



Neurotoxic Electrophile Interactions with Brain Selenoenzymes

Nicholas V. C. Ralston

Contents

1	Introduction	3
2	Biochemistry of Chalcogen Amino Acids	4
3	Brain Selenoenzymes and Selenium Interactive Proteins	8
3.1	Proteins Involved in Selenocysteine Formation, Degradation, and Transport	9
3.2	Selenoproteins Involved in Prevention, Reversal, and Regulation of Oxidation	10
3.3	Selenoprotein S, I, O, and V	14
3.4	Iodothyronine Deiodinases	14
4	Reactive Oxygen, Nitrogen, and Electrophile Species	15
4.1	Metallic Electrophiles	16
4.2	Organic Electrophiles	18
4.3	Kinetic and Thermodynamic Considerations	21
5	Conclusion	27
6	Cross-References	29
	References	30

Abstract

The physiological effects of selenium (Se) occur through the biochemical actions of selenocysteine (Sec), the twenty-first genetically encoded amino acid. The human genome includes 25 genes coding for Sec in enzymes (selenoenzymes) that control thyroid hormones, calcium activities, immune responses, prevent and reverse oxidative damage, and perform other vital functions. As the most potent intracellular nucleophile, Sec is vulnerable to binding by soft electrophiles (E^*), i.e., electron poor metallic elements and certain organic molecules. Soft electrophiles form covalent bonds with nucleophilic thiols and initially bind to cysteine (Cys). These adducts become suicide substrates which orient E^* to react with Sec in the active site and form Sec- E^* , thus irreversibly inhibiting the enzyme's activity. High E^* exposures diminish selenoenzyme activities and sequester tissue

N. V. C. Ralston (✉)

Earth System Science and Policy, University of North Dakota, Grand Forks, ND, USA

e-mail: nick.ralston@ndus.edu

Se, thus preventing synthesis of new selenoenzymes. Among those with poor Se status, toxicity can arise from even moderate E* doses. The adverse neurological effects of metallic electrophiles such as mercury correspond with their high Se-binding affinities. Organic electrophiles have lower Se-binding affinities, but their exposure sources are more abundant. Their inhibitory stoichiometries remain undefined, but the aggregate effects of multiple E* exposures may exceed the Se-recovery capabilities of certain populations. Soft electrophiles include well-known neurotoxic agents, but effects of common pharmaceutical, dietary, or environmental E* remain poorly studied. Establishing therapeutic versus toxic ranges for certain pharmaceutical agents should include consideration of the patient's Se status. The aggregate and cumulative effects of E* on brain selenoenzymes will require further evaluation in environmental risk assessments.

Keywords

Selenium · Selenoenzyme · Electrophile · Brain · Toxicity · Neurotoxicity

Abbreviations

ApoER2	Apolipoprotein E receptor 2
ATP	Adenosine triphosphate
CH ₃ Hg	Methylmercury
CNS	Central nervous system
Cys	Cysteine
DIO	Deiodinase
DIO	Iodothyronine deiodinase
GPx	Glutathione peroxidase
GRx	Glutathione reductase
GSH	Glutathione
GS–SG	Glutathione (oxidized form)
H ₂ O 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	Peroxyl radical
R–S•	Thiyl radicals
RSe [−]	Selenoate
R–SH	Thiol

Sec	Selenocysteine
SeMet	Selenomethionine
SeO ₃	Selenium trioxide
SeO ₃ ²⁻	Selenite
SeO ₄ ²⁻	Selenate
Ser	Serine
TGR	Thioredoxin-glutathione reductase
TRx	Thioredoxin reductase
Trx-(SH) ₂	Thioredoxin

1 Introduction

The brain requires selenium (Se)-dependent enzymes (selenoenzymes) to perform essential functions (Kühbacher et al., 2009; Chen & Berry, 2003; Zhang et al., 2008) through activities that depend on the unique redox range of selenocysteine (Sec), the twenty-first proteinogenic amino acid (Hatfield & Gladyshev, 2002; Kühbacher et al., 2009). Humans possess 25 Sec-containing genes encoded in proteins that are expressed in tissue-dependent patterns of occurrence in all cells of the body. However, brain tissues require uninterrupted activities of selenoenzymes and cannot survive without them. The functionally characterized selenoenzymes uniformly employ Sec as the primary catalytic agent in their active sites where it directly interacts with substrates to catalyze their enzymatic reactions (Arnér & Holmgren, 2000). All vertebrates and nearly all other forms of animal life express selenoenzymes to protect their brain and endocrine tissues (Hatfield & Gladyshev, 2002; Kühbacher et al., 2009). Because the selenoate of Sec is more nucleophilic than the thiol of cysteine (Cys), selenoenzymes are able to catalyze reactions to prevent or reverse oxidative damage, regulate calcium and thyroid hormone status, guide protein folding, control cytoskeletal structures, accomplish Sec synthesis, and communicate intracellular redox state conditions, while specialized transport molecules shuttle Se between tissue compartments (Reeves & Hoffmann, 2009; Reeves et al., 2010). High metabolic rates in brain tissues spontaneously produce reactive oxygen species (ROS), reactive nitrate species (RNS), and other metabolic byproducts which can damage lipids, proteins, and nucleic acids. To counteract these effects, the brain is preferentially supplied with Se to ensure that selenoenzyme synthesis is supported and their activities proceed without interruption (Kühbacher et al., 2009; Burk et al., 2013).

Unlike other dietarily essential trace elements, deficiencies in Se intakes are not accompanied by overt pathological consequences. This is largely due to the unparalleled homeostatic control of Se via kinetic mechanisms that evolved to ensure its preferential retention in the tissues that require it. Selenium concentrations in most dietary components are very low in comparison to most other essential elements. These generally low but variable levels in the diet may explain why the Se contents of brain tissues are so tightly controlled. Although feeding Se-deficient diets to laboratory animals rapidly depletes the Se contents of blood and most body tissues

to as little as ~2% of their normal concentrations, the brain is preferentially supplied with Se to ensure its contents remain largely unaffected and the activities of essential selenoenzymes continue without interruption. Tissue Se reserves are redistributed through somatic secretion of Se-transport proteins into the blood. These are selectively captured and internalized by specific receptor proteins exclusively expressed on cell surfaces of priority tissues such as the brain. This intricate homeostatic mechanism ensures brain selenoenzyme activities remain essentially constant regardless of how little or how much Se is present in the diet (Kühbacher et al., 2009; Burk et al., 2013). However, as the most potent intracellular nucleophile, Sec is vulnerable to binding by electron poor metallic and organic soft electrophiles (E*) which can irreversibly inhibit selenoenzymes in tissues that cannot survive without them. Other than genetic knockouts, the only environmental insults capable of substantially diminishing brain selenoenzyme activities are E* exposures in stoichiometric excess of tissue Se reserves, particularly when Se replenishment is limited due to Se-poor diets (Ralston & Raymond, 2018; Ralston, 2018; Ouyang et al., 2018; Ste. Marie et al., 2020).

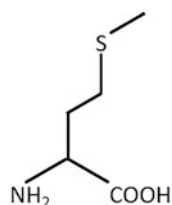
Therefore, the selenoenzyme-binding abilities of dietary, environmental, and pharmaceutical sources of E* need to be quantified and the anticipated range of their cooperative exposures evaluated. Further, they must be considered in relation to the Se status of E* exposed populations to properly assess the associated risks. Exposures to E* such as the various forms of mercury (Hg) on selenoenzyme activities are increasingly well characterized, but the magnitude of individual and combined exposures to other E* and the extents of their Se-binding interactions remain largely unstudied. While the Se-binding affinities of several metallic E* is high and their effects have been demonstrated, the binding affinities of organic E* and the potential effects of exposures on selenoenzyme activities remains conjectural at present. However, the available evidence suggests the combined effects of commonly encountered metallic and organic E* may contribute to the development and progression of neurological disorders and neurodegenerative diseases.

2 Biochemistry of Chalcogen Amino Acids

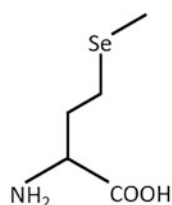
Selenium is an essential trace element that is present in variable amounts in soil. Factors such as the geologic source of the parent rock, how much leaching of soluble Se forms has occurred, and pH of the soil or water all affect plant uptake of Se from the soil. Due to the biochemical similarity between sulfur and Se, these elements are taken up by plants in amounts that reflect their relative molar abundance in soil (S:Se molar ratios typically range around 100,000:1). Plants use sulfur to synthesize methionine (Met) but will form selenomethionine (SeMet) when a Se atom is absorbed and incorporated in its structure instead of a sulfur (See Fig. 1). Because SeMet is not distinguished from Met at the mRNA level, it does not have a distinct three-letter code to denote its presence. These analogous forms are incorporated in plants, repeatedly used in cycles of plant protein synthesis and degradation, and repeat the same pattern in the proteins of grazing animals and their predators. The

Hydrophobic

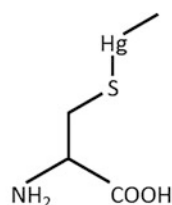
Amino Acid:
Letter codes:
Note:
mRNA Codons:



Methionine
 Met, M
Undifferentiated analogues
 AUG



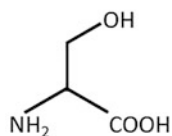
Selenomethionine
 SeMet, M
Undifferentiated analogues
 AUG



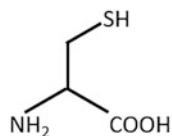
Pseudomethionine
 CH₃Hg-Cys

Nucleophilic

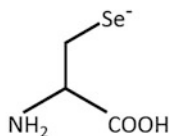
Amino Acid:
Letter codes:
Side group pK_a:
mRNA Codons:



Serine
 Ser, S
 pK_a = 13
 TCT, TCC,
 TCA, TCG



Cysteine
 Cys, C
 pK_a = 8.3
 TGT, TGC



Selenocysteine
 Sec, U,
 pK_a = 5.5
 UGA +3'UTR

Fig. 1 The chalcogenic amino acids include methionine and selenomethionine; analogous forms which are not physiologically differentiated in plant or animal biochemistry. SeMet is not distinguished from Met during protein translation and therefore does not have a unique 3 or 1 letter code. When CH₃Hg is bound to Cys, it resembles Met at a molecular level and apparently functions as a pseudo-Met until it is degraded. The nucleophilicity of these amino acids follows the rank order: Met < Ser << Cys < Sec

substitution of sulfur with Se appears to have very limited effects on protein structure and function. The biological relevance of Se in SeMet only becomes evident after the amino acid is degraded. While many proteins contain SeMet incorporated in place of Met, only those which are genetically encoded for inclusion of Sec in their primary sequence are called selenoproteins.

Oxygen, sulfur, and Se are chalcogens (members of group 16 of the periodic table) and thus share many chemical properties. Their similarities and differences are evident in the functionalities of the hydroxyl group of serine (Ser), the thiol of Cys, and the selenoate of Sec (See Fig. 1). The fates of S and Se sharply diverge once inorganic Se is released from SeMet. Selenium is rapidly reduced to selenide which can be phosphorylated by selenophosphate synthetase (SPS2) as described below. The amino acids which incorporate hydroxyls, thiols, or selenoates vary in the pK_a's of their side chains. The pK_a of serine is well above physiological pH, but in the endoplasmic reticulum, Golgi apparatus, and to a lesser extent in the cytoplasm, it undergoes posttranslational modifications including O-linked glycosylation. The oxygen atom of the hydroxyl groups of Ser (also threonine and tyrosine) is a

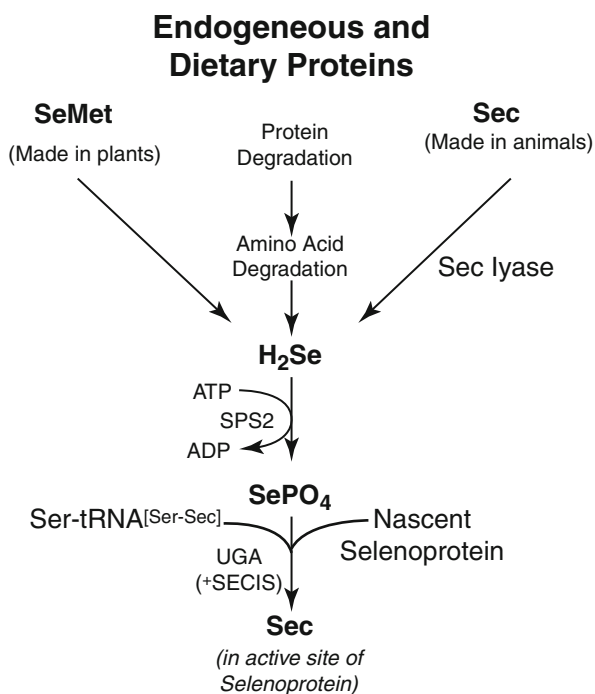
moderate nucleophile that can form esters, especially with inorganic phosphate. Phosphorylation is the replacement of the hydrogen of the hydroxyl moiety with a phosphoryl group (PO_3^{2-}) in reactions performed by a kinase and a co-substrate such as ATP. Serine is also particularly notable as the initial substrate used for *de novo* synthesis of Sec (see below).

The sulfur of the thiol side chain of Cys is functional in a diverse range of activities and metabolic reactions. Although it is a much better nucleophile than the hydroxyl of Ser, the Cys thiol's pK_a (8.3) indicates it would be mostly protonated (and thus uncharged) at physiological pH unless it was in a specialized environment. Thiols react with physiological oxidants through single and two electron oxidations of the thiolate anion (R-S^-), particularly within enzyme pockets where the pH is modulated to deprotonate the thiol. Single electron oxidations result in thiyl radicals (R-S^\bullet) as transient intermediates which react with thiolate anions in proteins such as Trx-(SH)₂ or peptides such as GSH. Because S-H bonds are ~20% weaker than C-H bonds, thiyl radicals are easily generated. In the presence of oxygen, the reaction proceeds toward the generation of disulfide forms (e.g., Trx(S)₂ or GS – SG) or superoxide anion radicals (O_2^\bullet) which prompt further oxidative reactions. Two-electron oxidations of a protein thiol (R-SH) yield sulfenic acid (R-SOH), a reactive intermediate that forms mixed disulfides with glutathione (R-SSG) or Cys and/or intramolecular or intermolecular disulfides. Single electron oxidant radicals generate the R-S^\bullet which under aerobic conditions will react with thiolate anions (GSH or R-SH) to yield the disulfide anion radical and transfer the radical reaction to neighboring molecules unless and until being quenched by scavenger molecules. Reaction of R-S^\bullet with nitric oxide (NO) leads to S-nitrosylation (R-SNO), a principal effector in redox-regulation of metabolic functions that occur through control of a myriad of S-nitrosylated proteins and highlight the importance of this reaction product. Dysregulation of S-nitrosylation and denitrosylation has been implicated in neurological disorders, chronic degenerative diseases, and other pathologies (Picón-Pagès et al., 2019).

Selenocysteine is a noncanonical amino acid that evolved in eukaryotes and prokaryotes ~2 billion years ago. As the most powerful intracellular nucleophile, the low pK_a of Sec's selenoate (5.5) enabled enzymes to work with thiols to protect against damaging effects of the oxygen being released by early photosynthetic life. Upon reaction with electrons, it can form reactive oxygen species (ROS) capable of inflicting nonspecific damage to proteins, lipids, and nucleic acids. Eukaryotes other than plants and yeast had to increasingly depend on the gradual refinement of selenoenzymes capable of protecting their vulnerable biomolecules against damage. As oxygen in the environment rose from near zero (weakly reducing) to the current strongly oxidizing levels, its damaging effects steadily increased. Gradually intensifying oxygen exposures over hundreds of millions of years selected for the survival of life forms that could make the metabolic adjustments necessary to endure. Species which were unable to avoid exposure to oxygen had to develop selenoenzymes as a means of maintaining intracellular reducing conditions or go extinct.

The development of selenoenzymes enabled sulfur-based antioxidants such as GSH, Trx-(SH)₂, and their accompanying reductase enzymes to protect against ROS.

Fig. 2 Dietary Se sources primarily consist of SeMet and Sec from plant and animal proteins. While SeMet can be repeatedly used in proteins, Sec is used only once. When these amino acids are degraded, they release an inorganic Se which can be phosphorylated and incorporated into new Sec molecules that are created de novo during selenoprotein synthesis



Gradually increasing survival pressures promoted continual development of the coordinated sequence of protein actions that is required to accomplish Sec synthesis and to work in concert with thiomolecules in preventing and reversing oxidative damage to cellular lipids, proteins, and nucleotides. Due to these evolutionary pressures, Sec synthesis and selenoenzyme activities are highly conserved and consistent in all cells of all vertebrates (Gladyshev & Hatfield, 1999; Hatfield & Gladyshev, 2002; Kühbacher et al., 2009).

Briefly, the UGA codon that otherwise serves as the “opal” termination signal has an alternate decoding when a specific stem-loop structure in the mRNA includes a 3′ untranslated region (3′-UTR) that signals for the synthesis and insertion of Sec. This is known as the selenocysteine insertion sequence (SECIS) element. These interact with a set of specific decoding protein factors and enzyme activities such as the selenophosphate displacement of the hydroxyl moiety of Ser brought into place by tRNA^{[Ser]Sec} to create Sec just as it is inserted into the protein. Unlike other amino acids, Sec is synthesized on its transfer RNA (tRNA^{[Ser]Sec}) with the anticodon to UGA which initially binds a Ser that is subsequently converted into Sec as it is inserted into the nascent protein sequence (Fig. 2). While the human selenoproteome include 25 human genes which express Sec (Gladyshev et al., 2004; Kryukov & Gladyshev, 2004), the expression levels of these gene products vary distinctly between cell types, change during stages of development, and, in certain cases, in response to specific stimuli. While all cells of all forms of vertebrate life display

similar tissue-dependent patterns of selenoenzyme expression, their roles in protecting brain tissues are particularly well conserved (Kühbacher et al., 2009; Chen & Berry, 2003; Zhang et al., 2008).

Following dietary intakes of normal levels of Se, a portion of the body's Se becomes excreted in urine in the form of selenosugars and monomethyl-Se (CH_3Se^-). Low dietary Se intakes result in greater retention of ingested Se and far less urinary excretion. Higher Se intakes increase formation of dimethyl-Se [$(\text{CH}_3)_2\text{Se}$], an uncharged molecule exhaled in the breath which has a distinct garlic odor in Se-intoxicated individuals. At toxic Se intakes, increasing amounts of trimethyl-Se [$(\text{CH}_3)_3\text{Se}^+$] are created. Since this is a charged form, it is excreted in the urine along with the normal selenosugars.

3 Brain Selenoenzymes and Selenium Interactive Proteins

The following sections describe human selenoproteins and certain other proteins with important roles in Se, Sec, or selenoprotein metabolism. Emphasis is placed on the brain-specific functions of the listed selenoproteins, Se-interactive proteins, and selenoproteins that are not expressed in the brain or central nervous system (CNS) will be only briefly mentioned. Instead of including specific references in each case, for convenience and clarity, the formal (IUPAC) gene name and UniProt database number are listed along with the short name for the protein to enable easy referencing. For the functionally characterized enzymes, the International Union of Biochemistry and Molecular Biology (IUBMB) enzyme nomenclature reference number is also provided. This is accompanied by brief mentions of the occurrence, distribution, metabolic roles, and functional relationships of these proteins sourced from Labunsky et al. (2014) and Avery and Hoffmann (2018).

The cerebral cortex, hippocampus, cerebellum, and olfactory bulb express the highest numbers of selenoproteins (Zhang et al., 2008). Since loss of certain brain selenoenzyme activities result in death or significant damage to neurological tissues, they are extraordinarily well protected against Se deficiency. The brain's highly effective homeostatic mechanisms can overcome nearly all environmental challenges to ensure adequate amounts of Se are received to support selenoenzyme activities. Diminishments or dysregulation of selenoenzyme functions contribute to neurological defects and neurodegenerative diseases. Brain selenoenzymes such as the GPx and TRx families are pivotal in preventing and reversing oxidative damage and regulating intercellular reducing conditions while others contribute to these processes directly or indirectly. Increased oxidative stress has been implicated in Parkinson's disease, Alzheimer's disease, stroke, and epilepsy, as well as other neurological disorders (Uttara et al., 2009). While the links between these diseases and Se status are mostly indirect and do not have clear causal connections, the pivotal role of selenoenzymes in preventing and reversing oxidative damage suggest their disruption may be part of the pathology and could contribute to the etiologies of these diseases. Although homeostatic regulatory processes ensure brain selenoenzyme activities are resilient to dietary Se deficiencies of extended or even

multigenerational durations, exposures to agents which irreversibly inhibit selenoenzymes or sequester sufficient Se to adversely affect brain physiology are likely to exhibit subtle latent effects which may not be observed until substantial damage has already been done.

3.1 Proteins Involved in Selenocysteine Formation, Degradation, and Transport

Unlike other amino acids, Sec must be created *de novo* as it is incorporated into a selenoprotein. This requires the activity of selenophosphate synthetase (SPS2) and the availability of inorganic Se. To provide the inorganic Se that will be required for Sec synthesis, selenocysteine lyase must cleave the carbon-Se bond. Unlike other essential trace elements, Se is distributed to placental, endocrine, and brain tissues. This is accomplished by selenoprotein P (SelP) being bound by a highly selective receptor expressed on these preferentially supplied tissues.

3.1.1 Selenophosphate Synthetase and Selenocysteine Lyase

Selenophosphate synthetase (SPS2, Gene name SEPHS2, UniProt: p49903, EC 2.7.9.3) is a selenoenzyme that acts upon selenide and ATP substrates to catalyze transfer of a phosphate from ATP to create the high energy selenophosphate (SePO_4^{2-}) precursor required for Sec synthesis during its co-translational incorporation into a nascent selenoprotein. Since all cells of all vertebrates create Sec, this selenoenzyme is ubiquitously expressed, and because SPS2 activity is the first step for formation of all selenoproteins, including its own, it may serve as an auto-regulator of selenoprotein synthesis. Because SPS2 is a selenoenzyme and is the required initiator of Sec synthesis during the first step in formation of all other selenoproteins, its loss would prevent formation of any further selenoenzymes in cells without its activities. For that reason, conditions which abolish SPS2 synthesis are expected to result in permanent loss of selenoprotein synthesis ► [Mercury's Neurotoxic Effects on Brain Selenoenzymes](#) (Ralston & Raymond, 2018).

Although it is not a selenoprotein, the activities of Sec lyase (Secly, Gene name SCLY, UniProt: q96i15, EC 4.4.1.16) are crucial for Sec metabolism. Depending on their form and function, the half-lives of selenoproteins range from a few minutes to several days, but eventually the molecule becomes damaged, marked for degradation, and is broken down to release its component amino acids. However, Sec cannot be used in subsequent cycles of protein synthesis. Instead, it is broken down to release inorganic Se which can be used to create Sec *de novo* when it is required in a nascent selenoprotein. Regardless of whether the Sec residue has been released from proteins of recently digested food or from endogenous cellular proteins, Sec lyase catalyzes the release of the inorganic Se which is rapidly reduced to selenide, the substrate required for SPS2 activity.

3.1.2 Selenoprotein P and the Selenoprotein P Receptor

Selenoprotein P (SelP, Gene name SELENOP, UniProt: p49908), the major plasma selenoprotein, represents >50% of the total Se present. Unlike all other selenoproteins which contain a single Sec per polypeptide chain, SelP contains ten Sec per molecule. Two of the Sec moieties are involved in selenylsulfide bridges with Cys residues. More than 80% of SelP is secreted by the liver, and internalized SelP along with the substantial quantities of SelP synthesized in the brain itself comprise a ready reserve of intracellular Se to support its needs (Burk et al., 2013). Prioritization of Se distribution to brain tissues occurs through a SelP being bound and internalized by the SelP-specific uptake receptor known as the apolipoprotein E2 receptor (ApoER2), named in reference to its first recognized binding partner. Selenoprotein P genetic knockouts show no phenotype until postnatal development when diminished growth, ataxia, cerebral symptoms, and sporadic fatalities become evident. These consequences can be prevented by feeding diets enriched with substantial amounts of Se to maintain supplies in the brain at levels capable of supporting normal selenoenzyme synthesis (Hill et al., 2003; Valentine et al., 2008).

Substantial fractions (as much as 25%) of the body's Se are cycled through SelP daily (Burk & Hill, 2005). As the main Se transport protein in plasma, SelP delivers Se to brain and a select set of endocrine and other indispensable tissues which express ApoER2 on their cell surfaces to ensure their Se needs are met.

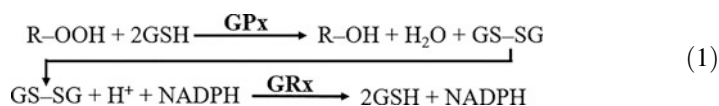
Although not a selenoenzyme, ApoER2 (Gene name APOE, UniProt: p02649) is required for the selective uptake of SelP by the brain and other preferentially supplied tissues with critical dependence on selenoenzyme activities (Burk et al., 2013). Genetic deletion of ApoER2 results in impaired motor and auditory functions unless dietary Se is greatly augmented to correct for the reduced amount of Se being supplied to the brain. This results in extensive damage and neurodegeneration of cerebellar, thalamic, and hippocampal regions which will respectively impair motor functions, sensory abilities, and learning behaviors. As is observed in the SelP knockout mouse, feeding Se-rich diets enables the brain to acquire and maintain Se at levels capable of supporting normal selenoenzyme synthesis and avoid these consequences, thus demonstrating these effects are due to loss of Se, not loss of any other components acquired and internalized by this receptor.

3.2 Selenoproteins Involved in Prevention, Reversal, and Regulation of Oxidation

Nine of the 25 selenoprotein genes (5 glutathione peroxidases, 3 thioredoxin reductases, and methionine sulfoxide reductase) are directly involved in control of oxidative damage and cell signaling through redox status. Based on structure-function predictions and homologies to functionally characterized selenoenzymes, an additional seven selenoproteins appear to perform similar roles, although their activities and substrates remain incompletely characterized. Thus, approximately two-thirds of selenoenzymes seem to be involved in protection against oxidative damage and control of intracellular redox activities.

3.2.1 Glutathione Peroxidases

Hydrogen peroxide (H_2O_2) and lipid peroxides (R-OOH) are ROS that form as consequence of normal metabolism and although excessive levels cause oxidative stress and damage cell components, low levels are necessary to contribute to signaling pathways. Peroxides are reactive species that must be quickly detoxified to prevent the damage they would otherwise cause. However, members of the glutathione peroxidase (GPx) family perform vital functions in regulating intracellular redox state as well as preventing and/or reversing oxidative damage. Eq. 1 is a generalized depiction of the glutathione (GSH)-dependent reduction of lipid peroxides (R-OOH) or H_2O_2 to an alcohol and water by GPx. This forms glutathione disulfide (GS-SG) which is subsequently reduced by glutathione reductase (GRx) which catalyzes the reduction of GS-SG back to 2GSH in the presence of NADPH.



Glutathione peroxidase 1 (GPx1, Gene name GPX1, UniProt: p07203, EC 1.11.1.9) is abundant in liver and erythrocytes with smaller amounts synthesized by the brain. It is a ubiquitous cytosolic homotetrameric (23 kDa/monomer) enzyme and its concentrations vary in relation to dietary Se intakes. No obvious phenotypes are apparent in GPx1 knockout mice, although they become more vulnerable to pathologies related to increased oxidative stress.

Glutathione peroxidase 2 (GPx2, Gene name GPX2, UniProt: p18283) is expressed in the liver and gastrointestinal system but its expression in brain tissues is comparatively low, so loss of its functions would be unlikely to be associated with any neurotoxic effects. It will not be discussed further in this chapter.

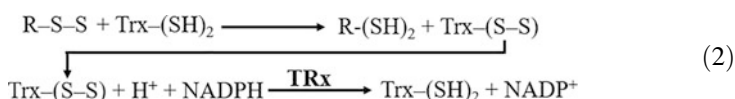
Glutathione peroxidase 3 (GPx3, Gene name GPX3, UniProt: p22352) is synthesized by kidney, liver, and other tissues, and is the second most abundant plasma Se form after selenoprotein P. It is an enzyme, but since GSH concentrations in plasma are insufficient to support catalysis, its primary role appears to be redistribution of Se among somatic tissues. Transport of Se via GPx3 is primarily from the digestive tract to the rest of the body. It also accomplishes homeostatic redistribution from somatic tissues to the liver and other tissues which can redeploy Se in SeIP to supply brain and endocrine tissues. However, since these tissues also synthesize GPx3, Se transport is not exclusively unidirectional.

Glutathione peroxidase 4 (GPx4, Gene name GPX4, UniProt: p36969, EC 1.11.1.12) is the most highly expressed selenoenzyme mRNA in the brain. It is localized to cytosol, mitochondria, and nucleus where it reduces phospholipid hydroperoxides but exhibits broad substrate specificity and may act as a universal antioxidant in the protection of biomembranes. It participates in redox signaling and regulatory processes including inhibiting lipoxygenases and apoptosis as well as assisting in preventing oxidation of low-density lipoproteins (LDL). GPx4 knock-outs have disrupted structural compartmentalization and are embryonically lethal at an early stage.

Glutathione peroxidase 6 (GPx6, Gene name GPX6, UniProt: p59796, EC 1.11.1.9) effectively detoxifies H_2O_2 , but its expression is restricted to embryos and adult olfactory epithelium with negligible or no expression occurring in brain tissues. Its functional significance remains undetermined, but since it is not important in the brain, its loss is unlikely to be associated with any neurotoxic outcomes.

3.2.2 Thioredoxin Reductases

Thioredoxin reductase (TRx) enzymes are named for their first recognized substrate; oxidized thioredoxin [$\text{Trx}-(\text{SH})_2$] which is itself an important antioxidative enzyme with vicinal thiols applied in reducing oxidized substrates. Although TRx enzymes have many substrates, a simplified depiction of the canonical reaction for which this family of enzymes was named proceeds is shown in Eq. 2. This generalized reaction between TRx and oxidized substrate disulfides (S-S) results in their reduction to thiols which are pivotal in reducing reactions throughout the cell.



The three TRx forms reduce a broad range of cognate substrates and deletion of their genes is embryonically lethal, indicating their functions are essential. Thioredoxin reductase 1 (TRx1: Gene name: TXNRD1, UniProt: q16881, EC 1.8.1.9), the cytosolic form, is a ubiquitously expressed homodimeric oxidoreductase flavoprotein containing a single Sec residue as penultimate amino acid of its C-terminus and 1 FAD per subunit. Localized to cytoplasm and the nucleus, TRx1 reduces a broad variety of oxidized endogenous substrates including $\text{Trx}-(\text{SH})_2$, dehydroascorbate (oxidized vitamin C), lipoic acid/lipoamide, H_2O_2 , lipid hydroperoxides, vitamin K, ubiquinone, S-nitrosoglutathione, selenodiglutathione, selenite, methylseleninate, protein disulfide isomerase, glutaredoxin, glutathione peroxidase, NK-lysin/granulysin, selenodiglutathione, selenocystine, and various oxidized molecular species of exogenous origin; e.g., HIV Tat protein, ninhydrin, juglone, alloxan, DTNB, as well as dietary polyphenols and additional molecular species (Arnér & Holmgren, 2000). Thioredoxin reductase 2 (TRx2, Gene name: TXNRD2, UniProt: q9nnw7, EC 1.8.1.9), the mitochondrial form, is a ubiquitous homodimeric pyridine nucleotide-disulfide oxidoreductase with Sec as its terminal amino acid and 1 FAD per subunit. The highest TRx2 expression levels are observed in prostate, testes, liver, uterus, and small intestine, with intermediate levels in brain, skeletal muscle, heart, and spleen. Thioredoxin glutathione reductase (TGR, Gene name: TXNRD3, UniProt: q86vq6) reduces GS-SG and contains an N-terminal 1-Cys glutaredoxin-like domain, with specific physiological functions that appear to link $\text{Trx}-(\text{SH})_2$ and GSH pathways.

The functions of the TRx family are intimately connected with vitamin E and vitamin C as they interactively protect against peroxidation and free radical damage. Once vitamin E has quenched a free radical chain reaction to terminate its propagation in lipids, it interacts with vitamin C (ascorbic acid) and is restored to its active

form. This converts ascorbate to dehydroascorbic acid which is subsequently acted upon by TRx to restore the active ascorbic acid form. Since many of TRx's substrates are important cellular antioxidants themselves, its activities are central in preserving the intracellular reducing environment required for normal metabolism. If synthesized without Sec at its terminus, TRx not only loses its enzyme activity but also becomes a potent apoptosis initiator known as GRIM-12 (Anestål & Arnér, 2003). This unique outcome may be an evolutionary strategy which sacrifices cells which have too little Se to thrive in order to redistribute their Se and thus support the survival of neighboring cells.

3.2.3 Methionine R-Sulfoxide Reductase

Methionine R-sulfoxide reductase (MsrB1: Gene name MSRB1, UniProt: q9nzh6, EC 1.8.4.14) reverses oxidative damage which results in formation of R-methionine to the methionine and its expression is stimulated by oxidative stress. MsrB1 possesses a single Sec which works together with a zinc that is bound to four Cys residues. It has the highest specific activity among the three principal types of Met-R-sulfoxide reductases, all of which employ Trx-(SH)₂ as the reductant, which requires TRx activities to maintain. MsrB1 appears to have functions which counteract aging and neurological disorders.

3.2.4 Selenoproteins M, F, N, W, T, H, and K

Selenoprotein M (SelM, Gene name SELENOM, UniProt: q8wwx9) is expressed in many tissues, with the highest expression occurring in the brain and notably high expression in a single cell layer of the cerebellum. Subcellularly localized in the endoplasmic reticulum (ER), it is a thioredoxin-like oxidoreductase that may assist in disulfide bond formation and protein folding. Selenoprotein F (SelF, Gene name SELENOF) is a thioredoxin-like oxidoreductase that has homology to SelM and improves protein quality control by correcting glycosylation and misfolding of glycoproteins via the calnexin-calreticulin endoplasmic reticulum protein 57 and pH-dependent endoplasmic reticulum protein 44 (ERp44) system. Selenoprotein N (SelN, Gene name SELENON, UniProt: q9nzh5) has low mRNA expression in the brain and is a glycoprotein retained in the ER that is more abundant during fetal development, but otherwise appears to be ubiquitous although expressed at very low levels. It occurs in two isoforms that protect against oxidative stress and regulate redox-related calcium homeostasis.

Selenoproteins W, T, and H appear to comprise a family of selenoenzymes that regulate the redox state of 14-3-3 proteins. These proteins comprise 1% of total brain protein, and their redox state is suspected to influence signaling functions. Selenoprotein W (SelW, Gene name SELENOW, UniProt: p63302) is a small (9.5 kDa) protein that is the second most abundantly expressed mRNA in the brain. It mainly resides in cytosol with small amounts being membrane associated. Selenoprotein T (SelT, Gene name SELENOT, UniProt: p62341) has low brain mRNA expression and its product is a thioredoxin-like enzyme anchored to the ER membrane and present in the Golgi complex. It has been reported to play a role in protection of dopaminergic neurons against oxidative stress in the mouse model of

Parkinson's disease. It is highly conserved during evolution and is particularly abundant in embryonic tissues. Although its functions are uncharacterized, they appear to be essential during development since knockout mouse models cannot survive past embryogenesis. Its expression is abundant in pituitary and other endocrine tissues, but it is repressed in most other adult tissues. It appears to have cytoprotective effects and its expression is induced after brain injury. For example, in Parkinson's disease, SelT is tremendously increased in the caudate putamen tissue. Selenoprotein H (SelH, Gene name SELENOH, UniProt: q8izq5) belongs to the SelW-T-H family and is moderately expressed in brain. It encodes a nucleolar protein which functions as an oxidoreductase that has been shown to protect neurons against UVB-induced damage by inhibiting apoptotic cell death pathways, promoting mitochondrial biogenesis and mitochondrial functions, and suppressing cellular senescence through redox regulation and genome maintenance.

Selenoprotein K (SelK; Gene name SELENOK, UniProt: q9y6d0) is expressed in the brain and in most other tissues. Localized in the ER, it appears to be an oxidoreductase enzyme that is involved in maintaining calcium homeostasis and assists in disulfide bond formation and protein folding.

3.3 Selenoprotein S, I, O, and V

Selenoprotein S (SelS, Gene name SELENOS, UniProt: q9bqe4) is a ubiquitously expressed protein with a single transmembrane helix with putative phosphorylation and glycosylation sites and a terminal Sec that is associated with plasma and ER membranes. Its expression appears to be inversely related to plasma glucose concentrations. Selenoprotein I (SelI, Gene name SELENOI, UniProt: q9c0d9, EC 2.7.8.1) catalyzes the transfer of phosphoethanolamine from CDP-ethanolamine to diacylglycerol to produce phosphatidylethanolamine, a phospholipid involved in vesicular membrane formation and maintenance, regulation of lipid metabolism, and protein folding. It is ubiquitously expressed in brain and other tissues. Selenoprotein O (SelO, Gene name SELENOO, UniProt: q9bvl4) is the largest selenoprotein expressed in mammals. Localized to mitochondria, it is ubiquitously expressed. Its activities are uncharacterized but possesses a structural motif that suggests it may be involved in redox functions. Selenoprotein V (SelV, Gene name SELENOV, UniProt: p59797) shows homology to SelW, but little information is available regarding its biochemical activities or physiological functions. Based on mRNA expression, it appears to be testes-specific, and the presence of SelV in brain tissues is negligible.

3.4 Iodothyronine Deiodinases

The development and function of tissues of the CNS are particularly sensitive to thyroid hormone supply and the expression of deiodinase enzymes. Iodothyronine deiodinase 1 (Dio1, Gene name DIO1; UniProt: p49895, EC 1.97.1.10) is a

homodimeric plasma membrane protein that cleaves the iodine-carbon bond of T_4 (thyroxine) to form T_3 in thyroid hormone activation. Its activity is high in the liver, kidney, and pituitary, although it occurs in only trace quantities in most tissues other than brain, where its concentrations are even lower. Iodothyronine deiodinase 2 (Dio2, Gene name DIO2; UniProt: q92813, EC 1.97.1.10) is a homodimeric plasma membrane protein present in the ER that cleaves the iodine-carbon bond of T_4 with a preference for T_4 over rT_3 . Dio2 is expressed in the CNS, pituitary and thyroid glands, skeletal and heart muscle, and placental and brown adipose tissue. Since there is minimal absorption of bloodstream T_3 across the blood–brain barrier, Dio2 is responsible for more than 75% of the T_3 production in the brain. Since circulating T_3 does not readily gain access to the intracellular nuclear receptors, Dio2 provides an important regulatory function in the brain and CNS. Iodothyronine deiodinase 3 (Dio3, Gene name DIO3, UniProt: p55073, EC 1.97.1.11), unlike Dio2, deiodinates the 5-position of the tyrosyl ring to inactivate thyroid hormone. The brain, placenta, and pregnant uterus express high amounts of Dio3. High Dio3 expression may protect the fetal CNS from disproportionately high levels of T_4 and T_3 but high Dio3 accompanied by low levels of T_3 may impair CNS development and functions.

4 Reactive Oxygen, Nitrogen, and Electrophile Species

Cells maintain intracellular reducing conditions and use redox state recognition and response pathways to regulate growth, apoptosis, and cell signaling. These critical physiological processes also control protein phosphorylation, ion channels, transcription factors, and crosslinking of extracellular matrix while also functioning in biosynthetic processes that regulate thyroid hormone production, blood pressure, immune functions, and cognition (Brieger et al., 2012). Hazardous ROS and reactive nitrogen species (RNS) include chemically active molecules that are naturally produced as metabolic byproducts (Bartos, 2009; Di Meo et al., 2016) which serve as molecular signals and chemical weapons of the immune system but also cause tissue damage and dysfunction. The ROS forms include the hydroxyl radical ($\cdot OH$), superoxide (O_2^-), hypochlorous acid ($HOCl$), and hydrogen peroxide (H_2O_2). The RNS forms include nitrogen dioxide NO_2^\bullet , nitric oxide (NO^\bullet), and its powerful oxidant derivative peroxynitrite ($ONOO^-$) which can damage many biological molecules (Radi, 2013).

Although ROS and RNS are spontaneously generated as physiological byproducts of normal cell biochemistry, metabolic imbalances, or environmental insults such as ionizing radiation, UV exposure, inflammation, and increased exposures to metal ions such as $Cu^{+/2+}$ or $Fe^{2+/3+}$ increase their production and result in oxidative stress which is associated with the cumulative damage of aging. Cardiovascular disease, cancer, and neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and ALS are also characterized by increased ROS and RNS (Battin & Brumaghim, 2009; Li et al., 2013). However, selenoenzymes

are not only important in preventing and reversing oxidative stress but also stress from reactions with electrophiles.

Electrophiles accept a pair of electrons from a nucleophile to form a covalent bond in one of the most fundamental chemical reactions in biochemistry. Reactive electrophiles (Lewis acids) are electron poor elements or molecules which can accept a pair of electrons from a nucleophile (Lewis base). When a molecule undergoes a reaction, electrons are either obtained from a nucleophile or donated to an electrophile. As the external potential experienced by the electrons in the molecule changes, they no longer interact with just the nuclei of their original atom or molecule but become increasingly attracted to the nuclei and repelled by the electrons of the new partner. The process and outcome of the chemical reaction will be determined by their mutual responses to changes in local electron density and external potential. Molecular interactions are therefore based on opposing charges, but also depend on the size and polarizability of the electron shells of the participants. The Pearson acid-base concept uses hard-soft acid-base (HSAB) terminology to qualitatively distinguish among the relative interactivities of electron shells of various elements. In this context, “hard” describes electrophiles (e.g., H^+ , Li^+ , Na^+) or nucleophiles (e.g., F^- , Cl^-) with more concentrated charges that are less polarizable and thus form ionic bonds. Meanwhile, “soft” electrophiles (E^*) have larger electron shells whose less concentrated charge distributions can be polarized and enable covalent bonds to form. Therefore, the HSAB perspective is a qualitative description that provides a generalized understanding of chemical interactions and factors which distinguish the chemical properties and reactions of transition metals with thiols and selenoates. Theoretical reactivity assessments performed with more advanced models of density functional theory (DFT) investigate these interactions and reactions with far greater intricacy and semiquantitative capabilities, but HSAB evaluations are sufficient to provide a reliable perspective for the current discussion (Pearson, 2005). Oxygen and reactive oxygen species (ROS) are just a subset of reactive electrophilic species (RES) that selenoenzymes encounter (Ste. Marie et al., 2020). Based on recent work in this area, it is evident that Sec confers resistance to irreversible oxidative inactivation by reactive oxygen species (ROS) as well as reactive electrophilic species (RES) since oxygen and related compounds are merely a subset of RES. As discussed below, metallic E^* have higher binding affinities while organic E^* contain an α , β -unsaturated carbonyl or other structures which are either electronegative or react with Se for other reasons. The following discussion includes representative examples provided for illustrative purposes, but a more complete list is provided in the review by Saccoccia et al. (2014).

4.1 Metallic Electrophiles

Metallic E^* are Lewis acids that exploit the increased accessibility that arises when bonds with electron withdrawing groups create a region with a low electron density which enables nucleophiles to approach. The E^* accepts an electron pair to form covalent bonds with oxides, sulfides, or selenides with the respective Lewis bases. In

Table 1 Representative metallic soft electrophiles

Form	Partial list of exposure source examples
Hg ⁰	Spills from outdated barometers, thermostats, artisanal mining activities
Hg ²⁺	Added to unapproved skin whitening creams and related cosmetics
CH ₃ HgCH ₃	Landfill emissions (very low levels), laboratory reagent spills
CH ₃ Hg–Cys	Fish, seafoods, large amounts in apex predator whale/shark meats
Cd	Contaminated water or foods in areas near pollution point sources
Au	Pharmaceutical agents used to treat inflammation
Ag	Dental amalgams and certain pharmaceuticals
Pb	Paint, contaminated water, dust, and air in certain regions
As	Contaminated water, food crops, livestock, cosmetics
Pt	Pharmaceutical agents used to treat various forms of cancer

this context, “heavy” metals are the thiophilic and even more selenophilic elements with broadly dispersed charge densities such as palladium (Pd), silver (Ag), cadmium (Cd), platinum (Pt), gold (Au), Hg, and thallium, and may include elements with lower binding affinities such as molybdenum, manganese, iron, cobalt, nickel, copper, zinc, lead, and bismuth (See Table 1). Certain pharmaceuticals specifically exploit the Se-binding capabilities of Au- or Ag-containing compounds (Saccoccia et al., 2014) whose therapeutic functions arise primarily because they inhibit TRx. Meanwhile, other compounds that incorporate an accessible metallic E* will be likely to have similar effects on TRx, GPx, and/or other selenoenzymes (Ouyang et al., 2018). While all E* interact with nucleophiles, the toxic mechanisms and interactions between Se and Hg are the best characterized (Ralston & Raymond, 2018).

Mercury’s association constant for sulfide is very high (10^{39}), but its affinity for selenide ($K_a = 10^{45}$) is ~one million-fold higher (Dyrssen & Wedborg, 1991). Meanwhile, methylmercury (CH₃Hg⁺) has a high binding affinity (10^{17}) with the thiol of Cys. The mass action effects of their high millimolar intracellular abundance results in >95% of intracellular CH₃Hg⁺ being present as CH₃Hg–Cys (Harris et al., 2003). However, because thiomolecules such as GSH, GS–SG, Trx–(SH)₂, and Trx–S₂ are selenoenzyme substrates and or cofactors (see Eqs. 1 and 2), adducts such as GS–E*–SG, GS–E*, Trx–(S₂–E*) and related forms are eventually brought into close proximity with the Se of Sec in the active sites of selenoenzymes. Since the higher affinity between these E* and Se is further accentuated by enzyme activation, the E* transfers from Cys to Sec, thus irreversibly inhibiting the selenoenzyme ► [Mercury’s Neurotoxic Effects on Brain Selenoenzymes](#) (Ralston & Raymond, 2018; Carvalho et al., 2011) and increasing amounts of Se becomes sequestered as insoluble HgSe in the brains of highly exposed individuals (Korbas et al., 2010).

Because the CH₃Hg–Cys resembles Met at the molecular level (see Fig. 1), the large neutral amino acid transporter (LAT1) readily binds and actively carries it across membranes (Aschner & Clarkson, 1988; Bridges & Zalups 2010) of maternal, placental, fetal, and brain cells. Once internalized by a cell, CH₃Hg–Cys is only slowly degraded or excreted. For this reason, it tends to persist and accumulate in

organisms in quantities that depend on the age, size, and trophic level. The $\text{CH}_3\text{Hg-Cys}$ within the organism is absorbed by the consumer and exhibits long-term retention in their tissues, especially once the Hg and Se have formed the crystalline HgSe that accumulates in lysosomes.

Similarly, Hg^0 vapor is readily absorbed and transported throughout the body. However, it is innocuous in this form and remains so until it is oxidized to Hg^+ by catalase (Clarkson & Magos, 2006). The inorganic Hg that is produced is capable of binding with thiols to form Cys-Hg^+ which can transfer the Hg to Sec and irreversibly inhibit selenoenzyme activities. Because Hg^+ and Hg^{+2} are usually present as inorganic forms, they are poorly absorbed. The Cys-Hg^+ adduct does not resemble any amino acids, so they are not transferred across the placental or blood-brain membranes by LAT1. This limits their neurotoxic effects, but high exposures could still sequester Se in somatic compartments and thus reduce the amounts available for redistribution to the brain.

4.2 Organic Electrophiles

In addition to metallic forms, the organic E^* are far more varied in form (See Table 2 and Figs. 3 and 4), include several classes with recognized neurotoxicity, and occur in forms that are associated with neurodegenerative diseases. The structure of α , β -unsaturated carbonyls are basically an alkene conjugated to a carboxylic acid. This is a common motif among organic molecules and includes many variations on the theme including methyl vinyl ketones, acrolein, acrylamide, methyl acrylate, maleic acid, fumaric acid, E-crotonaldehyde, testosterone, cinnamaldehyde, cyclohexanone, paraquinone, and others which either form endogenously, during preparation of foods, or arise from industrial processes, and occur as additives or accidental contaminants in foods. These include endogenously generated forms (e.g., acrolein, 4-hydroxy-2-nonenal), environmental toxicants (e.g., γ -diketones, quinones, unsaturated aldehydes), industrial pollutants (acrolein, acrylonitrile, methyl vinyl ketone), drug metabolites (e.g., acrolein metabolite of cyclophosphamide, NAPQI), and

Table 2 Representative organic soft electrophiles

Form	Partial list of exposure source examples.
Acrylamide	Foods cooked at high temperatures, partially burned materials, smoking
Acrolein	Ubiquitous in cooked foods, partially burned materials, smoking
NAPQI	Byproduct arising from cytochrome P-450 conversion of acetaminophen
Aflatoxin	Toxin produced by <i>Aspergillus flavus</i> mold growing on food
Hydroxyhexenal	Product of (exogenous and endogenous) lipid peroxidation
Neuroketal	Lipoxidation product that forms adducts with cellular proteins
Neuroprostane	Prostaglandin-like product of fatty acid peroxidation
2-Acetylpyrrolin	Maillard reaction product formed during baking
Dopamine quinone	Reactive metabolite formed from oxidation of dopamine
GalNAc	Endogenous carbohydrate of gangliosides and self-antigens

Endogenous Electrophiles

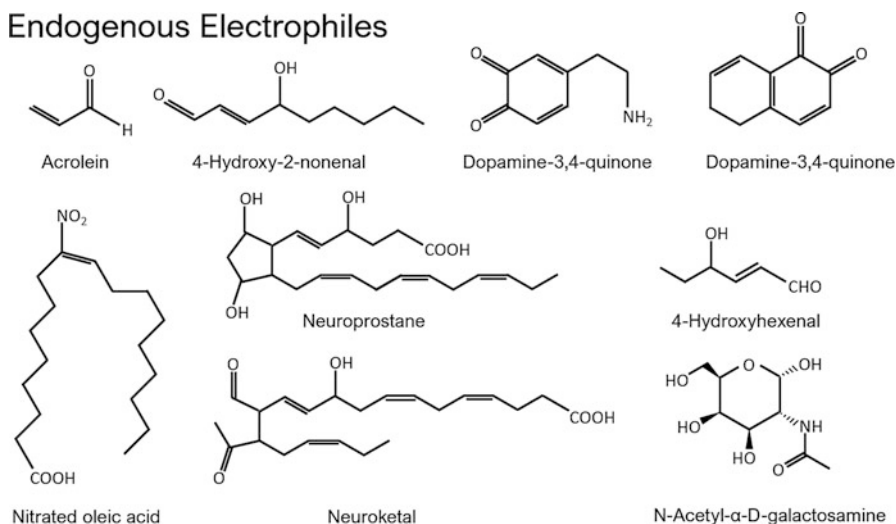


Fig. 3 The illustrative selection of endogenous E* forms shown above provides examples of organic molecules which may react with Se to form E*Se adducts. While molecules such as GalNAc lack features expected in a Se-binding partner, urinary Se is excreted as the selenosugar, 1 β -methylseleno-N-acetyl-D-galactosamine which forms through an incompletely described reaction pathway

Xenobiotic Electrophiles

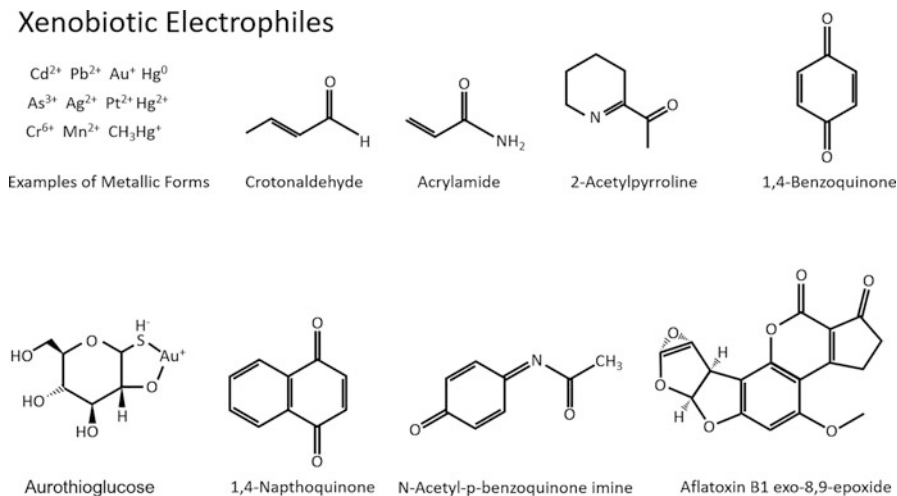


Fig. 4 The xenobiotic E* in the environment are increasing in the number of forms and concentrations in potential exposure sources. Certain E* are common products formed during cooking of foods, others are synthetic products, contaminants, and/or metabolites of pharmaceutical agents or other E* precursors

common components present in cooked foods (e.g., acrylamide) that can induce cell damage (LoPachin et al., 2007; LoPachin et al., 2012; LoPachin & Gavin, 2012). The unsaturated carbonyls of organic E* are known to form covalent bonds with nucleophilic Cys thiols but will have far higher affinities for Sec.

The organic forms of E* are far more varied in source and forms than the metallic forms, but their Se-binding affinities will be far lower and reversible in at least some cases (Ste. Marie et al., 2020). Thus, on a mole for mole basis, the risks of selenoenzyme impairment arising from exposures to α , β -unsaturated carbonyls of these E* may seem quite low, but with exposures arising from a broad range of sources and sometimes in larger quantities than is typically seen with metallic E*, there is a need for detailed study of both Se-binding affinities as well as the anticipated range of human exposures. These E*s include environmental/dietary contaminants, drug metabolites, partially combusted constituents of cigarette tobacco, wood smoke, and diesel exhaust, as well as a wider range of endogenously generated forms. Exposures to organic E* are pervasive, and although high exposures to several of these forms are known to be toxic, it remains unknown whether selenoenzyme inhibition and Se-sequestration are the primary cause of their toxicity.

Not all reactions between Se and organic molecules involve unsaturated carbonyls. The most common form of Se excreted in urine is 1 β -methylseleno-N-acetyl-D-galactosamine, a selenosugar formed from Se combining with GSH to form GS-SeH which reacts with N-acetyl-D-galactosamine (GalNAc), the amino sugar derivative of galactose (Suzuki et al., 2004). As a component of many O-linked and N-linked glycan structures throughout the body, GalNAc has important roles in regulation of intracellular proteins and transcription factors such as NF κ B, c-myc, and p53 in the nervous system. It is necessary for intercellular communication, and a common component of antigens and gangliosides as well as having structural roles at the cell surface and in the extracellular matrix. The binding of Se to GalNAc and its subsequent excretion increases with increasing dietary Se intakes. Although it diminishes when Se is less available, it only increases to a certain point as Se intakes rise above normal. At high Se intakes, homeostatic regulation is accomplished through exhalation of dimethyl and urinary excretion of trimethyl Se in younger animals, but it is not as inducible in older animals (Suzuki et al., 2004). Furthermore, while the source of the 1 β -methylseleno-N-acetyl-D-galactosamine has not been identified, it must either become more abundant with age or the mechanisms to reverse its formation become less competent.

The diminishing amount of 1 β -methylseleno-N-acetyl-D-galactosamine among those fed less dietary Se undoubtedly reflects lower production of the adducted form, but it may also reflect more complete degradation of the complex achieved when it is present in low abundance. This could be a homeostatic mechanism to limit the loss of Se during periods of dietary shortfalls. Such a Se recovery pathway would have significant advantages and degradation of a different Se-adduct was recently reported by Ste. Marie et al. (2020). They point out that enhanced catalytic efficiency may not have been the only evolutionary advantage associated with employing Sec in enzymes. They found that Sec is more readily recovered from complexes with, a common organic E* that originates from endogenous as well as exogenous sources.

Acrolein–Se adducts can be degraded to release Se, enabling it to continue participating in cycles of Sec synthesis. Similar reactions may release Se from selenosugars, other organic E*, and perhaps even degrade certain metallic E*Se complexes. However, it is unknown whether these are saturable processes that may be overwhelmed when E* exposures increase beyond a certain threshold.

The range of endogenous and exogenous E* in an individual's lifetime exposure profile will vary in relation to food choices, food preparation methods, pharmaceutical treatments, and environmental exposures originating from natural as well as pollutant sources. Until the Se-binding constants for these forms have been quantified and the concentrations associated with their toxicity defined, the level of risk associated with these exposures cannot be classified. The binding constants for organic E* are expected to be log orders lower than for metallic E*, but the plethora of forms and the mass action effects of the greater molar abundance of certain organic E* in exposed populations may compromise Se status and contribute to neurotoxic and other consequences. This will be especially true of populations with poor dietary Se intakes. While certain organic adducts that are formed can be degraded to release Se (Ste. Marie et al., 2020), further work will be required to establish the extent and limitations of these reactions.

4.3 Kinetic and Thermodynamic Considerations

Before the physiological significance of selenoenzyme metabolism had become generally recognized, toxicologists assumed that Hg toxicity arose as the result of its binding to thiomolecules and inhibition of enzyme thiols. This was perplexing since the blood concentration associated with Hg toxicity was $\sim 1 \mu\text{M}$, and severe Hg toxicity was observed at $2.5 \mu\text{M}$, but intracellular thiols are present at concentrations which are $\sim 100,000$ times greater. No toxic mechanism had been identified, but with a 1:100,000 molar ratio between Hg and sulfur, the interactions responsible for oxidative damage and other effects were assumed to follow pseudo-first order kinetics. Based on that mistaken assumption, it was expected that Hg exposures would be directly proportional to toxicity and that even low Hg exposures would have adverse effects. However, numerous epidemiological and laboratory animal studies have since proven that higher Hg exposures from increasing maternal seafood consumption were not associated with harm but were instead accompanied by ~ 7.7 IQ point improvements in their children (Hibbeln et al., 2019). While this initially seemed controversial and counterintuitive, these findings are entirely consistent with current understanding of the issue. Commonly consumed varieties of ocean fish contain far more Se than Hg, and although Hg exposures increase, the more fish that are eaten during pregnancy, the better the Se status of the mother and her child. Since other vital nutrients are present, additional physiological benefits arise that improve the health of the mother and the neurodevelopment of the child (Hibbeln et al., 2019).

Nearly all varieties of ocean fish have positive health benefit values (HBVs) that range between 10 and 20 (Ralston et al., 2019). Therefore, most commonly

consumed seafoods will improve rather than diminish the Se status of consumers. Because early studies of Hg exposures from seafood consumption involved highly exposed sentinel populations in the Faroe Islands and New Zealand where mothers were consistently eating pilot whale or great white shark meats, subtle adverse effects were observed. Since large specimens of these rarely eaten seafoods have high Hg and comparatively low Se concentrations, they often have seriously negative HBVs that range below -80 (Ralston et al., 2015, 2019). While only subtle adverse effects were noted in their children, mothers eating such hazardous seafoods might have caused serious damage to their children if they had not also been eating Se-rich varieties of ocean fish. As it was, the blood Hg levels of children of highly exposed mothers in the Faroe Islands were comparable to the concentrations associated with brain damage among adults during the catastrophic Hg poisoning incidents in Iraq during the 1950s and 1960s. While the beneficial effects of increasing Hg exposures associated with increasing ocean fish consumption may have initially seemed counterintuitive, now that the pivotal importance of seafood HBV's is recognized, the controversy is eliminated since these findings are consistent with expectations. The subtle diminishments among children of mothers that had eaten seafoods with negative HBVs and the substantial benefits among children of mothers that ate ocean fish with positive HBVs provide potent validation of the reliability of the HBV criterion.

The Se concentrations of most tissues in the body are $\sim 1 \mu\text{M}$ and Hg exposures that result in tissue contents which approach or exceed equimolar stoichiometries with Se will inhibit selenoenzyme activities in a concentration-dependent manner ► [Mercury's Neurotoxic Effects on Brain Selenoenzymes](#) (Ralston & Raymond, 2018). However, it is crucial to recognize that the effects of Hg exposures are not necessarily proportional to Hg dose or tissue concentrations, but are instead proportional to Hg:Se molar relationships in diet and tissues. If tissue Se reserves and dietary intakes are able to offset the losses due to Se sequestration by Hg and other E^* , the adverse effects associated with diminished brain Se availability would be averted. The effects of Se sequestration by multiple forms of E^* are expected to be additive, but there are intricacies to consider.

Although not all E^* will be as readily transported into the brain as Hg, and their sequestration of Se will seldom involve binding affinities that are as high, even small losses of Se from the body's tissue reserves will diminish Se redistribution to the brain. Several E^* sequestering fractions of the body's Se would be just as hazardous as a single E^* sequestering the same amount. However, when exposures to several E^* forms are involved, their contributions become harder to evaluate, especially since many arise from common and highly variable sources.

The toxicokinetics of E^* can differ at organ, tissue, cell, and subcellular levels, as can their toxicodynamics. For example, the effects of exposures to arsenic (As), Cd, or Hg have greater impact on the activities of mitochondrial TRx2 than cytosolic TRx1 (Branco et al., 2014). Although TRx activity can become impaired by processes unrelated to Se sequestration and selenoenzyme inhibition, interactions between Se and E^* such as Cd, Au, etc., form covalent adducts which are similar in character and almost as strong as those between Hg and Se. Because the HgSe which

forms is chemically resistant to all acids other than aqua regia, the bound Se is permanently retired from normal cycling in selenoenzymes.

Once a E* becomes bound to the thiol of GSH, Trx-(SH)₂, or other substrate or cofactor which directly interact with the Sec of a selenoenzyme, the E*-thiomolecule adduct will function as a suicide substrate (Ralston & Raymond, 2018) that orients the E* such that it is brought into close proximity with Sec's selenolate. The E* is transferred and a covalent bond between E* and Se is formed. If tissue concentrations of the various forms of Hg and/or other high Se-affinity E* occur in stoichiometric excess of Se, losses due to attrition would eventually exceed the ability of dietary Se intakes to offset them. The latency between E* exposures and onset of toxicity is proportional to the dietary Se status of the exposed individual and inversely related to the extent of the E* dose received and retained. Selenoenzyme catalytic rate constants, the half-lives of the enzyme forms, and the availability of Se in discrete tissues will all contribute to the rate of transfer of various metallic or organic E* from thiols to the Sec of selenoenzymes. Furthermore, distinctions between the molecular forms and Se-binding affinities of each E* will also need to be considered.

The kinetic profiles and biochemical interactions of E* are influenced by their high affinities for thiols, but their toxicodynamics are the direct effects of their irreversible inhibition of selenoenzymes and sequestration of Se. Although CH₃Hg toxicity is no longer mistakenly assumed to follow pseudo-first order reaction kinetics, the bimolecular reaction between E*-Cys and selenoenzymes is the first step in creating the suicide substrate which irreversibly inhibits selenoenzyme activities. In addition to forming adducts with substrates for the various functionally characterized GPx and TRx enzymes, E* toxicants may form inhibitory adducts for selenoenzymes whose activities and substrates are currently unknown. The inherent challenges are exacerbated by the extraordinary homeostatic regulation that normally protects the brain's Se reserves and selenoenzyme activities. In addition to being responsible for the pronounced latency period between E* exposures and observable toxicity, Se homeostasis may often manage to avert toxicity when sufficient Se can be mobilized from tissues or accessed from diet. As a further dilemma, forms of E* that may not be able to access the brain could still cause neurotoxic effects by sequestering enough Se in somatic tissues to preclude redistribution of sufficient Se to the brain. Since this would impair selenoenzyme synthesis, tissues which cannot survive loss of those activities would suffer from damage and dysfunctions.

Therefore, the toxicokinetics and toxicodynamics of E* assessments will require development of more thorough analytical assessments and application of advanced computational methods. Although physiologically based pharmacokinetic (PBPK) models may have seemed appropriate to apply in risk assessments when Hg toxicity was assumed to involve pseudo-first order reactions, a better-informed model is now required. Since neurotoxic outcomes are likely to involve the combined effects of multiple forms of E* acting cooperatively and perhaps synergistically in certain cases, the dynamic equilibrium between these substrates and the Se cycling that prevents the onset of toxicity is pivotally dependent on the rates of Sec synthesis and

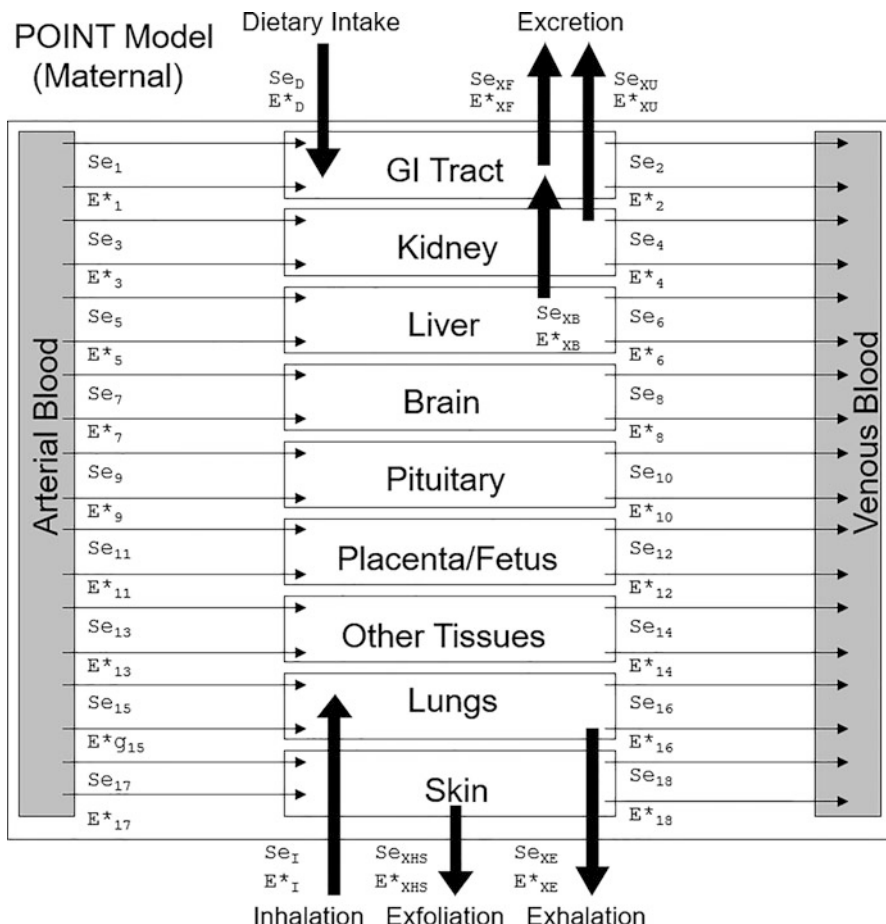


Fig. 5 Influx of Se and E* from diet (D), or inhalation (I) will influence their distributions into tissue compartments, as will their excretion via fecal (XF) and urinary (XU) routes, as well as exhalation (XE) and exfoliation through hair and sweat (XHS). Growing tissues accumulate both Se and E* and high binding affinity E* are likely to accumulate in lysosomes as stable E*Se complexes

activities in target tissues. Since those depend on the level of Se reserves in the somatic tissues and concurrent dietary Se intakes of the exposed subject, standard PBPK models are insufficient. To perform reliably accurate assessments of E*-related risks will require a physiologically oriented interactions of nutrients and toxicants (POINT) model. Early versions of these models have been demonstrated (Ralston & Raymond, 2006), but more refined forms are required. Since the brain is most vulnerable to the effects of Se deprivation during fetal development, the schematic shown in Fig. 5 represents an example of a maternal POINT model. Aside from obvious distinctions, the models for adults (and for the fetus in utero)

would be similar and reflect the influx and efflux of blood-borne Se in relation to the E* forms being assessed. Neurotoxic effects of high E* exposures would arise if Se availability was compromised, and selenoenzyme activities became significantly impaired.

A large and growing number of E* have already been identified (Saccoccia et al., 2014), but as new forms of synthetic molecules come into existence each year, their presence in the environment and their concentrations in biota and exposed human populations are likely to increase. In some cases, the original forms of these synthetic molecules are innocuous, but unexpected byproducts and metabolized forms of these novel molecules may not be recognized as hazardous until long after their use has resulted in environmental releases and exposures. These add to the cumulative exposures of an individual's lifetime known as the exposome (Vermeulen et al., 2020). The E* exposome includes all forms (natural and synthetic) which are absorbed from the environment, including exposures from food, water, air, or through the skin, and forms created within the body from various precursors. While the effects of high exposures to a single toxicant can be easily recognized, the potential consequences of the combined effects of multiple E* that cooperatively impact the same Se-dependent pathways will be challenging.

Those inherent challenges are exacerbated by the latency of E*-dependent Se-attrition effects and how this relates to the Se status of the exposed populations. The consequences of E* exposures will be inversely related to the individual's Se status and dietary intakes. A dose of E* that has serious consequences in Se-poor populations will have longer delays and far less serious effects on individuals with more Se in their diets and will be unlikely to have any effects in populations with Se-rich diets. Other variables such as differences in tissue compartment distributions of certain E* may introduce unique effects that may be similar, but distinct from the "SOS mechanisms" that characterize Hg toxicity. Co-exposures to toxicants that directly promote oxidative damage could synergistically increase the severity of the adverse effects that would otherwise accompany exposures to E* in amounts sufficient to diminish selenoenzyme activities. Since multiple forms of E* are encountered in various amounts per day, their molar concentrations [E*] must be assessed in relation to the molar concentrations of Se in the exposed tissues [Se] to evaluate the binding constants (k) for adducts [E*Se] of each form (See Eq. 3).

$$k_1 = \frac{[E^*_1Se]}{[Se][E^*_1]} \quad (3)$$

The sum of contributions of all the E* in the population's exposome must be assessed and evaluated to estimate a comprehensive Se-binding equivalent [E*_Σ] which incorporates consideration of the amounts and binding affinities of the E* of the individuals in the exposed population.

Their combined effects would need to be evaluated in relation to the Se status of the exposed population to establish a relative health value (HV_{Se}) using an equation based on the HBV criterion currently used to differentiate risks vs benefits associated with Hg exposures from maternal consumption of specific types of fish and/or

seafoods (Ralston et al., 2019). The HBV differentiates fish and/or seafoods with positive values that indicate their consumption would improve the Se status of consumers from meats of apex predators such as toothed whales or great white sharks whose consumption has been associated with harmful effects.

Epidemiological studies which have observed harm from seafood Hg exposures have uniformly involved mothers that had regularly eaten seafoods with negative HBV's, i.e., seafoods that contained more Hg than Se. Since such foods impair the ability of maternal tissues to redistribute their Se across the placenta to supply the needs of developing fetal brain tissues (Ralston et al., 2015), the findings of subtle harm observed in those studies was not surprising. Similar to the biochemically based HBV, the calculation to establish HV_{Se} (See Eq. 4) reflects the amount of Se available in tissues and (whole blood values are an acceptable index) should approach or slightly exceed 1 μM Se. The HV_{Se} provides a biochemically based index for defining risks associated with E^* exposures and is calculated:

$$HV_{Se} = \left(\frac{[Se] - [E^*_{\Sigma}]}{[Se]} \right) \cdot ([Se] + [E^*_{\Sigma}]) \quad (4)$$

Populations with a HV_{Se} of ~ 1 μM would not have health consequences from Se-sequestration aspects of their E^* exposures, but if there are additional toxic effects that arise, those would need to be separately considered. Vulnerable sub-groups and those with higher exposures and/or lower dietary Se intakes will need to be identified, evaluated, and protective interventions considered. Hazards associated with high E^* exposures will be greatest among populations that are known to have poor dietary Se intakes (See Fig. 6). If the HV_{Se} declines below ~ 0.5 μM , the risks of clinically significant physiological impairments steadily increase. Existing evidence indicates increasing dietary Se has a far greater protective effect than E^* has in compromising Se status. This undoubtedly reflects the contrast between the homeostatic regulation of Se versus the diffusion-driven distribution of E^* in tissues.

The present version of the HV_{Se} equation does not consider the amounts of E^* bound to thiomolecules or other binding partners. This is a purposefully precautionary measure to provide a conservative estimate of Se health status until sufficient data becomes available to justify an update of the provisional equation. Several knowledge gaps must be examined to establish: (1) the magnitude and effects of additive versus potentially synergistic effects of E^* forms and other toxicants, (2) the existence and protective effects of alternate ligands that compete for E^* binding, (3) the activities of enzymes that may release Se bound to E^* , (4) the pathological consequences occurring in neurological disorders or other organ systems, and (5) the contrasting magnitudes of benefits of increasing dietary Se versus the adverse effects of exposures to the various E^* . As further information becomes available on these aspects, the provisional iteration of the HV_{Se} criterion should be updated to reflect advances in understanding obtained through biochemical assessments, laboratory studies, epidemiological evaluations, mathematical models, and clinical perspectives.

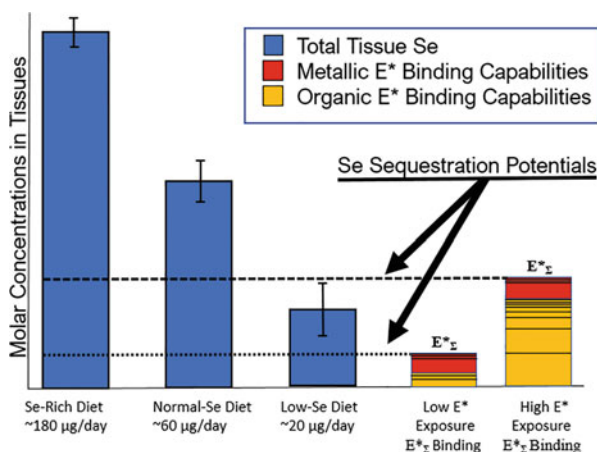


Fig. 6 Tissue Se concentrations reflect dietary Se intakes but portions of tissue Se are bound to E* in complexes that vary in stability and duration. Se sequestration potential (E^*_Σ) is combined total binding by discrete forms of metallic and organic E*. The E^*_Σ contribution by organic forms may be found to be minor and may not be associated with clinically significant effects. However, if their concentrations and Se-binding affinities are high enough to sequester sufficient Se to compromise the Se status of low Se individuals, the associated risks may contribute to a variety of pathological outcomes

5 Conclusion

To protect the vital but vulnerable tissues of the brain, individual selenoenzymes directly interact with thousands of substrates per second to detoxify ROS, reverse oxidative damage, and perform numerous other essential functions. Because its selenoate is deprotonated at neutral pH, Sec is the most powerful intracellular nucleophile, particularly when further empowered in the enzyme's active site. Although this elite ability enables it to catalyze otherwise challenging enzyme reactions, it also makes it likely to become bound with a wide range of E*. While low level attrition of Se is inconsequential, exposures to toxic quantities of E* with high Se-binding affinities irreversibly inhibit these activities and sequester Se in irretrievable forms. This results in a gradual attrition of bioavailable Se that can eventually abolish selenoenzyme synthesis. The delay between the exposure to the toxic dose and the onset of effects will directly depend on the Se status of the exposed individual. Doses of E* that are high enough to deplete tissue Se reservoirs and sequester bioavailable Se will impair selenoenzyme synthesis. Loss of the selenoenzyme activities required to prevent and reverse oxidative damage will subsequently result in the damage and death of brain tissues. While this toxic mechanism is increasingly well understood as a consequence of high exposures to E* with high Se-binding affinities, it is possible that concomitant exposures to multiple forms of E* with lower Se-affinities may have similar effects. If the combined effects of mass action and the unique Se binding affinities of the various

organic E* in an individual's exposome exceed the abilities of their dietary Se intakes to compensate, the loss of bioavailable Se in their tissues will mark the toxic threshold for that set of exposures.

Due to the diverse nature of E* exposure sources, the pathological consequences that may arise from the cooperative effects of multiple forms would be insidious and challenging to assess. The gradual onset and unpredictable latency of the cumulative effects of low level cooperative binding would most likely result in chronic accrual of damage which would be far less easily recognized than the acute effects of toxic exposures to E* with high binding affinities. Higher dietary Se intakes and tissue reserves increase the lag period between receiving a toxic dose and the onset of effects. This augmented Se status may also prevent development of toxic consequences. Although the lower Se-binding affinities of organic E* indicates they will not be as effective in inhibiting selenoenzyme activities, toxicity may still occur provided their binding affinities and mass action effects are sufficiently high.

There is a paucity of research in this area, so it is important to recognize that the risks potentially associated with organic E* exposures remain largely hypothetical. Scientific hypotheses can be improved or disproved, but never proved. Although it is clear that exposures to a wide range of low affinity E* from endogenous and xenobiotic sources is common, it remains unknown whether discrete or combined exposures to organic E* occur in sufficient quantities to impair selenoenzyme activities or synthesis.

For populations with normal or Se-enriched diets, losses of selenoenzyme activities or diminished Se bioavailability as a result of E* exposures are unlikely to cause significant impairments because tissue Se contents will typically be sufficient to offset any temporary deficits. Since populations that live in regions with adequate dietary Se intakes are unlikely to be exposed to organic E* pollutants in amounts capable of substantially impairing their tissue Se-reserves, it may be that only patients receiving certain E*-based pharmaceuticals or those with habitually poor diets will be at risk.

However, among populations whose subsistence primarily depends on locally obtained crops and livestock, poor environmental Se availability resulting in low Se intakes may fail to offset even minor losses due to E* exposures. Since exposures to environmental toxicants are often greatest among disadvantaged populations, the confluence of poor dietary Se intakes and high E* exposures may result in chronic as well as acute effects among populations living in Se-poor regions. Since many regions of the world are Se-poor, even subtle diminishments of tissue Se availability due to E* exposures might reduce brain selenoenzyme activities below thresholds required for optimal health. In economically disadvantaged areas, exposures to certain E* toxicants, metabolites, or other environmental insults which might accentuate production of ROS, RNS, or inflammation may also occur more frequently and pervasively.

If the binding affinities of organic E* are too low to have meaningful effects, or if the Se-adducts they form are too transient to induce clinically significant effects, there may be little risk associated with those exposures. In other cases, E* binding may seem sufficiently high to pose risks but adducts may be rendered transient by

competing ligands or release mechanisms as described by Ste. Marie et al. (2020). The objective of this chapter is to present these hypotheses for testing among populations which may be at risk and identify public and environmental health interventions which may alleviate harm.

In most tissues, selenoenzyme expression levels reflect dietary Se intakes, but the supply of Se to the brain is prioritized. Elegant homeostatic mechanisms evolved to ensure fluctuations due to dietary deficiency or excess are modulated to minimize harm to certain vital but vulnerable brain tissues. High E* exposures appear to be the only environmental insult capable of interrupting selenoenzyme activities of the brain. Since the cooperative effects of metallic and organic E* exposures from dietary, pharmaceutical and other sources will inevitably restrict Se availability and potentially inhibit selenoenzyme activities in these tissues, it is essential to establish what exposures would produce which effects and determine whether these factors may be an unrecognized initiator of neurological damage and disease.

To properly classify the toxic mechanisms associated with high exposures to an agent requires understanding the biochemistry of the relationships between the cause or causes of the pathology and its accompanying effects or symptoms. However, present diagnoses often reflect only the characteristic symptoms or defining syndrome that have been recognized in association with its pathology. A diagnostic label shared among individuals whose disorders are outwardly similar does not necessarily mean their pathologies are the same, let alone share the same etiology or prognosis. Similarly, subjects with differing pathological outcomes which have therefore received different diagnostic labels may be showing effects that arose in different tissues but have originated from a single cause or shared constellation of causes. Recognizing and defining the biochemical pathogenesis of a neurological disorder, disease, or degenerative process enables a more detailed definition of its initiators, progression, outcomes, and potential therapeutic interventions.

Without biochemically characterized mechanisms of toxicity, ascertaining the dose-dependent consequences of individual neurotoxic agents has always been a formidable challenge, but assessing the discrete, let alone potentially synergistic contributions of multiple toxicants which may cooperatively act upon the same biochemical pathway would be nearly impossible. Provided E* toxicants are found to share the same biochemical mechanism, these evaluations may become more manageable. While the effort required to assess the dose-dependent effects of E* on brain selenoenzyme activities is daunting, it is not intractable. The potential benefits of diminishing the incidence of debilitating neurological diseases and disorders are more than sufficient to warrant the endeavor.

6 Cross-References

- ▶ [Aluminum and Neurodegenerative Disease](#)
- ▶ [BMAA Neurotoxicity](#)
- ▶ [Lead and Excitotoxicity](#)
- ▶ [Manganese Neurotoxicity](#)

- ▶ Mercury's Neurotoxic Effects on Brain Selenoenzymes
- ▶ Methylmercury and Cellular Signal Transduction Systems
- ▶ Methylmercury Exposure and Developmental Neurotoxicity: New Insights from Neural Stem Cells
- ▶ Selenium Neuroprotection in Neurodegenerative Disorders
- ▶ Thallium Neurotoxicity

References

- Anestål, K., & Arnér, E. S. J. (2003). Rapid Induction of Cell Death by Selenium-compromised Thioredoxin Reductase 1 but not by the Fully Active Enzyme Containing Selenocysteine. *Journal of Biological Chemistry*, 278(18), 15966–15972.
- Arnér, E. S. J., & Holmgren, A. (2000). Physiological functions of thioredoxin and thioredoxin reductase. *European Journal of Biochemistry*, 267(20), 6102–6109.
- Aschner, M., & Clarkson, T. W. (1988). Uptake of methylmercury in the rat brain: Effects of amino acids. *Brain Research*, 462, 31–39.
- Avery, J. C., & Hoffmann, P. R. (2018). Selenium, Selenoproteins, and immunity. *Nutrients*, 10(9), 1203. <https://doi.org/10.3390/nu10091203>
- Bartosz, G. (2009). Reactive oxygen species: Destroyers or messengers? *Biochemical Pharmacology*, 77(8), 1303–1315.
- Battin, E. E., & Brumaghim, J. L. (2009). Antioxidant activity of sulfur and selenium: A review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. *Cell Biochemistry and Biophysics*, 55(1), 1–23.
- Branco, V., Santos, A. G., Rodrigues, J., Gonçalves, J., Lu, J., Holmgren, A., & Carvalho, C. (2014). Mitochondrial thioredoxin reductase inhibition, selenium status and Nrf-2 activation are determinant factors modulating the toxicity of mercury compounds. *Free Radical Biology and Medicine*, 73, 95–105.
- Bridges, C. C., & Zalups, R. K. (2010). Transport of inorganic mercury and methylmercury in target tissues and organs. *Journal of Toxicology and Environmental Health – Part B: Critical Reviews*, 13(5), 385–410.
- Brieger, K., Schiavone, S., Miller, F. J., Jr., & Krause, K.-H. (2012). Reactive oxygen species: From health to disease. *Swiss Medical Weekly*, 142, w13659. <https://doi.org/10.4414/smww.2012.13659>. PMID: 22903797.
- Burk, R. F., & Hill, K. E. (2005). Selenoprotein P: An extracellular protein with unique physical characteristics and a role in selenium homeostasis. *Annual Reviews of Nutrition*, 25, 215–235.
- Burk, R. F., Olson, G. E., Hill, K. E., Winfrey, V. P., Motley, A. K., & Kurokawa, S. (2013). Maternal-fetal transfer of selenium in the mouse. *FASEB Journal*, 27(8), 3249–3256.
- Carvalho, C. M., Lu, J., Zhang, X., Arnér, E. S. J., & Holmgren, A. (2011). Effects of selenite and chelating agents on mammalian thioredoxin reductase inhibited by mercury: Implications for treatment of mercury poisoning. *FASEB Journal*, 25(1), 370–381.
- Chen, J., & Berry, M. J. (2003). Selenium and selenoproteins in the brain and brain diseases. *Journal of Neurochemistry*, 86, 1–12.
- Clarkson, T. W., & Magos, L. (2006). The toxicology of mercury and its chemical compounds. *Critical Reviews in Toxicology*, 36, 608–662.
- Di Meo, S., Reed, T. T., Venditti, P., & Victor, V. M. (2016). Role of ROS and RNS sources in physiological and pathological conditions. *Oxidative Medicine and Cellular Longevity*, 2016, 1245049. <https://doi.org/10.1155/2016/1245049>
- Dyrssen, D., & Wedborg, M. (1991). The sulfur-mercury (II) system in natural waters. *Water, Air, Soil Pollution*, 56, 507–519.
- Gladyshev, V. N., & Hatfield, D. L. (1999). Selenocysteine-containing proteins in mammals. *Journal of Biomedical Science*, 6(3), 151–160.

- Gladyshev, V. N., Kryukov, G. V., Fomenko, D. E., & Hatfield, D. L. (2004). Identification of trace element-containing proteins in genomic databases. *Annual Review of Nutrition*, 24, 579–596.
- Harris, H. H., Pickering, I. J., & George, G. N. (2003). The chemical form of mercury in fish. *Science*, 301(5637), 1203.
- Hatfield, D. L., & Gladyshev, V. N. (2002). How selenium has altered our understanding of the genetic code. *Molecular and Cellular Biology*, 22(11), 3565–3576.
- Hibbeln, J. R., Spiller, P., Brenna, J. T., Golding, J., Holub, B. J., & Harris, W. S. (2019). Relationships between seafood consumption during pregnancy and childhood and neurocognitive development: Two systematic reviews. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 151, 14–36.
- Hill, K. E., Zhou, J., McMahan, W. J., Motley, A. K., Atkins, J. F., Gesteland, R. F., & Burk, R. F. (2003). Deletion of Selenoprotein P alters distribution of selenium in the mouse. *Journal of Biological Chemistry*, 278(16), 13640–13646.
- Korbas, M., O'Donoghue, J. L., Watson, G. E., Pickering, I. J., Singh, S. P., Myers, G. J., Clarkson, T. W., & George, G. N. (2010). The chemical nature of mercury in human brain following poisoning or environmental exposure. *ACS Chemical Neuroscience*, 1(12), 810–818.
- Kryukov, G. V., & Gladyshev, V. N. (2004). The prokaryotic selenoproteome. *EMBO Reports*, 5(5), 538–543.
- Kühbacher, M., Bartel, J., Hoppe, B., Alber, D., Bukalis, G., Bräuer, A. U., Behne, D., & Kyriakopoulos, A. (2009). The brain selenoproteome: Priorities in the hierarchy and different levels of selenium homeostasis in the brain of selenium-deficient rats. *Journal of Neurochemistry*, 110(1), 133–142.
- Labunskyy, V. M., Hatfield, D. L., & Gladyshev, V. N. (2014). Selenoproteins: Molecular pathways and physiological roles. *Physiology Reviews*, 94(3), 739–777.
- Li, J., Wuliji, O., Li, W., Jiang, Z.-G., & Ghanbari, H. (2013). Oxidative stress and neurodegenerative disorders. *International Journal of Molecular Sciences*, 14(12), 24438–24475.
- LoPachin, R. M., & Gavin, T. (2012). Acrylamide and related α,β -unsaturated carbonyl derivatives. In M. J. Aminoff & R. D. Daroff (Eds.), *Encyclopedia of neurological sciences* (2nd ed.). Elsevier. Chapter 244.
- LoPachin, R. M., Gavin, T., DeCaprio, A. P., & Barber, D. S. (2012). Application of the hard and soft, acids and bases theory to toxicant-target interactions. *Chemical Research Toxicology*, 25, 239–251.
- LoPachin, R. M., Gavin, T., Geohagen, B. C., & Das, S. (2007). Neurotoxic mechanisms of electrophilic type-2 alkenes: Soft–soft interactions described by quantum mechanical parameters. *Toxicological Sciences*, 98(2), 561–570.
- Ouyang, Y., Peng, Y., Li, J., Holmgren, A., & Lu, J. (2018). Modulation of thiol-dependent redox system by metal ions via thioredoxin and glutaredoxin systems. *Metallomics*, 10(2), 218–228.
- Pearson, R. G. (2005). Chemical hardness and density functional theory. *Journal of Chemical Sciences*, 117(5), 369–377.
- Picón-Pagès, P., Garcia-Buendia, J., & Muñoz, F. J. (2019). Functions and dysfunctions of nitric oxide in brain. *Biochimica et Biophysica Acta Molecular Basis of Disease*, 1865(8), 1949–1967.
- Radi, R. (2013). Peroxynitrite, a stealthy biological oxidant. *The Journal of Biological Chemistry*, 288(37), 26464–26472.
- Ralston, N. V. C. (2018). Effects of soft electrophiles on selenium physiology. *Free Radicals in Biology and Medicine*, 127, 134–144.
- Ralston, N. V. C., Azenkeng, A., Ralston, C. R., & Raymond, L. J. (2015). Chapter 19: Selenium-health benefit values as seafood safety criteria. In S.-K. Kim (Ed.), *Seafood science; advances in chemistry, technology and applications*. CRC Press/Taylor and Francis.
- Ralston, N. V. C., Kaneko, J. J., & Raymond, L. J. (2019). Selenium health benefit values: A more reliable index of seafood benefits vs. risks. *Journal of Trace Elements in Biology and Medicine*, 55, 50–57.

- Ralston, N. V. C., & Raymond, L. J. (2006). Physiologically based pharmacokinetic model of Hg-Se interactions. Center for Air Toxic Metals (CATM®) Annual Report to U.S. EPA., 2006, 172–184.
- Ralston, N. V. C., & Raymond, L. J. (2018). Mercury's neurotoxicity is characterized by its disruption of selenium biochemistry. *Biochimica et Biophysica Acta. General Subjects*, 1862, 2405–2416.
- Reeves, M. A., Bellinger, F. P., & Berry, M. J. (2010). The neuroprotective functions of selenoprotein m and its role in cytosolic calcium regulation. *Antioxidants & Redox Signaling*, 12(7), 809–818.
- Reeves, M. A., & Hoffmann, P. R. (2009). The human selenoproteome: Recent insights into functions and regulation. *Cellular and Molecular Life Sciences*, 66(15), 2457–2478.
- Saccoccia, F., Angelucci, F., Boumis, G., Carotti, D., Desiato, G., Miele, A. E., & Bellelli, A. (2014). Thioredoxin reductase and its inhibitors. *Current Protein & Peptide Science*, 15(6), 621–646.
- Ste. Marie, E. J., Wehrle, R. J., Haupt, D. J., Wood, N. B., van der Vliet, A., Previs, M. J., Masterson, D. S., & Hondal, R. J. (2020). Can selenoenzymes resist electrophilic modification? Evidence from thioredoxin reductase and a mutant containing α -methylselenocysteine. *Biochemistry*, 59(36), 3300–3315.
- Suzuki, K. T., Kurasaki, K., Okazaki, N., & Ogra, Y. (2004). Selenosugar and trimethylselenonium among urinary Se metabolites: Dose- and age-related changes. *Toxicology and Applied Pharmacology*, 206(1), 1–8.
- Uttara, B., Singh, A. V., Zamboni, P., & Mahajan, R. T. (2009). Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology*, 7(1), 65–74.
- Valentine, W. M., Abel, T. W., Hill, K. E., Austin, L. M., & Burk, R. F. (2008). Neurodegeneration in mice resulting from loss of functional selenoprotein P or its receptor apolipoprotein E receptor 2. *Journal of Neuropathology and Experimental Neurology*, 67(1), 68–77.
- Vermeulen, R., Schymanski, E. L., Barabási, A.-L., & Miller, G. W. (2020). The exposome and health: Where chemistry meets biology. *Science*, 367(6476), 392–396.
- Zhang, Y., Zhou, Y., Schweizer, U., Savaskan, N. E., Hua, D., Kipnis, J., Hatfield, D. L., & Gladyshev, V. N. (2008). Comparative analysis of selenocysteine machinery and selenoproteome gene expression in mouse brain identifies neurons as key functional sites of selenium in mammals. *Journal of Biological Chemistry*, 283(4), 2427–2438.