# Metallothioneins: Mercury Species-Specific Induction and Their Potential Role in Attenuating Neurotoxicity

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**Metallothionein (MT) proteins are widespread in bacteria, fungi, plants, and eukaryotic species. They are of low molecular weight (6–7 kDa) and of the 60+ amino acid residues, 20 are cysteines. Functions attributed to MTs include the sequestration and dispersal of metal ions, primarily in zinc and copper homeostasis; regulation of the biosynthesis and activity of zinc metalloproteins, most notably zinc-dependent transcription factors; and cellular cytoprotection from reactive oxygen species, ionizing radiation, electrophilic anticancer drugs and mutagens, and metals. Observations on the abundance of MTs within the central nervous system (CNS) and the identification of a brain-specific isoform, MT-III, suggest that it might have important neurophysiological and neuromodulatory functions. Reinforced by the potential involvement of MT-III in a number of neurodegenerative disorders, the role of MTs in the CNS has become an intense focus of scientific pursuit. This manuscript represents a survey on the ability of MTs to modulate mercury neurotoxicity, a neurotoxin that has been implied to play an etiologic role in Minamata disease, erethism, and autism, just to name a few.** Exp Biol Med 231:1468–1473, 2006

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Mercury (Hg) has been used extensively for centuries both commercially and medically. Today, exposure in the general population is associated predominantly with three major sources: fish consumption, dental amalgams, and multiuse vaccine vials. Dental amalgams emit inorganic mercury vapor ($Hg^0$) that is inhaled and absorbed into the bloodstream. Methylmercury (MeHg) is a significant environmental contaminant and excessive MeHg ingestion from fish-rich diets has been linked to neurobehavioral and cognitive changes. This ubiquitous environmental contaminant is capable of causing toxic effects as indicated by human poisoning epidemics following food-borne MeHg ingestion (1, 2). Thimerosal, an organomercurial, has been widely used in the past as an antifungal and antibacterial compound. It was used as an agricultural fungicide, but after a series of accidental Hg poisonings (3) and growing evidence of its environmental hazards (4) its use was discontinued. Since the 1930s, thimerosal has also been widely used as a preservative in a number of biological and pharmaceutical products, including many vaccines, where it has proven effective in preventing potentially life-threatening contamination with harmful microbes. In mammals, thimerosal, which is approximately 50% Hg by weight, dissociates to ethylmercury (EtHg) and thiosalicylate. Compared with MeHg, EtHg induces faster release of inorganic mercury within the central nervous system (CNS) (5, 6). However, EtHg should be distinguished from MeHg, because the toxic profiles of these two organomercurials differ in a number of respects (6, 7).

## Metallothioneins: Role in Brain Function

MTs have been implicated as regulator molecules in gene expression, homeostatic control of cellular metabolism of metals, and cellular adaptation to stress. MTs store and release essential metals such as zinc and copper, and maintain the low intracellular concentration of free essential metals. Thus, MTs fulfill a regulatory capacity and influence transcription, replication, protein synthesis, metabolism, as well as other zinc-dependent biological processes. Because MT-III is particularly abundant in zinc-containing neurons of the hippocampus, it is likely to play an important role in neuromodulation by zinc-containing neurons, and to act as a

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sink for free zinc. MTs may also play an etiologic role in various pathophysiological conditions associated with increased extracellular zinc. MTs (MT-III) prevent neuronal sprouting *in vitro*, and various MT-null (MT-I and MT-II) mice strains are more susceptible to physiological and chemical injury. It is well established that elevated concentrations of MT in tissues reduce the toxic effects of mutagens, electrophilic, heavy metals, as well as anticancer agents. The paper will commence with a brief discussion on the various MT isoforms, their structure and abundance (in brain), followed by a survey on the ability of MTs to modulate Hg neurotoxicity.

## Metallothioneins: Brain Distribution

The MTs have high cysteine content (~30%), expressing conserved Cys-$X_n$-Cys motifs, where X can be any amino acid other than cysteine. The proteins typically have a one- or two-domain structure and they bind to multiple monovalent and divalent metal ions (8). The structure of MTs and the nature of their metal binding reveal extensive evolutionary diversification. Fungi and early diverged metazoans have small, single-domain MTs that bind up to eight monovalent metal ions. However, most MTs consist of two domains, designated α and β. These domains are capable of binding metals independently and they are separated by a short linker region. Typically, the α domain contains 11 or 12 cysteinyl residues, binds four divalent metal cations, and conveys structure and stability to the protein. In contrast, the β domain contains nine cysteines, binds three divalent metal cations, and participates in metal exchange reactions involving glutathione (GSH) shuttling with zinc (Zn)- and copper (Cu)-requiring apoproteins.

In vertebrates, the MT-I and MT-II isoforms are expressed in all tissues. At neutral pH MT-1 and MT-II differ by a single negative charge. Each class of the MTs is composed of a number of different isoproteins, and these are commonly referred to or designated as MT-Ia, MT-Ib, MT-Ic, etc. MT-I and MT-II are characterized by a single chain protein containing 61 to 62 amino acids (species-dependent), of which about 20 are cysteinyl residues (9). The isoproteins of the MT-I and MT-II classes are often referred to collectively as MT-I/II because in the context of the CNS, as a group these isoproteins share properties that contrast with the other major brain MT isoform, MT-III.

In the brain, messenger RNA (mRNA) for MT-I and MT-II is particularly abundant in the olfactory bulb, and lowest in the eye. MT-I and MT-II transcripts are also abundant in the cerebellum, a region expressing conspicuously low levels of MT-III. The most conspicuous feature of MT-I and MT-II is their inducibility by a host of agents and conditions. The regulation of MT biosynthesis occurs primarily at the level of transcription, where *cis*-acting DNA elements respond to *trans*-acting transcriptional regulatory proteins. In most species MT-I and MT-II genes are rapidly induced both *in vitro* and *in vivo* by multiple stimuli including metals, hormones, cytokines, oxidants, stress, and irradiation (10, 11). A hallmark of MT-I and MT-II genes is that they are transcriptionally induced by various metals, and especially Zn and cadmium (Cd) (12). DNA motifs, referred to as metal response elements (MREs), which are present in multiple copies in the proximal promoters of MT genes, are essential for this induction. The MREs confer response to both Zn and Cd as well as to oxidative stress (12). A protein responsible for transactivation through the MRE has been cloned, and is referred to as MRE-binding transcription factor-1 (MTF-1). It is a zinc-finger transcription factor in the $Cys_2His_2$ family. Targeted disruption of both MTF-1 alleles in embryonic stem cells reveals its essential role for basal as well as heavy metal–induced MT gene expression and oxidative stress–induced MT-I gene expression in the mouse (13). MTF-1 is also important in regulating embryo survival. Embryos that lack MTF-1 die *in utero*. They show impaired development of hepatocytes as well as liver decay and generalized edema (13). Primary mouse embryo fibroblasts lacking MTF-1 show increased susceptibility to the cytotoxic effects of Cd or hydrogen peroxide. Accordingly, MTF-1 is likely to be involved in the control of metal homeostasis and probably cellular redox state, especially during liver development (13).

MT-III (referred to originally as growth-inhibiting factor; GIF), an isoform of MT that is largely restricted to the CNS (14), contains 68 amino acids (vs. 61–62 amino acids in mammalian MT-I and MT-II isoforms); 38 of the amino acids are identical both in alignment and type to the human MT-I and MT-II isoforms. MT-III possesses remarkable structural identity to other mammalian MTs, such that when aligned with MT-I and MT-II the position of all 20 constituent cysteine residues is completely conserved (9, 10). MT-III is expressed in glutamatergic neurons and associated astrocytes (15), and low expression has also been reported in pancreas and intestine. Expression of MT-III mRNA coincides with high concentrations of vesicular Zn, such as the hippocampus, pyriform cortex, and the amygdala, and accordingly, it has been postulated to factor in regulating glutamatergic neurotransmission and possibly glutamate neurotoxicity (15).

Another MT gene product, MT-IV, is solely expressed in squamous epithelial cells in skin and tongue. MT-III (at both the RNA and protein levels) and MT-IV (at the RNA level) isoforms are constitutively expressed, whereas MT-I and MT-II isoforms are both basally expressed and highly inducible by multiple chemical and physical triggers. Events that induce MT-I and MT-II gene expression, in general, do not enhance the expression of MT-III or MT-IV. The relative abundance of brain MT mRNA is as follows: MT-I > MT-III > MT-II (10). MT-III and MT-IV mRNAs are also both abundant in the maternal deciduum, and in experimentally induced deciduoma on Days 7 and 8 postcoitum (16).

## Inorganic Mercury and Metallothioneins

Given its chemical and physical properties, mercury vapor ($Hg^0$) is effectively absorbed by inhalation, easily crossing the blood-brain barrier and accumulating within the brain. Unlike inorganic mercury in the form of $Hg^{2+}$ (mercuric), $Hg^0$ poisoning is primarily associated with CNS symptoms. Aschner *et al.* (17) addressed developmental effects of exposure to $Hg^0$. Brain MT protein and mRNA levels were determined in the fetal rat following *in utero* (Gestational Days [GDs] 7–21) exposure to $Hg^0$ (300 μg $Hg/m^3$; 4 hrs/day). Expression of whole-brain MT-I mRNA in full-term fetal rats was significantly increased by exposure to $Hg^0$ compared with nonexposed controls. This corresponded to a 14-fold increase in fetal brain Hg concentration. Astrocyte cultures established from *in utero* $Hg^0$-exposed fetuses consistently expressed increased abundance of MT-I mRNA transcripts after 1, 2, and 3 weeks in culture compared with controls. Consistent with the increase in MT mRNA, an increase in astrocytic levels of MT proteins was noted by Western blot analysis and MT immunoreactivity. These authors (17) suggested that *in utero* exposure to $Hg^0$ induces brain MT gene expression, and that MT mRNAs and their respective proteins are useful quantitative biochemical markers of intrauterine exposure to $Hg^0$, a potentially cytotoxic challenge to astrocytes in the developing brain. It was concluded that induction of MT by fetal/neonatal astrocytes represents an attempt by astrocytes to protect against Hg cytotoxicity in maintaining cerebral homeostasis.

The disposition and toxicity of inhaled $Hg^0$ in rats, and potential adverse effects on reproductive outcome, were investigated by Morgan *et al.* (18). Rats were exposed to 0, 1, 2, 4, or 8 mg $Hg^0/m^3$ for 2 hrs/day from GD 6 through GD 15. The primary purpose of the experiments was to assess the effect of $Hg^0$ on reproductive parameters, such as the incidence of resorptions and litter size. However, the authors also measured MT levels in neonatal tissues. Although brain MT levels were not significantly increased by exposure to 4 $mg/m^3$ $Hg^0$, the manuscript lacks information on expression levels of MT at other exposure levels. Accordingly, it is unclear whether this exposure scenario was associated with changes in either maternal or neonatal MT levels. Nevertheless, the total amount of Hg in neonatal brain continued to increase after termination of $Hg^0$ exposure, and the possibility that this effect is associated with MT induction needs to be revisited.

In general, the literature supports the conclusion of the ability of Hg to induce brain MT (17). For example, Kramer *et al.* (19) examined the inducibility of MT-I and MT-II both in mouse neurons and astrocytes in response to Hg. In astrocyte cultures, induction of MT-I, MT-II, and MT-III at mRNA levels was examined 6 hrs following the addition of $Hg^{2+}$ or MeHg. Although $Hg^{2+}$ led to a ~4-fold increase in MT-I, MT-II, and MT-III mRNA levels, MeHg was reported to be ineffective. Similar results were obtained in neurons, but the basal level and induced level of MT protein is about one-third in neurons as in astrocytes (20). The studies are consistent with a more effective MT induction with exposure to $Hg^{2+}$.

Yasutake *et al.* (21) determined brain MT levels in rats exposed to MeHg or $Hg^0$. Rats treated with MeHg (40 μmol/kg per day × 5 days, po) showed neurological signs 10 days after final administration, but brain MT levels remained unchanged. However, in rats exposed to $Hg^0$ for 7 days, brain MT levels were about twice the levels in the control population. Although brain Hg levels fell gradually, MT levels induced by Hg exposure remained unchanged for >2 weeks. Gel fractionation revealed that most Hg was in the cytosol fraction and MT-bound. Hybridization analysis showed a significant increase in MT-I and MT-II mRNA in brain, and a smaller change in MT-III mRNA. Although significant Hg accumulation and MT induction were also observed in kidney and liver of $Hg^0$-exposed rats, these decreased more quickly than levels in brain. Yasutake *et al.* (21) suggested that the long-lived MT in brain might reflect the longer half-life of Hg in this tissue. In a follow-up study, the same authors (22) examined time-dependent alterations in the MT isomers, MT-I/II and MT-III, following 3 weeks of exposure to $Hg^0$ (8.3 $mg/m^3$ for 15 hrs in total over 5 consecutive days). Total MT levels in rat cerebrum and cerebellum increased significantly 24 hrs after the final exposure. The increased levels in both tissues remained unchanged for at least 2 weeks after termination of exposure. In MT-null mice, which lack MT-I/II but express MT-III (23), the effects of subchronic pulse exposure to $Hg^0$ (2 weeks at 0.1 mg $Hg/m^3$ for 1 hr/day for 3 days a week followed by 11 weeks at 4.1 mg $Hg/m^3$ for 30 mins/day for 3 days a week) was associated with lower accumulation of Hg in the brain (than in wild-type mice). $Hg^0$ exposure resulted in a 70% increase of brain MT in wild-type mice, which was mostly accounted for by the increase in MT-I/II. On the other hand, the brain MT in the MT-null (MT-I/II) mice increased by 19%, suggesting less reactivity of the MT-III gene to $Hg^0$. These studies are consistent with the ability of $Hg^0$ to induce MT, most likely as means of neuroprotection (see below), as well as the ability of MT to bind and retain CNS Hg.

The literature is replete with reports on the ability of MTs to function as antioxidant and scavenger free radicals, which have unpaired electrons and can be detected selectively and sensitively by electron spin resonance spectroscopy. The diffusible second-messenger nitric oxide (NO) serves as an intracellular messenger; however, when NO acts as an effector molecule and is synthesized in large amounts for prolonged periods of time (such as in inflammation or *N*-methyl-D-aspartate [NMDA] receptor activation), it may become cytotoxic due to inhibition of mitochondrial respiration (by the formation of iron-nitrosyl complexes with FeS-containing enzymes), inhibition of DNA synthesis, or initiation of DNA strand breaks. NO reacts rapidly with $O\bullet_2^-$ to produce the peroxynitrite anion

($ONOO^-$), which protonates at relevant pH (pKa 6.8) to form peroxynitrous acid (ONOOH), both of which are potent oxidizers. The rate constant of MT for its reaction with hydroxyl radicals is very high (24, 25). Overactivation of NMDA receptor can produce both NO and HO* (hydroxyl radical) and NO. NO can be chemically converted to peroxynitrite—a much more cytotoxic compound. Because thiols are known targets for both $ONOO^-$ and ONOOH, it is plausible that MT isoforms may intercept both these oxidizers.

It has also been suggested that the antioxidant properties of MTs may depend on their metal speciation; namely, the ability of oxygen free radicals to release zinc from MT thiolate (26). Enzymes that oxidize GSH to its disulfide (GSSG), such as GSH peroxidase, are thought to be coupled to events that signal release of zinc from MT (26). In toxic conditions involving oxidative stress zinc-sulfur bonds are also implicated in the mobilization and release of zinc from MT (27). It is noteworthy that the toxic effects of various mercury species have been purported to be associated with the generation of various reactive oxygen species both as a function of cytosolic phospholipase $A_2$ activation and glutamate excitotoxicity (28, 29). Thus the ability of MT to attenuate the neurotoxicity of $Hg^0$ is fully consistent both the binding of Hg to –SH groups within the cysteine backbone, as well as the ability of MTs to serve as potent antioxidant species. This is consistent with studies revealing that MT in the placenta has a defensive role as well as in preventing maternal-to-fetal Hg transfer (30).

Yoshida *et al.* (30) studied the role MT in the distribution and retention of Hg in the brain of MT-null (MT-I/II) and wild-type mice after exposure to $Hg^0$. Mice were exposed to $Hg^0$ vapor (5.5–6.7 mg/m$^3$ for 3 hrs and killed at 1, 24, 72 or 168 hrs after exposure). One hour after exposure to $Hg^0$ vapor, there were no differences in Hg concentrations in these organs from MT-null and wild-type mice. The elimination rate of Hg from the brain was identical in MT-null and wild-type mice. A large amount of Hg was bound to MT in both strains of mice immediately after exposure. Brain MT levels were slightly elevated in wild-type mice at 168 hrs after exposure but could not be detected in MT-null mice. These authors suggested that the absence of detectable MT-I and -II levels in the brain of MT-null mice indicates that Hg was primarily bound to MT-III, the latter playing an important role in the retention of Hg in the brain.

Yoshida *et al.* (31) also examined neurobehavioral effects of prolonged, low-level $Hg^0$ exposure in mice as well as the role of MT-I and MT-II in protecting the CNS. Adult female (MT-I–null and MT-II–null) and wild-type OLA129/C57BL6 mice were exposed to mercury vapor (0.06 mg/m$^3$ of $Hg^0$ for 8 hrs per day for 23 weeks). Neurobehavioral effects were evaluated at 12 and 23 weeks of exposure using open-field and passive avoidance tests. Subcellular distribution of Hg and the induction of MT were also assessed. $Hg^0$ exposure resulted in significantly enhanced locomotion in the open-field test and poorer performance in the passive avoidance test at a brain Hg concentration less than 1 part per million. These effects were slightly exaggerated in MT-null mice, which showed less induction of MT, lower brain Hg concentration, and lower concentration of MT-unbound cytosolic Hg. Similar studies were also conducted (32) in mice exposed to $Hg^0$ (50 or 500 μg/m$^3$ $Hg^0$ for 4 hrs a day for 5 days) in late pregnancy, and pups were sacrificed on Postnatal Day (PD) 1 or PD40. Neonatal mice were also exposed to $Hg^0$ [500 μg/m$^3$ $Hg^0$ for 2 hrs between PD1 and PD23], and were sacrificed 2 days later or at PD40. The authors reported that no Hg was observed in the nervous system of pups after fetal exposure to the 50 μg/m$^3$ $Hg^0$ dose rate. After fetal exposure to the 500 μg/m$^3$ $Hg^0$ dose rate, Hg was observed in nervous system blood vessels and sensory ganglia. No Hg was observed in the nervous system after neonatal exposure to 500 μg/m$^3$ $Hg^0$ for 2 hrs between PD1 and PD10. From this exposure at PD11 onward, Hg was detected in motor neurons. The lack of stainable Hg in early developing central neurons was taken to indicate that the fetal and neonatal nervous systems are somehow protected from $Hg^0$ uptake (32).

A recent study by Kameo *et al.* (33) examined the influences of the lack of MT-I and MT-II on Hg accumulation in the CNS after $Hg^0$ exposure by using MT-I, MT-II–null, and 129/Sv (wild-type) mice as an experimental model. MT-I–null, MT-II–null, and wild-type mice were exposed to $Hg^0$ (2 hrs) and sacrificed 24 hrs later. The brain was dissected into the cerebral cortex, the cerebellum, and the hippocampus. The concentrations of Hg in each brain section were determined. The Hg levels in MT-I–null and MT-II–null mice in each brain section were significantly higher than those in wild-type mice after $Hg^0$ exposure. From the results of high-performance liquid chromatography/inductively coupled plasma–mass spectrometry analyses, it was also determined that the Hg components of MT-III and high-molecular-weight metal-binding proteins in the cerebellum of MT-I–null and MT-II–null mice were much higher than those of wild-type mice. Kameo *et al.* (33) suggested that MT-III is associated with the storage of Hg in conditions lacking MT-I and MT-II. It was also suggested that the physiological role of MT-III and some kind of high-molecular-weight proteins might be impaired by exposure to $Hg^0$ and lack of MT-I and MT-II.

Prenatal exposure to $Hg^0$ and increased Hg brain levels are associated with neurobehavioral effects in both MT-null and wild-type mice (34). Exposure to $Hg^0$ (0.50 to 0.56 mg/m$^3$ for 6 hrs/day until Day 18 day of gestation) was shown at 12 weeks to be associated with a significant decrease in total locomotor activity in males, and a learning disability in the passive avoidance response and a retarded acquisition in the Morris water maze in MT-null females compared with the control, consistent with the notion that MT-null mice are more susceptible than wild-type mice to the behavioral neurotoxicity of prenatal $Hg^0$ exposure. Hg concentrations in the brain of both strains were slightly higher in the

exposed group than in the control group, indicating the retention of residual mercury even 12 weeks after the cessation of the exposure. Brain concentrations of Hg were also significantly higher in the exposed females than the exposed males in either strain. Thus, increased susceptibility of MT-null females to behavioral changes caused by prenatal $Hg^0$ exposure is likely due to a greater retention of Hg and a lack of MT-I and MT-II in the brain (34). The studies suggest that analogous to neurotoxicity resulting from a prolonged adult exposure to $Hg^0$ (31), the developmental toxicity of *in utero* exposure is more exaggerated in MT-null mice. The enhanced susceptibility of MT-null mice to the neurobehavioral sequelae of $Hg^0$ exposure confirms increased susceptibility of MT-null mice to $Hg^0$ and is consistent with MT induction in response to other metals such as cadmium and arsenic (35). The findings also suggest that the MT-I and MT-II also play some roles in alleviating Hg neurotoxicity.

## Methylmercury and Metallothioneins

There are relatively few studies on the interaction between MeHg and MT. As mentioned above, Kramer *et al.* (19, 20) found MeHg to be ineffective in inducing MTs, both in cultured neurons and astrocytes, consistent with the *in vivo* findings (21) in which brain MT levels in rats exposed to MeHg (40 μmol/kg per day × 5 days, po) 10 days after final administration remained unchanged, even in the presence of neurological symptoms.

Gonzalez *et al.* (36) examined effects of dietary MeHg on gene expression in three organs (liver, skeletal muscle, and brain) of the zebrafish. Adult male fish were fed over 7, 21, and 63 days on three different diets: one control diet and two diets contaminated by MeHg at 5 and 13.5 μg of Hg/g. Total Hg and MeHg concentrations were determined in the three organs after each exposure duration. Thirteen genes known to be involved in antioxidant defenses, metal chelation, active efflux of organic compounds, mitochondrial metabolism, DNA repair, and apoptosis were investigated by quantitative real-time reverse transcription–polymerase chain reaction and normalized according to actin gene expression. Surprisingly, no change in the expression levels of these genes, including MT-II, was observed in brain samples, although this organ accumulated the highest Hg concentration. Thus, all studies conducted to date are in support of the inability of MeHg to induce MT.

Leyshon-Sorland *et al.* (37) studied rats dosed with MeHg, either by gastric gavage (5 × 10 mg/kg body wt over a 15-day period), or in their drinking water (20 mg MeHgCl for 14 or 42 days). Hg was detected in structurally undamaged Purkinje neurons and adjacent Bergmann glial cells; no Hg was detected in granule cells even though these small cells nearest the Purkinje layer had a high incidence of pyknotic nuclei. In general, MT was detected primarily in Bergmann glial cells, Purkinje cells, astrocytes, and glial cells of white matter; no MT was detected in granule cells. The resistance of Purkinje cells to MeHg chloride was hypothesized to reflect their ability to transform organic mercurials to inorganic mercury, which in turn, induces the synthesis of radical-scavenging MT molecules.

## Ethylmercury and MTs

Studies have yet to be performed to address the effects of EtHg, a vaccine preservative containing 50% EtHg, on MTs in brain. EtHg decomposes faster than MeHg. Because passage through the blood-brain barrier favors small molecules and MeHg is actively transported, Hg concentrations in the brain are higher following exposure to MeHg (7). Hg also clears faster after administration of EtHg versus MeHg. Because metabolic rates (basic metabolism, rates of loss from the body burden) are related to the fractional power of body weight (allometric relationship), Hg clears faster from infants, thus blood Hg concentrations for MeHg underestimate the safe exposure range for EtHg (38). These observations suggest, but have yet to be verified, that MT induction upon EtHg is most likely relatively small in comparisons to $Hg^0$ and even inorganic mercury in the form of $Hg^{2+}$.

## Summary

MTs have important functions in metal metabolism and protection. Mammalian MTs are part of a gene cluster on human chromosome 16q13, which contains four isoforms (MT1, MT2, MT3, and MT4) and 17 subtypes of MT genes. MT1 and MT2 isoforms are ubiquitously expressed in most tissues, including the brain, whereas MT3 is only expressed in the brain, and MT4 is expressed in certain stratified squamous epithelia. MT-I and MT-II are induced following Hg exposure, presumably to protect essential cellular functions and enhance survival. MT-I and MT-II can function as antioxidants, and both are up-regulated in response to other divalent metals including $Cd^{2+}$, $Cu^{2+}$, $Ag^{2+}$, and $Zn^{2+}$. In addition, MT-I–null and MT-II–null mice exhibit increased susceptibility to Hg neurotoxicity Considerations should be given to the possibility that polymorphisms in MTs may prevent cells from mounting an optimal response to oxidative stress, rendering certain populations more susceptible to mercury exposure.

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