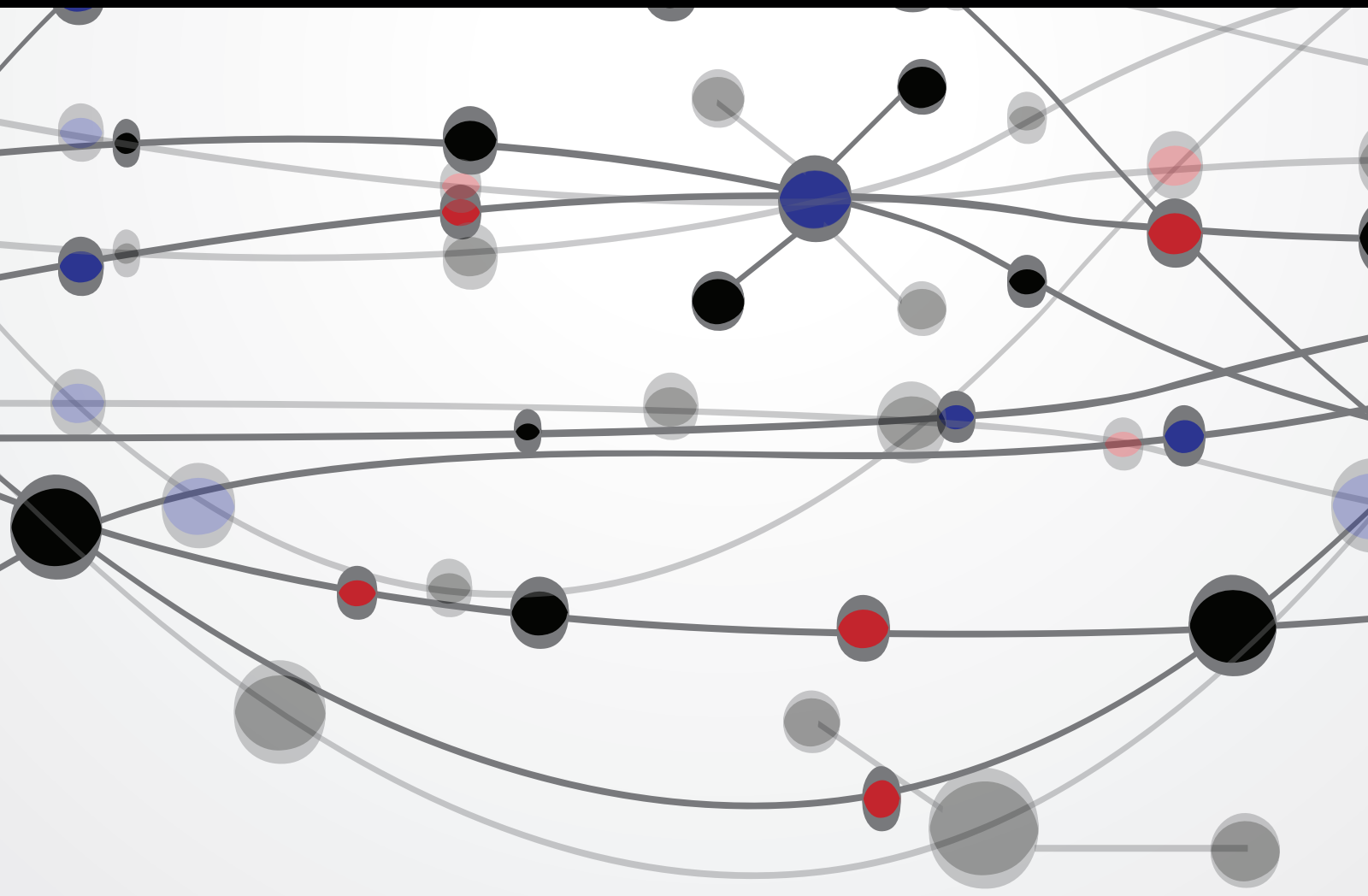
The Scientific World Journal

Clinical Detoxification: Elimination of Persistent Toxicants from the Human Body

Guest Editors: Stephen J. Genuis, Margaret E. Sears, Gerry Schwalfenberg, Janette Hope, and Robin Bernhoft



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Editorial

Clinical Detoxification: Elimination of Persistent Toxicants from the Human Body

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Underlying individuals' unique, invaluable, and enigmatic metaphysical qualities, the human organism is, in a physical sense, essentially a self-regulating biochemical machine. At any moment, our thoughts and feelings, our actions, metabolism and physical well-being all stem from the sum of dynamic, intricate biochemistry working within a distinctive genetic context; innumerable biochemical reactions are taking place to prepare the enzymes, hormones, neurotransmitters and all that we need to undertake the tasks required for daily life. We are truly wonderfully crafted. Like any functional system, however, in order to thrive we must receive the raw materials that we need to carry out our biological processes and we must stay away from influences that are harmful and which impair our machine from functioning normally. The widespread introduction of assorted toxic chemical agents into our intricate biochemical workings has the potential to disrupt sophisticated biochemical processes, becoming a widespread source of harm. Early life exposures can have life-long consequences, even at levels commonly experienced and thought to be safe [1, 2].

Attention to toxic chemical exposures and environmental health sciences has been expanding at an impressive rate. Extensive research by independent scientists as well as governments has prompted numerous toxicology, medical, public health and other scientific journals to report on the impacts of environmental determinants on human health. With the recognition that recent and emerging changes in the external environment have the potential to influence genetic function, hormonal biology, the gastrointestinal microbiome, and mitochondrial processes, as well as other important physiological parameters, the significance of environmental medicine on individual and population health is rapidly becoming a major area of study for scientists and public health officials.

A concern that has become increasingly manifest is that persistent toxicants are retained within the human body long after the primary exposure [3]. Many toxic compounds have long half-lives; they biomagnify up the food chain, and some are increasingly found in the air we breathe, water we drink, food we consume, and assorted personal care products we apply to our skin. Moreover, many persistent pollutants accumulate in developing children through vertical transfer from mother to child in utero and via breast milk [4]. As a consequence, many individuals now carry heavy body burdens of persistent toxicants, which often increase with advancing age as a result of ubiquitous exposures. Furthermore, despite some nations' regulations to restrict the ongoing use of some toxicants, historical contamination of persistent pollutants and regional release in other jurisdictions lacking restrictions have resulted in ongoing exposures and bioaccumulation throughout much of the world.

While the chemical revolution was birthed and grew prolifically over the last 5 to 6 decades, it appears that we will be spending much of the next few decades trying to deal

with the fallout of this revolution. Future generations may look back with astonishment and wonder how our culture thought it could stand by and tolerate the poisoning of its people and somehow not anticipate the ravages of widespread disordered biochemistry and ill health. With the mounting severity of the toxicant bioaccumulation problem, however, organizations such as the Pediatric Academic Societies have begun to speak out announcing that "low level exposure to environmental toxicity may be impacting the functioning of the current generation" [5]. Furthermore, with the recognition of the potential damage to children, the World Health Organization recently expressed the urgent need to build "Children's Environmental Health Capacity among Health Care Professionals" [6].

Despite recent recognition that accrual of toxicants is a major determinant in many chronic health problems, however, little attention in the mainstream medical literature has been devoted to mechanisms to address and resolve the problem of endogenous chemical accrual. Diminishing the influence of persistent harms has the potential to allow the biochemical machinery to be restored. Intervention to reduce the body burden of persistent toxicants—the field of clinical detoxification—constitutes a fundamental and urgently required approach to reducing toxicant-related health issues. It is rewarding indeed to witness remarkable recoveries from chronic illness that are made possible by removing the toxic etiological sources of harm that are disrupting human molecular biochemistry at a microscopic level and thus inducing clinical illness at a macroscopic level [7–10].

The main focus of this special issue is the translation of emerging scientific knowledge in clinical detoxification, in order to provide practical and useful information for clinical medicine as well as public health policy. The disciplines of environmental sciences, toxicology, epidemiology, clinical practice, and public policy mesh in this important field of science. This special issue was envisioned as a starting place for researchers and clinicians to summarize the most recent developments and ideas in the field of clinical detoxification, with a special emphasis given to practical methods to diminish the total load or body burden of toxicants within individuals.

We sent out a call for papers and, as expected in this nascent field, the response was not overwhelming. The reality is that we are in the early stages of knowledge translation in environmental health sciences. Thus far, there is a dearth of scientists and clinicians who are systematically researching interventions to eliminate persistent toxicants, and many clinicians in mainstream medicine have not yet been apprised of the issue of toxicant bioaccumulation.

Just the same, we received over a dozen submissions, of which five papers within the field of detoxification were chosen for publication. This represents a noble start, exploring a variety of topics. With the common and widespread problem of mold contamination in water damaged buildings, we are pleased to present a comprehensive review paper on the management of mold and mycotoxin exposure. As suspicion and confusion abound among some physicians regarding chelation, we present an insightful review of the broad field of chelation for detoxification of toxic elements. While various papers in the literature on toxic elements have explored the issues of mercury and lead contamination, we are grateful to share a paper on the rising problem of cadmium toxicity.

One of the predominant determinants of persistence of toxic compounds within the body is their level of lipophilicity—or their affinity for fat tissues. Many lipophilic compounds have prolonged half-lives in the body with ongoing potential for detriment. We are pleased to include a paper in this edition on novel interventions designed to facilitate the rapid elimination of some lipophilic compounds through the gastrointestinal tract. Finally, we present a paper which explores the effect of induced sweating on the release of phthalate plasticizers—one of the most common chemical exposures of contemporary society. Thus a variety of problems and potential management strategies are examined and discussed in this special edition.

With the ongoing chemical erosion of human health [11], we anticipate that there will be continued and escalating attention to the area of environmental health sciences in the medical community, as well as among the general population throughout the world. As the impact of toxic chemicals can include health afflictions involving many varied specialties, we also anticipate a need for clinicians from the spectrum of medical disciplines to become aware of this problem and to acquire the knowledge and skills necessary to intervene to diminish toxicant body burdens. As more clinical scientists become apprised of the reality of toxicant bioaccumulation and impacts on health, it is likely that elimination of toxicants or "clinical detoxification" will gain status as a foremost aspect of mainstream medical practice. We hope that this special issue will be a springboard for more research and attention to this field. Thank you for perusing and for considering the information we present in this special issue.

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Review Article

Cadmium Toxicity and Treatment

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Cadmium is a heavy metal of considerable toxicity with destructive impact on most organ systems. It is widely distributed in humans, the chief sources of contamination being cigarette smoke, welding, and contaminated food and beverages. Toxic impacts are discussed and appear to be proportional to body burden of cadmium. Detoxification of cadmium with EDTA and other chelators is possible and has been shown to be therapeutically beneficial in humans and animals when done using established protocols.

1. Introduction

Cadmium (Cd) is a naturally occurring metal situated in the Periodic Table of the Elements between zinc (Zn) and mercury (Hg), with chemical behavior similar to Zn. It generally exists as a divalent cation, complexed with other elements (e.g., $CdCl_2$). Cd exists in the earth's crust at about 0.1 part per million [1], usually being found as an impurity in Zn or lead (Pb) deposits, and therefore being produced primarily as a byproduct of Zn or Pb smelting.

Commercially, Cd is used in television screens, lasers, batteries, paint pigments, cosmetics, and in galvanizing steel, as a barrier in nuclear fission, and was used with zinc to weld seals in lead water pipes prior to the 1960s. Approximately 600 metric tons are produced annually in the United States, and about 150 metric tons are imported [2].

Human exposure to Cd occurs chiefly through inhalation or ingestion. Ten to fifty percent of inhaled cadmium dust is absorbed, depending on particle size. Absorption through skin contact is negligible. About five to ten percent of ingested Cd is absorbed, also depending on particle size. Intestinal absorption is greater in persons with iron, calcium, or zinc deficiency [3].

Cigarette smoking is considered to be the most significant source of human cadmium exposure [4]. Blood and kidney Cd levels are consistently higher in smokers than nonsmokers. Inhalation due to industrial exposure can be significant in occupational settings. for example, welding or soldering, and can produce severe chemical pneumonitis [3].

Cadmium exposure occurs from ingestion of contaminated food (e.g., crustaceans, organ meats, leafy vegetables, rice from certain areas of Japan and China) or water (either from old Zn/Cd sealed water pipes or industrial pollution) and can produce long-term health effects. Contamination of drugs and dietary supplements may also be a source of contamination [5].

2. Absorption and Distribution

After absorption, Cd is transported throughout the body, usually bound to a sulfhydryl group-containing protein like metallothionein. About 30% deposits in the liver and 30% in the kidneys, with the rest distributed throughout the body, with a clearance half-life of twenty-five years [6]. The half life of cadmium in the blood has been estimated at 75 to 128 days, but this half life primarily represents deposition in organs, not clearance from the body [7]. Consequently, blood, hair, and urine Cd levels are poor surrogates for body burden and chiefly reflect recent exposure, as is also true with the other heavy metals. Accurate estimate of body burden of Cd will require urine provocation testing [8].

3. Mechanisms of Toxicity

Cadmium toxicity has been demonstrated in several organs, as discussed later. Cadmium induces tissue injury through creating oxidative stress [9–11], epigenetic changes in DNA

expression [12–14], inhibition or upregulation of transport pathways [15–17] particularly in the proximal S1 segment of the kidney tubule [18]. Other pathologic mechanisms include competitive interference with the physiologic action of Zn or Mg [19–21], inhibition of heme synthesis [22], and impairment of mitochondrial function potentially inducing apoptosis [23]. Depletion of glutathione has been observed, as has structural distortion of proteins due to Cd binding to sulfhydryl groups [24]. These effects are magnified by interaction with other toxic metals such as Pb and As [25] and possibly ameliorated by Zn or Se (see later) and by factors increasing levels of Nrf2 [26, 27].

4. Clinical Toxicity

Clinical stigmata of cadmium toxicity depend on route, quantity, and rate of exposure. The chief organ of toxic impact in the human is the kidney, where the S1 segment of the proximal tubule is a major target of Cd deposition, with clinically observable defects in protein, amino acid, glucose, bicarbonate, and phosphate reabsorption (Fanconi syndrome) resulting from Cd-induced oxidative damage to transport proteins and mitochondria which may induce apoptosis of tubular cells [28–31]. Effective antioxidant therapies are being sought [32], and there is *in vitro* evidence that selenium [33] and zinc [34] may at least partially antagonize the toxic effects of cadmium. About 30% of body cadmium is deposited in the kidney tubule region, as discussed earlier, with tubular damage being proportionate to the quantity of cadmium not bound to metallothionein [35]. Diabetics are more susceptible to renal tubular damage from Cd exposure than controls [36].

Cadmium may also impair Vitamin D metabolism in the kidney [37], with deleterious impact on bone. This effect, coupled with direct Cd impairment of gut absorption of calcium and derangement of collagen metabolism, can produce osteomalacia and/or osteoporosis [3]. The most extreme example of this process is *itai-itai* disease in Japan, which combines severe pain from osteomalacia with osteoporosis, renal tubular dysfunction, anemia, and calcium malabsorption [38].

Mechanisms of Cd toxicity in bone include stimulation of fibroblast growth factor 23 which induces phosphaturia and decreases phosphate uptake, leading to osteomalacia [39]. Cd is toxic to MC3T3 osteoblasts by unknown mechanisms [40] and stimulates osteoclasts, thereby inducing osteoporosis [41]. Cd decreases serum osteocalcin levels in rats [42]. These factors apparently combine to induce calciuria, increase bone resorption and decrease bone mineral density in Cd-exposed children [43].

Cadmium affects the cardiovascular system in several ways. The literature is somewhat contradictory, but much of it supports a role for Cd in inducing hypertension [44] and diabetes [45], with apparent direct toxic impact on gene transcription in the vascular endothelium [46]. Epidemiological evidence links Cd with sudden cardiac death [47], peripheral arterial disease [48], increased vascular intima media thickness [49], and myocardial infarction [50]. Proposed mechanisms include disruption of calcium channels and direct vasoconstriction as well as inhibition of NO and possibly other vasodilators [51]. Cd also directly induces oxidative stress, increases lipid peroxidation and depletes glutathione [52–54]. Cadmium accumulates in the wall of the aorta [55]. Cadmium is apparently brought into the vascular wall by Cd-laden monocytes which differentiate into foam cells [56]. Cadmium is also deposited in vascular smooth muscle cells and produces apoptosis of endothelial cells [57]. Direct myocardial structural damage has also been documented [58].

Hematopoeisis is adversely affected, most notably in *itai-itai* disease where severe anemia is observed, in association with marked suppression of erythropoietin production [59]. Hemolysis may also be a factor in producing Cd-associated anemia, which may produce iron-deficient indices despite increased body Fe stores resulting from hemolysis and increased duodenal Fe absorption [60].

Similarly, the immune system suffers form Cd-induced impairment at several levels. Prenatal Cd exposure may impair postnatal T cell production and response to immunization [61], as well as dysregulated thymocyte development [62]. Post-natal Cd exposures induce cell cycle arrest and apoptosis in splenocytes [63]. Cd induces increased rates of autoimmunity, increased production of nonspecific antibodies, and decreased production of antigen-specific antibodies [64]. Lymphocyte proliferation and natural killer cell activity are also suppressed by Cd [65]. Metallothionein protects against Cd immune toxicity [66].

Cadmium has considerable endocrine disruption capacity, apparently disregulating all pituitary hormones [67]. In the 2007-8 NHANES survey, elevated blood Cd levels were associated with suppressed TSH production, while increased urine Cd was associated with elevated serum levels of T3 and T4 [68].

Cadmium is considered to be a metalloestrogen, but evidence to support that contention is stronger in *in vitro* and *in vivo* animal studies than in population-based human studies [69]. It is based partly on binding of Cd to breast cancer estrogen receptors [70]. It seems that estrogen-like effects of Cd result from a mechanism different from that of steroidal estrogens [71].

Male infertility in rats from Cd exposure is due to damage to the blood-testis barrier, decreasing germ cell adhesion leading to germ cell loss, reduced sperm count and subfertility or infertility [72]. Rat studies further suggest Cd may induce production of prostaglandin F2alpha which causes cavernosal vasoconstriction and suppressed testosterone synthesis and secretion in the male, as well as destruction of corpus luteum and fetus in the female. These occur perhaps through inhibition of steroidogenic acute regulatory protein (StAR) which is responsible for the rate limiting step in steroidogenesis [73]. Human epidemiological studies have not, however, supported Cd as a cause of male infertility or erectile dysfunction.

Cadmium exposure is a known risk factor for developing insulin resistance [74, 75]. In the Korean NHANES experience, there is a strong correlation between blood Cd and development of metabolic syndrome [76], the mechanisms of which remain unelucidated but may involve mechanical distortion of the insulin receptor. The Cd effect on insulin resistance may be minimized by supplementation of Fe, Ca, Mg,

and Zn (which also decreases the Cd-associated risks of cancers, fractures, vascular disorders, and total mortality) [77].

Cadmium has been observed to cause oxidative stress and histologically visible membrane disturbances in the central nervous system, with reduction in acetylcholinesterase activity, increase in oxidative stress markers, depletion of glutathione, superoxide dismutase 2, and other antioxidants, and depletion of catalase, glutathione peroxidase, and glutathione-S-transferase [78]. These changes have apparently led to apoptosis of cortical cells in the central nervous system, possibly due to phosphorylation of calcium/calmodulin-dependent protein kinase II [79]. Cd can also inhibit influx through calcium channels [80].

Clinically, humans with elevated blood or urine Cd demonstrate decreased attention level and memory [81]. Additionally, humans with high urinary Cd levels had significantly decreased low-frequency hearing [82]. Similarly, rats with high urinary Cd exhibit decreased learning ability. Intranasal cadmium destroys olfactory nerve function in the rat [83]. Cadmium raises the frequency of spontaneous cortical electrical activity in the rat, lengthens the latency of sensory-evoked potentials, and impairs frequency following ability even in rats without detectable Cd brain deposition [84].

The United States Environmental Protection Agency considers Cd to be a Class B1 carcinogen [85]. There is contradictory evidence linking Cd exposure to breast cancer [86–88] and denying that link [89]. Prostate cancer is also correlated with Cd consumption [90, 91] as is pancreatic cancer [92–94]. In the Third NHANES cohort, Cd was associated with pancreatic and lung cancer and non-Hodgkin's lymphoma [95]. Other investigators have found a plausible association between Cd and lung cancer [96–98] and weak evidence for a link between Cd and non-Hodgkin's lymphoma [99, 100].

5. Reduction of Body Burden

There is no agreement in the literature regarding treatment of Cd toxicity. Human studies are few and anecdotal. While clinical protocols exist for the use of EDTA, DMPS, and DMSA [101–104], they rely for the most part on clinical experience and on *in vitro* and animal studies [105, 106]. EDTA is the agent most widely accepted for clinical use. While it may seem axiomatic that reduction of body Cd burden would decrease its toxic effects, not all authorities agree that active measures beyond avoidance are indicated, at least for acute poisoning, where concern exists that chelation may aggravate damage to the kidney tubules [107, 108]. For chronic exposures, however, there is considerable evidence of chelation's clinical efficacy, in humans and in experimental animals. Several chelators have been used. Clinically available chelators include EDTA, DMPS, DMSA, and British Anti-Lewisite (BAL). BAL is more toxic than its derivatives, DMPS and DMSA, and is seldom used clinically. Several experimental chelators, including DTPA [109] (available from the National Strategic Reserve for radiation poisoning), NaB [110], and others [111, 112], are also being investigated but are not clinically available at present.

It is clear that EDTA [113, 114], DMPS [115], and DMSA [116] increase urinary excretion of Cd, but DMSA seems to have little impact on overall body burden of Cd [117, 118]. Studies *in vitro* [119] and *in vivo* [120] suggest that EDTA is superior to DMSA in mobilizing intracellular Cd. In clinical use, EDTA is credited with an anecdotal report of relief of rheumatoid arthritis [121], as well as reduction of oxidative stress [122], and reduction of general metal toxicity [123, 124]. The efficacy of EDTA is apparently improved with concomitant use of glutathione [125] which also protects against nephrotoxicity; efficacy may also be improved with concomitant use of antioxidants [126] including mannitol [127], as well as thiamine [128], methionine [129], or zinc [130]. DMPS has not been studied as extensively as EDTA and DMSA but appears effective in rats [131], is available over the counter in Germany, and may be compounded legally in the United States.

EDTA is approved by the FDA for lead and other heavy metals, and has a long history of safe use. It should not be given faster than one gram per hour nor in dosage greater than three grams per session. Sessions should be at least five days apart, and replacement of essential minerals should be done orally between sessions. Several effective protocols exist implementing these principles [101–104].

Cd is also significantly present in sweat during sauna, which appears to be a moderately successful modality for reducing body burden of Cd without risk of tubular damage [132], albeit at a rate slower than that of intravenous chelation with EDTA.

6. Conclusion

According to the Third National Report on Human Exposure to Environmental Chemicals (NHANES), Cd exposure is widespread in the general population [133]. No standards exist correlating blood or urine Cd measurements with clinical toxicity; so, no conclusions are drawn on the significance of blood or urine levels. This is also true since blood and urine levels do not correlate with body burden, as discussed earlier. Given the ubiquity of Cd in the environment, the multisystem toxicity of Cd as discussed previous, and the generally benign nature of EDTA treatment administered under any of the aforementioed clinical protocols, it would seem reasonable to screen high risk individuals (smokers, persons with industrial exposures, etc., as above) and those with potential clinical indications and treat those with elevated Cd levels on provocation.

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Review Article

A Review of the Mechanism of Injury and Treatment Approaches for Illness Resulting from Exposure to Water-Damaged Buildings, Mold, and Mycotoxins

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Physicians are increasingly being asked to diagnose and treat people made ill by exposure to water-damaged environments, mold, and mycotoxins. In addition to avoidance of further exposure to these environments and to items contaminated by these environments, a number of approaches have been used to help persons affected by exposure to restore their health. Illness results from a combination of factors present in water-damaged indoor environments including, mold spores and hyphal fragments, mycotoxins, bacteria, bacterial endotoxins, and cell wall components as well as other factors. Mechanisms of illness include inflammation, oxidative stress, toxicity, infection, allergy, and irritant effects of exposure. This paper reviews the scientific literature as it relates to commonly used treatments such as glutathione, antioxidants, antifungals, and sequestering agents such as Cholestyramine, charcoal, clay and chlorella, antioxidants, probiotics, and induced sweating.

1. Introduction

It has been estimated that up to 50% of illness results from exposure to indoor air pollution [1], with exposure to water-damaged indoor environments likely being a significant contributor to this. A number of treatment approaches have been used in the treatment of illness resulting from exposure to water-damaged buildings, molds, and mycotoxins [2, 3]. Symptoms and illness due to exposure result from varying mechanisms including infection, toxicity, allergy, irritant effects, and systemic inflammation. Additionally, individual responses to exposure vary based on genetic makeup, duration and severity of exposure, and underlying health and nutritional status. While it is often difficult to determine the contribution of the many components of water-damaged buildings, studies on illness from exposure to damp/water-damaged environments have been consistent in identifying the overall exposure itself as being the main factor associated with adverse health effects [4, 5]. Individual components of exposure that have been identified include: mold and mold spores, mycotoxins, bacteria, bacterial endotoxins and other cell wall components, protozoa (amoeba), increased presence of rodents, insects and dust mites, and increased deterioration of building materials with consequent offgassing of toxic fumes such as formaldehyde [4, 5]. While this paper focuses on individual mechanisms of illness for the purpose of reviewing treatment strategies, it is important to understand that the health consequences of exposure to water-damaged environments likely result from a combination of factors acting synergistically.

2. Molds and Mycotoxins

Amplified growth of mold in water-damaged, damp indoor environments contributes greatly to ill health effects and is extensively documented in the literature [4, 6–10]. Mold and mycotoxins are probably the best understood contaminants of water-damaged buildings and will be discussed throughout this paper. Exposure to water-damaged indoor environments has been shown to result in exposure to amplified growth of mold and mycotoxins including ochratoxin (OTA), aflatoxin, and trichothecene mycotoxins, all of which have been found

in indoor environments [11–14] and in the bodies of those exposed to these environments [15–18]. Exposure to mold and mold components are well known to trigger inflammation, oxidative stress, and inflammatory reactions in both human and animal studies and have frequently been found in association with air found in water-damaged indoor environments [13, 19–26]. Thousands of mycotoxins have been identified to date; however, we will limit discussion to those currently felt to have the most relevance to health effects resulting from water-damaged indoor environments.

Aflatoxins are produced by *Aspergillus parasiticus, Aspergillus flavus, Aspergillus nomius,* and various species of *Penicillium, Rhizopus, Mucor, and Streptomyces* [27]. Aflatoxin B1 (AFB1) is genotoxic, immunotoxic, hepatotoxic, mutagenic, and considered one of the most abundant, most toxic, and most potent naturally occurring carcinogenic substances known and is the leading cause of liver cancer in many developing countries [27–29]. Sterigmatocystin produced by multiple species of *Aspergillus* is considered only slightly less toxic than aflatoxin [29].

Ochratoxin A is produced by *Aspergillus ochraceus, Aspergillus niger, Aspergillus,* and species of *Penicillium, Petromyces,* and *Neopetromyces.* OTA is a nephrotoxic, genotoxic, immunotoxic, and [30] neurotoxic [22, 31] mycotoxin which is a known carcinogen in animals and a class 2B, possible human carcinogen. Associations have been found with human kidney disease [32, 33] including Balkan endemic nephropathy [34] and focal segmental glomerulosclerosis (FSGS) [35].

Trichothecenes are produced by *Stachybotrys, Fusarium,* and *Myrothecium* mold and include T2 toxin, deoxynivalenol (DON) and the macrocyclic trichothecenes satratoxin, and verrucarin [36]. Trichothecenes are distinguished by the presence of trichothecene ring and epoxide group at C-12 [37]. Trichothecenes are considered extremely toxic and have been used as biological warfare agents [37]. Much of the toxicity from trichothecenes is felt to result from inhibition of protein synthesis [36]. Trichothecene mycotoxins cause multiorgan effects including emesis and diarrhea, weight loss, nervous disorders, cardiovascular alterations, immunodepression, hemostatic derangements, skin toxicity, decreased reproductive capacity, and bone marrow damage [37]. A study of the trichothecene mycotoxin, satratoxin A, demonstrated that neurological system damage can occur in water-damaged buildings contaminated with fungal growth at levels that can occur indoors [38].

3. Bacteria and Endotoxins

Research continues to support the presence of bacteria and their components in water-damaged buildings. Gram positive bacteria found in water-damaged buildings often include species of *Actinobacter* including *Streptomyces*, *Mycobacteria,* and *Nocardia* which are capable of causing pulmonary diseases and other illnesses [39]. Gram negative bacteria, such as *Pseudomonas*, have also been identified in water-damaged buildings and are capable of causing illness through infections and the effects of endotoxins [39]. The extent to which an individual will be affected by exposure to bacteria and endotoxins will depend on the nature and severity of exposure as well as genetic makeup [40] and immune system function. Unfortunately, the immune system can be negatively affected by the exposure itself [41, 42] which will worsen illness.

Endotoxins, often referred to by their active component, lipopolysaccharides are formed in the cell walls of gram-negative bacteria and can trigger a profound inflammatory response mediated by cytokine release. In a recent study of healthy human volunteers administered *E. coli* endotoxin intravenously, elevations in cytokines, particularly TNFα, were noted compared to baseline and the authors noted that several of the criteria for systemic inflammatory response syndrome were observed after endotoxin administration, including elevations core temperature, heart rate, and white blood cell (WBC) count, and noted elevations of epinephrine and cortisol [43]. Furthermore, studies have shown that endotoxins act synergistically with mycotoxins to enhance the cytokine-mediated inflammatory response [24, 44].

Endotoxins have been found in water-damaged buildings with many of the studies occurring in flood-damaged homes in New Orleans. A study of homes affected by hurricanes Katrina and Rita showed that, in addition to elevated mold spore counts found in water-damaged homes, endotoxins and fungal glucans were detected at levels that can cause adverse health effects [45]. An additional study of eight flood-affected homes in New Orleans found endotoxins and beta-glucans and noted that smaller size particles (<1.0 and 1.8 microns) were found to have concentrations of endotoxin and glucans at the same level as particles greater than 1.8 microns [46]. One additional New Orleans study also quantified endotoxins indoors, however, at similar levels to the nonflooded homes also located in New Orleans [47].

Inhalational exposure to endotoxins has been found to be associated with physiologic abnormalities in animal studies. A study of intratracheal administration of lipopolysaccharide (LPS) to rat pups increased brainstem expression of inflammatory cytokine interleukin-1B (IL-1B) and is associated with impaired hypoxic ventilator response [48]. This could be particularly important in infants and young children as brain inflammation can not only have significant effects on early development but may play an important role in the development of neurodegenerative disorders later in life. Perinatal exposures to LPS have been shown to increase risk of dopaminergic disorders in animal models Parkinson's disease, autism, cerebral palsy, and schizophrenia [49]. A synergistic role of endotoxins and trichothecene mycotoxins is supported by studies in mice [24] as well as in human macrophages [44]. Endotoxins can also interact synergistically with other toxins worsening the consequences of exposure. In one study, LPS was injected intracerebrally into a neonatal rat to trigger brain inflammation and it was found that the application of the pesticide rotenone resulted in motor and neurobehavioral impairments in the rats exposed to LPS and not in the unexposed rats [50]. The authors speculated that perinatal brain inflammation may enhance adult susceptibility to environmental toxins. Similar results were seen in another study of exposure of neonatal rats to

LPS. The authors concluded that the chronic inflammation resulting from exposure may represent silent neurotoxicity and that compromised dendritic mitochondrial function might contribute to this [49].

4. Microbial Volatile Organic Compounds (MVOCs) and Other Sources of VOCs

MVOCs are found in water-damaged homes. However, their role as a marker for mold contamination and as a significant cause of human illness is still being elucidated. Mold VOCs have remained difficult to measure. Even when deliberate mold contamination occurred in a climate chamber, only 9 VOCs were detected and the authors concluded that even under those favorable conditions the MVOCs are hardly accessible and were not a reliable indicator of mold growth in water-damaged indoor environments [51]. One study of 23 homes showed a higher level of certain MVOCs in basements compared to the main floor of the house although overall MVOC levels did not significantly differ [52]. A Japanese study evaluated MVOC exposure and clinical symptoms [53]. It found that the presence of MVOCs and airborne fungi was only weakly correlated and that higher levels of the MVOC 1-octen-3-ol increased irritation of the nasal and ocular mucosa. Offgassing of VOCs, such as formaldehyde, from water-damaged building materials can also pose a risk to health [4].

5. Routes of Exposure

While foodborne exposure to mycotoxins and fungal contaminants has been well researched, substantial information about airborne and transdermal routes of exposure also exists. Airborne exposure is likely the most significant route of exposure in water-damaged indoor environments; however, transdermal and potentially foodborne exposure through contact with indoor mycotoxins can also occur in these settings.

The airborne presence of mycotoxins has been well documented in research studies and has been reported to cause human illness throughout the medical literature. Respirable particles (<1.0 micron) represent the majority of particulate material found in indoor air [54] and mycotoxins have been found to be present on these indoor particles which include hyphal fragments [55]. Trichothecene mycotoxins have been found to be present in the air of buildings contaminated by *Stachybotrys* mold. In one study, macrocyclic trichothecene mycotoxins were measurable in the air and concentrations increased with sampling time and short periods of air disturbance [11]. These conditions imitate the nature of human exposure which is typically long term, with normal activity repeatedly causing air disturbance which increases aerosolization of mold and mycotoxins. Toxicity from inhaled mycotoxins appears to be very significant. In a study involving rats and guinea pigs, toxicity from inhaled T2 mycotoxin was 20 times as toxic as intraperitoneally administered toxin in rats and at least twice as toxic in guinea pigs [56]. Of significance in that study the pathologic lesions resulting from exposure were similar whether the exposure was inhalational or systemic. Experiments studying effects of acute inhalation of T2 mycotoxins in both young and mature mice showed that inhalation of T2 mycotoxins is at least 10 times more toxic than systemic administrations and at least 20 times more toxic than dermal administration [57]. Clinical symptoms seen in these animals immediately after exposure were tremors, lethargy, stilted gait, and in some cases prostration [57]. These are common symptoms seen in humans exposed to water-damaged buildings, mold, and mycotoxins.

Other studies support clinical and serological effects of inhaled mycotoxins. A study of 44 individuals exposed to indoor *Stachybotrys chartarum* identified the presence of trichothecene mycotoxins by ELISA in the sera of 23 individuals while only 1 of the 26 controls tested positive [18]. In goats exposed to macrocyclic trichothecenes mycotoxins by tracheal installation, mycotoxins were detected in the sera 24 hours after exposure at similar levels whether the goats were exposed repeatedly or to a single dose [58]. Ochratoxin, aflatoxin, and zearalenone have been detected in the air of a poultry house [59]. The authors quantified the amounts a worker in this setting may inhale and expressed concern about the potential public health consequences of this exposure, as it can affect workers directly exposed to mycotoxins and the quality of the food. One study of a problematic household where occupants were experiencing symptoms known to be associated with ochratoxicosis in farm animals, such as increased thirst, polyuria, edema, skin rash, and lethargy, found elevations of OTA on all surfaces tested at concentrations up to 1500 ppb which was found on a heating duct dust [14]. OTA has been found in dust from other indoor settings as well [60]. Of great clinical significance is the identification of mycotoxins on items found in human living environments including building materials, air filters, and personal items [13]. ELISA techniques have detected the presence of mycotoxins in persons exposed to water-damaged environments in a number of tissues including urine, nasal polyps and secretions, cancerous breast tissue, spinal fluid, breast milk, gastric and colon tissue, bladder and transitional cell carcinomas, brain astrocytoma, lung, lymph nodes, especially those with granulomatous diseases and renal cell tumors [16]. Mycotoxins have been found in breast milk by clinicians treating patients exposed to mold and mycotoxins in indoor settings. A study of 113 breast-feeding moms in Sierra Leone found the presence of OTA and aflatoxins at levels which in some cases far exceeded those permissible in animal feed in developing countries [61].

Intact spores are not the only source of aerosolized exposure. It has been shown that fungal fragments, often submicron-sized, can be released at 320 times higher level than spores and that the number of released fragments cannot be predicted based on the number of spores [62]. Increased reactivity of smaller fragments has been documented as they have the potential to penetrate deeper into the respiratory tract than intact spores which are generally greater than 2.5 microns [38, 62].

Biotransformation of mycotoxins in nasal mucosa can also play a significant role in the consequence of aerosolized

exposure to mycotoxins. Nasal biotransformation of xenobiotics has been addressed in the literature as many biotransformation enzymes including cytochrome P450 1B1 and glutathione S transferase P1 have been detected in nasal mucosa of humans in levels at least as abundant as in the liver [63]. Rats exposed to intranasal aflatoxin B1 demonstrated high local bioactivation in the tissue and translocation of aflatoxin B1 and/or its metabolites to the olfactory bulb and also demonstrated mucosal injury [64].

Skin penetration of mycotoxins also occurs. Dermal contact with mycotoxin-contaminated items can also be a source of exposure which has the potential to occur even after a person has removed themselves from the contaminated environment since many people bring mold and mycotoxin-contaminated items to their new settings. One study showed that aflatoxin B1, OTA, citrinin, T2 toxin, zearalenone all penetrated human skin *in vitro* and that ochratoxin had the highest permeability [65].

6. Mechanisms of Illness

Illness resulting from exposure to water-damaged building can be caused by infection, toxicity, allergy, and inflammatory responses triggered by exposure to one or more of the agents present in water-damaged buildings and are often mediated by oxidative stress. Types of disorders that can be seen resulting from water-damaged environments, mold, mycotoxins and bacteria include, infections and mycoses, chronic and fungal rhinosinusitis, IgE-mediated sensitivity and asthma, other hypersensitivity reactions, pulmonary inflammatory disease, immune suppression and modulation, autoimmune disorders, mitochondrial toxicity, carcinogenicity, renal toxicity, neurotoxicity, and DNA adducts to nuclear and mitochondrial DNA causing mutations [39]. A significant mechanism of injury includes oxidative stress [23, 31, 66–69]. This becomes significant as it directs the approach to treatment, which focuses on removing ongoing sources of oxidative stress in the body, such as mycotoxins, as well as instituting treatments which focus on countering oxidative stress like glutathione and other antioxidants [70–74]. Inflammation triggered by exposure also appears to play a significant role in illness during and after exposure to water-damaged environments [24, 26, 75].

Most commonly, however, many mechanisms are interacting in an individual at any given time, making it imperative to address the illness with a comprehensive, multifaceted approach. Although respiratory symptoms are common from exposure to water-damaged indoor environments, it is important to note that a typical patient presents with multiple symptoms which are often debilitating, including fatigue, neurocognitive symptoms, myalgia, arthralgia, headache, insomnia, dizziness, anxiety, depression, irritability, gastrointestinal problems, tremors, balance disturbance, palpitations, vasculitis, angioedema, and autonomic nervous system dysfunction [76, 77]. The development of new onset chemical sensitivity is also commonly seen after exposure and can have a severe impact on a person's life [77].

It is always important to identify and address abnormalities that are found at increased frequency in persons exposed to water-damaged building such as thyroid, immune dysfunction and autoimmune conditions [78]. However, clinicians treating these conditions often see significant improvement with comprehensive treatment and detoxification [2].

Clearly, genetic individuality plays a role in response to exposure and response to treatment in ways that are still being elucidated. Human leukocyte antigen (HLA) patterns have been studied for their effects on response to exposure and genetic polymorphisms affecting detoxification pathways and inflammatory responses are also likely significant. For example, a human study looking at the effects of genetic polymorphisms on the effects of alpha tocopherol in 160 male volunteers evaluated polymorphisms in 15 pathways involved in inflammation or response to oxidative stress after exposure of peripheral blood mononuclear cells to lipopolysaccharides [40]. The authors found that the ability of alpha tocopherol to influence production of cytokines TNF α, IL β-6, and -10 was influenced by polymorphisms in GSTP 1 313 and genes encoding for TNFα and IL10 [40]. The study of genetic influences on illness and treatment after exposure to water-damaged buildings remains an exciting area for further research.

7. Neurocognitive Symptoms

Some of the most distressing symptoms encountered by patients following exposure to water-damaged indoor environments and toxigenic molds include neurocognitive disturbances. A disturbing study, conducted in Poland, measured IQ scores in children exposed to indoor mold for greater than two years, showed statistically significant IQ deficits in children exposed to indoor mold [79]. This study controlled for multiple variables and involved testing of 277 term babies at age 6 years using the WISC-R scale of intelligence and tests of neuropsychological function. Children exposed to indoor molds showed a statistically significant deficit of approximately 10 points. Additionally, it was shown in this study that longer exposure to indoor molds tripled the risk for low IQ scores defined as values below the 25th percentile. This is consistent with several other studies showing lower scores on cognitive testing [80, 80–83]. This is not surprising as several mycotoxins are known to be neurotoxic in animal studies including OTA, T2 toxin, macrocyclic trichothecene, and fumonisin [84]. Research has shown that satratoxin H can cause neurological system cell damage at levels found in water-damaged buildings, and it is believed that the constant activation of inflammatory and apoptotic pathways in human brain capillary endothelial cells, astrocytes, and neural progenitor cells can amplify the devastation of neurological tissues and lead to neurological system cell damage from indirect events triggered by the presence of trichothecenes [38]. Depression has also been shown to be increased in persons exposed to damp indoor environments [85]. Air quality can be a trigger for neuroinflammation as was seen in a highly disturbing study of healthy children and dogs exposed to outdoor air pollution. In this study the children

exposed to the polluted air of Mexico City were compared to controls and found to have significant deficits on cognitive testing and 56% of the Mexico City children were found to have prefrontal white matter hyperintense lesions similar to those seen in the exposed dogs who were found to have significant neuroinflammation based on brain studies assessing levels of proinflammatory cytokines [86].

Patients who have developed symptoms as a result of exposure to mold and mycotoxins can present similarly with several classic neurologic disorders including pain syndromes, movement disorders, delirium, dementia, and disorders of balance and coordination [87]. Abnormalities have been seen on standardized neurocognitive test batteries [77, 81]. These disturbances frequently include disturbances of balance as determined by patient history, examination (Romberg with eyes open and closed, tandem gait, and balance standing on toes with eyes open and closed), and, ideally, with objective testing including computerized sway balance testing [81, 88]. Worsening of these symptoms on testing repeated months to years after initial exposure is frequently seen [81]. However, it remains unclear whether patients have truly removed themselves from further exposure by avoiding contact with items that had been present in the water-damaged home. Studies have also shown abnormalities in quantitative EEG (QEEG) studies [83] and single-photon emission computed tomography (SPECT) scans [77, 89] in patients exposed to mold and mycotoxins in indoor settings. Clinicians working with patients with neurocognitive symptoms resulting from exposure to water-damaged environments have seen improvement with the comprehensive treatment approaches outlined above including use of intranasal glutathione [2].

A review of mycotoxins found to be neurotoxic in animal models, macrocyclic trichothecenes, T2 toxin, fumonisin B1 (FB1), and OTA found that FB1 induces neuronal degeneration in the cerebral cortex, T2 induces neuronal cell apoptosis in fetal and adult brains, OTA causes depletion of striatal dopamine and its metabolites, and macrocyclic trichothecenes cause neuronal cell apoptosis and inflammation in the olfactory epithelium and bulb [84].

8. Oxidative Stress

Oxidative stress is being increasingly understood as a significant mechanism of illness from exposure to water-damaged buildings. For example, ochratoxin A, which is commonly found at elevated levels in persons exposed to water-damaged homes, is a known cause of cellular changes associated with oxidative stress. A recent study of human blood mononuclear cells exposed to OTA showed increased levels of reactive oxygen species and 8-hydroxydeoxyguanasine (8-OHdG), a marker of oxidative stress [21]. Additionally, in this study, OTA-induced DNA strand breaks, G1 phase arrest, and apoptosis of human mononuclear cells, contributing to the immunotoxicity of OTA. Of significance in this study, application of N-acetyl cysteine, a significant glutathione precursor, was able to significantly block some of the negative effects of OTA on the human cells studied.

Additional support for the role of oxidative stress comes from several studies of OTA. A study of rat kidney cells confirmed oxidative stress as a key source of OTA-induced DNA damage [67]. Support for oxidative stress-mediated impairments of mitochondria in rats has also been seen for aflatoxin B1 [28, 68]. Neurotoxicity from oxidative stress mechanisms has also been seen from macrocyclic trichothecenes, OTA, fumonisin B1, and T2 toxin [84].

9. Allergy, Autoimmune Responses, and Nonallergic Respiratory Disease

Respiratory illnesses have consistently been found to be associated with exposure to water-damaged, damp indoor environments [4, 6, 90]. Examples of this include chronic rhinosinusitis, allergic rhinitis including allergic fungal rhinitis, and sinusitis, asthma (new onset and exacerbations), conjunctivitis, invasive, and allergic pulmonary aspergillosis (ABPA), hypersensitivity pneumonitis, and sarcoidosis [9, 90–94]. It has been estimated that 21% of asthma in the United States is attributable to dampness and mold exposure [7], exposure to mold odors at home increased the risk of developing asthma in children 2.4 times [8], and exposure to workplace mold increased the risk of new-onset asthma 4.6 times [95]. It is important to note that studies have found that allergic response to mold is often not IgE mediated. A study of adult asthma found that those who developed occupational asthma were significantly more likely to have been exposed to water-damage and mold at work [96]. Interestingly, though, in this study, only 33.1% of the probable occupational asthma patients were atopic to any environmental antigen and only 20% were sensitized to mold allergens suggesting mechanisms other than type 1 allergy are involved in this symptomatology. Similar results were seen in a Mayo clinic study where a high prevalence of nasal fungal growth was found in both symptomatic rhinosinusitis patients and controls [97] with the most significant difference being the development of eosinophilic mucin and not type 1 hypersensitivity since IgE positivity was not seen in the majority of patients [97].

After sensitization, avoiding exposure to allergenic triggers and decreasing the immune response to unavoidable exposures are the main principles of treatment. Allergy to mold clearly places an individual in a damp and moldy indoor environment at increased risk for illness. Allergy testing to mold varies by individual examiner and ranges from comprehensive to minimal panels. Allergy to dust mite can also place an individual in a damp indoor environment at increased risk as dust mites have been shown to grow at amplified levels in damp environments [4]. While avoidance of exposure is always essential, allergy treatment techniques have been used with good success including injection and sublingual immunotherapy [3]. While not all illness resulting from exposure to water-damaged indoor environments is due to mold, some disease clearly is.

10. Infection and Colonization

Fungal infections can occur throughout the body and can be a result of exposure to water-damaged indoor environments. These have traditionally been felt to be associated with immunosuppression but have also been seen in immunocompetent hosts [98, 99]. Direct exposure to elevated levels of mold spores indoors can contribute to fungal disease, either directly by allowing seeding for fungal growth such as that occurring in nasal mucosa or by direct toxic effects and immune system alteration resulting from exposure. Additionally, some treatments received by patients due to illness resulting from their exposure, such as antibiotics and steroids, can contribute to fungal growth throughout the body including in nasal, sinus, and gastrointestinal tissue.

Nasal infections and colonization deserve discussion as many patients develop respiratory symptoms during and after exposure to water-damaged indoor environments. Untreated nasal and sinus infections can be a cause of ongoing symptoms and should be addressed. Chronic respiratory symptoms resulting from exposure can lead to the use of antibiotics and steroids which can predispose a person to fungal infections. Allergic fungal rhinitis has long been identified as a cause of persistent nasal infection, receiving initial attention with a Mayo clinic study which found fungal growth in 96% of patients with chronic sinusitis [97]. Types of molds identified on culture in this study included *Aspergillus, Penicillium, Alternaria, Cladosporium, Fusarium, Cryptococcus,* and many other types of mold known to produce mycotoxins or have the potential to cause significant disease.

Treatment approaches include avoidance of exposure to elevated spore counts and particulates through remediation and the use of air filters, sinus irrigation, topical antifungals, and antimicrobials which can include agents such as EDTA for aiding in breaking down biofilm and rarely oral antibiotics or oral antifungals. Surgery is indicated in refractory cases and in the presence of mycetomas [54].

The results of clinical studies of the use of topical antifungals have been mixed. A study of 51 patients with chronic rhinosinusitis who were treated with intranasal amphotericin B found improvement in nasal obstruction and discharge in 75% of the patients and 25% reported complete resolution of symptoms after treatment with improvement starting after one to three months. The only reported side effect was burning on application in 20% of the patients although no patients discontinued therapy due to side effects [100].

A placebo-controlled double-blind trial of intranasal amphotericin B showed symptoms improved with treatment; however, it did not differ from symptom improvement seen with saline irrigation alone [101]. Another randomized placebo-controlled, double-blind trial found that intranasal amphotericin B reduced nasal inflammatory mucosal thickening on computed tomography (CT) and endoscopy and reduced intranasal markers for eosinophilic inflammation in patients with chronic rhinosinusitis [102]. A Cochrane review concluded there was no benefit to topical or systemic antifungals for chronic rhinosinusitis [103] which is consistent with a more recent meta-analysis [104]. Intranasal ketoconazole, fluconazole, and itraconazole are frequently used. However, more data is available for intranasal use of amphotericin B. Oral antifungals have been used for allergic fungal sinusitis [105]; however it is advised to use caution with oral antifungals given the risk of significant side effects, especially in those with cytochrome p450 polymorphisms affecting the metabolism of these drugs. A study of children undergoing bone marrow transplant found the use of intranasal amphotericin B to be a safe and effective intervention for preventing invasive aspergillosis in pediatric patients undergoing bone marrow transplantation [106].

While genetic polymorphisms of glutathione S transferase enzymes have been found to play a role in a number of illnesses, including asthma, a study evaluating the role of these enzymes in chronic rhinosinusitis did not find a correlation [107].

Gut microflora play an essential role in the immune system and detoxification of xenobiotics [108, 109]. Alterations in the gut flora are increasingly being seen to contribute to illness including allergic and autoimmune diseases such as asthma, eczema, and rheumatoid arthritis [108, 109]. Exposure to water-damaged environments, mold and mycotoxins can result in injury to the gastrointestinal tract [22, 37] and, again, treatments often used to treat illness, such as antibiotics and steroids, can result in profound alteration in gastrointestinal flora, thus decreasing metabolism of mycotoxins and other toxins. Identifying and treating these abnormalities can be significant in restoring health. The use of both bacterial and yeast probiotics, treating infections, and identifying and avoiding food allergens can be significant steps in treatment [110–112].

11. Treatment Modalities

12. Avoidance and Total Load Reduction

The most important component of treatment is avoidance of further exposure to water-damaged environments and items contaminated by those environments as ongoing exposure will thwart any efforts at detoxification and perpetuate a reactive state. Unfortunately, it is often difficult and expensive to test environments and items that have been exposed to those environments for mycotoxin contamination [4] and consequently this testing is often not done. Research has shown that none of the commonly used methods for cleaning water-damaged materials such as bleach, ammonia, ultraviolet (UV) light, heating, and ozone were found to completely remove mold and mycotoxins from water-damaged building materials [113]. For this reason, it is safest for patients who have become ill after exposure to water-damaged environments is to avoid exposure to items that were present in the contaminated environment. Air spore counts are frequently done and, unfortunately, have significant limitations as they typically collect over a short (5-minute) period and can easily result in false negative results. The presence of elevation on spore count testing can have significance, however, both in terms of total spore count and types of mold present. The author of one study of schools concluded that a building must be considered unhealthy at

spore counts over 1000 spores/m^3 [114]. A study of a water-damaged hospital highlights limitations of traditional limited testing. The researchers measured multiple markers including culturable fungi and bacteria, endotoxin, submicron-size particles, and markers of fungi (extracellular polysaccharides specific for *Penicillium* and *Aspergillus*, ergosterol, and beta-1–3 glucans) and found the presence of submicron-sized particles and markers of microbiological agents was positively associated with a building with historic water-damage and higher prevalence of reported occupant symptoms [115]. The authors proposed that marker compounds in air and floor dust samples may be better indicators of health risk than culturable fungi or bacteria in air or settled dust.

While abnormal test results can confirm the presence of a significant problem, they cannot be relied upon to ensure an environment is safe for rehabitation. Testing that can be useful in some situations includes environmental testing for bacteria and endotoxins, mycotoxins, VOCs and polymerase-chain-reaction (PCR) based mold testing such as ERMI to identify species of mold. It is important to note that an individual who had become ill in a water-damaged environment will likely be most sensitized to that specific environment and items present in the environment and may never be able to return to that environment or be exposed to items from that environment without getting ill. The ability of persons to return to a building without developing symptoms remains the most relevant indicator that a building has been properly remediated [116].

Unfortunately, common building remediation techniques have not been found to be successful in removing mold and mycotoxins from contaminated materials [113]. In this study, pine and gypsum were deliberately contaminated with *Stachybotrys chartarum* and *Aspergillus versicolor* and treated with either peroxide, hot air, flaming, two types of boron-based chemicals, drying, steam, UV light, ammonium chloride, or sodium-hypochlorite-based chemicals. No remediation treatment eliminated all the mycotoxins from the building materials. The study showed that none of the 10 different mold remediation agents and methods tested was able to totally remove mold from the infected materials and that they were ineffective in destroying mycotoxins. The authors conclude that there is a risk of inhaling mycotoxins in buildings even after mold remediation. Although not completely successful, boron, and ammonium-chloride-based chemicals were the most successful in reducing mold and mycotoxin levels. A common misperception is that killing mold, which is a relatively easy task, eliminates risk from contaminated environments or items. Unfortunately, this does little to decrease the risk as nonviable fungal spores, fragments, and mycotoxins remain present and, due to their structure, such as with an epoxide ring, [117] they can be extremely difficult to destroy.

As previously noted, mycotoxins can travel not just on spores, but on fungal fragments which can often be submicron-size [39, 62], allowing inhalation deep into lung tissue and preventing complete protection from occurring with the use of a protective mask. Paper and soft materials are particularly vulnerable and cannot be adequately remediated even with washing and HEPA vacuuming and often need to be replaced [116].

It is also important to be aware that disturbing contaminated material can significantly increase exposure to spores and mycotoxin-contaminated fragments [11], dramatically worsening exposure during attempts of remediation or packing of items. The potential for dermal penetration of mycotoxins is also important to keep in mind [65] during any contact with contaminated materials.

In addition to avoidance of further exposure to contaminated items, it is recommended to decrease exposure to other chemical xenobiotic agents including pesticides, heavy metals, volatile organic compounds and fragrances, vinyl chloride, plastics, perflourinates (nonstick cookware), and other toxins in an effort to reduce total load and improve the ability to detoxify from the exposure to a water-damaged environment. It is common for patients exposed to water-damaged indoor environments to become sensitive to and avoid many chemicals which frequently becomes noticeable after leaving the environment.

A review of the CDC's fourth national report on human exposure to chemicals showed that acrylamides, cotinine, trihalomethanes, bisphenol A, phthalates, chlorinated pesticides, triclosan, organophosphate pesticides, pyrethroids, heavy metals, aromatic hydrocarbons, polybrominated diphenyl ethers, benzophenone from sunblock, perfluorocarbons from nonstick coatings, and several polychlorinated biphenyls and solvents were found in the majority of individuals tested [118]. All of these potential exposures as well as other toxic exposures are important to address when treating patients.

13. Glutathione

Given the role of oxidative stress in illness from exposure to mold and mycotoxins, the use of glutathione and glutathione precursors plays a large part in treatment. Glutathione is an endogenously produced tripeptide (glycine, cysteine, and glutamate) that in its reduced state functions in several enzyme systems in the body to assist in the detoxification of fat-soluble compounds and as an antioxidant, quenching free radicals [119]. Many disease states including Alzheimer's, Parkinson's disease, and autism have been found to be associated with low glutathione levels and have been treated with glutathione precursors (N-acetyl cysteine and whey protein) or various forms of glutathione [120–126]. One study found a correlation of low brain GSH levels with negative symptoms of schizophrenia [127].

Glutathione deficiency, as is frequently seen by clinicians treating patients exposed to water-damaged buildings, can have far-reaching effects on the body. In addition genomics testing often shows abnormalities in glutathione transferases including GSTP transferase and the GSTM null genotype which has been found to be associated with increased toxicity from aflatoxin [128]. Marked glutathione deficiency induces cellular damage associated with severe mitochondrial degeneration in a number of tissues [129] and glutathione deficiency results in mitochondrial damage in the brain [130].

Glutathione deficiency leads to widespread mitochondrial damage which is lethal in newborn rats and guinea pigs. Ascorbate and glutathione function together in protecting mitochondria from oxidative damage [131].

Reduced glutathione (GSH) can be administered in an intravenous, nebulized, transdermal, oral liposomal, and nasal form.

Nebulized glutathione is the only known treatment for increasing glutathione levels in the epithelial lining fluid, thought to be one of the first lines of defense for oxidative stress [119]. Beneficial effects have been seen for a number of conditions including cystic fibrosis [132–134], emphysema [135], chronic otitis media with effusion [136], idiopathic pulmonary fibrosis [137], chronic rhinitis [138], and HIV disease [139]. Reduced glutathione is known to be decreased in the bronchoalveolar lavage (BAL) fluid of persons with cystic fibrosis [140]. Inhaled glutathione was shown to decrease the proinflammatory prostaglandin E2 and increase CD4+ and CD8+ lymphocytes in BAL [140] which is consistent with an observational study which showed improvement in FEV1 and body weight and a decline in bacteria cultured including *Pseudomonas aeuruginosa* [134]. An additional cystic fibrosis study showed improvement in FEV1 and that GSH levels in BAL fluid improved by 3 to 4 times 1 hour after inhalation and remained doubled 12 hours after inhalation [133]. The author of one review of nebulized and aerosolized glutathione concluded that there were many potential applications for its use given the number of conditions related to deficient antioxidant status and impaired host defenses and with theoretic uses that included farmers lung, pre- and postexercise, cigarette smoking, and chemical sensitivity [119].

Caution should be used, however, as there is evidence of increased bronchospasm seen in some asthmatics as noted in one small study [141]. In this 8-person study, bronchoconstriction was felt to be provoked by sulfite formation and was blocked by nebulized salbutamol given before nebulized GSH. Since bronchoconstriction is felt to occur primarily in sulfite-sensitive asthmatics, it has been suggested by one author that testing of sulfites in urine occurs before nebulized glutathione therapy [119]. Additionally, obtaining a history of sensitivity to sulfites in wines or dried fruits can be useful. It is important to note that sulfite sensitivity is different from sensitivity to sulfonamide antibiotics, which is felt to be, at least in part, a result of decreased glutathione levels in HIV patients [142]. A practice that is commonly used in clinical practice requires the initial dose of nebulized glutathione to be administered in the office. In the absence of bronchoconstriction after this dose, home therapy is typically initiated. While this risk of increased bronchospasm clearly exists in select patients, there are no reported cases of status asthmaticus or death resulting from the use of nebulized glutathione.

Since olfactory epithelium is the only place where dendritic processes are directly exposed to the environment in the cribiform plate of the ethmoid sinus, intranasal delivery of medications can bypass the blood-brain barrier [143]. It has long been studied as a means to achieve central nervous system effects and has been studied for multiple agents in an effort to treat diseases such as depression, schizophrenia, Alzheimer's and Parkinson's disease [144]. Medications administered intranasally have reportedly been detected in the cerebrospinal fluid (CSF) as fast as 1 minute after delivery [144]. Intranasal administration of neuropeptides has been studied and found to have the advantage of bypassing the blood-brain barrier, which has served to limit the effectiveness of systemic therapies on central nervous system (CNS) symptoms. A study of 36 humans administered insulin, vasopressin, and melanocortin (MSH/ACTH) intranasally found that they received direct access to the CSF within 30 minutes, bypassing systemic circulation, as measured by intraspinal and intravenous catheters [145]. Levels of these neuropeptides were found in the CSF within 10 minutes and remained increased for up to 80 minutes. More prolonged sampling of a subgroup of patients receiving MSH/ACTH and vasopressin intranasally found that levels of these neuropeptides in the CSF remained above that of placebos (administered intranasal saline) at 100 to 120 minutes after administration and the authors believed that intranasal administration of neuropeptides could be useful for the treatment of brain diseases such as Alzheimer's disease and obesity.

Given the powerful antioxidant properties of reduced glutathione, clinicians have taken advantage of the transnasal delivery route and have used intranasal glutathione to successfully treat neurocognitive symptoms resulting from exposure to water-damaged buildings [2, 13]. Additionally, reduced glutathione is a powerful antioxidant which can have significant beneficial effects on the nasal mucosa. A study of the use of nasal glutathione found it increased GSH levels in the nasal mucosa and produced statistically significant improvement in nasal obstruction, rhinorrhea, and ear fullness [138]. In a recent survey study of 70 patients who had used intranasal glutathione for conditions that included multiple chemical sensitivity, allergies/sinusitis, Parkinson's disease, Lyme disease, fatigue, and other symptoms, over 86% of the respondents found intranasal glutathione nasal spray to be comfortable and easy to use and 62.1% reported experiencing health benefits while 12.1% of respondents reported having experienced adverse effects, the most common of which were irritation of nasal passages, headache and bloody nose [146]. A recent rat study of intranasal milnacipran, a serotonin noradrenaline reuptake inhibitor used for depression and fibromyalgia, found both CSF and systemic levels of the drug to be higher when administered intranasally compared to orally and demonstrated an increased antidepressant effect from the transnasal administration [144]. There is evidence for carrier-mediated transport of glutathione across the blood-brain barrier in rats administered GSH via carotid artery catheterization [147] and in other animal models [148]. Increasing CNS delivery of antioxidants including glutathione remains an area of much interest given the broad role oxidative stress is felt to play in neurologic and neurodegenerative diseases including the neurocognitive deficits frequently seen as a result of exposure to water-damaged buildings, mold, and mycotoxins [149].

Studies have shown that cerebral GSH plays an important role in maintaining blood-brain barrier function. A rat study showed that glutathione depletion was associated with

blood-brain barrier dysfunction and that treatment with N-acetyl cysteine, methionine, and GSH provided a partial to full protection against GSH depletion and blood-brain barrier dysfunction, but treatment with α-tocopherol, ascorbic acid and turmeric was not effective [150]. Brain protection through the blood CSF interface involves a glutathione-dependent barrier system [151] and ischemic alterations of the glutathione redox system may unmask blood-brain barrier permeabilizing actions of leukotrienes [152] which could contribute to neurocognitive symptoms.

Glutathione at a low molecular weight of 307 Daltons appears to be a good candidate for intranasal delivery as studies have shown that the apparent cutoff weight for intranasal delivery is 1000 Daltons, with smaller molecules having better absorption [153]. The addition of the bioadhesive chitosan was seen to increase drug delivery into the CSF and plasma with increased drug effect, presumably due to increased residence time of the drug in the nasal cavity [144], a concept which is intriguing as a possibility of increasing the effect of intranasal medications including glutathione.

There is currently a phase 1 human study of nasal glutathione for the treatment of Parkinson's disease being conducted at Bastyr University [154].

Encapsulation in liposomes allows for systemic delivery of oral liposomal glutathione. An *in vitro* model of Parkinson's disease in rats showed that liposomal GSH was 100 times more potent than nonliposomal GSH at replenishing intracellular GSH [155]. Of interest in this study was that liposomal glutathione spared endogenous glutathione with exposure to paraquat plus maneb (used to induce Parkinson's disease) but did not require GSH biosynthesis for protection and no toxicity was seen at 200 times the half maximal effective concentration (EC (50)) needed for protection. A study in mice showed that oral liposomal glutathione had antioxidative and antiatherogenic properties towards macrophages [156]. Liposomal encapsulation was found to greatly increase bioavailability in a study involving administration of radioactive cobalt 60 to rats [157]. In this study orally administered liposomal glutathione was found to decrease Cobalt 60 levels in all tissues by 12–43% while the nonliposomal glutathione did not.

Transdermal glutathione is also an effective delivery method and may be particularly desirable in children, who may be less compliant with other methods. A clinical trial of the use of transdermal glutathione in children with autism demonstrated that the treatment group showed significant increases in plasma-reduced glutathione but not whole blood glutathione [158].

It is best to use GSH in conjunction with sequestering agents as administration of GSH appears to allow the mobilization of toxins, including mycotoxins as evidenced in a case of a woman with documented presence of mycotoxins who developed a reversible choreiform movement disorder after she discontinued sequestering agents and was using GSH for six weeks at high dose without sequestering agents [159]. Of note in this case was that levels of urinary mycotoxins increased dramatically during the time she was taking GSH without sequestering agents compared to when she was using both GSH and sequestering agents.

14. Sequestering Agents

Sequestering agents refer to nonabsorbable materials capable of binding toxins in the gastrointestinal tract, thus reducing enterohepatic recirculation and ultimately the body burden of toxins. These agents are not absorbed into systemic circulation; therefore, side effects are typically limited to gastrointestinal symptoms and potential malabsorption of medications and nutrients, especially if the dose is poorly timed. Sequestering agents have a large surface area to volume ratio, giving large absorptive capacity. Several agents have shown specific efficacy in lowering mycotoxin and endotoxin levels including cholestyramine, activated carbons, and chlorella. Additionally, these agents are nonspecific and can bind additional toxins, helping to lower total body burden of toxins. Of course they have the potential to bind medications, vitamins, and nutrients and should be taken several hours apart from medications and vitamins and ideally on an empty stomach.

Mycotoxins are sequestered in a variety of tissues and enter enterohepatic circulation [132, 160, 161]. For example, OTA has been found in liver, muscle, fat, as well as the adrenal medulla and cortex, skin, gastric mucosa, myocarium, and bone marrow in animals [162].

A review of the literature shows a successful use of a variety of sequestering agents. Activated carbons (charcoal) have long been used medically, both for acute and delayed effects of toxins. Studies of use of activated carbons for mycotoxins have shown several beneficial effects. An *in vitro* study of activated carbons' binding capacity to OTA and deoxynivalenol found them to have a high affinity for chemically different mycotoxins [163]. The authors felt they could be considered multimycotoxin sequestering agents. Another study of 14 absorbent materials to detoxify *Fusarium* mycotoxin deoxynivalenol and nivalenol found only activated carbon to have effective binding capacity and to produce a significant reduction in intestinal mycotoxin absorption [164]. A recent study of nanodiamond substrates found them to be comparably effective for absorption of mycotoxins and comparable to activated charcoal while outperforming clay for OTA absorption [165]. In contrast, a study of aflatoxin binders in cows showed good results for sodium bentonite and an esterified galactomannan, while showing no effect from activated carbon [166]. An *in vitro* study of charcoal and cholestyramine for endotoxin removal showed they were both effective with both agents removing about 90% of the endotoxin from solution [167]. Cytokines have also been shown to be removed by sequestering agents with charcoal and silica found to be more effective at removing the cytokines ILF beta 1 and TNF alpha than cholestyramine [168].

Clay has been extensively studied for its effect on reducing toxicity from aflatoxin exposure, with the sodium montmorilonite clay Novasil being frequently studied. A randomized human trial of the use of Novasil studied over 600 blood and urine specimens from Ghana and found significant reductions in aflatoxin B1 adducts and a decrease in urine aflatoxin M1 at both doses used at 3 months [169]. Another review of the use of clay for the prevention of aflatoxicosis in animals

found that Novasil clay binds aflatoxin with high affinity and capacity in the gastrointestinal tract resulting in noticeable reduction in aflatoxin bioavailability without interfering with the utilization of vitamins and other micronutrients including vitamins A and E, iron, and zinc [170, 171].

Chlorophyll and chlorophyllin, a water-soluble derivative of chlorophyll, have been found to be well studied as anticarcinogenic agents and have beneficial effects against aflatoxin toxicity. In a series of rat studies, Siminochich et al. showed that chlorophyl and chorophyllin provided potent chemoprotection against early and late biochemical and pathophysiological biomarkers of aflatoxin B1-induced carcinogenesis in rat livers and colons [172]. Chlorophyllin has the potential to reduce carcinogenicity of aflatoxins as it binds to aflatoxins and reduces their bioavailability which consequently significantly reduces AFB biomarkers in humans [173]. Caution should be exercised in the sourcing of marine-based supplements given the unfortunate known contamination of oceans with toxins and heavy metals. A recent study of contamination of natural supplements found contamination of some supplements including marine-sourced supplements like chlorella [174]. Techniques involving the growth of chlorella on filtered water would likely avoid this contamination and could provide a good option for treatment. Testing has shown at least one commercially available brand of chlorella to be free of contaminants [17].

Cholestyramine (CSM), an anion exchange resin that works as a bile acid sequestering agent, has been widely studied for its role in reducing a variety of toxins [175–177] including mycotoxins. CSM has generally been found to be safe and well tolerated even in children [178]. It has the ability to reduce enterohepatic recirculation of fat-soluble toxins and thus can be found throughout the literature as a treatment for many toxin exposures including mycotoxins and endotoxins. Animal studies involving the use of CSM for OTA exposure have shown it accomplishes the therapeutic goal of reducing plasma levels of OTA while enhancing fecal excretion of the toxin bound to CSM. A study was able to demonstrate decreased nephrotoxicity in rats exposed to OTA that were given CSM, by reducing plasma and urine values of OTA while increasing fecal excretion [179, 180]. An *in vitro* study showed the CSM had a higher affinity for OTA than bile salts and it was proposed that, in addition to effects on the enterohepatic circulation of OTA, alteration of the biliary bile salt pool may be a means in which CSM reduces OTA toxicity [181].

CSM has also been shown to bind endotoxins and was found to be more effective at removing endotoxin than charcoal or silica in an *in vitro* study [168] and has shown beneficial effects in preventing the suppression of the cellular immune system in rats after partial hepatectomy [182]. CSM has also been used in the treatment of infectious and non-infectious diarrhea including *Clostridium difficile* diarrhea [183, 184] and has been shown to be effective in the treatment of infectious diarrhea in the newborn with an *in vitro* study of CSM showing immediate binding of the toxins of *Vibrio cholera* and three strains of *E. coli* at a pH comparable to intestinal pH [185].

Clinicians typically treat with a combination of sequestering agents taken together 2 to 4 times a day, apart from medications and supplements [2].

15. Antioxidants and Nutritional Agents

In addition to glutathione, additional antioxidants and vitamins can be helpful. Patients seen in practice have often been ill for a prolonged period of time and identifying and correcting nutritional deficiencies essential for optimal detoxification and recovery. Common deficiencies encountered include vitamin D, magnesium, zinc, coenzyme Q10, and B vitamin deficiencies all, of which can adversely affect multiple pathways in the body necessary for detoxification, thereby perpetuating the effects of the toxin exposure.

Multiple animal studies have tested the effect of nutritional supplementation to counteract effects of cellular damage caused by oxidative stress and mycotoxins. A study of rats exposed to aflatoxin B1 found that multiple cellular changes including deoxyribonucleic acid (DNA) fragmentation and lipid peroxidation which resulted from aflatoxin could be restored towards normal with the use of whey protein concentrate, Korean ginseng, or a combination of whey protein concentrate and Korean ginseng, although they did not fully reverse the effects of aflatoxins. [73]. It was suggested by the authors that genotoxicity from aflatoxin can be prevented by supplementation of whey protein, Korean ginseng or their combination. Whey protein supplies cysteine, the rate limiting step in glutathione synthesis. Animal experiments showed that the concentrates of whey proteins exhibit anticarcinogenesis and anticancer activity through their effect on increasing GSH concentration in relevant tissues and may have antitumor effects [186]. Whey protein is used routinely for treatment of illness resulting from exposure to mold and mycotoxins both as a glutathione precursor and as an easily absorbed protein source for patients, many of whom have developed significant gastrointestinal symptoms as a result of their exposure.

Melatonin and licorice extract (*Glycyrrhiza glabra*) were evaluated for their effect on OTA-induced oxidative stress and histopathological damage in the testes of male rats [187]. Serum total antioxidant power and total thiol molecules were assessed and found to be decreased in OTA-exposed rats, while those that received melatonin or Glycyrrhiza glabra extract showed an enhancement in these levels. In this study, significant histopathologic changes were also seen in the OTA-exposed rat testes including testicular degeneration, seminiferous tubular atrophy, and vasodilation with vascular thrombosis and both melatonin and Glycyrrhiza glabra was found to have protective effects against these changes. It was proposed by the authors that the antioxidant effects of these agents exerted a protective effect agains OTA-induced oxidative stress. An additional rat study evaluated the antioxidant effects of melatonin and coenzyme Q10 in rats exposed to a single high dose of OTA. Malondialdehyde and glutathione levels were measured and kidneys and bone marrow were examined. Malondialdehyde levels were significantly higher and glutathione levels were significantly

lower in OTA-exposed rats compared to controls or those OTA-exposed rats receiving melatonin or coenzyme Q10. It was concluded by the authors that a single dose OTA administration caused oxidative damage and that melatonin or coenzyme Q10 appeared to ameliorate the OTA-induced tissue injuries [71]. Licorice was again evaluated for its effect on OTA-induced nephrotoxicity in rats [72]. In this study, rats exposed to 28 days of OTA showed elevated levels of serum creatinine, blood urea nitrogen, alkaline phosphatase, alanine aminotransferase, and malondialdehyde while the antioxidant power of the serum was significantly reduced and histopathological evaluation showed degenerative symptoms in proximal tubules, congestion in renal tissue, and remarkable infiltration of inflammatory cells. Liquorice plant extract was found to alleviate most of the biochemical alterations from OTA.

Melatonin was also evaluated for its role in aflatoxicosis in chicks [188]. The pathological changes seen in the aflatoxin-exposed chicks (vacuolar degenerations, necrosis, bile duct hyperplasia in liver, and mild tubular degeneration in kidney) were markedly reduced in the chicks given melatonin concurrently with their aflatoxin exposure. Additionally, it was noted that GSH levels were decreased and malondialdehyde levels were increased in aflatoxin exposed chicks, but with melatonin coadministration, the levels approached those of the controls. The authors concluded that the results indicated that nitrosative tissue degeneration induced by aflatoxin exposure could be greatly reduced by melatonin supplementation in chicks. An additional study of melatonin use in chicks exposed to aflatoxin B1-contaminated diets demonstrated that aflatoxin-exposed chicks showed an increase in lipid peroxidation in the liver, erythrocytes along with suppression of superoxide dismutase and catalase enzyme activities of erythrocytes, significant reduction of serum proteins, elevations in serum transaminases and decreasing of the humoral and cell-mediated immune responses in growing chicks. Simultaneous administration of melatonin showed an obvious improvement in all test parameters although long-term melatonin administration was more effective than short-term administration for countering aflatoxin B1-induced toxicity [74].

Vitamins A, C, and E were studied on human lymphocyte cultures exposed to a single dose of aflatoxin B1 with or without the addition of vitamins A, C, or E [70]. The experiment showed that aflatoxin B1 significantly reduced the level of GSH and activities of superoxide dismutase and glutathione peroxidase while increasing levels of malondialdehyde and that simultaneous supplementation with vitamins A, C, and E restored the parameters to normal range. It was felt that vitamins A, C, and E exhibited protective effects on human lymphocytes by inhibiting aflatoxin B1-induced reactive oxygen species generation. Additional support for the role of oxidative stress comes from a rat study involving the induction of glutathione deficiency by administration of L-buthionine-(S,R)-sulfoximine which was found to also decrease ascorbate levels in the kidney, liver, brain, and lung [189]. In this study, the administration of large doses of ascorbate to these glutathione-depleted rats decreased mortality, led to normal levels of ascorbate, and spared glutathione.

Cultured human lymphocytes exposed to aflatoxin B1 were assessed for the presence of chromosomal aberrations and sister chromatid exchanges after treatment with varying doses of resveratrol. The number of sister chromatid exchanges and micronuclei was reduced in the presence of resveratrol resulting in decreased genotoxicity of aflatoxin B1 [190]. In contrast, a study in aflatoxin B1-exposed rats, showed that resveratrol failed to protect against aflatoxin B1-induced liver injury [191]. In the same study, however curcumin showed a significant hepatoprotective activity by lowering the levels of serum marker enzymes and lipid peroxidation and by elevating the levels of reduced glutathione, superoxide dismutase, catalase, and glutathione peroxidase.

16. Probiotics and Dietary Interventions

Probiotics and various dietary interventions have been studied for their effects on modulating effects of toxins including mycotoxins. These treatments have the potential to have significant beneficial effects as much of the metabolism of toxins occurs via intestinal biotransformation. Ochratoxin A (OTA) undergoes hydroxylation to the less toxic ochratoxin alpha in the intestines. In fact, administration of radio labeled OTA to rats showed that effective metabolism of OTA was lacking in most tissues other than the intestines [192]. Antimicrobials can have a significant negative effect on gastrointestinal flora and detoxification process as was demonstrated in rats that were administered neomycin which resulted in decreased hydrolysis of OTA to ochratoxin alpha with elevated levels of OTA [193].

An *in vitro* study of various bacterial probiotics showed a reduction in bioaccessibility of Aflatoxin B1 and OTA with the use of these probiotics [194]. Fermented milk containing *Lactobacillus rhamnosus* GG and *Lactobacillus casei* strain Shirota alone and in combination with chlorophyllin demonstrated that the use of fermented milk with or without chlorophyllin was found to have a significant hepatoprotective effect against aflatoxin B1 by enhancing activities of glutathione-S transferase, glutathione peroxidase, catalase, and superoxide dismutase and lowering the levels of thiobarbituric acid-reactive substances [112].

The ability of *Lactobacillus plantarum* and *Lactobacillus rhamnosus* GAF01 to degrade or bind aflatoxin M1 *in vitro* was studied in mice [111]. They found both agents were able to remove aflatoxin M1 with superior removal being seen by *Lactobacillus rhamnosus*. Removal appeared to be by simple binding and the bacteria/aflatoxin M1 complex was stable and only a very small proportion of the mycotoxin was released back in solution. An additional study in quail exposed to aflatoxin B1 found the probiotic *Berevibacillus laterosporus* prevented the biochemical changes of decreased serum albumin, total protein, glucose, and cholesterol levels as well as the increase in serum uric acid, urea, creatinine, and phosphorus that was normally seen in the quail exposed to aflatoxin B1 [195].

A study of the yeast probiotic *Saccharomyces boulardii* in boiler chickens exposed to OTA showed improvement in the biochemical profiles of the *Saccharomyces boulardii*-treated group when compared to the untreated group which showed decreased values of total protein, albumin, and globulin and increased levels of serum creatinine and SGPT [110].

The *Fusarium* trichothecene mycotoxin, deoxynivalenol (DON) has been reported to be completely biotransformed by ruminal and intestinal microflora and eubacterium BSSH 797 was capable of DON degradation and counteracted the toxic effects of DON [196]. Additional studies on DON in crops have looked at promising effects from bacterial enzymes.

Some data exists on the ability of diet-derived factors to influence aflatoxin B (AFB) biotransformation and some dietary factors efficiently protect from AFB-induced genotoxicity with mechanisms including the induction of detoxification enzymes such as glutathione-S transferases (GST) [173]. Consideration is given in this review to dietary components that may decrease the rate of activation of AFB by inhibiting cytochrome p450 1A2 activity, which has been shown to occur in humans with apiaceous vegetables (carrot and parsley family) as well as sulforaphanes, which are found in cruciferous vegetables and have been shown to protect animals from AFB-induced tumors, to reduce biomarkers of AFB in humans *in vivo,* and to reduce AFB adduct formation in human hepatocytes, most likely through repression of human hepatic 3A4 expression [197]. A randomized clinical trial involving the use of a broccoli sprout tea in China showed a significant decrease in aflatoxin adducts in individuals receiving the tea [198].

A study in rats exposed to aflatoxin B1 showed that pretreatment and intervention with lycopene significantly reduced the toxic effect caused by AFB(1) and greatly modulated AFB(1) metabolism and metabolic activation, decreasing the urinary excretion of AFB(1) phase 1 metabolites, AFM(1), AFQ(1), and AFP(1) serum AFB(1)-albumin adducts. This was felt to be a result of inhibition of phase 1 metabolism and metabolic activation as well as induction of phase 2 detoxification by lycopene [199].

Phloretin, a natural phenol found in apple leaves, has been shown to have beneficial effects against aflatoxin with a strong chemopreventive effect against AFB1 through its inhibitory effect on CYP1A2 and CYP3A4 and its inductive effect on GST activity [200].

The identification of food allergies and avoidance of problematic foods are also beneficial. Gluten deserves special mention as it can contribute to an inflammation [201] and neurologic and psychiatric symptoms [202–204]. Beneficial effects can be seen from the avoidance of gluten, even in those not found to have celiac disease [205].

17. Sauna, Exercise, Weight Reduction

Sauna and sweat induction have been used safely in many cultures throughout history and have long been studied as a means of reducing the body burden of toxins [206]. The most frequently studied saunas are Finnish dry heat radiant saunas, although far infrared saunas are also frequently used effectively and have the advantage of potentially inducing sweating at a lower body temperature. Sauna has been found to have numerous benefits including the treatment of respiratory and cardiovascular diseases [207]. Sauna therapy has shown benefit for the treatment of hypertension, congestive heart failure, and post-myocardial infarction (MI) care and has also been used effectively for chronic obstructive pulmonary disease, chronic pain, rheumatologic disease, chronic fatigue, and addictions [207].

Clinical studies have shown that sauna was safe for patients with stable heart conditions (hypertension, coronary disease, and stable controlled chronic heart failure) [208], and some studies have shown benefits for persons with cardiac disease including congestive heart failure and hypertension [206, 207]. Studies of the risk of sudden cardiac death have not found an increased risk of sudden cardiovascular death except when alcohol was used [207]. Contraindications to sauna therapy include pregnancy, concurrent use of alcohol, unstable angina, aortic stenosis, severe orthostatic hypotension, fever, oozing skin conditions, urticarial, or recent myocardial infarction, although there are some studies supporting the safe use of saunas in persons with a recent history of myocardial infarction [209]. Sauna use was not only found to be safe but actually transiently improved pulmonary function in a study of men with obstructive pulmonary disease [210].

A study of 28 persons exposed to mold and mycotoxins included treatment with exercise, physical therapy and sauna as well as well as IV antioxidants, oxygen therapy and immunotherapy found improvement in all patients with 27 of the 28 returning to work [3].

Dr. Stephen Genuis has studied the excretion of a number of agents in his blood, urine, and sweat (BUS) studies and has found that a number of toxins are found in sweat, with some appearing to be preferentially excreted in sweat [211]. He has identified bisphenol A (BPA) in sweat, even, in some instances when it was not identified in blood or urine, supporting the use as of sauna as a possible means to induce excretion of BPA. The presence of phthalates and their metabolites have also been identified in sweat [212] as have heavy metals [213]. Ochratoxin has been found in human sweat [17]. Controlled studies to evaluate for the presence of mycotoxins in sweat would be useful. However, regardless of whether mycotoxins are found, induced sweating will likely reduce the total overall body burden of toxins and support recovery in persons made ill from exposure to water-damaged buildings.

Exercise, whether or not sweating is induced, can have numerous physiologic benefits and should be encouraged at whatever level is tolerated. At least in rats, exercise is shown to prevent oxidative stress and memory deficits with chronic cerebral hypoperfusion [214] and also reduces oxidative stress in hyperphenylalaninemic rats [215]. There are numerous benefits to exercise, and it should be initiated at whatever level is tolerated and gradually increased. Deconditioning, often severe, is frequently seen in those suffering from chronic illness, including illness resulting from exposure to

water-damaged buildings. A gradual, escalating approach to resuming exercise can be of great benefit in reversing this.

Weight gain is an unfortunate consequence of chronic symptoms resulting from long-term exposure to water-damaged indoor environments and can hinder health recovery. Obesity has been found to be associated with oxidative stress in humans and mice [216].

18. Conclusions

The treatment of patients who have become ill as a result of exposure to water-damaged buildings involves a comprehensive treatment approach utilizing available nutritional and detoxification strategies. Complete removal from exposure and contaminated items cannot be emphasized enough although it is often not sufficient for some people to regain health. Persistence of symptoms after exposure does occur, unfortunately, and is most likely related to genetic and nutritional factors as well as the severity, duration of exposure, and persistent exposure through cross contamination. The treatment approaches include the use of sequestering agents, antioxidant support, systemic, nebulized and intranasal glutathione, probiotics, nutritional support, and the correction of persistent fungal infections or symptomatic colonization. Also, the use of sauna and exercise can be invaluable in helping to restore the health of those injured from their exposure.

In 1989, the Massachusetts Department of Public Health estimated that indoor air pollution accounted for up to 50% of all illness [1]. It is likely this has increased since then and it would be expected that water-damaged indoor environments would be a significant contributor to this. Unfortunately, in spite of growing recognition of illness resulting from exposure to water-damaged environments, limited educational opportunities currently exist for medical students and residents to learn about the diagnoses and management of exposure-related conditions. Improving medical education as it relates to both indoor and outdoor air pollution would be a significant step in improving the quality of medical education and care for our patients.

Clearly much more research is needed to identify the best treatment options for these patients; however, an attempt was made to present the research currently available that addresses treatment options available to physicians. Limitations to the current research include the fact that many of the studies are limited to animals or are relatively small human studies or case reports. While some large studies in humans have occurred with promising results, great benefit could come from further investigation into the safety and efficacy of treatment options available to physicians treating patients with the complex array of symptoms that often result from exposure to water-damaged buildings. Since many of the agents used in the treatment of persons exposed to water damaged indoor environments are readily available and non-patentable, funding for large scale studies is often unavailable. It is important to note that a typical human exposure involves a complex mixture of biocontaminants, while much of the research occurs on single agents. Similar limitations exist to studying treatment outcomes, as typically patients have been ill with multiple symptoms for a prolonged period of time and are understandably eager to proceed with treatment, pursuing multiple treatment options concurrently. As understanding of illness resulting from exposure to water-damaged building increases, it is hoped that research into the best treatment approaches will allow physicians to provide optimal care for their patients.

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Review Article

Chelation: Harnessing and Enhancing Heavy Metal Detoxification—A Review

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Toxic metals such as arsenic, cadmium, lead, and mercury are ubiquitous, have no beneficial role in human homeostasis, and contribute to noncommunicable chronic diseases. While novel drug targets for chronic disease are eagerly sought, potentially helpful agents that aid in detoxification of toxic elements, chelators, have largely been restricted to overt acute poisoning. Chelation, that is multiple coordination bonds between organic molecules and metals, is very common in the body and at the heart of enzymes with a metal cofactor such as copper or zinc. Peptides glutathione and metallothionein chelate both essential and toxic elements as they are sequestered, transported, and excreted. Enhancing natural chelation detoxification pathways, as well as use of pharmaceutical chelators against heavy metals are reviewed. Historical adverse outcomes with chelators, lessons learned in the art of using them, and successes using chelation to ameliorate renal, cardiovascular, and neurological conditions highlight the need for renewed attention to simple, safe, inexpensive interventions that offer potential to stem the tide of debilitating, expensive chronic disease.

1. Introduction

The living body is full of chelates; metals bound with two or more coordination bonds. Metals of oxidation state greater than one (i.e., a charge of +2 or more) are predominantly bound in tissues by ionic (in skeletal minerals) or coordination bonds (e.g., bound to albumin, enzymes, small peptides, and amino acids such as cysteine, methionine, and selenomethionine). This was extensively reviewed by Apostoli et al. [1].

Cadmium [2–5], lead [6–8], and mercury [9–12] have no essential biochemical roles, but exert diverse, severe toxicities in multiple organ systems as they bind in tissues, create oxidative stress, affect endocrine function, block aquaporins, and interfere with functions of essential cations such as magnesium and zinc. Toxic metals pose particular risks to the very young, as exposures early in life compromise development, with lifelong physical, intellectual, and behavioural impairments. In adults, major chronic diseases [13], including cardiovascular and renal disease, and neurological decline, are also strongly associated with toxic elements. The International Agency for Research on Cancer (IARC) classifies cadmium as a known carcinogen, inorganic lead a probable carcinogen, and methylmercury a possible carcinogen [14].

As research progresses, harms more subtle than acute poisoning are seen at lower and lower body burdens of heavy metals. For example, early lead exposure is now found to cause IQ decrements at a blood level below 2 μg/dL [15]. The blood lead reference value at which the US Centers for Disease Control action recommends investigation and remediation of a child's environmental exposures is 5 μg/dL, while chelation is recommended at nine times that level above 45 μg/dL [16].

Modern mercury and cadmium exposures are frequently via oral routes, prompting advisories regarding fish (e.g., U.S. Environmental Protection Agency [17]), seafood and wildlife consumption (e.g., Canadian Aboriginal Affairs [18]), as well as cigarette smoke (also noted by Aboriginal Affairs; cadmium is but one toxic component). Lead may also originate in old drinking water supply pipes.

Toxic metals are ubiquitous in our environment, and thus in ourselves, at higher than historical levels. Exposures [5, 8, 12, 19] include the activities and legacies of mining and toxic wastes, lead in paint and gasoline, ongoing emissions from industrial and electricity-generating (particularly coal-burning) activities, chemicals in everyday products, and novel technologies such as nanomaterials containing toxic elements like cadmium [2].

Biological mobility, tissue concentrations, and excretion of metals are determined by oxidation state, solubility, a complex set of equilibria between complexing sites, as well as active transport through membranes [1]. Chelation is central to natural detoxification of heavy metals, via formation of complexes, particularly with glutathione and other small molecules, and their excretion [20].

This manuscript stems from a large scoping review regarding arsenic, cadmium, lead, and mercury, funded by the Canadian Institutes of Health Research. Multiple online literature searches included a comprehensive list of terms for the toxic elements and peer-reviewed search strategies, to search research publication databases, as well as governmental (e.g., Environment Canada, US Environmental Protection Agency) and nongovernmental (e.g., World Health Organization) sources, described previously [21]. Expert opinion was solicited via email, during a conference call, and during a two-day conference in Toronto (February 2011). Clinical toxicologists at Canadian Poison Control Centres were surveyed to gather information about screening, experiences, and preferred chelators for each toxic element. Ethics approval was obtained from the Children's Hospital of Eastern Ontario Research Institute, Ottawa, Canada.

In this paper, measures to support natural detoxification pathways involving chelation, as well as use of pharmaceutical chelators are examined. Historical adverse outcomes, lessons learned in the art of using chelators, and successes using chelation to ameliorate renal decline, cardiovascular disease, and autism in children are reviewed.

2. Chelation Background

"Chelation," from "chelos" the Greek word for claw, involves the incorporation of a mineral ion or cation into a complex ring structure by an organic molecule, the chelating agent. Typically, electron-donor atoms on the chelating molecule include sulphur, nitrogen, and/or oxygen.

The strength of the chemical bonds within coordination complexes that are formed between chelators and metal ions depends upon the elements involved and details of the stereochemistry. With a variety of metal ions that could bind competitively with the chelator (e.g., calcium, magnesium, zinc, copper, manganese, and other metals, that typically exceed concentrations of toxic elements), the identity of the metal predominately bound by a chelating agent depends both upon accessibility of the chelator to the tissues, how strongly the metal is already bound in the tissues, how strongly the metal binds to the chelator, and to some extent the relative quantities of various ions [1]. Chelators have the effect of mobilizing metals from tissues and maintaining the chelate moiety during circulation to the kidneys for excretion in the urine, and to the liver for excretion in the bile. There are significant concerns related to enterohepatic recirculation and reabsorption in the kidney [22].

Another consideration is solubility of the chelate, in water and in lipids. Aqueous solubility facilitates transport within the blood and excretion via the kidney, while a lipophilic chelator may exhibit greater penetration of cellular membranes (including those within the central nervous system) to chelate intracellular elements. A lipophilic chelator may also be excreted in greater quantities via the bile. These generalities may be modified by active transport of intracellular metal complexes via "drug resistance proteins" [23–26].

3. Roles of Chelation in Natural Toxicokinetics

Metal binding proteins, including metallothioneins, are potent chelators for heavy metals and are central to the natural response of the body to these toxic elements [27, 28]. Glutathione is another potent chelator involved in cellular response, transport, and excretion of metal cations and is a biomarker for toxic metal overload [29–31].

Not only animals, but also plants produce chelating compounds [32], and metallothionein content of foods may affect bioavailability as well as metabolism of toxic metals such as cadmium [33].

Some foods have been suggested to reduce absorption or reabsorption of toxic metals and to support natural detoxification pathways.

(i) Dietary fibres from various food products, including bran from grains as well as fruit, have been evaluated as an alternative or adjunct to chelation therapy with the aim to interrupt enterohepatic recirculation [34–36] and to modulate intestinal flora [37], with findings of reduced levels of mercury in the brain and blood. Caution is merited regarding soluble fibre; in contrast to protection offered by insoluble fibre, flax seed resulted in increased intestinal absorption of cadmium [38].

(ii) Other natural polymers have also been gaining attention as potential adsorbents of heavy metals, such as algal polysaccharides alginate [39] and chlorella [40]. Modified citrus pectin plus alginate products have been used successfully to reduce lead and mercury in case studies [39]. Poly(γ-glutamic acid), an edible and biodegradable biopolymer, has been produced extracellularly during fermentation of *Bacillus* species; its α-carboxyl groups conjugate a variety of compounds including metal cations [41].

(iii) Given that toxic metals have great affinity for sulphur-containing peptides, diets rich in sulphur-containing foods such as alliums (e.g. garlic [42]) and brassicas (e.g., broccoli [43]) have been suggested for effects on glutathione, with hopes for symptomatic improvement and enhanced excretion. Garlic prevented cadmium-induced kidney damage [44] and decreased the oxidative damage due to lead in rats [45].

(iv) Cilantro (leaves of *Coriandrum sativum*), a popular culinary and medicinal herb, gained attention when a soup

was reported to enhance mercury excretion following dental amalgam removal and remains popular despite limited evidence [46]. In animals, it decreased lead absorption into bone and inhibition of the delta-aminolevulinic acid dehydratase (ALAD) enzyme [47]. Less encouragingly, in a recent trial in 3- to 7-year old children exposed to lead, a cilantro extract was as effective as placebo in increasing renal excretion (improvements across treatment and placebo groups were ascribed to improved diet during the intervention) [48].

Several supplements are also in use to address metal toxicities.

(i) Taurine [49–51] and methionine [52] are sulphur-containing amino acids. They are rich in membranes particularly of excitable tissues, and they decrease oxidative stress markers resulting from heavy metal exposure. Practitioners also report using taurine for 6 weeks or so prior to hair analyses, to boost levels and improve detection.

(ii) Alpha lipoic acid is a powerful antioxidant that regenerates other antioxidants (e.g., vitamins E and C, and reduced glutathione) and has metal-chelating activity. Both fat and water soluble, it is readily absorbed from the gut and crosses cellular and blood-brain membrane barriers [22, 53]. Clinical experience is that it must be used carefully as it poses particular risks of redistribution of metals.

(iii) N-acetyl-cysteine (NAC), an orally available precursor of cysteine, is a chelator of toxic elements and may stimulate glutathione synthesis, particularly in the presence of vitamins C and E [54–56].

(iv) Glutathione is not recommended to be administered orally as it undergoes digestion; however novel modes of delivery such as liposomal and prodrug preparations are emerging [57]. It may be administered intravenously, in creams and via nebulizer. Glutathione is an important physiological chelator, and the reduced form of glutathione protects cells from reactive oxygen species associated with heavy metals [58–61].

(v) Selenium is an important essential element, that is present at a broad range of levels across populations. The selenide ion forms an extremely stable, insoluble compound with mercury, and provides relief of mercurialism symptoms. On the face of it, selenide might not be compatible with chelation, as the two agents may counter the effectiveness of one another [62]; however, selenium may be incorporated in organic molecules, and organic selenium/mercury complexes may be transported through membranes. Selenium depletion in the face of mercury exposures also depletes selenoenzymes. In humans, organic selenium supplementation was beneficial in a controlled trial among 103 mercury-exposed villagers [63]. A selenium yeast product increased mercury excretion and decreased oxidative stress-related biomarkers urinary malondialdehyde and 8-hydroxy-2-deoxyguanosine [63].

Overall, a number of studies have investigated the effects of micronutrients such as vitamins, sulphur-containing amino acids, antioxidants, and essential minerals on kinetics and adverse effects of toxic elements [64–68]. Nutritional status affects uptake, as toxic cations are transported by proteins for essential nutrients such as magnesium, zinc, and iron, putting those who are malnourished at greater risks for toxicity [2, 33]. This suggests potential for dietary preventive public health interventions. For example, in animals calcium deprivation enhanced absorption of lead and cadmium [69], while magnesium and zinc supplementation blunted absorption of cadmium [2]. Calcium supplementation reduced lead mobilization from maternal bones during pregnancy and lactation, protecting the newborn and infant [70–72]. In children, iron supplementation blunted lead accumulation [73]; however, mineral supplementation and school meal programs should not divert attention from the paramount importance of removal of the sources of exposure [74–76].

4. Pharmaceutical Chelators

Pharmaceuticals that chelate metal ions in solution are small organic molecules that typically form coordination complexes involving sulphur, oxygen, and/or nitrogen atoms.

Drug information from the US National Library of Medicine for five chelating agents used most commonly for the treatment of humans intoxicated with heavy metals and metalloids is summarized below, and in Table 1 [56].

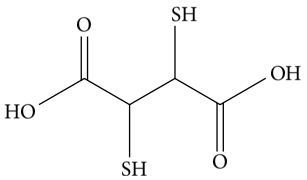
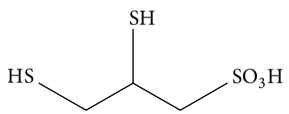
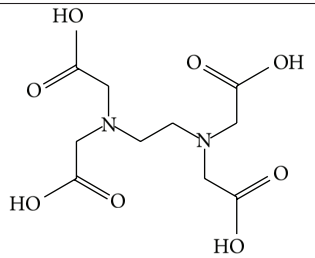
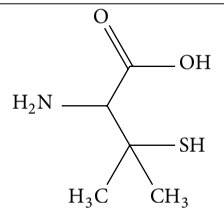
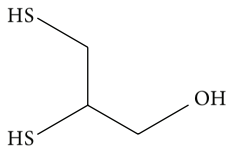
Dimercaprol (British Anti-Lewisite, BAL), the first antidote to an arsenical nerve gas, is a dithiol prepared in an oil base and given only by intramuscular injection (painful). It has a narrow therapeutic window and is commonly prepared with peanut oil, posing a risk of allergic reaction.

BAL has been largely supplanted by dimercaptosuccinic acid (DMSA or succimer) and dimercaptopropane sulfonate (DMPS), that were extensively researched in Russia, China, and Japan, a half century ago [77]. These dithiols, with greater water solubility, are being administered as oral, intravenous, suppository, or transdermal preparations. The absorbed dose is excreted with a half-life of approximately 3 hours; longer in children and people with mercury toxicity.

Oral administration of DMSA may be limited by intestinal dysbiosis. Oral absorption is approximately 20%, with most DMSA in plasma being protein bound (95%, mainly to albumin); only a very small amount is present as free drug. DMSA is extensively metabolized in humans to mixed disulfides of cysteine. Ten to 25% of an orally administered dose of DMSA is excreted in urine; the majority within 24 hours and most as DMSA-cysteine disulfide conjugates. The remainder is largely eliminated in the faeces [77–80]. DMSA increases urinary excretion of arsenic, cadmium, lead, methylmercury, and inorganic mercury, with removal from animals' brains of lead and methylmercury. Successful dialysis of methylmercury-DMSA complexes has been reported. Excretion of essential metals like zinc, iron, calcium, and magnesium is much less than with $CaNa_2EDTA$, with potentially higher losses of copper in humans. Although frequently administered orally, intravenous, rectal, and transdermal routes are in clinical use. A rare side effect is mucocutaneous eruptions and toxic epidermal necrosis, that resolves when the medication is stopped.

DMPS oral absorption is approximately 39%, higher than that of DMSA [81]. Solutions are relatively stable, so DMPS is administered intravenously more frequently than DMSA. DMPS is rapidly converted to a disulphide form

Table 1: Overview of chelation drugs.

Chemical name (common names, abbreviations)	Structure	Activation metabolism	Coordination (binding) groups	Elements chelated
2,3-bis(sulfanyl)butanedioic acid (Dimercaptosuccinic acid; Succimer; Dimercaptosuccinic acid; *DMSA*; Suximer; Tin Salt; Succicaptal; Chemet)		Excretion via urine >90% as DMSA—cysteine disulfide conjugates.	Oxygen and sulfhydryl	Lead Arsenic Mercury Cadmium Silver Tin Copper
Sodium 2,3-bis(sulfanyl)propane-1-sulfonate (Sodium Dimercaptopropanesulfonate; *DMPS*; Unithiol; Dimaval; Unitiol; (+)-DMPS; (−)-DMPS)		84% of IV dose excreted through urine	Oxygen and sulfhydryl	Mercury Arsenic Lead Cadmium Tin Silver Copper Selenium Zinc Magnesium
2-[2-[bis(carboxymethyl)amino]ethyl-(carboxymethyl)amino]acetic acid (Ethylenediaminetetraacetic acid; Edetic acid; *EDTA*; Edathamil; Endrate; Versene acid; Sequestrol; Titriplex; Havidote; Cheelox; Versene; Calcium Disodium Versenate (edetate calcium disodium injection, USP)		Not metabolized. Excreted unchanged, generally coordinated with a different divalent cation	Oxygen	Lead Cadmium Zinc (Mercury thought to be too strongly bound in tissues to be mobilized, but this is not clinical experience)
(2S)-2-amino-3-methyl-3-sulfanylbutanoic acid (3-Sulfanyl-D-valine; *Penicillamine*; D-Penicillamine; Cuprimine; Depen; Penicillamine; Mercaptyl; Artamine; Cuprenil; Perdolat; Trolovol		Rarely excreted unchanged; excreted mainly as disulfides	Oxygen, hydroxyl, sulfhydryl, and amine	Copper (Wilson's disease) Arsenic Zinc Mercury Lead
2,3-bis(sulfanyl)propan-1-ol (Dimercaprol; British Anti-Lewisite; *BAL*; 2,3-Dimercaptopropanol; Sulfactin; Dicaptol; Dimersol; Antoxol; Panobal; Dithioglycerine; Dithioglycerol)		Excreted unchanged in urine	Sulfhydryl and hydroxyl	Arsenic Gold Mercury Lead (BAL in combination with $CaNa_2EDTA$)

Information from US National Library of Medicine PubChem: http://pubchem.ncbi.nlm.nih.gov/search/search.cgi.

and is excreted largely in the urine as acyclic and cyclic disulfide chelates, with an overall half-life of approximately 20 hours following intravenous administration [81]. A significant proportion is also excreted in bile. DMPS increases urinary excretion of arsenic, cadmium, lead, methylmercury, and inorganic mercury. In a study of the DMPS challenge test there was significantly increased excretion of copper, selenium, zinc, and magnesium, necessitating replenishment of these essential minerals orally or intravenously before and after treatment [82].

In comparing the efficacy of the dithiol chelators in animals, DMSA was superior in removal of methylmercury, including from animal brains. Although DMPS did not affect levels in the brain, it was superior at removing methylmercury from the kidney [77]. In mice, cadmium was removed more effectively by DMSA than DMPS [83].

$CaNa_2EDTA$ is not metabolized and EDTA chelates are rapidly excreted, principally in the urine. With only oxygen atoms for coordination bonds, EDTA binds lead and cadmium strongly, eliminating them in the urine. Clinical experience is that $CaNa_2EDTA$ will result in increasing mercury excretion once other more well bound minerals such as lead and cadmium are depleted. Overall $CaNa_2EDTA$ causes greater losses of essential minerals than DMSA or DMPS.

Penicillamine binds with copper and is used for Wilson's disease. It will mobilize arsenic, cadmium, lead, and mercury, but it is generally not a drug of choice. It was inferior to DMSA and DMPS in removal of methylmercury from animals, with no effect on levels in the brain [77].

Canadian clinical toxicologist questionnaire respondents indicated that their preferences for chelation therapy for chronic toxicity would be DMPS or DMSA for arsenic; EDTA plus BAL, or as a second line medication penicillamine for cadmium; DMSA orally (or possibly EDTA plus BAL for acute exposure) for lead; and DMSA or DMPS (or possibly BAL for acute exposure) for mercury.

4.1. Roots of Chelation Controversies

4.1.1. EDTA Concerns. Three deaths associated with chelation therapy have been reported, related to hypocalcemia resulting in cardiac arrest after use of Na_2EDTA [84]. These were in fact drug errors and should not reflect on the safety of $CaNa_2EDTA$, the form generally indicated for chelation of toxic metals [85].

$CaNa_2EDTA$ is distributed mainly in the extracellular fluids and one of its major perceived drawbacks is that of redistributing lead from other tissues to the brain. In one study, treatment with DMSA after exposure to inorganic mercury caused an elevation of mercury in motor axons, likely due to redistribution of mercury, which was mobilized from nonneural tissues such as the kidneys and liver [86]. Mixed reports indicate that EDTA does not cross the blood-brain barrier, but this is in contrast to reports that EDTA may cause increased symptoms of lead poisoning or mercurialism [87].

Transient increases in hepatic transaminase activity have been reported with $CaNa_2EDTA$, DMSA, and DMPS, but hepatic toxicity resolves with discontinuation of the medication. Skin lesions associated with $CaNa_2EDTA$ may relate to zinc deficiency.

4.1.2. Chelation Therapy in Children. A single trial published in the New England Journal of Medicine (2001) is cited by authorities who recommend that chelation therapy be used only at highly elevated blood lead levels in children [88]. In an early, ambitious trial using DMSA chelation therapy in 780 children enrolled in the "Cincinnati cohort," blood lead levels were temporarily lowered in children receiving the medication compared with the control group; however, at 36-month followup blood lead levels in the treated children had rebounded. At this 3-year mark, there were no significant differences between treatment and control groups in terms of blood lead levels nor neurocognitive outcomes [89]. This trial used a very aggressive protocol, with 26 days of therapy for one, two, or three rounds. Currently, chelating agents are typically administered for multiple shorter periods, with time between courses for the body's minerals to become repleted. This aggressive therapy could very well have depleted essential minerals from this vulnerable population (poor, inner-city, and black/Hispanic children). The vitamin and mineral supplementation may have been inadequate and may have been countered by concomitant administration of the chelator (doses and adherence to treatment for supplemental minerals were not reported). Shannon et al. have offered similar criticisms [90].

A trial of chelation therapy to treat autism registered on the US National Institutes of Health (NIH) http://www.clinicaltrials.gov website is indicated as "completed," with the last update October 13, 2009 [91]. When contacted for an update, the NIH representative replied that the trial had been cancelled before recruitment, because some adverse effects were observed in a study of 120 rats [92]. This study by Stangle et al. clearly demonstrated that a single three-week course of high dose DMSA treatment ameliorated learning, attention, and arousal regulation in rats exposed to lead during a period from early postpartum to late adolescence. The treatment also reduced lead levels in both the blood and brain. What prompted cancellation of the autism trial was detection of a potential adverse drug effect in the form of adverse cognitive effects among *unexposed* rats that were treated with DMSA, compared with unexposed, untreated rats.

This pivotal animal study led to cancellation of a large, much-publicized trial in children. In assessing the relevance for the trial cancellation and to clinical practice, several issues are pertinent. Adverse effects of drugs are common, which is why drugs are not usually given without an indication that they are needed. (Pre- and postchelation challenge testing to assess excretion of both toxic and essential elements is discussed below). In the Stangle et al. study no mineral supplementation was provided, and no minerals other than lead were analysed. DMSA is well known to enhance excretion of many elements, notably zinc [93]. Zinc deficiency

impairs neurocognitive development in the young [94, 95]. In addition, Stangle et al.'s rats were treated using an "aggressive" protocol, with 50 mg/kg/day DMSA for 21 days; a dose that is much higher than the US Food and Drug approved maximum label dose of 30 mg/kg/day [93], that is typically used for less than a week at a time in children [96]. It is probable that detrimental effects attributed to DMSA resulted from deficiency of essential elements, an effect that is eminently avoidable.

In summary, the Stangle et al. study violated important current clinical practices by administering the drug at a high dose, over an extended period of time, when there was no indication of need; and failing to assess essential minerals loss and ensuring that minerals were appropriately supplemented to avoid health consequences.

4.2. Chelation in Various Tissues and Redistribution. Chelating agents are fairly rapidly excreted over a few hours or days. In contrast, toxic elements may have accumulated over long periods of time and partitioned into various bodily compartments, not all being equally accessible to chelating agents. Commonly a chelating agent will mobilize the most readily available metals first, typically in the plasma, kidney, liver and then to a lesser extent bone and central nervous system. As discussed above, toxic metals in the nervous system are best addressed conservatively, with repeated, modest treatments and the use of multiple agents. With repeated doses the most readily accessed "pools" of toxic elements will be depleted, but reequilibration slowly replenishes the toxic elements in more accessible body compartments. This is evident in the rebound of levels in the blood, following discontinuation of a chelator, which highlights two important facts.

(i) Blood and urine are poor surrogates to measure the toxins accrued over the lifetime (body burden). The common laboratory measures of urine, blood, and hair indicate exposures in recent days or months, and to a lesser extent kidney burden.

(ii) Toxic elements sequestered in bone and soft tissues are not completely immobilized; they migrate back to the bloodstream and hence to tissues where they will again exert toxic effects. It is important to gain a greater understanding of the quantities of biologically accessible toxic elements within the body that are not necessarily reflected in baseline blood or urine levels, before chelation provocation.

(iii) Introduction of a chelating agent into the body causes shifts of both essential and toxic cations. Increased symptoms commonly reported with aggressive initiation of chelation therapies are cited as a contraindication to any use of chelators. Improvements are nevertheless reported with low initial doses and gradual titration according to patient tolerance (characterized as a marathon rather than a sprint).

4.3. Testing to Identify Toxic Metals and to Follow Progress of Therapy. Toxicologically significant levels of toxic elements may not be predictable from exposure history, as relevant exposures may not be queried, recognized, or remembered. Furthermore, mobilization of metals from various compartments in the body could occur due to certain stressors such as disease, trauma, starvation, pregnancy, time of life (e.g., menopause), and extreme emotional impacts. Depending on a person's constitution, genetic make-up, diet, lifestyle, and sensitivities, a patient could be suffering from toxic metal effects without having a clear history of exposure. It is a common clinical experience that chronic conditions (e.g., neurological disturbances in a teacher who ate considerable quantities of tuna [97]) are linked to the causative toxic elements only following a test identifying elevated levels.

It is difficult to draw conclusions about adverse health effects of metals without assessing net retention, that is, the differences between the rates of assimilation and excretion of metals over the lifetime. In addition, clinicians require information to guide therapy. Most commonly, metals are analysed in urine, whole blood, red blood cells; less commonly hair; or rarely toenails.

One of the most effective methods to evaluate net retention, or at least the biologically readily available metal load, is to compare the levels of metals in urine before and after the administration of a pharmaceutical chelating agent such as $CaNa_2EDTA$, DMSA, or DMPS [98]. Variously known as "mobilization," "chelation challenge," or a "provocation" test, this procedure is not universally accepted as standard of care. Criticisms have included risks of the chelating drugs, and inappropriate comparisons of the provocation results with population norms rather than with patient baseline concentrations [85]. Indeed, some go so far as to say that any testing for metals when the exposure has not been identified; that is, when there is no reason for suspicion based upon known environmental history that toxins may be elevated, is inappropriate because of the possibility that false positives may lead to inappropriate, ineffective therapies and their attendant risks [99]. The use of chelation for diagnostic purposes, following dental amalgam removal or in asymptomatic patients with baseline urine or blood levels approximating population norms was deemed inappropriate in 2005 by staff of the Agency for Toxic Substances and Disease Registry [85]. Another criticism of use of a provocation test to judge net retention is the lack of a standard protocol, and laboratory reference ranges or guidance for interpretation of results [100]. Nevertheless, these shortcomings do not fundamentally invalidate the concept; work in this regard has started. Hansen et al. established such norms for protocol involving an oral DMPS test with four hour urine collection, among 2223 citizens in Luxembourg [101].

Pre- and postchallenge testing may allow the clinician to identify which chelating agent is the most effective for the patient, and if oral agents are employed, possible absorption or tolerance problems may be identified. An open research question has to do with changes in metals excreted over an extended course of chelation treatments; whether in a person with high levels of multiple metals, one will be preferentially chelated initially, with a second then third being excreted over time with repeated treatments. This research would aid interpretation of chelation challenge tests, as well as enhance knowledge of chelation therapy itself.

Comparison of baseline and provoked urine levels is entering standard practice and was used to determine inclusion in a trial of chelation therapy for children with autism

[96]. In this trial, however, a few children experienced worsening symptoms. Such worsening is ascribed to redistribution of toxic metals, with insufficient excretory mechanisms in place, leading some practitioners to prefer unprovoked analyses up front, in sensitive, fragile patients. Therapy may be guided by parental, caregiver, and patient observations.

4.4. Therapeutic Benefits. Chelation therapy is established as an effective treatment for acute and higher exposure poisoning, according to the drug labels. Examples of reports using chelation agents for high occupational or environmental exposures include the following.

(i) DMSA chelation therapy increased lead excretion on average by a factor of 12 and rapidly reversed lead related symptoms (largely neurological and gastrointestinal) in a case series of 17 lead-poisoned adults [102]; these authors also reviewed effectiveness of DMSA.

(ii) The same group reported a case of a jeweller with extensive neurological symptoms of mercury poisoning, reversed with DMPS treatment [103].

(iii) A trial of oral DMPS therapy in the Philippines provided two weeks of treatment in a community highly exposed to mercury used for artisanal gold mining [104]. Most participants experienced multiple significant neurological improvements. This trial was remarkable for the extensive testing conducted in this remote location, as well as near-perfect compliance, as the midwife distributed the medication. This report is high quality, with careful descriptions of the intervention, inclusion, dropouts, and results.

The effective use of chelation in patients with lower levels of accumulation of toxic elements is not as widely recognized, but positive trials are being reported.

(i) In a randomized, double-blind controlled trial conducted by Adams et al., reductions in measures of the severity of autism were associated with the difference in urinary excretion of toxic metals before and following treatment with DMSA, demonstrating both a significant positive association between the severity of autism and the body burden of toxic metals, and efficacy of reduction of this body burden in improving symptoms [96]. An inclusion criterion for the trial was elevated body burden of one or more toxic elements, determined using chelation challenge testing. The initial three days of treatment for this inclusion screening was sufficient to improve glutathione and platelet levels in children with autism [105].

(ii) A concern with chelation therapy is that renal insufficiency may be a contraindication for therapy. The opposite appears to be the case. In a randomized, controlled study of 64 patients with chronic renal insufficiency with elevated body burden of lead and without diabetes, three months of $CaNa_2EDTA$ weekly infusions resulted in slowing or reversing degeneration in the chelation group. Following 24 further months of treatment in 32 patients with elevated body lead burdens, glomerular filtration rate improved among the treatment group and decreased in controls. The rate of decline among those not treated during followup was lower among previously treated patients. Cost of therapy was approximately $3750 per patient, compared with a cost of $61,000 for hemodialysis over a similar time frame for end stage renal failure [106]. In a smaller trial in patients with type II diabetes, body lead burden was a strong predictor of rate of renal function decline. Chelation therapy halved decline during three months of treatment but kidney function worsened in both groups during nine months further followup without treatment [107]. Of note, no other toxic elements were measured during this research, so it is unknown to what extent other nephrotoxins such as cadmium or mercury may have also played roles.

(iii) A 1955 report that patients with ischemic heart disease had improvement in angina and other cardiovascular symptoms while undergoing EDTA chelation therapy for lead poisoning sparked long, ongoing interest in the prevention and treatment of cardiovascular disease [108]. EDTA chelation therapy treatment for atherosclerosis has been a controversial subject of debate. While early anecdotal evidence suggested significant clinical symptomatic improvements, the five clinical trials identified in a recent meta-analysis used small populations with different clinical syndromes, measured different outcomes, and yielded no overall evidence of benefit [109].

The Trial to Assess Chelation Therapy (TACT) [110] was a US National Institutes of Health sponsored, randomized, double blind, placebo-controlled clinical trial, evaluating the benefits and harms of EDTA chelation therapy in 1708 nonsmokers aged 50 and older who had an acute myocardial infarction more than 6 weeks prior to enrolment and were otherwise medically stable. The protocol was recommended by the American College for Advancement in Medicine, the largest physicians' organization in America practicing chelation. Treatment included 40 3-hour infusions of a multicomponent Na_2EDTA solution, plus an oral, high-dose multivitamin/mineral supplement on nonchelation days. The primary endpoint was a composite of all-cause mortality, myocardial infarction, stroke, coronary revascularization, and hospitalization for angina [111]. The success of this trial was reported at the American Heart Association meeting in November 2012. Three years after treatment, the risk of the combined endpoint was reduced by 18% in the group receiving EDTA ($P = 0.03$) compared with placebo. Among participants with diabetes and those who had experienced anterior myocardial infarctions, the combined endpoint was reduced by 39% ($P = 0.002$). Of equal importance, there was no difference between groups in serious adverse events. Hypocalcemia and transient renal function impairment, the two complications that had been reported in early studies using primitive protocols, did not occur at all. TACT proceeded despite detailed criticisms [112], but unfortunately excretion of toxic elements was not assessed during this trial. Thus, participants in whom chelation would potentially have been indicated on the basis of higher body burdens of toxic elements known to be associated with cardiovascular disease were not identified, and it is unknown if additional benefit may have accrued with additional treatment, among those with remaining significant body burdens of heavy metals.

4.5. Other Potential Pharmaceutical Chelators. Monoisoamyl DMSA (MiADMSA) is a potential drug candidate under development. In young rats exposed to lead or arsenic, MiADMSA was found to potentiate the synthesis of metallothionine in liver and kidneys and glutathione in liver and brain, along with significantly reducing the glutathione disulfide levels in tissues. MiADMSA is capable of mobilizing intracellularly bound cadmium and is seen to provide an indirect antioxidant effect by removing cadmium from the site of deleterious oxidation reactions [86].

Analogues of DMSA are capable of crossing biomembranes and are more effective in reducing arsenic burden in acute and subchronic intoxication. Monoesters may be preferred over DMSA diesters owing to their higher efficacy against arsenic intoxication and lower toxicity of the drug [86].

N-(alpha-L-Arabinofuranos-1-yl)-L-cysteine, stereoselectively prepared from L-arabinose and L-cysteine, is an experimental chelator which has been shown to have good intra- and extracellular mobility as well as little effect on the level of essential minerals when used in mice [113].

Older drugs known as "metal protein attenuating compounds" (MPACs) such as clioquinol are weaker chelators, thought to modulate copper and zinc in the brain, removing it from plaque and tangles. As with the TACT trial focusing on calcium, the focus has been on known physiologically essential metals, and little thought and research has been devoted to possible effects of MPACs on toxic metals. The hypothesis that MPACs may be acting on toxic as well as essential metals merits further investigation, as clioquinol and vitamin B were found to reduce lead accumulation and to rescue brain plasticity in rats [114].

4.6. Combination Therapies. Combination therapy is an approach to enhance metal mobilization from the body, reduce individual doses of chelators, and lessen redistribution of toxic metals from one site (e.g., bone or liver) to more sensitive sites such as the brain (discussed above). There are a large number of possible agents, which are being tested in animal research. This is an area ripe for research; here are a few examples.

Animals chronically exposed to lead experience redistribution from bone to soft tissues including the brain following $CaNa_2EDTA$. This is also seen in humans, leading to the recommendation that EDTA chelation be followed by a short course of DMSA [115]. Indeed, the recommendation to combine EDTA with thiol chelators was reported decades ago [116]. In lead-treated rats, a DMSA and $CaNa_2EDTA$ combination was superior to either drug on its own, or to DMPS alone or in combination with $CaNa_2EDTA$, in depleting organ and bone lead, normalizing lead-sensitive biochemical measures with no redistribution of lead to any other organ. DMSA was the only drug that resulted in decreased brain lead levels [117].

Coadministration of DMSA and monoisoamyl DMSA (MiADMSA) at lower doses was most effective not only in reducing arsenic-induced oxidative stress but also in depleting arsenic from blood and soft tissues compared to other treatments [86].

Supplementation with antioxidants and small molecules containing thiol groups, along with chelating agents may be beneficial in increasing toxic metal mobilization and excretion, with improvement of biochemical variables [118]. For example the following.

(i) Taurine, when coadministered with DMSA or MiADMSA, helped to further reduce total body burden of arsenic and lead [119].

(ii) NAC forms coordination bonds between metals and its thiol group. The thiol may also reduce free radicals. Combined administration of NAC and DMSA after arsenic exposure led to a significant reduction of oxidative stress biomarkers, as well as to removal of arsenic from organs [120].

(iii) The research group led by Flora has investigated toxic metals extensively in animals, and reviewed combinations of antioxidants and other agents in addition to chelators, including vitamins, NAC, taurine, lipoic acid [20], and liposomal glutathione [60, 61].

4.7. Clinical Approaches. Management of patients in whom low dose chronic toxic metal exposures are contributing to chronic illnesses presents a significant challenge to the health care provider. Irritable bowel syndrome, fatigue, autism spectrum disorders, cognitive impairment, allergies, environmental sensitivities, or soft neurological signs like tremor, imbalance or depression may be multifactorial in origin. Such patients' clinical situations are unique and complex, necessitating multiple therapeutic strategies. Individualized therapy is provided according to the best available evidence, clinical judgment, and patient preference, in order to maximize benefit and minimize risk [19]. A thorough work-up is used to identify underlying factors, such as allergens and gluten intolerance, that are addressed by avoidance, food intolerance identification and remediation (rotation and elimination diets) and pre- and probiotics for intestinal dysbiosis. As a result of intestinal malabsorption patients may present with nutritional deficiencies, which can be addressed through dietary counselling, oral supplementation with vitamins and minerals, and intravenous supplementation using a mixture such as Myers Cocktail, to which glutathione may be added. It should be noted that there is concern about endocrine disrupting di (2-ethylhexyl) phthalate (DEHP) leaching from vinyl intravenous bags and tubing [121].

An extensive environmental exposure history is used to identify xenobiotic exposures [122], so that sources may be recognized (e.g., occupational exposures), remediated (e.g., dust from lead-based paint) and avoided (e.g., consumption of high-mercury fish, or smoking). Once the sources of toxins are removed from the environment and diet, and if necessary the natural biochemistry is supported with replenishment of essential vitamins, minerals, and microbiota, many patients will improve with a healthy diet, exercise, and rest. Sweating with exercise or sauna may be of benefit, as toxic metals are excreted in sweat [21].

Toxic elements unfortunately build up over time in soft tissues and bone, and even when the external source is removed the bioaccumulated toxic elements represent an ongoing endogenous source of exposure, and measures to enhance excretion may be helpful.

Overall, during chelation therapy mobilization must equal excretion, so adequate hydration and bowel regularity are essential. A variety of products may assist in interrupting enterohepatic recirculation of toxicants, including cholestyramine, charcoal, psyllium, thiolized silica, and others [78]. Pharmaceutical chelating agents may also be considered, to assist with mobilization and excretion.

Chelation therapy, including nonabsorbed agents, should be initiated at a low dose and then gradually titrated to recommended doses according to the individual's response, to avoid the patient's health deteriorating with metal redistribution, other physiological perturbations, or drug intolerance. Mineral status must be monitored during chelation therapy, with panel assays of whole blood or red blood cell essential and toxic minerals, and possibly periodic pre- and postprovocation urinanalyses. Oral or intravenous vitamin and mineral supplementation are important, although mineral supplementation and chelation therapy are antagonistic so are generally not given concomitantly.

DMSA or DMPS are the oral drugs of choice, while EDTA with or without DMPS may be administered intravenously. Concomitant use of NAC or lipoic acid are best reserved until the patient tolerates the full chelator dose, and the metal quantities being excreted have fallen substantially (e.g., to a quarter or fifth of the initial levels).

Allergy-mediated adverse drug reactions have been reported with DMSA and DMPS, and less commonly with $CaNa_2EDTA$, so allergy testing may precede chelation therapy. In this context, it is interesting that anecdotally risk of allergy increases with frequency and degree of xenobiotic exposure, which adds further complexities to considerations of type, dose, and frequency of administration of a chelating agent. Clinical experience is that allergies decrease with reduction of the body burden of toxic elements.

5. Conclusion

Chelation is the basis of much of the physiology of multivalent cations and of the toxicokinetics and toxicodynamics of heavy metals. Recognizing toxicant contributors to chronic disease and conducting research to evaluate chelation strategies and protocols to assess and address toxic metal bioaccumulation offer potential for inexpensive, safe therapies addressing important root causes of today's most costly, prevalent chronic diseases. Future chelation research should include assessment of both essential and nonessential elements.

Conflict of Interests

The author declares that she has no conflict of interests.

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Review Article

An Assessment of the Intestinal Lumen as a Site for Intervention in Reducing Body Burdens of Organochlorine Compounds

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Many individuals maintain a persistent body burden of organochlorine compounds (OCs) as well as other lipophilic compounds, largely as a result of airborne and dietary exposures. Ingested OCs are typically absorbed from the small intestine along with dietary lipids. Once in the body, stored OCs can mobilize from adipose tissue storage sites and, along with circulating OCs, are delivered into the small intestine via hepatic processing and biliary transport. Retained OCs are also transported into both the large and small intestinal lumen via non-biliary mechanisms involving both secretion and desquamation from enterocytes. OCs and some other toxicants can be reabsorbed from the intestine, however, they take part in enterohepatic circulation(EHC). While dietary fat facilitates the absorption of OCs from the small intestine, it has little effect on OCs within the large intestine. Non-absorbable dietary fats and fat absorption inhibitors, however, can reduce the re-absorption of OCs and other lipophiles involved in EHC and may enhance the secretion of these compounds into the large intestine—thereby hastening their elimination. Clinical studies are currently underway to determine the efficacy of using non-absorbable fats and inhibitors of fat absorption in facilitating the elimination of persistent body burdens of OCs and other lipophilic human contaminants.

1. Introduction

The presence of organochlorine (OC) compounds in the environment and biosphere is a recent development in evolutionary terms. The intentional and inadvertent synthesis and widespread dispersion of OCs began in the twentieth century. Compounds including hexachlorobenzene, DDT, and PCBs were produced to meet industrial needs. Other OCs, including dioxins, have been produced as industrial byproducts. In addition to OCs, other lipophilic compounds including brominated hydrocarbons (to be used principally as flame retardants) have also been produced.

The magnitude of the industrial output and the chemical stability of OC compounds have resulted in their persistence in the environment. Even with planned reductions in the production and use of these compounds, they will remain in the environment for many decades.

The ubiquitous presence of OCs has resulted in their entry into the food chain, with accumulation in higher organisms. Their lipophilicity directs them to be stored in adipose depots of animals, including humans. Many OCs and their metabolites exit the body very slowly, resulting in long-storage half-lives.

There is a large body of evidence linking elevated levels of OCs to the risk of disease such as diabetes and hypertension [1, 2]. Some OCs are considered to be carcinogens in animals and humans. Although effort is underway to reduce the exposure of people to OCs, this work toward decreasing environmental levels will presumably not produce a significant reduction in the levels in the biosphere in the immediate future and does not address the bioaccumulated burden already present in many individuals.

Given the persistent and ubiquitous nature of OCs, and given the potential link to the risk of disease, it is desirable to consider strategies to reduce their level in the body. This paper considers the intestinal lumen as a site for intervention to reduce human exposure and the resulting detrimental effects on health.

It is generally thought that most of human exposure to OCs comes through ingestion of foods that contain OCs. Undoubtedly there are many situations in which entry is by inhalation or by the transdermal route, but food-borne OCs dominate the entry route for most people. It is clear that the interruption of absorption of OCs from the intestinal lumen into the systemic circulation can reduce the accumulation of OCs in the body.

It is also clear that many OCs undergo enterohepatic circulation; that is, they move from tissues in which they are stored to the blood, are taken up by the liver, and enter the intestine in bile. They may also enter the intestine directly from cells that line the intestinal lumen. Reabsorption from the intestine into systemic blood completes the enterohepatic circulation loop. As in the case for dietary OCs, inhibition of the reabsorption step can direct OCs to the large intestine where they will be excreted in the feces.

In the remainder of this paper, we discuss the means by which absorption from the intestinal tract might be reduced. We also discuss how this intervention might reduce the body's stores of OCs and comment on some considerations relating to clinical care. A schematic view of the involvement of the intestine in OC metabolism is presented in Figure 1.

2. Oral Absorption

For most people, the entry of OCs into the body is via oral ingestion. Their absorption is therefore from the lumen of the small intestine. Based on measurements of the level of OCs as a function of age, there is evidence that some people take in OCs more rapidly than they excrete them from the body [3, 4]. Reducing the intake of OCs from the diet is therefore a strategy that should be considered in high risk situations in light of the fact that the food chain will continue to contain OCs even if their production rates are curtailed.

Most OCs are very lipophilic, with partition coefficients, expressed as the $\log_{10}$ of the ratio of solubility in octanol to that in water of >6.0 [5]. This lipophilicity results in solubility in food lipids, and the presence of dietary triacylglycerol fat acts to facilitate the absorption of OCs. Dietary lipophilic OCs accompany triacylglycerol through absorption via the lymphatic route with little absorption into the portal vein [6]. They are incorporated into chylomicrons, and in this form a portion of the absorbed OC is delivered to peripheral tissues without encountering the liver for first pass metabolism.

It is possible to reduce of the absorption lipophiles from the intestine. Cholesterol absorption can be reduced by the addition of plant sterols to the diet or by ezetimibe, which blocks the internalization of the cholesterol NPC1L1 complex. This approach is specific for cholesterol and has little effect on other dietary lipophiles.

The addition of a nonabsorbable oil to the diet also hinders the absorption of dietary cholesterol. Olestra, which is comprised of sucrose bonded by ester links to 6–8 long-chain fatty acids, is not absorbed from the intestine. Its ingestion results in an intestinal lipophilic solvent sink that carries a portion of other dietary lipophiles into the large intestine and the feces. Olestra has been reported to significantly reduce the absorption of dietary lipophiles including cholesterol, retinol, vitamin A, vitamin D, vitamin E, vitamin K, β-carotene, and lycopene [7].

Olestra also interferes with the absorption of OCs. Carbon-14-labeled DDT dissolved either in soybean oil or a 50-50 blend of soybean oil, and olestra was given by gastric gavage to rats that had been surgically fitted with a cannula in the thoracic duct [8]. The percent of ^{14}C-DDT dosed in soybean oil that appeared in the lymph by the animals that received soybean oil was 66.6 ± 1.9, while the percentage recovered in the lymph of the animals that received the olestra-soybean oil blend was 21.0 ± 2.4.

Reductions in the absorption of two ^{14}C-labeled PCB congeners were also observed when olestra was included in the diet [9]. Six fasted mice were given a dose of either 2,2′,5,5′-tetrachlorobiphenyl or 3,3′,4,4′-tetrachlorobiphenyl in safflower oil. They then received a diet that contained either 19% (weight) butter fat, or the same diet to which 10 g of olestra was added for each 100 g of diet. Complete fecal collections were made for 48 hours after the gavage, and radioactivity was assayed. Based on these excretion data, the absorption of 2,2′,5,5′-tetrachlorobiphenyl was 81.0 ± 1.4% of the dose when the butter-based diet was fed, and 55.2 ± 4.3% when the diet contained olestra. Similarly, the absorption of 3,3′,4,4′-tetrachlorobiphenyl was 43.7 ± 2.0% during the butter-based diet, and 16.5 ± 4.7% with the diet containing olestra.

From the perspective of minimizing the body burden of OCs, an argument can be made that the most effective mean is that of reducing OC intake. Ideally this approach would be that of removing all OCs from the environment and food. Given the difficulty in reducing residual OCs in the environment, a reduction in intake by hindrance of intestinal absorption is an option that may be considered. The decrease in the absorption of the two PCB congeners noted above corresponds to reductions of 33% and 64% of that ingested. Whether that range of reduction is clinically meaningful depends on background intake of OCs, which is generally unknown for most people. However, in geographical area of known contamination, a prophylactic approach that reduces absorption could be beneficial.

The current state of knowledge does not allow us to determine an effective minimum dose of olestra to significantly reduce the absorption of OCs. Ingestion of 14 g/day of olestra significantly reduced the absorption of dietary cholesterol by 16% [10]. Only insignificant reductions in blood tocopherol levels were reported at levels of 10 and 20 g/day of olestra [11].

The consideration of the absorption of OCs in the diet is important since the ubiquitous nature of OCs makes it essentially impossible to completely exclude them from our diet even in geographical areas that have not been subject to extensive contamination. Also OCs that enter the intestine from the diet are in the same milieu as OCs and their lipophilic metabolites in enterohepatic circulation which enter the intestine in bile and by nonbiliary transport, as will be discussed below.

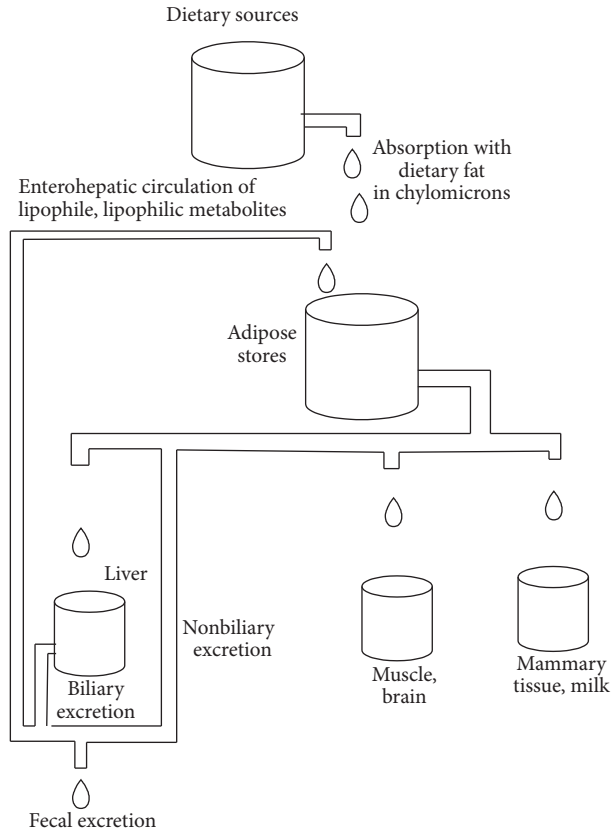

Figure 1: A schematic view of the entry, storage, and excretion of organochlorine compounds.

3. Enterohepatic Circulation

Enterohepatic circulation is a well-documented process that is generally associated with bile acid metabolism. Bile acids enter the intestinal lumen in bile, help to emulsify and solubilize dietary lipids and their digestion products, and then are actively reabsorbed in the distal part of the small intestine. This reabsorption is an efficient process that returns 90–95% of luminal bile acids back to the liver and ultimately to bile. This recycling, termed enterohepatic circulation, conserves the body's bile acid pool with only a small portion being fecally excreted with each of approximately 12 cycles per day.

There is evidence that other compounds undergo a similar enterohepatic circulation. This evidence includes the use of olestra in the diet and the measurement of fecal excretion. The first study of olestra in enterohepatic circulation was with cholesterol [12]. Rats were intravenously injected with ^{14}C-labelled cholesterol and then fed diets with or without olestra. Excretion of radioactivity in the neutral sterol fraction of feces was significantly higher in the animals fed olestra. Since the cholesterol had been given systemically, the excreted sterol had entered the intestine in bile or possibly directly through enterocytes, and the introduction of olestra slowed its reabsorption.

A similar study was carried out with ^{14}C-labelled DDT in gerbils [13]. The animals were dosed with DDT in corn oil, and fecal excretion of radioactivity was followed for 3 months. Olestra was provided in the diet at levels of 2.5, 5.0, and 10.0% by weight. Relative to the control group, increases of 2-3 fold in the rate of DDT excretion were seen in the olestra group. When a regimen of caloric restriction was included, the animals that received 10% olestra increased DDT excretion by 8-fold. Two weeks of this latter treatment resulted in a 50% reduction in total body burden. The data were consistent with the interruption of the enterohepatic circulation of DDE, the principal metabolite of DDT. Moreover, the data indicated that a regimen of olestra and caloric restriction would provide a clinically meaningful reduction in the body burden of an OC.

There is evidence that a significant part of nondietary OCs that enter the lumen of the small intestine is not in bile [14]. The mode of this transport is poorly understood but presumably involves sloughing of enterocytes and direct exudation of the OC from the enterocyte into the lumen. It seems reasonable to assume that OCs from intestinal cells enter the same pool in the lumen as OCs that enter in bile, and that they are absorbed from the intestine after dissolution in micellar forms. This kind of nonbiliary entry into the lumen of the intestine has also been seen for cholesterol although this process may be specifically related to ABCG5/G8 transporters [15].

The combination of olestra with weight loss was also studied in mice with body burdens with ^{14}C-labelled hexachlorobenzene (HCB) [16]. Caloric restriction combined with olestra resulted in a 30-fold increase in the rate of excretion of HCB.

Evidence that olestra can enhance the removal of dioxins from humans was presented by Geusau et al [17]. They found that daily ingestion of 15–66 g of olestra increased the excretion rate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by 8–10 fold. During the 38 days of this regimen, the patients did not experience gastrointestinal side effects. Based on these results, the use of olestra for the treatment of chloracne associated with dioxins was proposed by Sterling and Hanke [18].

Moser and McLachlan studied the effect of olestra on the excretion of PCBs, polychlorinated dibenzo-p-dioxins, and dibenzofurans in 4 healthy human volunteers who did not have a history of excessive exposure to these compounds [19]. The participants ate 25 g of olestra for each of 3 days, and fecal excretion of the OCs was measured. For essentially all comparisons with the nonolestra period, marked increases in the daily excretion rate were observed. The increases were 1.5–11-fold relative to the nonolestra period. PCB 153, for example, was excreted 6–10 times as rapidly during the olestra period. The excretion of this congener was 65–270 ng/d during the control period and 560–1790 ng/day during the olestra period.

These latter data allow us to estimate whether the inclusion of olestra can have a clinically meaningful effect on the body burden of OCs such as PCBs in a period of time that is practical for patient compliance. We can combine the data from Moser and MacLachlan with the known levels of PCBs in the population. As noted above, the relatively highly chlorinated congener, PCB 153, was studied in their trial and was also measured as a part of the National Health and Nutrition Examination Survey (NHANES) as recently as 2004 [20]. A 70 kg person with 20% body fat would have an adipose depot of 14000 g. We can estimate the concentration of PCBs in the adipose tissue from the lipid-adjusted concentration in serum. For a male in the 50th percentile in the NHANES data, this value is 19.7 ng/g (ppb), and the total amount of PCB 153 in the body would be 14000 g multiplied by 19.7 ng/g, which is equal to 0.28 mg. This rate of excretion corresponds to 8.2% of the body burden being excreted in a year and a half-life of 81 years.

Moser and McLachlan reported that olestra increased the rate of excretion of PCB 153 by factors ranging from 5.6 to 8.6. If the rate of excretion of PCB 153 is increased 5-fold, the excretion rate would be 43% of the body burden in a year with a corresponding half-life of 1.6 years. The results of these hypothetical calculations are consistent with clinically significant effects of intervention with olestra to reduce body burdens of OCs. Although this kind of calculation can be instructive, it does not answer questions about the variability in an individual's metabolism of OCs that are relevant to the practicality of a regimen that interferes with their enterohepatic circulation.

4. Mechanisms by Which Fecal Excretion Is Enhanced by Nonabsorbable Lipid

Two mechanisms have been proposed for the way that a nonabsorbable lipid enhances fecal excretion of stored OCs: (1) "using the gut wall as a dialysis membrane" [21] and (2) partitioning of OC in the lumen of the intestine into the nonabsorbable lipid [22]. Although both mechanisms may be valid, there are considerations that argue against the importance of the "dialysis" mechanism in the small intestine.

The "dialysis" mechanism is one in which the nonabsorbable lipid makes direct contact with the mucosal enterocytes and extracts OCs through and from the membrane of the intestinal cells. There are three principal reasons that argue against this mechanism as a major factor. First, if the enhancement of secretion through the cell wall results from contact with intestinal fat, then a normal diet would be expected to induce the same process. A typical diet in the US contains 70–100 g of triacylglycerol, a mass that is 3–5-fold times the mass of olestra that has been used in enhancing OC excretion [23, 24]. This fat is hydrolyzed rapidly into fatty acids and monoacylglycerol, but it enters the duodenum mostly as intact triacylglycerol.

A second reason to reject the idea that OCs are extracted from the enterocyte in the small intestine is the understanding of the barrier of the unstirred water layer that covers the intestinal cells [25]. The penetration of this layer by lipid is dependent on the incorporation of the lipid into bile salt-containing mixed micelles. Triacylglycerols and olestra are not incorporated into these micelles, presumably because of their molecular size. It therefore is very likely that the unstirred water layer will prevent an unhydrolyzed lipid from enhancing the secretion of a lipophile from enterocytes. The presence of 15 g of unhydrolyzed lipid in the intestine in the form of emulsified droplets of radius of 1 micrometer would have a surface area of 50 square meters. Although this area is large enough to interact with a part of the approximately 300 square meters of intestinal absorptive area, it should be noted that these droplets contain surfaces of bile salts and polar lipids. The presence of the unstirred water layer presumably will prevent efficient contact with the enterocyte membranes.

Finally, the effect of nonabsorbable dietary lipid on the absorption of OCs from the diet is consistent with a partitioning of OC into that lipid both in the stomach and in the intestine. OCs that enter the intestine in bile or by direct secretion from the enterocyte presumably enter the same pool of micellar and emulsified OCs as those from the diet. Nonabsorbable lipid affects both dietary and enterohepatic OCs in a similar manner in the milieu of the small intestinal lumen.

There is, however, support for the "dialysis" mechanism for nonabsorbable lipid in the large intestine. Rozman published results that argue strongly that hexachlorobenzene (HCB) enters the intestinal tract primarily in the large intestine [14]. Rats continued to excrete HCB even when bile flow into the intestine was diverted by ligation. Monkeys with bile diversion also excreted HCB in feces. It is possible that other OCs are also excreted through secretion and

desquamation into the large intestine. If this route accounts for a major part of fecal excretion, it is indeed possible that the presence of a nonabsorbable lipid in the colon may accelerate this process. The colon normally does not contain unabsorbed triacylglycerol since it is well absorbed in the small intestine. Most of the small amounts of fat that enter the colon are in the form of fatty acids and soaps after hydrolysis by pancreatic and bacterial lipases. It is possible that nonabsorbable lipid in the large intestine may facilitate the transport of OCs from intestinal cells to the lumen and thereby enhance their removal form the body in this manner. It should be noted that this mechanism does not depend on interrupting enterohepatic circulation of OCs.

There is, however, a significant question about how unabsorbed lipid in the intestine might interact with cells in contact with the lumen of the large intestine. If the lipid is in a phase that is separate from the other components in the milieu of the large intestine, then it may indeed interact with the cells and "extract" lipophilic compounds from the membrane. If, however, the unabsorbed lipid is dispersed with the other components that comprise fecal matter, it would seem unlikely that there would be enough contact between the surface of the lipid and the cells to affect a significant extraction of cellular OCs. Nevertheless, since colonic contents do not normally contain significant amounts of lipid that might act as a solvent, the presence of unhydrolyzed fat might provide a stimulus to move lipophiles into the lumen. Moreover, as pointed out by Schlummer et al., the residence time in the large intestine is long relative to that in the small intestine, and transport from tissue to lipid-laden fecal matter may take place [26].

There is no reason to conclude that the two mechanisms of interruption of enterohepatic circulation and of enhancing secretion throughout the small and large intestine are mutually exclusive. It is possible that nonabsorbable fat acts by both mechanisms.

5. The Influence of Body Fat

The hypothetical calculation presented previously assumed approximately normal body weight and body fat composition. A normal body mass index (BMI) of 25 corresponds to a range of 20–25% body fat [27]. Individuals with BMI of 35 may have a body composition with 30–55% fat.

In this instance, a mass of body fat might be 40% of 70 kg, or 28,000 g. Given the same total body burden in two individuals with 14 and 28 kg of adipose tissue, the concentration in adipose in the person with twice as much fat will presumably be half of that of the leaner individual. The concentration of OCs in the blood lipids will also be correspondingly reduced. Although the total amount of blood may also be greater in the person with higher body fat, it is likely that the total amount of OC in the blood circulation will be a smaller fraction of the total body burden when the fat depot is large relative to a smaller depot.

Support for this conclusion is seen in the study of half-lives of OCs reported by Milbrath et al. [28]. In a study summarizing reported OC half-lives, they found that the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) increased with increasing body fat. When body fat was expressed as percent of body mass, the half-life doubled from approximately 4 to 8 years when percent body fat increased from 20 to 35%. When body fat expressed as total mass was considered, the half-life increased from 4 to 8 years as body fat increased from approximately 18 to 28 kg. Increased morbidity and decreased longevity generally observed in obese individuals may, in some cases, be related to sequelae of persistent body burdens.

Presumably the fraction of an OC stored in the body that undergoes enterohepatic circulation is a function of the fraction of the OC that is carried in the blood to the liver and enterocytes. The efficacy of an intervention that interrupts enterohepatic circulation would therefore be predicted to be related to the amount of body fat that stores the OC and limits its appearance in the blood. A high level of body fat would be expected to limit the effects of interference with enterohepatic circulation if only a small fraction of the body burden of OCs appears in the lumen of the intestine.

Vigorous exercise, sauna therapy, and supplementation with glutathione enhancers may also facilitate mobilization of toxicants from adipose storage sites [29]. Cholagogues and choleretics can also be used clinically to stimulate the secretion of toxicant-containing bile into the intestinal lumen to enhance availability for potential removal.

6. Weight Loss and Mobilization of Adipose Tissue

Given the indications that high BMI and body adipose depot can reduce the elimination rate of stored OCs, it is important to consider the effects of weight loss on the normal elimination rate. It is also important to consider the potential for enhancing elimination by reducing enterohepatic circulation of the OC and its lipophilic metabolites. There is much evidence that a reduction in fat stored in adipose tissue results in an increase in OC concentration in adipose tissue, and also an increase in the appearance of OC in the blood. In addition, the mobilization of OCs from adipose tissue results in their distribution into other tissues.

We studied the effects of the interruption of enterohepatic circulation during weight loss [16]. In mice that lost weight during a regimen of caloric restriction, the concentration of hexachlorobenzene (HCB) in the brain more than tripled as adipose tissue mass decreased. In another group of calorically restricted mice that also ate olestra, the increased concentration of HCB in the brain was reduced by 50% relative to the increase in the animals that were calorically restricted without dietary olestra.

Also in that study we observed that olestra caused a dramatic increase in the fecal excretion of HCB. We later confirmed that the increase in excretion during the feeding of olestra was 25–60% greater during weight loss than during *ad lib* feeding [30]. Mutter et al. also had observed that fecal excretion of DDE in gerbils was markedly higher when olestra was fed during dietary restriction than during a period when the regimen was by dietary restriction alone [13].

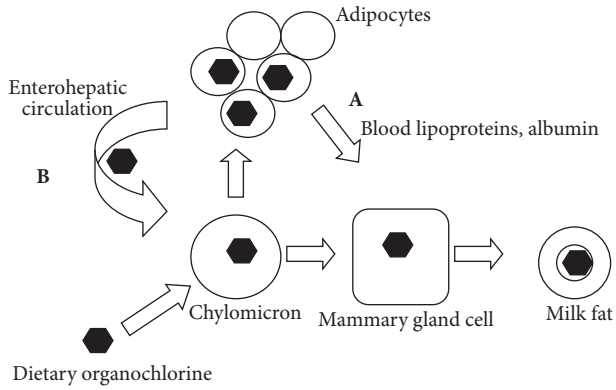

Figure 2: OC compounds (depicted by solid hexagons) are transported to mammary gland cells from adipose tissue carried by lipoproteins and albumin in blood (A). Chylomicrons also carry OCs from both the diet and enterohepatic circulation (B). Aim 1 addresses transport at (A).

Arguin et al. reported that the consumption of olestra during a 90-day weight loss regimen in humans reduced the increase in blood levels of β-hexachlorocyclohexane relative to that seen in during weight loss without olestra [31]. Although the relatively short duration of this trial limits conclusions about the effects of olestra, the observation is consistent with the results seen in excretion rates in mice and gerbils.

Given the continually increasing incidence of obesity seen in the United States and other developed countries, there is a high level of emphasis on the development of pharmaceuticals, dietary regimens, and surgery to reduce accumulated body fat. Diet and pharmaceuticals currently result only in modest fat reductions, but bariatric surgery has been repeatedly used to achieve large reductions in body weight and body fat. It is not clear how the rapid weight loss seen after bariatric surgery affects the distribution of OCs in patients. Some of these surgeries may result in malabsorption of OCs and interrupt enterohepatic circulation. Whether the interruption of enterohepatic circulation by other means may be of benefit in some of these cases is unknown.

7. Milk

There have been numerous reports that a primary excretory route for OCs from women is in breast milk [32]. OCs are readily mobilized from adipose tissue and transported into milk lipids during lactation. Studies in rats have found similar effects. For example, a single lactation cycle depleted a dam of 98% of her body burden of 2,4,5,2′,4′,5′-hexachlorobiphenyl [33].

Presumably the principal driving forces behind the appearance of OCs in milk are the concentration of the OC in adipose tissue, the mobilization of adipose tissue to provide triacylglycerol fat in the milk, and the movement of OCs with the mobilized fat to the mammary gland cells. The interruption of enterohepatic circulation of OCs would be predicted to have an effect on their appearance in milk fat primarily through a reduction in their adipose stores. A schematic view of how dietary and enterohepatic lipid and OCs can enter milk is presented in Figure 2.

It is not clear, however, if a biologically significant amount of OCs in the lumen of the intestine enters milk directly without first passing through storage in the adipocytes. There is evidence in humans that a small fraction of dietary fatty acids from the diet enters milk within a period of time that is consistent with direct entry into milk [34]. It is not known if other lipophilic nutrients follow the pattern of the fatty acids and also appear in milk soon after ingestion. If dietary OCs or OCs secreted into the intestine accompany dietary fat into milk, then it may be possible to reduce their entry into milk lipids by dietary nonabsorbable lipid or lipase inhibitor. At this time further study is needed to determine if this potential effect is clinically meaningful.

8. Emerging Clinical Considerations

Various medical bodies, such as the Pediatric Academic Societies, have recently concluded that "low level exposure to environmental toxicity may be impacting the functioning of the current generation [35]." With the recent emergence of abundant scientific literature correlating exposure to various toxicants with adverse clinical states and mounting awareness of the escalating chemical erosion of human health resulting from widespread bioaccumulation of chemical toxicants [36, 37], it is anticipated that intervention to diminish toxicant burdens in order to preclude and treat disease will eventually become a fundamental component of clinical medicine [38]. Numerous and varied disorders including congenital anomalies [39], neurodevelopmental conditions [40], autoimmune disorders [41], diabetes, [42], endocrine dysfunction [43], mental illness [44], cancer [45], neurodegenerative disease [46] and other assorted afflictions spanning the spectrum of medical specialties have now been directly linked, in some cases, to chemical toxicant exposures.

While some researchers and clinicians have pursued broad-based strategies to eliminate accrued toxicants from the human body in an effort to ameliorate illness [29, 47], no single practical mechanism has yet been identified to

eliminate the expansive range of persistent toxicants [38]. One of the main challenges facing researchers and clinicians, as discussed, has been the difficulty eliminating persistent lipophilic toxicants which often have prolonged half-lives because of their affinity to adipose tissue and their propensity for enterohepatic recycling. The clinical strategy of using nonabsorbable fats or other agents to impair fat absorption in conjunction with caloric restriction shows significant promise as a practical intervention to hasten the excretion of accrued lipophilic toxicants and to thus diminish the risks associated with toxicant persistence. Some concerns and novel ideas have recently emerged, however, with the clinical use of some aspects of this approach.

The use of tetrahydrolipstatin (Orlistat)—a pancreatic lipase inhibitor which adds nonabsorbable lipid to the lumen of the intestine by inhibiting lipid absorption—has recently come under scrutiny as a result of alleged adverse effects associated with the ingestion of this drug. Although the reported incidence of serious sequelae is decidedly low in relation to the amount of product that has been used (for purposes of weight loss), recent claims about serious hepatic injury [48–51] have recently prompted the US Food and Drug Administration to issue warnings about the potential risks associated with consumption of this compound [52, 53]. This caution and the associated media attention have resulted in diminished clinical use of Orlistat for weight loss. As a result, other pancreatic lipase inhibitors are being explored [53, 54], including components of grape seed extract (GSE) [55, 56], chitosan [57], and epigallocatechin-3-gallate (EGCG) found in some teas [58, 59]. Given the limited study of these materials for detoxification purposes, however, their long-term efficacy and safety profile has yet to be determined.

The use of olestra has been associated with gastrointestinal concerns and inhibition of fat-soluble vitamin absorption. In clinical trials, however, gastrointestinal events have been found not to differ from those experienced during consumption of foods with normal fats [60]. All olestra products are supplemented with vitamins A, D, E, and K to compensate for interference with the absorption of these nutrients. As noted below, during a one-year clinical trial testing 15 g/day of olestra in the removal of PCBs, gastrointestinal events were minimal and transient.

Acrylamide, a widespread contaminant formed in baked and fried starchy foods, is a significant component of potato chips—the primary medium currently used for therapeutic delivery of olestra. Acrylamide has evoked much attention of late with its recent classification as a "probable human carcinogen" [61]. In addition, developmental toxicity of acrylamide has been identified in human studies [62, 63], and other research suggests that exposure and potential bioaccumulation of acrylamide may also be associated with neurotoxicity [64] as well as genomic, hormonal, and testicular dysfunction in animals [65]. The use of potato chips as a delivery vehicle for olestra will not add to acrylamide ingestion when the chips are intentionally substituted for other baked and fried carbohydrate-based foods containing acrylamide. Other mediums to deliver olestra, however, may be considered in order to preclude this exposure.

There is also ongoing exploration of other lipid compounds that are not well absorbed and which might interrupt enterohepatic recycling of lipophilic compounds. Castor oil, for example, has been found to be therapeutic in some situations of toxicant overdose [66] while mineral oil has been noted to enhance fecal excretion of lipophilic DDT [67]. In review, the data suggests that clinical strategies to mobilize toxicants (such as caloric restriction) in combination with the provision of nonabsorbable lipid to the intestinal lumen may be useful in diminishing body burdens of OC compounds. Research is underway at various centers to explore safe and practical strategies that might have a clinical role in facilitating human elimination of lipophilic and other toxicants.

9. Studies in Progress

A one-year study of the effect of olestra on the body burden of PCBs in a cohort with elevated PCB levels has been completed. Blood levels of PCBs were measured during the trial, and the data are being evaluated. The trial began with fourteen participants in the control group receiving potato crisps made with vegetable oil, and fourteen in the olestra group receiving potato crisps made with olestra. The olestra dose was 15 g/day. Twelve participants in the control group completed the trial, and eleven, in the olestra group. Adverse events were modest and transient, with only one dropout reporting gastrointestinal problems, and that person received the vegetable oil crisps. This trial is registered in http://ClinicalTrials.gov/, with registration number NCT01261338.

Another study has been designed to determine if castor oil along with lipase inhibitors GSE and EGCG, in combination with other binders including pectin [68], clay [69], and activated charcoal [70] might be of clinical use. Taken after tissue toxicant mobilization and use of modalities to enhance biliary secretion into the small intestine, it is to be determined if this protocol used intermittently might facilitate the elimination of a broad range of adverse compounds including OCs. Chronically ill individuals with varying types of toxicant burdens will use this protocol, with pre- and posttreatment stool collections to be evaluated for comparative levels of a multiplicity of recognized toxicants.

10. Conclusion

The addition of nonabsorbable lipid to the intestinal lumen interferes with the absorption of OCs from the diet by sequestering the OC in the lipid phase and transporting it to the colon and feces. This nonabsorbable lipid phase also interferes with the reabsorption of OCs that enter the intestinal lumen in bile or through enterocyte secretion or sloughing. There is a possibility that the lipid phase enhances this transport from the enterocyte, but more study is needed to confirm this possibility. Weight loss mobilizes OCs from adipose tissue, and enhances their appearance in blood and intestine, and thereby increases the possibility for interaction with nonabsorbable lipid in the intestine. Nonabsorbable

lipid might affect the appearance of OCs in milk, but there currently are no data to support or refute this possibility.

It is possible to add nonabsorbable lipid to the lumen of the intestine, either as a dietary additive such as olestra or through the inhibition of lipase by a lipase inhibitor such as tetrahydrolipstatin. Established risks associated with either regimen are thus far limited to reductions in absorption of fat-soluble vitamins and loose stools which can often be moderated by the adjustment of dose. As of this time, our understanding is that the risk associated with olestra is small-based, in part, on outcomes of a one-year trial in subjects exposed to PCBs. The clinical use of nonabsorbable lipid on a long-term basis should, however, be dictated by the consideration of risk and benefit. Clinical strategies to mobilize toxicants (such as caloric restriction) in combination with the provision of nonabsorbable lipid to the intestinal lumen may be useful in diminishing body burdens of OC and other lipophilic compounds.

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Clinical Study

Human Elimination of Phthalate Compounds: Blood, Urine, and Sweat (BUS) Study

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Background. Individual members of the phthalate family of chemical compounds are components of innumerable everyday consumer products, resulting in a high exposure scenario for some individuals and population groups. Multiple epidemiological studies have demonstrated statistically significant exposure-disease relationships involving phthalates and toxicological studies have shown estrogenic effects in vitro. Data is lacking in the medical literature, however, on effective means to facilitate phthalate excretion. *Methods*. Blood, urine, and sweat were collected from 20 individuals (10 healthy participants and 10 participants with assorted health problems) and analyzed for parent phthalate compounds as well as phthalate metabolites using high performance liquid chromatography-tandem mass spectrometry. *Results*. Some parent phthalates as well as their metabolites were excreted into sweat. All patients had MEHP (mono(2-ethylhexyl) phthalate) in their blood, sweat, and urine samples, suggesting widespread phthalate exposure. In several individuals, DEHP (di (2-ethylhexl) phthalate) was found in sweat but not in serum, suggesting the possibility of phthalate retention and bioaccumulation. On average, MEHP concentration in sweat was more than twice as high as urine levels. *Conclusions*. Induced perspiration may be useful to facilitate elimination of some potentially toxic phthalate compounds including DEHP and MEHP. Sweat analysis may be helpful in establishing the existence of accrued DEHP in the human body.

1. Introduction

As a family of man-made chemical compounds, phthalates are a standard component of modern day plastics and are specifically used to create plastic products that are soft and malleable. First developed in the 1920s, some phthalates have also been found to maintain color and scent in various mediums and are thus used in a wide variety of consumer goods including fragrances, paints, and nail polish. As a result, production of phthalate compounds has exploded over the last half century and they have increasingly been incorporated into assorted household and medical materials [1]. Often referred to as plasticizers, phthalates can be found in medical devices such as intravenous tubing and blood collection bags. Moreover, they are extensively used in plastic wrapping for food and beverage packaging, and are a ubiquitous component of soft plastic toys as well as various other products including vinyl floor tiles, shower curtains, synthetic leather, cosmetics, shopping bags, and pharmaceuticals [2–5].

With the widespread use of phthalates in numerous everyday products, these compounds have become one of the most common synthetic chemical exposures, resulting in concern about the potential impact of phthalates on human health. Phthalates have recently been detected throughout large population samples in both North America and Europe, and more recently have been found in fetal samples [6–10]. Recent evidence demonstrates a link between phthalate exposure and adverse health effects in both animal and human models, raising the question of whether usage of these compounds requires regulation—a concern that recently prompted the signing of a European ban in 2005 on

certain phthalates in all childcare articles and toys [11]. An overview of the literature regarding the potential effects of phthalates on human health is presented, followed by data from 20 subjects whose blood, urine, and sweat were tested for phthalate compounds.

2. Background

Phthalates are synthesized as an ester of benzenedicarboxylic acid (also known as phthalic acid) and are valued for their ability to promote both flexibility and stability in plastics [2]. Diisononyl phthalate (DINP), diisodecyl phthalate (DIDP), and di-2-ethyl-hexyl phthalate (DEHP) are the most common types of compounds used within the phthalate family, with DEHP representing the highest proportion of produced phthalates as a component in the mass produced plastic, polyvinyl chloride (PVC) [12, 13]. Because phthalates are not covalently bound as plasticizers, they are able to migrate from phthalate-containing items into air, dust, water, soil, and sediment, leading to widespread human exposure through ingestion, inhalation, and dermal contact [5, 14].

Once they enter the body, phthalates undergo a series of phase I hydrolysis and phase II conjugation reactions and are subsequently excreted in feces and urine [15]. Existing literature suggests that phthalate clearance from the body is rapid and primarily via urinary excretion with only a slight cumulative potential. Thus, the major mechanism of detection is through screening urine for monoesters [16–18]. This was originally thought to be an accurate measure of all phthalate exposure; however, recent work regarding phthalate metabolism suggests it is likely to underestimate exposure to phthalates with long alkyl chains, such as DEHP and DINP, which undergo further metabolism prior to excretion [8]. Both primary and secondary phthalate metabolites are biologically active [19–22]. Conclusive evidence on levels of phthalate bioaccumulation within specific organs and tissues of the body has not been available thus far.

2.1. Human Exposure. Throughout the latter parts of the 20th century and the current 21st century, multiple urine samples analyzed from populations worldwide have consistently demonstrated phthalate exposure in up to 98% of participants, including pregnant women [6, 8–10, 23–25]. As phthalates are thought to have a relatively short half-life of less than 5 hours, this widespread detection is likely to indicate chronic exposure [15], rather than accrual within the body.

Sources and pathways of exposure may vary widely. In neonates, infants, and toddlers, exposure may come through vertical transmission or external sources. The most likely neonatal exposure pathway is vertical transmission through the placenta or breast-feeding. In utero, phthalates circulate through the placenta and into fetal blood, where they are found to have an extended half-life as compared to maternal serum (up to 6.2 and 64 hours in fetal serum and amniotic fluid, resp.) [15, 26]. Breast milk is also found to contain detectable levels of phthalates, particularly the most hydrophobic compounds, which include DEHP and DINP [27–30]. Infant formula, baby food, and children's toys are additional sources of exposure, a realization that has prompted Europe to enact legislation limiting use of these compounds in order to prevent adverse effects in development [8, 11, 31–34].

Other common sources of exposure in the general population include ingestion of contaminated food and dust. Phthalates are able to easily leech from plastics into proximal food and fluids and are found at highest concentration in foods with high fat concentrations, such as dairy, poultry, and oils [8, 14, 35]. Absorption of phthalates can also occur via dermal contact [5]. This is of concern with products such as deodorant, perfumes, aftershave, hair styling products, shampoo, skin and nail care products, as well as cosmetic products—which have been found to contain varying amounts of phthalates, ranging from 1–15,000 mg/kg [8]. Additionally, neonates or children who spent time in an intensive care unit and patients who are critically ill are exposed to high levels of phthalates through medical equipment including intravenous bags and tubing [36–38].

2.2. Potential Human Health Implications. A population analysis in Germany concluded that the average level of human exposure to DEHP was approximately 0.0024 mg/kg B.W/day, much below the current "No Observed Adverse Effect Level" (NOAEL) adopted by the European Food Safety Authority for DEHP at 5 mg/kg/day [39]. However, this is not adequate grounds for dismissing further study and regulation. DEHP levels, amongst other phthalates, are likely to be underestimated through monoester urine screening and the effects of various phthalates are thought to be cumulative [8, 40, 41]. Moreover, studies in human populations are increasingly associating phthalate exposure with adverse effects, highlighting the importance of a more complete and widespread understanding of the behavior, potential for bioaccumulation, and the adverse effects of phthalates in human populations.

The most widely studied adverse effect of phthalate exposure thus far suggests a potential disturbance in the development and function of reproductive organs through endocrine disruption [42–55]. Exposure of animals to high levels of phthalates results in a well-described change: the testis decrease in weight with atrophy of seminiferous tubules, progressive degeneration of germ cells, Sertoli cell dysfunction, and hormonal disruption in Leydig cells [19–21, 56–61]. Prepubertal and pubertal males appear to be more vulnerable at lower doses and a shorter duration of exposure leading to the changes described [62].

In developing male rats, phthalate metabolites were found to inhibit fetal testicular testosterone biosynthesis through changes in gene expression of enzymes and proteins necessary for fetal Leydig cell function [42–45]. This is especially prevalent with exposure to DEHP, dibutyl phthalate (DBP), and butyl benzyl phthalate (BBP) and results in anatomical anomalies consistent with the disruption of androgen-dependent development [63, 64]. Observed changes include cryptorchidism, hypospadias, reduced sperm production, permanent retention of nipples, atrophy or agenesis of sex accessory organs, and decreased anogenital distance [64, 65]. The severity and frequency of these

manifestations appear to be dose-dependent, with the most distorted malformations occurring at 750 mg DEHP/kg/day, and subtler manifestations at as low as 6 mg/kg/day in animal models [64, 66]. A recent study in male infants was the first of its kind in expressly demonstrating such an association in humans. Swan et al. found a significant correlation between increased levels of phthalates in maternal urine and a decreased (feminized) anogenital distance in their male offspring, suggesting that prenatal exposure to phthalates may be of real consequence for people [46].

Adult female rats have traditionally appeared less sensitive to phthalate exposure; however, there is evidence that at high levels (2000 mg/day), they develop reduced serum estradiol levels, prolonged estrous cycles, and at times may cease to ovulate [51]. Phthalate exposure has also been associated with a delay in the onset of puberty, a decrease in fertility, and an increased incidence of mid-gestation spontaneous abortion [50, 65, 67]. Metabolites are believed to target the ovary, where suppression of aromatase enzyme activity limits the synthesis of estradiol. Additionally, there is evidence to suggest phthalate exposure may have a teratogenic effect, resulting in both visceral and skeletal anomalies in animal models [4, 52, 68].

Given the emerging literature in this field, a series of observational studies have been undertaken in human populations. Recognizing that trials are not possible with humans—as exposing individuals or populations to potentially toxic compounds is unethical—more prolonged and academically challenging observational studies of cohorts found to be exposed is the primary method used to draw conclusions about associations between human exposures and health outcomes. Though unable to delineate causal relationships, preliminary research has associated phthalate exposure with reduced semen quality, endometriosis, shorter pregnancy duration, and reduced anogenital distance in males [26, 53, 54, 69–71]. More recent evidence, however, has demonstrated a definitive link between "DEHP concentration in ambient air and the adverse effects in sperm motility and chromatin DNA integrity [72]." Given the widespread use of compounds containing phthalates, the implications for reproductive toxicity are concerning.

Beyond reproductive outcomes, there has been much interest in the link between phthalate exposure and allergy and asthma symptoms in children, as well as the proposed association with an increased waist circumference and BMI [73–79]. Despite these emerging concerns, manufacturers are not obligated to include phthalates on the list of ingredients for children's products sold in Canada. Finally, it is not known whether the toxic effect of phthalates is dose dependent and whether there is a consistent threshold level where toxicity is manifest.

In this study, approved by the Health Research Ethics Board at the University of Alberta, we endeavored to increase the understanding of the behavior of phthalate compounds by assessing human excretion of various common members of the phthalate family into each of three body fluids: blood, urine, and sweat. Both parent compounds and their metabolites were studied.

3. Methods

3.1. Participant Recruitment. 9 males and 11 females with mean ages 44.5 ± 14.4 years and 45.6 ± 10.3 years, respectively, were recruited to participate in the study after appropriate ethical approval was received from the Health Research Ethics Board of the University of Alberta. 10 participants were patients with various clinical conditions and 10 were otherwise healthy adults. Participants with health issues were recruited from the first author's clinical practice by invitation and both healthy and sick individuals were selected as samples of convenience by availability, wish to participate, and ease of contact. Each participant in the study provided informed consent and volunteered to give one 200 mL random sample of blood, one sample of first morning urine and one 100 mL sample of sweat. Demographic and clinical characteristics of all research participants are provided in Table 1.

3.2. Samples Collection. All blood samples were collected at one DynaLIFE laboratory site in Edmonton, AB, Canada with vacutainer blood collection equipment (BD Vacutainer, Franklin Lakes, NJ 07417, USA) using 21-gauge stainless steel needles which were screwed into the "BD Vacutainer One-Use Holder" (REF 364815). The 10 mL glass vacutainer was directly inserted into the holder and into the back end of the needle. This process and the use of glass blood collection tubes were used to prevent contamination. Blood was collected directly into plain 10 mL glass vacutainer tubes, allowed to clot, and after 30 minutes was centrifuged for 10 minutes at 2,000 revolutions per minute (RPM). After serum was separated off, samples were picked up by ALS Laboratories (about 3 kilometres from the blood collection site) for storage pending analysis. When received at ALS, serum samples were transferred to 4 mL glass vials and stored in a freezer at -20°C, pending transfer to the analytical laboratory. We chose to analyze phthalates in serum rather than in whole blood, based on the fact that the matrix effect of serum is much lower than whole blood.

For urine collection, participants were instructed to collect a first morning midstream urine sample directly into a provided 500 mL glass jar container with Teflon-lined lid on the same day that blood samples were collected. Urine samples were delivered by the participants directly to ALS Laboratories, Edmonton. Samples were transferred to 4 mL glass vials and stored in a freezer at -20°C, pending transfer.

For sweat collection, participants were instructed to collect perspiration from any site on their body directly into the provided 500 mL glass jar container with Teflon-lined lid—by placing the jar against their prewashed skin (with toxicant-free soap, water, and nonplastic brush) when actively sweating or by using a stainless steel spatula against their skin to transfer perspiration directly into the glass jar (stainless steel—made up primarily of iron, chromium, and nickel—was chosen as it is the same material as the needles used in standard blood collections and is reported not to off-gas or leach at room or body temperature). In excess of 100 mL of sweat was provided in all but one case. Each of the glass bottles used for sampling in this study was provided by

Table 1: Participant demographics and general clinical characteristics.

Participant	Gender	Age	Clinical diagnosis	Technique used for sweat collection
1	M	61	Diabetes, obesity, hypertension	Exercise
2	F	40	Rheumatoid arthritis	Steam Sauna
3	M	38	Addiction disorder	Steam Sauna
4	F	25	Bipolar disorder	Steam Sauna
5	F	47	Lymphoma	Steam Sauna
6	F	43	Fibromyalgia	Steam Sauna
7	F	48	Depression	Steam Sauna
8	F	40	Chronic fatigue	Infrared Sauna
9	F	68	Diabetes, fatigue, obesity	Steam Sauna
10	M	49	Chronic pain, cognitive decline	Exercise
11	M	53	Healthy	Exercise
12	M	23	Healthy	Infrared Sauna
13	M	21	Healthy	Infrared Sauna
14	F	47	Healthy	Infrared Sauna
15	M	53	Healthy	Infrared Sauna
16	F	43	Healthy	Infrared Sauna
17	F	51	Healthy	Infrared Sauna
18	M	46	Healthy	Infrared Sauna
19	M	57	Healthy	Infrared Sauna
20	F	50	Healthy	Infrared Sauna

ALS laboratories and had undergone extensive cleaning and rinsing. The containers were deemed appropriate for sweat collection with negligible risk of contamination: laboratory-grade phosphate-free detergent wash; acid rinse; multiple hot and cold deionized water rinses; oven dried; capped and packed in quality-controlled conditions. Sweat was collected within 1 week before or after collecting the blood and urine samples. No specifications were given as to how long sweating had commenced before collection. 10 participants collected sweat inside a dry infrared sauna, 7 collected inside a steam sauna, and 3 collected during and immediately after exercise—no specific instruction was given regarding the type or location of exercise. Participants were educated about the research and phthalate sources and were asked to meticulously avoid exposure to any potential sources of phthalates (and other toxicants) around the time of collection. Sweat was delivered by the participants directly to ALS laboratories. Samples were transferred to 4 mL glass vials and stored in a freezer at −20°C, pending analysis. No preservatives were used in the jars provided for sweat and urine collection, nor in the serum storage vials.

3.3. Laboratory Method Description. The list of compounds tested for in this phthalate study—both parent and metabolites—are listed in Table 2. As parent compounds are metabolized prior to urine excretion, they were tested for in both sweat and blood but not in urine; metabolites were sought in all three fluid compartments—blood, urine, as well as sweat. High performance liquid chromatography/mass spectrometry (HPCL/MS) was used to determine phthalate metabolite concentrations while gas chromatography/mass spectrometry (GC/MS) was used to assess parent phthalates.

Table 2: Phthalate compounds tested.

Parent compounds	Corresponding metabolites
DMP (dimethyl phthalates)	MMP (monomethyl phtalate)
DEP (diethyl phthalates)	MEP (monoethyl phthalate)
DBP (dibutyl phthalates)	MBzP (mono-benzyl phthalate) MiBP (mono-iso-butyl phthalate)
BBP (benzyl butyl phthalates)	MBzP (mono-benzyl phthalate)
DCHP (dicyclohexyl phthalates)	MCHP (mono-cyclohexyl phthalate)
DEHP (di (2-ethylhexl)phthalates)	MEHP (mono(2-ethylhexyl) phthalate) MEHHP (mono-(2-ethyl-5-hydroxyhexyl)phthalate) MEOHP (mono-(2-ethyl-5-oxohexyl) phthalate)
DiNP (di-isononyl phthalates)	MINP (monoisononyl phthalate)
DOP (di-octyl Phthalate)	MOP (mono-n-octyl phthalate)

The methodology for determining parent phthalates in serum and sweat was as follows. Samples (serum and sweat) were weighed into glass tubes (ca 1 g) and 1 mL of acetonitrile was added in order to precipitate serum and plasma proteins. The resulting mixture was serially extracted twice with 5 mL portions of hexane : dichloromethane (8 : 1, v/v) using sonication as per Colon et al., [55]. The resulting extracts were

combined and concentrated to 200 microliters. Analysis was performed using gas chromatography/selected ion-monitoring mass spectrometry. Ions monitored include: dimethylphthalate (DMP), *m*/*z* 194/163; diethylphthalate (DEP), *m*/*z* 177/222; dibutylphthalate (DBP), *m*/*z* 223/278/205; benzylbutylphthalate (BBP), *m*/*z* 206/238; dicyclohexylphthalate (DCHP), *m*/*z* 249/330; diethylhexylphthalate (DEHP), *m*/*z* 279/390; disonylphthalate (DiNP), *m*/*z* 293/418. Prior to analysis all extracts were diluted 1 : 4 with hexane. Quantitation was performed using external standard calibration. Quality control was measured by analyzing method blanks, analyzing water fortified with the analytes of interest, as well as calf serum samples. The recovery of the phthalates from fortified water was 87–108% with a relative standard deviation of 1.9 to 9.0%. The relative percent difference for calf serum was 0.7 to 12% with the exception of DMP which was 23%. Instrument detection limits were determined to be 8 ng/g.

Serum, sweat, and urine were analyzed for phthalate metabolites following the general procedures established by the US Centers for Disease Control and Prevention [80, 81]. Briefly, 1.0 g of serum, sweat, or urine was fortified with 10 nanograms of isotopically-labelled phthalate metabolites, 20 micrograms of 4-methylumbelliferone glucuronide, 20-micrograms of labeled 4-methylumbelliferone, 500 microliters of ammonium acetate buffer (pH 6.5), and 10 microliters of β-glucuronidase (*Escherichia coli* K12, Roche Biomedical). The samples were mixed and incubated at 37°C for 90 minutes to allow for the deglucuronidation of the phthalate metabolites.

Following enzymatic hydrolysis, an aliquot (20 uL) was removed and analyzed for 4-methylumbilliferone to determine enzymatic hydrolysis efficiency. The remainder was removed and loaded onto a Zymark Rapid Trace Station for automated solid phase extraction (SPE). The 60 milligram/3 mL Oasis-HLB cartridges was conditioned with HPLC-grade methanol (2 mL) and 0.1 M formic acid (1 mL). The samples were diluted with 5 mL of 0.1 M formic acid and loaded onto the SPE cartridge at a rate of 0.5 mL/min. The cartridge was washed with water (1 mL) and 10% methanol in water (2 mL) at a flow rate of 1 mL/min. The phthalate metabolites and bisphenol A were eluted with 1.0 mL of acetonitrile at a flow rate of 0.5 mL/min. The eluate was evaporated to dryness under a stream of dry nitrogen and the residue reconstituted in 85% methanol in water (200 microliters) and transferred to glass autosampler vials prior to analysis. Prior to analysis, labeled sodium perfluoro-1-octanesulfinate (5 nanograms) was added as an internal standard.

Quality control for phthalate metabolites was maintained by analyzing a method blank (calf serum) and two spiked calf serum samples along with every 17 samples. The calf serum samples were spiked with phthalate metabolites at 20 ng/mL. The detection limit (0.2 ng/mL) for phthalate metabolites was based upon our lower calibration standard (0.5 ng/mL) which gave an instrument signal to noise response of 3 : 1. Analyses were performed using isotope dilution liquid chromatography/mass spectrometry. An API 4000 liquid chromatograph/tandem mass spectrometer was employed for the analysis of phthalate metabolites.

Table 3: Percentage of individuals with detection of parent phthalates in body compartments.

Parent compound	Serum ($n = 19$)	Sweat ($n = 18$)
DMP (dimethyl phthalate)	0	0
DEP (diethyl phthalate)	0	0
DBP (dibutyl phthalate)	84	22*
BBP (benzyl butyl phthalate)	0	0
DCHP (dicyclohexyl phthalate)	0	0
DEHP (di 2-ethylhexyl phthalate)	10	61**
DiNP (di-isononyl phthalate)	0	0
DOP (di-octyl Phthalate)	0	0

* In 3/4 of these participants where DBP was detected in sweat, this parent phthalate was not detectable in their serum samples.
** In all 11 individuals who are positive for DEHP in sweat, none of these had DEHP detected in their serum samples.

Table 4: Distribution of parent phthalate concentrations in serum (SE) and sweat (SW) (μg/g).

	SE-DBP	SW-DBP	SE-DEHP	SW-DEHP
n	19	18	18	18
Mean	35.1	*	*	49.9
Std. Dev.	28.3	*	*	133
Median	37.6	*	*	15.5
Range	<8–79.0	<8–58.6	<8–35.0	<8–576

* For the 18 individuals who had their sweat tested for DBP, only 4 were above detection limit (8 μg/L). Thus mean, SD and median are not reported for SW-DBP. Similarly for the 18 serum samples tested for DEHP only 2 were above the detection limit and mean, SD, and median are not reported.

4. Results & Discussion

Of the 7 parent compounds tested in 19 sera and 18 sweat samples, only DBP and DEHP were detected at all. DBP was detected in 16/19 sera and 4/18 sweat samples. In 3/4 of the participants where DBP was detected in sweat, this parent phthalate was undetectable in their sera. DEHP was detected in 2 sera and 11 sweat samples, yet out of the 11 individuals who were positive for DEHP in sweat, none had DEHP detected in their serum samples. The percentage detection of the parent compounds in human serum and sweat and their frequency distributions are given in Tables 3 and 4, respectively. No attempt was made to quantitate the parent compounds in the urine samples. The distinctive findings whereby the parent phthalates are detected in sweat but not in sera may be due to the fact that these compounds have sequestered in peripheral tissues and are mobilized during perspiration, but this explanation remains speculative.

The phthalate metabolites MEP, MiBP, and MEHP were detected in all samples of serum ($n = 19$), urine ($n = 20$), and sweat ($n = 18$), (the *n*-values differ for the differing body fluids as there were insufficient amounts of serum/sweat for testing in three samples). The percentage detection of

Table 5: Percentage of individuals with detection of phthalate metabolites in body compartments.

Metabolites	Serum	Urine	Sweat
MMP (monomethyl phtalate)	0	40	0
MEP (monoethyl phthalate)	100	100	100
MiBP (mono-iso-butyl phthalate)	100	100	100
MBzP (mono-benzyl phthalate)	0	100	0
MCHP (mono-cyclohexyl phthalate)	0	35	0
MEHP (mono(2-ethylhexyl) phthalate)	100	100	100
MEHHP (mono-(2-ethyl-5-hydroxyhexyl)phthalate)	0	100	0
MEOHP (mono-(2-ethyl-5-oxohexyl) phthalate)	0	100	0
MOP (mono-n-octyl phthalate)	0	0	0
MINP (monoisononyl phthalate)	0	0	0

the phthalate metabolites in the three body fluids and the frequency distributions of MEP, MiBP, and MEHP in the 3 body fluids are given in Tables 5 and 6, respectively. No phthalate metabolites other than MEP, MiBP, and MEHP were detected in the serum and sweat samples. For the 17 participants who had matched serum, urine, and sweat data for MEP, MiBP, and MEHP, we calculated the ratio of their concentrations in sweat to urine (S/U ratio) and found the following median values: MEP: 0.3, MiBP: 1.4, and MEHP: 4.6. This is suggestive of MEHP being more efficiently excreted in sweat, followed by MiBP, and urine being the best pathway of elimination of MEP. The sweat contribution to phthalate excretion may indicate release of bioaccumulated phthalates from storage sites or may originate from circulating phthalates.

Interestingly, 5 other phthalate metabolites were detected in the urine samples, with MBzP, MEHHP, and MEOHP found in all 20 samples, MMP in 8 samples, and MCHP in 7 samples. Various phthalate metabolites including MiBP, MCHP, and others were found exclusively in urine with none evident in sera or sweat. It is hard to conclude much from this other than these compounds are commonly found in people, and that they are excreted. General population figures for selected phthalate metabolites from the National Health and Nutrition Examination Survey (NHANES) data are provided in Table 7. Mean levels for urinary MEHP and MEP are considerably higher in our study than is found in the NHANES data. This would suggest that the exposure was higher in our sample, or possibly that some participants in our sample—perhaps those with illness—differed in their ability to metabolize or excrete phthalates. The fact that a high level of consistency of phthalate results exists between individuals suggests that contamination of skin at the time of collection is not likely to have been the source of detected phthalates.

As all participants had evidence of potentially toxic metabolite MEHP, the parent compound DEHP appears to be a ubiquitous contaminant. DEHP and its most notable metabolite MEHP have been associated with liver toxicity, testicular atrophy, hormone disruption, and cardiotoxicity in animals; these concerns have led to the banning of DEHP in toys in some parts of the world [24]. It is thus important that both DEHP and MEHP appear to be eliminated in sweat according to our results. While MEHP is thought to be responsible for much of DEHP's toxicity, however, many of the known secondary metabolites have not yet been studied for their toxicity [24].

It has previously been thought that after DEHP enters the body, it is readily metabolized into various metabolites that are readily excreted, including MEHP. Accordingly, it has been surmised that without bioaccumulation, DEHP toxicity is generally associated with repeated or chronic exposure. It is noteworthy in this study, however, that (i) DEHP was found in the sweat samples of a number of participants with no evidence of this compound in their serum, and that (ii) MEHP concentrations in sweat far exceed the concentration in urine. This may suggest that there is some accrual of DEHP in the tissues which is mobilized and eliminated in perspiration. It is unknown if the MEHP sweat concentration represents discharge of this circulating phthalate metabolite or the release of bioaccumulated MEHP from storage sites, such as adipose tissue.

5. Conclusion

This is the first study, to our knowledge, that examines the release of phthalates into sweat. Some parent phthalate compounds and metabolites appear to be readily excreted in sweat; others do not. As all participants had evidence of the potentially toxic metabolite MEHP, the parent compound DEHP appears to be a ubiquitous contaminant in some population groups. Considering that in a number of individuals, some phthalate compounds appeared in sweat but not in serum suggests that bioaccumulation of selected phthalate compounds such as DEHP and DBP may be occurring with uncertain human toxicity. Furthermore, the toxic metabolite MEHP appears to be well eliminated in sweat. For these reasons, there may be advantage to inducing perspiration through methods such as sauna use as a means (i) to eliminate some potentially toxic phthalates and (ii) to collect samples to possibly diagnose the presence of bioaccumulated phthalate compounds such as DEHP.

With the recognition that various persistent pollutants may be determinants of chronic illness, increasing attention is being directed toward research and study of potential techniques and interventions designed to facilitate removal of persistent toxicants from the human body [82–86]. Emerging evidence in the scientific literature suggests that various persistent pollutants may be excreted through induced thermal depuration techniques such as sauna therapy, use of steam rooms, or exercise within heated quarters [87–91]. As caloric restriction appears to mobilize toxicants from storage sites [84, 92] and the skin may act as an alternative storage compartment in the face of decreasing fat stores [92], measures to facilitate loss of adipose tissue may act synergistically to enhance toxicant mobilization through the skin. Recognizing the potentially toxic effect of DEHP

Table 6: Distribution of phthalate metabolite concentrations in serum (SE), sweat (SW), and urine (UR) (μg/g).

	SE-MEP	SW-MEP	UR-MEP	SE-MiBP	SW-MiBP	UR-MiBP	SE-MEHP	SW-MEHP	UR-MEHP
n	19	18	20	19	18	20	19	18	20
Mean	5.69	91.1	535	26.1	111	122	28.2	27.3	12.4
SD	8.61	172	1560	23.9	75.3	96.6	9.65	21.4	23.7
Median	3.88	29.9	107	17.8	100	74.4	27.6	12.4	35.1
Range	0.84 –39.2	3.94 –750	6.76–6978	4.0–77	46.0–378	20.7–342	17–52.6	2.68–68.6	1.11–108

Table 7: Urinary phthalate metabolite levels in the general population (μg/g) (national health and nutrition examination survey (NHANES) data) [3, 9].

	Above detection limit (%)	Geometric mean	95th percentile
MBzP	3	14.0	77.4
MEHP	22	3.1	18.5
MEOHP	N/A	13.6	118
MEHHP	N/A	20.4	182
MEP	0	63	1950

and MEHP, regular depuration through sweating may offer health benefits by precluding sequelae associated with bioaccumulated phthalates and toxic metabolites.

6. Key Findings

(i) DEHP and/or its metabolite MEHP were found in all participants, suggesting that exposure to potentially toxic phthalate compounds is very common.

(ii) Some parent phthalate compounds and some metabolites appeared to be readily excreted in sweat; others did not.

(iii) In several individuals, DEHP was found in sweat but not in serum, suggesting the possibility of some degree of phthalate retention and bioaccumulation.

(iv) Some toxic phthalate metabolites such as MEHP were eliminated comparatively well in sweat.

(v) Several phthalate metabolites were evident in urine with no evidence of the parent compound in either serum or sweat.

Conflict of Interests

There is no conflict of interests. No funding has been received for any part of this work.

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