average exposure of a nonsmoker and doubling the average amount of cadmium found stored in the kidneys.<sup>57</sup> Although absorption through the gastrointestinal tract is significantly lower, low dietary intakes of calcium, protein, zinc, iron, and copper may increase cadmium absorption in the gut.<sup>66</sup>

### *Iron Deficiency as a Risk Factor*

Iron deficiency creates a significant risk for increased cadmium exposure by increasing gastrointestinal absorption from five percent to as much as 20 percent.<sup>67</sup> Individuals with a serum ferritin less than 12 µg/L are considered to be high risk for cadmium-induced kidney lesions.<sup>57</sup>

A study of 57 nonsmoking Swedish women found those with a serum ferritin of less than 20 µg/L (indicating reduced body iron stores) had significantly higher blood cadmium levels than those with a serum ferritin above 30 µg/L.<sup>68</sup> The authors concluded that, since 44 percent of the women in the study had depleted bone marrow stores (serum ferritin less than 15 µg/L) and 23 percent had reduced body iron stores (serum ferritin less than 30 µg/L), iron deficiency appears to create a significant high-risk category for cadmium-induced renal damage in Swedish women. This is consistent with data on the Swedish female population in which 10-40 percent of women are reported to have depleted iron stores (serum ferritin less than 12 µg/L)<sup>57</sup> and is probably the reason why women, in general, tend to have higher blood cadmium levels than men.

Iron deficiency is an international health problem, with an estimated incidence of two billion, particularly young children and women of reproductive age.<sup>69</sup> Vitamin C, which has been shown to significantly increase iron uptake, may play a role in protecting against increased cadmium absorption. In a study of women in the United States, where the prevalence of low iron stores in females ages 12-49 is 10-19 percent, those who took vitamin C supplements had half the risk of low iron stores.<sup>69</sup>

### *Cadmium Metabolism and Mechanisms of Toxicity*

When cadmium is absorbed it circulates in erythrocytes or bound to albumin. In the liver it can induce and bind to metallothionein, a cysteine-rich protein that can concentrate cadmium up to 3,000-fold.<sup>70</sup> The metallothionein/cadmium complex is slowly released over time from the liver and circulates to the kidneys where it can accumulate in renal tissue. Cadmium also accumulates



in the bone, pancreas, adrenals, and placenta. The majority of accumulation, approximately 50 percent of total body stores, occurs in the liver and kidney.<sup>71</sup> The main pathologies related to chronic cadmium toxicity, renal disease and bone loss, are reflective of cadmium concentration in the kidney and the alteration of renal function that ultimately causes osteoporosis and osteomalacia.<sup>57</sup> Acute exposure (acute occupational exposure is common in jewelry braziers and soldering) can manifest as dysuria, polyuria, dyspnea, chest pain, irritability, fatigue, headache, and dizziness. Levels of urinary alpha1-microglobulin or beta2-microglobulin are often elevated in early cadmium-induced renal damage. A review of the symptoms of acute cadmium toxicity has been summarized by Wittman et al.<sup>59</sup>

The mechanisms of cadmium toxicity are not completely understood, but some of the cellular effects are known. Fifty to sixty percent of exposed populations have been shown to have chromosomal damage.<sup>72</sup> Cadmium is known to bind to the mitochondria of the cell and is capable of inhibiting both cellular respiration (by 75%) and oxidative phosphorylation (by 100%) at low concentrations. This mitochondrial toxicity can completely inhibit the hydroxylation of vitamin D in renal tissue at concentrations of 0.025 mmol.<sup>67</sup>

Some of the specific changes that lead to tissue damage and death in chronic exposure have been related to oxidative stress and thiol depletion.<sup>33</sup> Cellular damage results from cadmium binding to sulfhydryl groups in tissue, the production of lipid peroxides, and the depletion of glutathione. Cadmium also has a very high affinity for glutathione and can form a complex with glutathione that is eliminated in bile. Cadmium also inhibits the activity of antioxidant enzymes, including catalase, manganese-superoxide dismutase, and copper/zinc-superoxide dismutase.<sup>73</sup> Cadmium-induced lipid peroxidation has been seen in animal studies in liver, kidney, brain, lung, heart, and testes.<sup>33</sup> Cadmium can also substitute for zinc or selenium in metalloenzymes.<sup>71</sup> Lowered levels of selenium as well as lowered activity of glutathione peroxidase (a selenium-dependent enzyme) have been seen

in cadmium-exposed workers.<sup>74</sup> Cadmium's ability to generate free radicals also leads to the expression of inflammatory chemokines and cytokines,<sup>75</sup> the oxidation of nucleic acids, the alteration of DNA repair mechanisms, eventual cell death, and the mutagenic changes involved in cadmium-induced cancers.<sup>72</sup>

### *Metallothionein*

Metallothionein is a zinc-concentrating protein that contains 33-percent cysteine. Primarily induced and stored in the liver, it forms a complex with cadmium, sequestering it from inside the hepatic cytosol, thus reducing the amount of cadmium available to injure hepatocytes and preventing cadmium from depleting glutathione stores. Metallothionein has also been shown to prevent acute cadmium-induced hepatotoxicity and cell death in animal studies.<sup>70</sup> Mice with genetically-induced high levels of hepatic metallothionein and newborn animals with naturally high levels of metallothionein are resistant to cadmium-induced hepatotoxicity.<sup>76</sup> Metallothionein also has free-radical scavenging properties and is known to function like glutathione.<sup>77</sup> The ability of metallothionein to scavenge hydroxyl and superoxide radicals and function like superoxide dismutase in microorganisms has been demonstrated.<sup>78</sup>

Metallothionein, although it appears to assist in cadmium detoxification and prevent cadmium-induced damage, can also contribute to cadmium-induced renal damage. Cadmium bound to metallothionein can leak into plasma, leave storage sites in the liver, and be taken up by the kidney. The cadmium-metallothionein complex is dissolved and free cadmium is released in the kidney and reabsorbed in the proximal tubules. These free cadmium ions can again be bound by newly synthesized metallothionein. If production of kidney metallothionein and non-metallothionein defense and detoxification systems (glutathione) are not sufficient, free cadmium can damage cellular membranes in the renal tubules.<sup>70</sup> Mice that are genetically unable to produce metallothionein are much more susceptible to renal injury and hepatotoxicity resulting from long-term cadmium toxicity than metallothionein-producing mice.<sup>70</sup>

### *Renal Damage in Cadmium Toxicity*

An extensive review by Jarup et al includes an investigation of cadmium and renal damage.<sup>57</sup> The highest load of cadmium is found in the renal cortex. Renal concentrations in second trimester fetuses and infants compared to autopsy studies in adults show renal cadmium concentration increases about 5,000 times from birth to adulthood.<sup>79</sup> Studies of cortex concentrations have found that women have significantly higher concentrations than men, in spite of a higher male smoking rate.

The average cadmium exposure leads to kidney concentrations of 20 µg/g for nonsmokers and 40 µg/g for smokers.<sup>80</sup> At an average total intake of 30 µg/day, it is estimated that renal tubular damage occurs in one percent of the population. At an intake of 70 µg/day (the World Health Organization provisional tolerable weekly intake), seven percent of the adult population and up to 17 percent of high-risk groups would be expected to develop kidney lesions.<sup>57</sup> A Belgian study examining kidney cadmium and renal damage estimates that 10 percent of the Belgian population may currently have kidney cadmium concentrations of 50 µg/g, resulting in early signs of renal damage, proteinuria, and calcium loss.<sup>81</sup> In Japan, where cadmium exposure through environmental contamination of food and water has led to outbreaks of cadmium toxicity-related disease, cadmium-induced tubular lesions have been identified in more than 20,000 people. In Swedish studies, early signs of renal damage have appeared in those with urine cadmium levels of 0.5-2.0 µg/g creatinine, corresponding to renal cortex concentrations of 10-40 mg/kg, levels found in 50 percent of the adult Swedish population.<sup>57</sup> Glomerular damage and kidney stones have been seen in those with occupational exposure to cadmium. Studies of workers with cadmium-induced renal damage estimate 40-80 percent increased annual mortality risk as a result of cadmium exposure and renal damage. Once cadmium-induced nephropathy is initiated, it is accepted that it is irreversible.<sup>2</sup>

### *Cadmium and Bone*

The cadmium content of human bone in North America has increased by a factor of 50 in the last 600 years. The majority of that increase is believed to have occurred in the last 100 years.<sup>82</sup> Classic cadmium poisoning (known as itai-itai disease in Japan) has been characterized by multiple fractures, osteomalacia, bone pain, and osteoporosis that occurs along with renal disease.<sup>83</sup> Animal studies indicate postmenopausal women may be at greater risk for cadmium-related bone loss and that cadmium may increase bone loss in women with pre-existing postmenopausal osteoporosis.<sup>57</sup>

Epidemiological studies have found a positive correlation with elevated urinary cadmium levels and increased urinary calcium loss and elevated serum alkaline phosphatase levels.<sup>84</sup> Studies have also found correlations between cadmium-induced renal tubular damage and bone loss. A study of 1,021 men and women, who had either worked at a factory or lived in a community in Sweden where nickel-cadmium or lead batteries were produced, evaluated the relationship of cadmium and lead exposure to kidney and bone disease.<sup>85</sup> Those who were environmentally exposed and had the highest blood cadmium levels had a four-fold risk of tubular proteinemia. Older individuals (over 60 years) in that group had a three-fold risk of significant bone loss (Z-score < -1) compared to a same-age group with no known cadmium exposure. The Z score results from a comparison to the average bone density scores of a group of similar-aged individuals. A score of less than 0 indicates bone loss greater than the average of that same group.

The mechanisms behind cadmium and bone loss are related to renal tubular cell damage that results in elevated levels of urinary calcium and lowered levels of 1,25 dihydroxy-cholecalciferol, a consistent finding in women environmentally exposed to significant levels of cadmium.<sup>86</sup> Lower levels of activated vitamin D3 alter calcium homeostasis by decreasing absorption of calcium in the gut and altering deposition in bone.

### *Cadmium and Cancer, Heart Disease, and Reproduction*

Cadmium is classified as a group I human carcinogen, meaning sufficient evidence for carcinogenesis has been found in both animals and humans. Occupational and environmental exposure has been shown to increase risk for lung cancer with co-exposure to arsenic,<sup>87</sup> and renal cancer with cadmium exposure alone.<sup>88</sup> While animal studies support a role for cadmium-induced prostate cancer, inconsistent findings exist for cadmium's role in human prostate, breast, testicular, and bladder cancers.<sup>57</sup>

Cadmium appears to be completely filtered by the placenta when adequate zinc and copper are available for the induction of metallothionein. Studies with newborn rats reveal newborns whose cadmium-exposed mothers had been given adequate zinc and copper during pregnancy were cadmium-free at birth, as opposed to newborns whose cadmium-exposed mothers had a zinc and copper-deficient diet.<sup>89</sup> Maternal hypertension and low birth weight have been associated with elevated cadmium levels in infants.<sup>90</sup> Environmental exposure to lead, cadmium, and arsenic in pregnant women has been correlated with increased levels of lipid peroxides, and the incidence of threatened spontaneous abortion, toxemia, and anemia.<sup>91</sup> Only lead and cadmium exposure correlated with decreased levels of reduced glutathione.

Risk for hypertension and cardiovascular disease in nonpregnant women and in men is not conclusively a result of cadmium exposure. Studies have found both increased risk for cardiovascular mortality in one exposed group<sup>92</sup> and no increased risk for ischemic heart disease or hypertension in another large study.<sup>93</sup>

### *Antioxidants in Cadmium Toxicity Zinc and Metallothionein Induction as a Protective Mechanism*

Metallothionein production is induced by the presence of metals, including cadmium, mercury, copper, gold, bismuth, and most powerfully, zinc.<sup>94</sup> Low level zinc treatments have been used in animal studies to induce metallothionein and protect against acute cadmium-induced hepatotoxicity.<sup>95</sup> Similarly, hepatocyte cell lines treated with zinc became resistant to cadmium-induced cell death as a result of metallothionein induction.<sup>96</sup> In animals, both hepatic and intestinal metallothionein have been induced using oral zinc, and metallothionein induction using nontoxic zinc injections has been successful in reducing cadmium toxicity in animals.<sup>97</sup> The induction of intestinal metallothionein in humans, using zinc acetate, is the mechanism for the FDA-approved treatment of Wilson's disease, an inherited condition where accumulation of copper in the liver, brain, and other organs leads to copper toxicosis.<sup>98</sup>

The mechanisms of cadmium-induced renal damage result from the dissolution of the cadmium/metallothionein complex in the kidney, exposing renal tissue to unbound cadmium. Cadmium/cell membrane binding, cellular apoptosis of renal proximal tubules, increased calcium loss in the urine, and increased protein excretion are seen in animals given long-term doses of cadmium or repeated doses of cadmium/metallothionein complexes. Studies have also shown when the kidney is able to induce adequate *de novo* synthesis of metallothionein, no membrane damage occurs.<sup>70</sup>

Zinc has been used to induce renal metallothionein in animal studies and protects against cadmium/metallothionein-induced renal injury.<sup>99</sup> Rats pretreated with zinc or copper have shown less sensitivity to cadmium toxicity, specifically in renal proximal tubule cells. Proteinuria caused by cadmium-metallothionein injections was more effectively reduced by pretreatment injections with zinc than with copper.<sup>100</sup> Although there have been no human clinical trials with zinc or copper to assess metallothionein induction, zinc acetate, used to stimulate intestinal

**Table 3.** Effects of Dietary Selenium Levels on Whole Body Retention of Cadmium Chloride

	Low selenium diet, cadmium in drinking water	Normal selenium diet, cadmium in drinking water
Whole-body retention	14.4	6.4*
Liver	0.70	0.48
Kidneys	0.72	0.52

\*  $p < 0.05$  compared to low Se diet in the same experiment.

Adapted from: Andersen O, Nielsen JB. Effects of simultaneous low-level dietary supplementation with inorganic and organic selenium on whole-body, blood and organ levels of toxic metals in mice. *Environ Health Perspect* 1994;102:321-324.

metallothionein in the treatment of Wilson's disease, is nontoxic in 150 mg daily doses and has minimal side effects.<sup>98</sup> In those without Wilson's disease, the possibility of inducing a copper deficiency with high doses of zinc is preventable with copper supplementation.

### Alpha-Lipoic Acid

Alpha-lipoic acid (ALA), injected in cadmium-exposed murine hepatocytes, was shown to protect cells from toxic effects of cadmium, including hepatocyte membrane damage, lipid peroxidation, and depletion of intracellular glutathione.<sup>101</sup> These protective effects have also been seen in rats who had experimentally-depleted glutathione stores prior to cadmium exposure.<sup>102</sup> Although the acute toxicity induced in these studies (150  $\mu$ M or about 17 mg cadmium) is vastly different than low level chronic exposure in humans, oxidant stress and glutathione depletion are also recognized toxic mechanisms of low level exposure.<sup>103,104</sup> The authors of the first study concluded that dihydrolipoic acid (the reduced form of alpha-lipoic acid) is an effective extra- and intracellular chelator of cadmium in hepatocytes as a

result of a significant decrease in intra- and extracellular levels of cadmium after ALA was added to the cells.<sup>101</sup> The authors measured both intra- and extracellular cadmium/lipoate and cadmium/dihydrolipoate complexes to conclude that cadmium was actually being removed from the hepatocytes by lipoic acid compounds themselves and not glutathione generated by lipoic acid. They also noted, however, that these effects occurred only at low levels of cadmium exposure and high levels of lipoic acid concentration.<sup>101</sup>

### Selenium

The theory that selenium and cadmium can form complexes has been substantiated by researchers in animal studies with concomitant selenium and cadmium exposure.<sup>105,106</sup>

In a study with acute cadmium toxicity (8 mg/kg oral cadmium) and concomitant oral selenium supplementation (350  $\mu$ g/kg sodium selenite), rats who received both had a 25-percent reduction in kidney cadmium. The ability of selenium to decrease the tissue burden of cadmium has been repeated in other animal studies.<sup>105</sup> Acute toxicity studies have found that, as a result of dosing selenium and cadmium at the same time, organ tissue levels of both metals increased and the toxic burden of cadmium decreased, possibly as a result of the inert nature of the cadmium/selenium complex.<sup>107</sup> A low-level cadmium exposure study in mice (1 ng/L drinking water) with a varied selenium diet revealed significant differences in cadmium retention.<sup>108</sup> In mice that received a normal selenomethionine/sodium selenite diet (99.25  $\mu$ g selenomethionine and 68  $\mu$ g sodium selenite/kg food) the whole body retention of cadmium was less than half of the retention in mice on a low selenium diet (31.25  $\mu$ g selenomethionine/kg food) (Table 3). For comparison, the average American gets approximately 65  $\mu$ g of selenium per day through diet and supplementation.

**Table 4.** Effect of Chelator and/or Methionine on Tissue Cadmium Levels

Treatment	Liver ( $\mu\text{g/g}$ fresh tissue)	Kidney ( $\mu\text{g/g}$ fresh tissue)	Brain ( $\mu\text{g/g}$ fresh tissue)
Normal-control	1.67 $\pm$ 0.19	0.55 $\pm$ 0.12	0.05 $\pm$ 0.02
Cd (control)	43.47 $\pm$ 1.77 <sup>a</sup>	47.68 $\pm$ 3.72 <sup>a</sup>	2.59 $\pm$ 0.45 <sup>a</sup>
CaNa <sub>3</sub> DTPA	31.81 $\pm$ 1.83 <sup>c</sup>	29.24 $\pm$ 4.13 <sup>c</sup>	1.54 $\pm$ 0.35 <sup>c</sup>
DMPS	24.60 $\pm$ 1.29 <sup>c</sup>	22.36 $\pm$ 1.20 <sup>c</sup>	1.75 $\pm$ 0.44 <sup>c</sup>
Methionine	19.77 $\pm$ 2.54 <sup>c</sup>	17.06 $\pm$ 2.68 <sup>c</sup>	1.42 $\pm$ 0.28 <sup>c</sup>
CaNa <sub>3</sub> DTPA + Methionine	15.20 $\pm$ 2.76 <sup>c**</sup>	12.74 $\pm$ 3.22 <sup>c***</sup>	1.47 $\pm$ 0.46 <sup>c</sup>
DMPS + Methionine	13.76 $\pm$ 2.60 <sup>c*</sup>	7.79 $\pm$ 2.62 <sup>c*</sup>	1.04 $\pm$ 0.07 <sup>c***</sup>

Values are mean ( $\pm$  S.D.; n=6).

\* p < 0.001; \*\* p < 0.01; \*\*\* p < 0.05 versus CaNa<sub>3</sub> DTPA or methionine/DMPS or methionine at 5% level of significance (ANOVA).

The Cd removal in % control, has been given in parenthesis.

<sup>a</sup> p < 0.001; <sup>b</sup> p < 0.05 versus normal-control (Student's t-test); <sup>c</sup> p < 0.01 versus Cd (control).

Adapted from: Tandon SK, Singh S, Prasad S. Influence of methionine administration during chelation of cadmium by CaNa<sub>3</sub>DTPA and DMPS in the rat. *Environ Toxicol Pharmacol* 1997;3:159-165.

Selenium supplementation has a known antioxidant action in cadmium toxicity. Selenium supplementation in acute cadmium toxicity has been shown to decrease lipid peroxidation in rat studies<sup>109</sup> and has also been shown to increase the production of glutathione S-transferase and glutathione peroxidase in rhesus monkeys.<sup>106</sup> The dosage of selenium in the primate study was far beyond what would be used in human trials, 500  $\mu\text{g/kg}$  body weight, but the cadmium exposure was also elevated beyond possible environmental or occupational human exposure to 5 mg/kg body weight/day. Similar results – elevations of glutathione peroxidase and decreased whole body and renal burden of cadmium – were found in rats given daily selenium supplementation of 350  $\mu\text{g/kg}$  body weight.<sup>105</sup> Selenium also appears to act in conjunction with other antioxidants. When selenium was given to rats simultaneously with vitamin E and glutathione, the cadmium uptake in liver

and kidneys was significantly inhibited.<sup>110</sup> Selenium has also been shown to decrease lipid peroxidation in testicular tissue of rats,<sup>111</sup> an effect relevant to human health due to the correlation of blood cadmium levels in men with decreases in sperm motility and alterations in sperm morphology.<sup>112</sup>

### Plant Triterpenes

Triterpenes are common plant compounds shown to have antioxidant,<sup>113</sup> hepatoprotective,<sup>114</sup> anti-inflammatory,<sup>115</sup> and antitumor<sup>116,117</sup> properties. Triterpenoids also induce metallothionein in cadmium toxicity. Oleanolic acid, a triterpenoid present in many plants and one of the active constituents of *Ligustrum lucidum*, is used in China to treat hepatitis. It has also been shown to induce hepatic metallothionein in cadmium toxicity.<sup>118</sup> Doses of 100 mg/kg of oleanolic acid were given to mice for three days prior to cadmium injections

**Table 5. Influence of Cysteine or N-acetylcysteine on the Efficacy of DMPS**

Treatment	Liver ( $\mu\text{g g}^{-1}$ fresh tissue)			Kidney ( $\mu\text{g g}^{-1}$ fresh tissue)		
	WT	SCF	NMF	WT	SCF	NMF
Normal Animal	ND	ND	ND	ND	ND	ND
Cd (control)	80.24 $\pm$ 10.55	72.46 $\pm$ 11.95	6.83 $\pm$ 1.69	57.93 $\pm$ 7.87	48.57 $\pm$ 9.02	8.80 $\pm$ 1.87
Cd-cysteine	37.41 $\pm$ 3.43***	31.29 $\pm$ 6.85***	6.01 $\pm$ 2.03	33.83 $\pm$ 3.90***	24.13 $\pm$ 6.83***	9.38 $\pm$ 1.47
Cd-N-acetylcysteine	55.36 $\pm$ 3.36***	49.28 $\pm$ 5.35***	5.95 $\pm$ 0.79	31.82 $\pm$ 4.64***	24.34 $\pm$ 5.46***	7.55 $\pm$ 2.16
Cd-DMPS	39.79 $\pm$ 5.82***	32.19 $\pm$ 5.95***	6.61 $\pm$ 0.54	27.08 $\pm$ 1.52***	19.62 $\pm$ 2.04***	5.52 $\pm$ 0.80***
Cd-DMPS + cysteine	42.85 $\pm$ 4.96***	37.68 $\pm$ 3.45***	4.60 $\pm$ 0.69***	48.90 $\pm$ 4.89***	43.27 $\pm$ 4.50***	5.38 $\pm$ 1.10***
Cd-DMPS + N-acetylcysteine	37.13 $\pm$ 3.86***	33.99 $\pm$ 3.28***	2.16 $\pm$ 0.74***	45.72 $\pm$ 5.01***	41.28 $\pm$ 5.85***	5.21 $\pm$ 0.33***

<sup>a</sup> Values are means  $\pm$  SD(n=6); WT, whole tissue; SCF, supernatant cytosol fraction; NMF, nuclear mitochondrial fraction; ND, not detected; \* p < 0.05, \*\*\* p < 0.001 and \*\*\*\* p < 0.001 vs Cd-control and \*\* p < 0.01 and \*\*\* p < 0.001 vs Cd + DMPS at 5% level of significance (ANOVA).

Adapted from: Tandon SK, Prasad S, Singh S. Chelation in metal intoxication: influence of cysteine or N-Acetyl cysteine on the efficacy of 2,3-dimercaptopropane-1-sulphonate in the treatment of cadmium toxicity. *J Appl Toxicol* 2002;22:67-71.

in doses known to induce acute liver injury. Oleanolic acid resulted in a 30-fold increase in hepatic metallothionein and a significant increase in the mobilization of cadmium, preventing cadmium-binding to intracellular proteins. Liver injury was also significantly reduced, as indicated by reductions in ALT and sorbitol dehydrogenase levels.

Specific triterpenoids, including oleanolic acid, betulinic acid, ursolic acid, soyasapogenol A and B (all present in *Glycyrrhiza glabra* and *Betula alba* L.), uvaol (present in *Betula alba* and *Syzgium* sp.), and glycyrrhizin have been found to be effective in reducing the hepatotoxicity of cadmium.<sup>117</sup> Betulin, present in high levels in white birch bark (*Betula alba* L.), was found to have the strongest ability to reduce the cytotoxicity of cadmium-poisoned hepatocytes and completely prevented toxicity at doses as minimal as 0.1 µg/mL.<sup>119</sup> The mechanism of betulin appears to be a gene-promoting effect in hepatocytes that eliminates the toxic effects of cadmium.

### Glycyrrhizin

Glycyrrhizin, a triterpenoid saponin, is known to act in hepatic tissues as an antioxidant by reducing lipid peroxidation.<sup>120</sup> The Japanese drug, Stronger Neo-Minophagen C (SNMC), which contains glycyrrhizin, glycine, and cysteine, has also been shown to protect against acute cadmium toxicity-related hepatic damage and renal damage in animal studies.<sup>104,121</sup> The dosage of the glycyrrhizin compound used in these studies was small – 2 mg glycyrrhizin, 20 mg glycine, and 1 mg cysteine/kg – yet was able to reverse the nephrotoxicity brought on by 19 weeks of daily high-level cadmium injections. As a result of the SNMC studies, researchers investigated the effect of glycine to differentiate therapeutic effects of glycine from those of glycyrrhizin. Studies with glycine (12 mmol/L hepatic perfusion) alone found it could prevent the decrease in bile flow caused by cadmium, but glycine was unable to reverse the 30-90 percent decrease in hepatic glutathione caused by cadmium.<sup>122</sup>

**Table 6. Influence of Zinc Supplementation during Chelation Treatment on Cadmium Concentration in Liver and Kidneys**

	Liver (µg g <sup>-1</sup> )	Kidneys (µg g <sup>-1</sup> )
Normal Animal	0.02 ± 0.004	0.016 ± 0.004
Cd (control)	48.2 ± 4.14 <sup>b</sup>	71.4 ± 3.94 <sup>b</sup>
Cd + DTPA	38.2 ± 3.59	54.5 ± 2.30 <sup>c</sup>
Cd + DMSA	42.7 ± 2.89	66.2 ± 3.94
Cd + Zn	50.1 ± 3.08	78.4 ± 3.28
Cd + Zn + DTPA	32.7 ± 1.82 <sup>c</sup>	35.8 ± 1.76 <sup>d</sup>
Cd + Zn + DMSA	43.4 ± 2.14	64.2 ± 3.37

<sup>a</sup> values are mean ± SEM (n=6)

<sup>b</sup> p < 0.001 compared to normal animals

<sup>c</sup> p < 0.01 compared to Cd-exposed control

<sup>d</sup> p < 0.001 compared to Cd-exposed control

Adapted from: Flora SJ, Gubrelay U, Kannan GM, Mathur R. Effects of zinc supplementation during chelating agent administration in cadmium intoxication in rats. *J Appl Toxicol* 1998;18:357-362.

### Melatonin

Melatonin, a known antioxidant, has been studied as a preventive agent in cadmium-induced lipid peroxidation. Pretreatment by single injection of 15 mg/kg body weight in hamsters completely prevented lipid peroxidation in the brain and the kidney induced by cadmium injection.<sup>123</sup>

### Antioxidants and Chelating Agents

Cadmium is known to bind tightly to metallothionein in complexes stored intracellularly in the liver and kidney.<sup>124</sup> Because standard chelating agents do not work intracellularly, many sources state there is no clinically effective treatment for cadmium poisoning.<sup>2,59</sup> While DMSA is effective for both lead and mercury toxicity, it is not an intracellular chelator. DMPS has limited ability to enter the cell and chelate cadmium.<sup>124</sup>

**Table 7. Influence of Zinc Supplementation during Chelation Treatment on the Levels of Biochemical Variables in Liver of Cadmium-exposed Rats**

	AST	ALT	ALP	γ-GT	Urea	Creatinine
Normal Animal	25.7 ± 1.98	31.6 ± 4.25	4.0 ± 0.85	6.52 ± 0.32	26.3 ± 1.72	1.95 ± 0.52
Cd (Control)	32.8 ± 0.07 <sup>b</sup>	44.8 ± 1.60 <sup>c</sup>	8.5 ± 0.65 <sup>b</sup>	8.29 ± 0.21 <sup>d</sup>	28.0 ± 0.44	2.41 ± 0.58
Cd + DTPA	26.2 ± 1.03 <sup>***</sup>	29.1 ± 2.18 <sup>***</sup>	4.8 ± 0.48 <sup>**</sup>	6.49 ± 1.13	29.0 ± 2.24	1.97 ± 0.41
Cd + DMSA	39.2 ± 2.60 <sup>**</sup>	47.3 ± 6.06	7.7 ± 0.75	9.31 ± 0.45	22.7 ± 1.61	1.83 ± 0.52
Cd + Zn	29.5 ± 0.76	36.4 ± 2.50 <sup>*</sup>	5.0 ± 0.47 <sup>**</sup>	8.17 ± 1.23	33.5 ± 0.61	2.03 ± 0.87
Cd + Zn + DTPA	20.5 ± 0.81 <sup>**</sup>	32.5 ± 3.12 <sup>*</sup>	3.8 ± 78.0 <sup>**</sup>	9.97 ± 1.25	29.4 ± 5.45	1.92 ± 0.37
Cd + Zn + DMSA	29.5 ± 2.50	39.4 ± 0.59 <sup>**</sup>	2.4 ± 0.08 <sup>***</sup>	7.75 ± 0.74	29.08 ± 2.11	1.86 ± 0.26

<sup>a</sup> Values are means ± SEM(n=6); \* p < 0.05 and \*\* p < 0.01 and \*\*\* p < 0.001 compared to Cd-exposed control.

<sup>b</sup> p < 0.001 compared to normal animals.

<sup>c</sup> p < 0.05 compared to normal animals.

Adapted from: Flora SJ, Gubrelay U, Kannan GM, Mathur R. Effects of zinc supplementation during chelating agent administration in cadmium intoxication in rats. *J Appl Toxicol* 1998;18:357-362.

Diethylenetriaminepentaacetate (DTPA), a chelating agent used to chelate uranium isotopes, binds tightly to cadmium.<sup>125</sup> Prior animal studies with

lead toxicity have shown methionine (a glutathione precursor), used in conjunction with chelating agents, increased lead elimination.<sup>126</sup> Two studies have followed looking at the co-administration of DMPS and DTPA using the glutathione precursors methionine, cysteine, and NAC in cadmium chelation.<sup>125,127</sup> In the first study, rats pre-exposed to cadmium were given a combination of oral methionine and injections of either DMPS or DTPA as chelating agents.<sup>125</sup> After three days of methionine/chelation treatment the cadmium content of organ tissue was compared to rats receiving either chelating agent or methionine alone, or controls who had received only cadmium and controls who had not received any treatment (Table 4). DMPS plus methionine was significantly more effective in removing cadmium from the liver, kidney, and brain than DTPA plus methionine or any of the treatments alone.

A second study evaluated a three-day oral dosing of the antioxidants cysteine and NAC with oral DMPS in acute cadmium-exposed rats.<sup>127</sup> Because DMPS is 60-percent orally bioavailable, it was administered by mouth. The combination treatments were more effective than any agent alone at mobilizing cadmium from body stores and delivering it to the



*Table 8. Summary of Nutrients and their Effects on Arsenic and Cadmium*

SAME	Necessary for As methylation <sup>30</sup>
Selenium	Forms insoluble complexes with As <sup>28,37-39</sup> <i>In vitro</i> with GSH and methylcobalamin; supports As methylation <sup>40</sup> Forms inert complexes with Cd and decreases toxicity <sup>105,106</sup> ↓ lipid peroxidation, <sup>109,110</sup> ↑ glutathione-recycling enzymes <sup>106</sup> ↓ tissue retention of Cd <sup>108</sup> + GSH and vitamin E results in ↓ Cd uptake in liver <sup>110</sup>
NAC	+ DMSA ↑ hepatic GSH and normalizes RBC GSH in As toxicity <sup>53</sup> + DMPS ↑ hepatic metallothionein and ↑ Cd chelation from intracellular hepatic stores <sup>127</sup>
Lipoic acid	Hepatic protection from Cd-induced damage <sup>101</sup> Mobilizes Cd by forming ALA-Cd complex <sup>101</sup>
Zinc	Increases metallothionein levels in liver, kidney, intestines <sup>93</sup> + DTPA ↓ hepatic Cd stores <sup>128</sup>
Oleanolic acid	↑ hepatic metallothionein and mobilizes Cd <sup>118</sup>
Betulin	↓ hepatotoxicity of Cd <sup>117</sup>
Glycyrrhizin	Prevents hepatic and renal damage from Cd toxicity <sup>104,121</sup>
Melatonin	Prevents lipid peroxidation in acute Cd toxicity <sup>123</sup>
Methionine	+ DMPS improves ability of DMPS to chelate Cd from liver and kidneys <sup>125</sup>
Cysteine	+ DMPS ↑ hepatic metallothionein and ↑ Cd chelation from intracellular hepatic stores <sup>127</sup>

from the intracellular hepatic compartments. DMPS/NAC was significantly more effective ( $p < 0.001$ ) than the DMPS/cysteine ( $p < 0.01$ ) combination. The authors credited the glutathione precursor status of cysteine and NAC in their ability to decrease cadmium levels in the nuclear fractions of hepatic tissue compared to chelation alone. They also theorized that both cysteine and NAC were able to induce metallothionein, explaining

the significant elevations in renal and hepatic metallothionein in rats receiving combination treatment as opposed to those receiving DMPS alone.

Zinc has also been given in combination with either DMSA or DTPA to rats pre-exposed to low levels of cadmium (10 ppm/liter water).<sup>128</sup> Zinc sulphate (20 mg/100 g body weight) was

given with either chelating agent for two five-day periods, with a seven-day rest period in between (to prevent side effects from the chelating agents). Only DTPA and zinc had any significant lowering effect on liver concentrations of cadmium, and only DTPA alone or DTPA with zinc had any effect on renal cadmium (Table 6). Zinc supplementation alone, however, was able to normalize serum AST and ALT levels, reflecting cadmium-induced hepatic damage (Table 7). And zinc, added to DMSA, was able to significantly reduce serum AST and ALT levels compared to DMSA alone. Zinc alone resulted in a significant increase in both hepatic and renal metallothionein levels. The authors suggest from the results of this study that zinc-induced metallothionein is capable of binding cadmium and reducing cadmium toxicity, and that zinc aids in the mobilization of cadmium from intracellular storage depots.

## Conclusion

Arsenic and cadmium are ubiquitous and dangerous environmental toxins. Arsenic in groundwater and concentrated in soil and food is a Group A human carcinogen. Exposure can cause a variety of cancers, most commonly nonmelanoma skin cancers, and chronic toxicity may manifest as diffuse symptoms not easily recognizable as chronic heavy metal toxicity. Arsenic is metabolized via a methylation sequence that uses glutathione and SAME, eventually being eliminated through the intestines and kidneys. Recent research suggests the end products of methylation are also carcinogenic. Methylation is aided by methylcobalamin and possibly selenium, which has long been known to both aid in arsenic elimination and bind arsenic in a nontoxic selenium-arsenic complex.

Oxidant stress and lipid peroxidation are well-defined mechanisms of arsenic toxicity related to arsenic-induced skin cancers and the reduction of whole-blood glutathione stores in humans with environmental exposure.

*In vivo* studies with animals and fetal cell cultures exposed to arsenic have shown that antioxidants, particularly NAC, vitamin E, and vitamin C, given in conjunction with a chelating agent (DMSA), have been able to restore glutathione

levels and reduce damage secondary to oxidative stress.

Cadmium, through contaminated soil, food, and tobacco smoking, may have significantly increased toxicity in those with iron, zinc, or calcium deficiency due to increased gastrointestinal absorption and increased calcium loss. Chronic cadmium toxicity has been linked to lung and kidney cancers, irreversible renal damage, osteoporosis, and osteomalacia. Cadmium is stored primarily in the kidneys and liver, tightly bound to metallothionein in an intracellular complex. Metallothionein has protective effects on cadmium toxicity, but may also facilitate renal damage if it is not produced in renal tissues in sufficient quantities. Zinc induces hepatic and renal metallothionein, and has been shown to protect both organs from cadmium-induced damage. Lipoic acid, selenium, naturally occurring triterpenoid compounds, and melatonin have been shown to inhibit oxidant production secondary to cadmium exposure and to mitigate cadmium toxicity in animal studies. NAC, methionine, cysteine, and zinc improve the efficacy of the chelating agents DMPS and DMSA by allowing removal of cadmium from intracellular stores and raising metallothionein levels.

## References

1. National Priorities List. U.S. Environmental Protection Agency. December 24, 2002. <http://www.epa.gov/superfund/sites/query/queryhtm/npltotal.htm>
2. Hu H. Exposure to metals. *Prim Care* 2000;27:983-996.
3. Centeno JA, Mullick FG, Martinez L, et al. Pathology related to chronic arsenic exposure. *Environ Health Perspect* 2002;110:883-886.
4. Agency for Toxic Substances and Disease Registry (ATSDR). *2000 Toxicological Profile for Arsenic*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2001:244.
5. Agency for Toxic Substances and Disease Registry (ATSDR). *2000 Toxicological Profile for Arsenic*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2001:244-246.

6. Ryker SJ. Mapping arsenic in groundwater. *Geotimes* 2001;46:34-36.
7. Lewis DR, Southwick JW, Ouellet-Hellstrom R, et al. Drinking water arsenic in Utah: a cohort mortality study. *Environ Health Perspect* 1999;107:359-365.
8. Warner ML, Moore LE, Smith MT, et al. Increased micronuclei in exfoliated bladder cells of individuals who chronically ingest arsenic-contaminated water in Nevada. *Cancer Epidemiol Biomarkers Prev* 1994;3:583-590.
9. US EPA Notice of Proposed Rulemaking. National primary drinking water regulations; arsenic and clarifications to compliance and new source contaminants monitoring 65. *Federal Register*. June 22, 2000;38:904.
10. National Academy of Sciences. *Arsenic in Drinking Water*. Washington, DC: National Academies Press; 1999:223.
11. Agency for Toxic Substances and Disease Registry (ATSDR). *2000 Toxicological Profile for Arsenic*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2000:243-269.
12. Agency for Toxic Substances and Disease Registry (ATSDR). *2000 Toxicological Profile for Arsenic*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2000:A-5.
13. Helgesen H, Larsen EH. Bioavailability and speciation of arsenic in carrots grown in contaminated soil. *Analyst* 1998;123:791-796.
14. Agency for Toxic Substances and Disease Registry (ATSDR). *2000 Toxicological Profile for Arsenic*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2000:282.
15. Agency for Toxic Substances and Disease Registry (ATSDR). *2000 Toxicological Profile for Arsenic*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2000:276.
16. Franzblau A, Lilis R. Acute arsenic intoxication from environmental arsenic exposure. *Arch Environ Health* 1989;44:385-390.
17. Rahman M, Tondel M, Ahmad SA, et al. Hypertension and arsenic exposure in Bangladesh. *Hypertension* 1999;33:74-78.
18. Engel RR, Smith AH. Arsenic in drinking water and mortality from vascular disease: an ecologic analysis in 30 counties in the United States. *Arch Environ Health* 1994;49:418-427.
19. Tseng CH, Tai TY, Chong CK, et al. Long-term arsenic exposure and the incidence of non-insulin-dependant diabetes mellitus: a cohort study in arseniasis-hyperendemic villages in Taiwan. *Environ Health Perspect* 2000;108:847-851.
20. Wu MM, Kuo TL, Hwang YH, Chen CJ. Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. *Am J Epidemiol* 1989;130:1123-1132.
21. Chen CJ, Wang CJ. Ecological correlation between arsenic level in well water and age-adjusted mortality from malignant neoplasms. *Cancer Res* 1990;50:5470-5474.
22. Concha G, Vogler G, Lezcano D, et al. Exposure to inorganic arsenic metabolites during early human development. *Toxicol Sci* 1998;44:185-190.
23. Concha G, Vogler G, Nermell B, Vahter M. Low-level arsenic excretion in breast milk of native Andean women exposed to high levels of arsenic in the drinking water. *Int Arch Occup Environ Health* 1998;71:42-46.
24. Agency for Toxic Substances and Disease Registry (ATSDR). *2000 Toxicological Profile for Arsenic*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2000:18.
25. Vahter M, Friberg L, Rahnster B, et al. Airborne arsenic and urinary excretion of metabolites of inorganic arsenic among smelter workers. *Int Arch Occup Environ Health* 1986;57:79-91.
26. Agency for Toxic Substances and Disease Registry (ATSDR). *2000 Toxicological Profile for Arsenic*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2000:134.
27. Gyurasics A, Varga F, Gregus Z. Effect of arsenicals on biliary excretion of endogenous glutathione and xenobiotics with glutathione-dependent hepatobiliary transport. *Biochem Pharmacol* 1991;41:937-944.
28. Aposhian HV, Zakharyan RA, Wildfang EK, et al. How is inorganic arsenic detoxified? In: Chappell WR, Abernathy CO, Calderon RL, eds. *Arsenic: Exposure and Health Effects*. New York, NY: Elsevier Science Ltd.; 1999:289-297.
29. Agency for Toxic Substances and Disease Registry (ATSDR). *2000 Toxicological Profile for Arsenic*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 2000:140.

30. Vahter M, Marafante E. Effects of low dietary intake of methionine, choline or proteins on the biotransformation of arsenite in the rabbit. *Toxicol Lett* 1987;37:41-46.
31. Goering PL, Aposhian HV, Mass MJ, et al. The enigma of arsenic carcinogenesis: role of metabolism. *Toxicol Sci* 1999;49:5-14.
32. National Academy of Sciences. *Arsenic in Drinking Water*. Washington, DC: National Academies Press; 2001:81.
33. Ercal N, Gurer-Orhan H, Aykin-Burns N. Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Curr Top Med Chem* 2001;1:529-539.
34. National Academy of Sciences. *Arsenic in Drinking Water*. Washington, DC: National Academies Press; 1999:229.
35. National Academy of Sciences. *Arsenic in Drinking Water*. Washington, DC: National Academies Press; 1999:238.
36. Hopenhayn-Rich C, Biggs ML, Kalman DA, et al. Arsenic methylation patterns before and after changing from high to low concentrations of arsenic in drinking water. *Environ Health Perspect* 1996;104:1200-1207.
37. National Academy of Sciences. *Arsenic in Drinking Water*. Washington, DC: National Academies Press; 1999:240.
38. Levander OA. Metabolic interrelationships between arsenic and selenium. *Environ Health Perspect* 1977;19:159-164.
39. Kenyon EM, Hughes MF, Levander OA. Influence of dietary selenium on the disposition of arsenate in the female B6C3F1 mouse. *J Toxicol Environ Health* 1997;51:279-299.
40. Zakharyan RA, Aposhian HV. Arsenite methylation by methylvitamin B12 and glutathione does not require an enzyme. *Toxicol Appl Pharmacol* 1999;154:287-291.
41. National Academy of Sciences. *Arsenic in Drinking Water*. Washington, DC: National Academies Press; 2001:91.
42. National Academy of Sciences. *Arsenic in Drinking Water*. Washington, DC: National Academies Press; 2001:108-109.
43. Matsui M, Nishigori C, Toyokuni S, et al. The role of oxidative DNA damage in human arsenic carcinogenesis: detection of 8-hydroxy-2'-deoxyguanosine in arsenic-related Bowen's disease. *J Invest Dermatol* 1999;113:26-31.
44. National Academy of Sciences. *Arsenic in Drinking Water*. Washington, DC: National Academies Press; 2001:75-202.
45. National Academy of Sciences. *Arsenic in Drinking Water*. Washington, DC: National Academies Press; 2001:76-77.
46. National Academy of Sciences. *Arsenic in Drinking Water*. Washington, DC: National Academies Press; 2001:207-208.
47. National Academy of Sciences. *Arsenic in Drinking Water*. Washington, DC: National Academies Press; 2001:207-210.
48. Pi J, Yamauchi H, Kumagai Y, et al. Evidence for induction of oxidative stress caused by chronic exposure of Chinese residents to arsenic contained in drinking water. *Environ Health Perspect* 2002;110:331-336.
49. Wu MM, Chiou HY, Want TW, et al. Association of blood arsenic levels with increased reactive oxidants and decreased antioxidant capacity in a human population of northeastern Taiwan. *Environ Health Perspect* 2001;109:1011-1017.
50. Hsueh YM, Wu WL, Huang YL, et al. Low serum carotene level and increased risk of ischemic heart disease related to long-term arsenic exposure. *Atherosclerosis* 1998;141:249-257.
51. Personal communication. Walter Crinnion, ND.
52. Aposhian HV, Maiorino RM, Gonzalez-Ramirez D, et al. Mobilization of heavy metals by newer, therapeutically useful chelating agents. *Toxicology* 1995;97:23-38.
53. Flora SJ. Arsenic-induced oxidative stress and its reversibility following combined administration of N-acetylcysteine and meso 2,3-dimercaptosuccinic acid in rats. *Clin Exp Pharmacol Physiol* 1999;26:865-869.
54. Tseng CH, Chong CK, Chen CJ, Tai TY. Lipid profile and peripheral vascular disease in arseniasis-hyperendemic villages in Taiwan. *Angiology* 1997;48:321-335.
55. Chaudhuri AN, Basu S, Chattopadhyay S, Das Gupta S. Effect of high arsenic content in drinking water on rat brain. *Indian J Biochem Biophys* 1999;36:51-54.
56. Chattopadhyay S, Bhaumik S, Purkayastha M, et al. Apoptosis and necrosis in developing brain cells due to arsenic toxicity and protection with antioxidants. *Toxicol Lett* 2002;136:65-76.
57. Jarup L, Berglund M, Elinder CG, et al. Health effects of cadmium exposure – a review of the literature and a risk estimate. *Scand J Work Environ Health* 1998;24:1-51.

58. Agency for Toxic Substances and Disease Registry (ATSDR). *1999 Toxicological Profile for Cadmium*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1999:242.
59. Wittman R, Hu H. Cadmium exposure and nephropathy in a 28-year-old female metals worker. *Environ Health Perspect* 2002;110:1261-1266.
60. Agency for Toxic Substances and Disease Registry (ATSDR). *1999 Toxicological Profile for Cadmium*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1999:258.
61. Agency for Toxic Substances and Disease Registry (ATSDR). *1999 Toxicological Profile for Cadmium*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1999:249.
62. *Imported cadmium-contaminated zinc sulfate used in fertilizer and other products*. Washington State Department of Ecology. July 2000. Document 00-04-025.
63. *Final Report. Screening survey for metals and dioxins in fertilizer products and soils in Washington state*. Washington State Department of Ecology. April 1999. Publication No. 99-309.
64. Agency for Toxic Substances and Disease Registry (ATSDR). *1999 Toxicological Profile for Cadmium*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1999:259.
65. Agency for Toxic Substances and Disease Registry (ATSDR). *1999 Toxicological Profile for Cadmium*. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service; 1999:166.
66. Fox S. Nutritional factors that may influence bioavailability of cadmium. *J Environ Qual* 1988;17:175-180.
67. Nordberg GF, Kjellstrom T, Norberg M. Kinetics and metabolism. In: Friberg L, Elinder CG, Kjellstrom T, et al, eds. *Cadmium and Health: A Toxicological and Epidemiological Appraisal, Vol 1: Exposure Dose and Metabolism*. Boca Raton: CRC Press; 1985:103-178.
68. Berglund M, Akesson A, Nermell B, Vahter M. Intestinal absorption of dietary cadmium in women depends on body iron stores and fiber intake. *Environ Health Perspect* 1994;102:1058-1066.
69. Ramakrishnan U, Frith-Terhune A, Cogswell M, Kettle Khan L. Dietary intake does not account for differences in low iron stores among Mexican American and non-Hispanic white women: Third National Health and Nutrition Examination Survey, 1988-1994. *J Nutr* 2002;132:996-1001.
70. Klaassen CD, Liu J, Choudhuri S. Metallothionein: an intracellular protein to protect against cadmium toxicity. *Annu Rev Pharmacol Toxicol* 1999;39:267-294.
71. Pope A, Rall DP. *Environmental Medicine. Integrating a Missing Element into Medical Education*. Washington, DC: National Academy Press; 1995:230-231.
72. Fowler BA. General subcellular effects of lead, mercury, cadmium, and arsenic. *Environ Health Perspect* 1978;22:37-41.
73. Casalino E, Calzaretto G, Sblano C, Landriscina C. Molecular inhibitory mechanisms of antioxidant enzymes in rat liver and kidney by cadmium. *Toxicology* 2002;179:37-50.
74. Wasowicz W, Gromadzinska J, Ryzdzyński K. Blood concentration of essential trace elements and heavy metals in workers exposed to lead and cadmium. *Int J Occup Med Environ Health* 2001;14:223-229.
75. Dong W, Simeonova PP, Gallucci R, et al. Toxic metals stimulate inflammatory cytokines in hepatocytes through oxidative stress mechanisms. *Toxicol Appl Pharmacol* 1998;151:359-366.
76. Liu J, Liu Y, Michalska AE, et al. Metallothionein plays less of a protective role in cadmium-metallothionein-induced nephrotoxicity than in cadmium chloride-induced hepatotoxicity. *J Pharmacol Exp Ther* 1996;276:1216-1223.
77. Klaassen CD, Cagen SZ. Metallothionein as a trap for reactive organic intermediates. *Adv Exp Med Biol* 1981;136:633-646.
78. Thornalley PJ, Vasak M. Possible role for metallothionein in protection against radiation-induced oxidative stress. Kinetics and mechanism of its reaction with superoxide and hydroxyl radicals. *Biochim Biophys Acta* 1985;827:36-44.
79. Elinder CG, Lind B, Kjellstrom T, et al. Cadmium in kidney cortex, liver, and pancreas from Swedish autopsies. Estimation of biological half time in kidney cortex, considering calorie intake and smoking habits. *Arch Environ Health* 1976;31:292-302.

80. Elinder CG, Kjellstrom T, Lind B, et al. Cadmium exposure from smoking cigarettes: variations with time and country where purchased. *Environ Res* 1983;32:220-227.
81. Buchet JP, Lauwerys R, Roels H, et al. Renal effects of cadmium body burden of the general population. *Lancet* 1990;336:699-702.
82. Ericson JE, Smith DR, Flegal AR. Skeletal concentrations of lead, cadmium, zinc, and silver in ancient North American Pecos Indians. *Environ Health Perspect* 1991;93:217-223.
83. Noda M, Kitagawa M. A quantitative study of iliac bone histopathology on 62 cases with itai-itai disease. *Calcif Tissue Int* 1990;47:66-74.
84. Staessen J, Bernard A, Buchet JP, et al. Effects of cadmium exposure on the cardiovascular system and on calcium metabolism: results of a cross-sectional population study. *IARC Sci Publ* 1992;118:263-269.
85. Alfven T, Jarup L, Elinder CG. Cadmium and lead in blood in relation to low bone mineral density and tubular proteinuria. *Environ Health Perspect* 2002;110:699-702.
86. Nogawa K, Tsuritani I, Kido T, et al. Mechanism for bone disease found in inhabitants environmentally exposed to cadmium: decreased serum 1 alpha, 25-dihydroxyvitamin D level. *Int Arch Occup Environ Health* 1987;59:21-30.
87. Sorahan T, Lancashire RJ. Lung cancer mortality in a cohort of workers employed at a cadmium recovery plant in the United States: an analysis with detailed job histories. *Occup Environ Med* 1997;54:194-201.
88. Mandel JS, McLaughlin JK, Schlehofer B, et al. International renal-cell cancer study. IV: Occupation. *Int J Cancer* 1995;61:601-605.
89. Goyer RA, Cherian MG. Role of metallothionein in human placenta and rats exposed to cadmium. In: Nordberg GE, Herber RF, Alessio L, eds. *Cadmium in the Human Environment: Toxicity and Carcinogenicity*. Lyon, France: IARC; 1992:199-210.
90. World Health Organization (WHO). *Cadmium. Environmental Health Criteria*. Geneva, France: WHO; 1992:134:351.
91. Tabacova S, Little RE, Balabaeva L, et al. Complications of pregnancy in relation to maternal lipid peroxides, glutathione, and exposure to metals. *Reprod Toxicol* 1994;8:217-224.
92. Nishijo M, Nakagawa H, Morikawa Y, et al. Mortality of inhabitants in an area polluted by cadmium: 15 year follow up. *Occup Environ Med* 1995;52:181-184.
93. Staessen JA, Buchet JP, Ginucchio G, et al. Public health implications of environmental exposure to cadmium and lead: an overview of epidemiological studies in Belgium. Working Groups. *J Cardiovasc Risk* 1996;3:26-41.
94. Coyle P, Philcox JC, Carey LC, Rofe AM. Metallothionein: the multipurpose protein. *Cell Mol Life Sci* 2002;59:627-647.
95. Leber AP, Miya TS. A mechanism for cadmium- and zinc-induced tolerance to cadmium toxicity: involvement of metallothionein. *Toxicol Appl Pharmacol* 1976;37:403-414.
96. Shimoda R, Nagamine T, Takagi H, et al. Induction of apoptosis in cells by cadmium: quantitative negative correlation between basal or induced metallothionein concentration and apoptotic rate. *Toxicol Sci* 2001;64:208-215.
97. Onosaka S, Tetsuchikawahara N, Min KS. Paradigm shift in zinc: metal pathology. *Tohoku J Exp Med* 2002;196:1-7.
98. Brewer GJ. Recognition, diagnosis, and management of Wilson's disease. *Proc Soc Exp Biol Med* 2000;223:39-46.
99. Liu YP, Liu J, Palmiter RD, Klaassen CD. Metallothionein-I-transgenic mice are not protected from acute cadmium-metallothionein-induced nephrotoxicity. *Toxicol Appl Pharmacol* 1996;137:307-315.
100. Liu X, Jin T, Nordberg GF, et al. Influence of zinc and copper administration on metal disposition in rats with cadmium-metallothionein-induced nephrotoxicity. *Toxicol Appl Pharmacol* 1994;126:84-90.
101. Muller L, Menzel H. Studies on the efficacy of lipoate and dihydrolipoate in the alteration of cadmium<sup>2+</sup> toxicity in isolated hepatocytes. *Biochim Biophys Acta* 1990;1052:386-391.
102. Sumathi R, Baskaran G, Varalakshmi P. Relationship between glutathione and DL alpha-lipoic acid against cadmium-induced hepatotoxicity. *Jap J Med Sci Biol* 1996;49:39-48.
103. Badgchi D, Vuchetich PJ, Bagchi M, et al. Induction of oxidative stress by chronic administration of sodium dichromate [chromium VI] and cadmium chloride [cadmium II] to rats. *Free Radic Biol Med* 1997;22:471-478.

104. Shaikh ZA, Vu TT, Zaman K. Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. *Toxicol Appl Pharmacol* 1999;154:256-263.
105. Nehru LB, Bansal MP. Effect of selenium supplementation on the glutathione redox system in the kidney of mice after chronic cadmium exposures. *J Appl Toxicol* 1997;17:81-84.
106. Sidhu M, Sharma M, Bhatia M, et al. Effect of chronic cadmium exposure on glutathione S-transferase and glutathione peroxidase activities in rhesus monkey: the role of selenium. *Toxicology* 1993;83:203-213.
107. No authors listed. Factors influencing metabolism and toxicity of metals: a consensus report. *Environ Health Perspect* 1978;25:3-41.
108. Andersen O, Nielsen JB. Effects of simultaneous low-level dietary supplementation with inorganic and organic selenium on whole-body, blood and organ levels of toxic metals in mice. *Environ Health Perspect* 1994;102:321-324.
109. Yiin SJ, Chern CL, Sheu JY, Lin TH. Cadmium-induced liver, heart, and spleen lipid peroxidation in rats and protection by selenium. *Biol Trace Elem Res* 2000;78:219-230.
110. Rana SV, Verma S. Protective effects of GSH, vitamin E, and selenium on lipid peroxidation in cadmium-fed rats. *Biol Trace Elem Res* 1996;51:161-168.
111. Sugawara N, Sugawara C. Selenium protection against testicular lipid peroxidation from cadmium. *J Appl Biochem* 1984;6:199-204.
112. Telisman S, Cvitkovic P, Jurasovic J, et al. Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. *Environ Health Perspect* 2000;108:45-53.
113. Kleijnen J, Knipschild P. *Ginkgo biloba*. *Lancet* 1992;340:1136-1139.
114. Liu J, Liu Y, Mao Q, Klaassen CD. The effects of 10 triterpenoid compounds on experimental liver injury in mice. *Fundam Appl Toxicol* 1994;22:34-40.
115. Mahato SB, Nandy AK, Roy G. Triterpenoids. *Phytochemistry* 1992;31:2199-2249.
116. Yasukawa K, Takido M, Matsumoto T, et al. Sterol and triterpene derivatives from plants inhibit the effects of a tumor promoter, and sitosterol and betulinic acid inhibit tumor formation in mouse skin two-stage carcinogenesis. *Oncology* 1991;48:72-76.
117. Pisha E, Chai H, Lee IS, et al. Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. *Nat Med* 1995;1:1046-1051.
118. Liu Y, Kreppel H, Liu J, et al. Oleanolic acid protects against cadmium hepatotoxicity by inducing metallothionein. *J Pharmacol Exp Ther* 1993;266:400-406.
119. Miura N, Matsumoto Y, Miyairi S, et al. Protective effects of triterpene compounds against the cytotoxicity of cadmium in HepG2 cells. *Mol Pharmacol* 1999;56:1324-1328.
120. Kiso Y, Tohkin M, Hikino H, et al. Mechanism of antihepatotoxic activity of glycyrrhizin. I: Effect on free radical generation and lipid peroxidation. *Planta Med* 1984;50:298-302.
121. Nomiyama K, Nomiyama H. Cadmium-induced renal dysfunction was improved by treating hepatic injury with glycyrrhizin. *J Trace Elem Exp Med* 1993;6:171-178.
122. Deters M, Siegers CP, Strubelt O. Influence of glycine on the damage induced in isolated perfused rat liver by five hepatotoxic agents. *Toxicology* 1998;128:63-72.
123. Karbownik M, Gitto E, Lewinski A, Reiter RJ. Induction of lipid peroxidation in hamster organs by the carcinogen cadmium: melioration by melatonin. *Cell Biol Toxicol* 2001;17:33-40.
124. Aposhian HV, Maiorino RM, Rivera M, et al. Human studies with the chelating agents, DMPS and DMSA. *J Toxicol Clin Toxicol* 1992;30:505-528.
125. Tandon SK, Singh S, Prasad S. Influence of methionine administration during chelation of cadmium by CaNa3DTPA and DMPS in the rat. *Environ Toxicol Pharmacol* 1997;3:159-165.
126. Tandon SK, Singh S, Flora SJ. Influence of methionine and zinc supplementation during chelation of lead in rats. *J Trace Elem Electrolytes Health Dis* 1994;8:75-77.
127. Tandon SK, Prasad S, Singh S. Chelation in metal intoxication: influence of cysteine or N-acetyl cysteine on the efficacy of 2,3-dimercaptopropane-1-sulphonate in the treatment of cadmium toxicity. *J Appl Toxicol* 2002;22:67-71.
128. Flora SJ, Gubrelay U, Kannan GM, Mathur R. Effects of zinc supplementation during chelating agent administration in cadmium intoxication in rats. *J Appl Toxicol* 1998;18:357-362.