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1 Mercury's Neurotoxicity is Characterized by its Disruption of Selenium Biochemistry
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17 **Abstract:**
18
19 Background:
20 Methylmercury (CH_3Hg^+) toxicity is characterized by challenging conundrums: 1) "selenium (Se)-protective"
21 effects, 2) undefined biochemical mechanism/s of toxicity, 3) brain-specific oxidative damage, 4) fetal
22 vulnerability, and 5) its latency effect. The "protective effects of Se" against CH_3Hg^+ toxicity were first
23 recognized >50 years ago, but awareness of Se's vital functions in the brain has transformed understanding of
24 CH_3Hg^+ biochemical mechanisms. Mercury's affinity for Se is ~1 million times greater than its affinity for sulfur,
25 revealing it as the primary target of CH_3Hg^+ toxicity.
26
27 Scope of Review:
28 This focused review examined research literature regarding distinctive characteristics of CH_3Hg^+ toxicity to
29 identify Se-dependent aspects of its biochemical mechanisms and effects.
30
31 Conclusions:
32 Research indicates that CH_3Hg^+ irreversibly inhibits the selenoenzymes that normally prevent/reverse oxidative
33 damage in the brain. Unless supplemental Se is provided, consequences increase as CH_3Hg^+ approaches/exceeds
34 equimolar stoichiometries with Se, thus forming HgSe and inducing a conditioned Se deficiency. As the
35 biochemical target of CH_3Hg^+ toxicity, Se-physiology provides perspectives on the brain specificity of its
36 oxidative damage, accentuated fetal vulnerability, and latency. This review reconsiders the concept that Se is a
37 "tonic" that protects against CH_3Hg^+ toxicity and recognizes Se's role as Hg's molecular "target". As the most
38 potent intracellular nucleophile, the selenoenzyme inhibition paradigm has broad implications in toxicology,
39 including resolution of conundrums of CH_3Hg^+ toxicity.
40
41 General Significance:
42 Mercury-dependent sequestration of selenium and the irreversible inhibition of selenoenzymes, especially those
43 required to prevent and reverse oxidative damage in the brain, are primarily responsible for the characteristic
44 effects of mercury toxicity.
45

Abbreviations:

46 **Abbreviations:**
47
48 apolipoprotein E receptor 2; (ApoER2)
49 cysteine (Cys)
50 deiodinase (DIO)
51 dimethylmercury (CH_3HgCH_3)
52 elemental mercury (Hg^0)
53 glutathione peroxidase 4 (GPX4)
54 iodothyronine deiodinase 1 (DIO1)
55 large neutral amino acid transporter (LAT1)
56 methylmercury (CH_3Hg^+)
57 methionine (Met)
58 methionine sulfoxide reductase B1 (MSRB1)
59 oxidized mercury (Hg^+ or Hg^{2+})
60 selenate (SeO_4^{2-})
61 selenium trioxide (SeO_3)
62 selenide (HSe^-)
63 selenite (SeO_3^{2-})
64 selenoate (RSe^-)
65 selenocysteine (Sec)
66 selenomethionine (SeMet)
67 selenophosphate synthetase 2 (SEPHS2)
68 selenoprotein F (SELENOF)
69 selenoprotein K (SELENOK)
70 selenoprotein M (SELENOM)
71 selenoprotein N (SELENON)
72 selenoprotein P (SELENOP)
73 selenoprotein W (SELENOW
74 serine (Ser)
75 sulfhydrl (RSH)
76 thioredoxin reductase 1 (TXNRD1)
77 thioredoxin reductase 2 (TXNRD2)
78
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80
81

82 **1.0 Introduction**

83 Mercury (Hg) occurs in elemental (Hg^0), oxidized (Hg^+, Hg^{2+}), and organic forms such as methylmercury

84 (CH_3Hg^+) and dimethylmercury (CH_3HgCH_3). Each form is distinguished by differences in sources, tissue

85 distributions, and risks of neurotoxicity (Clarkson and Magos, 2006; Syversen and Kaur, 2012). Since ~75% of

86 inhaled Hg^0 is absorbed (Syversen and Kaur 2012), this can be a significant source of Hg exposure in locations

87 where ambient concentrations of this volatile form are high. Once incorporated, Hg^0 passes into tissues where it

88 can either be exhaled or become oxidized to form Hg^+ or Hg^{2+} with the assistance of catalase (Halbach and

89 Clarkson, 1978). Anthropogenic and natural sources release 6,500-8,200 Mg yr^{-1} of Hg^0 into the global

90 atmospheric pool that remain airborne until it becomes oxidized to form water-soluble $Hg^{+/2+}$ that can be

91 deposited with rain (Driscoll et al., 2013). These inorganic forms are poorly absorbed by vertebrates, however

92 anaerobic bacteria can methylate $Hg^{+/2+}$ into CH_3Hg^+, a neurotoxicant which bioaccumulates and biomagnifies in

93 marine and freshwater food webs. Thus, ocean and freshwater fish are the dominant sources of dietary CH_3Hg^+

94 exposures. The addition of a second methyl group to CH_3Hg^+ creates the CH_3HgCH_3 form consistently observed

95 in deep ocean waters, but not in freshwater systems (Hintelmann, 2010). Although chemically unreactive,

96 CH_3HgCH_3 is readily absorbed and becomes distributed throughout vertebrate tissues (Ostland, 1969). However,

97 only the minor fraction which has been demethylated to CH_3Hg^+ is retained, whereas the majority of incorporated

98 CH_3HgCH_3 is exhaled in the first 48 hours (Ostland, 1969). Low level exposures to Hg^0 or CH_3Hg^+ are ubiquitous

99 and without adverse consequences, but high exposures are neurotoxic because they can readily cross the blood-

100 brain barrier and preferentially bind with nucleophilic chalcogens such as sulfur or selenium (Se).

101   Cysteine (Cys) is abundant in tissues, and its thiol is capable of binding with CH_3Hg^+ to form CH_3Hg-Cys

102 (Harris et al., 2003), an adduct with a molecular structure resembling methionine (Met) and other uncharged

103 amino acids (Hoffmeyer et al., 2006). As a molecular mimic of these amino acids (Bridges and Zalups, 2005)

104 CH_3Hg-Cys is transported into cells by the large neutral amino acid transporter (LAT1). Biota in aquatic

105 ecosystems acquire CH_3Hg-Cys in place of Met and retain it in their tissue proteins. Predators absorb the majority

106 of the CH_3Hg-Cys present in their prey, thus bioaccumulating increasing amounts at each trophic level, resulting

107 in the highest quantities in oldest, largest, and most voracious fish of marine and freshwater food webs as well as

108 in piscivorous mammals. Fish consumption is the primary source of human exposures to CH_3Hg^+ and are of

109 concern in relation to the potential risks that maternal exposures might have on fetal neurodevelopment. High

110 CH_3Hg^+ exposures following catastrophic poisoning incidents resulted in a well characterized syndrome of motor

111 and sensory deficits associated with extensive oxidative damage to brain, with the fetal brain being particularly

112 vulnerable to harm (Clarkson and Magos, 2006). However, the potential for risks being associated with lower

113 CH_3Hg^+ exposures, such as those associated with fish consumption, have remained controversial. This is largely

114 due to uncertainties regarding its molecular mechanism/s and the vulnerability of population subgroups.

115 The mistaken idea that CH_3Hg^+ localizes in association with lipids persists in some current literature. This

116 originated from observations in protein free suspensions (Nakada et al., 1978; Giraultab et al., 1997), but is not

117 true in tissues (Prohaska and Ganther, 1977; Harris et al., 2003), where it is predominantly bound to thiols. The

118 sulfhydrl (RSH) or thiol group has a high affinity for Hg compounds ($K_a = 10^{39}$) (Dyrrsen and Wedborg, 1991)

119 and for this reason, thiomolecules are often referred to as mercaptans (from the Latin; *mercurium captāns* -

120 meaning mercury capturing) (Cremlyn, 1996). Mercury's affinity for thiols suggested this could be related to the

121 mechanism of its toxicity. However, intracellular thiol concentrations are in the mM range, ~10,000 times greater

122 than the 1-2.5 µM blood Hg level associated with toxicity, so defining the stoichiometry of its reaction

123 mechanism was elusive. Interactions between Hg and thiols are bimolecular, but because thiol concentrations are

124 saturating, their reactions follow pseudo-first order kinetics proportional to the amount of Hg present. However,

125 interactions with thiols fail to provide compelling rationales for Hg's brain specificity, the reactions responsible

126 for their damage, why fetal brains are more vulnerable than their mother's (Clarkson and Magos, 2006), nor the

127 prolonged silent latency between toxic exposures and the onset of effects (Weiss et al., 2002). However, CH_3Hg-

128 dependent interruptions of Se-metabolism provide a coherent rationale that is consistent with these consequences.

129 Although Se's "protective effect" against Hg toxicity was first noted by Pařízek and Oštádalová (1967)

130 over 50 years ago, the pivotal importance of this finding remained overlooked or widely misunderstood. The

131 protective effect was thought to involve Se binding to Hg, thus acting as a "tonic" that sequestered Hg in a form

132 that no longer harmed important biomolecules, but instead of acting as a "tonic" that dilutes Hg's effects, Se is the

133 biochemical "target" of CH_3Hg^+ toxicity. Methylmercury binding to thiols is kinetically labile, readily exchanging

134 between thermodynamically equivalent partners (Erni and Geier, 1979). However, Hg compounds have an affinity

135 for Se ($K_a = 10^{45}$) that is ~1 million-fold higher than for sulfur (Dyrrsen and Wedborg, 1991). Based on their high

136 binding affinities, one might expect that Hg should be predominantly bound to selenomolecules. Due to mass

137 action effects, >95% of cellular Hg is associated with thiols (Harris et al., 2003). This would have minimal

138 influence on sulfur metabolism since intracellular thiols are 10,000 times more abundant than toxic levels of Hg.

139 In contrast, tissue Se ranges are between 1-2 µM, concentrations which are stoichiometrically consistent with the

140 ranges associated with CH_3Hg^+ toxicity. Thiomolecules function as vehicles that conduct CH_3Hg^+ into metabolic

141 pathways where it can disrupt or interrupt normal Se-metabolism.

142   This focused review discusses the biochemistry of CH_3Hg^+ and Se in relation to distinctive characteristics

143 of CH_3Hg^+ toxicity: 1) the mechanism/s of the "Se-protective" effect, 2) the biochemical mechanisms responsible

144 for its pathology, 3) the oxidative damage specific to the brain, 4) the accentuated vulnerability of fetal brain, and

145 5) the biochemical basis for the latency effect. These aspects are sequentially considered from the perspective of

146 the past 50 years of research that reveal Se as a primary target of CH_3Hg^+ toxicity and the importance of dietary

147 Se in relation to CH_3Hg^+ exposure risks.

148

149

150 **2.0 The "Selenium-Protective" Effect**

151 The biological functions of Se arise through the activities of Sec in 25 proteins expressed by the human proteome

152 (Gladyshev et al., 2004; Kryukov and Gladyshev, 2004). The majority of the selenoproteins are enzymes in which

153 Sec is the primary catalytic actor in the active site. Selenoproteins are expressed in all vertebrates, and are

154 especially important in the brain for prevention and reversal of oxidative damage that might otherwise occur due

155 to its high metabolic activities. Therefore, the tissue occurrence and distributions of these unique selenoproteins

156 (See Table 1) are tightly controlled and preferentially preserved in brain and neuroendocrine tissues (Chen and

157 Berry 2003; Schweizer et al., 2004; Whanger 2001; Köhrle, 2006). To understand how Se "protects" against Hg

158 toxicity, it is necessary to understand Se physiology.

159

160   *2.1 Selenocysteine Synthesis and Selenoprotein Activities*

161   Selenium was identified as an element in 1817 by Jöns Jakob Berzelius. The chalcogens of group 16, oxygen (O),

162   sulfur (S), and Se are chemically similar and form analogous compounds. With six valence electrons, two of them

163   unpaired ([Ar] $3d^{10}4s^{2}4p^{4}$), Se can form six covalent bonds due to 4d orbitals. In association with oxygen, its

164   oxidation state is +6 in selenium trioxide (SeO_3), +4 in selenates (SeO_4^{2-}), and +2 in selenites (SeO_3^{2-}). In

165   combination with other elements, it forms binary compounds with an oxidation state of -2, e.g., in selenide (HSe^{-}),

166   hydrogen selenide (H_2Se), and organic selenides.

167   Sulfur and Se are chemically similar and indistinguishable to the plants or bacteria that incorporate them

168   into various molecules including the amino acids methionine (Met) and selenomethionine (SeMet) (see Figure 1).

169   The SeMet and Met are incorporated into proteins nonspecifically from one another in plants and in the cells of

170   animals that consume them. However, an important distinction between these two amino acids is the release of

171   inorganic selenide (HSe^{-}) following degradation of SeMet. Since Se^{2-} is the required precursor for Se-

172   biochemistry in animals, this is the crucial first step of Se-physiology.

173

Methionine (Met)   Selenomethionine (SeMet)   Serine (Ser)   Cysteine (Cys)   Selenocysteine (Sec)
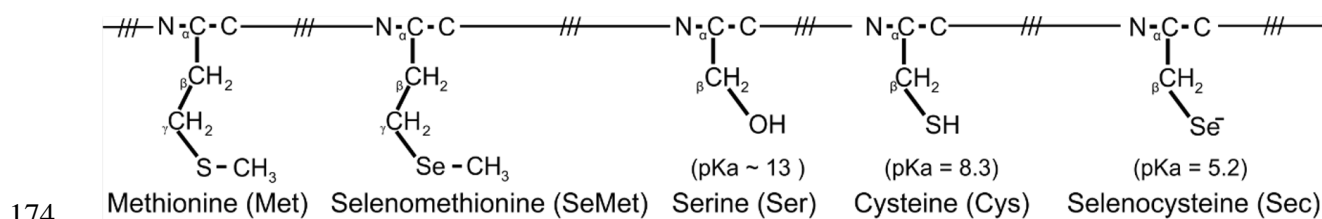
175 Figure 1. The structural analogues of biologically significant chalcogen amino acids and their pK_a's.

176

177   The synthesis, reactivities, and functions of the chalcogen amino acids; serine (Ser), Cys, and

178   selenocysteine (Sec), the 21st proteinogenic amino acid, are vastly different (See Figure 2). With a pKa of ~13, the

179   hydroxyl proton of Ser is stable and unreactive. However, with displacement of its hydroxyl, Ser can serve as the

180   precursor for biosynthesis of Sec, Cys and glycine (Umbarger, 1978). In contrast to the hydroxyl of Ser, the thiol

181   of Cys is a nucleophile in enzymes that adjust its pKa from 8.3 to nearly neutral. The Cys thiol is easily oxidized

182   to form the disulfides that contribute to folding and confer structural stability to proteins. Disulfide formation is

183   an important aspect of Cys participation in reactions, such as those that help preserve intracellular reducing

184   conditions. Incorporation of Ser and Cys into proteins involve specific ligases to form a L-seryl-tRNA[Ser] and L-

185   cysteinyl-tRNA[Cys] to designate insertion into nascent polypeptides during synthesis. Like other amino acids, Ser

186   and Cys can be repeatedly used in continuous cycles of protein synthesis, activity, and degradation. In contrast,

187   Sec cannot be reused, and must be degraded to inorganic Se^{2-} by a Sec-specific lyase (Raman et al., 2012) so that

188   it can be used to synthesize a new Sec, which is created as it becomes incorporated in nascent selenoproteins

189   (Hatfield and Gladyshev, 2002).

190   In response to UGA (normally a stop codon) acting in concert with a specific stem-loop structure in the 3'

191   untranslated region, the Sec Insertion Sequence (SECIS) initiates *de novo* synthesis of Sec as it is co-

192   translationally inserted into the protein sequence. Mammalian Sec synthase (SecS), a pyridoxal phosphate-

193   containing protein, functions together with selenophosphate synthetase-2 (SEPHS2-which is itself a Sec-

194   dependent enzyme) to form selenophosphate ($SePO_3^{3-}$), a high energy molecule that displaces the hydroxyl of the

195   Ser moiety of O-phosphoseryl-tRNA[Ser]Sec to be replaced with a Se atom (Papp et al., 2007; Turanov et al., 2013),

196   thus generating the selenocysteyl-tRNA[Ser]Sec for incorporation of Sec in the polypeptide chain (Berry et al., 2001;

197   Xu et al., 2006).

198   The importance of Se-metabolism is evident through the significant functions of its proteins (Table 1).

199   For example, selenoenzymes such as glutathione peroxidase (GPX1, 4, and 6) intercept and detoxify hydroxyl

200   radicals while thioredoxin reductase (TXNRD1-3) restores vital cellular redox molecules (thioredoxin, ascorbate,

201   and numerous others) that have become oxidized, such as vitamin C, vitamin E, ubiquinol, and polyphenols, back

202   into their functional (reduced) forms in cytosol (TXNRD1) and within mitochondria (TXNRD2) for the

203   prevention of oxidative damage in the cell (see Figure 2). Selenoprotein M (SELENOM), selenoprotein N

204   (SELENON), and selenoprotein W (SELENOW) appear to have similarly significant intracellular antioxidant

205   functions while still other selenoenzymes restore oxidized Met (methionine sulfoxide reductase B1; MSRB1) and

206   long chain fatty acids of phospholipids (GPX4) back to their reduced forms, using glutathione (GSH) as a

207   cofactor. Selenoprotein P (SELENOP), the most common selenoprotein in the plasma, possesses 10 Sec residues

208   which are preferentially delivered to the brain, placenta and endocrine tissues to supply their Se requirements

209 (Burk and Hill, 2005; Burk et al., 2013). The deiodinases (DIO1-3) regulate thyroid hormone metabolism. DIO1

210 cleaves the iodine-carbon bond of T_4 (thyroxine) to activate thyroid hormone (T_3) in tissues other than brain,

211 while DIO2 is responsible for more than 75 % of T_3 production in the brain, and is also active in pituitary/thyroid

212 glands, skeletal/heart muscle, and placenta. The brain, placenta, and pregnant uterus express higher amounts of

213 DIO3 and may protect the fetal central nervous system from disproportionately high levels of T_4 and T_3 (Köhrle,

214 2000; 2009). Selenoprotein K (SELENOK) and selenoprotein T (SELENOT) are located on the membrane of the

215 endoplasmic reticulum and are involved with calcium release and maintaining intracellular calcium homeostasis

216 (Grumolato et al., 2008; Wang et al., 2017). For comprehensive reviews of selenoproteins and their functions see

217 Reeves and Hoffman (2009), Whanger (2001), Rayman (2000), Köhrle (2000; 2009), and Kühbacher (2009).

218   The names of the 25 selenoprotein genes expressed in the human proteome reflect the recognized

219 activities of the functionally characterized selenoenzymes while the nomenclature of the rest are standardized to

220 employ the root symbol SELENO followed by a letter designating the individual gene (Gladyshev et al., 2016).

221 For convenience and clarity, the gene names are used as the short name for the proteins throughout this article.

222
223 Table 1. Mammalian selenoproteins[1]

| Gene 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 brain, testis and other tissues
GPX6	Expressed in embryos and olfactory epithelium, catalyzes reduction of peroxides
TXNRD1	Cytosolic form, reduces multiple antioxidant substrates, regulates metabolic pathways
TXNRD2	Mitochondrial form, reduces multiple antioxidant substrates, controls redox pathways
TXNRD3	Reduces both glutathione disulfide and oxidized Trx, highest expression in testis
SELENOF	Oxidoreductase that may assist in disulfide formation and protein folding
SELENOH	Oxidoreductase, protects neurons against apoptosis, promotes mitochondrial biogenesis
SELENOI	Ethanolamine-phosphotransferase 1 that synthesizes phosphatidylethanolamine
SELENOK	Participates in detoxification in endoplasmic reticulum, involved in calcium regulation
SELENOM	Perinuclear, highly expressed in brain, may be involved in calcium metabolism
SELENON	Protect against oxidative stress, regulates redox-related calcium homeostasis
SELENOO	Mitochondrial, largest mammalian selenoprotein, potentially active in redox control
SELENOP	Transports Se (10 Sec/molecule in humans) to brain, endocrine tissues, and placenta.
SELENOS	Participates in detoxification in the endoplasmic reticulum, may control inflammation
SELENOT	Thioredoxin-like protein expressed during development, and in adult endocrine tissues
SELENOV	Possesses GPX and TXNRD activities, expressed specifically in testis, may be redox active
SELENOW	Highly expressed in skeletal muscle, heart, and brain neurons, appears to be an oxidoreductase

245	DIO1	Activates thyroid hormone, converts T_4 into T_3 (thyroxine) predominant in liver, kidney
246	DIO2	Activates thyroid hormone, converts T_4 into T_3 thyroid, placenta, pituitary and brain
247	DIO3	Deactivates thyroid hormone in brain, placenta, and pregnant uterus, important in fetus
248	SEPHS2	Catalyzes formation of Se-phosphates required for synthesis of Sec to all selenoproteins
249	MSRB1	Repairs oxidatively damaged Met-R-sulfoxides back into native reduced Met conformation
250		

251 [1]Information presented in this table was compiled from Reeves and Hoffmann, 2009 using the newly approved
252 names for the selenoproteins National Center for Biotechnology Information https://www.ncbi.nlm.nih.gov/;
253 Gladyshev et al., 2016;)
254

255
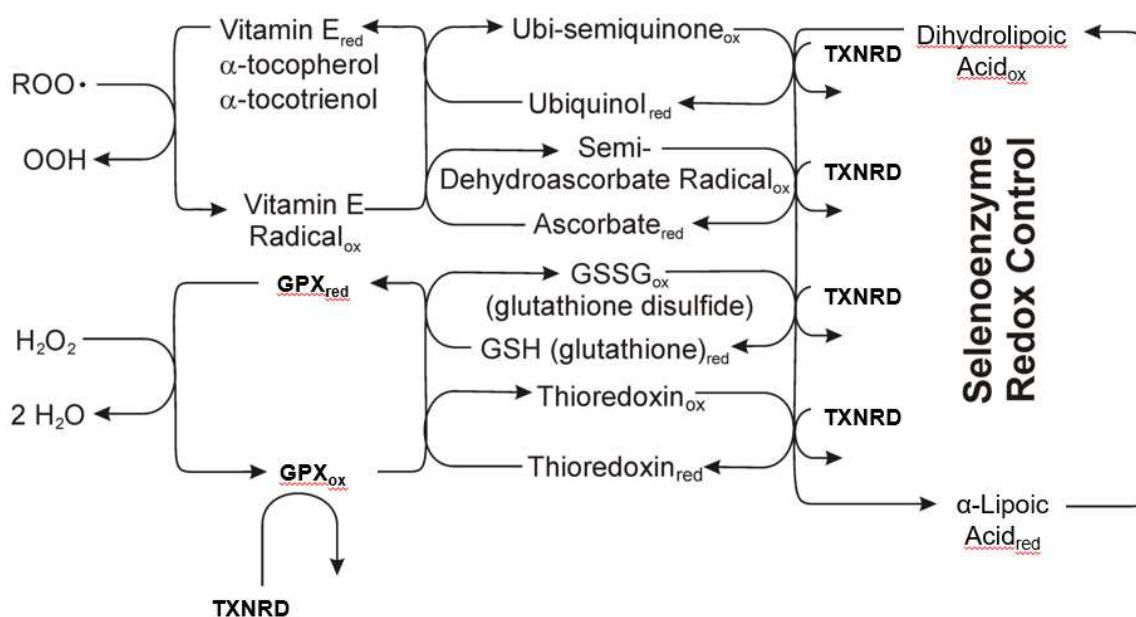
256

257 Figure 2. Schematic of thioredoxin reductase (TXNRD) and glutathione peroxidase (GPX) activities in concert
258 with some of the most important agents they interact with to restore oxidized (ox) forms back to their functional
259 reduced (red) states as they cooperate in preventing and reversing oxidative damage.
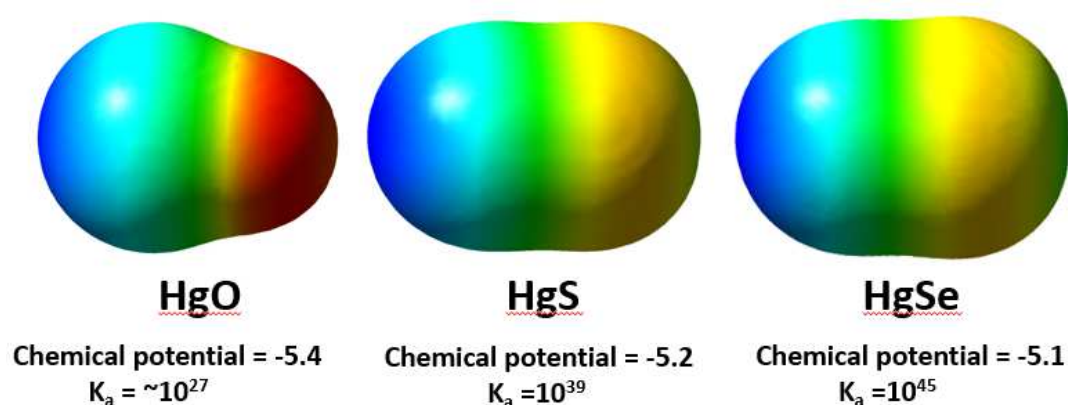260

261 *2.3 Studies of Mercury-Selenium Interactions*

262   The role of selenoenzyme dependent prevention and reversal of oxidative damage in the brain was

263 generally overlooked in earlier studies of Hg toxicity. Unaware that supplemental Se offset losses due to Hg

264 sequestration, thus preventing interruption of the activities of selenoenzymes necessary to prevent oxidative

265 damage to the brain and perform other vital functions, early investigators described Se as having a "protective

266 effect" against Hg toxicity, -terminology which is convenient, but unacceptably imprecise. However, since Hg has

267 greater affinity for Se than sulfur, readily exchanging association with a thiolate ligand for selenolate ligand in

268   aqueous medium (Sugiura et al., 1978; Dyrrsen and Wedborg, 1991; Khan and Wang, 2009; Melnick et al., 2010),

269   that results in formation of HgSe within cells (Huggins et al., 2009; Korbas et al., 2010; Gajdosechova et al.,

270   2016), this misapprehension is understandable. In silico calculations of quantum chemical interactions studies

271   confirm CH_3Hg^+ complexes with Sec are thermodynamically favored (ΔG of formation from model reactants) in

272   comparison to Cys (Asaduzzaman et al., 2010). These findings are consistent with qualitative predictions based

273   on the Hard-Soft Acid Base concept (Pearson, 1997), an approach which is useful for inferring interactions

274   between electrophilic Hg and nucleophilic chalcogens. Mercury affinities would be predicted to follow the order:

275   O << S < Se, reflecting their relative reactivities consistent with results shown in Figure 3. Mercury continually

276   exchanges association between thermodynamically equivalent binding partners such as thiols, but will readily

277   exchange a bond with sulfur to form a new, higher affinity bond with Se.

278

HgO
Chemical potential = -5.4
$K_a = \sim 10^{27}$

HgS
Chemical potential = -5.2
$K_a = 10^{39}$

HgSe
Chemical potential = -5.1
$K_a = 10^{45}$

279

280   Figure 3. Depictions of electrostatic potential surfaces of mercury in covalent association with the biologically
281   significant chalcogens, their chemical potentials, and binding affinity constants. The electron cloud depicted in
282   blue indicates a lower e⁻ abundance and a more positive charge, while yellow shading to red indicates increasingly
283   negative charge. The balance of the HgSe charges stabilize the molecule, contributing to their remarkably high
284   binding affinities. Images were generated using GaussView software, courtesy of Dr. Alexander Azenkeng, UND.
285

286   *2.4 Dietary Selenium Counteracting Mercury Toxicity*

287   Selenium-containing molecules must first be degraded into inorganic Se before it can be used for de novo synthesis of

288   Sec, regardless of whether it is obtained from the diet or originates from the breakdown of endogenous intracellular

289   molecules. The major metabolic difference between inorganic and organic sources of Se is their rate of selenide

290 formation. Selenite is quickly transformed into selenide once it enters the reducing environment of the cell (Cupp-

291 Sutton and Ashby, 2016) and Sec lyase degrades Sec to form selenide almost as rapidly, therefore, both promptly

292 provide Se for Sec synthesis. Since SeMet becomes incorporated into proteins nonspecifically from Met and can engage

293 in many cycles of protein synthesis before it is eventually degraded, the eventual release of its Se can be substantially

294 delayed.

295   Numerous studies have shown Se counteracts Hg toxicity. Pařízek J, Oštádalová (1967) reported that lethal toxicity

296 of mercuric chloride was alleviated by sodium selenite simultaneously administered to rats. Work by Ganther et

297 al., (1972) showed that inorganic Se diminished the toxicity of CH_3Hg^+, reducing mortality and restoring weight gain in

298 rats. Friedman et al. studied the protective effect of Se present in freeze-dried swordfish against CH_3Hg^+, toxicity in rats

299 (Freidman et al., 1978). Rats fed CH_3Hg^+ containing diets that were not supplemented with Se from fish exhibited

300 symptoms of neurotoxicity, but rats that were fed CH_3Hg^+ in a diet enriched with Se from swordfish showed no signs of

301 toxicity. The molar concentrations of Se in the swordfish were approximate 5 times higher than the Hg concentrations.

302 In a similar study, Japanese quail that were given 20 ppm CH_3Hg^+ in diets containing 17% (by weight) tuna survived

303 longer than quail given the same concentration of CH_3Hg^+ in a corn–soya diet. Methylmercury toxicity was also

304 reduced when Se was added to the corn–soya diets at concentrations equivalent to the tuna diets (Ganther et al., 1972).

305 In both these studies, the authors suggested that the additional dietary Se protected against the negative consequences

306 that otherwise accompanied the high levels of dietary CH_3Hg^+ that were administered. It has also been shown that

307 maternal exposure to CH_3Hg^+ decreased Se concentration and impaired GPX and DIO activities in the brain of neonatal

308 mice (Watanabe et al., 1999a). Watanabe reported that CH_3Hg^+ exposure of Se-deficient perinatal mice resulted in

309 retarded neurobehavioral development and persistent learning disabilities. Prenatal CH_3Hg^+ exposure affected several

310 fetal mouse neurobehavioral and biochemical end points when their mothers were fed various amounts of dietary Se

311 and all toxicity effects were exacerbated by perinatal Se deficiency. To determine whether CH_3Hg^+ exposure induces

312 local Se deficiency in the fetal brain, Se concentrations and the activity of GPX were measured in the neonatal brain

313 and other organs. Although the dietary level of Se did not affect brain Hg concentrations, the Se concentration and the

314 activity of GPX were severely depressed by CH_3Hg^+ in fetal, but not maternal neural tissue (Watanabe et al., 1999b),

315 demonstrating that CH_3Hg^+ affects Se metabolism more severely in the fetal than adult brain.

316   Recently, dietary Se was used to successfully treat a previously healthy and athletic 70 kg (154 pound)

317   15-year-old patient that had been exposed to large amounts of Hg^0 vapor over a period of several weeks (Spiller,

318   2017). The patient had developed hypertension, muscular, testicular, and abdominal pain, insomnia, delusions,

319   hallucinations, tachycardia, palmar desquamation, diaphoresis, tremor, loss of 17 kg (38 pounds), and increasingly

320   severe ataxia leading to hospitalization. Examination revealed an elevated blood Hg level of 23 µg/L (~0.11 µM)

321   that was below the concentration range associated with CH_3Hg^+ toxicity. Chelation with 2,3-Dimercaptosuccinic

322   acid (DMSA) was initiated, but the patient's health continued to deteriorate. Dietary Se supplementation with 500

323   µg Se (~0.1 µMol/kg BW) along with 50 mg of N-acetylcysteine per day was initiated to support SELENOP and

324   GSH synthesis. Within 3 days, the patient showed noticeable improvement, and by day 11, delusions, delirium,

325   tachycardia, and abdominal pain had resolved and since he was once again ambulatory and eating normally, he

326   was released from the hospital but maintained on the Se and NAC supplement. After 3 months, all symptoms had

327   resolved except hypertension and after an additional 2 months, he regained 35 pounds, his hypertension resolved,

328   and he returned to athletic activities, returning to his position on the football team soon after. Selenium

329   supplementation was continued for 8 months, but did not result in elevated serum Se levels. The treating

330   physician indicated this may suggest a systemic Se deficit and/or continued Se sequestration as HgSe.

331

332

333   **3.0 The Biochemical Mechanisms of Mercury Toxicity**

334   Pařízek and Oštádalová (1967) were the first to report that rats treated with otherwise lethal doses of HgCl were

335   protected when provided supplemental Se. Since then, the ability of Se compounds to decrease the toxicity of

336   various forms of Hg has been established in all investigated species of mammals, birds, and fish (Whanger, 1992;

337   Beijer and Jernelov, 1978; Friedman et al., 1978; Ohi et al., 1980) and described in comprehensive reviews by

338   Cuvin-Aralar and Furness (1991), Gailer (2007), Bjørklund et al., (2017) and Spiller et al., (2017b).

339   The toxic effects of CH_3Hg^+ (see Figure 4) involve a sequence of biochemical reactions referred to as the

340   "SOS" Mechanisms (Ralston and Raymond, 2015). The consequences of these metabolic disruptions become

341 increasingly apparent as CH_3Hg^+ concentrations approach, and especially as they exceed, equimolar

342 stoichiometries with brain Se.
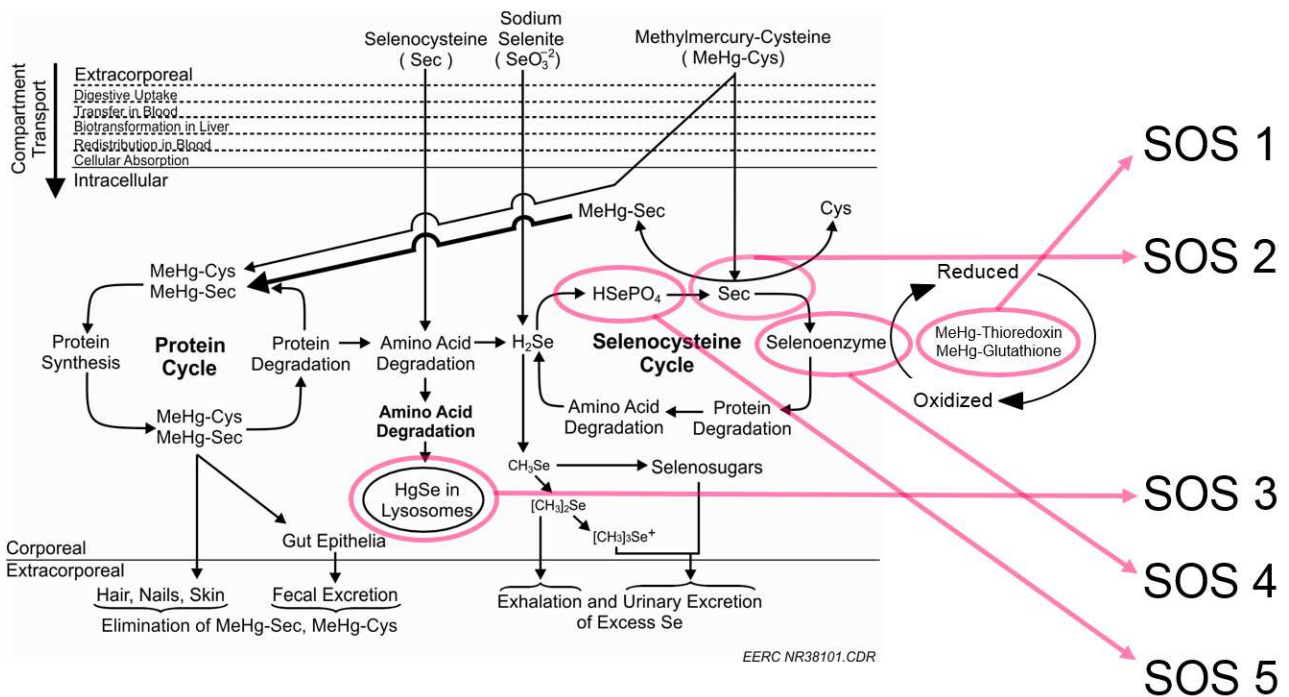
343

344 Figure 4. Schematic of the "SOS Mechanisms" of Mercury Toxicity. Disruptions of the biochemical pathways of
345 selenoenzyme activities, synthesis and related physiological outcomes, are indicated in the sequence in which
346 they are expected to occur.
347

348 *3.1 Synthesis of Suicide-substrates (SOS-1)*

349 The placental and blood brain barriers do not prevent passage of CH_3Hg-Cys, which is taken up by the LAT1

350 transporter and carried across cell membranes. Protein synthesis demands require higher importation of Met and

351 other large nonpolar amino acids, explaining the higher concentrations of fetal CH_3Hg-Cys accumulation relative

352 to maternal blood. Once across placental and blood brain barriers, CH_3Hg promiscuously exchanges binding

353 partners among Cys residues of other molecules. As Figure 2 illustrates, the binding sites of the three forms of

354 TXNRD interact with thioredoxin and a broad variety of other thiomolecule substrates (Arnér, 2009) to reduce

355 their oxidized disulfides. The GPX enzymes employ 2 GSH molecules as reducing agent co-substrates to reduce

356 cellular peroxides. In these and other thioreactive selenoenzymes, the Cys residue of the substrate enters into close

357 proximity with the active site Sec that catalyzes the proton exchange. Formation of CH_3Hg-bound thiomolecules

358   during SOS 1 is the precipitating first step towards toxicity. If the various thiomolecules were not specific

359   substrates for selenoenzymes, SOS-2 and all the subsequent consequences of CH_3Hg^+ toxicity would not occur.

360   However, because SOS-1 results in CH_3Hg^+ binding to the Cys residues of these thiomolecules, they become

361   "suicide substrates" that subsequently deliver the bound Hg to the selenoenzyme's active site.

362

363   *3.2 Silencing of Selenoenzymes (SOS-2)*

364   The high vulnerability of selenoenzymes to CH_3Hg^+ exposures was proposed by Ganther et al., (1972), and

365   demonstrated by Prohaska and Ganther (1977). The development of oxidative damage as a result of Hg-dependent

366   inhibition of selenoenzymes was described by Seppanen et al., (2004). With an IC_{50} of ~19.7 nM, TXNRD

367   activities are especially prone to inhibition by CH_3Hg^+ (Carvalho et al., 2008) and numerous *in vitro* and *in vivo*

368   studies have examined time and dose dependent effects (Carvalho et al., 2008; Branco et al., 2014; Rodrigues et

369   al., 2015; Ralston and Raymond, 2015; Branco et al., 2017). Mercury dependent inhibition of GPX is well

370   documented (Prohaska et al., 1977; Watanabe, 1999a; 1999b; Seppanen et al., 2004; Stringari et al., 2008; Ralston

371   and Raymond, 2015) and supplemental Se has been shown to prevent interruption of these selenoenzyme

372   activities in the brains of laboratory animals (Watanabe et al., 1999; Stringari et al., 2008; Ralston and Raymond,

373   2015).

374   Upon binding with the CH_3Hg-Cys adduct formed via SOS 1, the CH_3Hg^+ exchanges its covalent

375   association from the substrate Cys to the activated Sec of the enzyme's active site (See Figure 4) resulting in

376   formation of an extremely stable CH_3Hg-Sec inhibitor-enzyme complex. Unlike Cys, the partnership of CH_3Hg

377   with Sec is permanent due to the high binding affinity between Hg and Se. Therefore, the enzyme can no longer

378   perform its essential functions because its catalytic Sec is blocked by CH_3Hg^+. Thus, by biochemical definition,

379   CH_3Hg^+ is a highly selective irreversible inhibitor of selenoenzymes. In addition to coinciding with observations

380   of increased oxidative damage, inhibition of SELENOT results in increased cellular free calcium and increased

381   catecholamine release (Grumolato et al., 2008; Uezono et al., 2006; Baoukhzar et al., 2016). This could arise

382   through direct binding to the active site Sec of SELENOT or as a result of depletion of biologically available Se

383   resulting it its absence. Since increases in catecholamine release appear to occur between>1 and 10 mmol of Hg

384 (Weinberg et al., 1995), this mechanism may explain the tachycardia and hypertension observed in acrodynia as

385 well as in the Hg^0 exposed patient described in section 2.4.

386

387 *3.3 Sequestration of Selenium (SOS-3)*

388 Loss of selenoenzyme activities due to irreversible inhibition is augmented by CH_3Hg's uniquely insidious ability

389 to induce a conditioned Se-deficiency in the brain. Methylmercury is the only environmental insult has been

390 shown to diminish brain Se below the otherwise impenetrable minimum threshold of ~60% of normal (Behne et

391 al., 2000). Sequestration of Hg together with Se as the result of CH_3Hg^+ binding to the Sec of TXNRD is

392 particularly evident in kidney and liver (Wagner et al., 2010). Following catastrophically high CH_3Hg exposures,

393 there is an ongoing attrition of Se in somatic and brain (Korbas et al., 2010) tissues due to the continual formation

394 biologically unavailable mercury selenide (HgSe). This complex is resistant to decomposition by acids other than

395 aqua regia or by heating in excess 300°C. Therefore, lysosomal HgSe accumulates in equimolar precipitates that

396 exhibit long-term retention (Falnoga et al., 2000; Falnoga et al., 2006). It is important to recognize that high Hg

397 accumulations of Hg (e.g., 10-100 µM) in brain and endocrine tissues appear to be without toxicological

398 consequences (Falnoga et al., 2006), provided at least ~1 µM of "free Se" remains available for selenoenzyme

399 synthesis, thus ensuring their activities can proceed without interruption.

400

401 *3.4 Suicide Of Selenium-deprived cells (SOS-4)*

402 Following Hg-sequestration of cellular Se, insufficient bioavailable Se may produce truncated molecules that lack

403 the terminal Sec residue (Anestål and Arnér, 2003). Truncated forms of TXNRD, known as GRIM-12; are potent

404 apoptosis (cell suicide) initiators. Sequestration of cellular Se by CH_3Hg^+ may not only deprive cells of the

405 selenoenzymes they need to prevent and reverse oxidative damage, but may also transform of TXNRD into a

406 potent apoptosis initiator. Observations of phosphorylation of apoptosis signaling kinase 1 (ASK1), caspase-3

407 activity, and the increase apoptotic cells following high CH_3Hg^+ exposures are supportive of this mechanism

408 (Branco et al., 2017), although further work is clearly needed to establish its validity. The consequences of

409 GRIM12-dependent and other mechanisms that contribute to neuronal apoptosis could be especially damaging

410   during fetal brain development, and might also be a contributing factor in adult CH_3Hg^+ poisoning. Furthermore,

411   impairment of the thioredoxin and glutaredoxin systems results in proliferation of reactive oxygen and nitrogen

412   species in cytosol and mitochondria which lead to mitochondrial injury/loss, lipid peroxidation, calcium

413   dyshomeostasis, impairment of protein repair, and apoptosis (Lu and Holmgren, 2014; Glasser et al., 2014;

414   Mendelev et al., 2012).

415

416   *3.5 Sustained Oblivion of Sec Synthesis (SOS-5)*

417   Selenophosphate synthetase (SEPHS2), the enzyme that makes the $SePO_3^{3-}$ required for Sec production, is itself a

418   selenoenzyme. If SEPHS2 activities are abolished, production of Sec may never be restored in that cell since there

419   is no way to create the Sec required in its own active site. Although this mechanism remains hypothetical, once

420   SEPHS2 has been abolished by high CH_3Hg^+ exposures in a cell, this biochemical "catch-22" could permanently

421   prevent restoration of Sec synthesis. Stringari et al., (2008), found that high CH_3Hg^+ exposures during fetal

422   growth had a sustained effect on brain selenoenzyme activities. If confirmed, it appears that the damaging effects

423   of CH_3Hg^+ toxicity are not only extensive, they are likely to endure.

424

425   **4.0 The Brain Specificity of Mercury-Dependent Oxidative Damage**

426   Oxygen consumption in the brain is ~10 fold higher than in other tissues, placing the brain at an increased risk of

427   oxidative damage due to formation of reactive oxygen and nitrogen species. This risk is accentuated by the brain's

428   limited antioxidant enzyme pathways that are abundantly available in other tissues; its high iron contents could

429   potentiate oxidative damage via the Fenton reaction; and the brains increased abundance of long chain

430   polyunsaturated fatty acids, which are vulnerable to lipid oxidation (Schweizer et al., 2004a; 2004b). These

431   factors emphasize the importance of selenoenzymes that prevent as well as reverse oxidative damage in the brain.

432   To ensure these essential functions are not interrupted, brain Se concentrations are homeostatically controlled to

433   maintain Se availability for selenoenzyme synthesis and activities (Cui et al., 2004; Nazioglu, 2009). During

434   extended periods of dietary Se deficiency in laboratory animals (Prohaska and Ganther, 1977; Behne et al., 2001),

435   the Se contents of somatic tissues such as liver, muscle, and blood, diminish to less than 2% of their normal

436 contents. Selenium-transport molecules redistribute Se from somatic tissues to preferentially supply brain and

437 endocrine tissues. When Se-deficient rats were provided radiolabeled $^{75}SeO_3^{2-}$, brain was preferentially labelled

438 before other tissues (Burk et al., 1972). Brain reserves in the form of cellular SELENOP serve as accessible

439 reservoirs since Sec lyase rapidly degrades Sec to supply inorganic Se for utilization in each cycle of *de novo*

440 synthesis of new Sec molecules. These sources will maintain brain Se concentrations at a minimum plateau level

441 of 60% of normal (Behne et al., 2001), while retaining essential selenoenzyme activities at near-normal levels.

442 This pattern has been shown to continue in offspring, even after many generations of continual Se-deficiency.

443   Homeostatic regulation of selenoenzyme expression and activities in the brain varies by tissue, cell layer,

444 and cell type (Zhang et al., 2008; Zhang et al., 2010). Although all selenoprotein mRNAs are expressed in brain,

445 GPX4, SELENOK, SELENOM, SELENOW, and SELENOF are exceptionally rich in neurons of the olfactory

446 bulb, hippocampus, cerebral cortex, and cerebellar cortex. The preferential expression of certain selenoproteins in

447 the brain suggests a hierarchy of need for brain activities (Nakayama et al., 2007). Because the distal

448 compartments of dendrites and axons are remote from the soma of the neuron, it is difficult for the cell to repair

449 damage to cellular components in the highly active regions of their synapses. Therefore, selenoenzyme-dependent

450 maintenance of reduced ascorbate and other antioxidant molecules are essential for the prevention and reversal of

451 oxidative damage in the synaptic interface, and homeostatic mechanisms have evolved to ensure their expression

452 and activities proceed without interruption (Behne et al., 2000). The only environmental insult known to severely

453 impair brain selenoenzyme activities is high CH_3Hg^+ exposure (Prohaska and Ganther, 1977; Watanabe et al.,

454 1999; Stringari et al., 2008; Ralston and Raymond, 2015). As examples of mRNA expression differences in brain,

455 the distributions of two selenoproteins, SELENOM and SELENOW, are shown in Figure 5.

456
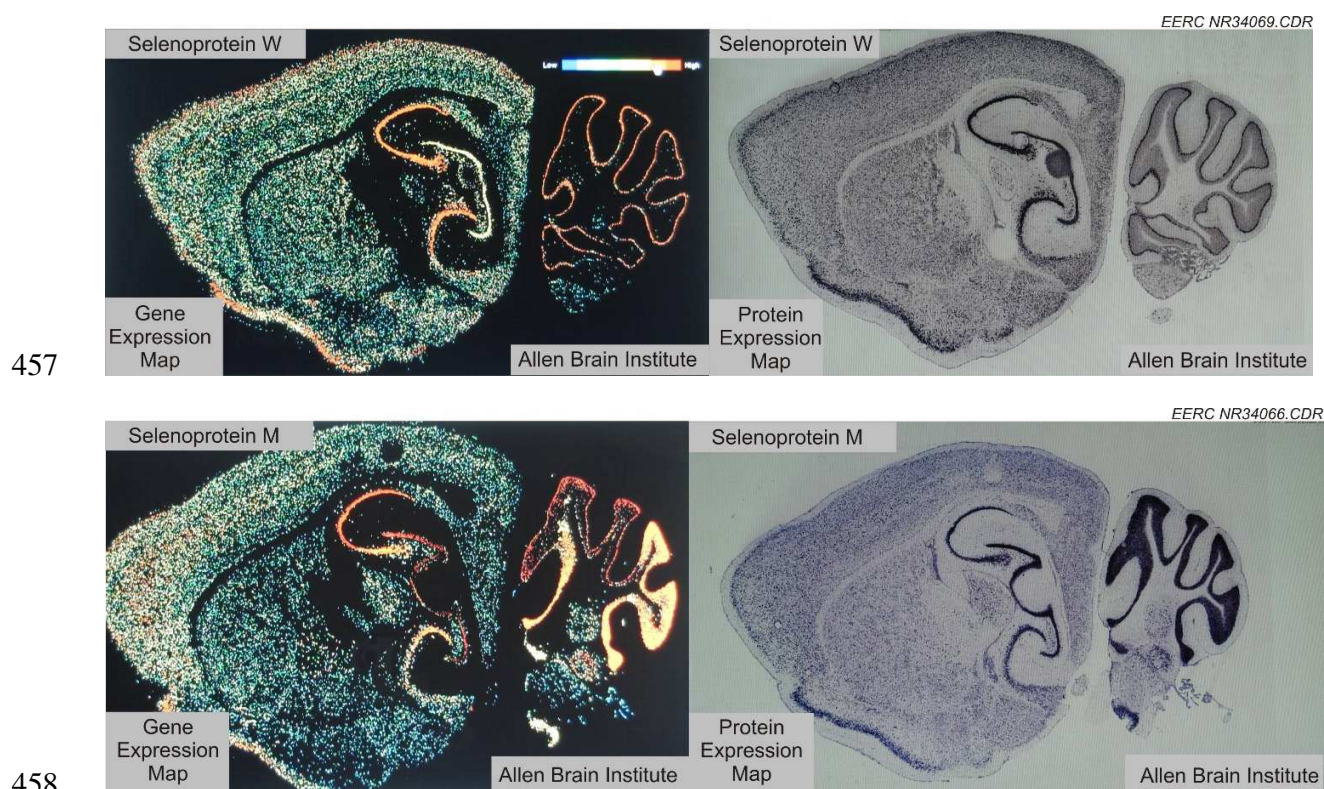
Figure 5. Images from the Allen Brain Atlas depicting *in situ* hybridization of mRNA (left side) and protein distributions (right side) for SELENOW and SELENOM observed in sagittal sections of mouse brain cerebrum and cerebellum. High levels of mRNA expression are in red, moderate levels in yellow, low levels in green, while where mRNA below detection limits are black. Distinctive patterns of expression are evident in discrete cell layers with mRNA expression correlated with high protein concentrations. Image credit: Allen Institute.

Using synchrotron X-Ray absorption spectroscopy (XAS), Korbas et al., (2010) found high concentrations of HgSe in brains of individuals that had been poisoned with high CH_3Hg^+. During CH_3Hg^+ poisoning, availability of free Se in the brain declines and brain selenoenzyme activities diminish, resulting in extensive damage to the most active neurons. These neurons are destroyed as a result of SOS 1-3 and/or apoptosis as a result of SOS 4. Meanwhile, the less vulnerable cells survive the crisis because they have maintained sufficient Se for selenoenzyme activities. The severity of the pathology will be proportional to neuronal cell damage and death, even though the brain cells that survive may gradually recover to normal levels of selenoenzyme activity, as was observed by Korbas, et al.

Postmortum examination of the brains of victims of CH_3Hg^+ poisoning show varying degrees of neuronal cell loss, especially in the sensory regions of the cortex, cerebellar granular cells, primary motor cortex (Castoldi

475   et al., 2003), and peripheral nerves (Eto et al., 2002), a pattern also seen in laboratory animals (Sakamoto et al.,

476   1998). The loss of coordination (ataxia) that occurs during severe CH_3Hg^+ poisoning is due to cerebellar damage

477   to small granule cells being destroyed, but Purkinje cells and other neighboring cells from the same region remain

478   mostly unaffected. Similarly, loss of neurons from the visual cortex is responsible for the constriction of visual

479   fields. The reasons for different sensitivities of neurons from different brain regions is currently unknown.

480   However, distinctions in neuron sensitivity to high CH_3Hg^+ exposures are likely to be due to variances in the

481   turnover rates of essential selenoenzymes, different efficiencies of ApoER2-mediated uptake of SELENOP from

482   plasma by certain brain cell types, and discrepancies in relative abilities of each cell type to preserve their internal

483   Se reservoirs.

484

485   **5.0 Accentuated Fetal Vulnerability to Mercury Exposures**

486   The fetus is without significant tissue Se reserves, so loss of maternal Se imports to the rapidly growing

487   brain can result in impaired selenoenzyme activities and damage. The three main families of selenoenzymes

488   (iodothyronine deiodinases, thioredoxin reductases, and glutathione peroxidases) have critical roles in fetal brain

489   development, growth, thyroid and calcium metabolism, protein folding, and prevention/reversal of oxidative

490   damage, particularly in neuroendocrine tissues (See Table 1 and reviews mentioned above). The SELENOP

491   molecule, the most abundant form of Se in plasma, is the primary carrier of Se to the placenta where it is taken up

492   by the SELENOP specific receptor: ApoER2 (Burk et al., 2007; Burk et al., 2013). Approximately 25% of the

493   body's total Se is cycled through SELENOP daily (Burk et al., 2005) Mice that have been genetically modified to

494   delete SELENOP or ApoER2 (the cell surface receptor that captures and internalizes SELENOP) suffer severe

495   neurodegeneration in brain regions that are associated with auditory and motor functions (Valentine et al., 2008;

496   Burk et al., 2007). SELENOP knockout models demonstrate ataxia and Se-deficient diets result in lethality (Hill

497   et al., 2003). Additional studies indicate that high CH_3Hg^+ exposures diminish maternal Se distribution to the

498   fetus by ~70% (Parizek et al., 1971). The combination of high Hg and low dietary Se was shown to diminish fetal

499   brain Se to ~23% of normal, with a portion of that sequestered as HgSe, and brain GPX activities diminishing to

500   ~14% of normal (Watanabe et al., 1999).

501   Fetal vulnerability was first observed in association with catastrophic poisoning events in Minamata

502   Japan where 75-150 tons of Hg were dumped into the local bay of the Yatsushiro Sea, a shallow semi-enclosed

503   inland sea separating the island of Kyūshū from the Amakusa Islands. The contaminated fish that were consumed

504   obtained levels of CH_3Hg^+ concentrations as high as 50 mg/kg (Takeuchi and Eto, 1999). This amounts to ~250

505   µM, a quantity 25-50-fold greater than their Se contents. Umbilical cords saved from births were found to contain

506   ~40 times more Se than Hg prior to the poisoning events (1927-1937). However, the umbilical cords of children

507   with high Hg-exposures contained ~3 times more Hg than Se during the poisoning event (1939-1959).

508   In addition to the poisoning events in Japan (Takeuchi and Eto, 1999) and Iraq (Marsh et al., 1987),

509   epidemiological studies in New Zealand (Crump et al., 1998), which is a Se deficient region, and the Faroe

510   Islands (Grandjean et al., 1997, 1998 Debes et al., 2006a; 2006b) reported associations of CH_3Hg^+ exposures with

511   slight, concentration dependent adverse effects on fetal development. These studies involved mothers eating

512   seafoods during pregnancy with high Hg:Se ratios such as great white shark and pilot whale (~5:1) respectively.

513   Although Se-rich cod fish was consumed in greater quantities, more than 95% of total CH_3Hg^+ exposure in the

514   Faroe Islands originated from pilot whale consumption. The authors of this study later concluded that the cod fish

515   offered substantial benefits that offset the otherwise expected neurodevelopmental damage from pilot whale

516   consumption. Due to their high Hg content, advisories against pilot whale consumption have since been evoked

517   and its meats removed from consumer markets (Weihe and Debes-Joensen 2012).

518   Conversely, epidemiological studies have consistently found that increasing CH3Hg-Cys exposures from

519   maternal consumption of typical varieties of ocean fish result in neurological benefits rather than deficits in their

520   children (Hibbeln et al., 2007; Davidson et al., 1998, 2011; Myers et al., 1998; 2000; Avella-Garcia and Julvez,

521   2014; Julvez et al., 2016, Llop et al., 2017; Golding et al., 2017). These studies report beneficial associations with

522   neurological development, motor development, verbal intelligence quotient, perception, social behavior, and

523   reduced inattention and hyperactivity. A partial attenuation of these positive associations was noted in the highest

524   seafood intake category (Avella-Garcia and Julvez, 2014), but it is uncertain whether CH3Hg-Cys was actually

525   responsible for the slight decrease in the net beneficial effects, or if higher exposures to other persistent

526   bioaccumulative toxicants were responsible.

527   In the Seychelles study, mean prenatal CH_3Hg^+ exposure was higher than in the Faroe Islands study, but

528 no adverse associations were found between CH_3Hg^+ and 21 endpoints (Davidson et al., 2011). Instead, increasing

529 prenatal CH_3Hg^+ was associated with improved scores on four neurological endpoints, as well as fewer reports of

530 substance abuse and incidents of problematic behaviors in school. Furthermore, increasing maternal seafood

531 consumption was shown to be associated with up to 5 points of child IQ benefits in the United Kingdom (Hibbeln

532 et al., 2007) and nearly 10 points in the United States (Lederman et al., 2008), even though MeHg exposures were

533 greatest among mothers with the highest seafood intakes. Children of mothers who avoided fish consumption

534 during pregnancy displayed developmental impairments of a magnitude ~60 times greater than the worst-case

535 effects associated with the highest pilot whale consumption (thus the highest CH_3Hg^+ exposures) in the Faroes

536 (Hibbeln et al., 2007). Additionally, the children of mothers who complied with the 2004 U.S. Environmental

537 Protection Agency reference dose (RfD) for CH_3Hg^+ exposure from fish consumption had an increased risk of

538 scoring in the lowest quartile for verbal IQ, compared to children of mothers exceeding the recommended fish

539 intake. Maternal compliance with diminished fish consumption also increased children's risks for pathological

540 scores in fine motor, communication, and social skills.

541   The findings of these studies suggest that CH_3Hg^+ exposure from ocean fish which contain Se in excess of

542 CH_3Hg^+ (a characteristic shared by nearly all commercial marine fish species) does not result in developmental

543 harm, but diminished maternal consumption of ocean fish during pregnancy is associated with significant risks.

544 Ocean fish are a significant source of Se and other important nutrients required for the health and development of

545 children and avoiding ocean fish consumption during pregnancy is associated with the loss of these benefits.

546

547 **6.0 The Biochemical Basis for the Latency Effect in Mercury Toxicity**

548 Mercury toxicity is characterized by (an unexplained) silent latency; a prolonged delay between ingestion of a

549 harmful or lethal dose and the onset of symptoms, which in some cases may take months to develop (Hunter,

550 1969; Bakir et al., 1973; Tsubaki and Irukayama, 1977; Rice, 1996; Nierenberg et al., 1998; Weiss et al., 2002).

551 The onset of clinical symptoms following high CH_3Hg^+ exposures display a similar sequence: paresthesia (a

552 tingling sensation in lips and extremities) is the first symptom to arise followed by ataxia (loss of motor

553   coordination gradually intensifying to severe disruption of functions), dysarthria (difficulty in pronouncing

554   words), vision constriction, deafness, and if the dose is overwhelming, ultimately death. However, CH_3Hg^+ has a

555   physiological half-life of ~74-days (Syversen and Kaur, 2012) and symptoms often don't arise until much of the

556   ingested dose has left the body (Nierenberg et al., 1998; Weiss et al., 2002). The severity of Hg-associated brain

557   damage is directly related to the magnitude of the dose, but the latency period is not (Weiss et al., 2002). For

558   example, a researcher that died following an accidental laboratory exposure to CH_3HgCH_3 (which is rapidly

559   demethylated to CH_3Hg^+) showed no symptoms for ~150 days (Nierenberg et al., 1998), whereas among Iraqis

560   exposed to similar amounts of CH_3Hg^+ the latency period was only 16-38 days (Bakir et al., 1973). The influence

561   of Se status on latency of CH_3Hg^+ effects are apparent in animal studies where laboratory rats fed low-Se diets

562   rapidly show physiological, biochemical, and neurofunctional defects while those fed normal-Se diets show these

563   effects later and to a lesser degree, and those fed rich-Se diets showed no consequences during the course of the 9

564   or 18 week study (Ralston et al., 2007; Ralston et al., 2008)

565   If CH_3Hg^+ occurred through pseudo-first order reactions, the latency period should be uniformly brief,

566   inversely related to dose, and comparable among those exposed to similar doses. It would also be only marginally

567   affected by supplementation with Se in quantities that are considerably smaller than the CH_3Hg^+ dose. Likewise,

568   latency would be inversely related to the received dose if the mechanism involved gradual accumulation of toxic

569   metabolites to some threshold level causing the damage, e.g., demethylation of CH_3Hg^+ to form inorganic $Hg^{+/2+}$.

570   However, the latency period which characterizes CH_3Hg^+ poisoning is strong evidence in support of the concept

571   that Hg's effects arise primarily if not exclusively from inhibition of Se-metabolism. Provided Se is available to

572   support essential brain selenoenzyme activities, the adverse consequences of toxic levels of CH_3Hg^+ will not

573   develop. However, CH_3Hg^+ in stoichiometric excess of the exposed individual's total Se reserves are likely to

574   eventually overwhelm their ability to offset systemic losses of Se-sequestration as HgSe. Differences in individual

575   Se status will influence the duration of latency since the effects of biomolecular reactions are proportional to

576   tissue concentrations of both CH_3Hg^+ and Se. Continual attrition of Se reservoirs will gradually diminish

577   availability of mobilized Se for the brain to maintain enzymatic function in the neurons. As the availability of

578   selenoenzyme activities that prevent and reverse oxidative damage diminishes below a critical threshold, the

579   damage to cellular lipids, proteins, and other important biomolecules will become increasingly evident, resulting

580   in the symptoms which characterize Hg toxicity (Spiller et al., 2017). The extent of the delay in onset of these

581   damaging effects are predicted to be directly proportional to the Se-reserves of the exposed individual, while the

582   severity of the effects will be proportional to the molar ratio of CH_3Hg^+ dose in relation to total Se.

583   Because Americans typically consume Se rich foods, Se reserves tend to be more extensive. The tissue Se

584   reserves and daily dietary Se intakes of the American researcher were apparently sufficient to preserve her brain's

585   selenoenzyme activities for 5 months before the consequences of the onetime toxic dose became evident. But

586   because the diets consumed by the Iraqi population are not as Se rich as those of Americans, it is likely their Se

587   reserves were overwhelmed more quickly by their CH_3Hg^+ exposures, resulting in more rapid onset of symptoms.

588   These possibilities are being evaluated by a Physiologically Oriented Interactions of Nutrients and Toxicants

589   (POINT) model. This computational method incorporates dietary Se intakes, CH_3Hg^+ exposures, and their relative

590   rates of retention/excretion, tissue distributions and complex formation to assess Se-attrition as a result of Hg

591   sequestration in comparison to Se-redistribution through the homeostatic mechanisms which preferentially supply

592   Se to brain and endocrine tissues.

593

594   **7.0 Discussion**

595   Recognition of the biochemical interactions between CH_3Hg^+ and selenoenzymes provides a consistent basis for

596   understanding the distinctive aspects of Hg toxicity and previous discrepancies between results of various studies.

597   The consequences of the SOS mechanisms appear sufficient to account for the adverse effects that have been

598   reported in association with toxic CH_3Hg^+ exposures. The possibility of additional mechanisms should not be

599   excluded; however, care must be applied to distinguish potentially Se-independent consequences from those that

600   may occur secondary to loss of selenoenzyme activities.

601   Failing to adhere to laboratory study designs that properly reflect the normal physiological ranges of

602   dietary CH_3Hg^+ exposures and Se intakes have contributed to mistakes and misunderstandings of the effects that

603   are expected to accompany Hg-Se interactions in human exposures. Prior to recognition of Se's metabolic

604   functions, Se was only known as a toxicant, so its protective mechanism was attributed to mutual detoxification of

605　two poisonous elements. Early attempts to examine effects of supplemental Se in protecting against Hg toxicity

606　have sometimes used equivalent mass quantities (e.g., 10 mg/kg) of Hg and Se, rather than physiologically

607　appropriate molar concentrations. Although 10 mg Hg/kg is ~50 µmol Hg/kg, 10 mg Se/kg (~126 µmol Se/kg), is

608　in tremendous excess of the normal ~1 µmol Se/kg in laboratory animal diets, and ~5 times Se's toxic threshold.

609　Such unfortunate oversights were common in early experimental studies. Later research studies have employed

610　physiologically appropriate amounts of Se (e.g., 10 µmol Se/kg, -approximating the average Se concentration in

611　ocean fish) and found it effective in eliminating the otherwise toxic effects of 50 µmol Hg/kg on the development

612　and neurological functions of growing rats (Ralston et al., 2007; 2008).

613　　　　Throughout this review, our focus has been on the loss of cellular redox control that arise as a result of

614　CH_3Hg-dependent inhibition of selenoenzymes that prevent and reverse oxidative damage. However, intracellular

615　Se-deficiencies due to Hg-dependent Se-sequestration seem likely to impair other Se-dependent metabolic

616　pathways, including some which could greatly exacerbate oxidative damage (Spiller et al., 2017). Loss of

617　SELENOK results in calcium release from the endoplasmic reticulum (Wang et al., 2017), coinciding with effects

618　noted in cell culture experiments (Tan et al., 1993; Limke et al., 2003). SELENOM, SELENON, SELENOT also

619　have been linked to calcium homeostasis, supporting the concept that (See Table 1; Grumolato et al., 2008)

620　　　　As the biochemical target of CH_3Hg^+ toxicity, Se-physiology provides perspective on the brain specificity

621　of its oxidative damage, accentuated fetal vulnerability, and latency. However, current seafood risk assessments

622　are based solely on the CH_3Hg^+ levels in the fish, but actual risks increase in direct relation to Hg:Se molar ratios

623　(Ralston et al., 2008, Ralston and Raymond 2015). Ocean fish are among the richest sources of Se in the U.S. diet,

624　and although their CH_3Hg^+ concentrations vary in relation to their trophic level, their tissue Se concentrations generally

625　remain constant regardless of size (Kaneko and Ralston 2007). Conversely, MeHg is nonspecifically bioaccumulated

626　in fish as a molecular mimic of methionine, so the amount they bioaccumulate increases as they grow older and

627　larger. Fish at the top of the food web can harbor tissue mercury concentrations $>10^6$-fold higher than that of the

628　water in which they live (Lindqvist et al, 1991).

629　　　　Similar to all other vertebrates, fish homeostatically regulate their tissue concentrations of Sec, so their

630　brain and endocrine tissues are well protected against decrements due to poor Se intakes. Selenium is abundant in

631 the marine food web, so regional differences in tissue Se are unlikely to be observed in pelagic fish. However, fish that

632 inhabit estuaries of rivers whose watershed have poor soil Se availability are likely to have diminished Se in their

633 fillets. The abundance of Se available in aquatic ecosystems is directly related to the abundance of Se in

634 surrounding soils, but it is also dependent on pH of their soil-water environment. Even when Se is present in soil,

635 its availability for uptake by plants becomes compromised in regions with low pH levels. Waterbodies with low Se

636 have been shown to accentuate MeHg accumulation and retention in the fish inhabiting these areas (Paulsson et al,

637 1989; Chen, et al, 2001; Southworth et al, 2000; Turner and Rudd, 1983; Mailman et al., 2014; Raymond and

638 Ralston 2018). Therefore, the CH_3Hg^+ and Se levels in freshwater fish can differ considerably.

639 Enhanced CH_3Hg^+ bioaccumulation in fish from Se-poor watersheds has the potential for an adverse

640 synergy of increasing CH_3Hg^+ exposures while simultaneously increasing the risks associated with those

641 exposures since the fish fail to provide adequate Se to offset losses due to Se-sequestration. Subsistence

642 consumers of fresh water fish are at particular risk of toxic effects from such high exposures. For example, in a

643 subsistence freshwater fish consuming population in the Amazon, motor function abilities were inversely related

644 to blood Hg concentrations, but directly related to Se status (Lemire et al., 2011). Because locally grown foods in

645 Se-deficient regions fail to provide background dietary sources of Se, the effect of other soft metallic or organic

646 (e.g., Ali et al., 2014) electrophiles will accentuate risks associated with CH_3Hg^+ exposures. Risk assessments that

647 simply assess CH_3Hg^+ exposures cannot adequately address these other important considerations.

648

649 *7.1 Conclusions*

650 Toxicology is a rapidly evolving field which continually disproves dogma, overcomes mistaken assumptions, and

651 steadily improves understandings of biochemical mechanisms of toxicity. The conundrums of CH_3Hg^+ toxicity:

652 the basis for the "selenium (Se)-protective" effect, the absence of a biochemically defined mechanism, its tissue

653 specificity, enhanced fetal vulnerably, and latency effect, all arose from a misunderstanding of Hg-Se interactions.

654 This review, compiled from over 50 years of research progress, indicates that the distinctive characteristics of

655 CH_3Hg^+ toxicity are consistent with its unique ability to impair brain selenoenzyme activities, thus resolving these

656 conundrums. The SOS mechanisms result in selenoenzyme inhibition, thus providing a consilient perspective of

657 the commonalities between predicted and observed reaction stoichiometries, biochemical products, tissue

658 sensitivities, and the pathological effects that arise as a result of CH_3Hg^+ dependent impairments.

659   These findings have clear implications for risk assessment research, policy, and regulations. Predatory

660 whales, certain varieties of shark, large specimens of swordfish, halibut, or any other types of fish that contain

661 more Hg than Se should not be consumed by children or pregnant women. However, nearly all other seafoods and

662 ocean or freshwater fish provide far more Se than CH_3Hg^+ to consumers and will therefore improve, rather than

663 diminish, maternal and fetal Se status while offering additional nutritional benefits required for health and

664 development. To enhance the reliability of CH_3Hg^+ risk assessments, dietary Se intakes must be considered in

665 relation to CH_3Hg^+ exposures.

666

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678
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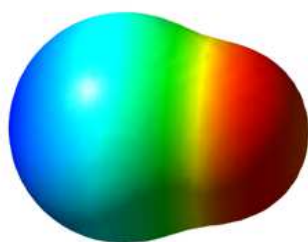
HgO
Chemical potential = -5.4
$K_a = \sim 10^{27}$

HgS
Chemical potential = -5.2
$K_a = 10^{39}$
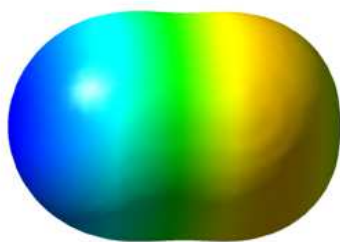
HgSe
Chemical potential = -5.1
$K_a = 10^{45}$

The electrostatic potential surfaces of mercury in covalent association with the biologically significant chalcogens (oxygen, sulfur, and selenium), their chemical potentials, and binding affinity constants.

The electron cloud depicted in blue indicates a lower e^- abundance and a more positive charge, while yellow shading to red indicates increasingly negative charge. The balance of the HgSe charges stabilize the molecule, contributing to their remarkably high binding affinities.