Mercury's Neurotoxic Effects on Brain Selenoenzymes

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Contents

Abstract

Toxic mercury (Hg) exposures inhibit selenium (Se)-dependent enzymes (selenoenzymes) required in the brain. Selenocysteine (Sec), the 21st genetically encoded amino acid, is the most powerful intracellular nucleophile, making it vulnerable to electrophiles such as Hg. The human genome includes 25 genes which express selenoenzymes with essential functions that control cellular redox status, thyroid hormone and calcium-dependent processes, immune responses, and other vital processes. Methyl-Hg (CH_3Hg) binds to cysteine (Cys) to form the CH_3Hg-Cys adducts which predominate in tissues. Due to molecular similarities between methionine (Met) and CH_3Hg-Cys, cellular membrane transporters actively mobilize this species across membranes and into proteins. Because

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many selenoenzymes directly act upon or work in concert with thiomolecules, the CH_3Hg-Cys in these molecules function as a suicide substrate that brings Hg into direct contact with the selenoenzyme's active site Sec. Since Hg's affinity for Se is far higher than its affinity for sulfur, CH_3Hg exchanges partners to form CH_3Hg-Sec, irreversibly inhibiting the enzyme and subsequently forming insoluble HgSe. Exposures to CH_3Hg can impair Se availability in maternal, placental, and fetal tissues, but provided tissue reservoirs and intakes of Se are sufficient to ensure Sec synthesis proceeds without hindrance, the oxidative damage and other pathological consequences of high exposures are averted. Exposure to toxic amounts of Hg overcome the ability of these sources to offset Se losses. Mercury toxicity's biochemical mechanisms, Se-dependent protective effects, variability in dose effects, prolonged latency, tissue specificity, and enhanced fetal vulnerability to high exposures are becoming increasingly well understood.

Keywords

Mercury · Selenium · Methylmercury · Selenoenzyme · Brain · Toxicity · Neurotoxicity

Abbreviations

AP-1	Activator protein 1
ApoER2	Apolipoprotein E receptor 2
CH_3Hg	Methylmercury
CH_3HgCH_3	Dimethylmercury
Cys	Cysteine
DIO	Deiodinase
DIO1	Iodothyronine deiodinase 1
GPx	Glutathione peroxidase
GSH	Glutathione
GSSH	Glutathione (oxidized form)
H_2O_2	Hydrogen peroxide
Hg^+	Oxidized mercury
Hg^+, Hg^{2+}	Inorganic mercury
Hg^0	Elemental mercury
HSe^-	Selenide
LAT1	Large neutral amino acid transporter
Met	Methionine
MsrB	Methionine sulfoxide reductase B
NF-κB	Nuclear factor kappa light-chain enhancer of activated B cells
OOH	Hydroperoxo species
Prx	Peroxyredoxins
RNR	Ribonucleotide reductase
$ROO^{\cdot}$	Peroxyl radical
RSe^-	Selenoate
RSH	Sulfhydryl

Sec	Selenocysteine
SeMet	Selenomethionine
SeO_3	Selenium trioxide
SeO_3^{2-}	Selenite
SeO_4^{2-}	Selenate
Ser	Serine
TGR	Thioredoxin-glutathione reductase
Trx-$(SH)_2$	Thioredoxin
TRx1	Thioredoxin reductase 1
TRx2	Thioredoxin reductase 2

1 Introduction

In recent years, the neurotoxic effects of mercury (Hg) have progressed from being poorly understood to being among the best characterized of all toxic agents (Ralston & Raymond, 2018). High exposures to elemental (Hg^0), oxidized (Hg^+, Hg^{2+}), as well as organic forms such as methyl-Hg (CH_3Hg) result in Hg accumulation in brain parenchyma leading to oxidative damage (increase of lipoperoxidation and nitrite), cytotoxicity, and apoptosis of neurons and astrocytes. Neurotoxic symptoms and signs of localized oxidative damage are the most visible aspects of Hg poisoning, but relationships between their severity and the Hg exposures or Hg concentration in bioindicators such as blood, urine, or hair are highly variable (Clarkson & Magos, 2006). Interindividual variability in susceptibility to acute or chronic exposures to toxicants is not uncommon, but rationalizing why symptom severity appeared to be strongly dose-dependent among certain individuals or populations while little or no effects were observed among others with similar or even higher Hg exposures and tissue concentrations was difficult. The reasons why similar Hg doses were associated with toxic effects in one population but no effects or even beneficial effects in others seemed to defy explanation. The fundamental reason underlying these mysteries was the lack of a defined biochemical mechanism of Hg toxicity and a failure to understand why supplemental dietary selenium (Se) provided such potent protection against its effects. Additional mysteries included the extended period of latency (up to several months) between receiving a harmful dose and the onset of its effects, the reasons for Hg's damaging effects in brain tissues, and the greater vulnerability of the exposed fetus to its harmful effects. Insights regarding Hg's molecular pathology required improved understanding of its kinetic and thermodynamic interactions, particularly in relation to Se (Spiller, 2017; Ralston & Raymond, 2018).

Depending on the tissue, total thiols are present at concentrations ranging between ~60 and 100 mM with ~90% of protein thiols (10–50 mM) maintained in their reduced state, representing ~70% of the total pool of reduced thiols (Hansen et al., 2009). Cytosolic glutathione (GSH) is almost exclusively in the reduced form as is 91% of the total (2–17 mM) GSH in the cell. Thiol/disulfide networks include GSH (and its oxidized form GSSG), glutaredoxin, and the glutathione reductase

system, as well as the thioredoxin system acting upon many substrates and in turn acted upon by thioredoxin reductase and other important antioxidants. Intracellular thiols and the numerous oxidation repair and regulation molecules responsible for homeostatic balance of metabolic activities are ultimately kept reduced mainly by the action of NADPH. Because thiols such as cysteine (Cys) occur in millimolar concentrations in tissues while the toxic and severely toxic concentrations of Hg in blood (~1 μM and 2.5 μM, respectively) are ~5 log orders lower, it was difficult to conceive of a toxic mechanism consistent with these stoichiometries. Still, since thiols would be saturating when intracellular Hg is present in toxic amounts, it was assumed that the mechanism/s involved in the damage caused by Hg must follow pseudo-first-order reaction kinetics and could therefore be expected to be proportional to Hg dose. While Hg binding to thiomolecules was clearly involved, it was not until the physiological importance of Se-dependent enzyme pathways in the brain became known that more useful perspectives became available.

In recognition of the biochemical mechanisms of Hg toxicity, the development, refinement, and application of the health benefit value (HBV) criterion was funded by the US EPA and NOAA to provide a more reliable means of clearly differentiating between seafood and freshwater fish that might be hazardous and those that would protect against Hg toxicity and be expected to benefit maternal and fetal health outcomes (Ralston et al., 2015, 2019).

The following describes the molecular mechanisms of Hg toxicity, the origins of Se-dependent "protective' effects, the brain specificity of Hg-dependent oxidative damage, the latency between Hg exposures and its toxic effects, and the reasons for accentuated fetal vulnerability. The outcomes of studies which reported seemingly contradictory outcomes are now easily understood based on the HBVs of the seafood that were consumed, and the findings of these and other studies are consistent with expectations based on the current understanding of Hg's toxicokinetics and toxicodynamics.

2 Selenium Physiology

It is necessary to have a basic understanding of Se physiology to understand the molecular mechanisms of Hg toxicity and the defining aspects of its pathology. In contrast to other essential elements which act as cofactors that assist proteins, Se is incorporated in molecules formed de novo during synthesis of the polypeptide chain. The metabolic roles of Se are accomplished through the activities of selenoenzymes which have pivotal roles in antioxidant metabolism, regulatory mechanisms, fetal development, homeostasis, and other functions which will be discussed below.

2.1 Selenoenzymes in Brain Development and Functions

The human proteome includes 25 genes which encode for expression of selenocysteine (Sec), the 21st naturally occurring amino acid (Hatfield & Gladyshev,

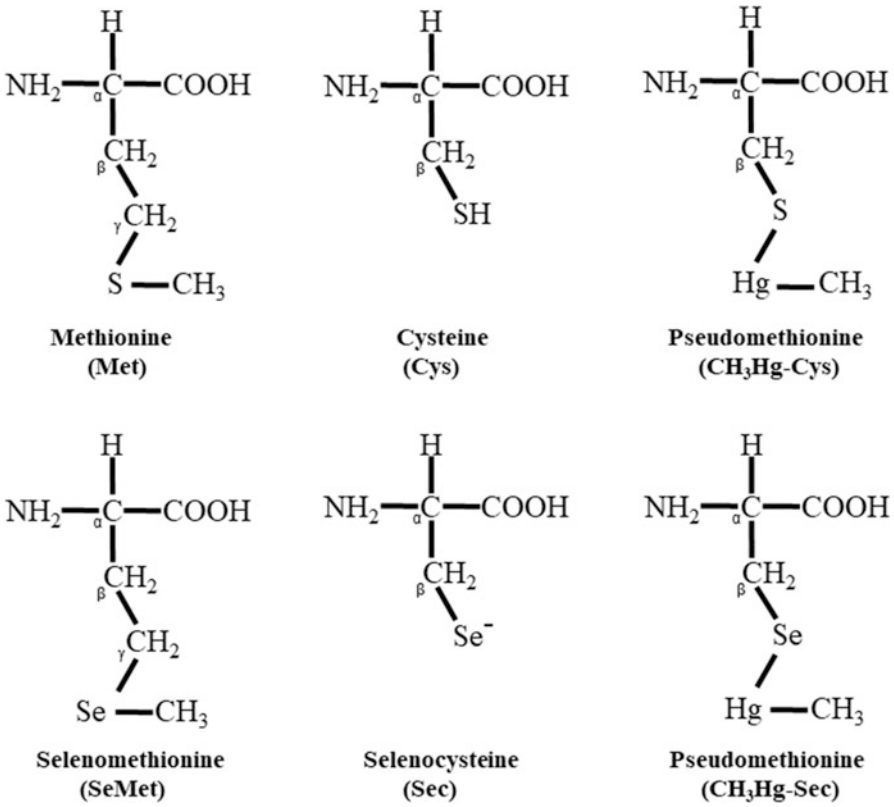

Fig. 1 The structures of sulfur and Se amino acid homologs. Since the selenol of Sec (pKa 5.5) is almost entirely deprotonated at physiological pH (~7.4), it is shown in its ionized form. For convenience, the CH_3Hg adducts with Cys and Sec are referred to as pseudomethionine in reference to their molecular resemblance to Met and their resulting expedited import into tissues. The CH_3Hg can subsequently transfer from Cys to form CH_3Hg-Sec whose breakdown product results in formation of inorganic HgSe, a permanently sequestered form of Se

2002). The pKa of the R group thiols of Cys range around ~8.3, and it is predominantly protonated and uncharged at physiological pH (7.4). However, interactions with its microenvironment (e.g., when acting as an enzyme cofactor) can make it more nucleophilic and easily oxidized. In contrast, the selenol of Sec has a pKa of ~5.5 which means it is almost exclusively unprotonated and more reactive at physiological pH (see Fig. 1). The selenol group of Sec is the most powerful intracellular nucleophile, and its higher reduction potential of Se as the interacting nucleophile in the catalytic sites of selenoenzymes is more efficient in catalyzing redox reactions (Arnér, 2009). Certain selenoproteins occur in families with similar genes and biochemical functions (i.e., 3 deiodinases, 5 glutathione peroxidases, and 3 thioredoxin reductases), others are unique in their form and function, but nearly all have critical roles in brain function and fetal development. Selenoproteins are

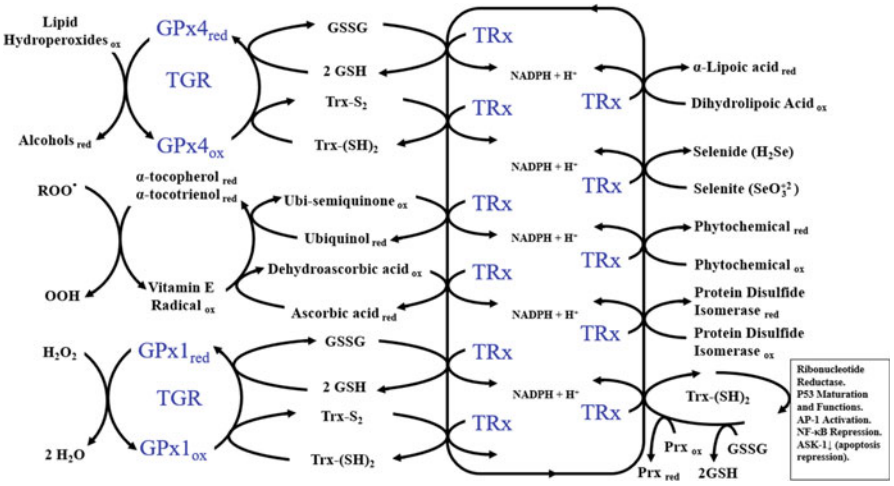

Fig. 2 Simplified schematic of thioredoxin reductase (TRx) and glutathione peroxidase (GPx) activities in concert with representative antioxidants they act upon to restore oxidized (ox) forms back to reduced (red) states. NADPH + H^+ provides the reducing equivalents to restore TRx between each cycle. A partial listing of the independent actions of thioredoxin (Trx-$(SH)_2$) is shown for reference

expressed in all cells of all vertebrates in tissue-dependent occurrence and distributions, but they are especially important in the brain (Chen & Berry, 2003; Reeves & Hoffmann, 2009).

Thyroid hormones have important roles in embryogenesis, fetal maturation, and regulation of key biochemical reactions and appear to regulate processes associated with terminal brain differentiation such as dendritic and axonal growth, synaptogenesis, neuronal migration, and myelination (Köhrle & Gartner, 2009). The three Se-dependent deiodinases regulate thyroid hormone metabolism in different ways. DIO1 cleaves the iodine-carbon bond of T_4 (thyroxine) to activate thyroid hormone (T_3) in somatic tissues, while DIO2 creates >75% of T_3 production in the brain and is also active in pituitary and thyroid glands, skeletal/heart muscle, and placenta. DIO3 is expressed in higher amounts in the brain, placenta, and pregnant uterus and may protect the fetal central nervous system from disproportionately high levels of T_4 and T_3 (Köhrle & Gartner, 2009).

The Se-dependent glutathione peroxidase (GPx) enzymes intercept and detoxify hydroxyl radicals using GSH as a cofactor as they perform their functions in cytosol (GPx1) and upon membrane lipids (GPx4). These reactions occur in conjunction with thioredoxin reductase (TRx) enzymes (see Fig. 2) which also restore a host of other antioxidant molecules such as vitamin C, vitamin E, ubiquinol, and polyphenols to their functional (reduced) forms in cytosol (TRx1) and in mitochondria (TRx2), thus preventing and reversing oxidative damage while controlling redox regulated cell signaling through multiple pathways (Arnér, 2009). In addition, selenoprotein M, selenoprotein N, and selenoprotein W appear to have significant

Table 1 Mammalian selenoproteins[a]

Name	Functions and/or comments regarding tissue and/or subcellular localization
GPx1	Detoxifies peroxides in aqueous compartment of mitochondria and cytosol
GPx2	Expressed in cytosol of liver and tissues of the digestive system
GPx3	Primarily synthesized in kidney; active in plasma Se transport to other tissues
GPx4	Prevents and reverses oxidative damage to lipids in the brain, testis, and other tissues
GPx6	Expressed in embryos and olfactory epithelium; catalyzes reduction of peroxides
TRx1	Cytosolic form; reduces multiple antioxidant substrates, regulates metabolic pathways
TRx2	Mitochondrial form; reduces multiple antioxidant substrates, controls redox pathways
TGR	Reduces oxidized Trx and GSSG in nucleus and cytosol; highest expression in testis
Selenoprotein F	Oxidoreductase that may assist in disulfide formation and protein folding
Selenoprotein H	Oxidoreductase; protects neurons against apoptosis, promotes mitochondrial biogenesis
Selenoprotein I	Ethanolamine-phosphotransferase 1 that synthesizes phosphatidylethanolamine
Selenoprotein K	Participates in detoxification in endoplasmic reticulum; involved in calcium regulation
Selenoprotein M	Perinuclear, highly expressed in the brain; may be involved in calcium metabolism
Selenoprotein N	Protects against oxidative stress, regulates redox-related calcium homeostasis
Selenoprotein O	Mitochondrial, largest mammalian selenoprotein, potentially active in redox control
Selenoprotein P	Transports Se (10 s/molecule in humans) to the brain, endocrine tissues, and placenta
Selenoprotein S	Participates in detoxification in the endoplasmic reticulum, may control inflammation
Selenoprotein T	Thioredoxin-like protein expressed during development and in adult endocrine tissues
Selenoprotein V	Possesses GPx and TRx activities, expressed specifically in the testis, may be redox active
Selenoprotein W	Highly expressed in the skeletal muscle, heart, and brain neurons; appears to be an oxidoreductase
DIO1	Activates thyroid hormone, converts T_4 into T_3 (thyroxine) predominant in the liver, kidney
DIO2	Activates thyroid hormone, converts T_4 into T_3 thyroid, placenta, pituitary, and brain
DIO3	Deactivates thyroid hormone in the brain, placenta, and pregnant uterus; important in fetus
SPS2	Catalyzes formation of Se-phosphates required for synthesis of Sec to all selenoproteins
MsrB1	Repairs oxidatively damaged Met-R-sulfoxides back into the native reduced Met conformation

[a]Information presented in this table is from Reeves and Hoffmann (2009) with additional details as described in Ralston and Raymond (2018)

roles in prevention of oxidative damage (see Table 1), but their reaction substrates and products currently remain incompletely characterized. Methionine sulfoxide reductase B1 (MsrB1) is a selenoenzyme that restores oxidized Met to its reduced form using GSH as a cofactor (Reeves & Hoffmann, 2009).

Selenoprotein M is highly expressed in the brain and appears to be involved in calcium metabolism, as is selenoprotein N, a selenoenzyme that also is involved in protecting against oxidative stress. Selenoprotein W is highly expressed in brain neurons and other tissues with high metabolic activity rates and has oxidoreductase characteristics. Although the potential brain-specific functions of selenoprotein K and selenoprotein T are not known, they are located on the membrane of the endoplasmic reticulum and are also involved with calcium release and in maintaining intracellular calcium homeostasis. Since high CH_3Hg exposures are associated with loss of intracellular calcium (Ca^{2+}) homeostasis, it is possible that compromised functions of these selenoproteins may have a role in the neurological defects associated with its toxicity. However, differentiating effects due to loss of Ca^{2+} related activities from those secondary to loss of other selenoenzymes will be difficult.

Selenoprotein P (SelP), the most abundant selenoprotein in the plasma, delivers ten Sec residues per molecule to the brain, endocrine, placenta, and a few additional tissues that express the SelP-specific receptor protein (ApoER2). While the preferential supply of Se to certain tissues has been recognized for decades (Behne et al., 2000), we now recognize that expression of this receptor by these tissues is how their needs are met (Burk et al., 2014). A remarkably large fraction of the body's total Se cycles through SelP daily, up to ~25% in the rat model (Burk & Hill, 2005). Plasma SelP docks with ApoER2 at the blood-brain barrier before being endocytosed by brain capillary endothelial cells and choroid plexus epithelial cells. Since Sec is not reutilized, it is degraded and recycled into Sec which is synthesized de novo by astrocytes and inserted into a newly created SelP for transport as well as serving as a distinct Se reservoir. ApoER2 is required for uptake of SelP by neurons and is essential for neurodevelopment and brain function. ApoER2 is expressed in choroid plexus and on parvalbumin positive (PV^+) interneurons in the hippocampus, inferior colliculus, medial septum, red nucleus, reticular thalamus, and cerebellum. In addition to tissue specificity, ApoER2 expression is regulated by developmental stage since the constitutive mRNA expression of ApoER2 by fetal brain tissues is >9 times greater than in adult brain (Burk et al., 2014; Pitts et al., 2012).

2.2 Selenocysteine Synthesis and Selenoprotein Activities

Sulfur and Se are chemically similar and are generally indistinguishable to the plants, bacteria, and fungi that incorporate them nonspecifically. Thus, the presence of Se in plant and animal proteins will vary in proportion to its presence and availability in soils. The amounts of thiomolecules such as methionine (Met) and selenomolecule homologs such as selenomethionine (SeMet) that are formed reflect the environmental abundance of sulfur to Se (generally ~100,000:1). Animals which

consume plant products are similarly unable to distinguish between these forms, and no biochemical distinctions are made between Met and SeMet during repeated cycles of protein synthesis and breakdown in their tissues. However, once these amino acids are degraded in animal tissues, the biochemical pathways of sulfur and Se sharply diverge. Once SeMet is degraded, it releases inorganic selenide (HSe^-), the required precursor for Sec synthesis and the crucial first step of Se physiology.

The structures of the chalcogen amino acids – serine (Ser), Cys, and Sec – are similar (see Fig. 1), yet their synthesis, chemical reactivities, and biological functions are different. The hydroxyl proton of Ser is stable and unreactive (pKa of ~13), but since its hydroxyl can be displaced, it is a precursor in the biosynthesis of Cys and Sec. The thiol of Cys is a poor nucleophile (pKa ~8.3), but enzymes can adjust it to nearly neutral to enable it to be oxidized and form the disulfides that contribute to protein folding and preserve the vitally important intracellular reducing conditions (e.g., oxidation of two GSH molecules to GSSG). Serine and Cys incorporation into proteins involve ligases to, respectively, form a L-seryl-$tRNA^{Ser}$ and L-cysteinyl-$tRNA^{Cys}$ to designate insertion into nascent polypeptides during synthesis. Upon protein degradation, these amino acids can be repeatedly used in subsequent cycles of protein synthesis, activity, degradation, and reincorporation. In contrast, Sec cannot be reused but must instead be degraded to by a Sec-specific lyase (Raman et al., 2012) to release inorganic Se^{2-} which can be used to synthesize a new Sec.

Every cycle of selenoprotein synthesis requires an inorganic Se to be released from a SeMet or Sec that has been acquired from exogenous proteins provided by diet or from endogenous proteins which have been degraded. Regardless of the source, inorganic Se is incorporated into Sec de novo as it is inserted in nascent selenoproteins (Hatfield & Gladyshev, 2002). This occurs in response to UGA (normally the "opal" stop codon) acting in concert with a Sec insertion sequence (SECIS) (Berry et al., 2001)-specific stem-loop structure in the 3′ untranslated region. Mammalian Sec synthase, a pyridoxal phosphate-containing protein, acts together with selenophosphate synthetase-2 (SPS2 which is a Sec-dependent enzyme) to form selenophosphate ($SePO_3^{3-}$), a high energy molecule that is used to displace the hydroxyl of the Ser moiety of O-phosphoseryl-$tRNA^{[Ser]Sec}$ with a Se atom, thus generating the selenocysteyl-$tRNA^{[Ser]Sec}$ which inserts Sec into the nascent polypeptide (Berry et al., 2001; Hatfield & Gladyshev, 2002).

Several neurological disorders involve alterations in selenoprotein expression or activity. Insufficient Se availability in tissues, genetic polymorphisms, or mutations in selenoproteins and/or proteins and cofactors which are required for selenoprotein expression, synthesis, are involved in the pathophysiology of additional diseases, dysfunctions, inappropriate endocrine functions, and neurological disorders. High exposures to various soft electrophilic metals decrease selenoprotein activities with Hg toxicity being the best characterized.

3 Mercury Toxicity

The role of selenoenzyme-dependent prevention and reversal of oxidative damage in the brain was unfamiliar to toxicologists performing early studies of Hg toxicity. Unaware of the importance of Se physiology in the brain or that supplemental Se involved offsetting losses due to Hg sequestration and thus preventing interruption of the synthesis and activities of selenoenzymes necessary to prevent oxidative damage to the brain, early investigators described Se as having a "protective effect." This terminology was convenient, but imprecise. Mercury has a million times greater affinity for Se than sulfur (see Table 2) and will exchange a thiol for a selenol in aqueous medium (Dyrssen & Wedborg, 1991). Upon degradation of these molecules, their carbon bonds are released, and inorganic HgSe forms and demonstrates long-term retention in brain tissues (Korbas et al., 2010). Therefore, the commonly accepted was assumption that Se sequestered Hg and thus prevented it from causing damage.

The distinct "soft acid" character of the Hg(II) ion shows a strong binding affinity for ligands with "soft base" donor atoms such as sulfur but has far greater affinities for Se. Thiolates are referred to as mercaptans because of their tendency to capture Hg. Accordingly, Hg forms stable covalent bonds with the thiol group of cysteine (Cys) but will readily transfer to the far more nucleophilic selenol group of selenocysteine (Sec). Binding behaviors of Hg (an electrophile) with oxygen, sulfur, and Se are consistent with qualitative predictions based on the hard-soft acid-base concept that Hg affinities would follow the order O<<S<Se, reflecting their relative nucleophilic reactivities. Mercury continually exchanges association between thermodynamically equivalent binding thiols but will exchange a bond with sulfur to form a new, higher affinity bond with Se.

3.1 Dose-Dependent Effects of Mercury Toxicity

Recognition that Hg affinities for Se were several log orders higher than its affinity for sulfur was an important finding, and dose-effect relationships made complete sense once their molecular interactions were properly evaluated using molar concentrations instead of using outdated and relatively uninformative mass-based units (e.g., mg/kg). While Hg toxicity could not cause significant impairments of thiols present in concentrations ~100,000 times higher, once it was realized that 1–2.5 μM

Table 2 Mercury and methylmercury binding behavior with chalcogens

Element	Inorganic Hg form	Mineral name	~K_a	Amino acid	~Log K_f
Oxygen	Mercury (II) oxide (HgO)	Montroydite	10^{27}	Ser	–
Sulfur	Mercury (II) sulfide (HgS)	Cinnabar	10^{39}	Cys	16.7[1]
Selenium	Mercury (II) selenide (HgSe)	Tiemannite	10^{45}	Sec	17.4[2]

The Log K_f binding data for CH_3Hg-Cys and CH_3Hg-Sec are from (1) Rabenstein et al., 1981 and (2) Arnold et al., 1986

Hg was equimolar to or in stoichiometric excess of Se (~1 μM most body tissues), the reasons for Hg toxicity becoming apparent at that dose could readily be discerned. Since Se sequestration as HgSe results in gradual attrition of tissue Se reservoirs, the reasons for the characteristic latency effect also became obvious. Because the metabolic consequences from the loss of Se-dependent enzyme (selenoenzyme) activities will only arise once the exposed individual's tissue Se reserves have become sufficiently depleted to induce a conditioned Se deficiency, the duration of the latency effect would be directly proportional to their Se status and dietary intakes and inversely proportional to the magnitude of the Hg dose they received.

3.2 Dietary Selenium Counteracting Mercury Toxicity

Regardless of whether a selenomolecule is obtained from the diet or originates from the breakdown of endogenous intracellular proteins, Se must be released as inorganic selenide before it can be used for de novo synthesis of Sec. Therefore, the major metabolic difference between inorganic and organic sources of dietary Se is their rate of selenide formation. Selenite is quickly transformed into selenide once it enters the reducing environment of the cell, and Sec lyase degrades Sec to form selenide for Sec synthesis. Since SeMet becomes incorporated into proteins nonspecifically from Met and can engage in many cycles of protein synthesis, the release of its Se is delayed until it is degraded.

Numerous studies in experimental animals have shown Se counteracts Hg toxicity since Pařízek and Oštádalová (1967) first reported that the near 100% lethality of a high Hg exposures was almost entirely eliminated in rats provided with supplemental Se. Work by Ganther et al. (1972) showed that supplemental Se diminished the lethality of CH_3Hg and restored weight gain. Studies showed the Se present in ocean fish prevented or alleviated toxic (Freidman et al., 1978) effects of diets prepared with large quantities of CH_3Hg. Rats fed diets that were not supplemented with Se showed signs of neurotoxicity, but those fed CH_3Hg in a diet which had swordfish added (and would therefore have had even higher CH_3Hg exposures) showed no signs of toxicity. The molar concentrations of Se present in the swordfish were ~5 times higher than its Hg contents. Pařízek et al. (1971) also showed that high Hg exposures severely limited maternal Se transport to the placenta, thus limiting the amounts that would be delivered to the developing fetus. Watanabe et al. (1999) found that high maternal exposures to CH_3Hg which did not affect maternal health or their brain Se concentrations still severely decreased fetal brain Se and impaired their brain GPx and DIO activities, thus limiting their neurobehavioral development and resulting in persistent learning disabilities. Prenatal CH_3Hg exposure affected several neurobehavioral and biochemical end points of the offspring of mothers, and all toxic effects were exacerbated by perinatal Se deficiency.

3.3 The Mechanisms of Mercury Toxicity

The toxic effects of CH_3Hg occur through a sequence of biochemical reactions referred to as the "SOS Mechanisms" (Ralston & Raymond, 2018). These disruptions and their pathological consequences become increasingly apparent as CH_3Hg concentrations approach and especially as they exceed equimolar stoichiometries with brain Se (see Fig. 3).

3.3.1 Synthesis of Suicide Substrates (SOS-1)

Instead of preventing passage of CH_3Hg-Cys, LAT1 transporters in the placenta and brain selectively acquire it as a molecular mimic of Met (see Fig. 1) and other large neutral amino acids and actively distribute it across cell membranes (Bridges & Zalups, 2005). Therefore, the higher concentrations of fetal CH_3Hg-Cys accumulation relative to maternal blood appear to reflect the accentuated importation of Met and other large nonpolar amino acids to support increased protein synthesis in developing tissues. Once across placental and blood-brain barriers, CH_3Hg exchanges binding partner thiols among Cys residues of other molecules. As shown in Fig. 2, TRx interacts with thioredoxin, GPx, and other thiomolecule substrates (Arnér, 2009) to reduce their oxidized forms. Glutathione peroxidase enzymes employ two GSH molecules to reduce cellular peroxides. The oxidized Cys residues of these substrates enter the selenoenzyme binding pocket to orient them for insertion into proximity with the active site Sec where proton exchange normally occurs. However, CH_3Hg-bound thiomolecules act as suicide substrates that accentuate the transfer of CH_3Hg from Cys to the active site Sec.

3.3.2 Silencing of Selenoenzymes (SOS-2)

The vulnerability of selenoenzymes to CH_3Hg was proposed by Ganther et al. (1972) and demonstrated by Prohaska and Ganther (1977). Mercury-dependent inhibition of GPx has since become well documented (Ralston & Raymond, 2015; Seppänen et al., 2004; Stringari et al., 2008; Watanabe et al., 1999), and supplemental dietary Se has been shown to prevent and/or reverse loss of GPx activities in the brains of laboratory animals (Ralston & Raymond, 2015; Stringari et al., 2008; Watanabe et al., 1999). That oxidative damage did not occur due to Hg interactions with lipids but instead from Hg-dependent inhibition of selenoenzymes that protect lipids from oxidation as described by Seppänen et al. (2004). Carvalho et al. (2011) demonstrated that TRx activities are inhibited by CH_3Hg (IC_{50} of ~19.7 nM) and numerous in vitro and in vivo studies have since confirmed these findings (Branco et al., 2014, 2017; Ralston & Raymond, 2015; Rodrigues et al., 2015).

Upon reacting with the adduct, the CH_3Hg exchanges from the substrate Cys to the activated Sec of the active site to form the extremely stable CH_3Hg-Sec (see "► CH_3Hg Binding Affinity Comparison of Cys Versus Sec"). The enzyme can no longer perform its biochemical functions since its catalytic Sec is blocked by CH_3Hg. Therefore, by definition, CH_3Hg is a highly selective irreversible inhibitor of selenoenzymes. In addition to explaining why high Hg exposures are associated with increased oxidative damage, the eventual loss of biologically available Se

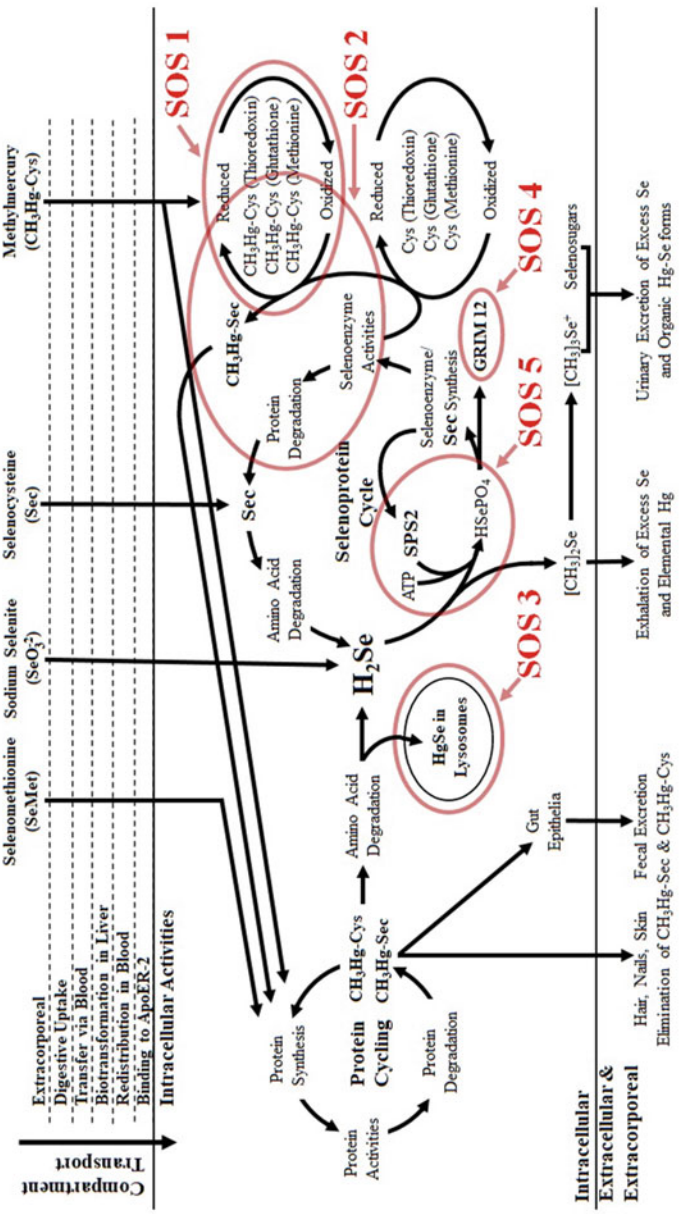

Fig. 3 Simplified schematic of the "SOS Mechanisms" of mercury toxicity. Mercury-dependent disruptions of the biochemical pathways of selenoenzyme synthesis and activities result in the pathological outcomes associated with high mercury exposures

through attrition of tissue reserves will subsequently result in loss of selenoprotein T, increasing intracellular free calcium levels and catecholamine release.

3.3.3 Sequestration of Selenium (SOS-3)

In addition to irreversible inhibition of selenoenzyme activities, high Hg exposures have the insidious ability to induce a conditioned Se deficiency in the brain. High CH_3Hg exposures have been shown to diminish brain Se below ~60% of normal (Behne et al., 2000), the minimum threshold ever achieved after feeding the lowest achievable dietary Se concentration (0.1 μmole Se/kg) for multiple generations. Poisonously high Hg exposures induce an ongoing attrition of Se in somatic and brain tissues (Korbas et al., 2010) due to formation of HgSe. Since HgSe resists decomposition by even concentrated acids (aqua regia, a mixture of hydrochloric and nitric acid is the only acid known to degrade it), HgSe accumulates in cellular lysosomes in precipitates that exhibit long-term retention (Falnoga et al., 2006). However, even high Hg (e.g., 10–100 μM) in brain and endocrine tissues appear to be without toxicological consequences provided enough unbound Se (~1 μM) remains available for selenoenzyme synthesis (Falnoga et al., 2006). Since this is equivalent to the "normal" concentration of Se in these tissues, it is evident that so long as selenoenzyme synthesis and activities can proceed, even these extraordinarily high Hg concentrations are without observable effect.

3.3.4 Suicide of Selenium-Deprived Cells (SOS-4)

Sequestration of cellular Se as insoluble HgSe because of high exposures to elemental or organic Hg would be expected to limit the amounts of Se which remain bioavailable, particularly if dietary Se intakes are not sufficient to offset the amount of Se being lost. If Se deprivation interrupts de novo Sec synthesis, it may result in truncated molecules that lack the terminal Sec residue. If de novo Sec synthesis cannot proceed normally, it may result in truncated molecules that lack the terminal Sec residue. Truncated forms of TRx are potent apoptosis initiators known as GRIM-12 (Anestål & Arnér, 2003). Since apoptosis of Se-deprived cells would result in the release of their remaining bioavailable Se to support the survival of neighboring cells, this suggests a potential evolutionary driver selecting for this pathway. Therefore, sequestration of cellular Se by high Hg exposures may not only deprive cells of the selenoenzymes they need to prevent and reverse oxidative damage but may also initiate apoptosis. Phosphorylation of apoptosis signaling kinase 1 (ASK1), caspase-3 activity, and increase apoptotic cells following high CH_3Hg exposures appear supportive of this mechanism (Branco et al., 2017), although further work is needed to test its validity. Neuronal apoptosis initiated by GRIM12 may be a contributing factor in the tissue destruction observed in adult Hg poisoning and would be particularly damaging if it occurred during fetal development. Since the most rapidly reproducing cells would be at greatest risk from diminished Se availability, entire lineages of neuronal development could be lost if their progenitors are lost. Since Hg exposures high enough to induce GRIM-12 production would impair the entire range of Se-dependent enzymes, excess production of reactive oxygen and nitrogen species (in cytosol and mitochondria), lipid peroxidation, calcium dyshomeostasis,

and impaired protein repair appear likely to be accompanied by initiation of apoptosis in highly exposed cells. While this proposed mechanism of GRIM-12 initiation has not been demonstrated in Hg toxicity, the required causes and observed effects appear to align in support of this mechanism contributing to Hg's pathological effects.

3.3.5 Sustained Oblivion of Sec Synthesis (SOS-5)

The selenophosphate synthetase (SPS2) that makes the $SePO_3^{3-}$ required for Sec production is itself a selenoenzyme. Therefore, if its activities are abolished in a cell, production of Sec may never be restored since there appears to be no other way to create the Sec required in its active site. This potential mechanism remains untested, but evidence in support of this hypothetically permanent loss Sec synthesis is already available. High CH_3Hg exposures during fetal growth in laboratory animals had a sustained effect since their brain selenoenzyme activities showed no signs of recovery later in life (Stringari et al., 2008). The outcomes of multiple human cases of Hg poisoning indicate that brain damage that occurs is extensive and enduring (Clarkson & Magos, 2006).

However, if supplemental Se is provided soon enough, it may be possible to therapeutically intervene and support full recovery from Hg toxicity which has progressed to serious loss of function. Dietary Se was recently used to successfully treat Hg toxicity (Spiller et al., 2017). Several weeks after a substantial spill of liquid Hg contaminated his bedroom and exposed a healthy and athletic 154 pound 15-year-old subject to large amounts of Hg^0 vapor, he developed hypertension, insomnia, delusions, hallucinations, tachycardia, palmar desquamation, diaphoresis, tremor, as well as muscular, testicular, and abdominal pain. Having lost 38 pounds, his increasingly severe ataxia led to hospitalization. Chelation with 2,3-dimercaptosuccinic acid (DMSA) was initiated, but the patient's health continued to deteriorate. Dietary Se supplementation with 500 μg Se (~0.1 μmol/kg body weight) along with 50 mg of N-acetylcysteine per day was initiated to replete tissue Se and support GSH synthesis. Within 3 days of starting treatment, the patient showed noticeable improvement, and delusions, delirium, tachycardia, and abdominal pain had resolved by day 11. He was once again ambulatory, eating normally, and was released from the hospital but maintained on the Se and NAC supplement. After 3 months, all symptoms had resolved except hypertension. After 2 more months, he had regained 35 pounds, his hypertension resolved, and he returned to athletic activities. Similar results have been observed in animal studies in which supplemental Se restored health, weight gain, and neurofunctional activity among experimental animals fed otherwise lethal amounts of CH_3Hg. Interestingly, supplemental Se added to the diet restored health and weight gain even among animals that were kept on the high daily intakes of dietary CH_3Hg (Ralston, unpublished results).

3.4 Brain Specificity of Mercury-Dependent Oxidative Damage

Antioxidant activities of specific selenoproteins are of particular interest in the central nervous system. Although the brain represents only 2% of the total body mass, its high metabolic activities account for about 20% of the oxygen and 25% of the glucose consumed by the human body (Bélanger et al., 2011). Its high metabolic activity generates increased amounts of reactive oxygen and nitrogen species and free radicals. Since brain tissues are characterized by abundant amounts of long-chain polyunsaturated fatty acids which are particularly vulnerable to peroxidation, there is an even greater need to ensure oxidative stress is properly controlled. The importance of selenoenzymes that prevent and reverse oxidative damage in the brain is emphasized by the selective vulnerability of certain neurons to oxidative stress from deficiencies in antioxidant enzyme activity (Wang & Michaelis, 2010). The cerebral cortex, hippocampus, cerebellum, and olfactory bulb have exceptional selenoprotein expression patterns (Zhang et al., 2008). However, reductions of brain selenoenzyme activities induced by genetic defects in Se transport (Burk et al., 2014) or high Hg exposures (Ralston et al., 2008; Ralston & Raymond, 2018) are sufficient to impair Se transport to the brain, and loss of selenoenzyme activities results in impaired functions (Burk et al., 2014; Ralston & Raymond, 2018).

Dietary Se deficiency in laboratory animals depletes the Se contents of the liver, muscle, and blood to less than 2% of their normal contents (Behne et al., 2000; Prohaska & Ganther, 1977) because Se transport molecules (plasma SelP and GPx3) redistribute Se from somatic tissues to brain and endocrine tissues. Brain reserves in the form of cellular SelP serve as accessible reservoirs since Sec lyase rapidly degrades Sec to supply inorganic Se for utilization in each cycle of de novo synthesis of new Sec molecules. These sources maintain brain Se concentrations at a minimum plateau level of ~60% of normal (Behne et al., 2000), while brain selenoenzyme activities remain nearly normal. This pattern of brain Se contents and selenoenzyme activity levels continue in offspring, even after many generations of continual Se deficiency. However, mice that have been genetically modified to delete ApoER2 suffer severe neurodegeneration in brain regions that are associated with auditory and motor functions (Burk et al., 2014). Homeostatic regulation of brain selenoenzyme expression and activities varies by tissue, cell layer, and cell type. Although all selenoproteins are expressed in the brain, GPx4, SelK, SelM, SelW, and SelF are exceptionally rich in neurons of the olfactory bulb, hippocampus, cerebral cortex, and cerebellar cortex (Zhang et al., 2008). Neurons are unique in that the distal compartments of their dendrites and axons are quite remote from the cell soma, making difficult for them to repair damage that occurs in the highly active regions of their synapses. Thus, the selenoenzyme-dependent maintenance of reduced ascorbate and other antioxidant molecules in the synaptic interface is essential for the prevention and reversal of oxidative damage. Evolution of homeostatic mechanisms to ensure selenoenzyme expression and activities proceed in these neuronal regions without interruption appears to be nearly flawless since protection is assured under almost all conditions. High CH_3Hg exposures are the only environmental insult

known to seriously impair brain selenoenzyme activities (Prohaska & Ganther, 1977; Ralston & Raymond, 2015; Stringari et al., 2008; Watanabe et al., 1999). To illustrate the patterns of selenoprotein levels and mRNA expression in the brain, the distributions of SelM and SelW in mouse brains are shown as examples in Fig. 4.

Using synchrotron X-Ray absorption spectroscopy (XAS), Korbas et al. (2010) found HgSe accumulated at high concentrations in brains of individuals that had been poisoned with high CH_3Hg. High CH_3Hg exposures steadily diminish the availability of free Se in the brain, and the resulting diminishment of selenoenzyme activities causes extensive damage to the most active neurons. Neurons are destroyed through the combined actions of SOS 1–3 and/or apoptosis initiated via SOS 4. Meanwhile, less vulnerable brain cells survive the crisis because they do not have the high rates of oxidative damage or else have maintained sufficient Se to maintain their selenoenzyme activities. Although certain brain cells will survive and may gradually recover to normal levels of selenoenzyme activity, as was observed by Korbas et al., the magnitude of neuronal cell damage and death will be reflected in the severity of the loss of cognitive and neurofunctional abilities.

Postmortem examination of the brains of victims of CH_3Hg poisoning shows varying degrees of neuronal cell loss, especially in the sensory regions of the cortex, cerebellar granular cells, primary motor cortex (Castoldi et al., 2003), and peripheral nerves. The loss of coordination (ataxia) that occurs during severe CH_3Hg poisoning is due to cerebellar damage to small granule cells even though Purkinje cells and other neighboring cells from the same region remain mostly unaffected. Similarly, loss of neurons from the visual cortex results in constriction of visual fields. Distinctions in neuron sensitivity to high CH_3Hg exposures appear likely to be due to variances in the turnover rates of essential selenoenzymes, different efficiencies of ApoER2-mediated uptake of SelP, and differences in their abilities to preserve their Se reserves.

3.5 Enhanced Fetal Vulnerability to Mercury

Health benefit values are the only seafood safety criterion based on biochemical interactions of the toxicants and nutrients for which it provides guidance (Ralston et al., 2015, 2019). The fetus grows without significant tissue Se reserves and depends on maternal Se imports to ensure the rapidly growing brain has adequate selenoenzyme activities to prevent oxidative damage and ensure proper regulation of its activities. Damage or destruction of an early generation of brain cells precludes normal outcomes for future generations of cells that would otherwise have grown in correct relation to them, thus constraining proper development of fetal brain tissues. A newborn child's brain has a complement of ~100 billion neuronal cells with ~100 trillion interconnections (Ackerman, 1992). Averaged out during the ~40 weeks of fetal development and not accounting for the extensive controlled apoptosis and pruning that continually occurs in the progressive revising of brain architecture throughout development, the fetal brain grows at a rate of ~250,000 new neurons per minute that form new interconnections at a rate of ~250 million/minute. These

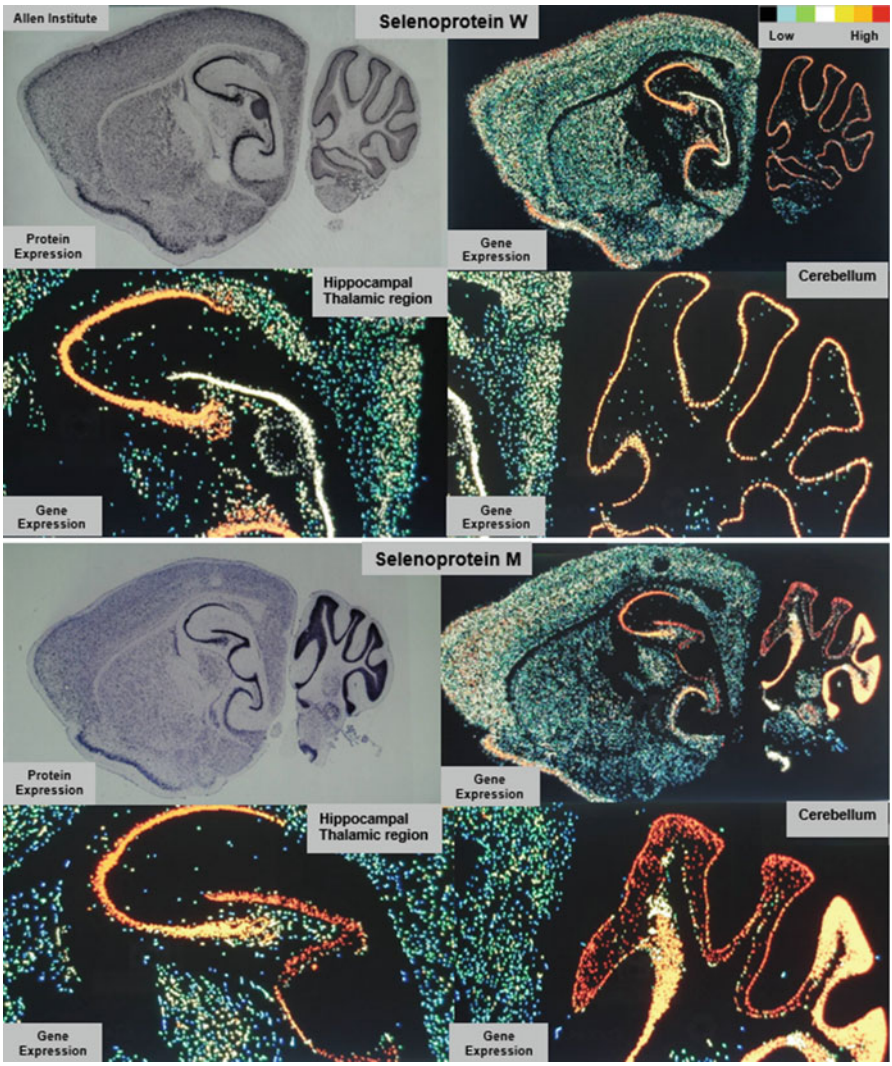

Fig. 4 Selenoprotein W and selenoprotein M protein and gene expression levels in adjacent midsagittal sections of mouse brain. Expanded views of the hippocampal/thalamic region and cerebellum are provided to enable closer examination of differences in expression in individual cell layers. Image credit: Allen Institute

proliferating brain cells are vulnerable to Se depletion since sufficient amounts must continually be present in each precursor cell to support normal Se-dependent enzyme activities in the daughter cells. The severity and duration of a transient Hg-induced Se deficiency will depend on the relative amounts of Hg in excess of Se inputs from maternal supplies and available Se reserves in fetal tissues.

As summarized by Ralston et al. (2015), fetal vulnerability was first observed in association with catastrophic poisoning that occurred in Minamata, Japan, where 75–150 tons of Hg were dumped into a shallow, semi-enclosed inland sea separating the island of Kyūshū from the Amakusa Islands. The fish that were consumed were contaminated with up to 50 mg/kg (~250 μM), which would have been 25–50 times more than their Se contents. Umbilical cords saved from children born during the time of the poisoning contained ~3 times more Hg than Se (1939–1959) which contrasted with those collected from children before the poisoning events (1927–1937) which contained ~40 times more Se than Hg. The findings of studies performed in Iraq confirmed the increased fetal vulnerability to Hg and also provided some of the first indications of a latency period between Hg exposure and effects. Maternal exposures to Hg in a New Zealand population that had been eating great white shark meats during pregnancy and in the Faroe Islands where mothers had eaten pilot whale organ and muscle meats as well as blubber found subtle Hg dose-dependent effects among their children.

Increased Hg contents in umbilical cord blood samples from the Faroes study were associated with subtle decreases in language, attention, and to a lesser extent, sensory and motor functions with the lowest observed adverse effect level (LOAEL) being 58 ppb (0.28 μM). As a precautionary measure, the results of the Faroes study were used to establish Hg reference dose (RfD) at 1/10th of the LOAEL: 5.8 ppb (0.028 μM). However, because pilot whale meats contain far more Hg than Se, the cord blood Hg in Faroes children approached a 1:1 stoichiometry with Se, and several approached or exceeded the 1 μM threshold considered toxic to adults.

In an assessment of molar relationships between Hg and Se in cord blood samples from the Faroes, Ralston et al. (2015) found the slope of the linear equation relating the increase in cord blood Se in relation to the increase in cord blood Hg was $Y = 0.467X + 1.374$ in the Faroes children but was $Y = 9.378X + 1.704$ in cord blood samples from children of an ocean fish eating population in Hawaii (data not shown). The relationship between cord blood Se to cord blood Hg indicates blood Se in the Faroes was adequate (~1.374) but significantly lower than the 1.705 μM intercept for Se for the cord bloods of children whose mothers ate ocean fish and did not eat any muscle or organ meats from pilot whales. The more important finding was that increasing Hg exposures from eating Se-poor pilot whale meats in the Faroe Islands resulted in cord blood Se rising ~half as fast as Hg (slope = 0.467X) while Se rose ~9 times faster (slope = 9.378X) than Hg in cord bloods of children from the Hawaii study.

The high Hg concentrations in great white shark and pilot whale meats were present at as much as a fivefold molar excess of their Se contents. In the Faroes, more than 85% of total seafood Hg exposure originated from pilot whale consumption, but over 80% of their seafood-derived Se came from eating ocean fish. The Faroes study research team concluded that the cod fish eaten by the mothers in the Faroes provided substantial benefits that counteracted much of the neurodevelopmental damage from pilot whale consumption that would have otherwise occurred (Budtz-Jørgensen et al., 2007). Due to their high Hg contents, advisories against pilot whale consumption have since been evoked and its meats removed from

consumer markets. The findings of harm in the epidemiological studies that involved consumption of pilot whale or great white shark meats with negative HBVs (Faroe Islands, New Zealand) confirm the HBV reliably identifies seafood that should be avoided. The findings of all the other studies that found benefits (e.g., the Seychelles, UK, USA, Spain) associated with eating ocean fish with positive HBVs also coincide with expectations.

Unaware that Se from ocean fish had already repeatedly been shown to protect against the toxic effects of Hg instead of contributing to causing it, several well-intentioned efforts added to mistaken concerns regarding the safety and benefits of ocean fish consumption during pregnancy. Confusion undoubtedly increased after the National Research Council (NRC, 2000) report "Toxicological Effects of Methylmercury" inadvertently conflated exposure source effects. The first footnote in the document stated: "In this report, the term fish includes shellfish and marine mammals, such as pilot whales that are consumed by certain populations." It was easy to overlook this footnote, and many casual readers may have assumed that the subtle adverse effects noted among children of seafood eating mothers in the Faroe Islands had originated from eating ocean fish since pilot whales were little mentioned when adverse effects were discussed in the rest of the report. Although researchers in the Faroes pointed out that most of the Hg exposures of their study population came from eating pilot whale products (muscle, kidney, liver, and blubber were the source of ~85% of the total Hg exposures in that population), authors of the NRC report incorrectly expected that Hg exposures from eating ocean fish would be as harmful as from eating pilot whale products. Since the molecular mechanism of Hg toxicity was not widely understood at that time, this is understandable.

The 2020 Dietary Guidelines Advisory Committee (2020) examined associations between seafood consumption during pregnancy and during childhood in relation to the child's subsequent neurocognitive development. Efforts were made to identify any adverse effects from neurotoxicant exposures and/or benefits from nutrients present in ocean fish. After a review of 44 publications reflecting the findings of studies of 106,237 mother-offspring pairs and 25,960 ocean fish eating children, the committee concluded that increased consumption of commercially available seafood during pregnancy is associated with improved neurocognitive development of offspring as compared to eating no seafood. Maternal seafood consumption was associated with improved neurocognitive outcomes in their children at even the lowest amounts of seafood consumed (~4 oz/wk) which continued to increase through the highest levels of consumption (12–100 oz/wk) which were associated with an average increase of 7.7 IQ points (Hibbeln et al., 2019). These studies report beneficial associations with neurological development, motor development, verbal intelligence quotient, perception, social behavior, and reduced inattention and hyperactivity. The studies reporting benefits are more numerous, included far larger numbers of mother-child pairs, corrected for more potential confounders, studied more performance indices, and report the effects of maternal consumption of typical varieties of ocean fish instead of unusual and seldom consumed varieties such as great white shark or pilot whale organ meats, muscle meats, and blubber. The findings of the epidemiological studies of seafood consumption that found harm

from eating pilot whale or great white shark meats with negative HBVs (Faroe Islands, New Zealand) confirm the reliability of this index in predicting risks from high Hg exposures. The findings of the far more numerous studies that have found substantial benefits from eating ocean fish with positive HBVs demonstrate that the index accurately predicts benefits from eating these more typically consumed varieties of seafood.

3.6 The "Silent" Latency of Mercury Toxicity

Mercury toxicity is characterized by a previously unexplained delay between ingestion of a harmful or lethal dose and the onset of symptoms which can take months to develop. The eventual onset of clinical symptoms following high exposures follows a similar sequence: of paresthesia (tingling/numbness in the lips and extremities), ataxia (loss of motor coordination and gradual loss of motor control), dysarthria (difficulty in properly pronouncing words), vision constriction, deafness, and in the most severe cases, ultimately death. It seemed mysterious that CH_3Hg has a physiological half-life of ~74-days but that initial symptoms did not become evident until much of the ingested dose had already left the body. While it is now realized that ingested Hg is gradually sequestering Se from tissue reservoirs during the latency period, it was difficult for earlier researchers to explain this delay or the reasons why the magnitude of the dose was directly related to the severity of the exposed individual's brain damage while the latency period leading up to the onset of symptoms was independent of the dose.

An accidental laboratory exposure to dimethylmercury (CH_3HgCH_3) provided the strongest evidence of the latency effect of Hg toxicity. Since the CH_3HgCH_3 is uncharged, it readily passes through membranes and distributes throughout body tissues. Although 90–95% of the acquired CH_3HgCH_3 dose is exhaled during the first 48 h after exposure, the portion that becomes demethylated to CH_3Hg remains distributed throughout the body and gradually sequesters Se in somatic as well as neuroendocrine tissues. During the ~150 days between the actual exposure and onset of symptoms that eventually resulted in death, there was no indication of Hg toxicity. Since this individual lived in the United States where foods tend to be more Se-rich than in much of the rest of the world, the exposed individual's tissue Se and daily intake of Se from food were sufficient to offset the attritional losses caused by CH_3Hg for the intervening months. But once tissue Se reserves were exhausted, the loss of selenoenzyme activities to protect neurological tissues resulted in their rapid decline and demise.

In contrast, villagers in Iraq that had been exposed to similar amounts of CH_3Hg had a latency period that lasted only 16–38 days. The Se status and dietary Se intakes tend to be lower in that region of the world so both tissue reservoirs and daily intake of Se from diet would have been far lower, so the attritional losses of Se would have exhausted Se far more quickly. If therapeutic amounts of supplemental dietary Se had been provided to reverse the effects of Se sequestration and restore enough tissue Se to support selenoenzyme activities as they did in the case of the 15-year-old

patient treated by Spiller et al. (2017), this therapy may have saved the lives of individuals exposed to lethal amounts of CH_3Hg.

The influence of Se status on latency of CH_3Hg effects has been confirmed in laboratory studies where growing rats fed low-Se diets showed rapid development of physiological, biochemical, and neurofunctional deficits while exposed animals fed normal-Se diets did not show any effects until much later and to a far lesser degree. Meanwhile, those fed Se-rich diets showed no signs of Hg toxicity from the otherwise lethal amounts they were fed during the study. Since these rats continued growing normally, they consumed larger amounts of diet and therefore their total CH_3Hg exposures were significantly greater. However, even with their higher exposures, there were no effects on their physiological, biochemical, and neurofunctional health outcomes (Ralston et al., 2008).

4 Future Directions

While ocean fish consumption is associated with benefits instead of harms, it should be noted that Hg exposures from eating freshwater fish (and other sources) may be more hazardous than might be assumed based on the findings of the Faroes study. Maternal Hg exposures far below the LOAEL observed in the Faroes populations could seriously impair fetal neurodevelopment in populations with poor Se status. Because the Faroese mothers regularly ate ocean fish which were rich in Se, their children were mostly protected from harm. Therefore, it is inappropriate to use observations based on the Faroes study to guide risk assessments for freshwater fish consumers, particularly in Se-poor regions where such fish are depended upon for subsistence. The effects of CH_3Hg exposures in populations with a poor Se status and low dietary Se are likely to be far worse than expected but may currently be overlooked because risk assessments are presently based on Hg alone. It is important to note that Se contents of freshwater fish are exclusively dependent on the amounts and availability of Se from the soils in their watershed of origin. The Hg/Se ratios in freshwater fish are far more variable than in ocean fish, and high Hg inputs from point sources can result in disproportionately high Hg concentrations in exposed fish, particularly in regions where environmental Se availability is poor. Since Hg bioaccumulation in fish is significantly augmented in water bodies where Se availability is low, higher CH_3Hg exposures and greater risks of harm from those exposures are likely to occur in regions where the Se status is low. Studies reporting Hg levels in fish and blood samples of populations that fail to concurrently assess Se cannot provide a meaningful indication of associated risks. Risk assessments based on maternal Hg exposures must determine the relative and absolute amounts of Hg and Se present in food sources and include the HBVs of the freshwater fish in their assessments. While not discussed in this chapter, there are numerous other Se-binding metallic or organic soft electrophiles that should also be considered when assessing Hg exposure risks (Ralston, 2018). In populations where these exposures to any other soft electrophiles are expected to be meaningful, there should be an assessment of their total combined Se-binding potential. Assessments that only

assess CH_3Hg without considering the Se status of the exposed population or the presence of significant exposures to any other soft electrophiles in their environment or diet cannot provide a reliable index of the actual level of risk.

Irreversible enzyme inhibitors are defined as those that covalently bind to the active site, thus denaturing it and preventing any further enzyme-substrate complexes to form. Mercury's irreversible inhibition of selenoenzymes is definitive but unique in that it is the only case where the bond formed between the inhibitor and the catalytic actor truly is irreversible. The HgSe bond is impervious to all concentrated acids other than aqua regia and will only decompose when heated to temperatures in excess of 350 °C. As the most irreversible of all irreversible inhibitors, this will almost assuredly become the textbook example for this class of enzyme inhibitors.

While the findings of epidemiological studies confirm that Hg exposures from ocean fish consumption are beneficial rather than harmful, there is a distinct need to identify the nutrients from seafood that contribute to the 7.7 IQ point enhancements in child neurodevelopment. An early analysis suggested that the harms attributed to CH_3Hg exposure from ocean fish consumption cost the United States an estimated $8.7 billion per year due to loss of ~0.1 IQ points in an estimated 450,000 children whose mothers had eaten sufficient seafood to result in a Hg dose which exceeded the 5.8 ppb Hg RfD limit (Trasande et al., 2005). Based on current understanding of the issue, those mothers with elevated blood Hg levels represent the minor fraction of US mothers who ate enough ocean fish to potentially enhance their children's neurodevelopmental outcomes by 7.7 IQ points. Assuming the $8.7 billion estimate of harm from loss of 0.1 IQ points from 450,000 affected children was calculated correctly, applying those factors linearly to the loss of ~7.7 IQ points per child for ~3,500,000 children born to mothers that did not eat enough ocean fish to obtain those benefits, the cost to the US economy from inadequate fish consumption during pregnancy would be estimated to result in losses amounting to over $500 trillion per year. While unlikely to reflect reality, this calculation emphasizes the importance of ensuring that women receive the amounts of beneficial nutrients that are needed to optimize their children's neurodevelopment.

5 Conclusion

Understanding of the neurotoxic effects of Hg has progressed rapidly in recent years. As in all areas of science, new insights and perspectives displace mistaken assumptions. Mysteries, misunderstandings, and myths regarding sources of Hg-dependent risks, its toxicokinetics, inaccurate ideas about its toxicodynamics, and other early mistaken perspectives regarding CH_3Hg toxicity can be set aside. Recognizing its effects on Se physiology has clarified details regarding its kinetics, thermodynamics and biochemical mechanisms, tissue specificity, latency, and harmful effects on fetal neurodevelopment. Recognition of the biochemical interactions between CH_3Hg and selenoenzymes provides a consistent basis for understanding the distinctive aspects of its toxicity. These insights enable researchers, policy makers, and regulatory agencies to better understand and respond to the adverse effects reported in

association with various CH_3Hg exposures. Rather than considering supplemental dietary Se as a "tonic" that somehow protected against Hg, it is more appropriate to consider Se as the molecular "target" of Hg toxicity. Predatory whales, certain varieties of shark, and large specimens of certain other ocean and freshwater fish have negative HBVs (contain more Hg than Se) and should therefore not be consumed by children or pregnant women. The potential for high Hg exposures from consuming negative HBV freshwater fish is a particularly urgent issue in the case of subsistence consumers in the many Se-poor regions of the world, and their health risks demand further study. However, virtually all commercially supplied seafood provide far more Se than CH_3Hg to consumers and therefore improve rather than diminish the Se status of the consumer. Since seafood consumption is associated with substantial neurodevelopmental benefits, it is important to optimize maternal nutrition during pregnancy.

The past 50 years of research has refined our understanding and now provides a consilient perspective of the importance of Se in relation to the risks of Hg exposures. To enhance the reliability of risk assessments related to Hg exposures, dietary Se intakes must be properly considered. Furthermore, assessing the potential for various other metallic and organic soft electrophiles which might cooperate with Hg in sequestering Se will require a more sophisticated approach to evaluating public and environmental health issues associated with these exposures. These findings have obvious implications for risk assessment research, policy, and regulations.

6 Cross-References

- ▶ Methylmercury and Cellular Signal Transduction Systems
- ▶ Methylmercury Exposure and Risk of Developmental Toxicity
- ▶ Reactive Electrophile Species and Their Effects on Brain Selenoenzymes
- ▶ Selenium Neuroprotection in Neurodegenerative Disorders

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