HHS Public Access

Author manuscript

Biol Trace Elem Res. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Biol Trace Elem Res. 2015 July ; 166(1): 13–23. doi:10.1007/s12011-015-0267-x.

Lead, arsenic and manganese metal mixture exposures: focus on biomarkers of effect

VL Andrade[a], **ML Mateus**[a], **MC Batoréu**[a], **M Aschner**[b], and **AP Marreilha dos Santos**[a,*]

[a]Instituto de Investigação do Medicamento, iMed.UL, Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal

[b]Department of Molecular Pharmacology, Albert Einstein College of Medicine, 10461 NY, USA

Summary

The increasing exposure of human populations to excessive levels of metals continues to represent a matter of public health concern. Several biomarkers have been studied and proposed for the detection of adverse health effects induced by lead (Pb), arsenic (As) and manganese (Mn); however, these studies have relied on exposures to each single metal, which fails to replicate real-life exposure scenarios. These 3 metals are commonly detected in different environmental, occupational and food contexts and they share common neurotoxic effects, which are progressive and once clinically apparent may be irreversible. Thus, chronic exposure to low levels of a mixture of these metals represents an additive risk of toxicity. Building upon their shared mechanisms of toxicity, such as oxidative stress, interference with neurotransmitters and effects on hematopoietic system, we address putative biomarkers, which may be assist in assessing onset of neurological diseases associated with exposure to this metal mixture.

Keywords

Metal mixtures; lead; arsenic; manganese; biomarkers

1. Metals in the environment

Pollution is a worldwide problem with immeasurable health consequences, resulting in overexposure to toxic metals. Globally, environmental contamination of heavy metals continues to grow and exposure in the general population, by air, food or drinking water has become an increasing global phenomenon [1–3].

1.1. Environmental exposure to metal mixtures

Metals are often introduced into the environment as mixtures [4]. Accordingly, the U.S. Environmental Protection Agency (EPA) recommends greater emphasis on understanding the combined toxic effects of metals [4, 5]. To date, emphasis has been focused largely on single metal exposures [6, 7]. Given that mixtures are commonly viewed as a simple collection of chemicals, understanding the magnitude of toxicological interactions has yet to

*Corresponding author – apsantos@ff.ul.pt, Tel – 351217946400, Fax - 351217946470.

be fully appreciated [5]. Notably, the practical consequence of studying single metal exposures likely underestimates their health risks in affected populations [6, 8].

Exposures to metal mixtures may synergize or be additive thus leading to adverse health effects exceeding those noted upon exposures to a single metal [9, 10]. Furthermore, while low doses of a single metal may not cause health effects, when combined with other metals, they may present increased health risk [9]. This matter was recently recognized by the U.S. EPA as a key gap in metal risk assessment [11].

1.2. Criteria for the selection of metal mixtures

Given the almost infinite number of chemical mixtures, regulators are faced with the problem of how to assess and regulate their toxicity [6, 12]. Recently it has been argued that grouping criteria should focus on common adverse outcomes, with less emphasis on similarity of mechanisms [6], and that attention should be focused on those having the greatest potential impact on human health [12]. In addition, for practical purposes, criteria are needed to define the relevant components of a mixture. Such criteria cannot rely simply on the concentrations of the compounds in the mixture, but must also take note of their expected contribution to relevant endpoints of toxicity [6]. In this context exposure to neurotoxic agents represents a concern of high priority, given the increased prevalence of a number of neurodegenerative diseases [13, 14].

1.3. Sources of lead (Pb), arsenic (As) and manganese (Mn)

A recent assessment on the global health impacts of contaminants, identified As and Pb among the six most toxic pollutants threatening human health [15]. Mn is also of great environmental and public health significance due to its broad usage in the ferroalloy industry [16].

Pb is naturally present in the earth’s crust [17]. Its anthropogenic releases to ambient air from gas, coal, oil and waste have persisted, representing a continued health risk. It is estimated that body burdens of Pb in humans in the modern era are 1000-fold higher than during the preindustrial era [18]. Fortunately, over the last few decades, Pb emissions in developed countries have markedly decreased, largely due to the abolishment of leaded gasoline, which has been paralleled by a decrease in blood Pb levels in the general population, especially children [19]. However, since Pb is a persistent element [20] and given its long half-life, past emissions remain a matter of concern. Airborne Pb can be deposited in soil and water, reaching humans via the food chain. Drinking water is also a potential source of Pb exposure [20]. Occupational cohorts may be exposed to Pb in the process of manufacturing of batteries, sheet lead, bronze plumbing, ceramic glazes, caulking, radiation shields, circuit boards and military equipment [20], as well as the glass industry [19].

The metalloid As is particularly difficult to characterize as a single element because it has a complex chemistry and there are numerous As compounds, with different oxidation states, and differential toxicities. This element naturally occurs in rocks, soil, water and air [19, 21] where it is largely present in the trivalent or pentavalent forms; the trivalent form is the most toxic. Organic As compounds may also be trivalent or pentavalent and they occur in

methylated forms; when compared with inorganic As, the methylated metabolites are less reactive with tissue constituents, less toxic upon acute exposures, likely reflecting their fast excretion in urine [22, 23]. Arsenical compounds are known since primordial times as poisons [21, 24, 25]. Exposure to As-contaminated water is a major concern in several Asian countries, where groundwater may be highly contaminated with As [19, 26, 27]. Smelting of non-ferrous metals and the production of energy from fossil fuel represent two major industrial processes that lead to As contamination of air, water and soil. Occupational exposures to As occur as well in several industries, particularly in nonferrous smelting, electronics, wood preservatives, glass manufacturing and application of arsenical pesticides [27].

Mn is the 12th most abundant element in the earth's crust [2] and is an essential element. Few cases of Mn deficiency have been reported in humans, its deficiency can lead to serious health disorders [28]. Excess Mn may cause a wide range of deleterious effects [17]. Diet, contaminated drinking water and inhalation represent the main sources of exposure [29]. In recent years, Mn has received increased attention, since organic Mn has been used as an antiknock gasoline additive - methylcyclopentadienyl manganese tricarbonyl (MMT) in several countries [30, 31]. The most prominent occupations where Mn exposures are common include mining, ore-crushing, ferromanganese production, smelting and welding [32]. Mn is also found at high concentrations in various other industrial settings, such as the manufacturing of dry-cell batteries, production of Mn-containing organic pesticides, fireworks, ceramics, glass, leather, textiles, paint and cosmetics [2, 33].

Despite the existence of a reasonable amount of information pertaining to the health effects induced by Pb, As or Mn exposure [17, 20, 26], the majotiry of the studies focus on solitary exposures to these metals. However, Pb, As and Mn are frequently found as mixtures [34–38], and whenever mixtures are treated as a single complex substance, uncertainties may range from inexact descriptions of exposure to inadequate toxicity outcome information [5]. Therefore, the toxic effects induced Pb, As and Mn alone and also as binary mixtures, will be described.

2. Toxicity of metals

Pb is a potent health hazard [39] that may cause neurotoxicity, hypertension, anemia, renal impairment and interfere with sperm production [16, 20, 40]. Pb compounds may also be carcinogens [16]. The metalloid As can induce cancer, genotoxicity and affect the hematopoietic system, liver, kidneys, skin and brain [41]. Exposure to As has also been reported to increase the risk of cancer in skin, liver, bladder and lungs. Long-term Mn exposure is commonly associated with central and peripheral nervous system disorders. Reproductive outcomes might be affected, with decreased libido, impotence and sexual dysfunction. There is no evidence that Mn causes cancer in humans or animals [17].

Lead

Exposure to high Pb levels may result in acute encephalopathy with compromised blood-brain barrier (BBB) function [42]. This structure is highly vulnerable to the toxic effect of Pb and once damaged, Pb itself and other toxicants can easily enter in the brain [43]. *In vivo*

experiments have established that the cerebral cortex and the basal ganglia are affected by Pb [44, 45]. Because Pb exposure may affect several neurotransmitters (dopaminergic, cholinergic and glutamatergic), most frequently early symptoms of human Pb neurotoxicity include irritability, fatigue, depression, headache, decreased attention, memory loss and low-level cognitive impairment [20]. Other concern related to chronic Pb exposures is associated with the propensity of Pb to accumulate in bones [22] where it has a long half-life. Accordingly, bone acts as a reservoir for Pb with continuous release of this metal, when bones are remodeled [46].

Arsenic

To date, limited information exists on the effects of As exposure on cellular and molecular mechanisms of the induced neurological effects [47]. This metal easily crosses the BBB. The basal ganglia are particularly vulnerable; As can also have marked effects on the hippocampus and cortex [48]. As exposure has been suggested as a risk factor for Alzheimer's disease (AD) [21] as well as Parkinson's disease (PD) [49]. Children exposed to As show impaired learning and memory, sleep disturbances, abnormal performances and altered latency of auditory evoked potentials [48, 50]. Patients with occupational exposure to As may develop encephalopathy, characterized by impairments of higher neurological functions, such as learning, memory and concentration [21, 50].

Manganese

Epidemiological data suggest that high Mn concentrations in drinking water may be associated with neurological impairment [32]. Nevertheless neurotoxic effects induced by Mn in humans emerge mostly subsequent to inhalation exposure [51] where the metal can enter the brain through the olfactory pathways, providing a direct path into brain tissue [32, 52]. Inhaled Mn at exceedingly high levels may lead manganism, which is characterized by motor and postural signs consistent with those inherent to Parkinson's disease [53]. The subclinical deficits observed in Mn-exposed subjects are consistent with damage of the basal ganglia [51]. In the early stages of intoxication the symptoms may be reversible [54].

2.1.2. Binary mixtures—Practically no reports are found on the neurotoxic effects induced by the mixture of Pb, As and Mn, except a few experimental studies on binary mixtures, demonstrating interactions between these metals. The co-administration of Pb and Mn to rats has been associated with reduced brain weight to a greater extent than upon exposure to either metal alone [55]. Pb levels were also increased in several brain regions, such as the cerebellum, cerebral cortex, corpus striatum, hippocampus and midbrain, as compared with the administration of Pb alone [56]. ln addition, Pb increased the concentration of Mn in corpus striatum and midbrain as compared with Mn alone [12, 56]. Low doses of Pb plus Mn raised the striatal Mn and Pb concentrations [56]. Behavioral changes were also observed, such as a significant hypoactivity and aggravation of Pb-induced aggressive behavior [57]. Tests on learning ability, where conditioned avoidance responses were measured, showed that a mixture of Pb and Mn impaired learning to a greater extent than the single administration of Pb. These results suggest a synergistic effect of binary metal mixtures even at subclinical levels [56]. Accordingly, the EPA highlighted the need for further refined assessment of the potential hazard to public health of combined

Mn/Pb mixtures, with emphasis on neurological endpoints [12]. In humans, a positive correlation between Mn and Pb in blood was detected, while co-exposure to environmental Pb and Mn has been shown to affect the intelligence of school-aged children [58]. Several studies were performed with other binary mixtures, such as Pb and As, resulting in increased Pb brain levels, along with decreased As concentrations [50]. Neurotransmitter changes were also noted, which were absent when animals were treated with either metal alone [59]. The mixture of As and Pb was shown to cause neuropsychological effects in children living in Morales (Mexico); however, no conclusive results were reached regarding the interactions between these metals [59]. Several studies have also addressed the effects of a mixture of As and Mn [37], noting that As and Mn exposures led to a greater accumulation of these metals in rats' brains when compared to animals exposed to single metals. In addition, higher hair As and Mn levels in humans were associated with significantly lower scores on IQ test, verbal learning and memory. In several cases, a significant interaction was noted for Mn and As [29]. With respect to the mixture of the three elements one study reported that when combined, Pb, As and Mn caused changes in neurotransmitter levels. The authors concluded that the findings were complex, but the data supported the concept that co-exposure to multiple metals may cause neurotoxic effects which is absent with exposure to a single metal at similar doses [60].

3. Mechanisms of toxicity induced by lead (Pb), arsenic (As) and manganese (Mn)

3.1. Oxidative stress

Oxidative stress is a shared mechanism of Pb, As and Mn toxicity [61–64, 40]. The 3 metals can generate reactive oxygen species (ROS) [23, 61, 64–67] and affect ROS-metabolizing enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) [20, 23, 40, 61, 66, 68–70] and interfere with cellular redox status, characterized by reduction in intracellular glutathione (GSH) levels [61, 66, 23, 69]. Decreased levels of reduced GSH levels render cells more susceptible to oxidative damage [63] and may sensitize cells to toxicity associated with other metals [23].

3.2. Interference with the nervous system

Several chemicals inhibit acetylcholine esterase (AChE), causing neurotoxicity [71] via ACh accumulation in the synaptic cleft with an ensuing overstimulation of muscarinic and nicotinic ACh receptors. AChE is anchored to the plasma membrane and there is evidence that lipid peroxidation arising from oxidative stress may alter its activity [72–74]. Indeed, and although much less studied than the dopaminergic system, cholinergic functions are altered by Pb [72], As [21] and Mn [17]. Block of ACh release by Mn and Pb has been proposed as a putative toxic mechanism for interference with cholinergic functions [18, 75]. Furthermore, Pb, As and Mn can affect dopaminergic functions [37, 40, 76–80]. Several studies have demonstrated that interference with neurotransmission in cholinergic or dopaminergic neurons is accompanied by oxidative events [40, 48, 65, 74, 77, 81, 82].

3.3. Interference with the hematopoietic system

Pb, As and Mn exert several effects on the hematopoietic system [83–85], including interference with heme synthesis [40, 62, 86]. Several enzymes that catalyze the heme synthesis metabolic pathway [87, 88] are susceptible to As and Pb [89–91], secondary to their affinity for the functional thiol (-SH; sulfhydryl) groups [61, 92]. Limited information exists on its potential to alter the activity of heme synthesizing enzymes [93], but it has been established that Mn can alter heme metabolism in an *in vitro* assay in rat neurons [94]. Interference with heme biosynthetic pathway is commonly characterized by excessive accumulation and excretion of delta-aminolevulinic acid (ALA) and/or porphyrins [95, 96]. Because individual porphyrins differ in their side-chain substituents, different metals may induce signature-specific changes in porphyrin excretion patterns [92].

Delta-aminolevulinic acid dehydratase (ALAD) is highly sensitive to Pb and As by virtue of its sulfhydryl moiety, and the inhibition of ALAD leads to the accumulation of delta-ALA [61, 63]. At physiological pH, metal catalyzed autoxidation of delta-ALA generates a carbon centered radical (ALA•) as well as O_2^{-}•. The ALA• further oxidizes to the imino form of delta-ALA, producing O_2^{-}•, which dismutates to H_2O_2 [40, 63, 86, 97, 98]. Delta-ALA-generated O_2^{-}• also promotes Fe release from the endoplasmic reticulum, which may contribute to the generation of •OH through the Haber-Weiss reaction [99]. Furthermore, the coupled autoxidation of delta-ALA with oxyhemoglobin [Hb-Fe (II)] produces methemoglobin and H_2O_2 [98]. Disruption of mitochondrial membrane potential and promotion of Ca^{2+} (II) release from the intramitochondrial matrix, with subsequent mitochondrial damage may also occur [95, 99].

Pb can also interfere with coproporphyrinogen oxidase and ferrochelatase (FECH) [97, 63, 100,] and coproporphyrinogen oxidase is also affected by As [91]. Porhyrins, when in excess may also act themselves as toxins [87, 101] given their lipophilic nature and propensity to generate ROS upon exposure to light (430–635 nm), via photochemical reactions [98, 101]. Porphyrins may also form a porphyrin anion radical, which may substitute for O_2^{-}• in Fenton-type reactions or produce O_2^{-}• by reducing O_2 [98]. These events may cause oxidative damage to membrane components, with proteins and lipids as the main targets of photo-damage, leading eventually to cell death [101, 102].

4. Human control of chemical exposure

Neurological diseases induced by the exposure to neurotoxic agents are most frequently chronic progressive disorders, where traditional early warning symptoms of the development of the disease may be lacking. They may be only clinically apparent long after exposure to the initiating factor/s, at a point where the disease is irreversible [27]. Accordingly, great effort has been made to identify neuropathological and biochemical biomarkers of neurotoxicity as early indicators of disease [27,103]. A common concept underlying biomarkers of effect is that they should reflect early and reversible biochemical modifications that precede structural or functional damage and may also be predictive of later responses. Still, challenges and imitations exist concerning biomarkers of neurotoxicity [104].

5. Biomarkers for lead (Pb), arsenic (As) and manganese (Mn)

To date several biomarkers have been studied and proposed for exposure and susceptibility assessment, as well as the detection of adverse health outcomes induced by Pb, As or Mn

5.1. Biomarkers of exposure

Biomarkers of exposure are often obtained from measurement of the parent compound or its metabolites in biological samples [105]. Determination of metal levels in biological samples has been the most common surrogate of exposure [106] with body burdens generally determined in blood or urine [27].

Biomarkers of exposure to Pb are characterized by measurements of total Pb levels in tissues or body fluids, and from these, blood Pb concentration is the most widely used parameter in general clinical practice and public health surveillance [16, 107, 108]. The elimination half-life of Pb in blood is approximately 30 days, and therefore, Pb concentration in blood reflects mainly the exposure history of the previous few months [16].

Several attempts were made to use urinary Pb as a surrogate of blood Pb levels [16, 108], but caution is advised: i) urinary Pb concentration exhibits relatively high intra-individual variability even at similar concentrations of Pb in the blood [16, 109]; ii) estimation of Pb in blood from urinary Pb may be possible on a group basis, but has limited value on an individual basis [108]; iii) for low level exposures urinary Pb levels are close to the detection limit of the analytical methods; iv) Pb urinary excretion reflects mainly recent exposure; and v) the determination of Pb in urine is further complicated by altered kidney function, in association with the nephrotoxic Pb effects [16].

The metalloid As is rapidly cleared from blood, which is the reason why measurements of blood As reflect recent exposures or exposures to high As levels [26, 110]. Urinary As measurements have been considered more reliable than blood, because urinary elimination is the major route for the excretion of this metalloid [26, 110, 111]. Therefore, urinary As has been the most used biomarker in epidemiological and occupational studies as indicator of recent As exposure [110]. Speciation of urinary As may also indicate the extent of past cumulative exposure to the metalloid, more specifically by measuring the levels of monomethylarsenic (MMA) and dimethylarsenic (DMA) [26], resulting from metabolic methylation of ingested or inhaled inorganic As [112]. Total urinary levels of As are used as exposure biomarkers [110].

Mn levels in biological samples, such as blood and urine, have been investigated as biomarker of exposure [17], but their suitability is highly controversial [106]. While they may indicate average levels of exposure on a group basis [17] they are not suitable for individual assessment [17, 113, 114]. Another limiting factor is the rapid rate of Mn clearance from the body, because excess Mn in blood is rapidly removed by the liver and excreted into the bile. Thus, levels of Mn in blood have been used as an indicator of recent exposure, generally less than one month [17, 28, 107]. Since the urinary excretion of Mn is approximately 3% of total excretion urinary Mn has limited clinical validity [107, 28]. Some authors recommend that urinary Mn should be abandoned as a biomarker of exposure [114].

5.2. Biomarkers of susceptibility

Biomarkers of susceptibility reflect an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific chemical substance [115].

It has been known that there is large variation in susceptibility to Pb exposure, with some individuals experiencing toxic effects at levels that others can sustain without any ill effects. Some of these differences may have a genetic basis, namely polymorphisms in ALAD, which is the major Pb-binding protein in the blood [116, 117]. Indeed ALAD2 carriers have a lower risk of toxic effects than ALAD1 homozygotes at the same level of exposure, and some authors suggest that the effects of Pb on neurobehavioral functions tends also to be worse among ALAD1 homozygotes [118].

Much of the susceptibility to As-related health effects is determined by large interindividual variation in As methylation [119]. The trivalent metabolites (particularly MMA III) are most commonly associated with adverse health outcomes and the distribution of urinary As metabolites is determined to a greater extend by genetic variations and is illustrated by the aggregation of methylation patterns within families [120, 121]. Mn secretion in the bile may vary according to individuals' genotypic status. A significant association between the CYP2D6*2 polymorphism (a biomarker of susceptibility) and the latency of chronic Mn poisoning has been reported [122].

5.3. Biomarkers of effect

The knowledge on the toxicity mechanisms of metals at tissue, cellular and molecular levels are crucial for the discovery of new biomarkers. On the basis of oxidative stress, dopaminergic, cholinergic and heme synthesis alterations induced by Pb, As or Mn, several potential biomarkers of effect are summarized in Table 1 and will be described below.

Lead—Serum prolactin (PRL) has been mentioned as an indicator of the dopaminergic function, which is a target of Pb [123]. While Pb's ability to affect PRL levels has been inconsistent across various studies, PRL is generally increased by exposure to this metal [80, 124]. Pb exposure has been also positively associated with PRL levels in occupationally exposed men [123]. However, in other studies PRL levels failed to change upon exposure to Pb [16].

The effects of Pb on cholinergic functions have been consistently reported, with alterations preceding the well-known neurobehavioral and neurophysiological endpoints associated with Pb neurotoxicity [72]. It was suggested that AChE activity in erythrocytes may serve as a peripheral surrogate dose–effect index of neurotoxicity on cholinergic function in occupational exposure to Pb [72]; indeed, decreased AChE erythrocytic levels were observed in workers exposed to this metal [32]. Free radicals can decrease AChE activity, leading to speculation that the inhibitory effect of Pb on AChE observed in the workers may be due to free radicals produced by Pb [75]. Pb effects on heme synthesis are well documented [125, 126] and delta-ALA and ALAD levels in blood are considered the most reliable indicators of Pb intoxication [40, 127, 128], even at relatively low Pb blood concentrations [40]. Since inhibition of ALAD activity results in elevated delta-ALA levels

in blood, consequential increase of urinary delta-ALA excretion is expected, and in fact, urinary delta-ALA has been used in clinical diagnosis of chronic occupational Pb intoxication [129]. However, urinary delta-ALA does not seem to be a sensitive indicator when Pb exposure is low [130]. Patients with Pb poisoning also show accumulation of porphyrins due to the inhibition by Pb of heme biosynthetic enzymes. These enzymes are ALAD, coproporphyrinogen oxidase and FECH [63, 97, 100]. Typically increased urinary excretion of coproporphyrin, as well as accumulation of protoporphyrin in erythrocytes is observed [90].

Arsenic—There are practically no studies evaluating the effects of As exposure on PRL release, but a few have documented that exposure to As leads to reductions in dopamine (DA) content in brain areas involved in the regulation of PRL release [131] and recent reports mention that sodium arsenite can cause a reduction in plasma PRL levels [132]. Both arsenite and arsenate can cause cholinergic dysfunctions in rats in a dose dependent manner, with concomitant AChE inhibition [21, 133, 134] and consequent disturbances in nervous activity [135]. Increased blood ALAS levels [26] and changes in ALAD activity have been noted upon exposure to both As (III) and (V) [136] and it has been posited that ALAD activity in blood may be used to estimate its enzymatic brain activity [40]. Increases in urinary coproporphyrins were found in smelter workers exposed to arsenic trioxide dust, but the mean concentration of uroporphyrin was analogous to that of controls [101]. In contrast, increased urinary uroporphyrin and coproporphyrin levels were observed in individuals exposed to As released from a burning contaminated coal [91]. Further studies are required to clarify this apparent discrepancy in the effect of As among human populations [137].

Manganese—The investigation of Mn effects on PRL has led to inconsistent results. Serum PRL has been proposed as a biomarker of occupational Mn exposure [76], reflecting Mn's effect on DA neurotransmission [138]. Negative results have also been reported [17, 55, 139]. Some salient features of manganism, such as the intensity of mood disturbances cannot be explained only by the disruption of dopaminergic systems, and likely encompasses the cholinergic system as well. The latter is known to play a crucial role in modulating emotional response and higher cognitive functions. [18]. Miners chronically exposed to exceedingly high levels of Mn may exhibit changes in the activity of blood AChE and ACh [17], as well as red blood cells (RBC)-AChE [18]. Inconsistent finding exist regarding Mn-induced hematological effects. *In vivo* studies in rats exposed to Mn by dietary intake have documented decreased hematocrit and hemoglobin levels [12]. Even less information is available on Mn's propensity to affect heme biosynthetic pathway. One such study suggested that Mn can interfere with heme biosynthesis by inhibiting ALAS activity [93]. In addition, Mn has been shown to inhibit liver and erythrocytes ALAD [93] and competitively inhibit FECH [87].

5.4. Biomarker(s) for metal mixtures

In real life scenarios people are co-exposed to an almost infinite number of chemical mixtures and thus, it is barely impossible to determine all the components of a mixture present in an environmental setting [12]. For practical purposes, if mixtures' components are not usually well known, criteria are needed to define the relevant components of a mixture.

Such criteria cannot rely simply on the concentrations of the compounds in the mixture, but must also take note of the expected contribution to relevant endpoints of toxicity [6].

Recently, it has been argued that grouping criteria should focus on common adverse outcomes [6]. This approach could allow the use of shared effect biomarkers, even unspecific, since when used in combination it may reflect specific patterns of biochemical changes (signatures) induced by a mixture of chemicals. However to date, most of the outcomes pertaining to Pb, As and Mn biomarkers have relied on single exposure studies, and they do not permit extrapolation to their combined effects in humans (see Table 1).

With respect to biomarkers of effect induced by metal mixtures, even less progress has been achieved [146], despite the knowledge on interactions between metals at numerous biological levels [4]. Interactions between metals may also result in altered toxicokinetics and toxicodynamic processes [147]. Added effects of Pb and As on the monoaminergic systems in have been shown [50]. Depressed levels of DA were found in the brain of rats co-exposed to Pb and Mn [51]. Changes in the dopaminergic marker serum PRL in children were associated with the environmental exposure to a mixture of four metals that included Pb, As and Mn [145]. Concomitantly the experimental co-exposure to Pb and As caused synergistic inhibition of ALAD and increased ALA urinary excretion as compared to a single exposure either to Pb or As [143, 144].

Concluding Remarks

The Pb, As and Mn mixture is present in several environmental, occupational and food contexts. This mixture may induce synergetic or added toxic effects to those inherent to single metal exposure, rendering the risk of increased toxicity a likely scenario. The selection of biomarkers based on analogous endpoints of toxicity should be a useful tool for predicting and preventing the risk of toxicity. Induction of oxidative stress, interference with cholinergic or dopaminergic systems and altered heme synthesis are shared mechanisms of Pb, - As- and Mn-induced toxicity. A better understanding on the interactive toxic mechanisms is necessary to further delineate a rational basis for the their biomarkers of effect, thus enhancing the tool-kit available for risk assessment, and ultimately limiting exposure and preventing health risks in exposed populations.

Acknowledgments

The authors wish to acknowledge financial support from FCT strategic project PEst-OE/SAU/UI4013/2011, Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa and from the National Institute of Health (NIH R01ES10563).

References

1. Agency for Toxic Substances and Disease Registry (ASTDR), U.S. Department of Health and Human Sciences. Issue paper on the human health effects of metals. 2004
2. Martinez-Finley EJ, Chakraborty S, Fretham SJB, Aschner M. Cellular transport and homeostasis of essential and nonessential metals. Metallomics. 2012; 4(7):593–605. [PubMed: 22337135]
3. Wong CSC, Li X, Thornton I. Urban environmental geochemistry of trace metals. Environ Pollut. 2006; 142:1–16. [PubMed: 16297517]

4. Fairbrother A, Wenste R, Sappington K, Wood W. Framework for metals risk assessment. Ecotoxicol Environ Safety. 2007; 68:145–227. [PubMed: 17889701]
5. Agency for Toxic Substances and Disease Registry (ASTDR), U.S. Department of Health and Human Sciences. Supplementary guidance for conducting health risk assessment of chemical mixtures. 2000
6. Kortenkamp; Faust. State of the Art Report on Mixture Toxicity - Final Report. UE Commission; 2009. http://ec.europa.eu/environment/chemicals/effects/pdf/reportmixture_toxicity.pdf [13th June 2014 2 pm]
7. Pohl HR, Hansen H, Chou CHSJ. Public health guidance values for chemical mixtures: current practice and future directions. Regul Toxicol Pharmacol. 1997; 26:322–329. [PubMed: 9441922]
8. Kordas K, Queirolo EI, Ettinger AS, Wright RO, Stoltzfus RJ. Prevalence and predictors of exposure to multiple metals in preschool children from Montevideo, Uruguay. Sci Total Environ. 2010; 408:4488–4494. [PubMed: 20619443]
9. Calderon J, Ortiz-Perez D, Yanez L, Díaz-Barriga F. Human exposure to metals. Pathways of exposure, biomarkers of effect, and host factors. Ecotoxicol Environ Saf. 2003; 56:93–103. [PubMed: 12915143]
10. Lister LJ, Svendsen C, Wright J, Hooper HL, Spurgeon DJ. Modelling the joint effects of a metal and a pesticide on reproduction and toxicokinetics in Lumbricid earthworms. Environ Int. 2011; 37:663–670. [PubMed: 21329984]
11. Abboud P, Wilkinson KJ. Role of metal mixtures (Ca, Cu and Pb) on Cd bioaccumulation and phytochelatin production by Chlamydomonas reinhardtii. Environ Poll. 2013; 179:33–38.
12. Agency for Toxic Substances and Disease Registry (ASTDR), U.S. Department of Health and Human Sciences. Interaction profile for: Lead, Manganese, Zinc and Copper. 2004
13. Lucchini R, Zimmerman N. Lifetime cumulative exposure as a threat for neurodegeneration: need for prevention strategies on a global scale. NeuroToxicology. 2009; 30 (6):1144–1148. [PubMed: 19835910]
14. Emerit J, Edeas M, Bricaire F. Neurodegenerative diseases and oxidative stress. Biomed Pharmacother. 2004; 58:39–46. [PubMed: 14739060]
15. Csavina J, Field J, Taylor MP, Gao S, Landázuri A, Betterton EA, Sáez AE. A review on the importance of metals and metalloids in atmospheric dust and aerosol from mining operations. Sci Total Environ. 2012; 433:58–73. [PubMed: 22766428]
16. Agency for Toxic Substances and Disease Registry ASTDR), U.S. Department of Health and Human Sciences. Toxicological profile for Lead. 2007b
17. Agency for Toxic Substances and Disease Registry ASTDR), U.S. Department of Health and Human Sciences. Toxicological profile for Manganese. 2007c
18. Finkelstein Y, Milatovic D, Aschner M. Modulation of cholinergic systems by manganese. NeuroToxicology. 2007; 28:1003–1014. [PubMed: 17920128]
19. Järup L. Hazards of heavy metal contamination. Br Med Bull. 2003; 68:167–182. [PubMed: 14757716]
20. Patrick L. Lead toxicity, a review of the literature. Part I: exposure, evaluation, and treatment. Altern Med Rev. 2006; 11 (1):2–22. [PubMed: 16597190]
21. Rodríguez VM, Jiménez-Capdeville ME, Giordano M. The effects of arsenic exposure on the nervous system. Toxicol Lett. 2003; 145:1–18. [PubMed: 12962969]
22. Casarett; Doull's. Toxicology: The Basic Science of Poisons. 8. McGraw-Hill; 2013.
23. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology. 2011; 283 (2–3):65–87. [PubMed: 21414382]
24. Hughes MF, Beck BD, Chen Y, Lewis AS, Thomas DJ. Arsenic exposure and toxicology: a historical perspective. Toxicol Sci. 2011; 123(2):305–332. [PubMed: 21750349]
25. Moinuddin, M. Drinking Death in Groundwater: Arsenic Contamination as a Threat to Water Security for Bangladesh. ACDIS Occasional Paper. 2004. http://acdis.illinois
26. Agency for Toxic Substances and Disease Registry (ASTDR), U.S. Department of Health and Human Sciences. Toxicological profile for Arsenic. 2007a

27. Kakkar P, Jaffery FN. Biological markers for metal toxicity. Environ Toxicol Pharmacol. 2005; 19:335–349. [PubMed: 21783494]
28. Santamaria AB. Manganese exposure, essentiality & toxicity. Indian J Med Res. 2008; 128:484–500. [PubMed: 19106442]
29. Wright RO, Amarasiriwardena C, Woolf AD, Jime R, Bellinger DC. Neuropsychological correlates of hair arsenic, manganese, and cadmium levels in school-age children residing near a hazardous waste site. NeuroToxicology. 2006; 27:210–216. [PubMed: 16310252]
30. Gulson B, Mizon K, Taylor A, Korsch M, Stauber J, Davise JJM, Louie H, Wu M, Swan H. Changes in manganese and lead in the environment and young children associated with the introduction of methylcyclopentadienyl manganese tricarbonyl in gasoline—preliminary results. Environ Res. 2006; 100:100–114. [PubMed: 16337847]
31. Menkes DB, Fawcett JP. Too easily lead? Health effects of gasoline additives. Environ Health Perspect. 1997; 105 (3):270–273. [PubMed: 9171982]
32. Weiss B. Economic implications of manganese neurotoxicity. NeuroToxicology. 2006; 27:362–368. [PubMed: 15936082]
33. Nelson G, Criswell SR, Zhang J, Murray J, Racette BA. Research capacity development in South African manganese mines to bridge exposure and neuropathologic outcomes. NeuroToxicology. 2012; 27:315–326.
34. Basu N, Nama DH, Kwansaa-Ansah E, Renne EP, Nriagu JO. Multiple metals exposure in a small-scale artisanal gold mining community. Environ Res. 2011; 111:463–467. [PubMed: 21397224]
35. Choudhury H, Mudipalli A. Potential considerations and concerns in the risk characterization for the interaction profiles of metals. Indian J Med Res. 2008; 128:462–483. [PubMed: 19106441]
36. Dhatrak SV, Nandi SS. Risk assessment of chronic poisoning among Indian metallic miners. Indian J Occup Environ Med. 2009; 13(2):60–64. [PubMed: 20386621]
37. Rodriguez VM, Dufour L, Carrizales L, Diaz-Barriga F, Jimenez-Capdeville ME. Effects of oral exposure to mining waste on in vivo dopamine release from rat striatum. Environ Health Perspect. 1998; 106 (8):487–491. [PubMed: 9681976]
38. Yim JH, Kim KW, Kim SD. Effect of hardness on acute toxicity of metal mixtures using Daphnia magna: Prediction of acid mine drainage toxicity. J Hazard Mater. 2006; 38:16–21. [PubMed: 16806685]
39. Sansar W, Ahboucha S, Gamrani H. Chronic lead intoxication affects glial and neural systems and induces hypoactivity in adult rat. Acta Histochem. 2011; 113:601–607. [PubMed: 20656334]
40. Reckziegel P, Dias VT, Benvegnú D, Boufleur N, Barcelos RCS, Segat HJ, Pase CS, dos Santos Flores CMM, Bürger ME. Locomotor damage and brain oxidative stress induced by lead exposure are attenuated by gallic acid treatment. Toxicol Let. 2001; 203:74–81. [PubMed: 21402136]
41. Halatek T, Sinczuk-Walczak H, Rabieh S, Wasowicz W. Association between occupational exposure to arsenic and neurological, respiratory and renal effects. Toxicol Appl Pharmacol. 2009; 239:193–199. [PubMed: 19410594]
42. Lockitch G. Perspectives on lead toxicity. Clin Biochem. 1993; 26:371–381. [PubMed: 8299207]
43. Zheng W, Aschner M, Ghersi-Egeac JF. Brain barrier systems: a new frontier in metal neurotoxicological research. Toxicol Appl Pharmacol. 2003; 192(1):1–11. [PubMed: 14554098]
44. Mameli O, Caria MA, Melis F, Solinas A, Tavera C, Ibba A, Tocco M, Flore C, Randaccio FS. Neurotoxic effect of lead at low concentrations. Brain Res Bull. 2001; 55(2):269–275. [PubMed: 11470326]
45. Moreira EG, Vassilieff I, Vassilieff VS. Developmental lead exposure: behavioral alterations in the short and long term. Neurotoxicol Teratol. 2001; 23:489–495. [PubMed: 11711252]
46. van Wijngaarden E, Winters PC, Cory-Slechta DA. Blood lead levels in relation to cognitive function in older U.S. adults. NeuroToxicology. 2011; 32:110–115. [PubMed: 21093481]
47. García-Chávez E, Jiménez I, Segura B, Del Razo LM. Lipid oxidative damage and distribution of inorganic arsenic and its metabolites in the rat nervous system after arsenite exposure: Infuence of alpha tocopherol supplementation. NeuroToxicology. 2006; 27:1024–1031. [PubMed: 16797074]
48. Yadav RS, Chandravanshi LP, Shukla RK, Sankhwar ML, Ansari RW, Shukla PK, Pant AB, Khanna VK. Neuroprotective efficacy of curcumin in arsenic induced cholinergic dysfunctions in rats. NeuroToxicology. 2011; 32(6):760–768. [PubMed: 21839772]

49. de Vizcaya-Ruiza A, Barbiera O, Ruiz-Ramos R, Cebrian ME. Biomarkers of oxidative stress and damage in human populations exposed to arsenic. Mut Res. 2009; 674:85–92. [PubMed: 18984063]
50. Mejía JJ, Díaz-Barriga F, Calderón J, Ríos C, Jiménez-Capdeville ME. Effects of lead-arsenic combined exposure on central monoaminergic systems. Neurotoxicol Teratol. 1997; 19(6):489–497. [PubMed: 9392784]
51. Health Canada, Water Air & Climate Change Bureau. [2th May 2014 4 pm] Human health risk assessment for inhaled manganese. Draft. 2008. http://www.hc-sc.gc.ca/ewh-semt/alt_formats/hecs-sesc/pdf/air/out-ext/_consult/draft_ebauche/manganese-eng.pdf
52. Tjälve H, Henriksson J. Uptake of metals in the brain via olfactory pathways. Neurotoxicology. 1999; 20(2–3):181–195. [PubMed: 10385882]
53. Normandin L, Beaupre LA, Salehi F, St-Pierre A, Kennedy G, Mergler D, Butterworth RF, Philippe S, Zayed J. Manganese distribution in the brain and neurobehavioral changes following inhalation exposure of rats to three chemical forms of manganese. NeuroToxicology. 2004; 25:433–441. [PubMed: 15019306]
54. Bowler RM, Gysens S, Diamond E, Nakagawa S, Drezgic M, Roels HA. Manganese exposure: neuropsychological and neurological symptoms and effects in welders. NeuroToxicology. 2006; 27:315–326. [PubMed: 16343629]
55. Kim HY, Lee CK, Lee JT, Moon CS, Ha SC, Kang SG, Kim DH, Kim HD, Ahn JH, Lee SB, Kang MG. Effects of manganese exposure on dopamine and prolactin production in rat. NeuroReport. 2009; 20(1):69–73. (a). [PubMed: 19057282]
56. Shukla GS, Chandra SV. Concurrent exposure to lead, manganese, and cadmium and their distribution to various brain regions, liver, kidney, and testis of growing rats. Arch Environ Contam Toxicol. 1987; 16:303–310. [PubMed: 3592755]
57. Chandra SV, Mohd Ali M, Saxena DK, Murthy RC. Behavioral and neurochemical changes in rats simultaneously exposed to manganese and lead. Arch Toxicol. 1981; 49:49–56. [PubMed: 7325800]
58. Kim Y, Kim BN, Hong YC, Shin MS, Yoo HJ, Kim JW, Bhang SY, Cho SC. Co-exposure to environmental lead and manganese affects the intelligence of school-aged children. NeuroToxicology. 2009; 30:564–571. (b). [PubMed: 19635390]
59. Carrizales L, Razoa I, Tellez-Hernandez J, Torres-Nerioa R, Torres A, Batres LE, Cubillas AC, Dáaz-Barriga F. Exposure to arsenic and lead of children living near a copper-smelter in San Luis Potosi, Mexico: Importance of soil contamination for exposure. Environ Res. 2006; 101:1–10. [PubMed: 16171795]
60. Wright RO, Baccarelli A. Metals and neurotoxicology. J Nutr. 2009; 137(12):2809–2813. [PubMed: 18029504]
61. Flora SJS. Arsenic-induced oxidative stress and its reversibility. Free Rad Biol Med. 2011; 51:257–281. [PubMed: 21554949]
62. Flora SJS, Bhadauria S, Pant SC, Dhaked RK. Arsenic induced blood and brain oxidative stress and its response to some thiol chelators in rats. Life Sci. 2005; 77:2324–2337. [PubMed: 15964026]
63. Gurer H, Ercal N. Can antioxidants be beneficial in the treatment of lead poisoning? Free Rad Biol Med. 2000; 29 (10):927–945. [PubMed: 11084283]
64. Milatovic D, Milatovic SZ, Gupta RC, Yu Y, Aschner M. Oxidative damage and neurodegeneration in manganese-induced neurotoxicity. Toxicol Appl Pharmacol. 2009; 240:219–225. [PubMed: 19607852]
65. Erikson KM, Dobson AW, Dorman DC, Aschner M. Manganese exposure and induced oxidative stress in the rat brain. Sci Total Environ. 2004; 1:334–335. 409–416.
66. Franco R, Sánchez-Olea R, Reyes-Reyes EM, Panayiotidis MI. Environmental toxicity, oxidative stress and apoptosis: Ménage à Trois. Mutat Res. 2009; 674:3–22. [PubMed: 19114126]
67. Zhang S, Fu J, Zhou Z. In vitro effect of manganese chloride exposure on reactive oxygen species generation and respiratory chain complexes activities of mitochondria isolated from rat brain. Toxicol in Vitro. 2004; 18:71–77. [PubMed: 14630064]

68. Jain A, Yadav A, Bozhkov AI, Padalko VI, Flora SJS. Therapeutic efficacy of silymarin and naringenin in reducing arsenic-induced hepatic damage in young rats. Ecotoxicol Environ Saf. 2011; 74:607–614. [PubMed: 20719385]
69. Liccione JJ, Maines MD. Selective vulnerability of glutathione metabolism and cellular defense mechanisms in rat striatum to manganese. J Pharmacol Exp Ther. 1988; 247(1):156–161. [PubMed: 2902211]
70. Malecki EA, Lo HC, Yang H, Davis CD, Ney DM, Greger JL. Tissue manganese concentrations and antioxidant enzyme activities in rats given total parenteral nutrition with and without supplemental manganese. J Parenter Enteral Nutr. 1995; 19(3):222–226.
71. Ali N, Hoque A, Haque A, Salam KA, Karim R, Rahman A, Islam K, Saud ZA, Khalek A, Akhand AA, Hossain M, Mandal A, Karim R, Miyataka H, Himeno S, Hossain K. Association between arsenic exposure and plasma cholinesterase activity: a population based study in Bangladesh. Environ Health. 2010; 9(36):1–9. [PubMed: 20064246]
72. Ademuyiwa O, Ugbaja RN, Rotimi SO, Abama E, Okediran BS, Dosumu OA, Onunkwor BO. Erythrocyte acetylcholinesterase activity as a surrogate indicator of lead-induced neurotoxicity in occupational lead exposure in Abeokuta, Nigeria. Environ Toxicol Pharmacol. 2007; 24:183–188. [PubMed: 21783808]
73. Rosemberg DB, da Rocha RF, Rico EP, Zanotto-Filho A, Dias RD, Bogo MR, Bonan CD, Moreira JCF, Klamt F, Souza DO. Taurine prevents enhancement of acetylcholinesterase activity induced by acute ethanol exposure and decreases the level of markers of oxidative stress in zebrafish brain. Neuroscience. 2010; 171:683–692. [PubMed: 20884336]
74. Santos D, Milatovic D, Andrade V, Batoréu MC, Aschner M, Marreilha dos Santos AP. The inhibitory effect of manganese on acetylcholinesterase activity enhances oxidative stress and neuroinflammation in the rat brain. Toxicology. 2012; 292(2–3):90–98. [PubMed: 22154916]
75. Amal EA, Mona HM. Protective effect of some antioxidants on the brain of adult male albino rats, Rattus rattus, exposed to heavy metals. Biosci Res. 2009; 6(1):12–19.
76. Ellingsen DG, Haug E, Gaarder PI, Bast-Pettersen R, Thomassen Y. Endocrine and immunologic markers in manganese alloy production workers. Scand J Work Environ Health. 2003; 29(3):230–238. [PubMed: 12828393]
77. Jones DC, Miller GW. The effects of environmental neurotoxicants on the dopaminergic system: A possible role in drug addiction. Biochem Pharmacol. 2008; 76:569–581. [PubMed: 18555207]
78. Prabhakarana K, Ghoshb D, Chapmana GD, Gunasekara PG. Molecular mechanism of manganese exposure-induced dopaminergic toxicity. Brain Res Bull. 2008; 76:361–367. [PubMed: 18502311]
79. Rodríguez VM, Limón-Pacheco JH, Carrizales L, Mendoza-Trejo MS, Giordano M. Chronic exposure to low levels of inorganic arsenic causes alterations in locomotor activity and in the expression of dopaminergic and antioxidant systems in the albino rat. Neurotoxicol Teratol. 2010; 32:640–647. [PubMed: 20699118]
80. Roses OE, Alvarez S, Conti MI, Nobile RA, Villaami EC. Correlation between lead and prolactin in males exposed and unexposed to lead in Buenos Aires (Argentina) area. Bull Environ Contam Toxicol. 1989; 42:438–442. [PubMed: 2706355]
81. Ghareeb DA, Hussien HM, Khalil AA, El-Saadani MA, Ali AN. Toxic effects of lead exposure on the brain of rats: Involvement of oxidative stress, inflammation, acetylcholinesterase, and the beneficial role of flaxseed extract. Toxicol Environ Chem. 2010; 92:187–195.
82. Shavali S, Sens DA. Synergistic neurotoxic effects of arsenic and dopamine in human dopaminergic neuroblastoma SH-SY5Y cells. Toxicol Sci. 2008; 102(2):254–261. [PubMed: 18079140]
83. Case AJ, Madsen JM, Motto DG, Meyerholz DK, Domann FE. Manganese superoxide dismutase depletion in murine hematopoietic stem cells perturbs iron homeostasis, globin switching, and epigenetic control in erythrocyte precursor cells. Free Radical Biol Med. 2013; 56:17–27. [PubMed: 23219873]
84. Cory-Slechta DA. Alterations in tissue Pb distribution and hematopoietic indices during advanced age. Arch Toxicol. 1990; 64(1):31–37. [PubMed: 2306191]

85. Pereira JA, Das P, Chaklader M, Chatterjee S, Basak P, Chaudhuri S, Law S. Effects of inorganic arsenic on bone marrow hematopoietic cells: an emphasis on apoptosis and Sca-1/c-Kit positive population. J Stem Cells. 2010; 5(3):117–27. [PubMed: 22314828]

86. Demasi M, Penatti CAA, Delucia RT, Bechara EJH. The prooxidant effect of 5-aminolevulinic acid in the brain tissue of rats: implications in neuropsychiatric manifestations in porphyrias. Free Radical Biol Med. 1996; 20(3):291–299. [PubMed: 8720899]

87. Hift RJ, Thunell S, Brun A. Drugs in porphyria: From observation to a modern algorithm-based system for the prediction of porphyrogenicity. Pharmacol Ther. 2011; 132(2):158–169. [PubMed: 21704073]

88. Kauppinen R. Porphyrias. Lancet. 2005; 365:241–52. [PubMed: 15652607]

89. Bleiberg J, Wallen M, Brodkin R, Applebaum IL, Newark NJ. Industrially acquired porphyria. Arch Dermatol. 1967; 89:793–797. [PubMed: 14164959]

90. Quintanilla-Vega B, Hernandez A, Mendoza-Figueroa T. Reduction in porphyrin excretion as a sensitive indicator of lead toxicity in primary cultures of adult rat hepatocytes. Toxicol in Vitro. 1996; 10:675–683. [PubMed: 20650251]

91. Ng JCT, Wang JP, Zheng B, Zhai C, Maddalena R, Liu F. Urinary porphyrins as biomarkers for arsenic exposure among susceptible populations in Guizhou province, China. Toxicol Appl Pharmacol. 2005; 206:176–184. [PubMed: 15967206]

92. Woods JS, Martin MD, Leroux BG, DeRouen TA, Bernardo MF, Luis HS, Leitão JG, Simmonds PL, Rue TC. Urinary porphyrin excretion in normal children and adolescents. Clin Chim Acta. 2009; 405:104–109. [PubMed: 19394319]

93. Maines MD. Regional distribution of the enzymes of haem biosynthesis and the inhibition of 5-aminolaevulinate synthase by manganese in the rat brain. Biochem J. 1980; 190:315–321. [PubMed: 6894089]

94. Qato MK, Maines MD. Regulation of heme and drug metabolism activities in the brain by manganese. Biochem Biophys Res Commun. 1985; 128(1):18–24. [PubMed: 3921022]

95. Adhikari A, Penatti CAA, Resende RR, Ulrich H, Britto LRG, Bechara EJH. 5-Aminolevulinate and 4, 5-dioxovalerate ions decrease GABAA receptor density in neuronal cells, synaptosomes and rat brain. Brain Res. 2006; 1093:95–104. [PubMed: 16701578]

96. Guolo M, Stella AM, Melito V, Parera V, Batle AMC. Altered 5-aminolevulinic acid metabolism leading to pseudophorphyria in hemodialysed patients. Int J Biochem Cell Bid. 1996; 28:311–317.

97. Ahamed M, Siddiqui MKJ. Low level lead exposure and oxidative stress: Current opinions. Clin Chim Acta. 2007; 383:57–64. [PubMed: 17573057]

98. Ryter SW, Tyrrell RM. The heme synthesis and degradation pathways: role in oxidant sensitivity. Heme oxygenase has both pro- and antioxidant properties. Free Radical Biol Med. 2000; 28 (2): 289–309. [PubMed: 11281297]

99. Onuki J, Chen Y, Teixeira PC, Schumachera RI, Medeiros MHG, Van Houten B, Di Mascio P. Mitochondrial and nuclear DNA damage induced by 5-aminolevulinic acid. Arch Biochem Biophys. 2004; 432:178–187. [PubMed: 15542056]

100. Moore MR. The biochemistry of heme synthesis in porphyria and in the porphyrinurias. Clin Dermatol. 1998; 16:203–223. [PubMed: 9554234]

101. Krishnamohan M, Qi L, Lam PKS, Moore MR, Ng JC. Urinary arsenic and porphyrin profile in C57BL/6J mice chronically exposed to monomethylarsonous acid (MMAIII) for two years. Toxicol Appl Pharmacol. 2007; 224:89–97. [PubMed: 17707874]

102. Ricchelli F. New Trends in Photobiology - Photophysical properties of porphyrins in biological membranes. J Photochem Photobiol. 1995; 29:109–118.

103. Rachakonda V, Pan TH, Le WD. Biomarkers of neurodegenerative disorders: How good are they? Cell Res. 2004; 14(5):349–360.

104. Costa LG, Manzo L. Biochemical markers of neurotoxicity: research and epidemiological applications. Toxicol Lett. 1995; 77:137–144. [PubMed: 7618127]

105. Nielsen GD, Øvrebø S. Background, approaches and recent trends for setting health-based occupational exposure limits: A minireview. Regul Toxicol Pharmacol. 2008; 51:253–269. [PubMed: 18502550]

106. Phoon HW. Manganese exposure and biological indicators. Sing Med J. 1998; 29:93–94.

107. Batterman S, Su FC, Jia C, Naidoo RN, Robins T, Naik I. Manganese and lead in children's blood and airborne particulate matter in Durban, South Africa. Sci Total Environ. 2011; 409:1058–1068. [PubMed: 21211823]
108. Fukui Y, Miki M, Ukai H, Okamoto S, Takada S, Higashikawa K, Ikeda M. Urinary lead as a possible surrogate of blood lead among workers occupationally exposed to lead. Int Arch Occup Environ Health. 1999; 72:516–520. [PubMed: 10592003]
109. Moreira MFR, Neves EB. Uso do chumbo em urina como indicador de exposição e sua relação com chumbo no sangue. Cad Saúde Pública, Rio de Janeiro. 2008; 24(9):2151–2159.
110. Marchiset-Ferlay N, Savanovitch C, Sauvant-Rocha MP. What is the best biomarker to assess arsenic exposure via drinking water? Environ Int. 2012; 39:150–171. [PubMed: 22208756]
111. Morton J, Mason H. Speciation of Arsenic Compounds in urine from occupationally unexposed and exposed persons in the U.K. using a routine LC-ICP-MS Method. J Anal Toxicol. 2006; 30:293–301. [PubMed: 16839464]
112. Chen CJ, Hsu L, Wang CH, Shihl WL, Hsu YH, et al. Biomarkers of exposure, effect, and susceptibility of arsenic-induced health hazards in Taiwan. Toxicol Appl Pharmacol. 2005; 206:198–206. [PubMed: 15967209]
113. Bader M, Dietz MC, Ihrig A, Triebig G. Biomonitoring of manganese in blood, urine and axillary hair following low-dose exposure during the manufacture of dry cell batteries. Int Arch Occup Environ Health. 1999; 72:521–527. [PubMed: 10592004]
114. Cowan, DM. Doctoral thesis. 2008. Exploring biomarkers of manganese exposure in humans and animals: the manganese-iron ratio as a potential tool for identification of early-onset manganism.
115. Slikker TW Jr, Bowyer JF. Biomarkers of adult and developmental neurotoxicity. Toxicol Appl Pharmacol. 2005; 206:255–260. [PubMed: 15967216]
116. Bergdahl IA, Grubb A, Schütz A, Desnick RJ, Wetmur JG, Sassa S, Skerfving S. Lead binding to delta-aminolevulinic acid dehydratase (ALAD) in human erythrocytes. Pharmacol Toxicol. 1997; 81(4):153–158. [PubMed: 9353844]
117. Scinicariello F, Murray HE, Moffett DB, Abadin HG, Sexton MJ, Fowler BA. Lead and delta-aminolevulinic acid dehydratase polymorphism: where does it lead? A meta-analysis. Environ Health Perspect. 2007; 115(1):35–41. [PubMed: 17366816]
118. Tian T, Ali B, Qin Y, Malik Z, Gill RA, Ali S, Zhou W. Alleviation of lead toxicity by 5-aminolevulinic acid is related to elevated growth, photosynthesis, and suppressed ultrastructural damages in oilseed rape. Biomed Res Int. 2014 Epub 2014 Feb 11. 10.1155/2014/530642
119. Loffredo CA, Aposhian HV, Cebrian ME, Yamauchi H, Silbergeld EK. Variability in human metabolism of arsenic. Environ Res. 2003; 92(2):85–91. [PubMed: 12854687]
120. Antonelli R, Shao K, Thomas DJ, Sams R, Cowden J. AS3MT, GSTO, and PNP polymorphisms: Impact on arsenic methylation and implications for disease susceptibility. Environ Res. 2014; 132:156–167. [PubMed: 24792412]
121. McClintock TR, Chen Y, Bundschuh J, Oliver JT, Navoni J, Olmos V, Lepori EV, Ahsan H, Parvez F. Arsenic exposure in Latin America: biomarkers, risk assessments and related health effects. Sci Total Environ. 2012; 1(429):76–91. [PubMed: 22119448]
122. Vinayagamoorthy N, Krishnamurthi K, Devi SS, Naoghare PK, Biswas R, Biswas AR. GSTM1, NQO1 genes and their correlation with biomarkers in manganese miners of Central India. Chemosphere. 2850; 81(10):1286–1291. [PubMed: 20851451]
123. Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, Paneth N, Wirth JJ. Multiple metals predict prolactin and thyrotropin (TSH) levels in men. Environ Res. 2009; 109(7):869–873. [PubMed: 19595304]
124. Alessio L, Luchini R. Prolactin Changes as a Consequence of Chemical Exposure. Environ Health Perspect. 2006; 114(10):573–574. [PubMed: 16581548]
125. Goering PL, Fowler BA. Metal constitution of metallothionein influences inhibition of 5-aminolaevulinic acid dehydratase (porphobilinogen synthase) by lead. Biochem J. 1987; 245:339–345. [PubMed: 3663161]
126. Seth TD, Agarwal LN, Satija NK, Hasan MZ. The effect of lead and cadmium on liver, kidney, and brain levels of cadmium, copper, lead, manganese, and zinc, and on erythrocyte ALAD activity in mice. Bull Environ Contam Toxicol. 1976; 16(2):190–196. [PubMed: 963322]

127. Chibe M, Sinohara A, Matsushita K, Watanabe H, Inaba Y. Indices of Lead expousure in blood and urine of lead exposed workers and concentrations of major trace elements and activities of SOD, GSH-Px and catalase in their blood. J Exp Med. 1996; 178:49–62.

128. Rocha JBT, Pereira ME, Emanuell T, Christofari RS, Souza DO. Effect of treatment with mercury chloride and lead acetate second stage of rapid postnatal brain growth on 6-during the aminolevulinic acid dehydratase (ALA-D) activity in brain, liver, kidney and blood of suckling rats. Toxicology. 1995; 100:27–37. [PubMed: 7624881]

129. Wang Q, Zhao HU, Zhao, Chen JW, Hao QL, Gu KD, Zhu YX, Zhou YK, Ye LX. d-Aminolevulinic acid dehydratase activity, urinary d-aminolevulinic acid concentration and zinc protoporphyrin level among people with low level of lead exposure. Int J Hyg Environ Health. 2010; 213:52–58. [PubMed: 19733117]

130. Makino S, Tsruta H, Takata T. Relationship between blood lead level and urinary ALA level in workers exposed to very low levels of lead. Ind Health. 2000; 38:95–98. [PubMed: 10680317]

131. Bardullas U, Limón-Pacheco JH, Giordano M, Carrizales L, Mendoza-Trejo MS, Rodríguez VM. Chronic low-level arsenic exposure causes gender-specific alterations in locomotor activity, dopaminergic systems, and thioredoxin expression in mice. Toxicol Appl Pharmacol. 2009; 239:169–177. [PubMed: 19121333]

132. Jahan S, Ahmed S, Razzaq S, Amed H. Adverse effects of arsenic exposure in the mammary glands of adult female rats. Pakistan J Zool. 2012; 44(3):691–697.

133. Kobayashi H, Yuyama A, Ishihara M, Matsusaka N. Effects of arsenic on cholinergic parameters in brain in vitro. Neuropharmacology. 1987; 26 (12):1707–1713. [PubMed: 3437937]

134. Roy S, Chattoraj A, Bhattacharya S. Arsenic-induced changes in optic tectal histoarchitecture and acetylcholinesterase-acetylcholine profile in Channa punctatus: Amelioration by selenium. Comp Biochem Physiol. 2006; 144:16–24.

135. Yousef MI, El-Demerdash FM, Radwan FME. Sodium arsenite induced biochemical perturbations in rats: Ameliorating effect of curcumin. Food Chem Toxicol. 2008; 46:3506–3511. [PubMed: 18809455]

136. Bhadauria S, Flora SJ. Arsenic induced inhibition of delta-aminolevulinate dehydratase activity in rat blood and its response to meso 2,3-dimercaptosuccinic acid and monoisoamyl DMSA. Biomed Environ Sci. 2004; 17(1):101–8. [PubMed: 15202869]

137. Wu H, Manonmanii K, Lam PKS, Huang SH, Wang JP, Ng JC. Urinary arsenic speciation and porphyrins in C57Bl/6J mice chronically exposed to low doses of sodium arsenate. Toxicol Lett. 2004; 154:149–157. [PubMed: 15475189]

138. Takser L, Mergler D, de Grosbois S, Smargiassi A, Lafond J. Blood manganese content at birth and cord serum prolactin levels. Neurotoxicol Teratol. 2004; 26:811–815. [PubMed: 15451044]

139. Aschner M. Manganese as a Potential Confounder of Serum Prolactin. Environ Health Perspect. 2006; 114 (8):A458. [PubMed: 16882508]

140. Govonia S, Lucchia L, Battainia F, Spanoa PF, Trabucchia M. Chronic lead treatment affects dopaminergic control of prolactin secretion in rat pituitary. Toxicol Lett. 1984; 20 (3):237–241. [PubMed: 6701910]

141. Adaudi AO, Aliu YO. Urinary delta-aminolevulinic acid (ALA) excretion in humans and cattle as an index of exposure to lead. Vet Hum Toxicol. 1980; 22(6):403–5. [PubMed: 7210469]

142. Marreilha dos Santos AP, Santos ML, Batoréu MC, Aschner M. Prolactin is a peripheral marker of manganese neurotoxicity. Brain Res. 2011; 1382:282–290. [PubMed: 21262206]

143. Wang G, Fowler BA. Roles of biomarkers in evaluating interactions among mixtures of lead, cadmium and arsenic. Toxicol Appl Pharmacol. 2008; 233:92–99. [PubMed: 18325558]

144. Whittaker MH, Wang G, Chen XQ, Lipsky M, Smith D, Gwiazda R, Fowler BA. Exposure to Pb, Cd, and As mixtures potentiates the production of oxidative stress precursors: 30-day, 90-day, and 180-day drinking water studies in rats. Toxicol Appl Pharmacol. 2010; 254(2):154–166. [PubMed: 21034764]

145. de Burbure C, Buchet JP, Leroyer A, Nisse C, Haguenoer JM, Mutti A, et al. Renal and neurologic effects of cadmium, lead, mercury, and arsenic in children: evidence of early effects and multiple interactions at environmental exposure levels. Environ Health Perspect. 2006; 114(4):584–590. [PubMed: 16581550]

146. Zhai R, Su S, Lu X, Liao R, Ge X, He M, Huang Y, Mai S, Lu X, Christiani D. Proteomic profiling in the sera of workers occupationally exposed to arsenic and lead: identification of potential biomarkers. BioMetals. 2005; 18:603–613. [PubMed: 16388400]

147. Jadhav SH, Sarkar SN, Kataria M, Tripathi HC. Subchronic exposure to a mixture of groundwater-contaminating metals through drinking water induces oxidative stress in male rats. Environ Toxicol Pharmacol. 2007; 23:205–211. [PubMed: 21783759]

Table 1

Proposed biomarkers of effect in peripheral samples for Pb, As, Mn and mixtures of these elements

Exposure	Biological Sample	Biomarker	References
Pb	Serum	PRL	[123, 140]
	Blod erythrocytes	AChE	[32, 72]
	Blood	ALA and ALAD	[40,127,128]
	Urine	ALA	[141]
	Blood erythrocytes	Protoporphyrin	[100]
	Urine	Coproporphyrin	[100]
As	Serum	PRL	[132]
	Blood	ALA, ALAS, ALAD	[26,40,136]
	Urine	Uro- and coprophophyrin	[91,101,137]
Mn	Serum	PRL	[17,76, 123,138,142]
	Blood and blood erythrocytes	Ach and AChE	[17, 18]
Pb + As	Urine	ALA	[143, 144]
Pb+As+Mn+others	Serum	PRL	[145]