Published in final edited form as:

J Toxicol Environ Health B Crit Rev. 2017 ; 20(3): 119–154. doi:10.1080/10937404.2017.1289834.

Biomarkers of mercury toxicity: Past, present and future trends

Vasco Branco[1], **Sam Caito**[2], **Marcelo Farina**[3], **João Batista Teixeira da Rocha**[4], **Michael Aschner**[2], and **Cristina Carvalho***[1]

[1]Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

[2]Department of Molecular Pharmacology, Albert Einstein College of Medicine, Bronx, USA

[3]Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Florianópolis, Brazil

[4]Departamento Bioquímica e Biologia Molecular, Universidade Federal de Santa Maria, RS, Brazil

Abstract

Mercury (Hg) toxicity continues to represent a global health concern. Given that human populations are mostly exposed to low chronic levels of mercurial compounds (methylmercury through fish, mercury vapor from dental amalgams and ethylmercury from vaccines) the need for more sensitive and refined tools to assess the effects and/or susceptibility to adverse metal-mediated health risks remains. Traditional biomarkers, such as hair or blood Hg levels, are practical and provide a reliable measure of exposure, but given intra-population variability it is difficult to establish accurate cause-effect relationships. It is therefore important to identify and validate biomarkers that are predictive of early adverse effects prior to adversehealth outcomes becoming irreversible.This review describes the predominant biomarkers used by toxicologists and epidemiologists to evaluate exposure, effect and susceptibility to Hg compounds, weighing on their advantages and disadvantages. Most importantly, and in light of recent findings on the molecular mechanisms underlying Hg-mediated toxicity, potential novel biomarkers that might be predictive of toxic effect are presented and the applicability of these parameters in risk assessment is examined.

Keywords

Biomarkers; Mercury; Toxicity; Risk assessment

Mercury toxicity

Mercury (Hg) is a ubiquitous environmental pollutant to which humans are exposed to in varying amounts and chemical forms (Clarkson and Magos, 2006). The three main forms of Hg that are a cause of concern to non-occupationally exposed human populations include

Address for correspondence: Cristina Carvalho, Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, Lisboa 1649-003, Portugal, cristina.carvalho@ff.ulisboa.pt.

methylmercury (MeHg), mercury vapor (Hg^0) and ethylmercury (EtHg) (Clarkson et al., 2003). Ingestion of fish containing MeHg is by far the most common route of exposure to Hg, inhalation of Hg^0 occurs due to its release from dental amalgams whereas exposure to EtHg results from the use of Thimerosal (Ethyl (2-mercaptobenzoato-(2-)-O,S) mercurate(1-) sodium)-containing vaccines (TCV) (Dórea et al. 2013). In addition, given that Hg compounds are still in use in many human activities, occupational exposure is also of relevance to metal-induced toxicity and an important driving force for the discovery of new putative biomarkers. It is well-known that Hg^0 was employed in artisanal small-scale gold mining (ASSGM) mainly in developing countries (Armah et al., 2016; Bose-O'Reilly et al., 2016; Castilhos et al. 2015; Gibb & O'Leary, 2014; Kristensen et al. 2014) and the number of humans exposed to this element is still high (Gibb & O'Leary, 2014).

All Hg forms generate Hg^{2+}. In the case of Hg^0 oxidation is facilitated by catalase (CAT), a process first described in red blood cells by Halbach and Clarkson (1978). Demethylathion of MeHg to Hg^{2+} is also known to occur (Mottet et al., 1997; Vahter et al., 1995; Watanabe, 2002). Several hypotheses suggest the involvement of selenium (Se) and free radical attack in this process but the precise mechanism has yet to be determined (Yang et al., 2008).

The main adverse health effects of Hg include neurotoxicity, teratogenicity, nephrotoxicity and immunotoxicity (Ratcliffe et al, 1996; Sweet and Zelikoff, 2001; Counter et al, 2002). The magnitude of these toxic effects may vary considerably, depending upon the life-stage, dose and duration of exposure (Clarkson and Magos, 2006), and symptoms may intensify, become irreversible and eventually lead to death (WHO, 2003). Even though the adverse health effects attributed to Hg compounds are well-known, the molecular basis underlying the development of toxicity is still not fully understood. Given the adverse effects of Hg compounds it is imperative to assess exposure and detect early effects predictive of toxicity, i.e. an early stage of an adverse effect, especially in vulnerable populations. This is of extreme importance for risk assessment, particularly when dealing with exposure levels at the lower-end of the dose-response curve, since biomarkers of early effects exhibit greater sensitivity when compared to classical assessment of clinical symptoms, functional tests, or morbidity (Bernard, 2008; Ratcliffe et al., 1996).

Biomarkers are indicator-signaling events in biological systems or samples (body fluids, cells or tissues) (NRC, 1987). The usefulness of biomarkers in identifying a postulated adverse health effect is intrinsically related to their role (Benford et al., 2000) within the mode of action of the toxicant.

The objective of this review is to describe the principal biomarkers used in the evaluation of Hg-induced toxicity weighing their predictiveness and/or insufficiencies. In addition, recent discoveries on the mechanisms underlying Hg-mediated toxicity are presented and examine whether these findings, for which there may be none or limited epidemiological data available, may potentially prove useful as novel biomarkers of metal-induced toxicity.

Biomarker Applications

Biomarkers have been defined as measures in cellular or biochemical components or processes, structures or functions (including neurobehavioral) that can be measured in a biological system (Gil and Pla, 2001; Kendall et al., 2001; Paustenbach, 2001; Silbergeld and Davis, 1994; Tan et al, 2012; Wallace et al, 2016). These alterations may occur at any stage along the causal pathway, which varies from minimal molecular changes resulting from exposure to the toxic compound until establishment of overt toxicity, when evident end-points of toxicity become apparent from a clinical perspective (Figure 1A) (Bendford et al., 2000; Wallace et al, 2016). Ideally, biomarkers should be non-invasive, sensitive and specific for the xenobiotic that is being assessed (Bernard, 2008; Grandjean et al., 1994; Paustenbach, 2001; Tan et al, 2012). Biomarkers play an essential role in risk assessment by reducing overall uncertainty (Maier et al., 2004).

Classically, biomarkers have been divided into biomarkers of exposure, effect or susceptibility (NRC, 1987), although the boundaries between these are difficult to establish (WHO, 1993; Grandjean et al., 1994) (Figure 1A). Currently, the use of biomarkers has broadened, encompassing employment of pharmacogenomics and other biomarkers in medicine as shown in Figure 1B.

Exposure biomarkers may be markers of internal dose, i.e. the direct measurement of the compound, its metabolites or conjugates in a biological sample such as urine, blood, hair, and markers of biologically effective dose (BED) when they evaluate interaction between xenobiotics and macromolecules, such as proteins or DNA (WHO, 1993). Although markers of BED are not crucial for development of toxicity as they are not linked to the events that trigger the neurotoxicity nephrotoxicity, or other biological system, these markers share a common etiology (NRC, 1987; Timbrell, 1998; Woods, 1995).

Biomarkers of effect represent alterations including biochemical, physiological or behavioral that can be measured in an organism and attributed to exposure to a xenobiotic. Hence, biomarkers of effect are directly linked to toxicity (this needs biomarker validation). Ideally, these biomarkers should be predictive of effect, as these measures should identify initial alterations or adverse effects prior to becoming irreversible or considered pathological evidence (Bendford et al., 2000; Bernard, 2008; Gil and Pla, 2001; WHO, 1993). Biomarkers of susceptibility reflect characteristics that increase individual sensitivity to a given compound by either increasing its internal dose or by lowering the threshold toxic dose, for example, as a result of genetic polymorphisms (Schmidt, 2006).

Mercury biomarkers

Biomarkers of Exposure

Monitoring of exposure levels in the human population, in particular in groups at risk, such as pregnant women, newborns, children and occupationally exposed workers, has been the basis of Hg-associated risk assessment. Mercury enters the human body by various routes and in different chemical forms and each of these has its own distinctive toxicological features. Thus, not all biological indicators have the same reliability as biomarkers of

exposure for all Hg compounds. Consequently, it is important that the biological indicator used as a biomarker of exposure translates the Hg burden in the organism especially at the target organ(s). In addition, the appropriate biomarker of exposure depends upon the type of exposure (acute *vs.* chronic) (Clarkson et al., 2007).

Biomarkers of Exposure: Internal Dose

The most common biomarkers of exposure to mercurials are internal dose markers tha encompass measurements of total Hg levels in hair, urine and blood (Berglund et al., 2005), in the absence of speciation analysis. Other biomarkers, such as the levels of Hg in placental cord-blood, feces, breast-milk or nails, are seldom used although in particular situations they have been proven quite useful (Bose-O'Reilly et al., 2010; Cooke, 2014; LaKind et al., 2005; Ohono et al., 2007). Despite their common use in Hg-induced toxicity evaluation (Maier et al., 2004) it should be stressed that, due to the inter-individual human variability, the levels of Hg in hair, blood or urine do not necessarily reflect the presence or absence of toxicity (Bose O'Reilly et al., 2010).

Mercury Levels in the Hair

MeHg binds sulfhydryl (SH-) groups of keratin and is integrated in the hair (Mottet et al., 1997) constituting more than 80% of the hair metal burden (Berglund et al., 2005). Hair follicles are thought to accumulate the same transportable species that reach the brain (Clarkson et al., 2007). In fact, hair Hg levels correlate well with levels in the brain and whole blood at a ratio of 250:5:1, respectively (Clarkson and Magos, 2006) (Figure 3). However, the ratio of Hg levels between blood and brain, and blood and hair may vary according to individual's characteristics such as age, gender and genetics (Bartell et al., 2000; Doi and Tagawa, 1983).

The concentration of Hg in hair is an appropriate biomarker to address exposure to MeHg in a non-invasive manner (Berglund et al., 2005; Satoh, 2000). Normal levels of Hg in hair range between 1 and 2 μg/g, but individuals who consume large amounts of fish may have hair Hg levels in excess of 10 μg/g (WHO/UNEP, 2008) with the lowest observable adverse effect level (LOAEL) for neurotoxic effects (paresthesia) in adults set at 50 μg/g (Clarkson and Magos, 2006).

Another advantage of quantifying Hg levels in hair is that it allows for retrospective studies by evaluation of Hg in different sections of the hair strand (Mottet et al., 1997). Additionally, levels of Hg in maternal hair correlate well with the blood Hg levels in the fetus, which makes it a useful biomarker of pre-natal exposure for risk assessment purposes (Cooke et al., 2014; WHO/UNEP, 2008). The no observable adverse effect level (NOAEL) for developmental effects is estimated to be 10 μg Hg/g in maternal hair (Clarkson and Magos, 2006).

During pregnancy, maternal hair Hg levels may decrease up to 20%, indicating placental transfer of Hg to the fetus (Barbosa et al., 1998). In fact, placental transfer of MeHg is far more important than transfer by breast-milk (Barbosa et al., 1998; Björnberg et al., 2005) in determining the relationship between maternal and newborn hair Hg levels, in fish eating populations (Barbosa et al., 1998; Díez et al., 2009; Kim et al., 2008; Marques et al., 2013;

2015; Oliveira et al., 2010; Ou et al., 2015; Savabieasfahani et al., 2012; Sikorski et al, 1986). Infant hair Hg concentration is a reliable biomarker for *in utero* MeHg exposure. Notably, breast-fed infants receiving Thimerosal-containing vaccines (TCV) show asymmetric changes in Hg hair levels relatively to their mother, indicating that hair Hg levels also reflect exposure to EtHg in addition to MeHg (Marques et al., 2007). Speciation analysis demonstrated that EtHg levels in hair are positively correlated to the number of TCV vaccinations and negatively correlated to the time elapsed since the last vaccination (Dórea et al., 2011). This additional burden of EtHg from TCV further adds to the pre and post-natal Hg burden (Dórea et al., 2012). However, it should be stressed that several epidemiological studies (Andrews et al., 2004; Fombonne et al., 2006; Heron and Golding, 2004; Hviid et al., 2003; Madsen et al., 2003; Stehr-Green et al., 2003; Taylor et al., 2014; Uno et al., 2015; Verstraeten et al., 2003) failed to detect any positive association between TCVs and impaired neurodevelopment.

Hair Hg levels are normally not considered an appropriate biomarker of exposure to Hg^0 (Mottet et al., 1997), as the Hg^{2+} found in hair is likely the result of MeHg demethylation within hair follicles (Berglund et al., 2005) or external deposition (Bose-O' Reilly et al., 2008). Moreover, in pregnant women living in areas where ASSGM is practiced, Hg levels in hair did not accurately reflect concentrations found in other biological media such as breast-milk, which might underestimate infant exposure (Bose-O' Reilly et al., 2008). Barbosa et al. (1995) compared populations from the Brazilian Amazon that were exposed to Hg by different routes (ASSGM vs. fish consumption) and showed that in general, levels in hair were a more reliable descriptor for exposure to Hg from fish (mainly MeHg) than for Hg vapor from ASSGM activities. However, *in utero* exposure to Hg^0 from maternal dental amalgams was claimed to contribute increased levels of Hg in baby hair (Lindow et al., 2003). To overcome the possibility of mixed sources of exposure (dietary MeHg *vs.* occupational Hg^0) the measurement of Hg stable isotopic signatures in hair was applied as a useful technique to distinguish between different sources of Hg in the organism (Laffont et al., 2011).

Mercury Levels in Urine

The concentration of Hg in urine (U-Hg) is the most common biomarker of exposure to Hg^0 both in occupational exposures as well as to dental amalgams (Magos, 1997). Its utilization in monitoring workers exposed to Hg dates back to the first half of the 20^{th} century (Buckell et al., 1946; Neal, 1938). Urinary mercury is derived from Hg that accumulates in kidney cells after acute (Bose-O' Reilly et al., 2010) or chronic exposure to Hg^0 (Cherian et al., 1976), and consequently in the mid- to long- term this reflects the Hg^{2+} body burden (Clarkson and Magos, 2006; Zalups, 2000). Neurotoxic effects attributed to Hg^0 are evident in subjects with U-Hg levels exceeding 35 μg/g creatinine (SCOEL, 2007). However, several investigators indicated the occurrence of neurobehavioral effects in the 20–30 μg of Hg /g creatinine range or lower (Echeverria et al., 1998; Meyer-Baron et al., 2002).

Experiments with rats showed that even in chronic exposures the urinary excretion of MeHg is low (Zalups et al., 1992), since the MeHg-Cys conjugate is slowly excreted in urine (Yasutake et al., 1989). In addition, in human populations exposed to different sources of

Hg, urine levels were noted to be a less sensitive biomarker for MeHg exposure through fish consumption in comparison to Hg^0 derived from ASSGM activities (Barbosa et al., 1995). Accordingly, U-Hg levels are not considered a useful biomarker for assessing MeHg exposure (Berglund et al., 2005).

Mercury Levels in Blood

The blood is responsible for the distribution of all forms of Hg to target organs (Clarkson et al., 2007). Thus, the concentration of Hg in blood (B-Hg) is considered a reliable biomarker of exposure. In fish eating populations, metal levels in whole blood are usually interpreted as reflecting exposure to MeHg (Berglund et al, 2005; Bose-O' Reilly et al., 2010). B-Hg levels in individuals with no consumption of highly contaminated fish are normally between 5 to 10 μg/L (WHO/UNEP, 2008), while in populations with limited fish consumption B-Hg normally does not exceed 2μg/L (NRC, 2000). In blood, approximately 90% of MeHg is found in red blood cells (RBC) bound to hemoglobin (Figure 3), while inorganic Hg (Hg^0 and Hg^{2+}) is evenly distributed between RBCs and plasma (Berglund et al., 2005; Clarkson et al., 2007). Plasma Hg accounts for a smaller fraction of whole blood Hg that may range from 5–35%, depending upon the characteristics of the population such as age and diet (Carneiro et al., 2014). Hg^{2+} is normally the dominant species, although large variations exist between individuals concerning the ratio between Hg^{2+} and MeHg in plasma (Carneiro et al., 2014). Since Hg kinetics in blood is relatively fast, this biomarker has the limitation of only being useful for a short time after an acute exposure, or in the case of continuous (chronic) exposure (Satoh, 2000).

Concerning pre-natal development, Hg cord-blood levels may be employed to quantify fetal exposure to MeHg (Aylward et al., 2014; Cooke, 2014). However, since fetal blood levels are known to be 1.5 to 2-fold higher than maternal blood concentrations, the latter is the preferred biomarker for assessing pre-natal exposure (WHO/UNEP, 2008).

Biomarkers of exposure: biologically effective dose (BED)

As referred to above, biomarkers of biologically effective dose (BED) indicate the interaction of the xenobiotic with macromolecules such as proteins including albumin or hemoglobin in blood or nucleic acids but these interactions are mainly a consequence of the absorption distribution metabolism excretion (ADME) processes and not necessarily related to adverse effects development. Due to the electrophilic nature of Hg, once absorbed into the organism, it binds nucleophilic groups, such as thiols (SH) and selenols (SeH) in cysteine (Cys) and selenocysteine (Sec), respectively. Consequently, peptides and proteins containing these moieties are targeted by mercurials (Carvalho et al., 2008a; Farina et al. 2009; Rooney 2007; Silva de Paula et al, 2016).

Conjugation of Hg compounds with cysteine-containing compounds such as GSH, primarily facilitates detoxification (Clarkson and Magos, 2006; Bridges and Zalups, 2010); however, it may also trigger toxicity upon metabolism. Simmon-Willis et al (2002) showed that by binding to the thiol in Cys, MeHg gains access to the central nervous system (CNS), since the MeHg-Cys complex mimics the amino acid methionine and thus transported across membranes by neutral amino acid transporters. Similarly, Hg^{2+} is known to bind two Cys or

GSH molecules forming complexes similar to cystine (Cys-Cys) and glutathione disulfide (GSSG). Formation of these types of conjugates has been involved in Hg^{2+} and MeHg uptake and excretion from the CNS, kidneys, liver, intestine, placenta (Bridges and Zalups, 2010). In addition, inhibition of Cys- and Sec-containing enzymes produced by covalent binding of mercurials (discussed below) may lead to increased reactive oxygen species (ROS) generation and oxidative stress.

Taken together, Hg compounds have several potential binding targets and it becomes challenging to determine which of these interactions are critical for development of toxicity. The BED biomarkers end-points which have been thoroughly studied in relation to Hg toxicity are presented; however, these have not been directly linked to the main pathways of toxicity that culminate in cell death. Some of the important BED biomarkers for Hg exposure and their potential value are discussed below.

Depletion of Intracellular Glutathione (GSH)

Glutathione is the most abundant non-protein cellular thiol, present at millimolar concentrations in several organs including liver, kidneys and brain (Meister, 1988; Pastore et al., 2003). Conjugation with GSH is an important step in the toxicokinetics and systemic transport of mercurials, and several *in vitro* and *in vivo* studies (Bo et al., 1993; Franco et al., 2007; Fujiyama et al., 1994; Piccoli et al., 2012) demonstrated GSH depletion following exposure to MeHg and Hg^{2+}. However, for the case of Hg^{2+}, the binding to free Cys is more important than GSH, because Hg-Cys complexes are more efficiently transported than Hg-GSH complexes in tubular cells (Zalups and Barfuss, 2002a).

An excessive Hg burden may result in marked depletion of reduced GSH, with serious consequences to the cell antioxidant capacity (Farina et al., 2011). However, it is difficult to understand how the equimolar interaction between Hg and GSH might lead to GSH depletion and, consequently, oxidative stress. In fact, GSH is present in some mammalian cells at concentrations as high as 10 millimolar (Cooper and Kristal, 1997) and Hg compounds (particularly, MeHg) were reported to induce oxidative damage in cultured cells when present at concentrations as low as 1 micromolar (Farina et al., 2009). In addition, experimental studies based on *in vivo* approaches noted reduced cerebellar (Franco et al., 2006), cerebral (Stringari et al., 2008) and blood (Silva de Paula et al., 2016) GSH levels in MeHg-exposed animals when Hg levels in these organs were in the low micromolar range. Thus, it is noteworthy that decreased GSH levels following Hg exposure do not necessarily indicate direct interaction between Hg and thiol groups, but may represent the consequence of direct oxidative effects of reactive species (i.e. hydrogen peroxide) whose levels are increased after Hg exposure (Franco et al., 2007).

A comparison of blood GSH levels in populations from the Amazon showed an association with hair Hg concentrations (Pinheiro et al., 2008). In fact, hair Hg levels above 10 μg/g typically led to higher GSH (20 to 60%) quantities than following lower exposure (hair Hg levels below <4 μg/g on average), even tough the correlation is scattered (Pinheiro et al., 2008). Similarly, elevated GSH levels in blood (+45%) were associated with seasonal variation in hair Hg concentrations in sports fishermen (1.4 μg/g vs. 2.8 μg/g) (Bélanger et al., 2008).

In miners, previously exposed to Hg^0, a 10% increase in erythrocyte GSH levels was noted (Kobal et al., 2008). Nonetheless, in older, retired miners levels of GSH were 20% lower than in controls, suggesting the existence of age related factors influencing overall GSH blood levels (Kobal et al., 2008). However, many other compounds, such as paraquat (Djukic et al., 2012), arsenic (Jain et al., 2012), benzo(a)pyrene (Romero et al., 1997) and acetaminophen (McMurtry et al., 1978) may also decrease GSH levels. Consequently, in a multi-contaminant or therapeutic context this end-point does not permit distinction of Hg related toxicity from that produced by other compounds. Thus, the usefulness of GSH as a BED is quite limited.

Impairment of Heme- Biosynthesis and Coproporphyrin Accumulation

Porphyrin metabolism has been proposed as an indicator of metal exposure and toxicity (Woods, 1995). The synthesis of the prosthetic group heme- is an important physiological process that occurs in all tissues and involves several product intermediates and enzymatic processes, from the initial substrate (Succinil CoA) to the final heme group (Figure 4) (Woods et al., 2009). In the normal unfolding of the heme biosynthesis process, overproduction of intermediate porphyrinogens is followed by their oxidation to porphyrins, which are then excreted in the urine (Woods et al., 1993). Several metals, including lead (Pb), arsenic (As) and mercury (MeHg and Hg^{2+}) (Fowler and Mahaffey, 1978; Pingree et al., 2001; Sakai, 2000; Woods and Fowler, 1977; 1978), interfere in a dose-dependent manner with the normal course of the heme biosynthetic pathway by inhibiting the activity of several of the specific enzymes involved and promoting porphyrin accumulation (Woods et al., 2009). These accumulated prophyrins are then excreted and their rise in urine can be utilized as a biomarker of exposure to metals. Mercury targets the enzymes uroporphyrinogen decarboxylase (UROD), and especially coproporphyrinogen oxidase (CPOX), thus leading to accumulation and excretion of 4 and 5 carboxyl porphyrines (Woods, 1995; Woods et al., 2005). Specifically, in the case of Hg, there is also excretion of an atypical “precoproporphyrin” known as keto-isocoproporphyrin (KICP) (Woods et al., 1991), which was proposed as a biomarker of exposure for both MeHg and Hg^0 (Marks, 1985; Woods, 1995). Moreover, such alterations occur prior to the onset of target tissue injury, suggesting that porphyrines may be used as biomarkers predictive of early toxic effects (Woods, 1995; Woods et al., 2009). Animal studies (Pingree et al., 2001) have supported the usefulness of coproporphyrines in predicting nephrotoxic effects. Further, a relationship between urinary porphyrin changes and alterations in neurobehavioral function resulting from prolonged Hg exposure were described by Echeverria et al (1994). However, alterations in porphyrin excretion do not necessarily represent a step in the casual pathway between exposure and effect and thus, its value as a predictive biomarker of neurotoxic effects has yet to be established.

Induction of Metallothioneins

Metallothioneins (MTs) are low-molecular weight proteins (6–7 kDa) rich in Cys residues (30% of amino acid residues of MT are cysteine) and without aromatic amino acids (Eroglu et al., 2005). Therefore, MTs contain several thiol groups, which may be complexed by heavy metal ions (up to 7 molecules). Hg^0 and MeHg are known to induce MT synthesis after conversion to Hg^{2+} in tissues, especially in the kidney and liver (Gerson and Shaik,

1982; Piotrowski et al., 1974; Yasutake and Nakamura, 2011), and binding to its SH groups concurrently enhances urinary excretion of Cu^{2+} and Zn^{2+} (Chmielnicka et al. 1986, Liu et al. 1992). However, other metals, both essential (Zn^{2+} and Cu^{2+}) and non-essential (Ag^{2+}; Cd^{2+}) also induce MT synthesis (Zalups, 2000); thus, it becomes exceedingly difficult to discern between Hg-induced alterations in the presence of other metals with this biomarker.

Oxidative stress indicators (ROS and NOS)

Mercurials are known to enhance production of reactive oxygen/nitrogen species (ROS/RNS), such as hydrogen peroxide (H_2O_2), the superoxide anion (O_2^-) and nitrous oxide (NO^-) (Farina et al., 2011; Grotto et al. 2010; Lund et al., 1991; 1993). Increased ROS production enhances lipid peroxidation (LPO), which has been widely used as an indicator of Hg-mediated oxidative stress (Huang et al 2011; Kong et al. 2012; Mahboob et al., 2001; Suda and Takabashi, 1992). However, many compounds in the environment increase ROS production including pesticides, cadmium, and PAHs to name a few. Thus, analogous to GSH, these biochemical changes are not ideal markers of selective Hg-induced toxicity in a multi-contaminant scenario due to the lack of specificity.

Biomarkers of Effect

Neurobehavioral alterations—In order to correlate exposure to mercurials with development of neurotoxic effects, many studies relied on clinical observations of subtle alterations in neuromotor, neuropsychological and neurophysiological functions (Bose O'Reilly et al., 2010). These include assessment of various endpoints such as language skills, attention deficit, memory, hand–eye coordination, reaction time, IQ tests and neurological and cardiac function (Bose O'Reilly et al., 2010; Cordier et al., 2002; Dahl et al., 1996; Grandjean et al., 1997; 1999; Lebel et al., 1998; Myers et al., 1997; 2003). In toddlers, frequently assessed endpoints include the age-related achievement of developmental milestones such as sitting, standing up, walking and talking (Dórea et al., 2014; Grandjean et al., 1995; Myers et al., 1997).

The strength of association between Hg exposure and neurobehavioral alterations varies according to the endpoint analyzed (Wigle et al., 2008), which correlates with the fact that inherent to this type of endpoints is the occurrence of confounding factors that main interpretation of results difficult (Grandjean et al., 1995). In addition, these alterations may represent either reversible effects or already established manifestations of toxicity; thus, for risk prevention, it is imperative to identify biomarkers predictive of early toxic manifestations (Santos et al., 2007). Of note, several of the aforementioned behavioral impairments, which are detected in Hg-exposed humans, were also observed in rodent-based models following *in vivo* exposures (Dietrich et al., 2005; Haut et al., 1999; Moreira et al., 2012; Watanabe et al., 199b; Zimmermann et al., 2014). Notably, these behavioral changes were also noted after exposures to xenobiotics other that Hg compounds, thus not enabling for specific use as biomarkers of Hg associated effects. Nonetheless, neurobehavioral alteration may be employed as tools for quantifying the extent of toxicity (mainly neurotoxicity) in subjects knowingly exposed to Hg compound.

Renal dysfunction—The kidneys are the main organs of Hg accumulation following exposure to Hg^0 and Hg^{2+} and all mercuric forms produce nephrotoxicity (Zalups, 2000; Bridges and Zalups, 2010). The primary site of Hg deposition in the kidney is the proximal tubule with segments S2 and S3 being affected first (Bridges and Zalups, 2010). Unless exposure to Hg compounds occurs acutely, in which case kidney accumulation the metal rapidly (Zalups and Barfuss, 2002b), alterations in renal function often remain clinically undectected (Bernard and Lauwerys, 1989). Thus, the need arises for reliable biomarkers capable of detecting changes in kidney function prior to their irreversible damage.

Early toxicity to the proximal tubule is identified by measuring the activity in urinary enzyme activities from the luminal brush-border of the proximal tubule, such as γ-glutamiltransferase (γ -GT) (Diamond and Zalups, 1998). As toxicity and cell death progress, intracellular enzymes such as lactate dehydrogenase (LDH), N-acetyl-β-D-glucosaminidase (NAG) and β-galactosidase are found in urine (Bernard and Lauwerys, 1989). Levels of α1, β2 microglobulins and retinol-binding protein (RBP) also represent sensitive markers of tubular dysfunction (Franko et al., 2005). Kidney injury molecule-1 (Kim-1) has also been associated with Hg-mediated nephrotoxicity in animal studies (Wang et al., 2015; Zalups et al., 2014; Zhang et al., 2008; Zhou et al., 2008) and is claimed to allow detection of early tubular damage (Zhou et al., 2008). Neutrophile gelatinase-associated lipocalin (Ngal) was utilized as a predictor of early metal nephrotoxicity (Zalups et al., 2014). Altered, glomerular kidney function is often assessed by measuring urinary levels of high molecular proteins, such as albumin, IgG and transferrin (Bernard and Lauwerys, 1989) or by evaluating glomerular filtration rate (eGFR) by measuring plasma creatinine or blood urea nitrogen (Zalups et al., 2014).

Several studies in humans used different combinations of these biomarkers to establish a connection between nephrotoxic effects and Hg exposure. In chloroalkali workers exposed to Hg (U-Hg range: 1.1–124 μg/g creatinine), a significant increase in NAG activity was observed as well as in α1-microglobulin excretion (U-Hg > 35 μg/g creatinine) (Jarosinska et al., 2008).The same study reported elevated albumin levels in urine, indicating altered glomerular function. Similarly, workers exposed to Hg^0 with U-Hg above 50 μg Hg/g creatinine showed signs of glomerular proteinuria as evidenced by enhanced excretion of albumin, IgG and transferrin (Bernard and Lauwerys, 1989). In miners, previously exposed to Hg (average U-Hg: 68.24 μg/L), glomerular and tubular dysfunction was noted with elevated excretion of albumin, IgG and α1-microglobulin, suggesting that nephrotoxic effects remain long after cessation of exposure (Franko et al., 2005).

In non-occupationally exposed subjects, where excretion of U-Hg is considerably lower, the response observed in renal function markers is unclear. In subjects living near chloralkali plants, no significant association between U-Hg (median 1.2 μg Hg/g creatinine) and renal markers (α1-microglobulin, NAG, albumin) was observed (Jarosinska et al., 2008). In contrast, in individuals residing in the vicinity of a ASSGM area in China (average 1.24 μg Hg/L urine) concentrations of β2 microglobulin were increased relative to control subjects (Tian et al., 2009).

The effect of Hg^0 release from dental amalgams on kidney function has also been addressed in several studies. NAG activity in urine demonstrated significant positive correlation with Hg^0 release from dental amalgam fillings in children from Saudi Arabia (average 2.75 μg Hg/g creatinine) (Al-Saleh et al. 2012). However, in a similar study in US children, no marked effect of Hg^0 was found with NAG activity in urine (Barregard et al., 2008). In both studies, no significant correlation was noted between Hg and α1 and β2-microglobulin, RBP or albumin levels. In addition, in a study analyzing renal function markers in a population with low exposure to different metals, including Hg (median urine levels= 1.4 μg Hg/g creatinine), no marked correlation between urinary Hg with eGFR and Kim-1 (Callan et al., 2015) was noted. Assessment of the relationship between Hg exposure (blood Hg) and renal function in the US population (aged over 40 years) by Lin and co-workers (2014) showed no significant association between exposure (<10 μg Hg/L) and albuminuria. Nevertheless, Hg blood levels correlated negatively with eGFR. A prospective cohort examining premenopausal women with low-level exposure to metals reported that a 2-fold elevation in Hg was associated with increased protein levels in serum, whereas alkaline phosphatase was reduced (Pollack et al., 2015). The significance of these biomarker responses to low exposure levels findings is unclear; however, it is evident that the abundance of markers of renal function allowing for identification of early nephrotoxicity effects, contrasts with the absence of measurable biochemical endpoints predictive of early neurotoxic effects.

Indicators of susceptibility—Common indicators of susceptibility to Hg include life-stage, socio-economic and geographic situation, fish consumption habits and occupation (WHO/UNEP, 2008). The *in utero* developing fetus and first stages of infancy are the life-stages most sensitive to mercurials (Clarkson and Magos, 2006) and thus these population groups constitute the core target of Hg risk assessment (Carvalho et al., 2008b; Grandjean et al., 1992; 1997, 2004; Myers et al., 2003, Nunes et al., 2014a; 2014b). Further, the constraints imposed by geographical and socio-economic context (Burger et al., 1999; Carvalho et al., 2008b) of a population, such as food habits (high fish consumption) or occupation (ASSGM workers) might alter vulnerability to Hg-mediated toxicity and have been used in epidemiological studies as indicators of susceptibility (Bose O' Reilly 2008; Carvalho et al., 2008b; Cordier et al, 2002; Grandjean, 2004).

New perspectives: mechanistic-based biomarkers

Research linking genetic polymorphisms to susceptibility to mercuric compounds has brought a new perspective into identifying vulnerable population sub-groups. These genetic factors are subsequently discussed.

New biomarkers of effect

As previously mentioned, biomonitoring of Hg exposure is predominantly based upon quantitative analyses of Hg in tissues and biological fluids such as urine, nails, blood and hair. In addition, there are specific proteins (enzymes, transporters and transcriptional factors) and signaling pathways that have been linked to exposure to Hg compounds. Albeit, there is no consensus whether these proteins and pathways might be adequate to quantify the

extent of toxicity in Hg-exposed subjects, some of these “end-points” may potentially be useful as biomarkers.

Conjugation of mercury compounds with cysteine (Cys)-containing compounds and Se (e.g. SeCys-containing proteins), may lead to toxicity. Our knowledge regarding metal speciation in relation to binding to both low molecular mass molecules containing selenol and thiol groups or high molecular mass proteins is limited. The identification of single- or, preferentially, multi-targets of Hg that might represent early markers of effect to low levels of mercurial exposure is highly desirable, yet a difficult task to achieve. “Proteomic profiling” has recently emerged as a strategy capable of identifying early targets of Hg that may potentially be employed as new biomarkers (de Oliveira Souza et al., 2016) albeit this toxicoproteomic approach remains incipient (Merrick, 2006). Thus, future studies need to be conducted in experimental models of intoxication with mercurials encompassing Hg speciation in plasma, blood and target tissues. This requires advances in high throughput accurate mass and time (AMT) proteomic analytical techniques, mass spectroscopy techniques (MS), particularly inductively coupled plasma-MS (ICP-MS), MALDI and ESI-MS to identify proteins covalently bound to Hg. It is noteworthy that micro-liquid chromatography (μLC)-ICP-MS, ESI-MS and MALDI-MS techniques were utilized to quantify the labeling of ovalbumin by para-hydroxy-mercurybenzoate (Kutscher et al., 2008). The development of new biomarkers is particularly important to enable prediction of adverse effects to overcome insufficiencies in risk assessment analysis, particularly inter-individual variations in sensitivity to Hg compounds.

Assessment of Hg-induced nephrotoxicity relies on biomarkers present in urine that reflect directly early target organ (kidney) toxicity. The equivalent in neurotoxicity evaluation would be to analyze biomarkers in the cerebrospinal fluid, which is not feasible for routine analysis. Thus, the major challenge in Hg-mediated toxicity is to develop biomarkers that are predictive of neurotoxic events, similar to those existing for nephrotoxicity.

Glutamine/glutamate changes

Mercuric compounds affect multiple neurotransmitter systems. Neurotoxic effects associated with MeHg were shown to involve the glutamate/glutamine cycle. Glutamate is the predominant excitatory neurotransmitter in the CNS, but in excess is a neurotoxin. For this reason, glutamate is actively removed from the synapse. While both neurons and astrocytes take up glutamate, the majority is removed by astrocytes. Excitatory amino acid carrier 1 (EAAT3), excitatory amino acid transporter 4 and 5 (EAAT4 and EAAT5), glutamate/aspartate transporter (GLAST), and glutamate transporter 1 (GLT1) are implicated in the removal of glutamate from the synapse by astrocytes (GLT and GLAST) and neurons (EAAT3, EAAT4, and EAAT5). MeHg preferentially accumulates in astrocytes and increases extracellular glutamate levels by reducing glutamate uptake by GLT and GLAST (Aschner et al., 1993; Brookes, 1992; Qu et al., 2003). In mice exposed to MeHg through milk, there was nearly 50% reduction in glutamate uptake in cerebellar slices compared to controls (Manfroi et al., 2004). Increased levels of synaptic glutamate resulted in *N*-methyl-D-aspartate (NMDA) receptor-dependent excitotoxicity. Park et al (1996) found that blockade of the NMDA receptor with antagonists prevented death in cultured cerebral

neurons. In addition, MeHg primes neurons for excitotoxic death by enhancing expression of NMDA receptor NR-2B subunit in the hippocampus of exposed rats (Baraldi et al., 2002).

Glutamate released from neurons is normally taken up by astrocytes, where it is converted into glutamine to be transported back to the neuronal terminal. In addition to inhibiting uptake of glutamate, Hg compounds disrupt glutamine synthesis and transport. All Hg compounds were reported to decrease glutamine synthetase (GS) activity and inhibit glutamine transport in cultured astrocytes (Allen et al., 2001a; Yin et al., 2007) and in rat brain (de Oliveira Souza et al., 2016). Further, MeHg inhibits GS activity in brains of rats, mice and sheep, where elevated blood levels of MeHg correlated to reduced GS activity (Kwon and Park, 2003). Both MeHg and $HgCl_2$ were found to inhibit the glutamine/amino acid transporter (ASCT2), which shuttles glutamine into neurons from astrocytes (Oppedisano et al., 2010).

In summary, the use of glutamatergic transmission as a marker of Hg exposure in humans is complicated due to lack of sensitivity, selectivity or predictability as a biological indicator of Hg exposure per se. The presence of NMDA-like receptors and high levels of glutamate in platelets (Franconi et al., 1996; 1998; Kalev-Zylinska et al., 2014) indicates that NMDA-mediated responses do not necessarily reflect exposure to Hg, as these receptors are modifified by a myriad of factors including genetic, epigenetiv metabolic and external stressors including other metals as well as age and gender.

Glial fibrillary acidic protein

Injury to the CNS by multiple stimuli, including Hg compounds, results in reactive gliosis, a condition where there is hypertrophy and activation of glial cells, particularly astrocytes. Glial fibrillary acidic protein (GFAP) is one of many intermediate filament proteins that are up-regulated in glial cells during reactive gliosis. Exposure to non-cytotoxic concentrations of MeHg increased the expression of GFAP in both primary rat astrocytes and microglia *in vitro*, which correlated with decreased glutamate and serotonin reuptake by astrocytes and enhanced IL-6 release from microglia (Dave et al., 1994; Eskes et al., 2002). Interestingly, Jebbett et al (2013) found that MeHg increased ciliary neurotrophic factor (CNTF)-induced expression of STAT3-target genes, including GFAP, in neural progenitor cells enabling glial differentiation.

Data suggest that developmental exposure to MeHg may affect proper glial differentiation *in vivo*. In rat pups exposed to MeHg *in utero*, elevated levels of GFAP were detected in the corpus callosum, brain stem, cerebellum and hippocampus (Barone et al., 1998; El-Fawal et al., 1996; Gao et al., 2006). Increased GFAP following MeHg exposure was also observed in zebrafish (Cambier et al., 2012), suggesting its use as a biomarker for analyzing wild-life specimens. It is noteworthy that exposure to organophosphates or neoniocotinoid also results in enhanced GFAP staining in humans rats (Abou-Donia et al, 2008; 2013). In summary, the use of GFAP as a marker of Hg exposure in humans lacks sensitivity, selectivity or predictability as a biological indicator of Hg exposure per se.

Changes in the activity of enzymatic systems

As previously mentioned, many critical enzymes including those involved in antioxidant defense rely on the presence of reactive thiol and/or selenol groups in the active site. Binding of mercurials to these groups impairs their catalytic activity and leads to toxicity. Enzymes containing reactive thiol and/or selenol groups are potential targets for Hg compounds and thus may constitute useful biomarkers.

Notably, selenols (SeH) have generally higher nucleophilicity than thiols and are present in mammalian cells as selenocysteine (Sec) in the active site of selenoproteins catalyzing redox reactions (Hatfield et al. 2014; Lu and Holmgren, 2009). The selenol group is generally more reactive than thiols (lower pKa) towards electrophilic forms of Hg (Sugiura et al., 1976). In addition, abundance of selenoproteins is lower. Accordingly, selenoproteins represent important (and relatively “more specific”) targets for Hg-mediated toxicity. Among the enzymes known to be altered by exposure to Hg compounds, one needs to consider ROS scavenging enzymatic systems, such as superoxide dismutase (SOD), catalase (CAT)and glutathione peroxidase (GPx), and redox active proteins from the thioredoxin (Trx) system.

Catalase and Superoxide Dismutase

Changes in CAT and SOD activities were proposed as biomarkers of Hg-induced oxidative stress. However, reported findings are inconsistent regarding the effect of mercurials on the activity of these enzymes *in vivo*. In workers exposed to Hg^0, Perrin-Nadif et al. (1996) observed a rise in CAT activity in plasma similar to the observations by Kobal et al. (2004; 2008) comparing miners with past exposure to Hg (U-Hg: 2.1 μg/L) and control subjects (U-Hg: 1.4 μg/L). On the other hand, Pinheiro et al (2008) found a negative association between blood CAT activity and Hg exposure as seen in hair levels in women living in the Amazon. Similarly, Carneiro and co-workers (2014) observed a negative association between plasma MeHg and CAT activity. These contradictory results are also inherent in animal studies. In rats Nath et al. (1996) and more recently Silva de Paula et al. (2016) noted no significant changes in CAT activity following exposure to Hg compounds, whereas Kong et al. (2012) reported a decrease in activity levels during the embryonic development of a fish-model.

The lack of a clear pattern was also demonstrated for SOD activity levels following exposure to Hg (Hussain et al., 1997; Perrin-Nadif et al., 1996). A numerical decrease in erythrocyte SOD activity (5%) was observed in workers exposed to Hg^0 (U-Hg: 77.44 ± 48.15 μg/L) (Zabínski et al., 2000). In Hg^0 exposed dental personnel (B-Hg: 7.74 ± 1.03 μg/L vs 4.79 ± 0.84 μg/L in control subjects), Samir and Aref (2011), reported that erythrocyte SOD activity was reduced by 40% and that this decrease was inversely correlated to the number of working years. Kobal et al (2004) compared RBC SOD activity between former Hg miners (U-Hg: 2.1 μg/L) and control subjects (U-Hg: 1.4 μg/L), and noted no significant difference.

In fish exposed to MeHg no marked effect on SOD activity was found in liver and brain (Branco et al., 2011), whereas in brain of rats exposed to MeHg a significant increase in activity was observed (de Oliveira Souza et al., 2016). In the blood of rats chronically

exposed to MeHg SOD activity remained unchanged relative to controls (Silva de Paula et al., 2016)

Glutathione peroxidase

Selenols are present in the active site of selenoproteins catalyzing redox reactions (Lu and Holmgren, 2009; Hatfield et al. 2014) and are preferential targets for Hg-mediated toxicity. An experimental *in vitro* study with primary cultures of cerebellar neurons showed that the activity of the selenoenzyme glutathione peroxidase (isoform 1; GPx1), was decreased by low-dose MeHg (Farina et al., 2009), prior to changes in several biochemical parameters that are normally affected by high-dose MeHg exposures. The diminished enzyme activity was a consequence of direct inhibitory effects, which were probably related to Hg-selenol interactions. Interestingly, these events were responsible for enhanced susceptibility to peroxides, enhanced lipid peroxidation and neuronal death (Farina et al., 2009). In agreement, another study established that GPx also serves as a crucial molecule involved with MeHg-induced neurotoxicity (Franco et al., 2009).

Zemolin and coworkers (2012) showed that the protein levels of GPx4, an important isoform of GPx family whose activity is crucial to detoxify lipid peroxides in cell membranes was decreased in encephalic structures of MeHg-exposed mice. Thus, in addition to direct interaction between MeHg and the selenol group of GPx, the fall in GPx expression also contributes to loss of activity. In agreement, cultured cells exposed to MeHg demonstrated a “selenium-deficient-like” condition, which affects GPx1 synthesis through a posttranscriptional action (Usuki et al., 2011). Collectively, these effects indicate that the selenoenzyme GPx as an important molecular target of MeHg-induced neurotoxicity. Nevertheless, studies in humans are less clear. In dental workers exposed to Hg^0 (B-Hg: 7.74 ± 1.03 μg/L vs 4.79 ± 0.84 μg/L in control subjects), a negative correlation was found between Hg exposure and GPx activity (40% reduction) in erythrocytes (Samir and Aref, 2011). However, for higher exposure levels (average B-Hg: 79.1 μg/L), Jayaprakash (2009) reported a reduction of only 30% in RBC GPx activity. Interestingly, Kobal et al. (2008), found no marked differences in RBC GPx activity between former Hg miners (average U-Hg: 2.53 μg/g creatinine) and control subjects (average U-Hg: 1.31 μg/g creatinine), even though Se excretion was higher in miners as evidenced by lower plasma and higher urinary Se (Kobal et al., 2004). In contrast, in Chinese workers, Hg^0 exposure (U-Hg: 86.8±65.2 vs 1.25±1.5 μg/L) was associated with higher (+13%) plasma activity of GPx (Chen et al., 2006). In addition, in sports fishermen consuming Hg via dietary fish, a positive effect of exposure was seen in the activity of blood GPx (+10%), albeit with only numerical rise in blood Se (Bélanger et al., 2008) whereas in a fish-eating population from the Brazilian Amazon, a negative association between plasma MeHg and GPx activity was detected (Carneiro et al., 2014).

Thioredoxin and Thioredoxin Reductase

Carvalho et al., (2008a; 2011) showed *in vitro* that mercurials target the enzymes of the thioredoxin system – thioredoxin reductase (TrxR) and thioredoxin (Trx) - binding to their active site thus decreasing activity. The thioredoxin system is found in all tissues and involved in multiple functions associated with cellular maintenance and survival including

protein repair, regulation of the cell cycle, cell signaling (Lillig and Holmgren, 2007). Since the Trx system is upstream of several biochemical pathways, its inhibition by mercurials represents a key step in mediation of Hg-induced toxicity (Carvalho et al., 2008a).

Several compounds are known to produce inhibition of both enzymes of the Trx system, with TrxR being particularly prone to interaction with electrophilic agents due the presence of selenol (selenolate at physiological pH) in the open C-terminus of its active site (Arnér, 2009). As depicted in Table 1, many of the compounds known to target TrxR include soft metals, such as gold and platinum (e.g. aurofin and cisplatin) (Mustacich and Powis, 2000; Rigobello et al., 2004; Witte et al., 2005) cadmium, arsenic and zinc (Lu et al., 2007; Rigobello et al., 2004).

It is noteworthy that Hg compounds especially of Hg^{2+}exhibit an inhibitory capacity against purified TrxR1 which is remarkable among metallic compounds (Table 1). Further, the inhibitory effect of mercurial compounds is particularly fast, with complete blockade of TrxR activity after only 5 min incubation (Carvalho et al., 2008a). These results clearly show that TrxR is highly susceptible to inhibition by mercurials (Carvalho et al., 2008a; 2011). In addition, it is significant that Hg^{2+} displays greater potency as an inhibitor of TrxR, suggesting that MeHg upon demethylation produces an even greater toxic outcome (Carvalho et al., 2008a). Trx activity was also found to decrease upon exposure to Hg *in vitro*, with both Hg^{2+} and MeHg targeting the dithiol at the active site of Trx leading to loss of activity, albeit with higher IC_{50} than the one observed for TrxR (Carvalho et al. 2008a).

Mercurials also affect TrxR in HeLa and HEK293 cells in a time- and concentration-dependent manner (Carvalho et al., 2008a). Inhibition of TrxR was detected after only 7 hr exposure (Carvalho et al., 2008a), correlating well with observations *in vitro* showing a rapid inhibition of TrxR by mercurials. Interestingly, the activity of glutathione reductase (GR) was not affected by mercurials in HeLa cells, reinforcing the fact that the Sec residue in the C-terminus of TrxR is a target for Hg (Carvalho et al., 2008a). Experiments in a fish model (Branco et al., 2011; 2012a) further corroborated the importance of targeting the Trx system and in particular TrxR as a key step in mediating Hg toxicity. This fact was further supported by the findings that the activities of GR and GPx1 were less sensitive to Hg compounds (Branco et al., 2011; 2012a). Experiments with rodents also demonstrated that TrxR activity is modified by MeHg administration; however, the mangnitude of the effects varied depending upon the schedule of intoxication, the species, developmental period, animal gender and subcellular fraction considered (Dalla Corte et al. 2013; Ruszkiewicz et al. 2016; Wagner et al. 2010). Trx was also reported to be affected by mercurials in cells and animal models but not as severely as TrxR (Carvalho et al. 2008a, Branco et al., 2011; 2012a; Ruszkiewicz et al. 2016).

Results in human THP1 monocytic cells exposed to Hg^{2+} suggested that Hg induced transcription of the enzymes of the Trx system, via an increase in nuclear factor (erythroid-derived 2)-like 2(Nrf2) levels (Wataha et al., 2008). Recently, it was shown that induction of cytosolic thioredoxin reductase (TrxR1) via Nrf-2 is faster during exposure of liver cells to Hg^{2+} - leading to elevated mRNA and enzyme expression – than to MeHg, which may explain the higher cytotoxicity of this compound (Branco et al., 2014). Most importantly

mitochondrial thioredoxin reductase (TrxR2) is not regulated by Nrf-2 and its activity is markedly affected by both Hg^{2+} and MeHg, suggesting its importance as a target for Hg-associated toxicity (Branco et al., 2014) (Figure 6). The targeting of the mitochondria and the effects of Hg in the overall function of this organelle may therefore constitute a promising field of research in the discovery of additional novel biomarkers.

Overall, results to date indicate that both TrxR and Trx are important targets for mercurials and their inhibition is likely upstream of development of Hg-initiated toxic outcomes (Branco et al, 2012b). Accordingly, evaluation of TrxR and Trx in accessible matrices, such as blood and plasma may provide a useful tool for the prediction of Hg-induced adverse effects but to our best knowledge this possibility has not been addressed in human subjects.

Only few studies evaluated changes in the activity of selenoenzymes in humans exposed to Hg (Chen et al. 2006; Hagmar et al., 1998); however, the status of Se may be critical in limiting the toxicity of Hg (Farina et al., 2011; Khan and Wang, 2009; Watanabe, 2002). Consequently, the use of selenoenzymes and/or selenoproteins as biomarkers of effect for Hg might require concomitant Se status determination.

Pentose Phosphate Pathway dehydrogenases

NADPH is mostly generated by the thiol-containing enzymes glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH) of the pentose phosphate pathway (PPP). NADPH functions as one of the main intracellular reducing agents and an essential co-factor required for the normal function of antioxidant cycles, such as GSH and Trx systems (Horecker, 2002). Targeting of these enzymes by Hg compounds was reported for several mercurials in HepG2 and SH-SY5Y cells with inhibition intensity of Hg^{2+}>MeHg≈EtHg>Thimerosal (Rodrigues et al., 2015). EtHg and MeHg produced equivalent and significant inhibition of G6PDH activity and to a lesser extent 6PGDH, especially in neuroblastoma cells. In MeHg-incubated fibroblasts Amoli et al (2011) noted a decrease in the activity of the oxidative branch of PPP impairs ribose synthesis which is essential for nucleotide production causing DNA damage.Interestingly, Zabinski et al. (2000) observed that in workers exposed to Hg^0 (U-Hg: 77.44 ± 48.15 μg Hg/L) G6PDH activity in erythrocytes was redeuced by 25% relative to non-exposed controls.

During the last decade evidence has been accumulating concerning the role of selenoenzymes, namely TrxR and GPx, as targets for Hg compounds. The importance of these enzymes as well as of Trx and GSH for cellular function and homeostasis stresses the importance of a more comprehensive understanding of their role in Hg-associated toxicity. It is worthwhile noting that it possible to measure in plasma the activity of these enzymes, and thus it is necessary to determine if activity of selenoenzymes in blood reflects Hg exposure and whether enzymic activity is affected in brain. In the case of the thioredoxin system (TrxR and Trx), since their inhibition is an early event in Mg-mediated toxicity, this system may serve as a potential predictive biomarkers of toxic effect.

Transcription factors

Altered gene transcription following MeHg exposure has been documented in many recent toxicogenomics studies (Hwang et al., 2011a; Robinson et al., 2011; Theunissen et al., 2011;

Yadetie et al., 2013). Most of these studies were performed using cell lines; however, their application to *in vivo* exposures is relevant as demonstrated by studies investigating the roles of the transcription factors nuclear factor (erythroid-derived 2)-like 2(Nrf2) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) as discussed below.

Nrf2

Nrf2 is a basic leucine zipper transcription factor that responds to oxidative stress. Under basal conditions, Nrf2 resides in the cytoplasm bound by kelch-like ECH-associated protein1 (Keap1). Keap1 is associated with Cullin 3, an E3 ubiquitin ligase, which targets Nrf2 for proteasomal degradation (Baird and Dinkova-Kostova, 2011).

Oxidative stress in general, including exposure to MeHg, enhances Nrf2 to be released from Keap1 by disruption of cysteine residues on Keap1 or through oxidant-dependent kinase signaling. Unbound Nrf2 translocates into the nucleus, where it can bind to antioxidant response element (ARE) promoters as a heterodimer with Maf proteins (Baird and Dinkova-Kostova, 2011). Nrf2 induces the expression of several cytoprotective proteins, including NAD(P)H quinine oxidoreductase (NQO1), glutamate-cysteine ligase catalytic subunit and modifier subunit (GCLC and GCLM), and heme oxygenase-1 (HO-1) (Baird and Dinkova-Kostova, 2011). Nrf-2 also enhances transcription of the TXNRD1 gene, increasing TrxR1 mRNA build up in the cytosol and TrxR1 *de novo* synthesis. In HepG2 cells Branco et al (2014) demonstrated that this process is faster upon exposure to Hg^{+2} than to MeHg (Figure 6).

In addition to redox regulation, Nrf2 is also regulated by protein kinase pathways (Sherratt et al., 2004). Nrf2 interacts with p38 mitogen-activated protein kinase (Alam et al., 2000; Yu et al., 2000), ER-resident kinase PERK (Kobayashi et al., 2006) and a Src family tyrosine kinase, Fyn (Jain and Jaiswal, 2006). The PI3K/Akt pathway controls Nrf2's function upstream of Fyn (Niture et al., 2014). Activation of Nrf2 by MeHg was noted in cell lines and primary cells allowing for up-regulation of NQO1 and HO-1 (Ni et al., 2010; Toyama et al., 2010; 2011b; Wang et al., 2009). Further, MeHg induced Nrf2 activity in rodents and fish (Deng et al., 2014; Toyama et al., 2011a; Yadetie et al., 2013). Interestingly, levels of Cullin 3 were decreased following MeHg exposure in SH-SY5Y cells (Toyama et al., 2011b), suggesting that there is coordinated control to prevent degradation of Nrf2. While oxidative stress derived from MeHg exposure might activate Nrf2, direct Nrf2 activation was observed following MeHg binding to recombinant Keap1 and by MeHg-GSH adducts (Toyama et al., 2007; Yoshida et al., 2014). Kinase regulation of MeHg-induced Nrf2 activation has yet to be demonstrated, although microarray studies of Atlantic cod found upregulation of MAP kinase MEK4 by MeHg, which potentially may activate Nrf2 by JNK activation (Yadetie et al., 2013). Nrf2 was shown to be protective in a *Caenorhabditis elegans* (*C. elegans*) model of dopaminergic neurodegeneration induced by MeHg, where reduction of SKN-1, the Nrf2 homologue, increases the vulnerability for neurodegeneration following either an acute or a chronic exposure to this compound (Martinez-Finley et al., 2013; Vanduyn et al., 2010). Knockdown of Nrf2 in worms displays a elevated levels of ROS and increased measures of dopaminergic cell dysfunction (Martinez-Finley et al.,

2013). Activation of Nrf2 by MeHg represents a protective response; however, MeHg often alters gene expression resulting in neurotoxic effects.

NF-κB

NF-κB is a transcription factor that controls the regulation of inflammatory cytokines (IL-6 and IL-8) and chemokines (CCL2 and CCL4), as well as anti-apoptotic proteins (Bcl2, TRAF1 and TRAF2). NF-κB consists of a heterodimer of a class I NF-κB protein (p50 or p52) and a class II NF-κB protein (p65/RelA, RelB, or c-Rel) (Hoesel and Schmid, 2013). Combinations of the subunits vary by cell type and development stage. NF-κB is bound in the cytoplasm by the inhibitor IκB where it is sequestered from activating transcription. When stimulated, IκB kinase (IKK) phosphorylates IκB, targeting IκB for proteasomal degradation, leaving NF-κB free to translocate to the nucleus and initiate gene transcription. There are several stimuli that result in activation of NF-κB. Binding of cytokines or bacterial or viral antigens to cell surface receptors activate kinase cascades, which ultimately phosphorylate IKK, leading to its activation. Many of these kinases (IKK, PI3K, AKT, MEKK-1) are redox sensitive (Pantano et al., 2006), enabling activation by oxidative stressors, such as ROS and oxidized lipids. Therefore, both lipid peroxidation end products and ROS derived from MeHg exposure possess the potential to activate NF-κB.

Exposure to MeHg leads to increased markers of inflammation mediated by NF-κB gene trascription. MeHg exposure enhanced NF-κB activation as measured by elevated levels of the RelA/p65 subunit and DNA bound NF-κB, in both cerebrum and cerebellum of rats and mice (Dong et al., 2001; Hwang et al., 2011). This leads to increased expression of chemokines CCL2 and CCL4 and anti-apoptosis protein Bcl2a1b (Hwang et al., 2011; Lee et al., 2012). *In vitro* MeHg elevated both CCL2 and IL-6 release from glial cells (Chang, 2007; Kim et al., 2012).

Even though there is sufficient evidence reporting a relationship between Hg exposure and changes in Nrf2 and NF-κB pathways, most of the available data are derived from experimental (mainly *in vitro*) studies. Further, these responses lack specificity and are highly pleiotropic which diminishes their value as biomarkers of Hg-induced toxicity. Thus, it is difficult to predict the usefulness of these transcription factors for employment as human biomarkers. In sunmmary, due to the numerous stimuli activate NF-kB, these are not specific biomarkers for Hg exposure.

Immunological outcomes

Immunotoxicity due to mercurial compounds has been described in a variety of animal species, including humans (Sweet and Zelikoff, 2001). Immunotoxic effects arise at Hg concentrations that are below those reported to damage the CNS and kidneys (Vas and Monestier, 2008) and consequently, may be considered as a critical effect for development of toxicity. The mercuric ion exerts a general stimulatory effect on the immune system and several studies in rodents (Fournie et al., 2001; Nielsen and Hultman, 2002; Pollard and Hultman, 1997) demomstrated the ability of Hg^{2+} to promote autoimmunity, which is primarily mediated via NF-kB signaling (Pollard and Kono, 2013).

In vitro results suggest that MeHg exerts a similar effect on the immune system as Hg^{2+} with induction of pro-inflammatory cytokines. However *in vivo*, the immune response to MeHg appears to be more complex, since it acts initially as an immunosuppressor, but as exposure continues and it is converted to Hg^{2+}, a stimulating effect on the immune system occurs inducing production of autoantibodies and increasing serum pro-inflammatory cytokine levels (Havarinasab and Hultman, 2005).

Data in cells indicate that EtHg appears to yield an atypical pattern of pro- and anti-inflammatory cytokines (Gardner et al., 2010b). Dendritic cells exposed to increasing levels of Thimerosal, showed a reduction in secretion of pro-inflammatory cytokines associated with GSH depletion. Consequently, Th2 activation is enhanced while Th1 dependent inflammatory response is reduced (Agrawal et al. 2007). In contrast, in H2 mice exposed to Thimerosal, the immune response was skewed towards Th1 activation similarly to a typical Hg^{2+} exposure (Havrinasab et al., 2007) and resulted in production of antinucleolar antibodies towards a 34-kDa nucleolar protein fibrillarin (Havarinasab et al., 2005). This autoimmune response is significantly more potent when mice are exposed to EtHg (as Thimerosal) compared to MeHg, which might be attributed to a faster conversion to Hg^{2+} (Havarinasab et al., 2004; 2005).

Occupational exposure to Hg^0 has also been associated with a rise in serum levels of both antinuclear (ANA) and antinucleolar (ANoA) autoantibodies and pro-inflammatory cytokines (IFN-γ, TNF-α and IL-1β) (Gardner et al., 2010a; Motts et al., 2014). Accordingly, in sensitized individuals, exposure to low levels of Hg^0 or MeHg from fish deregulate the immune response, altering serum levels of pro-inflammatory cytokines (e.g. IL-1β and TNF-α) and anti-inflammatory cytokines such as IL-4 and IL-17 (Nyland et al., 2011).

In summary, immune responses to mercurial compounds have not been thoroughly investigated and knowledge of molecular mechanisms is still elusive. However, a limitation exists in that these responses occur with numerous compounds and thus could not be reasonably employed as biomarkers of Hg-mediated toxicity. Nevertheless, potential for discovery and validation of new biomarkers is possible.

Other Biological Responses to Mercury

Protein adducts—Protein adducts may occur in response to Hg exposure by two mechanisms. Mercury, given its electrophilicity, directly interacts with protein thiol and selenol groups leading to a loss in residues nucleophilic potential. This may result in decreased protein activity. As indicated previously, MeHg binds the selenol group in GPx1 contributing to ROS generation (Farina et al., 2009). MeHg was also found to directly modify cysteine groups of delta-aminolevulinate dehydratase (ALAD), an enzyme important for heme biosynthesis (Rocha et al., 1993). ALAD inhibition was reduced in both brain and liver of suckling rats exposed to MeHg; however, the enzyme was not inhibited in kidney. There is potential to use ALAD inhibition as a biomarker for MeHg exposure, as ALAD activity assays are commonly performed in blood (Fujita, 2001). However it is unknown whether there is enzymatic inhibition of ALAD in this tissue by mercury. It should be

stressed that measurement of this enzyme is affected by other metals and not a specific indicator of Hg --mediated toxicity.

In addition, oxidative stress derived from Hg exposure may lead to modification of proteins. MeHg induces inhibition of glutamate, cystine and cysteine uptake, thus adversely affecting intracellular GSH content and redox status in astrocytes (Alen et al., 2001b; 2001c; Aschner et al., 1993; Shanker and Aschner, 2001; Shanker et al., 2001, 2003). MeHg also stimulates cytosolic phospholipase A2 (cPLA2) resulting in arachidonic acid (AA) release from astrocytes, the latter directly inhibiting glutamate transporters and glutamate uptake into astrocytes (Aschner, 2000; Shanker et al., 2003). ROS interact with cysteine thiol groups on proteins. Nucleophilic Sec and Cys residues readily react with electrophilic ROS to form sulfenic (-SOH) or selenenic acids (-SeOH) which are short lived, either reacting with other cysteine residues to form disulfide bonds or further oxidized to sulfinic ($-SO_2H$) and sulfonic acids ($-SO_3H$) by potent oxidants such as superoxide anion (Janssen-Heininger et al., 2008). Both sulfinic and sulfonic acid formation are irreversible, and their intracellular accumulation may lead to altered protein function, protein degradation and even cell death. RNS formed from MeHg-induced activation of nNOS produces nitrosylation of amino acids, such as tyrosine (Caito et al., 2014; Miyamoto et al., 2001). NMDA, one such protein nitrosylated by MeHg, altered synaptic activity of cortical neurons (Miyamoto et al., 2001).

Surprisingly, there are no data in humans concerning this type of interactions with Hg compounds. However, these interactions lack specificity and thus diminish theirv utility as bionmarkers solely for Hg-associated toxicity.

Lipid peroxidation and F(2)-isoprostanes—Lipid peroxidation (LPO) is the process by which reactive species may damage lipid membranes. Mercury-induced lipid peroxidation is mainly dependent upon the ability of this metal to increase, directly or indirectly, the levels of H_2O_2 and superoxide anion. Although the induction of lipid peroxidation by H_2O_2 or superoxide is relatively low, both molecules may contribute to the formation of the hydroxyl radical by the Fenton reaction, allowing for initiation of LPO. Indeed, the chelator deferoxamine is protective against MeHg-induced lipid peroxidation in rat brain (LeBel et al., 1992), by preventing the Fenton reaction secondary to ROS and biological metals, such as iron. Organs such as the brain contain large amounts of polyunsaturated fatty acids, which are highly susceptible to LPO (Grintal et al., 2009), which seems to occur not only is the cell membrane susceptible to peroxidation, but also intracellular organelles. Damage to both lysosomal and the mitochondrial membranes by Hg compounds might lead to apoptotic cell death (Dare et al., 2001; Franco et al., 2010). Evidence for Hg compounds eliciting LPO has been extensively reported in both cultured cells and rodent models (Huang et al., 2011; Lin et al., 1996; Martinez et al., 2014; Silva de Paula et al., 2016; Vendrell et al., 2007; Watanabe et al., 1999a; Yin et al., 2007). Several studies demonstrated a link between enhanced LPO and Hg exposure in humans. Chen and co-workers (2006) analyzed serum samples from miners exposed to Hg^0 and found a positive correlation between MDA levels and exposure. Further, Kobal et al (2004) found higher urinary levels of MDA in miners exposed to Hg^0 even though the total amount of lipid peroxides did not change. In addition, LPO has been used for biomonitoring wild-life exposure to MeHg (Berntssen et al., 2003; Hoffman et al., 2005). In these samples, LPO is

typically measured by levels of individual LPO species, such as malondialdehyde, or by thiobarbituric acid reactive species (TBARS) assay (Huang et al., 2011; Watanabe et al., 1999a). However, the limitation to the utilization of LPO as a biomarker for Hg exposure lies in the fact that a vast amount of chemicals act via oxidative stress and consequent LPO that this measure could not be reasonably used to address the issue of mercury-related toxicity.

F(2)-isoprostanes (F2-IsoPs) are specific LPO products non-enzymatically formed from peroxidation of arachidonic acid by ROS. Due to their chemical stability and prevalence in all human tissues and biological fluids, F2-IsoPs are regarded as the gold standard for quantification of oxidative damage in mammals (Morrow et al., 1990). Exposure to MeHg *in vivo* and *in vitro* showed increased levels of F2-IsoPs (Farina et al., 2009; Jin et al., 2012; Stringari et al., 2008; Wormser et al., 2012). There are few studies that examined the use of F2-IsoPs in human tissues. In a study of premenopausal women in New York State found no significant relationship between MeHg blood levels (mean of 1.1 μg/l) and F2-IsoPs (Pollack et al., 2012). Similarly, a study of Canadian Inuits noted an inverse correlation between blood Se levels and F2-IsoPs, but no correlation with Hg. Interestingly in the same study Alkazemi et al (2013) found a correlation between blood Hg levels and isofuran (IsoFs) levels. IsoFs are also formed by LPO from archidonic acid under conditions of elevated oxygen tension that occur during mitochondrial dysfunction (Fessel and Roberts, 2005). Although more research is needed to verify IsoFs as biomarkers for Hg exposure, this parameter would unfortunately lack specificity.

Paraoxonase—Paraoxonase-1 (PON1), is an enzyme present on high-density lipoproteins that prevents oxidation of blood lipids and their deposition in vascular endothelium. PON1 is potentially useful as a clinical biomarker of cardiovascular risk, as well as a critical enzyme in detoxification of certain organophosphate oxons. MeHg and other metals are known to inhibit PON1 activity *in vitro.* MeHg was associated with lowered serum PON1 activity in a fish-eating population and this maybe related to the MeHg-induced cardiovascular risk (Ginsberg et al., 2014).

New Biomarkers of susceptibility to mercury

As research on the interaction between genes and environment evolves, it has become increasingly apparent that toxicity and disease risk are the result of complex interactions between genetic and environmental (epigenetic) factors that determine a high degree of variability in individual response to a given exposure (Basu et al., 2014).

Polymorphisms

A field of research that has grown in recent years is the study in human populations of the relationship between Hg exposure biomarker levels and genetic polymorphisms namely, single nucleotide polymorphisms (SNPs) in genes linked to xenobiotic ADME. The influence of these particular genes on Hg accumulation may vary considerably depending upon the level of exposure, the Hg species to which the population is exposed and also degree to which the SNP is inherent in the population or if the trait is poligenic (Barcelos et

al., 2013; Llop et al., 2015). Further, false-positive findings cannot be totally ruled out (Schläwicke-Engström et al., 2008).

Among the several SNPs related to Hg exposure, those concerning GSH synthesis and metabolism are the best studied. Several investigators (Barcelos et al., 2013; Custodio et al., 2004; 2005; Goodrich et al., 2011; Gundacker et al., 2009; Parajuli et al., 2016. Schläwicke-Engström et al., 2008) showed that Hg accumulation was altered by SNPs in genes encoding for glutamyl cysteine ligase (GCL) and glutathione synthetase (GS), enzymes involved in GSH synthesis. Depending upon the resulting phenotype (increase or decreased expression), such SNPs may lead to different levels of GSH, affecting Hg retention/excretion. similarly, SNPs in genes controlling the synthesis of different classes of glutathione S-transferases (GST), which are responsible for mediating GSH-xenobiotic conjugation reactions, have a well-documented link to individual variability in excretion of mercuric compounds (Barcelos et al., 2013; Custodio et al., 2004; 2005; Goodrich et al., 2011; Gundacker et al., 2009; Lee et al., 2010; Parajuli et al., 2016; Schläwicke-Engström et al., 2008, Woods et al., 2014a).

Polymorphisms of genes encoding for ATP binding cassette (ABC) membrane transporters, have been linked to elevated uptake of MeHg across the placenta and increased cord-blood Hg levels (Llop et al., 2014), and altered urinary excretion of Hg in ASSGM exposed populations (Engström et al., 2013). Other membrane transporters such as organic anion transporters (OATs) and L-amino acid transporters (LATs) are known to be involved in Hg uptake through membranes (Bridges and Zalups, 2005; Zalups, 1998), and SNPs in their genes have also been related to changes in Hg excretion and accumulation (Engström et al., 2013). Similarly, SNPs in genes encoding for metallothioneins (MTs), especially the isoforms MT1, MT2 and MT4, have been associated to alterations in concentrations of exposure biomarkers, namely Hg levels in urine and hair (Gundacker et al., 2009; Wang et al., 2012), and neurobehavioral endpoints (Woods et al., 2013; 2014a). The precise significance of these SNPs for MT function has not been clarified, but it is reasonable to postulate that SNPs might lead to structural changes in MTs that consequently alter protein ability to bind metals (Wang et al., 2012).

A polymorphism in the gene encoding for inositol 1,4,5-triphosphate kinase C (ITPKC) has been related to development of Kawasaki syndrome (KS) in susceptible children, following exposure to EtHg after TCV immunization (Yeter and Deth, 2012). This SNP results in increased Ca release from cells, which is at the basis for observed autoimmunity and coronary arterial lesions in KS (Yeter and Deth, 2012).

In a study on the effect of Hg^0 exposure via dental amalgams in children, it was found that a genetic variant of coproporphyrinogen oxidase (CPOX), CPOX4, was associated with enhanced susceptibility to Hg, as evidenced by lower performance in neurobehavioral testing (Woods et al., 2012; 2014a). Similar outcomes were noted in subjects with SNPs in catechol-O-methyltransferase (COMT) (Woods et al., 2014a; 2014b). Notably, both effect of CPOX4 and COMT SNPs were only evident in boys and not girls suggesting gender related differences in susceptibility. Specific polymorphisms of COMT (Val158Met), brain-derived neurotrophic factor (BDNF) and serotonin transporter gene promoter region (5-HTTLPR)

have been associated with elevated Hg levels and moodiness in dental workers exposed to Hg^0 (Heyer et al., 2004, 2008, 2009, Parajuli et al., 2016, Woods et al., 2014a).

It is of interestin that SNPs in the gene encoding for selenoprotein P1 (SelP1) were linked to changes in Hg^{2+} excretion in urine and MeHg incorporation in hair (Goodrich et al., 2011). SelP1 contains multiple Sec residues and secreted from liver to plasma and thought to be involved in Se distribution throughout the body (Burk and Hill, 2005; Papp et al., 2007) as well as known to bind Hg (Suzuki and Ogra, 2001). Thus, changes in SelP1expression or Sec content resulting from SNPs may exert an important impact in Hg distribution, excretion and affect the pool of Se available for metabolism. In this sense it would be of interest to extend this analysis to the effects of other critical molecular targets for mercurials. In addition to SelP1, Parajuli et al. (2016) found a positive association between an SNP in the gene encoding for TrxR2 and internal dose makers for Hg. Given the relevance of this enzyme for Hg toxicity this association is worth further detailed analysis.

Several other SNPs have been related with alterations in levels of Hg exposure biomarkers even though correlations are often absent or non-existent. Among others, these include apolipoprotein E (Ng, 2013; Woods et al., 2014a), eNos (de Marco et al., 2011), paraoxonase 1 progesterone receptor, transferrin (Julvez et al., 2013), kidney and brain expressed protein (KIBRA), and glutamate receptors (Woods et al., 2014a).

One of the main limitations of SNPs analysis is related to sample size, which needs to be sufficient to incorporate a significant number of the less frequent alleles in the population, in order to enable an adequate statistical analysis and understanding of the actual relevance of the polymorphism to Hg toxicity. It should be noted that prospective birth-cohort studies, with appropriate confounding controls aimed at addressing the link between SNPs and neurotoxicity in children remain to be done (Llop et al., 2015). Nevertheless, this is a promising field of research and may serve a great value at identifying more vulnerable populations or its subsets, which could then be the focus of risk management/ communication strategies.

Epigenetic Markers

The effect of Hg compounds on factors affecting gene expression other than direct modification of DNA sequences is the most recently emerging field of research in this metal toxicology. Altough there a few studies concerning the influence of Hg exposure on epigenetic markers in wildlife and lab animals (Basu et al., 2014) data from human studies are scarce.

Hypermethylation of CpG islands, and epigenetic trait associated to gene silencing, was described by Cardenas and co-workers (2015) in umbilical cord-blood of infants exposed *in utero* to Hg. In addition, both GSTM1 (Hanna et al., 2012) and SEPP1 genes (Goodrich et al., 2013) were shown to be hypermethylated. Sanders et al (2015) examined cervical swabs from pregnant women and found that the expression of several miRNA was negatively associated with Hg exposure, but the significance of such findings is not totally clear.

Remarks and Perspectives

The available knowledge concerning Hg toxicity and biomarkers suggests that:

- Among the possibilities for putative biomarkers in Hg toxicity, promising endpoints include selenoprotein (such as TrxR and GPx isoforms) activities in non-invasive biological samples. The latter may serve in the future as potential early indicators of cellular damage, i.e. predictive of damage and eventual disease.
- Although transcription factors such as Nrf2 and NF-kappa B, as well as proteins such as GFAP and glutamate transporters, are considered as important molecules affected by and modulating MeHg-induced neurotoxicity, their use as biomarkers is likely not practical because of the lack of specificity with respect to solely Hg, as well as dificulty of properly assessing samples from the CNS.
- Immunotoxicity, including autoimmune effects have been established at metal concentrations well below the adverse threshold for CNS and kidney toxicity, and this issue seems to have been overlooked as a critical effect; however, the responses noted again are not practical due to lack of specificity.
- Protein adducts with mercuric species are presently amenable to analysis by sophisticated methods based on mass spectrometry and this undoubtedly provide a future focus for the discovery of protein targets with critical function(s) as predictive biomarkers of Hg-induced toxicity. Finally, new developments in genomics and SNPs identification of target proteins and epigenetic markers may lead to individualized prediction of Hg-induced toxicity;

Taken together, a critical need exists for validation of reliable and specific biomarkers that represent the earliest signs of Hg exposure in an attempt to reduce or eliminate exposure and protect the most vulnerable populations from this metal. New insights on the mechanistic pathways affected by mercurial compounds might further stimulate discovery in this fast evolving field.

Acknowledgments

Cristina Carvalho and Vasco Branco are supported by Fundação para a Ciência e Tecnologia, Portugal (www.fct.pt) through MERTOX project (PTDC/QUI-BIQ/117281/2010) and the Post-Doc fellowship (SFRH/BPD/85219/2012). The work was funded, in part, by iMed.ULisboa through project UID/DTP/04138/2013 also from FCT. MA was supported in part by grants from the NIH, R01 ES10563 and R01 ES07331, and R01 ES020852. MF was supported in part by grants from the Brazilian Agencies CNPq, CAPES and FAPESC. Finaly, the authors would like to thank the reviewers of this manuscript whose comments and suggestions contributed to significantly improve the original submission.

REFERENCES

Abou-Donia MB, Goldstein LB, Bullman S, Tu T, Khan WA, dechkovskaia AM and Abdel-Rahman AA, 2008 Imidacloprid induces neurobehavioral deficits and increases protein expression of glial fibrillary acidic protein in the motor cortex and hippocampus in offspring rats following in utero exoposure. J. Toxicol. Environ. Health, A 71: 119–130. [PubMed: 18080902]

Abou-Donia MB, Abou-Donia MM, ElMasry EM, Monro JA and Mulder MFA 2013 Autoantibodies to nervous system specific proteins are elevated in sera of flight crew members: Biomarkers for nervous sytem injury. J. Toxicol. Environ. Health, A 76: 363–380. [PubMed: 23557235]

Agrawal A, Kaushal P, Agrawal S, Gollapudi S, and Gupta S 2007 Thimerosal induces Th2 responses via influencing cytokine secretion by human dendritic cells. J. Leukocyte Biol 81: 474–482. [PubMed: 17079650]

Alam J, Wicks C, Stewart D, Gong P, Touchard C, Otterbein S, Choi AM, Burow ME, and Tou J 2000 Mechanism of heme oxygenase-1 gene activation by cadmium in MCF-7 mammary epithelial cells. Role of p38 kinase and Nrf2 transcription factor. J. Biol. Chem 275: 27694–27702. [PubMed: 10874044]

Alkazemi D, Egeland GM, Roberts LJ, 2nd, Chan HM, and Kubow S 2013 New insights regarding tissue Se and Hg interactions on oxidative stress from plasma IsoP and IsoF measures in the Canadian Inuit population. J. Lipid Res 54: 1972–1979. [PubMed: 23670530]

Allen JW, Mutkus LA, and Aschner M 2001a Mercuric chloride, but not methylmercury, inhibits glutamine synthetase activity in primary cultures of cortical astrocytes. Brain Res 891: 148–157. [PubMed: 11164818]

Allen JW, Mutkus LA, and Aschner M 2001b Methylmercury-mediated inhibition of 3H-d-aspartate transporter in cultured astrocytes is reversed by the antioxidant catalase. Brain Res 902: 92– 100. [PubMed: 11376598]

Allen JW, Shanker G, and Aschner M 2001c Methylmercury inhibits the in vitro uptake of the glutathione precursor, cystine, in astrocytes, but not in neurons. Brain Res 894: 131– 140. [PubMed: 11245823]

Al-Saleh I, Al-Sedairi A, and Elkhatib R 2012 Effect of mercury (Hg) dental amalgam fillings on renal and oxidative stress biomarkers in children. Sci. Total Environ 431: 188–196. [PubMed: 22683759]

Amoli JS, Barin A, Ebrahimi-Rad M, and Sadighara P 2011 Cell damage through pentose pohosphate pathway in fetus fibroblast cells exposed to methyl mercury. J. Appl. Toxicol 31: 685–689. [PubMed: 21433036]

Andrews N, Miller E, Grant A, Stowe J, Osborne V, and Taylor B 2004 Thimerosal exposure in infants and developmental disorders: A retrospective cohort study in the United Kingdom does not support a causal association. Pediatrics 114: 584–591. [PubMed: 15342825]

Armah FA, Boamah SA, Quansah R, Obiri S and Luginaah I 2016 Unsafe occupational health behaviours: Understanding mercury-related environmental health risks to artisanal gold miners in Ghana. Front. Environ. Sci 4: 29.

Arnér ESJ 2009 Focus on mammalian thioredoxin reductases - important selenoproteins with versatile functions. Biochim. Biophys. Acta 1790: 495–526. [PubMed: 19364476]

Aschner M 2000 Astrocytic swelling, phospholipase A2, glutathione and glutamate: Interactions in methylmercury-induced neurotoxicity. Cell Mol. Biol 46: 843–854. [PubMed: 10875445]

Aschner M, Du YL, Gannon M, and Kimelberg HK 1993 Methylmercury-induced alterations in excitatory amino acid transport in rat primary astrocyte cultures. Brain Res 602: 181–186. [PubMed: 8095428]

Aylward LL, Hays SM, Kirman CR, Marchitti SA, Kenneke JF, English C, Mattison DR, and Becker RA 2014 Relationships of chemical concentrations in maternal and cord blood: A review of available data. J. Toxicol. Environ. Health, B 17:175–203.

Baird L, and Dinkova-Kostova AT 2011 The cytoprotective role of the Keap1-Nrf2 pathway. Arch. Toxicol 85: 241–272. [PubMed: 21365312]

Baraldi M, Zanoli P, Tascedda F, Blom JM, and Brunello N 2002 Cognitive deficits and changes in gene expression of NMDA receptors after prenatal methylmercury exposure. Environ. Health Persp 110 Suppl 5: 855–858.

Barbosa AC, Boischio AA, East GA, Ferrari I, I., Gonçalves A,Silva PRM, and Cruz TME 1995 Mercury contamination in the Brazilian Amazon. Environmental and occupational aspects. Water Air Soil Pollut 80: 109–121.

Barbosa AC, Silva SRL, and Dórea JG 1998 Concentration of mercury in hair of indigenous mothers and infant from the Amazon basin. Arch. Environ. Contamin. Toxicol 34: 100–105.

Barcelos GR, Grotto D, de Marco KC, Valentini J, Lengert Av., de Oliveira AA, Garcia SC, Braga GÚ, Schläwicke- Engström K, Cólus IM, Broberg K and Barbosa F, Jr 2013 Polymorphisms in glutathione-related genes modify mercury concentrations and antioxidant status in subjects environmentally exposed to methylmercury. Sci. Total Environ 463–464: 319–25.

Barone S, Jr., Haykal-Coates N, Parran DK, and Tilson HA 1998 Gestational exposure to methylmercury alters the developmental pattern of Trk-like immunoreactivity in the rat brain and results in cortical dysmorphology. Dev. Brain Res 109: 13–31. [PubMed: 9706388]

Bartell SM, Ponce RA, Sanga RN, and Faustman EM 2000 Human variability in mercury toxicokinetics and steady state biomarker ratios. Environ. Res 84: 127–132. [PubMed: 11068925]

Barregard L, Trachtenberg F, and McKinlay S 2008 Renal effects of dental amalgam in children: The New England children's amalgam trial. Environ. Health Persp 116: 394–399.

Basu N, Goodrich JM, and Head J 2014 Ecogenetics of mercury: From genetic polymorphisms and epigenetics to risk assessment and decision making. Environ. Toxiol. Chem 33: 1248–1258.

Bélanger M-C., Mirault M-E, Dewailly E, Plante M, Berthiaume L, Noel M, and Julien P 2008 Seasonal mercury and oxidant-antioxidant status of James Bay sport fishermen. Metabolism 57: 630–636. [PubMed: 18442625]

Bendford DJ, Hanley AB, Bottrill K, Oehlshlager S, Balls M, Branca F, Castegnaro JJ, Descotes J, Hemminiki K, Lindsay D, and Schilter B 2000 Biomarkers as predictive tools in toxicity testing. ATLA - Altern. Lab. Animal 28: 119–131.

Berglund M, Lind B, Björnberg KA, Palm B, Einarsson Ö, and Vahter M 2005 Inter-individual variations of human mercury exposure biomarkers: a cross-sectional assessment. Environ. Health 4: 20. [PubMed: 16202128]

Bernard A 2008 Biomarkers of metal toxicity in population studies: Research potential and interpretation issues. J. Toxicol. Environ. Health A 71: 1259–1265. [PubMed: 18654897]

Bernard A, and Lauwerys R 1989 Epidemiological application of early markers of nephrotoxicity. Toxicol. Lett 46: 293–306. [PubMed: 2705200]

Berntssen MH, Aatland A, and Handy RD 2003 Chronic dietary mercury exposure causes oxidative stress, brain lesions, and altered behavior in Atlantic salmon (*Salmo salar*) parr. Aquat. Toxicol 65: 55–72. [PubMed: 12932701]

Björnberg KA, Vahter M, Berglund B, Niklasson B, Blennow M, and Sandborgh-Englund G, G. 2005 Transport of methylmercury and inorganic mercury to the fetus and breast-fed infant. Environ. Health Persp 113: 1381–1385.

Bo L, Miller DM, and Woods JS 1993 Studies on Hg(II)-induced H_2O_2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem. Pharmacol 45: 2017–2024. [PubMed: 8512585]

Bose-O'Reilly S, Lettmeier B, Roider G, Sieberta U, and Drasch G, G. 2008 Mercury in breast milk – a health hazard for infants in gold mining areas? Int. J. Hyg. Environ. Health 211: 615–623. [PubMed: 18262466]

Bose-O'Reilly S, McCarty KM, Steckling N, and Lettmeier B 2010 Mercury exposure and children's health. Curr. Probl. Pediatr. Adolesc. Health Care 40:186–215. [PubMed: 20816346]

Bose-O'Reilly S, Schierl R, Nowak D, Siebert U, William JF, Owi FT, and Ir YI 2016 A preliminary study on health effects in villagers exposed to mercury in a small-scale artisanal gold mining area in Indonesia. Environ. Res 149: 274–281. [PubMed: 27174690]

Branco V, Canário J, Holmgren A, and Carvalho C 2011 Inhibition of the thioredoxin system in the brain and liver of zebra-seabreams exposed to waterborne methylmercury. Toxicol. Appl. Pharmacol 251: 95–103. [PubMed: 21168431]

Branco V, Canário J, Lu J, Holmgren A, and Carvalho C 2012a Mercury and selenium interaction in vivo: Effects on thioredoxin reductase and glutathione peroxidase. Free Radic Biol. Med 52: 781–793. [PubMed: 22198265]

Branco V, Godinho-Santos A, Gonçalves J, Lu J, Holmgren A, and Carvalho C 2014 Mitochondrial thioredoxin reductase inhibition, selenium status and Nrf-2 activation are determinant factors modulating the toxicity of mercury compounds. Free Radic Biol. Med 73: 95–105. [PubMed: 24816296]

Branco V, Ramos P, Canário J, Lu J, Holmgren A, and Carvalho C 2012b Biomarkers of adverse response to mercury: histopathology vs. thioredoxin reductase activity. J. Biomed. Biotechnol 2012: 359879. [PubMed: 22888199]

Bridges CC, and Zalups RK 2005 Molecular and ionic mimicry and the transport of toxic metals. Toxicol. Appl. Pharmacol 204: 274–308. [PubMed: 15845419]

Bridges CC, and Zalups RK 2010 Transport of inorganic mercury and methylmercury in target tissues and organs. J. Toxicol. Environ. Health B 13: 385–410.

Brookes N 1992 In vitro evidence for the role of glutamate in the CNS toxicity of mercury. Toxicology 76: 245–256. [PubMed: 1361692]

Buckell M, Hunter D, Milton R, and Perry KMA 1946 Chronic mercury poisoning. Br. J. Ind. Med 3: 55–63. [PubMed: 20991165]

Burger J, Stephens WL, Boring CS, Jr., Kuklinski M, Gibbons JW, and Gochfeld M 1999 Factors in exposure assessment: ethnic and socioeconomic differences in fishing and consumption of fish caught along the Savannah River. Risk Anal 19: 427–438. [PubMed: 10765415]

Burk RF, and Hill KE 2005 Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis. Annu. Rev. Nutr 25: 215–235. [PubMed: 16011466]

Caito S, Zeng H, Aschner JL, and Aschner M 2014 Methylmercury alters the activities of Hsp90 client proteins, prostaglandin E synthase/p23 (PGES/23) and nNOS. PLoS One 9: e98161. [PubMed: 24852575]

Callan AC, Devine A, Qi L, Ng JC, and Hinwood AL 2015 Investigation of the relationship between low environmental exposure to metals and bone mineral density, bone resorption and renal function. Int. J. Hyg. Environ. Health 218: 444–451. [PubMed: 25857756]

Cambier S, Gonzalez P, Mesmer-Dudons N, Brethes D, Fujimura M, and Bourdineaud JP 2012 Effects of dietary methylmercury on the zebrafish brain: Histological, mitochondrial, and gene transcription analyses. Biometals 25: 165–180. [PubMed: 21947502]

Cardenas A, Koestler DC, Houseman EA, Jackson BP, Kile ML, Karagas MR, and Marsit CJ 2015 Differential DNA methylation in umbilical cord blood of infants exposed to mercury and arsenic in utero. Epigenetics 10: 508–515. [PubMed: 25923418]

Carneiro MF, Grotto D, and Barbosa F, Jr. 2014 Inorganic and methylmercury levels in plasma are differentially associated with age, gender, and oxidative stress markers in a population exposed to mercury through fish consumption. J. Toxicol. Environ. Health A 77: 69–79. [PubMed: 24555648]

Carvalho CML, Chew E, Hashemy SI, Lu J, and Holmgren A 2008a Inhibition of human thioredoxin system: A molecular mechanism of mercury toxicity. J. Biol. Chem 283: 11913–11923. [PubMed: 18321861]

Carvalho CML, Matos AINM, Mateus ML, Santos APM , and Batoréu MCC 2008b High-fish consumption and risk prevention: Exposure assessment to methylmercury in Portugal. J. Toxicol. Environ. Health A 71: 1279–1288. [PubMed: 18654900]

Carvalho C, Lu J, Zhang X, Arnér E, and Holmgren A 2011 Effects of selenite and chelating agents on mammalian thioredoxin reductase inhibited by mercury: implications for treatment of mercury poisoning. FASEB J 25: 370–381. [PubMed: 20810785]

Castilhos Z, Rodrigues-Filho S, Cesar R, Rodrigues AP, Villas-Bôas R, de Jesus I, Lima M, Faial K, Miranda A, Brabo E, and Beinhoff C 2015 Human exposure and risk assessment associated with mercury contamination in artisanal gold mining areas in the Brazilian Amazon. Environ. Sci. Pollut. Res. Int 22: 11255–11264. [PubMed: 25797016]

Chang JY 2007 Methylmercury causes glial IL-6 release. Neurosci. Lett 416: 217–220. [PubMed: 17368937]

Chen C, Yu H, Zhao J, Li B, Qu L, Liu S, Zhang P, and Chai Z 2006 The roles of serum selenium and selenoproteins on mercury toxicity in environmental and occupational exposure. Environ. Health Persp 114: 297–301.

Cherian MG, and Clarkson TW 1976 Biochemical changes in rat kidney on exposure to elemental mercury vapor: effect on biosynthesis of metallothionein. Chem.-Biol. Interact 13: 109–120.

Chmielnicka J, Brzeźnicka E, and Sniady A 1986 Kidney concentrations and urinary excretion of mercury, zinc and copper following the administration of mercuric chloride and sodium selenite to rats. Arch. Toxicol 59: 16–20. [PubMed: 3741138]

Clarkson TW, Magos L and Myers GJ 2003 Human exposure to mercury: The three modern dilemmas. J. Trace Elem. Exp. Med 16: 321–343.

Clarkson TW and Magos L 2006 The toxicology of mercury and its chemical compounds. Crit. Rev. Toxicol 36: 609–662. [PubMed: 16973445]

Clarkson TW, Vyas JB and Ballatori N 2007 Mechanisms of mercury disposition in the body. Am. J. Ind. Med 50: 757–764. [PubMed: 17477364]

Cooke GM 2014 Biomonitoring of human fetal exposure to environmental chemicals in early pregnancy. J. Toxicol. Environ. Health B 17: 205–224.

Cooper AJ, and Kristal BS 1997 Multiple roles of glutathione in the central nervous system. Biol. Chem 378: 793–802. [PubMed: 9377474]

Cordier S, Garel M, Mandereau L, Morcel H, Doineau P, Gosme-Seguret S 2002 Neurodevelopmental investigations among methylmercury exposed children in French Guiana. Environ. Res 89: 1–11. [PubMed: 12051779]

Counter SA, Buchanan LH, Ortega F and Laurell G 2002 Elevated blood mercury and neuro-otological observations in children of the Ecuadorian gold mines. J Toxicol Environ Health A 65: 149–163. [PubMed: 11820503]

Custodio HM, Broberg K, Wennberg M, Jansson JH, Vessby B, Hallmans G, Stegmayr B, and Skerfving S 2004 Polymorphisms in glutathione-related genes affect methylmercury retention. Arch. Environ. Health 59: 588–595. [PubMed: 16599007]

Custodio HM, Harari R, Gerhardsson L, Skerfving S, and Broberg K 2005 Genetic influences on the retention of inorganic mercury. Arch. Environ. Occup. Health 60: 17–23. [PubMed: 16961004]

Dahl R, White RF, Weihe P, Sørensen N, Letz R, Hudnell HK, Otto DA, and Grandjean P 1996 Feasibility and validity of three computer-assisted neurobehavioral tests in 7-year-old children. Neurotoxicol. Teratol 18: 413–419. [PubMed: 8866532]

Dalla Corte CL, Wagner C, Sudati JH, Comparsi B, Leite GO, Busanello A, Soares FA, Aschner M, and Rocha JB 2013 Effects of diphenyl diselenide on methylmercury toxicity in rats. Biomed Res. Int 2013: 983821. [PubMed: 24459674]

Dare E, Li W, Zhivotovsky B, Yuan X, and Ceccatelli S 2001 Methylmercury and H_2O_2 provoke lysosomal damage in human astrocytoma D384 cells followed by apoptosis. Free Radic Biol. Med 30: 1347–1356. [PubMed: 11390179]

Dave V, Mullaney KJ, Goderie S, Kimelberg HK, and Aschner M 1994 Astrocytes as mediators of methylmercury neurotoxicity: Effects on D-aspartate and serotonin uptake. Dev. Neurosci 16: 222–231. [PubMed: 7705225]

de Marco KC, Braga GU, and Barbosa F, Jr 2011 Determination of the effects of eNOS gene polymorphisms (T-786C and Glu298Asp) on nitric oxide levels in a methylmercury-exposed population. J. Toxicol. Environ. Health A 74: 1323–1333. [PubMed: 21899406]

de Oliveira Souza VC, de Marco KC, Laure HJ, Rosa JC, Barbosa F, Jr. 2016 A brain proteome profile in rats exposed to methylmercury or thimerosal (ethylmercury). J. Toxicol. Environ. Health A 79: 502–512. [PubMed: 27294299]

Deng Y, Xu Z, Xu B, Liu W, Wei Y, Li Y, Feng S, and Yang T 2014 Exploring cross-talk between oxidative damage and excitotoxicity and the effects of riluzole in the rat cortex after exposure to methylmercury. Neurotoxicol. Res 26: 40–51.

Diamond GL, and Zalups RK. 1998 Understanding renal toxicity of heavy metals. Toxicol. Pathol 26: 92–103. [PubMed: 9502391]

Dietrich MO, Mantese CE, dos Anjos G, Souza DO, and Farina M 2005 Motor impairment induced by oral exposure to methylmercury in adult mice. Environ. Toxicol. Pharmacol 19:169–175. [PubMed: 21783473]

Díez S, Delgado S, Aguilera I, Astray J, Pérez-Gómez B, Torrent M, Sunyer J, and Bayona JM 2009 Prenatal and early childhood exposure to mercury and methylmercury in Spain, a high-fish-consumer country. Arch. Environ. Contam. Toxicol 56: 615–622. [PubMed: 18836676]

Djukic MM, Jovanovic MD, Ninkovic M, Stevanovic I, Ilic K, Curcic M, and Vekic J 2012 Protective role of glutathione reductase in paraquat induced neurotoxicity. Chem.-Biol. Interact 199: 74–86. [PubMed: 22721943]

Doi R, and Tagawa M. 1983 A study on the biochemical and biological behavior of methylmercury. Toxicol. Appl. Pharmacol 69: 407–416. [PubMed: 6879610]

Dong L, Li Z, Bi X, and Ling L 2001 Effects of methyl mercury chloride on nuclear factor-kappa B DNA binding activities of nuclear protein extracts from developing rat cerebra and cerebella. Wei Sheng Yan Jiu 30: 7–9. [PubMed: 11255770]

Dórea JG, Bezerra VL, Fajon V, and Horvat M 2011 Speciation of methyl- and ethyl-mercury in hair of breastfed infants acutely exposed to Thimerosal-containing vaccines. lin. Chim. Acta 412:1563–1566.

Dórea JG, Farina M, and Rocha JBT 2013 Toxicity of ethylmercury (and Thimerosal): a comparison with methylmercury. J. Appl. Toxicol 33: 700–711. [PubMed: 23401210]

Dórea JG, Marques RC, and Abreu L 2014 Milestone achievement and neurodevelopment of rural Amazonian toddlers (12 to 24 Months) with different methylmercury and ethylmercury exposure. J. Toxicol. Environ. Health A 77: 1–13. [PubMed: 24555642]

Dórea JG, Marques RC, and Isejima C 2012 Antenatal and postnatal exposure to methyl- and ethylmercury. J. Biomed. Biotechnol 2012: 9.

Echeverria D, Aposhian HV, Woods JS, Heyer NJ, Aposhian MM, Bittner AC, Jr, Mahurin RK, and Cianciola M 1998 Neurobehavioral effects from exposure to dental amalgam Hg0: new distinctions between recent exposure and Hg body burden. FASEB J 12: 971–980. [PubMed: 9707169]

Echeverria D, Heyer NJ, Martin MD, Naleway CA, Woods JS, and Bittner AC 1994 Behavioral effects of low-level exposure to elemental mercury among dentists. Neurotoxicol. Teratol 17: 161–168.

El-Fawal HA, Gong Z, Little AR, and Evans HL 1996 Exposure to methylmercury results in serum autoantibodies to neurotypic and gliotypic proteins. Neurotoxicology 17: 531–539. [PubMed: 8856747]

Engström K, Ameer S, Bernaudat L, Drasch G, Baeuml J, Skerfving S, Bose-O'Reilly S, and Broberg K 2013 Polymorphisms in genes encoding potential mercury transporters and urine mercury concentrations in populations exposed to mercury vapor from gold mining. Environ. Health Persp 121: 85–91.

Eroglu K, Atli G, and Canli M 2005 Effects of metal (Cd, Cu, Zn) interactions on the profiles of metallothionein-like proteins in the Nile fish *Oreochromis niloticus*. Bull. Environ. Contam. Toxicol 75: 390–399. [PubMed: 16222515]

Eskes C, Honegger P, Juillerat-Jeanneret L, and Monnet-Tschudi F 2002 Microglial reaction induced by noncytotoxic methylmercury treatment leads to neuroprotection via interactions with astrocytes and IL-6 release. Glia 37: 43–52. [PubMed: 11746782]

Farina M, Aschner M, and Rocha JBT 2011 Oxidative stress in MeHg-induced neurotoxicity. Toxicol. Appl. Pharmacol 256: 405–417. [PubMed: 21601588]

Farina M, Campos F, Vendrell I, Berenguer J, Barzi M, Pons S, and Sunol C 2009 Probucol increases glutathione peroxidase-1 activity and displays long-lasting protection against methylmercury toxicity in cerebellar granule cells. Toxicol. Sci 112: 416–426. [PubMed: 19770487]

Fessel JP, and Roberts LJ, II 2005 Isofurans: novel products of lipid peroxidation that define the occurrence of oxidant injury in settings of elevated oxygen tension. Antioxid. Redox Signal 7: 202–209. [PubMed: 15650408]

Fombonne E, Zakarian R, Bennett A, Meng L, and McLean-Heywood D 2006 Pervasive developmental disorders in Montreal, Quebec, Canada: Prevalence and links with immunizations. Pediatrics 118: e139–150. [PubMed: 16818529]

Fournie GJ, Mas M, Cautain B, Savignac M, Subra JF, Pelletier L, Saoudi A, Lagrange D, Calise M, and Druet P 2001 Induction of autoimmunity through bystander effects. Lessons from immunological disorders induced by heavy metals. J. Autoimmun 16: 319–326. [PubMed: 11334498]

Fowler BA, and Mahaffey KR 1978 Interactions among lead, cadmium, and arsenic in relation to porphyrin excretion patterns. Environ. Health Persp 25: 87–90.

Franco JL, Braga HC, Stringari J, Missau FC, Posser T, Mendes BG, Leal RB, Santos AR, Dafre AL, Pizzolatti MG, and Farina M. 2007 Mercurial-induced hydrogen peroxide generation in mouse brain mitochondria: protective effects of quercetin. Chem. Res. Toxicol 20: 1919–1926. [PubMed: 17944542]

Franco JL, Posser T, Dunkley PR, Dickson PW, Mattos JJ, Martins R, Bainy AC, Marques MR, Dafre AL, and Farina M 2009 Methylmercury neurotoxicity is associated with the inhibition of the antioxidant enzyme glutathione peroxidase. Free Radic Biol. Med 47: 449–457. [PubMed: 19450679]

Franco JL, Posser T, Missau F, Pizzolatti MG, dos Santos AR, Souza DO, Aschner M, Rocha JBT, Dafre AL, and Farina M 2010 Structure-activity relationship of flavonoids derived from medicinal plants in preventing methylmercury-induced mitochondrial dysfunction. Environ. Toxicol. Pharmacol 30: 272–278. [PubMed: 21127717]

Franco JL, Teixeira A, Meotti FC, Ribas CM, Stringari J, Garcia Pomblum SC, Moro AM, Bohrer D, Bairros AV, Dafre AL, Santos AR, and Farina M 2006 Cerebellar thiol status and motor deficit after lactational exposure to methylmercury. Environ. Res 102: 22–28. [PubMed: 16564521]

Franconi F, Miceli M, De Montis MG, Crisafi EL, Bennardini F and Tagliamonte A 1996 NMDA receptors play an anti-aggregating role in human platelets. Thromb Haemost 76: 84–87. [PubMed: 8819257]

Franconi F, Miceli M, Alberti L, Seghieri G, De Montis MG and Tagliamonte A 1998 Further insights into the anti-aggregating activity of NMDA in human platelets. Br J Pharmacol 124: 35–40. [PubMed: 9630340]

Franko A, Budihna MV, and Dodic-Fikfak M 2005 Long.term effects of elemental mercury on renal function in miners of the Idrija mercury mine. Ann. Occup. Hyg 49: 521–527. [PubMed: 15964879]

Fujita H 2001 Measurement of delta-aminolevulinate dehydratase activity. In: Current Protocols in ToxicologyCosta LG, Hodgson E, Lawrence DA, Reed DJ, and GreenLee WF, eds. USA: John Wiley & Sons Inc, 8.6:8.61–8.66.11.

Fujiyama J, Hirayama K, and Yasutake A 1994 Mechanism of methylmercury efflux from cultured astrocytes. Biochem. Pharmacol 47: 1525–1530. [PubMed: 8185663]

Gao Y, Yan CH, Yu XD, and Wu SH. 2006 Effects of perinatal exposure to methylmercury on the structure of hippocampus and cerebellum in young rats. Wei Sheng Yan Jiu 35: 402–405. [PubMed: 16986508]

Gardner RM, Nyland JF, Silva IA, Ventura AM, de Souza JM, and Silbergeld EK 2010a Mercury exposure, serum antinuclear/antinucleolar antibodies, and serum cytokine levels in mining populations in amazonian Brazil: A cross-sectional study. Environ. Res 110: 345–354. [PubMed: 20176347]

Gardner RM, Nyland JF, and Silbergeld EK 2010b Differential immunotoxic effects of inorganic and organic mercury species in vitro. Toxicol. Lett 198: 182–190. [PubMed: 20600710]

Gerson RJ, and Shaikh ZA. 1982 Uptake and binding of cadmium and mercury to metallothionein in rat hepatocyte primary cultures. Biochem. J 208: 465–472. [PubMed: 7159412]

Gibb H, and O'Leary KG 2014 Mercury exposure and health impacts among individuals in the artisanal and small-scale gold mining community: a comprehensive review. Environ. Health Persp 122: 667.

Gil F, and Pla A 2001 Biomarkers as biological indicators of xenobiotic exposure. J. Appl. Toxicol 21: 245–255. [PubMed: 11481655]

Ginsberg G, Sonawane B, Nath R, and Lewandowski P 2014 Methylmercury-induced inhibition of paraoxonase-1 (PON-1)—implications for cardiovascular risk J. Toxicol. Environ. Health A 77: 1004–1023 [PubMed: 25072822]

Goodrich JM, Basu N, Franzblau A, and Dolinoy DC 2013 Mercury biomarkers and DNA methylation among Michigan dental professionals. Environ. Mol. Mutagen 54: 195–203. [PubMed: 23444121]

Goodrich JM, Wang Y, Gillespie B, Werner R, Franzblau A, and Basu N 2011 Glutathione enzyme and selenoprotein polymorphisms associate with mercury biomarker levels in Michigan dental professionals. Toxicol. Appl. Pharmacol 257: 301–308. [PubMed: 21967774]

Grandjean P, Brown SS, Reavey P, and Young DS 1994 Biomarkers of chemical exposure: State of the art. Clin. Chem 40: 1360–1362. [PubMed: 8013119]

Grandjean P, Budtz-Jørgensen E, White RF, Jørgensen PJ, Weihe P, Debes F, and Keiding N 1999 Methylmercury exposure biomarkers as indicators of neurotoxicity in children aged 7 years. Am. J. Epidemiol 150: 301–305. [PubMed: 10430235]

Grandjean P, Murata K, Budtz-Jorgensen E, and Weihe P 2004 Cardiac autonomic activity in methylmercury neurotoxicity: 14-years follow-up of a Faroese birth cohort. J. Pediatr 144: 189–176.

Grandjean P, Weihe P, Jorgensen PJ, Clarkson T, Cernichiari E, and Videro T 1992 Impact of maternal seafood diet on fetal exposure to mercury, selenium and lead. Arch. Environ. Health 47: 185–195. [PubMed: 1596101]

Grandjean P, Weihe P, and White RF 1995 Milestone development in infants exposed to methylmercury from human milk. Neurotoxicology 16: 27–33. [PubMed: 7603642]

Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sørensen N, Dahl R, and Jørgensen PJ 1997 Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol. Teratol 19: 417–428. [PubMed: 9392777]

Grintal B, Champeil-Potokar G, Lavialle M, Vancassel S, Breton S, and Denis I 2009 Inhibition of astroglial glutamate transport by polyunsaturated fatty acids: Evidence for a signaling role of docosahexaenoic acid. Neurochem. Int 54: 535–543. [PubMed: 19428799]

Grotto D, Valentini J, Fillion M, Passos CJS, Garcia SC, Mergler D, and Barbosa F, Jr 2010 Mercury exposure and oxidative stress in communities of the Brazilian Amazon. Sci. Total Environ 408: 806–811. [PubMed: 19914681]

Gundacker C, Wittmann KJ, Kukuckova M, Komarnicki G, Hikkel I, and Gencik M 2009 Genetic background of lead and mercury metabolism in a group of medical students in Austria. Environ. Res 109: 786–796. [PubMed: 19515364]

Hagmar L, Persson-Moschos M, Åkesson B, and Schütz A 1998 Plasma levels of selenium, selenoprotein P and glutathione peroxidase and their correlations to fish intake and serum levels of thyrotropin and thyroid hormones: A study on Latvian fish consumers. Eur. J. Clin. Nutr 52: 796–800. [PubMed: 9846591]

Halbach S, and Clarkson TW 1978 Enzymatic oxidation of mercury vapour by erythrocytes. Biochim. Biophys. Acta 523: 522–531. [PubMed: 656439]

Hanna CW, Bloom MS, Robinson WP, Kim D, Parsons PJ, vom Saal FS, Taylor JA, Steuerwald AJ, and Fujimoto VY 2012 DNA methylation changes in whole blood is associated with exposure to the environmental contaminants, mercury, lead, cadmium and bisphenol A, in women undergoing ovarian stimulation for IVF. Human Reprod 27:1401–1410.

Hatfield DL, Tsuji PA, Carlson BA, and Gladyshev VN 2014 Selenium and selenocysteine: roles in cancer, health, and development. Trends Biochem Sci 39:112–120. [PubMed: 24485058]

Haut MW, Morrow LA, Pool D, Callahan TS, Haut JS, and Franzen MD 1999 Neurobehavioral effects of acute exposure to inorganic mercury vapor. Appl Neuropsychol 6: 193–200. [PubMed: 10635433]

Havarinasab S and Hultman P 2005 Organic mercury compounds and autoimmunity. Autoimmun. Rev 4: 270– 275. [PubMed: 15990073]

Havarinasab S, Häggqvist B, Björn E, Pollard KM and Hultman P 2005 Immunosuppressive and autoimmune effects of Thimerosal in mice. Toxicol. Appl. Pharmacol 204: 109–121. [PubMed: 15808517]

Havarinasab S, Björn E, Ekstrand J, and Hultman P 2007 Dose and Hg species determine the T-helper cell activation in murine autoimmunity. Toxicology 229: 23–32. [PubMed: 17084957]

Havarinasab S, Lambertson L, Qvarnström J, and Hultman P 2004 Dose-response study of Thimerosal-induce murine systemic autoimmunity. Toxicol. Appl. Pharmacol 194: 169–179. [PubMed: 14736497]

Heron J, and Golding J 2004 Thimerosal exposure in infants and developmental disorders: A prospective cohort study in the United Kingdom does not support a causal association. Pediatrics 114: 577–583. [PubMed: 15342824]

Heyer NJ, Echeverria D, Bittner AC, Jr., Farin FM, Garabedian CC, and Woods JS 2004 Chronic low-level mercury exposure, BDNF polymorphism, and associations with self-reported symptoms and mood. Toxicol. Sci 81:354–363. [PubMed: 15254338]

Heyer NJ, Echeverria D, Farin FM, and Woods JS 2008 The association between serotonin transporter gene promoter polymorphism (5-HTTLPR), self-reported symptoms, and dental mercury exposure. J. Toxicol. Environ. Health A 71:1318–1326. [PubMed: 18686203]

Heyer NJ, Echeverria D, Martin MD, Farin FM, and Woods JS 2009 Catechol O-Methyltransferase (COMT) VAL158MET functional polymorphism, dental mercury exposure, and self-reported symptoms and mood. J. Toxicol. Environ. Health A 72: 599–609. [PubMed: 19296409]

Hoesel B, and Schmid JA 2013 The complexity of NF-kappaB signaling in inflammation and cancer. Mol. Cancer 12: 86. [PubMed: 23915189]

Hoffman DJ, Spalding MG, and Frederick PC 2005 Subchronic effects of methylmercury on plasma and organ biochemistries in great egret nestlings. Environ. Toxicol. Chem 24: 3078–3084. [PubMed: 16445088]

Horecker BL 2002 The pentose phosphate pathway. J Biol. Chem 277: 47965–47971. [PubMed: 12403765]

Huang CF, Liu SH, Hsu CJ, and Lin-Shiau SY 2011 Neurotoxicological effects of low-dose methylmercury and mercuric chloride in developing offspring mice. Toxicol. Lett 201: 196–204. [PubMed: 21195143]

Hussain S, Rodgers DA, Duhart HM, and Ali SF 1997 Mercuric chloride-induced reactive oxygen species and its effect on antioxidant enzymes in different regions of rat brain. J. Environ. Sci. Health, Part B 32: 395–409.

Hviid A, Stellfeld M, Wohlfahrt J, and Melbye M 2003 Association between thimerosal-containing vaccine and autism. JAm Med Assoc 290: 1763–1766.

Hwang GW, Lee JY, Ryoke K, Matsuyama F, Kim JM, Takahashi T, and Naganuma A 2011 Gene expression profiling using DNA microarray analysis of the cerebellum of mice treated with methylmercury. J. Toxicol. Sci 36: 389–391. [PubMed: 21628967]

Jain AK, and Jaiswal AK 2006 Phosphorylation of tyrosine 568 controls nuclear export of Nrf2. J. Biol. Chem 281: 12132–12142. [PubMed: 16513647]

Jain A, Flora GJ, Bhargava R, and Flora SJ 2012 Influence of age on arsenic-induced oxidative stress in rat. Biol. Trace Elem. Res 149: 382–390. [PubMed: 22614922]

Janssen-Heininger YM, Mossman BT, Heintz NH, Forman HJ, Kalyanaraman B, Finkel T, Stamler JS, Rhee SG, and van der Vliet A 2008 Redox-based regulation of signal transduction: Principles, pitfalls, and promises. Free Radic Biol. Med 45: 1–17. [PubMed: 18423411]

Jarosínska D, Horvat M, Sällsten G, Mazzolai B, Dąbkowska B, Prokopowicz A, Biesiada M, and Barregård L 2008 Urinary mercury and biomarkers of early renal dysfunction in environmentally and occupationally exposed adults: A three-country study. Environ. Res 108: 224–232. [PubMed: 18684440]

Jayaprakash K 2009 Mercury vapor inhalation and its effect on glutathione peroxidase in goldsmiths exposed occupationally. Toxicol. Ind. Health 25: 463–465. [PubMed: 19648214]

Jebbett NJ, Hamilton JW, Rand MD, and Eckenstein F 2013 Low level methylmercury enhances CNTF-evoked STAT3 signaling and glial differentiation in cultured cortical progenitor cells. Neurotoxicology 38: 91–100. [PubMed: 23845766]

Jin X, Hidiroglou N, Lok E, Taylor M, Kapal K, Ross N, Sarafin K, Lau A, de Souza A, Chan HM, and Mehta E 2012 Dietary selenium (Se) and vitamin E (V(E)) supplementation modulated methylmercury-mediated changes in markers of cardiovascular diseases in rats. Cardiovasc. Toxicol 12: 10–24. [PubMed: 21822669]

Julvez J, Smith GD, Golding J, Ring S, Pourcain BS, Gonzalez JR, and Grandjean P 2013 Prenatal methylmercury exposure and genetic predisposition to cognitive deficit at age 8 years. Epidemiology 24: 643–650. [PubMed: 23903878]

Kalev-Zylinska ML, Green TN, Morel-Kopp MC, Sun PP, Park YE, Lasham A, During MJ, and Ward CM 2014 N-methyl-d-aspartate receptors amplify activation and aggregation of human platelets. Thromb. Res 133: 837–847. [PubMed: 24593912]

Khan MAK, and Wang F 2009 Mercury-selenium compounds and their toxicological significance: Toward a molecular understanding of the mercury-selenium antagonism. Environ. Toxicol. Chem 28: 1567–1577. [PubMed: 19374471]

Khandelwal PJ, Herman AM, and Moussa CE. 2011 Inflammation in the early stages of neurodegenerative pathology. J. Neuroimmunol 238: 1–11. [PubMed: 21820744]

Kendall RJ, Anderson TA, Baker RJ, Bens CM, Carr JA, Chiodo GPC, Dickerson RL, Dixon KR, Frame LT, Hooper MJ, Martin CF, McMurry ST, Patino R, Smith EE, and Theodorakis CW 2001 Ecotoxicology. In: Casarett and Doull's Toxicology. The Basic Science of Poisons. Chapter 29, Klaassen CD, ed., McGraw-Hill, 6th Edition, New York, pp. 1013–1045.th

Kim MS, Takahashi T, Lee JY, Hwang GW, and Naganuma A 2012 Methylmercury induces CCL2 expression through activation of NF-kappaB in human 1321N1 astrocytes. J. Toxicol. Sci 37: 1275–1278. [PubMed: 23208443]

Kim SA, Jeon CK, and Paek DM. 2008 Hair mercury concentrations of children and mothers in Korea: implication for exposure and evaluation. Sci. Total Environ 402: 36–42. [PubMed: 18502474]

Kobal AB, Horvat M, Prezelj M, Briski AS, Krsnik M, Dizdarevic T, Mazej D, Falnoga I, Stibilj V, Arneric N, Kobal D, and Osredkar J 2004 The impact of long-term past exposure to elemental mercury on antioxidative capacity and lipid peroxidation in mercury miners. J. Trace Elem. Med. Biol 17: 262–274.

Kobal AB, Prezelj M, Horvat M, Krsnik M, Gibicar D, and Osredkar J 2008 Glutathione level after long-term occupational elemental mercury exposure. Environ. Res 107: 115–123. [PubMed: 17706633]

Kobayashi A, Kang MI, Watai Y, Tong KI, Shibata T, Uchida K, and Yamamoto M 2006 Oxidative and electrophilic stresses activate Nrf2 through inhibition of ubiquitination activity of Keap1. Mol. Cell. Biol 26: 221–229. [PubMed: 16354693]

Kong X, Wang S, Jiang H, Nie G, and Li X 2012 Responses of acid/alkaline phosphatase, lysozyme, and catalase activities and lipid peroxidation to mercury exposure during the embryonic development of goldfish *Carassius auratus*. Aquat. Toxicol 120/121: 119–125.

Kristensen AKB, Thomsen JF, and Mikkelsen S 2014 A review of mercury exposure among artisanal small-scale gold miners in developing countries. Int Arch Occup Environ Health 87: 579–590. [PubMed: 23979147]

Kutscher DJ, del Castillo Busto ME, Zinn N, Sanz-Medel A, Bettmer J 2008 Protein labelling with mercury tags: fundamental studies on ovalbumin derivatised with p-hydroxymercuribenzoic acid (pHMB). J. Anal. Atom. Spectrom 23:1359–1364.

Kwon OS, and Park YJ 2003 In vitro and in vivo dose-dependent inhibition of methylmercury on glutamine synthetase in the brain of different species. Environ. Toxicol. Pharmacol 14: 17–24. [PubMed: 21782658]

Laffont L, Sonke JE, Maurice L, Monrroy SL, Chincheros J, Amouroux D, and Behra P 2011 Hg speciation and stable isotope signatures in human hair as a tracer for dietary and occupational exposure to mercury. Environ. Sci. Technol 45: 9910–9916. [PubMed: 22003970]

LaKind JS, Brent RL, Dourson ML, Kacew S, Koren G, Sonawane B, Tarzian AJ, and Uhl K 2005 Human milk biomonitoring data: Interpretation and risk assessment issues. J. Toxicol. Environ. Health A 68:1713–1769. [PubMed: 16176917]

LeBel CP, Ali SF, and Bondy SC 1992 Deferoxamine inhibits methyl mercury-induced increases in reactive oxygen species formation in rat brain. Toxicol. Appl. Pharmacol 112: 161–165. [PubMed: 1310167]

Lebel J, Mergler D, Branches F, Lucotte M, Amorim M, and Larribe F 1998 Neurotoxic effects of low-level methyl mercury contamination in the Amazonian Basin. Environ. Res 79: 20–32. [PubMed: 9756677]

Lee BE, Hong YC, Park H, Ha M, Koo BS, Chang N, Roh YM, Kim BN, Kim YJ, Kim BM, Jo SJ, and Ha EH 2010 Interaction between GSTM1/GSTT1 polymorphism and blood mercury on birth weight. Environ Health Persp 118: 437–443.

Lee JY, Hwang GW, Kim MS, Takahashi T, and Naganuma A 2012 Methylmercury induces a brain-specific increase in chemokine CCL4 expression in mice. J. Toxicol. Sci 37: 1279–1282. [PubMed: 23208444]

Lillig CH, and Holmgren A 2007 Thioredoxin and related molecules – from biology to health and disease. Antioxid. Redox Signal 9: 25–47. [PubMed: 17115886]

Lin TH, Huang YL, and Huang SF 1996 Lipid peroxidation in liver of rats administrated with methyl mercuric chloride. Biol. Trace Elem. Res 54: 33–41. [PubMed: 8862759]

Lin YS, Ginsberg G, Lin JW, and Sonawane B 2014 Mercury exposure and omega-3 fatty acid intake in relation to renal function in the US population. Int. J. Hyg. Environ. Health 217: 465–472. [PubMed: 24189320]

Lindow SW, Knight R, Batty J, and Haswell SJ 2003 Maternal and neonatal hair mercury concentrations: the effect of dental amalgam. Br J Obstet Gynaecol 110: 287–291.

Liu X, Nordberg GF, and Jin T 1992 Increased urinary excretion of zinc and copper by mercuric chloride injection in rats. Biometals 5: 17–22. [PubMed: 1392467]

Llop S, Ballester F, and Broberg K 2015 Effect of gene-merury interactions on mercury toxicokinetics and neurotoxicity. Curr. Environ. Heath Rep 2: 179–194.

Llop S, Engström K, Ballester F, Franforte E, Alhamdow A, Pisa F, Tratnik JS, Mazej D, Murcia M, Rebagliato M, Bustamante M, Sunyer J, Sofianou-Katsoulis A, Prasouli A, Antonopoulou E, Antoniadou I, Nakou S, Barbone F, Horvat M, and Broberg K 2014 Polymorphisms in ABC transporter genes and concentrations of mercury in newborns--evidence from two Mediterranean birth cohorts. PLoS One 9: e97172. [PubMed: 24831289]

Lu J, and Holmgren A 2009 Selenoproteins. J. Biol. Chem 284: 723–727. [PubMed: 18757362]

Lu J, Chew EH, and Holmgren A 2007 Targeting thioredoxin reductase is a basis for cancer therapy by arsenic trioxide. Proc. Natl. Acad. Sci. U. S. A 104: 12288–12293. [PubMed: 17640917]

Lund B, Miller DM, and Woods JS. 1991 Mercury-induced H_2O_2 production and lipid peroxidation in vitro in rat kidney and mitochondria. Biochem. Pharmacol 42: S181–187. [PubMed: 1768276]

Lund B, Miller DM, and Woods JS 1993 Studies on Hg(II)-induced H_2O_2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem. Pharmacol 45: 2017–2024. [PubMed: 8512585]

Madsen KM, Lauritsen MB, Pedersen CB, Thorsen P, Plesner A-M, Andersen PH, and Mortensen PB 2003 Thimerosal and the occurrence of autism: negative ecological evidence from Danish population-based data. Pediatrics 112: 604–606. [PubMed: 12949291]

Magos L 1997 Physiology and toxicology of mercury. In: Metal Ions in Biological Systems, Volume 34: Mercury and its Effects on Environment and Biology, Sigel A and Sigel H, eds., Marcel Dekker Inc, New York, pp 321–370.

Mahboob M, Shireen KF, Atkinson A, and Khan AT 2001 Lipid peroxidation and antioxidant enzyme activity in different organs of mice exposed to low level of mercury. J. Environ. Sci. Health, Part B 36: 687–697.

Maier A, Savage RE, Jr., and Haber LT 2004 Assessing biomarker use in risk assessment--a survey of practitioners. Assessing biomarker use in risk assessment - a survey of practitioners. J. Toxicol. Environ. Health A 67: 687–695. [PubMed: 15192862]

Manfroi CB, Schwalm FD, Cereser V, Abreu F, Oliveira A, Bizarro L, Rocha JBT, Frizzo ME, Souza DO, and Farina M 2004 Maternal milk as methylmercury source for suckling mice: Neurotoxic effects involved with the cerebellar glutamatergic system. Toxicol. Sci 81: 172–178. [PubMed: 15201443]

Marks GS 1985 Exposure to toxic agents: The heme biosynthetic pathway and hemoproteins as indicator. Crit. Rev. Toxicol 15: 151–179. [PubMed: 3899520]

Marques RC, Dórea JG, , Fonseca MF, Bastos WR, and Malm O 2007 Hair mercury in breast-fed infants exposed to Thimerosal-preserved vaccines. Eur. J. Pediatr 166: 935–941. [PubMed: 17237965]

Marques RC, Bernardi JV, Dórea JG, Leão RS, and Malm O. 2013 Mercury transfer during pregnancy and breastfeeding: hair mercury concentrations as biomarker. Biol. Trace Elem. Res 154: 326–332. [PubMed: 23836367]

Marques RC, Bernardi JV, Abreu L, and. Dórea JG 2015 Neurodevelopment outcomes in children exposed to organic mercury from multiple sources in a tin-ore mine environment in Brazil. Arch. Environ. Contam. Toxicol 68: 432–441. [PubMed: 25425160]

Martinez CS, Escobar AG, Torres JG, Brum DS, Santos FW, Alonso MJ, Salaices M, Vassallo DV, Pecanha FM, Leivas FG, and Wiggers GA 2014 Chronic exposure to low doses of mercury impairs sperm quality and induces oxidative stress in rats. J. Toxicol. Environ. Health A 77: 143–154. [PubMed: 24555655]

Martinez-Finley EJ, Caito S, Slaughter JC, and Aschner M 2013 The role of skn-1 in methylmercury-induced latent dopaminergic neurodegeneration. Neurochem. Res 38: 2650–2660. [PubMed: 24194349]

McMurtry RJ, Snodgrass WR, and Mitchell JR 1978 Renal necrosis, glutathione depletion, and covalent binding after acetaminophen. Toxicol. Appl. Pharmacol 46: 87–100. [PubMed: 725953]

Meister A 1988 Glutathione metabolism and its selective modification. J. Biol. Chem 263: 17205–17208. [PubMed: 3053703]

Merrick BA 2006 Toxicoproteomics in liver injury and inflammation. Ann. N Y Acad. Sci 1076: 707–717. [PubMed: 17119248]

Meyer-Baron M, Schaeper M, and Seeber A 2002 A meta-analysis for neurobehavioural results due to occupational mercury exposure. Arch. Toxicol76: 127–136. [PubMed: 11967617]

Miyamoto K, Nakanishi H, Moriguchi S, Fukuyama N, Eto K, Wakamiya J, Murao K, Arimura K, and Osame M 2001 Involvement of enhanced sensitivity of N-methyl-D-aspartate receptors in vulnerability of developing cortical neurons to methylmercury neurotoxicity. Brain Res 901: 252–258. [PubMed: 11368974]

Moreira EL, de Oliveira J, Dutra MF, Santos DB, Goncalves CA, Goldfeder EM, de Bem AF, Prediger RD, Aschner M, and Farina M. 2012 Does methylmercury-induced hypercholesterolemia play a causal role in its neurotoxicity and cardiovascular disease? Toxicol Sci 130: 373–382. [PubMed: 22903822]

Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, and Roberts LJ, 2nd 1990 A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc. Natl. Acad. Sci. U. S. A 87: 9383–9387. [PubMed: 2123555]

Mottet NK, Vahter ME, Charleston JS, and Friberg LT 1997 Metabolism of methylmercury in the brain and its toxicological significance. In: Metal Ions in Biological Systems, Volume 34: Mercury and its Effects on Environment and Biology, Sigel A and Sigel H, eds., Marcel Dekker Inc, New York, pp 371–403.

Motts JA, Shirley DL, Silbergeld EK, and Nyland JF 2014 Novel biomarkers of mercury-induced autoimmune dysfunction: A cross-sectional study in Amazonian Brazil. Environ. Res 132: 12–18. [PubMed: 24742722]

Mustacich D, and Powis G 2000 Thioredoxin reductase. Biochem. J 346: 1–8. [PubMed: 10657232]

Myers GJ, Davidson PW, Cox C, Shamlaye C, Palumbo D, Cernichiari E, Sloane-Reeves J, Wilding GE, Kost J, Haung L-S, and Clarkson TW. 2003 Prenatal methylmercury exposure from ocean fish consumption in the Seychelles Child Development Study. Lancet 361: 1686–1692. [PubMed: 12767734]

Myers G, Davidson PW, Shamlaye CF, Axtell CD, Cernichiari E, Choisy O, Choi A, Cox C, and Clarkson TW 1997 Effects of prenatal methylmercury exposure from a high fish diet on developmental milestones in the Seychelles Child Development Study. Neurotoxicology 18: 819–830. [PubMed: 9339828]

Nath KA, Croatt AJ, Likely S, Behrens TW, and Warden D 1996 Renal oxidant injury and oxidant response induced by mercury. Kidney Int 50: 1032–1043. [PubMed: 8872981]

National Research Council (NRC). 1987 Biological markers in environmental health research. Environ. Health Persp 74: 3–9.

National Research Council (NRC). 2000 Toxicological effects of Methylmercury Washington, DC: National Academy Press.

Neal PA 1938 Mercury poisoning from the public health viewpoint. Am. J. Public Health 28: 907–915.

Ng S, Lin CC, Hwang YH, Hsieh WS, Liao HF, and Chen PC 2013 Mercury, APOE, and children's neurodevelopment. Neurotoxicology 37: 85–92. [PubMed: 23603214]

Ni M, Li X, Yin Z, Jiang H, Sidoryk-Wegrzynowicz M, Milatovic D, Cai J, and Aschner M 2010 Methylmercury induces acute oxidative stress, altering Nrf2 protein level in primary microglial cells. Toxicol. Sci 116: 590–603. [PubMed: 20421342]

Nielsen JB, and Hultman P 2002 Mercury-induced autoimmunity in mice. Environ. Health Persp 110: 877–881.

Niture SK, Khatri R, and Jaiswal AK 2014 Regulation of Nrf2-an update. Free Radic Biol. Med 66: 36–44. [PubMed: 23434765]

Nunes E, Cavaco A, and Carvalho C 2014a Children's health risk and benefits of fish consumption: risk indices based on a diet diary follow-up of two weeks. J. Toxicol. Environ. Health A 77: 103–114. [PubMed: 24555651]

Nunes E, Cavaco A, and Carvalho C 2014b Exposure assessment of pregnant Portuguese women to methylmercury through the ingestion of fish: Cross-sectional survey and biomarker validation. J. Toxicol. Environ. Health A 77: 133–142. [PubMed: 24555654]

Nyland JF, Fillion M, Barbosa F, Shirley DL, Chine C, Lemire M, Mergler D, and Silbergeld EK 2011 Biomarkers of methylmercury exposure immunotoxicity among fish consumers in Amazonian Brazil. Environ. Health Persp 119: 1733–1738.

Ohno T, Sakamoto M, Kurosawa T, Dakeishia M, Iwata T, and Murataa K 2007 Total mercury levels in hair, toenail, and urine among women free from occupational exposure and their relations to renal tubular function. Environ. Res 103: 191–197. [PubMed: 16890218]

Oliveira RC, Dórea JG, Bernardi JV, Bastos WR, Almeida R, and Manzatto AG 2010 Fish consumption by traditional subsistence villagers of the Rio Madeira (Amazon): impact on hair mercury. Ann. Human Biol 37: 629–642. [PubMed: 20132080]

Oppedisano F, Galluccio M, and Indiveri C 2010 Inactivation by Hg^{2+} and methylmercury of the glutamine/amino acid transporter (ASCT2) reconstituted in liposomes: Prediction of the involvement of a CXXC motif by homology modelling. Biochem. Pharmacol 80: 1266–1273. [PubMed: 20599776]

Ou L, Chen C, Chen L, Wang H, Yang T, Xie H, Tong Y, Hu D, Zhang W, and Wang X 2015 Low-level prenatal mercury exposure in north China: An exploratory study of anthropometric effects. Environ. Sci. Technol 49: 6899–6908. [PubMed: 25936461]

Papp LV, Lu J, Holmgren A, and Khanna KK. 2007 From selenium to selenoproteins: Synthesis, identity and their role in human health. Antioxid. Redox Signal 9: 775–806. [PubMed: 17508906]

Pantano C, Reynaert NL, van der Vliet A, and Janssen-Heininger YM. 2006 Redox-sensitive kinases of the nuclear factor-kappaB signaling pathway. Antioxid. Redox Signal 8: 1791–1806. [PubMed: 16987032]

Parajuli RP, Goodrich JM, Chou HN, Gruninger SE, Dolinoy DC, Franzblau A, and Basu N Genetic polymorphisms are associated with hair, blood, and urine mercury levels in the American Dental Association (ADA) study participants. Environ. Res 149: 247–258. [PubMed: 26673400]

Park ST, Lim KT, Chung YT, and Kim SU 1996 Methylmercury-induced neurotoxicity in cerebral neuron culture is blocked by antioxidants and NMDA receptor antagonists. Neurotoxicology 17: 37–45. [PubMed: 8784817]

Pastore A, Federici G, Bertini E, and Piemonte F 2003 Analysis of glutathione: implication in redox and detoxification. Clin. Chim. Acta 333: 19–39. [PubMed: 12809732]

Paustenbach DJ 2001 The practice of exposure assessment; In: Principles and Methods of Toxicology, Chapter 9; Hayes AW, ed., Taylor& Francis, Philadelphia, pp. 387–448.

Perrin-Nadif R, Dusch M, Koch C, Schmitt P, and Mur JM 1996 Catalase and superoxide dismutase activities as biomarkers of oxidative stress in workers exposed to mercury vapors. J. Toxicol. Environ. Health 7: 107–119.

Piccoli C, D'Aprile A, Scrima R, Ambrosi L, Zefferino R, and Capitanio N. 2012 Subcytotoxic mercury chloride inhibits gap junction intercellular communication by a redox- and phosphorylation-mediated mechanism. Free Radic Biol. Med 52: 916–927. [PubMed: 22240155]

Pingree SD, Simmonds PL, Rummel KT, and Woods JS 2001 Quantitative evaluation of urinary porphyrins as a measure of kidney mercury content and mercury body burden during prolonged methylmercury exposure in rats. Toxicol. Sci 61: 234–240. [PubMed: 11353132]

Pinheiro MC,. Macchi BM, Vieira JL, Oikawa T, Amoras WW, Guimarães GA, Costa CA, Crespo-López ME, Herculano AM, Silveira LC, and do Nascimento JLM. 2008 Mercury exposure and

antioxidant defenses in women: A comparative study in the Amazon. Environ. Res 107: 53–59. [PubMed: 17905226]

Piotrowski JK, Trojanowska B, Wiśniewska-Knypl JM, and Bolanowska W 1974 Mercury binding in the kidney and liver of rats repeatedly exposed to mercuric chloride: induction of metallothionein by mercury and cadmium. Toxicol. Appl. Pharmacol 27: 11–19. [PubMed: 4853165]

Pollack AZ, Mumford SL Mendola P, Perkins NJ,Rotman Y, Wactawski-Wende J, and Schisterman EF 2015 Kidney biomarkers associated with blood lead, mercury, and cadmium in premenopausal women: A prospective cohort study. J. Toxicol. Environ. Health A 78: 119–131. [PubMed: 25424620]

Pollack AZ, Schisterman EF, Goldman LR, Mumford SL, Perkins NJ, Bloom MS, Rudra CB, Browne RW, and Wactawski-Wende J 2012 Relation of blood cadmium, lead, and mercury levels to biomarkers of lipid peroxidation in premenopausal women. Am. J. Epidemiol 175: 645–652. [PubMed: 22302120]

Pollard KM, and Hultman P 1997 Effects of mercury in the immune system. In: Metal Ions in Biological Systems, Volume 34 : Mercury and its Effects on Environment and Biology, Sigel A and Sigel H, eds., Marcel Dekker Inc, New York, pp 421–460.

Pollard KM, and Kono DH 2013 Requirements for innate immune pathways in environmentally induced autoimmunity. BMC Med 11: 100. [PubMed: 23557436]

Qu H, Syversen T, Aschner M, and Sonnewald U 2003 Effect of methylmercury on glutamate metabolism in cerebellar astrocytes in culture. Neurochem. Int 43: 411–416. [PubMed: 12742086]

Ratcliffe HE, Swanson GM, and Fischer LJ 1996 Human exposure to mercury: a critical assessment of the evidence of adverse health effects. J. Toxicol. Environ. Health 49: 221–270. [PubMed: 8876653]

Rigobello MP, Scutari G, Folda A, and Bindoli A 2004 Mitochondrial thioredoxin reductase inhibition by gold(I) compounds and concurrent stimulation of permeability transition and release of cytochrome c. Biochem. Pharmacol 67: 689–696. [PubMed: 14757168]

Robinson JF, Theunissen PT, van Dartel DA, Pennings JL, Faustman EM, and Piersma AH 2011 Comparison of MeHg-induced toxicogenomic responses across in vivo and in vitro models used in developmental toxicology. Reprod. Toxicol 32: 180–188. [PubMed: 21664453]

Rocha JB, Freitas AJ, Marques MB, Pereira ME, Emanuelli T, and Souza DO. 1993 Effects of methylmercury exposure during the second stage of rapid postnatal brain growth on negative geotaxis and on delta-aminolevulinate dehydratase of suckling rats. Braz. J. Med. Biol. Res 26: 1077–1083. [PubMed: 8312839]

Rodrigues J, Branco V, Lu J, Holmgren A, and Carvalho C. 2015 Toxicological effects of thiomersal and ethylmercury: Inhibition of the thioredoxin system and NADP+-dependent dehydrogenases of the pentose phosphate pathway. Toxicol. Appl. Pharmacol 286: 216–223. [PubMed: 25981166]

Romero DL, Mounho BJ, Lauer FT, Born JL, and Burchiel SW 1997 Depletion of glutathione by benzo(a)pyrene metabolites, ionomycin, thapsigargin, and phorbol myristate in human peripheral blood mononuclear cells. Toxicol. Appl. Pharmacol 144: 62–69. [PubMed: 9169070]

Rooney JPK 2007 The role of thiols, dithiols , nutritional factors and interacting ligands in the toxicology of mercury. Toxicology 234: 145–156. [PubMed: 17408840]

Ruszkiewicz JA, Bowman AB, Farina M, Rocha JB, and Aschner M 2016 Sex-and structure-specific differences in antioxidant responses to methylmercury during early development. NeuroToxicology 56: 118–126. [PubMed: 27456245]

Sakai T 2000 Biomarkers of lead exposure. Ind. Health 38: 127–142. [PubMed: 10812836]

Samir AM, and Aref WM 2011 Impact of occupational exposure to elemental mercury on some antioxidative enzymes among dental staff. Toxicol. Ind. Health 27: 779–786. [PubMed: 21427135]

Sanders AP, Burris HH, Just AC, Motta V, Amarasiriwardena C, Svensson K, Oken E, Solano-Gonzalez M, Mercado-Garcia A, Pantic I, Schwartz J, Tellez-Rojo MM, Baccarelli AA, and Wright RO 2015 Altered miRNA expression in the cervix during pregnancy associated with lead and mercury exposure. Epigenomics 7: 885–896 [PubMed: 26418635]

Santos APM, Mateus ML, Carvalho CML, and Batoréu MCC 2007 Biomarkers of exposure and effect as indicators of the interference of selenomethionine on methylmercury toxicity. Toxicol. Lett 169: 121–128. [PubMed: 17267146]

Satoh H 2000 Occupational and environmental toxicology of mercury and its compounds. Ind. Health 38: 153–164. [PubMed: 10812838]

Savabieasfahani M, Hoseiny M, and Goodarzi S 2012 Toxic and essential trace metals in first baby haircuts and mother hair from Imam Hossein Hospital Tehran, Iran. Bull. Environ. Contam. Toxicol 88: 140–144. [PubMed: 22139296]

Schläwicke-Engström K, Strömberg U, Lundh T, Johansson I, Vessby B, Hallmans G, Skerfving S, and Broberg K 2008 Genetic variation in glutathione-related genes and body burden of methylmercury. Environ. Health Persp 116: 734–739.

Schmidt CW 2006 Signs of the times: Biomarkers in perspective. Environ. Health Persp 114: A700–705.

Scientific Committee on Occupational Exposure Limits (SCOEL). 2007 Recommendation from the Scientific Committee on Occupational Exposure Limits for elemental mercury and inorganic divalent mercury compounds. European Commission, SCOEL/SUM/84 (available at: ec.europa.eu/social/BlobServlet?docId=3852&langId=en)

Shanker G, and Aschner M 2001 Identification and characterization of uptake systems for cystine and cysteine in cultured astrocytes and neurons: Evidence for methylmercury-targeted disruption of astrocytic transport. J. Neurosci. Res 66: 998–1002. [PubMed: 11746429]

Shanker G, Allen JW, Mutkus LA, and Aschner M 2001 Methylmercury inhibits cysteine uptake in cultured primary astrocytes, but not in neurons. Brain Res 914: 159–165. [PubMed: 11578608]

Shanker G, Syversen T, and Aschner M. 2003 Astrocyte-mediated methylmercury neurotoxicity. Biol. Trace Elem. Res 95: 1 – 10. [PubMed: 14555794]

Sherratt PJ, Huang HC, Nguyen T, and Pickett CB. 2004 Role of protein phosphorylation in the regulation of NF-E2-related factor 2 activity. Meth Enzymol 378: 286–301. [PubMed: 15038976]

Sikorski R, Paszkowski T, and Szprengier-Juszkiewicz T 1986 Mercury in neonatal scalp hair. Sci. Total Environ 57: 105–110. [PubMed: 3810136]

Silbergeld EK, and Davis DL 1994 Role of biomarkers in identifying and understanding environmentally induced disease. Clin. Chem 40: 1363–1367. [PubMed: 8013120]

Silva de Paula E, Carneiro MF, Grotto D, Hernandes LC, Antunes LM, and Barbosa F, Jr 2016 Protective effects of niacin against methylmercury-induced genotoxicity and alterations in antioxidant status in rats. J. Toxicol. Environ. Health, A 79: 174–183.

Simmons-Willis TA, Koh AS, Clarkson TW, and Ballatori N 2002 Transport of a neurotoxicant by molecular mimicry: the methylmercury–L-cysteine complex is a substrate for human L-type large neutral amino acid transporter (LAT) 1 and LAT2. Biochem. J 367: 239–246. [PubMed: 12117417]

Srivastava M, Singh S, and Self WT 2012 Exposure to silver nanoparticles inhibits selenoprotein synthesis and the activity of thioredoxin reductase. Environ. Health Persp 120: 56–61.

Stehr-Green P, Tullb P, Stellfeld M, Mortenson P, and Simpson D 2003 Autism and thimerosal-containing vaccines: Lack of consistent evidence for an association. Am. J. Prev. Med 25:101–106. [PubMed: 12880876]

Stringari J, Nunes AK, Franco JL, Bohrer D, Garcia SC, Dafre AL, Milatovic D, Souza DO, Rocha JB, Aschner MM, and Farina M 2008 Prenatal methylmercury exposure hampers glutathione antioxidant system ontogenesis and causes long-lasting oxidative stress in the mouse brain. *Toxicol.* Appl. Pharmacol. 227: 147–154. [PubMed: 18023834]

Suda I, and Takahashi H 1992 Degradation of methyl and ethyl mercury into inorganic mercury by other reactive oxygen species besides hydroxyl radical. Arch. Toxicol 66: 34–39. [PubMed: 1316115]

Sugiura Y, Hojo Y, Tamai Y, and Tanaka H 1976 Selenium protection against mercury toxicity. Binding of methylmercury by the selenohydryl-containing ligand. J. Am. Chem. Soc 98: 2339–2341. [PubMed: 1254870]

Suzuki KT, and Ogra Y 2001 Metabolism of selenium and its interaction with mercury: mechanisms by a speciation study. Phosphorus, Sulfur Silicon Relat. Elem 171: 135–169.

Sweet LI, and Zelikoff JT 2001 Toxicology and immunotoxicology of mercury: A comparative review in fish and humans. J. Toxicol. Environ. Health B 4:161–205.

Tan Y-M, Sobus J, Chang D, Tornero-Velez R, Goldsmith M Pleil J and Daty C 2012Reconstructing human exposures using biomarkers and other “clues’. J. Toxicol. Environ. Health B 15: 22–38.

Taylor LE, Swerdfeger AL, and Eslick GD. 2014 Vaccines are not associated with autism: An evidence-based meta-analysis of case-control and cohort studies. Vaccine 32: 3623–3629. [PubMed: 24814559]

Theunissen PT, Pennings JL, Robinson JF, Claessen SM, Kleinjans JC, and Piersma AH 2011 Time-response evaluation by transcriptomics of methylmercury effects on neural differentiation of murine embryonic stem cells. Toxicol. Sci 122: 437–447. [PubMed: 21613230]

Tian L, Guo HF, Gao A, Lu XT, and Li QY 2009 Effects of mercury released from glold extraction by amalgamation on renal function and environment in Shanxi, China. Bull. Environ. Contam. Toxicol 83: 71–74. [PubMed: 19387524]

Timbrell JA 1998 Biomarkers in toxicology. Toxicology 129:1–12 [PubMed: 9769106]

Toyama T, Shinkai Y, Sumi D, and Kumagai Y 2010 Carbon monoxide derived from heme oxygenase-2 mediates reduction of methylmercury toxicity in SH-SY5Y cells. Toxicol. Appl. Pharmacol 249: 86–90. [PubMed: 20807546]

Toyama T, Shinkai Y, Yasutake A, Uchida K, Yamamoto M, and Kumagai Y 2011a Isothiocyanates reduce mercury accumulation via an Nrf2-dependent mechanism during exposure of mice to methylmercury. Environ. Health Persp 119: 1117–1122.

Toyama T, Sumi D, Shinkai Y, Yasutake A, Taguchi K, Tong KI, Yamamoto M, and Kumagai Y 2007 Cytoprotective role of Nrf2/Keap1 system in methylmercury toxicity. Biochem. Biophys. Res. Commun 363: 645–650. [PubMed: 17904103]

Toyama T, Yoshida E, Shinkai Y, and Kumagai Y 2011b DNA microarray analysis of human neuroblastoma SH-SY5Y cells exposed to methylmercury. J. Toxicol. Sci 36: 843–845. [PubMed: 22129749]

Uno Y, Uchiyama T, Kurosawa M, Aleksic B, and Ozaki N 2015 Early exposure to the combined measles–mumps–rubella vaccine and thimerosal-containing vaccines and risk of autism spectrum disorder. Vaccine 33: 2511–2516. [PubMed: 25562790]

Usuki F, Yamashita A, and Fujimura M 2011 Post-transcriptional defects of antioxidant selenoenzymes cause oxidative stress under methylmercury exposure. J. Biol. Chem 286: 6641–6649. [PubMed: 21106535]

Vahter ME, Mottet NK, Friberg LT, Lind SB, Charleston JS, and Burbacher TM 1995 Demethylation of methylmercury in different brain sites of *Macaca fascicularis* monkeys during long-term subclinical methylmercury exposure. Toxicol. Appl. Pharmacol 134: 273–284. [PubMed: 7570604]

Vanduyn N, Settivari R, Wong G, and Nass R 2010 SKN-1/Nrf2 inhibits dopamine neuron degeneration in a *Caenorhabditis elegans* model of methylmercury toxicity. Toxicol. Sci 118: 613–624. [PubMed: 20855423]

Vas J, and Monestier M 2008 Immunology of mercury. Ann. N. Y. Acad. Sci 1143: 240–267. [PubMed: 19076354]

Vendrell I, Carrascal M, Vilaro MT, Abian J, Rodriguez-Farre E, and Sunol C 2007 Cell viability and proteomic analysis in cultured neurons exposed to methylmercury. Human Exp. Toxicol 26: 263–272.

Verstraeten T, Davis RL, DeStefano F, Lieu TA, Rhodes PH, Black SB, Shinefield H, and Chen RT 2003 Safety of thimerosal-containing vaccines: A two-phased study of computerized health maintenance organization databases. Pediatrics 112:1039–1048. [PubMed: 14595043]

Wagner C, Sudati JH, Nogueira CW, and Rocha JB 2010 In vivo and in vitro inhibition of mice thioredoxin reductase by methylmercury. Biometals 23: 1171–1177. [PubMed: 20717703]

Wallace MAG, Kormos TM and Pleil J 2016Blood-bornebiomarkers and bioindicators for linking exposure to health effects in environmental health science. J. Toxicol. Environ. Health B 19: 380–409.

Wang L, Jiang H, Yin Z, Aschner M, and Cai J 2009 Methylmercury toxicity and Nrf2-dependent detoxification in astrocytes. Toxicol. Sci 107: 135–143. [PubMed: 18815141]

Wang Y, Goodrich JM, Gillespie B, Werner R, Basu N, and Franzblau A. 2012 An investigation of modifying effects of metallothionein single-nucleotide polymorphisms on the association between mercury exposure and biomarker levels. Environ. Health Persp 120: 530–534.

Wang Y, Wang D, Wu J, Wang B, Wang L, Gao X, Huang H, and Ma H 2015 Cinnabar induces renal inflammation and fibrogenesis in rats. BioMed Res. Int 2015: ID280958.

Wataha JC, Lewis JB, McCloud VV, Shaw M, Omata Y, Lockwood PE, Messer RLW, and Hansen JM 2008 Effect of mercury (II) on Nrf2, thioredoxin reductase-1 and thioredoxin-1 in human monocytes. Dent. Mater 24: 765–772. [PubMed: 17959236]

Watanabe C 2002 Modification of mercury toxicity by selenium: Practical importance? Tohoku J. Exp. Med 196: 71–77. [PubMed: 12498318]

Watanabe C, Kasanuma Y, Dejima Y, and Satoh H 1999a The effect of prenatal methylmercury exposure on the GSH level and lipid peroxidation in the fetal brain and placenta of mice. Tohoku J. Exp. Med 187: 121–126. [PubMed: 10228983]

Watanabe C, Yin K, Kasanuma Y, and Satoh H 1999b In utero exposure to methylmercury and Se deficiency converge on the neurobehavioral outcome in mice. Neurotoxicol. Teratol 21: 83–88. [PubMed: 10023805]

Wigle DT, Arbuckle TE, Turner MC, Bérubé A, Yang Q, Liu S, and Krewski D 2008 Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. J. Toxicol. Environ. Health B 11: 373–517

Witte AB, Anestål K, Jerremalm E, Ehrsson H, and Arnér ES 2005 Inhibition of thioredoxin reductase but not of glutathione reductase by the major classes of alkylating and platinum-containing anticancer compounds. Free Radic Biol. Med 39: 696–703. [PubMed: 16085187]

Woods JS 1995 Porphyrin metabolism as indicator of metal exposure and toxicity. In: Handbook of Experimental Pharmacology, Vol. 105 – Toxicology of Metal—Biochemical Aspects; Goyer RA and Cherian MG, eds., Springer-Verlag, Berlin, Germany, pp.19–52.

Woods JS, and Fowler BA 1977 Renal porphyrinuria during chronic methyl mercury exposure. J. Lab. Clin. Med 90: 266–272. [PubMed: 886212]

Woods JS, and. Fowler BA 1978 Altered regulation of mammalian hepatic heme biosynthesis and urinary porphyrin excretion during prolonged exposure to sodium arsenate. Toxicol. Appl. Pharmacol 43: 361–371. [PubMed: 635923]

Woods JS, Bowers MA, and Davis HA 1991 Urinary porphyrin profiles as biomarkers of trace metal exposure and toxicity: studies on urinary porphryin excretion patterns in rats during prolonged exposure to methylmercury. Toxicol. Appl.Pharmacol 110: 464–476. [PubMed: 1949014]

Woods JS, Echeverria D, Heyer NJ, Simmonds PL, Wilkerson J, and Farin FM 2005 The association between genetic polymorphisms of coproporphyrinogen oxidase and an atypical porphyrinogenic response to mercury exposure in humans. Toxicol. Appl.Pharmacol 206: 113–120. [PubMed: 15967199]

Woods JS, Heyer NJ, Echeverria D, Russo JE, Martin MD, Bernardo MF, Luis HS, Vaz L, and Farin FM 2012 Modification of neurobehavioral effects of mercury by a genetic polymorphism of coproporphyrinogen oxidase in children. Neurotoxicol. Teratol 34: 513–521. [PubMed: 22765978]

Woods JS, Heyer NJ, Russo JE, Martin MD, and Farin FM 2014a Genetic polymorphisms affecting susceptibility to mercury neurotoxicity in children: summary findings from the Casa Pia children's amalgam clinical trial. Neurotoxicology 44: 288–302. [PubMed: 25109824]

Woods JS, Heyer N, Russo JE, Martin MD, Pillai PB, and Farin FM 2013 Modification of neurobehavioral effects of mercury by genetic polymorphisms of metallothionein in children. Neurotoxicol. Teratol 39: 36–44. [PubMed: 23827881]

Woods JS, Heyer NJ, Russo JE, Martin MD, Pillai PB, Bammler TK, and Farin FM 2014b Genetic polymorphisms of catechol-O-methyltransferase modify the neurobehavioral effects of mercury in children. J. Toxicol. Environ. Health A 77: 293–312. [PubMed: 24593143]

Woods JS, Martin MD, Leroux BG, DeRouen TA, Bernardo MF, Luis HS, Leitão JG, Simmonds PL, Echeverria D, and Rue TC 2009 Urinary porphyrin excretion in children with mercury amalgam treatment: Findings from the Casa Pia children's dental amalgam trial. J. Toxicol. Environ. Health A 72: 891–896. [PubMed: 19557617]

Woods JS, Martin MD, Naleway CA, and Echeverria D 1993 Urinary porphyrin profiles as a biomarker of mercury exposure: studies on dentists with occupational exposure to mercury vapor. J. Toxicol. Environ. Health, 40: 235–246. [PubMed: 8230299]

World Health Organization (WHO). 1993 Biomarkers and Risk Assessment: Concepts and Principles. International Programme on Chemical Safety, Environmental Health Criteria 155, Geneva, 82 pp (available at: http://www.inchem.org/documents/ehc/ehc/ehc155.htm)

World Health Organization (WHO). 2003 Elemental Mercury and Inorganic Mercury Compounds: Human Health Aspects. Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives, WHO Technical Report Series 922, Geneva, 176 pp (available at: http://www.who.int/ipcs/publications/cicad/en/cicad50.pdf)

World Health Organization/United Nations Environment Programme (WHO/UNEP). 2008 Guidance for Identifying Populations at risk from Mercury Exposure. Inter-Organization Programme for the Sound Management of Chemicals, Geneva, 170 pp.

Wormser U, Brodsky B, Milatovic D, Finkelstein Y, Farina M, Rocha JB, and Aschner M 2012 Protective effect of a novel peptide against methylmercury-induced toxicity in rat primary astrocytes. Neurotoxicology 33: 763–768. [PubMed: 22186600]

Yadetie F, Karlsen OA, Lanzen A, Berg K, Olsvik P, Hogstrand C, and Goksoyr A 2013 Global transcriptome analysis of Atlantic cod (Gadus morhua) liver after in vivo methylmercury exposure suggests effects on energy metabolism pathways. Aquat. Toxicol 126: 314–325. [PubMed: 23103053]

Yang D, Yu-Wei C, Gunn JM, and Belzille N 2008 Selenium and mercury in organisms: Interactions and mechanisms. Environ. Rev 16: 71–92.

Yasutake A, Hirayma K, and Inoue M 1989 Mechanism of urinary excretion of methylmercury in mice. Arch. Toxicol 63: 479–483. [PubMed: 2575893]

Yasutake A, and Nakamura M 2011 Induction by mercury compounds of metallothioneins in mouse tissues: Inorganic mercury accumulation is not a dominant factor for metallothionein induction in the liver. J. Toxicol. Sci 36: 365–372. [PubMed: 21628964]

Yeter D, and Deth R 2012 ITPKC susceptibility in Kawasaki syndrome as a sensitizing factor for autoimmunity and coronary arterial wall relaxation induced by Thimerosal's effects on calcium signaling via IP3. Autoimmun. Rev 11: 903–908. [PubMed: 22498790]

Yin Z, Milatovic D, Aschner JL, Syversen T, Rocha JB, Souza DO, Sidoryk M, Albrecht J, and Aschner M 2007 Methylmercury induces oxidative injury, alterations in permeability and glutamine transport in cultured astrocytes. Brain Res 1131: 1–10. [PubMed: 17182013]

Yoshida E, Abiko Y, and Kumagai Y. 2014 Glutathione adduct of methylmercury activates the Keap1-Nrf2 pathway in SH-SY5Y Cells. Chem. Res. Toxicol 27: 1780–1786. [PubMed: 25271560]

Yu R, Mandlekar S, Lei W, Fahl WE, Tan TH, and Kong AN 2000 p38 mitogen-activated protein kinase negatively regulates the induction of phase II drug-metabolizing enzymes that detoxify carcinogens. J. Biol. Chem 275: 2322–2327. [PubMed: 10644681]

Zabiński Z, Dabrowski Z, Moszczyński P, and Rutowski J 2000 The activity of erythrocyte enzymes and basic indices of peripheral blood erythrocytes from workers chronically exposed to mercury vapours. Toxicol. Ind. Health 16: 58–64. [PubMed: 10798623]

Zalups RK 1998 Basolateral uptake of mercuric conjugates of N-acetylcysteine and cysteine in the kidney involves the organic anion transport system. J. Toxicol. Environ. Health A 55:13–29. [PubMed: 9747601]

Zalups RK, Barfuss DW, and Kostyniak PJ 1992 Altered intrarenal accumulation of mercury in uninephrectomized rats treated with methylmercury chloride. Toxicol. Appl. Pharmacol 115: 174–182. [PubMed: 1641852]

Zalups RK, and Barfuss DW 2002a Renal organic anion transport system: A mechanism for the basolateral uptake of mercury-thiol conjugates along the pars recta of the proximal tubule. Appl. Pharmacol 182: 234–243.

Zalups RK, and Barfuss DW 2002b Simultaneous coexposure to inorganic mercury and cadmium: A study of the renal and hepatic disposition of mercury and cadmium. J. Toxicol. Environ. Health A 65: 1471–1490. [PubMed: 12396877]

Zalups RK 2000 Molecular interactions with mercury in the kidney. Pharmacol. Rev 52: 113–143. [PubMed: 10699157]

Zalups RK, Joshee L, and Bridges CC 2014 Novel Hg^{2+}-induced Nephropathy in rats and mice lacking Mrp2: Evidence of axial heterogeneity in the handling of Hg^{2+} along the proximal tubule. Toxicol. Sci 142: 250–260 [PubMed: 25145654]

Zemolin AP, Meinerz DF, de Paula MT, Mariano DO, Rocha JB, Pereira AB, Posser T, and Franco JL. 2012 Evidences for a role of glutathione peroxidase 4 (GPx4) in methylmercury induced neurotoxicity in vivo. Toxicology 302: 60–67. [PubMed: 22885222]

Zhang J, Brown RP, Shaw M, Vaidya VS, Zhou Y, Espandiari P, Sadrieh N, Stratmeyer M, Keenan J, Kilty CG, Bonventre JV, and Goering PL 2008 Immunolocalization of Kim-1, RPA-1, and RPA-2 in kidney of gentamicin-, mercury-, or chromium- treated rats: relationship to renal distributions of iNOS and nitrotyrosine. Toxicol. Pathol 36: 397–409. [PubMed: 18441258]

Zhou Y, Vaidya VS, Brown RP, Zhang J, Rosenzweig BA, Thompson KL, Miller TJ, Bonventre JV, and Goering PL 2008 Comparison of kidney injury molecule-1 and other nephrotoxicity biomarkers in urine and kidney following acute exposure to gentamicin, mercury and chromium. Toxicol.Sci 101: 159–170. [PubMed: 17934191]

Zimmermann LT, dos Santos DB, Colle D, dos Santos AA, Hort MA, Garcia SC, Bressan LP, Bohrer D, and Farina M 2014 Methionine stimulates motor impairment and cerebellar mercury deposition in methylmercury-exposed mice. J. Toxicol. Environ. Health A 77: 46–56. [PubMed: 24555646]

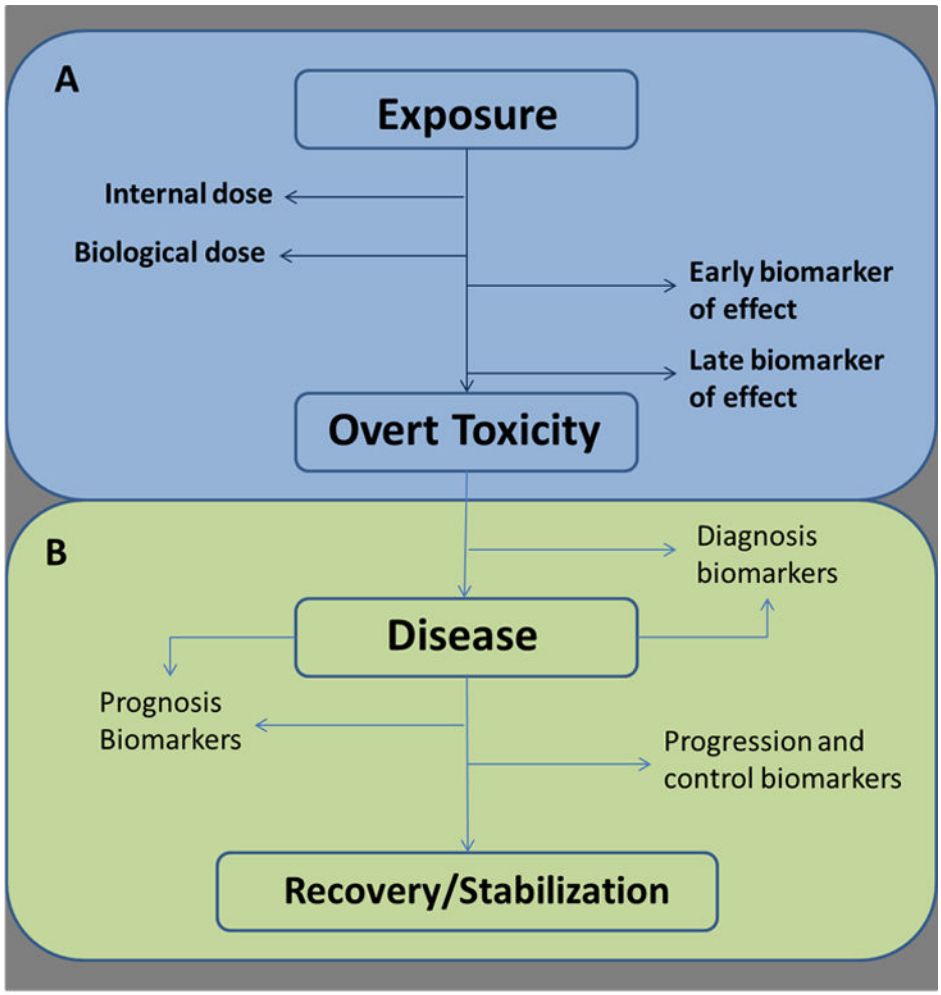

Figure 1. Applications of biomarkers in different areas of Toxicology and Medicine.

Biomarkers can be used to evaluate exposure and predict the toxicity (A) of a xenobiotic by measuring the internal dose, the dose interacting with molecular targets or molecular effects that reflect early changes or that represent an established toxic response. If toxicity persists, disease ensues (B) and it may be diagnosed by standard clinical markers. New approaches make use of biomarkers for the development of new medicines and moreover the identification of biomarkers that follow disease progression will become a key element for personalized medicine.

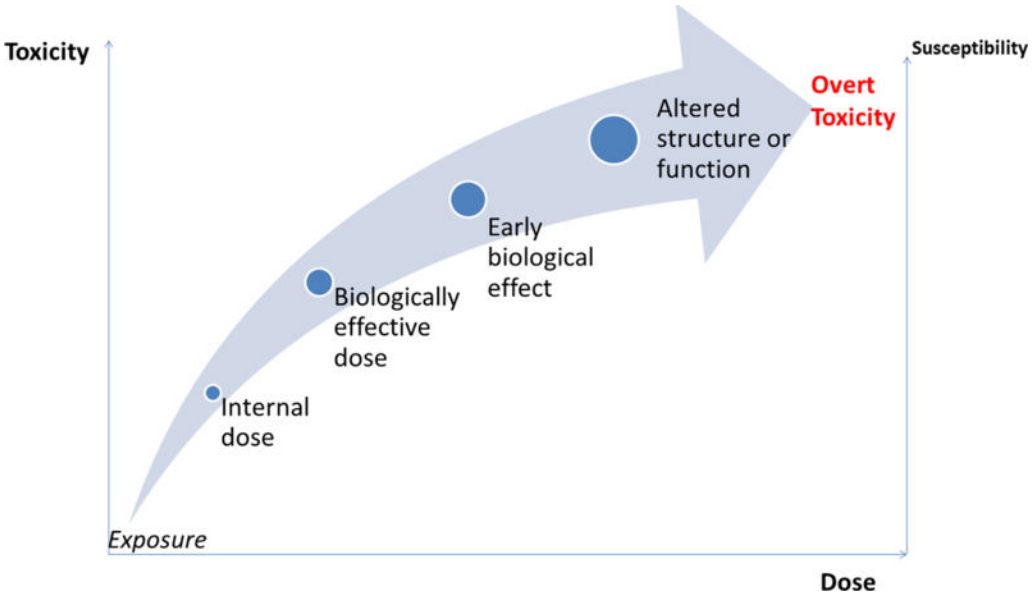

Figure 2.
Pathway leading from exposure to a xenobiotic to toxic manifestations, and the relative position of exposure and effect biomarkers.

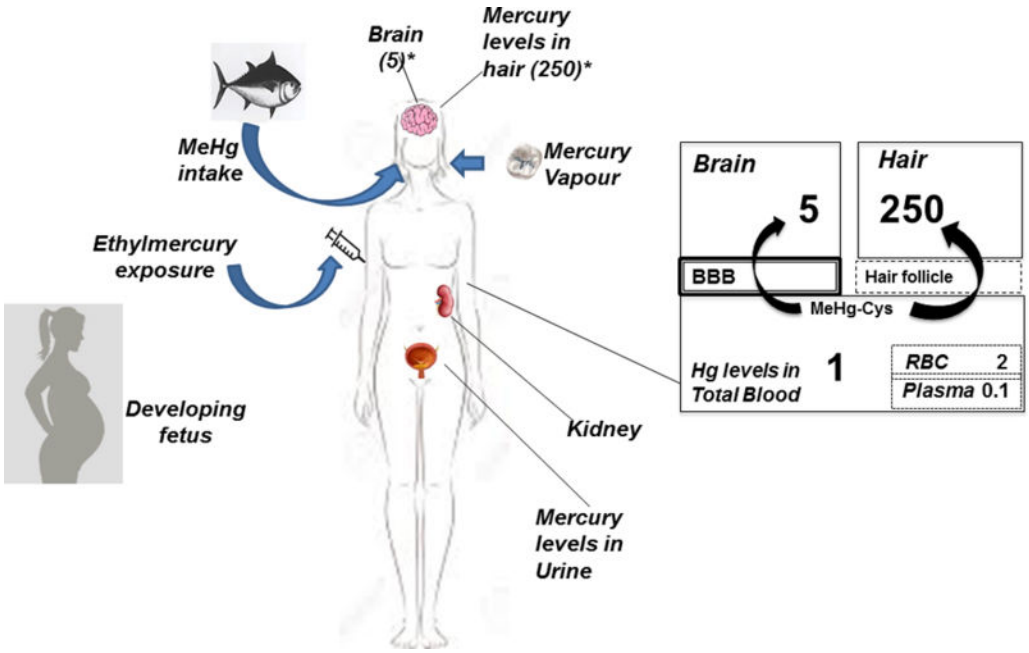

Figure 3. Main pathways of exposure to mercury compounds, exposure biomarkers and relative concentrations of MeHg in hair, brain and blood.

Humans are exposed to different mercury compounds by different routes, such as MeHg (fish consumption), Hg^0 (dental amalgams), and EtHg (TCV). The major target organs include the brain, kidney and the developing fetus, and exposure levels are normally assessed by measuring mercury levels in hair, blood and urine. In the case of MeHg levels in hair and blood correlate well with the values found in the brain in the proportion 250:5:1. (adapted from Clarkson et al., 2007)

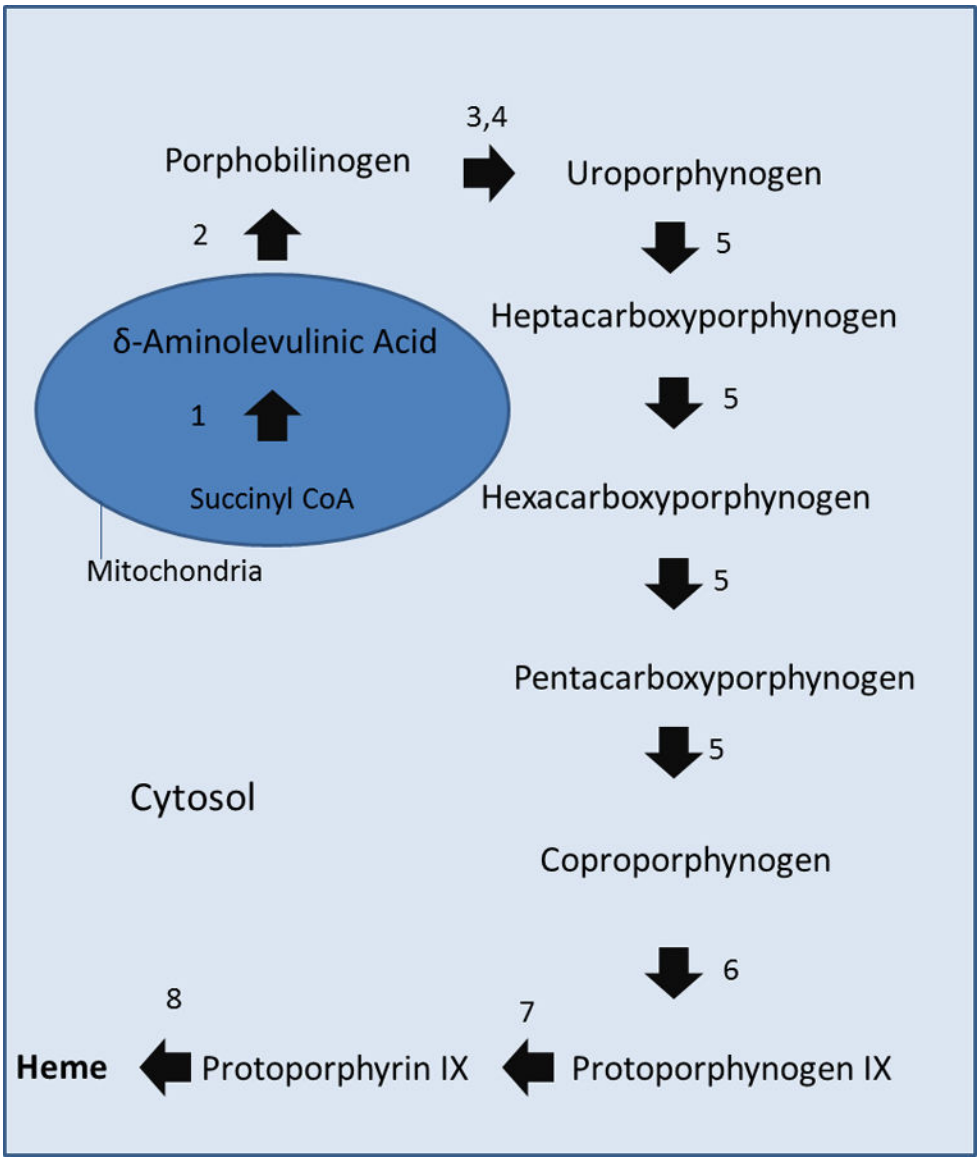

Figure 4. The Heme Biosynthetic Pathway.

The enzymes involved in heme biosynthesis include: 1- δ-aminolevulinic acid (ALA) synthetase; 2- ALA dehydratase; 3- uroporphyrinogen I synthetase; 4- uroporphyrinogen III cosynthetase; 5- uroporphyrinogen decarboxylase; 6- coproporphyrinogen oxidase; 7- protoporphyrinogen oxidase; 8- ferrochelatase. Mercury targets the enzymes uroporphyrinogen decarboxylase (UROD; 5), and especially coproporphyrinogen oxidase (CPOX; 6), thus leading to the accumulation and excretion of 4 and 5 carboxylporphyrinogens which afterwards are oxidized to porphyrins and excreted in urine (adapted from Pingree et al., 2001).

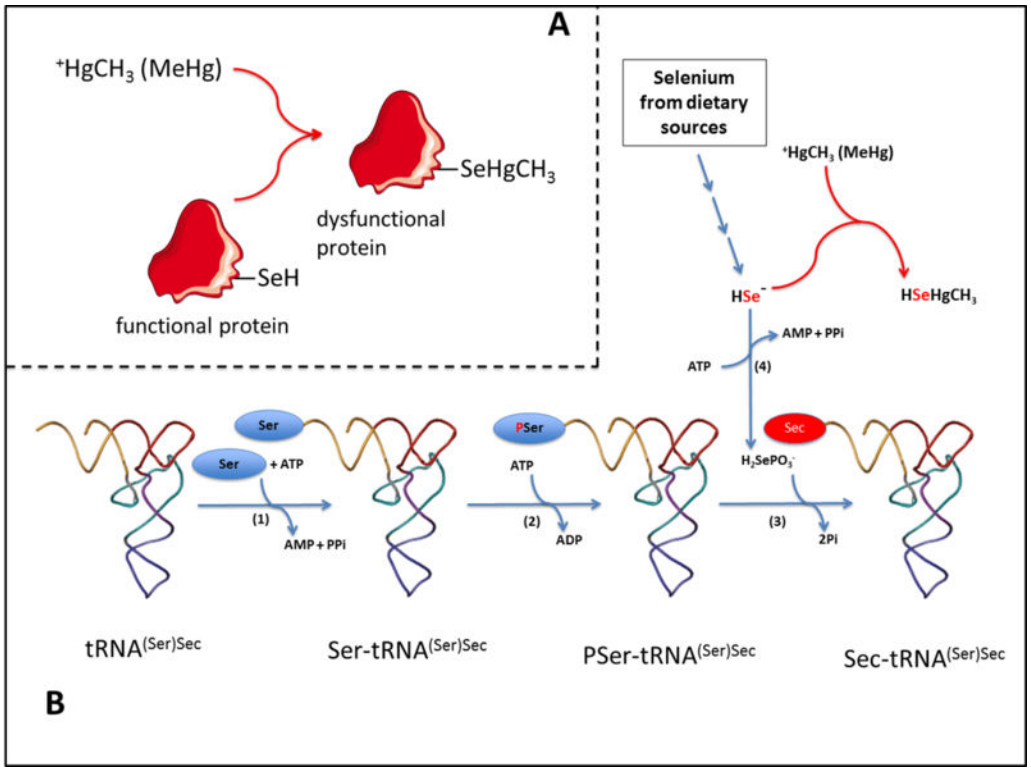

Figure 5. Potential mechanisms by which MeHg decreases selenoproteins' homeostasis.

(A) MeHg directly interacts with the selenol group of selenocysteine. Due to its high electrophilicity, MeHg directly interacts with the selenol group (–SeH) of selenoccysteine (red arrow). Because this group is classically responsible for catalytic activity in several selenoproteins, loss of protein function is observed. **(B) MeHg may impair the biosynthesis of selenocysteine.** Selenocysteine (Sec) is crucial for the proper synthesis and functioning of selenoproteins. The biosynthesis of Sec occurs on its tRNA, and the pathway begins with the attachment of serine to Sec $tRNA^{[Ser]Sec}$ (**event 1**; catalyzed by seryl-tRNAsynthetase) in the presence of ATP. Phosphoseryl-tRNA kinase (**event 2**) phosphorylates the serine moiety to form an intermediate, phosphoseryl-$tRNA^{[Ser]Sec}$ (PSer-$tRNA^{[Ser]Sec}$). Sec synthase (**event 3**) catalyzes the formation of Sec-$tRNA^{[Ser]Sec}$, which is subsequently incorporated into selenoproteins during protein synthesis. This metabolic step requires the presence of selenophosphate ($H_2SePO_3^-$), whose precursor is selenide (HSe^-) (**event 4**). MeHg (CH_3Hg^+) directly interacts with selenide (red arrow), leading to a "selenium-deficient-like" condition, which lead to inappropriate selenoprotein synthesis (Usuki et al., 2011). For additional details on Sec biosynthesis, see Hatfield et al., 2014. Abbreviations: P_i, inorganic phosphate; PP_i, inorganic pyrophosphate.

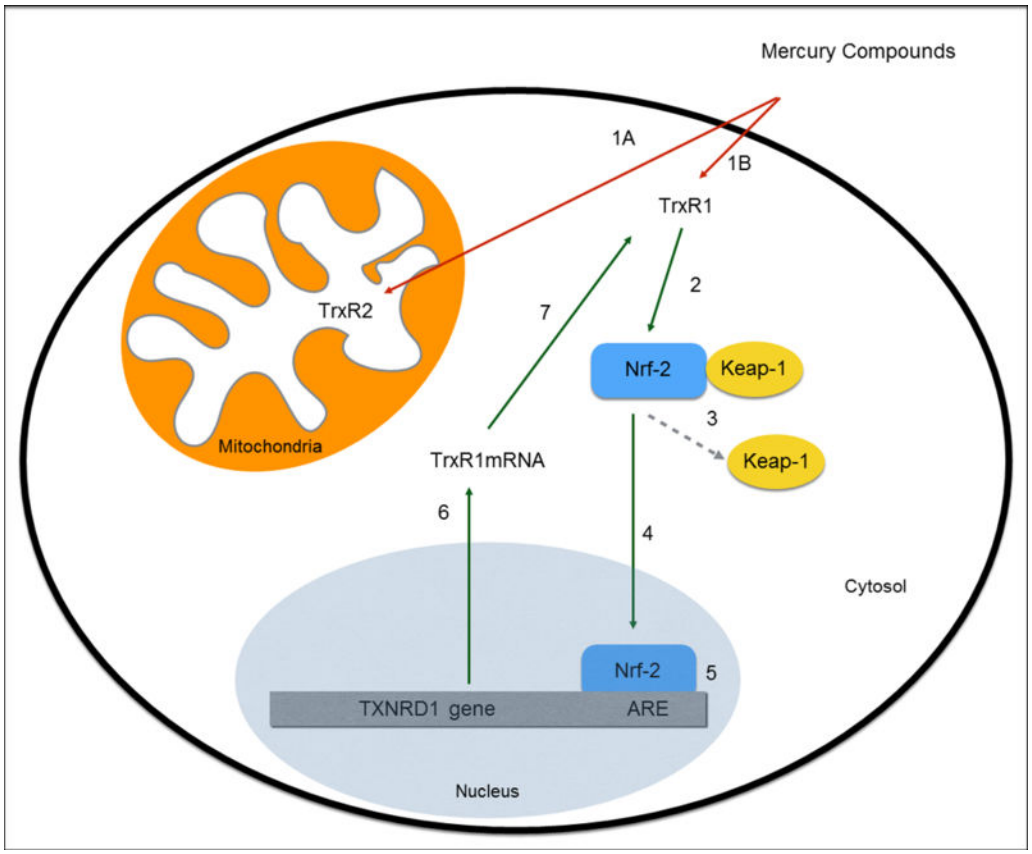

Figure 6. Interaction between mercury compounds and TrxR1 and TrxR2 at the cytosol and mitochondria.

Mercury compounds enter liver cells and target TrxR1 and 2 at the cytosol and mitochondria, respectively (1A and 1B), leading to a decrease in activity. Loss of TrxR1 promotes Nrf-2 release from Keap-1 (2 and 3) and its subsequent translocation to the nucleus of the cell (4) where it binds the ARE element in the promoter region of the TXNRD1 gene (5). This process is faster upon exposure to Hg^{2+} than to MeHg (see Branco et al., 2014 for detailed explanation). Transcription of the gene is enhanced and TrxR1 mRNA builds up in the cytosol (6) and TrxR1 is de novo synthetized (7)

Table 1:

Comparison of IC_{50} values for the inhibition of purified TrxR by different metals.

Compound		[TrxR] (nM)	IC_{50} (μM)	incubation time (min)	Reference
Mercury	$HgCl_2$	50	0.0072	5	Carvalho et al., 2008a
	MeHgCl	50	0.0197	5	
Gold (I)	Auranofin	2	0.020	10	Rigobello et al., 2004
	TEPAu	2	0.065	10	
	Aurothiomalate	2	0.280	10	
Gold (III)	Aupy	2	1.42	10	
	Audien	2	0.42	10	
	Aubipy	2	0.28	10	
	Aubipyxil	2	0.21	10	
Tin	Tributyltin	2	76.1	10	Rigobello et al., 2004
Cadmium	Cadmiumacetate	2	23.5	10	
Zinc	ZincAcetate	2	19.5	10	
	Zincpyrithione	2	11.8	10	
Platinum	Cisplatin	300	~200	20	Witte et al., 2005
	Oxaliplatin	300	~100	20	
Silver	Ag_2SO_4	25	~0.025/5	5	Srivastava et al., 2012
Arsenic	As_2O_3	50	0.25/30	30	Lu et al., 2007