Brazilian Journal of Medical and Biological Research (2007) 40: 285-291
ISSN 0100-879X Review

Involvement of glutamate and reactive oxygen species in methylmercury neurotoxicity

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Abstract

This review addresses the mechanisms of methylmercury (MeHg)-induced neurotoxicity, specifically examining the role of oxidative stress in mediating neuronal damage. A number of critical findings point to a central role for astrocytes in mediating MeHg-induced neurotoxicity as evidenced by the following observations: a) MeHg preferentially accumulates in astrocytes; b) MeHg specifically inhibits its glutamate uptake in astrocytes; c) neuronal dysfunction is secondary to disturbances in astrocytes. The generation of reactive oxygen species (ROS) by MeHg has been observed in various experimental paradigms. For example, MeHg enhances ROS formation both *in vivo* (rodent cerebellum) and *in vitro* (isolated rat brain synaptosomes), as well as in neuronal and mixed reaggregating cell cultures. Antioxidants, including selenocompounds, can rescue astrocytes from MeHg-induced cytotoxicity by reducing ROS formation. We emphasize that oxidative stress plays a significant role in mediating MeHg-induced neurotoxic damage with active involvement of the mitochondria in this process. Furthermore, we provide a mechanistic overview on oxidative stress induced by MeHg that is triggered by a series of molecular events such as activation of various kinases, stress proteins and other immediate early genes culminating in cell damage.

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Research supported in part by Public Health Service Grant ES07331 to M. Aschner.

Received August 21, 2006
Accepted January 16, 2007

Key words
- Methylmercury neurotoxicity
- Oxidative stress
- Glutamate and selenocompounds
- Reactive oxygen species
- Astrocytes

Introduction

Methylmercury (MeHg) is a major neurotoxicant that continues to pose appreciable risk to human health as evidenced by the tragic epidemics of MeHg poisoning in Japan and Iraq (1,2). All sources of environmental mercury represent a potential risk for poisoning in humans through the methylation of inorganic mercury to MeHg in waterways, resulting in MeHg accumulation in the sea food chain and representing the most prevalent source for human consumption. Excessive MeHg ingestion from a diet high in fish is associated with

aberrant central nervous system (CNS) functions (3-5). Recent studies in human populations support the earlier findings that maternal exposure to mercury during pregnancy is associated with neurological as well as neuropsychological deficits detectable in the child at 6 to 7 years of age (6,7). Another recent study has pointed to the selective detrimental effects of MeHg on neurogenesis. However, despite these observations, the issue remains controversial, as exemplified by other studies (8,9) in which no association was noted between MeHg and neurodevelopmental outcomes in children at 66 months of age. Thus, the ultimate effects of MeHg in the human population remain unknown and clearly there is an urgent need to understand the mechanisms and consequences of MeHg exposure for CNS function. The current literature suggests that no single mechanism can explain the multitude of effects observed in MeHg-induced neurotoxicity.

The role of astrocytes in brain function

Among various cell types, astrocytes represent a major cell type occupying approximately 50% of the CNS volume and their dysfunction following toxicant exposure has been implicated as a main cause of the observed neurotoxicity (10). The "foot" processes of these cells are known to be closely associated with synapses, axonal tracts, nodes of Ranvier, and capillaries. Astrocytes perform important duties and some of their functions include the maintenance of normal extracellular ion concentrations, the uptake of potassium (K^+) and the control of extracellular pH. They express a pantheon of receptors and uptake systems for neurotransmitters, properties that were formerly thought to be exclusively neuronal. Thus, astrocytes can respond to and prevent the build-up of potentially dangerous levels of neurotransmitters (e.g., glutamate) in the extracellular fluid (11). Important roles of astrocytes during early brain development include the synthesis and elaboration of cues for neuronal migration and the production of neurotrophic factors important for neuronal division and differentiation. Astrocytes also induce the high electrical resistance (tightness) of the blood-brain barrier that limits the transport of noxious substances entering the brain, and modulates optimal transport of nutrients and metabolites (12).

Mechanisms of methylmercury-induced neurotoxicity and glutamate

Although not the only cell type to be adversely affected by MeHg, a number of studies have established a key role for astrocytes in mediating MeHg neurotoxicity: a) astrocytes represent a preferential cellular site for MeHg accumulation (13-16); b) MeHg selectively inhibits astrocytic transport of cystine and cysteine (Figure 1), thereby adversely affecting their redox status and attenuating glutathione (GSH) content (17-21); c) MeHg inhibits astrocytic glutamate (and aspartate) uptake (Figure 1) and stimulates its efflux, thereby increasing glutamate concentrations in the extracellular fluid and sensitizing neurons to excitotoxic injury (17,18,22-24); d) MeHg-induced neuronal dysfunction is secondary to disturbances in astrocytes (25), and the *in vitro* co-application of non-toxic concentrations of mercury with glutamate results in the appearance of typical neuronal lesions found with excitotoxic stimulation (26); e) MeHg causes the activation of cytosolic phospholipase A_2 leading to arachidonic acid release and further inhibition of the glutamate transporter (Figure 1), setting in motion an unimpeded cytotoxic cycle (27,28).

The role of reactive oxygen species in methylmercury-induced neurotoxicity

Though free radicals are known to play a

physiological role in optimal cell function, excessive oxidative stress has been implicated in a variety of neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis (29-31). Oxidative stress also plays an important role in other degenerative conditions such as autoimmune and inflammatory diseases (i.e., ischemia and rheumatoid arthritis), cancer, diabetes mellitus, and atherosclerosis (32-34), as well as in metal-induced toxicity (35,36). The balance between oxidative and reductive cellular processes is known to be adversely affected in these various disorders (37). Oxidative stress is associated with the accumulation of high levels of toxic reactive species, such as reactive oxygen species (ROS), reactive nitrogen species, reactive nitrogen oxygen species, as well as unbound metal ions (38). Typical ROS include oxygen radicals such as superoxide radical, hydroxyl radical, as well as non-radical derivatives of oxygen including hydrogen peroxide (H_2O_2). Reactive nitrogen species include nitric oxide radical, and reactive nitrogen oxygen species include the highly reactive oxidant species peroxynitrite, which is a product of reaction between nitric oxide and the superoxide radical. These reactive species are highly oxidizing and potently damaging to cellular redox-sensitive proteins, enzymes

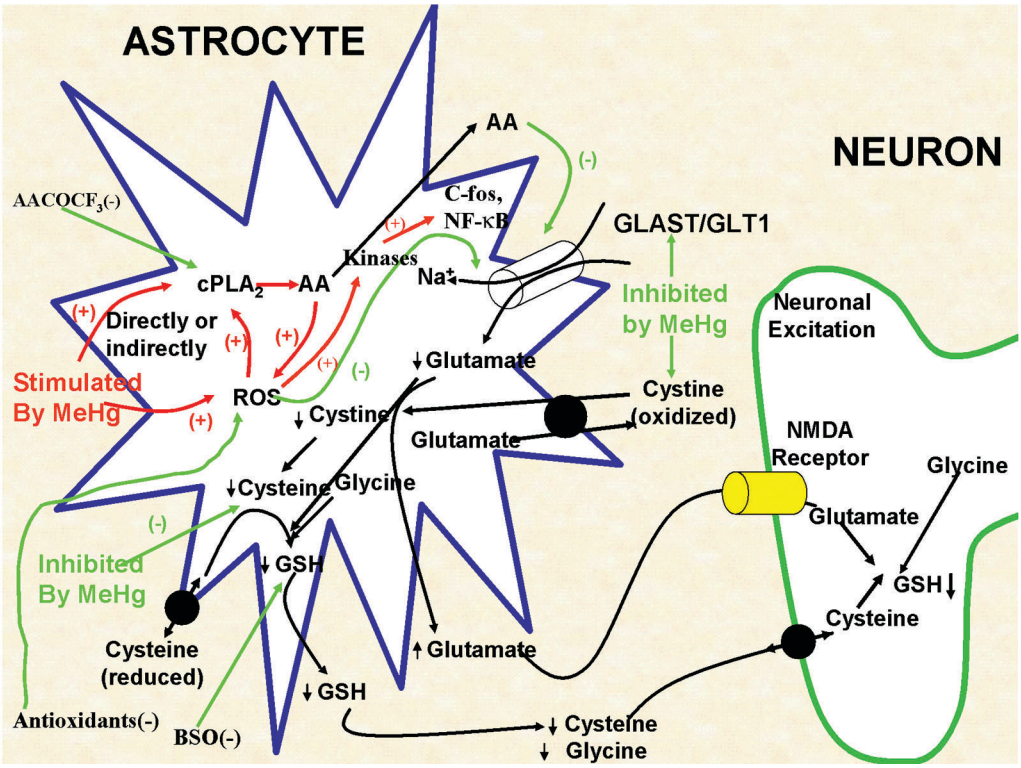

Figure 1. A schematic model of some of the currently proposed as well as some of our previously studied processes resulting in methylmercury (MeHg)-dependent neurotoxicity. Red lines and text indicate processes where MeHg stimulates cellular processes, whereas green lines and text indicate targets for MeHg-induced inhibition. For example, MeHg is known to increase the release of arachidonic acid (AA) from astrocytes and to stimulate glutamate release, increasing its synaptic levels. At the same time MeHg also inhibits glutamate uptake, as well as a number of the amino acids that are associated with the synthesis of astrocytic glutathione (GSH). Combined, these effects lead to a reduction in intracellular GSH levels and increased synaptic glutamate levels, which in turn activate NMDA receptors on adjacent neurons, leading to excitotoxicity. $cPLA_2$ = cytosolic phospholipase A_2; ROS = reactive oxygen species; NF-κB = nuclear factor kappa B; BSO = L-buthionine-[*S,R*]-sulfoximine; NMDA = N-methyl D-aspartate; GLAST = glutamate aspartate transporter; GLT1 = glutamate transporter 1.

and DNA, and they also cause peroxidation of membranes.

ROS are known to mediate MeHg-induced neurotoxicity in multiple experimental models. For example, MeHg induces ROS formation *in vivo* (rodent cerebellum), and *in vitro* (isolated rat brain synaptosomes) (39), as well as in cerebellar neuronal cultures, a hypothalamic neuronal cell line and in mixed reaggregating cell cultures (40-42). In addition, an increase in ROS has been observed in a) mitochondria isolated from MeHg-injected rat brains (36), b) mitochondria isolated *in vitro* from rat brain and then exposed to MeHg (43), and c) mitochondria from Hg- and glutamate-exposed astrocytes and neurons (44,45). MeHg-induced ROS production and MeHg-induced glutamate dyshomeostasis are connected phenomena affecting each other. In fact, MeHg-induced inhibition of astrocyte glutamate transporters leads to increased glutamate concentrations in the extracellular fluid, causing hyperactivation of N-methyl D-aspartate type glutamate receptors and leading to an increase in Na^+ and Ca^{2+} influx (46). Increased intracellular Ca^{2+} levels are associated with the generation of ROS (47). On the other hand, MeHg-induced ROS (mainly H_2O_2) production appears to directly inhibit astrocyte glutamate transporters, leading to increased glutamate concentrations in the extracellular fluid (17,18). This ROS formation resulting from MeHg- and glutamate-induced oxidative stress contributes to mitochondrial dysfunction. Overproduction of ROS is mediated, at least in part, by glutamate, since this toxicity can be attenuated by N-methyl D-aspartate receptor antagonists. The source of glutamate is likely to be astrocytic, given the effect of MeHg on glutamate uptake and release (Figure 1) from astrocytes (17,18,48). Therefore, although the toxic damage caused by MeHg might be most prevalent in neurons, a large body of literature suggests that neuronal damage in response to MeHg most likely represents aberrant control of the extracellular milieu by astrocytes.

Experimental studies have investigated the potential protective effects of antioxidant molecules (i.e., GSH precursors and antioxidant selenocompounds) against MeHg-induced neurotoxicity (49,50). Since hydrogen peroxide has been shown to be an important hazardous molecule involved in MeHg toxicity (17,18,50), such protective effects appear to be related, at least in part, to the ability of these compounds to mitigate the deleterious effects of hydrogen peroxide. MeHg exhibits a direct inhibitory effect on the activity of glutathione peroxidase in mouse CNS, leading to increased lipid peroxidation and decreased glutamate uptake into cerebrocortical slices (50,51). Organoseleno compounds with thiol peroxidase activity show protective effects that appear to be related to the maintenance of H_2O_2 status at low physiological levels in MeHg-exposed systems (50,52). Although the GSH precursor N-acetylcysteine can contribute to the maintenance of GSH intracellular homeostasis, which is crucial for the detoxification of hydrogen peroxide by glutathione peroxidase, part of the beneficial effects elicited by N-acetylcysteine under *in vivo* conditions is also related to its ability to accelerate urinary MeHg excretion in poisoned animals (53). However, even though antioxidant molecules have been showing protective effects against MeHg-induced neurotoxicity under experimental conditions (21,50), their use as possible therapeutic agents in MeHg poisoning is far from becoming a reality. Currently, the only way to prevent or ameliorate toxicity in MeHg poisoning is to accelerate its elimination from the body. Strategies for removing MeHg include hemodialysis, exchange transfusion, and chelation therapy (54,55).

Signaling pathways mediating methylmercury-induced neurotoxicity

A number of reports indicate oxidative stress-induced activation of some signaling molecules (i.e., distinct kinases/transcription factors (nuclear factor kappa B, NF-κB),

(activator protein-1, etc.)/early response genes (c-fos, c-jun, etc.)). This activation leads to induction of various target genes (i.e., inducible nitric oxide synthase, cyclooxygenase II, manganese-superoxide dismutase, inducible form (HSP-72), cytokines, etc.), which contribute to cell damage (56-58). Studies have also shown that distinct kinases mediate the metal/toxicant-induced toxicity both downstream and upstream of generated ROS (43). For example, the ROS-generating nicotinamide-adenine dinucleotide phosphate oxidase enzyme is stimulated by zinc in astrocytes and neurons in a protein kinase C (PKC)-dependent manner. Furthermore, the upstream involvement of tyrosine kinase, PKC, and mitogen-activated protein kinase (MAPK) pathway kinases in MeHg-induced generation of ROS in synaptosomes has been demonstrated with selective inhibitors (43). The generated ROS resulting from oxidative stress (induced by a variety of agents like copper, arsenic, chromium, H_2O_2, angiotensin II, and from degenerative conditions) causes the downstream activation of a variety of kinases (p38MAPK, ERK and JNK), as well as transcription factors such as NF-κB, leading to a cytotoxic response (59,60).

There are no detailed and systematic studies examining how MeHg-induced ROS formation in astrocytes ultimately leads to cytotoxicity, and what, if any, is the role of various signal transduction pathways in this process. Figure 2 (see legend for a detailed description) depicts various targets that may be affected by MeHg. Future studies with astrocytes should be designed to answer these issues and to determine if the neurotoxic effects of MeHg are, at least in part, due to the generation of ROS and may activate signaling pathways involving distinct kinases (i.e., PKC, tyrosine protein kinase, p38MAPK, and ERK MAPK), phospholipase A_2, as well as immediate early genes (c-fos) and transcription factor NF-κB (please refer to Figure 2). Since mitochondria are known mediators of ROS generation, future studies on the efficacy of mitochondrial permeability transition pore inhibitors and mitochondrial cytochrome C release in attenuating MeHg-induced cellular damage should be profitable. Another consequence of increased oxidative stress is the induction of the mitochondrial permeability transition, a Ca^{2+}-dependent process characterized by the opening of the permeability transition pore in the inner mitochondrial membrane. This causes increased permeability to protons, ions and other solutes ≤1500 Da, in turn, leading to a

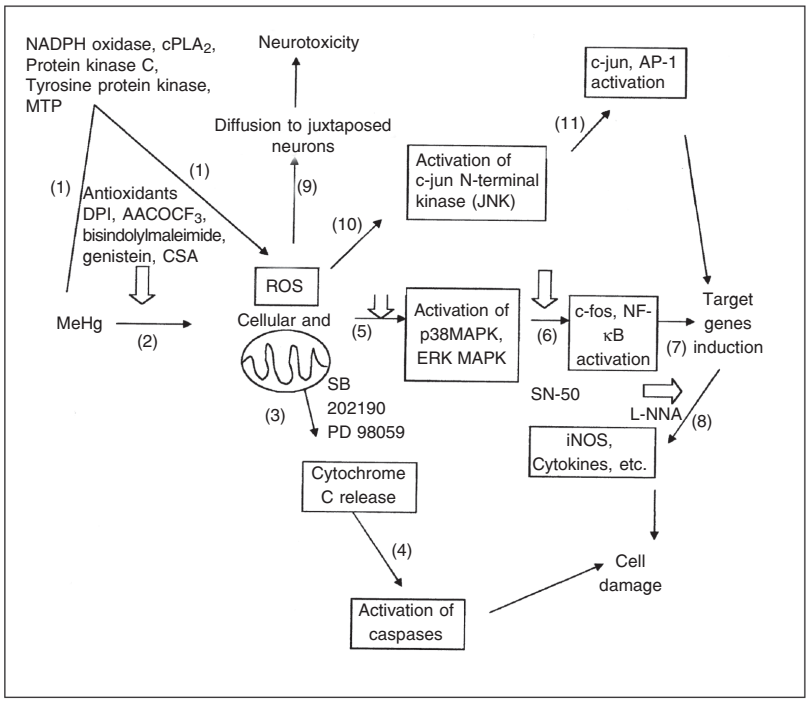

Figure 2. A model depicting the role of oxidative stress in methylmercury (MeHg) neurotoxicity: 1) by involvement of various enzymes; 2) increased reactive oxygen species (ROS) can potentially be prevented with a) antioxidants, b) nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase inhibitor (DPI), c) cytosolic phospholipase A_2 ($cPLA_2$) inhibitor ($AACOCF_3$), d) protein kinase C inhibitor (bisindolylmaleimide), e) tyrosine protein kinase inhibitor (genistein), and e) microsomal triglyceride transfer protein (MTP) inhibitor (CSA); 3,4) release of cytochrome C into cytosol from mitochondria; 5) reactive oxygen species (ROS) formed may lead to the activation of p38 mitogen-activated protein kinase (p38MAPK) and extracellular-signal regulated kinase (ERK) MAPK, which may be inhibited by pretreatment with inhibitors SB 202190 and PD 98059; 6) stimulated kinases may mediate the activation of c-fos and nuclear factor kappa B (NF-κB); 7,8) possible induction of target proteins like inducible nitric oxide (iNOS) may be blocked with nitro-L-arginine (L-NNA), an iNOS inhibitor); 9) the diffusion of ROS from astrocytes to adjacent neurons can lead to possible mitochondrial damage in neurons; 10,11) there may be involvement of other signaling (i.e., JNK, c-jun, and AP-1) pathways in modulating MeHg cytotoxicity. SN-50 = inhibitory peptide derived from the p50 protein.

collapse of the mitochondrial inner membrane potential ($\Delta\Psi_m$). Loss of the $\Delta\Psi_m$ results in colloid osmotic swelling of the mitochondrial matrix, movement of metabolites across the inner membrane, defective oxidative phosphorylation, cessation of ATP synthesis, and further generation of ROS. The concentration-dependent deleterious effects of MeHg on mitochondrial $\Delta\Psi_m$ in cultured astrocytes suggest that $\Delta\Psi_m$ is a very sensitive endpoint for MeHg toxicity, and these effects are consistent with increased $(Ca^{2+})_i$ triggering ROS formation and increased oxidative stress. What has yet to be established is the temporal sequence of events reported here and whether changes in membrane potential precede the changes in oxidative stress. This would be consistent with early reports from our laboratory showing that MeHg increases cellular permeability to ions such as Na^+ (and K^+), and that an increase in Na^+ permeability via Na^+/H^+ exchange, offsetting K^+ loss, is the primary mechanism in its inhibition of regulatory volume decrease in astrocytes.

References

1. Takeuchi T. Biological reactions and pathological changes in human beings and animals caused by organic mercury contamination. In: Hartung R, Dinman BD (Editors), *Environmental mercury contamination*. Ann Arbor: Ann Arbor Science; 1972. p 247-289.
2. Bakir F, Damluji SF, Amin-Zaki L, Murtadha M, Khalidi A, al-Rawi NY, et al. Methylmercury poisoning in Iraq. *Science* 1973; 181: 230-241.
3. Kjelstrom T, Kennedy P, Wallis S, Stewart A, Friberg L, Lind B, et al. *Physical and mental development of children with prenatal exposure to mercury from fish. Stage II: Interviews and psychological tests at age 6*. Solna: National Swedish Environmental Protection Board Report; 1989.
4. Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, et al. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol* 1997; 19: 417-428.
5. Grandjean P, Budtz-Jorgensen E, White RF, Jorgensen PJ, Weihe P, Debes F, et al. Methylmercury exposure biomarkers as indicators of neurotoxicity in children aged 7 years. *Am J Epidemiol* 1999; 150: 301-305.
6. Steuerwald U, Weihe P, Jorgensen PJ, Bjerve K, Brock J, Heinzow B, et al. Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. *J Pediatr* 2000; 136: 599-605.
7. Grandjean P, White RF, Weihe P, Jorgensen PJ. Neurotoxic risk caused by stable and variable exposure to methylmercury from seafood. *Ambul Pediatr* 2003; 3: 18-23.
8. Myers GJ, Davidson PW, Shamlaye CF, Axtell CD, Cernichiari E, Choisy O, et al. Effects of prenatal methylmercury exposure from a high fish diet on developmental milestones in the Seychelles Child Development Study. *Neurotoxicology* 1997; 18: 819-829.
9. Davidson PW, Myers GJ, Cox C, Axtell C, Shamlaye C, Sloane-Reeves J, et al. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study. *JAMA* 1998; 280: 701-707.
10. Chen SH, Liu SH, Liang YC, Lin JK, Lin-Shiau SY. Death signaling pathway induced by pyrrolidine dithiocarbamate-$Cu^{(2+)}$ complex in the cultured rat cortical astrocytes. *Glia* 2000; 31: 249-261.
11. Aschner M, Kimelberg HK. *The role of glia in neurotoxicity*. Boca Raton: CRC Press; 1996.
12. Aschner M, Allen JW, Kimelberg HK, LoPachin RM, Streit WJ. Glial cells in neurotoxicity development. *Annu Rev Pharmacol Toxicol* 1999; 39: 151-173.
13. Garman RH, Weiss B, Evans HL. Alkylmercurial encephalopathy in the monkey (*Saimiri sciureus* and *Macaca arctoides*): a histopathologic and autoradiographic study. *Acta Neuropathol* 1975; 32: 61-74.
14. Charleston JS, Body RL, Bolender RP, Mottet NK, Vahter ME, Burbacher TM. Changes in the number of astrocytes and microglia in the thalamus of the monkey *Macaca fascicularis* following long-term subclinical methylmercury exposure. *Neurotoxicology* 1996; 17: 127-138.
15. Aschner M. Methylmercury in astrocytes - what possible significance? *Neurotoxicology* 1996; 17: 93-106.
16. Aschner M, Yao CP, Allen JW, Tan KH. Methylmercury alters glutamate transport in astrocytes. *Neurochem Int* 2000; 37: 199-206.
17. Allen JW, Mutkus LA, Aschner M. Methylmercury-mediated inhibition of 3H-D-aspartate transport in cultured astrocytes is reversed by the antioxidant catalase. *Brain Res* 2001; 902: 92-100.
18. Allen JW, Shanker G, Aschner M. Methylmercury inhibits the *in vitro* uptake of the glutathione precursor, cystine, in astrocytes, but not in neurons. *Brain Res* 2001; 894: 131-140.
19. Shanker G, Allen JW, Mutkus LA, Aschner M. The uptake of cysteine in cultured primary astrocytes and neurons. *Brain Res* 2001; 902: 156-163.
20. Shanker G, Aschner M. Identification and characterization of uptake systems for cystine and cysteine in cultured astrocytes and neurons: evidence for methylmercury-targeted disruption of astrocyte transport. *J Neurosci Res* 2001; 66: 998-1002.
21. Shanker G, Aschner M. Methylmercury-induced reactive oxygen species formation in neonatal cerebral astrocytic cultures is attenuated by antioxidants. *Brain Res Mol Brain Res* 2003; 110: 85-91.
22. Brookes N, Kristt DA. Inhibition of amino acid transport and protein synthesis by $HgCl_2$ and methylmercury in astrocytes: selectivity and reversibility. *J Neurochem* 1989; 53: 1228-1237.
23. Aschner M, Du YL, Gannon M, Kimelberg HK. Methylmercury-induced alterations in excitatory amino acid transport in rat primary

astrocyte cultures. *Brain Res* 1993; 602: 181-186.
24. Dave V, Mullaney KJ, Goderie S, Kimelberg HK, Aschner M. Astrocytes as mediators of methylmercury neurotoxicity: effects on D-aspartate and serotonin uptake. *Dev Neurosci* 1994; 16: 222-231.
25. Brookes N. *In vitro* evidence for the role of glutamate in the CNS toxicity of mercury. *Toxicology* 1992; 76: 245-256.
26. Matyja E, Albrecht J. Ultrastructural evidence that mercuric chloride lowers the threshold for glutamate neurotoxicity in an organotypic culture of rat cerebellum. *Neurosci Lett* 1993; 158: 155-158.
27. Shanker G, Mutkus LA, Walker SJ, Aschner M. Methylmercury enhances arachidonic acid release and cytosolic phospholipase A2 expression in primary cultures of neonatal astrocytes. *Brain Res Mol Brain Res* 2002; 106: 1-11.
28. Shanker G, Syversen T, Aschner M. Astrocyte-mediated methylmercury neurotoxicity. *Biol Trace Elem Res* 2003; 95: 1-10.
29. Kaltschmidt B, Baeuerle PA, Kaltschmidt C. Potential involvement of the transcription factor NF-kappa B in neurological disorders. *Mol Aspects Med* 1993; 14: 171-190.
30. Hunot S, Brugg B, Ricard D, Michel PP, Muriel MP, Ruberg M, et al. Nuclear translocation of NF-kappaB is increased in dopaminergic neurons of patients with Parkinson disease. *Proc Natl Acad Sci U S A* 1997; 94: 7531-7536.
31. Kitamura Y, Shimohama S, Ota T, Matsuoka Y, Nomura Y, Taniguchi T. Alteration of transcription factors NF-kappaB and STAT1 in Alzheimer's disease brains. *Neurosci Lett* 1997; 237: 17-20.
32. Kehrer JP, Smith CV. Free radicals in biology: sources, reactivities, and roles in the etiology of human diseases. In: Frei B (Editor), *Natural antioxidants in human health and disease*. San Diego: Academic Press; 1994. p 25-62.
33. Halliwell B. Free radicals and antioxidants: a personal view. *Nutr Rev* 1994; 52: 253-265.
34. Betteridge DJ. What is oxidative stress? *Metabolism* 2000; 49: 3-8.
35. LeBel CP, Ischiropoulos H, Bondy SC. Evaluation of the probe 2',7'-dichlorofluorescin as an indicator of reactive oxygen species formation and oxidative stress. *Chem Res Toxicol* 1992; 5: 227-231.
36. Yee S, Choi BH. Oxidative stress in neurotoxic effects of methylmercury poisoning. *Neurotoxicology* 1996; 17: 17-26.
37. Sayre LM, Perry G, Smith MA. Redox metals and neurodegenerative disease. *Curr Opin Chem Biol* 1999; 3: 220-225.
38. Davis KL, Martin E, Turko IV, Murad F. Novel effects of nitric oxide. *Annu Rev Pharmacol Toxicol* 2001; 41: 203-236.
39. Ali SF, LeBel CP, Bondy SC. Reactive oxygen species formation as a biomarker of methylmercury and trimethyltin neurotoxicity. *Neurotoxicology* 1992; 13: 637-648.
40. Sarafian TA, Vartavarian L, Kane DJ, Bredesen DE, Verity MA. bcl-2 expression decreases methyl mercury-induced free-radical generation and cell killing in a neural cell line. *Toxicol Lett* 1994; 74: 149-155.
41. Park ST, Lim KT, Chung YT, Kim SU. Methylmercury-induced neurotoxicity in cerebral neuron culture is blocked by antioxidants and NMDA receptor antagonists. *Neurotoxicology* 1996; 17: 37-45.
42. Sorg O, Schilter B, Honegger P, Monnet-Tschudi F. Increased vulnerability of neurones and glial cells to low concentrations of methylmercury in a prooxidant situation. *Acta Neuropathol* 1998; 96: 621-627.
43. Myhre O, Fonnum F. The effect of aliphatic, naphthenic, and aromatic hydrocarbons on production of reactive oxygen species and reactive nitrogen species in rat brain synaptosome fraction: the involvement of calcium, nitric oxide synthase, mitochondria, and phospholipase A. *Biochem Pharmacol* 2001; 62: 119-128.
44. Dugan LL, Sensi SL, Canzoniero LM, Handran SD, Rothman SM, Lin TS, et al. Mitochondrial production of reactive oxygen species in cortical neurons following exposure to N-methyl-D-aspartate. *J Neurosci* 1995; 15: 6377-6388.
45. Brawer JR, McCarthy GF, Gornitsky M, Frankel D, Mehindate K, Schipper HM. Mercuric chloride induces a stress response in cultured astrocytes characterized by mitochondrial uptake of iron. *Neurotoxicology* 1998; 19: 767-776.
46. Choi DW. Excitotoxic cell death. *J Neurobiol* 1992; 23: 1261-1276.
47. Lafon-Cazal M, Pietri S, Culcasi M, Bockaert J. NMDA-dependent superoxide production and neurotoxicity. *Nature* 1993; 364: 535-537.
48. Aschner M, Mullaney KJ, Wagoner D, Lash LH, Kimelberg HK. Intracellular glutathione (GSH) levels modulate mercuric chloride (MC)- and methylmercuric chloride (MeHgCl)-induced amino acid release from neonatal rat primary astrocytes cultures. *Brain Res* 1994; 664: 133-140.
49. Ballatori N, Lieberman MW, Wang W. N-acetylcysteine as an antidote in methylmercury poisoning. *Environ Health Perspect* 1998; 106: 267-271.
50. Farina M, Frizzo ME, Soares FA, Schwalm FD, Dietrich MO, Zeni G, et al. Ebselen protects against methylmercury-induced inhibition of glutamate uptake by cortical slices from adult mice. *Toxicol Lett* 2003; 144: 351-357.
51. Farina M, Franco JL, Ribas CM, Meotti FC, Missau FC, Pizzolatti MG, et al. Protective effects of *Polygala paniculata* extract against methylmercury-induced neurotoxicity in mice. *J Pharm Pharmacol* 2005; 57: 1503-1508.
52. Moretto MB, Funchal C, Santos AQ, Gottfried C, Boff B, Zeni G, et al. Ebselen protects glutamate uptake inhibition caused by methyl mercury but does not by Hg^{2+}. *Toxicology* 2005; 214: 57-66.
53. Koh AS, Simmons-Willis TA, Pritchard JB, Grassl SM, Ballatori N. Identification of a mechanism by which the methylmercury antidotes N-acetylcysteine and dimercaptopropanesulfonate enhance urinary metal excretion: transport by the renal organic anion transporter-1. *Mol Pharmacol* 2002; 62: 921-926.
54. Clarkson TW, Magos L, Cox C, Greenwood MR, Amin-Zaki L, Majeed MA, et al. Tests of efficacy of antidotes for removal of methylmercury in human poisoning during the Iraq outbreak. *J Pharmacol Exp Ther* 1981; 218: 74-83.
55. Lund ME, Banner W Jr, Clarkson TW, Berlin M. Treatment of acute methylmercury ingestion by hemodialysis with N-acetylcysteine (Mucomyst) infusion and 2,3-dimercaptopropane sulfonate. *J Toxicol Clin Toxicol* 1984; 22: 31-49.
56. Kumagai Y, Mizukado S, Nagafune J, Shinyashiki M, Homma-Takeda S, Shimojo N. Post-transcriptional elevation of mouse brain Mn-SOD protein by mercuric chloride. *Brain Res* 1997; 769: 178-182.
57. Hsieh HJ, Cheng CC, Wu ST, Chiu JJ, Wung BS, Wang DL. Increase of reactive oxygen species (ROS) in endothelial cells by shear flow and involvement of ROS in shear-induced c-fos expression. *J Cell Physiol* 1998; 175: 156-162.
58. Goering PL, Fisher BR, Noren BT, Papaconstantinou A, Rojko JL, Marler RJ. Mercury induces regional and cell-specific stress protein expression in rat kidney. *Toxicol Sci* 2000; 53: 447-457.
59. Rosenberger J, Petrovics G, Buzas B. Oxidative stress induces proorphanin FQ and proenkephalin gene expression in astrocytes through p38- and ERK-MAP kinases and NF-kappaB. *J Neurochem* 2001; 79: 35-44.
60. Chen F, Shi X. Signaling from toxic metals to NF-kappaB and beyond: not just a matter of reactive oxygen species. *Environ Health Perspect* 2002; 110 (Suppl 5): 807-811.