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Behavioral Effects of Developmental Methylmercury Drinking Water Exposure in Rodents

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Abstract

Early methylmercury (MeHg) exposure can have long-lasting consequences likely arising from impaired developmental processes, the outcome of which has been exposed in several longitudinal studies of affected populations. Given the large number of newborns at an increased risk of learning disabilities associated with *in utero* MeHg exposure, it is important to study neurobehavioral alterations using ecologically valid and physiologically relevant models. This review highlights the benefits of using the MeHg drinking water exposure paradigm and outlines behavioral outcomes arising from this procedure in rodents. Combination treatments that exacerbate or ameliorate MeHg-induced effects, and possible molecular mechanisms underlying behavioral impairment are also discussed.

Keywords

Mercury; Developmental neurotoxicity; Behavior; Rodents; Learning

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Conflict of Interest Statement

None of the authors have any perceived conflict of interest with the content of this manuscript.

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Introduction

Mercury [Hg] is a global pollutant that knows no environmental boundaries. Even the most stringent control of anthropogenic (Hg) sources will not eliminate exposure given its ubiquitous presence. Exposure to Hg occurs primarily via the food chain due to the accumulation of its methylated form, methylmercury (MeHg), in fish. Latest United States statistics indicate that 46 states have fish consumption advisories. In addition, Hg is a common pollutant in hazardous waste sites, with an estimated 3–4 million children living within one mile of at least one of the 1,300+ active hazardous waste sites in the United States [1]. The effects on intellectual function in children prenatally exposed to MeHg via maternal fish consumption have been the subject of two on-going major, prospective, longitudinal studies in the Seychelles [2–7] and the Faroe Islands [8–11]. A National Academy of Sciences [12] expert panel reviewed these studies, concluding that the weight-of-evidence supports MeHg's adverse health effects, recommending that levels of Hg not exceed 5.0 µg/L in whole blood or 1.0 µg/g in hair, corresponding to a reference-dose (RfD) of 0.1 µg/kg body weight/day. It is important to recognize that the risk for MeHg exposure is *not* limited only to islanders with high fish consumption. The US Environmental Protection Agency's (EPA) Mercury Study Report to Congress [13] estimates that, in the United States, 8% of women of childbearing age have blood Hg concentrations exceeding this RfD. Based on the annual number of births, an estimated 300,000 newborns in the United States alone may be at increased risk of learning disabilities associated with *in utero* MeHg exposure.

MeHg is a highly toxic agent that can cause irreversible damage to the central nervous system (CNS) and, since MeHg easily crosses the placental barrier, the developing brain is especially vulnerable to its adverse effects. Fetuses and neonates are particularly susceptible since MeHg can be transferred both through the placenta and breast milk [14] and the levels of cerebral Hg in fetuses after *in utero* MeHg exposure can be higher when compared with those of the exposed dams [15]. Additionally, exposure to MeHg during early development can be associated with subtle brain damage at levels much lower than those affecting the mature brain [16]. Evidence of developmental neurotoxicity from environmental exposure to MeHg was evident following the Minamata disease epidemic of the 1950s in Japan. Consumption of highly contaminated seafood led to minimal symptoms in pregnant women who later gave birth to infants exhibiting diffuse and severe cortical damage. Children displayed severe neurological disabilities, including impaired vision, speech and hearing, altered gait, paresthesias, mental retardation, and cerebral palsy [17]. Similar symptoms presented in Iraq following poisoning of seed grain used in homemade bread [18]. Populations with high fish intake have been the subjects of several epidemiological studies [see 19 for review]. Briefly, children exposed to MeHg *in utero* in New Zealand and the Faroe Islands exhibited decreased intelligence quotient (IQ) scores and impairment in memory, attention, language and visuospatial perception [20]. These epidemiological studies have reported greater developmental effects in males than in females [21], which are in line with experimental studies showing lower sensitivity of female mice to MeHg when compared with males [22], as well as the protective effect of 17β-estradiol against MeHg-induced neurotoxicity in male mice [23, 24]. In contrast, a longitudinal study of the

Seychelles showed no evidence of developmental effects on cognition following prenatal MeHg exposure [25].

Animal models have been useful for studying the deleterious effects of MeHg across the lifespan. Although the neurological, sensory, and motor deficits observed in humans have been replicated in primates following developmental or adult exposure [26, 27], the current paper will focus on the neurobehavioral effects of MeHg exposure in rodents. In particular, studies using the MeHg drinking water exposure paradigm will be highlighted, as this *in vivo* procedure parallels the most common form of prenatal exposure in humans.

MeHg Drinking Water Exposure Paradigm

The MeHg drinking water exposure paradigm allows the administration of chronic, low dose MeHg to pregnant dams during periods of gestation and lactation, leading to indirect developmental exposure of MeHg to offspring. Prolonged exposure to low doses of MeHg (typically methylmercuric chloride in tap water) through drinking water is constant and maternally mediated, mimicking potential exposure occurring in human infants. Drinking water provides a convenient means for exposure because 95% of MeHg can be absorbed from the gastrointestinal (GI) tract [28] with its continuous cycling via the enterohepatic circulation. A disadvantage of this procedure is that because water is available *ad libitum*, the amount of consumption is not fixed. Daily and cumulative intake is determined both by the experimentally controlled concentration and individual differences in fluid consumption, which can also vary between phases of pregnancy [29]. Daily water consumption measurements are necessary to account for individual differences in fluid intake between subjects, and the addition of an empty cage containing a filled water bottle is useful for measuring fluid loss through spillage or leakage. It is also important to avoid high concentrations of MeHg in drinking water because of its metallic taste, which can cause decreased liquid consumption and, consequently, dehydration in long-term or chronic studies. Experimental evidence has shown that the liquid ingestion of MeHg-exposed Swiss adult mice is not different from controls when MeHg concentrations do not exceed 40 mg/L [30].

Length of exposure varies among studies, with some reports of exposure beginning 3 to 7 weeks before mating [29, 31–34], and others focusing on gestation [35, 36] and lactation [37–42]. Some reports of exposure beginning prenatally and continuing throughout the lifespan have also been undertaken [43, 44].

Hg Levels in Blood and Brain

In choosing MeHg treatment paradigms, an important consideration is the estimated blood and brain levels of Hg following the exposure protocol, as well as the extent to which levels are physiologically relevant to human exposures noted in the clinical literature. The concentration of Hg in blood and brain can be used as a biomarker of exposure and allows comparison of adverse effects across studies and different species [29]. *In vivo* rodent studies demonstrated at birth neuropathologic damage and neurobehavioral alterations at brain [Hg] of 4.5 and 0.5 mg/kg, respectively [45]. Perinatal MeHg treatment resulted in neonatal rat brain [Hg] of 3–11 mg/kg [46]. In rats, behavioral alterations after continual

pre- plus postnatal exposure [until postnatal day (PND 16)] to 40 µg MeHg/kg/day led to brain [Hg] of 0.5 mg/kg at birth and 0.04 mg/kg at weaning [47]; 6 months exposure to 0.5 mg/kg MeHg in drinking water resulted in brain [Hg] of 5 mg/kg [48]. In children, delayed psychomotor development occurred at brain [Hg] < 3 mg/kg [46]. The threshold for observable clinical effects approximated brain [Hg] of 1 mg/kg [46] and the lowest-observed adverse effects level is at 0.5–1.0 mg/kg [Hg] [49]. In Seychelloid asymptomatic neonates, brain total [Hg] ranged from 0.026 to 0.295 mg/kg [50]. [Hg] of 0.1–0.4 mg/kg were detected in infants from Minamata [51, 52] and in two Iraqi babies who died from *in utero* MeHg intoxication, brain [Hg] were ~1 and 13.7 mg/kg [53].

Newland and Reile [29] assessed the quantitative relationship between blood and brain [Hg] following the MeHg drinking water exposure paradigm in rat neonates (PND 0) and weanlings (PND 21). Using this procedure, 4.5-month-old dams were exposed to MeHg for 4 or 7 weeks prior to mating and throughout gestation up to PND 16, in order to avoid direct exposure to the pups once they were able to drink from the water bottle. Blood and brain [Hg] were higher than those reported for less chronic exposure paradigms and paralleled maternal consumption during gestation but not during lactation. Brain [Hg] in offspring decreased between birth and weaning from 0.49 to 0.045 mg/kg in the low-dose (0.5 mg/kg) and from 9.8 to 0.53 mg/kg in the high-dose (6 mg/kg) groups. These brain [Hg] are well within those described in the human literature detailed above. MeHg concentrations of 0, 0.5, or 6 mg/kg resulted in exposures approximating 0, 40 or 400 µg/kg/day for the 3 groups, respectively. This protocol reproduces [Hg] levels that are toxicologically relevant to the human exposures detailed above, although indirect exposure to offspring may be more reliable during gestation than through nursing. Similarly, Stern et al. [43] measured blood and brain [Hg] concentrations following exposure to 0, 1, or 3 mg/kg MeHg across the mouse lifespan, with only half of the subjects continuing exposure post-weaning. Blood and brain levels closely resembled maternal consumption shortly following birth (PND 4) but concentrations declined between PND 4 and 21, with a greater decrease occurring in the perinatal only exposure group compared to the lifetime exposure group. Between months 14 and 26, brain [Hg] increased modestly in the 1 mg/kg group, but both brain and blood [Hg] increased significantly in the 3 mg/kg group. Therefore, MeHg exposure through drinking water is an effective way to increase [Hg] to toxicologically relevant levels, either through direct or indirect exposure, across the rodent lifespan.

Neurobehavioral Effects

Sensory and Motor Deficits

The relationship between MeHg-induced motor deficits and cerebellar damage in rodents has been well described [54, 55], including impaired rotarod performance [55], open-field activity, motor coordination, retarded or abnormal walking ability, delayed development of swimming ability, hind-limb dysfunction, delayed spatial alternation, radial arm maze learning and severe movement and postural disorders [see 56 for review]. Some of these effects are sex-specific [37] and the type and severity of the behavioral changes are dose- and time- dependent, with the most deleterious effects occurring subsequent to exposure in late gestation [57, 58]. These behavioral deficits resemble symptoms observed in humans in

their scope, developmental timing, and affected systems [12]. Although some studies using the MeHg drinking water exposure paradigm during gestation support MeHg-induced alterations in offspring on measures of motor coordination [40, 44], somatosensory sensitivity [59] and locomotor activity [35, 37, 41], others have noted no effect in these areas, with rotarod performance (a measure of motor coordination) being the most inconsistent [35, 37, 38, 41].

Several studies have examined the effects of MeHg exposure on developmental milestones and reflexes, with mixed results. Dose- and time-dependent impairment on measures of grip strength, hind-limb cross (clasp reflex), and flexion have been found in rats following chronic exposure to 0.5, 5, or 15 mg/kg MeHg in drinking water for 16 months [59]. MeHg exposure through maternal drinking water and continuing until weaning (0.5 or 10 mg/kg) led to delayed negative geotaxis, an orienting response considered diagnostic of vestibular and/or proprioceptive function [38, 41, 60]. In contrast, rats exposed *in utero* to 0.5 or 5 mg/kg MeHg doses did not exhibit any deficits on measures of surface righting, elevation of head, gait, eye opening, onset of walking, startle reflex, or negative geotaxis [61].

Learning and Memory

Learning and memory deficits are a concern following developmental exposure to MeHg, as prospective studies of clinical populations have reported impaired scores on neuropsychological tests of memory, attention, and language [20]. Preclinical evaluation of a variety of learning and memory assays has revealed task-dependent effects following *in utero* low dose MeHg exposure through maternal drinking water (see Table 1). For instance, no significant impairment was found in mice or rats on measures of spatial memory as assessed by the Morris water maze or Y-maze [35, 38, 40, 41], reference memory as assessed by the modified T-maze [37], object discrimination [38], or passive avoidance [38]. Working memory impairment on the modified T-maze was noted for females, but not males, at 6 and 8 mg/kg MeHg pre- and postnatal exposure [37].

Although little evidence supports impairment on preclinical assays reflecting memory processes, findings support a greater role for early MeHg exposure on operant behavior tasks assessing acquisition of a response-reinforcer relationship [see 62 for review]. In general, effects are most robust on tasks related to resistance to choice, behavioral flexibility versus perseveration, and reinforcement efficacy [31, 33, 34, 61, 63–66], and behavioral effects are evident on several schedules of reinforcement.

Compared to controls, rats *in utero* exposed to MeHg (0.5 or 5 mg/kg) displayed significantly higher response rates under large fixed ratio (FR) schedules of reinforcement [61]. Typically, high rates of responding decrease as the FR requirement, or number of responses required for receiving a reinforcer, increases. A lack of ratio strain in the MeHg exposed group indicated by an increasing rate of responding is indicative of perseveration. Alterations in response patterns have also been noted on fixed interval (FI) schedules of reinforcement, in which the first response after a specified period of time is reinforced [31]. Similarly, rats exposed to the same MeHg exposure procedure showed no deficit in acquisition of a differential reinforcement of high-rate behavior (DRH) schedule of reinforcement [34], which reinforces short inter-response time between responses, but

showed delayed acquisition of a differential reinforcement of low-rate behavior (DRL) schedule of reinforcement, which reinforces long inter-response time between responses [61]. Perseveration of high-rate operant behavior was particularly evident when responding on a DRH schedule was stabilized before switching to a DRL, suggesting that perinatal MeHg exposure impairs response inhibition and interferes with the acquisition of new behavior [34]. Rats exposed to MeHg through drinking water during gestation also demonstrated impaired response inhibition and preservation on spatial and visual discrimination reversal procedures [63, 64], as well as impaired acquisition of choice between concurrently available reinforcement schedules [66]. A motivational explanation for such behavioral changes arises from MeHg-induced increases in breakpoint, or maximum ratio obtained, under a progressive ratio (PR) schedule of reinforcement, since breakpoint is used as a measure of reinforcer efficacy [61, 65]. It has been proposed that a MeHg-induced enhancement of reinforcer efficacy may lead to behavioral rigidity [65].

Maternal exposure to low level MeHg produced deranged and reduced outgrowth of neurites in the cerebral cortex of offspring [67], a possible mechanism for the long-lasting behavioral consequences observed following gestational MeHg exposure. In addition, a role for disrupted dopamine neurotransmission is supported by a selective increased sensitivity of behavior to cocaine under a FI schedule of reinforcement following gestational MeHg (5 mg/kg) exposure [33].

Emotional behavior, which can influence learning and memory processes, has also been assessed in rodents. While no effect was noted on the elevated plus maze [38], a measure of anxiety, evidence of depression-like behavior has been reported. Perinatal exposure to low dose (0.5 mg/kg/day) MeHg through maternal drinking water led to significantly longer immobility time on the forced swimming test and the tail suspension test [35], which was later reversed by chronic treatment with the antidepressant fluoxetine [68].

Antagonistic, Additive or Synergistic Interactions

The MeHg drinking water exposure paradigm is useful for examining the interaction (antagonistic, additive or synergistic) between MeHg and other contaminants or nutrients. The interaction between MeHg and perfluorooctanoic acid (PFOA) has been examined, as both contaminants are found in the same food sources [41]. Rats from dams receiving drinking water containing both MeHg and PFOA (10 mg/kg of each) had decreased [Hg] concentrations in all tissue, compared to the MeHg only group. The combination treatment also blocked a MeHg-induced increase in locomotor activity. Interactions can also be examined by manipulating diet along with the addition of MeHg to the drinking water. The effect of polychlorinated biphenyls (PCBs), also found in fish, combined with MeHg was evaluated through MeHg (0.5 mg/kg/day) in maternal drinking water and PCB126 (100 mg/kg/day) in food. Although assessment included developmental milestones, attention, spatial learning, motor coordination, object discrimination, anxiety, conditioned learning, and locomotor activity, hyperactivity in female offspring was the only difference found in the combination treatment group at 4 months of age [38]. This effect persisted when rats were tested at 20 months of age [39].

The evaluation of MeHg administered along with nutrients has revealed mixed results. Along with MeHg drinking water (0.5 or 5 mg/kg), fish oil or coconut oil was added to the diet of pregnant dams in order to examine the interaction between MeHg and *n*-3 fatty acids, which are found in fish and important for neural development [61]. Neither diet modified the effects of MeHg. A purified diet containing selenium (Se), an essential nutrient required for the activity of many antioxidant enzymes [69], significantly delayed or blunted the effects of MeHg on measures of somatosensory sensitivity, grip strength, hind-limb cross, flexion, and voluntary wheel-running when administered 100 days before exposure to MeHg drinking water in adult rats [59]. However, studies examining the effects of an identical Se diet (0.06 or 0.6 mg/kg) fed to pregnant dams on *in utero* exposure to MeHg through drinking water have reported no effect of Se diet on MeHg-induced behavioral alterations [31, 34]. *Chlorella*, a unicellular green algae that has been eaten as a nutritional food in Japan, enhances the tissue elimination of MeHg through stimulation of excretion [70, 71]. In addition, along with MeHg drinking water, continuous intake of 10% *chlorella* powder (CP) in the diet of mice for 4 weeks before mating through birth suppressed MeHg transfer to offspring, with both mothers and neonates in the CP diet group showing significantly lower blood and brain [Hg], compared to controls, 24h after birth [72]. No measures of behavior were included in this study.

MeHg-Induced Oxidative Stress and Nrf2-Dependent Neuroprotection

The deleterious effects of pre- and postnatal MeHg exposure may be due to transitory or permanent neurochemical changes during critical periods of brain development. However, the precise connection between these neurochemical changes and behavioral deficits is still elusive. Nevertheless, there is strong evidence linking MeHg toxicity and oxidative damage [73, 74]. In the developing brain, the glutathione (GSH) antioxidant system has been identified as a possible molecular target for MeHg toxicity. MeHg (1, 3, or 10 mg/l) in the drinking water of pregnant mice during gestation led to a dose-dependent inhibition of normally increasing GSH, glutathione peroxidase (GPx), and glutathione reductase (GR) in offspring [36]. A developmental increase in these antioxidant enzymes observed during the early postnatal period is proposed to protect the brain from a surge in oxygen concentration, and increased reactive oxygen species (ROS) generation, after delivery [75]. Increases in cerebral F₂-isoprostane levels suggest that MeHg-induced disruption of the GSH system is correlated with increased lipid peroxidation in the developing brain. Alterations in the GSH system remained even after brain [Hg] levels decreased, leading to MeHg-induced long-term disruption of the GSH antioxidant system and pro-oxidative damage, rendering the brain more susceptible to the deleterious effects of ROS [36].

In addition to oxidative stress, glutamate dyshomeostasis has also been reported as an important event related to the deleterious neurotoxic effects elicited by MeHg during the perinatal period. Using the MeHg drinking water exposure paradigm in mouse dams, Manfroi et al. [42] investigated the exclusive contribution of MeHg exposure through maternal milk. The authors showed that the exposure of lactating mice to MeHg causes inhibition of glutamate uptake in the offspring's cerebella, although this event was not observed in dams. The inhibition of glutamate uptake was correlated with increased oxidative stress, which is in line with the idea that MeHg-dependent glutamatergic

hyperstimulation might result in excitotoxicity, leading to calcium dyshomeostasis and increased ROS generation [see 76 for review].

Evidence also supports a potential role for Nrf2 and the PI3K/Akt pathway in mediating MeHg-induced neurotoxicity (see Figure 1), particularly during embryonic development [77–83]. Nrf2 is a leucine zipper transcription factor belonging to the cap ‘n’ collar family; it is involved in the induction of genes encoding antioxidant proteins, including those in the GSH family. When bound to its inhibitory protein Kelch-like ECH-associated protein 1 (Keap1), Nrf2 is targeted for ubiquitination. Upon exposure to oxidative stress, Nrf2 is liberated from Keap1. Keap1’s phosphorylation by protein kinase C (PKC) at serine 40 promotes Nrf2 dissociation and nuclear translocation [84, 85]. Nrf2 heterodimerizes with the small Maf family members of transcription factors and binds to the cis-acting antioxidant antioxidant response element (ARE) sequence in the regulatory regions of target genes encoding detoxifying and antioxidant enzymes/proteins, such as g-glutamylcysteine ligase (GCL), GSH transferase (GST) A1 and A2, GSH peroxidase (GPx), heme oxygenase (HO), NAD(P)H:quinone reductase and the glutamate (Glu)-Cys exchanger (Xc⁻) [86]. Nrf2-knockout mice show increased sensitivity to a variety of pharmacological and environmental toxicants [86] and inhibition of SKN-1 (the mammalian homologue of Nrf2 in *C. elegans*) leads to shortened lifespan and increased sensitivity to stress [87]; *skn-1* deletions or loss-of-function mutations also suppress oxidative stress resistance in the nematode [88]. Nrf2 activation directly inhibits Fas-mediated apoptosis, a substrate for caspase-3-like proteases and an effector of PK-like ER kinase-mediated cell survival [89].

A crosstalk exists between the protein kinase pathways and the Nrf2-dependent antioxidant system [90], with Nrf2 function controlled upstream by the PI3K/Akt pathway. Consistent with antioxidant response element (ARE)-driven gene expression, sub- μ M MeHg concentrations trigger ROS production and increase PI3K activity and its downstream effector, phospho-Akt (p-Akt) [91]. PI3K is activated by a G-protein-coupled receptor or receptor tyrosine kinase, such as the insulin receptor (Ins-R) [92]. Once activated, PI3K phosphorylates PtdIns(4,5)P₂ to form PtdIns(3,4,5)P₃. Akt functions downstream of PI3K and is activated by phosphorylation at Thr308 and Ser473 [92]. There are three Akt isoforms (Akt 1, 2 and 3), members of the serine/threonine-specific protein kinase family [92]. Results from knockout and transgenic mice demonstrate that Akt isoforms play critical roles in development [93]. Depending on the tissue-specific promoters used to construct the transgenic mice, the animals with constitutively active Akt develop tissue hypertrophy and tumors [94]. Akt knockouts show defects in placental development [95], glucose metabolism, adipogenesis [96], and brain development [95, 97]. The three Akt isoforms show tissue specificities with Akt1 being highly expressed in the brain [95, 97]. Mice with targeted disruption of Akt3 demonstrate a distinct phenotype with a 30% reduction in brain volume and neonatal lethality [98]. The latter observations are consistent with MeHg’s widespread and diffuse damage in fetal and neonatal brain, characterized by hypoplastic and symmetrical brain atrophy, and reflective of aberrant cell division, migration, differentiation and synaptogenesis [17, 99–102].

Although a potential role for Nrf2 and the PI3K/Akt pathway in mediating MeHg-induced neurotoxicity has been indicated in the literature, the majority of results were obtained *in*

vitro (see above). Rand and collaborators [78] elegantly demonstrated in intact flies an indirect role for a neuroprotective effect of Nrf2 against MeHg. In this study, they demonstrated that the ectopic expression of Nrf2 decreased the morphological deleterious effect caused by MeHg exposure during critical periods of nervous system development in *D. melanogaster* embryo [78]. Thus, detailed studies with rodents are needed to establish a clear connection between MeHg exposure during critical periods of development with transitory or permanent changes in Nrf2 and the PI3K/Akt pathway and long-lasting morphological and behavioral deleterious effects in young and adult animals.

Conclusion

Early MeHg exposure can have long-lasting consequences likely arising from impaired developmental processes, the outcome of which has been exposed in several longitudinal studies of affected populations. Given the large number of newborns at an increased risk of learning disabilities associated with *in utero* MeHg exposure, it is important to study neurobehavioral alterations using an environmentally valid model/s. The MeHg drinking water exposure paradigm allows *in vivo* analysis of developmental exposure to MeHg at physiologically relevant levels.

The extent of neurobehavioral alterations found in rodents following perinatal MeHg exposure is task-dependent, with evidence in support of sensory, motor and learning deficits. Of particular interest are operant behavior tasks related to behavioral flexibility and reinforcement efficacy, which have important clinical implications for the acquisition of new information and rate of learning. Assessment at multiple time points should be undertaken to examine for how long these deficits persist. Furthermore, the majority of studies on MeHg-induced neurobehavioral function have used metal concentrations that are closely related to environmentally toxic levels. Studies with lower levels of exposure, approximating relevant exposures in the general population, are sparse and have not been systematically addressed. In addition, it is not known how genetic variation segregates with the severity of MeHg-induced effects. Possible therapeutic strategies for ameliorating these adverse effects can be examined using the drinking water exposure procedure. This model also allows for analysis of cellular and molecular correlates of behavioral impairment, such as alterations in the GSH antioxidant system, oxidative stress and glutamate dyshomeostasis. Future studies correlating biochemical and behavioral endpoints would be a valuable addition to the growing literature of MeHg-induced developmental neurotoxicity.

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Figure 1.

Schematic of MeHg -induced oxidative stress and Nrf2-dependent neuroprotection. MeHg causes increased production of reactive intermediates and GSH depletion. These events activate Nrf2 and upregulate the transcription of downstream antioxidant genes. Other signaling pathways, such as the PI3K/Akt, can modulate Nrf2 function. Variations in these upstream pathways determine susceptibility to MeHg.

Table 1

Neurobehavioral effects caused by developmental MeHg exposure through maternal drinking water in rodent models

Animal model	Exposure time	Dose	Behavioral effects in offspring	References
Rat, Long-Evans	Se diet 3 weeks before MeHg exposure; MeHg exposure 2.5 weeks before mating to PND 16	0.5 or 5 mg/kg MeHg alone or in combination with 0.06 or 0.6 mg/kg Se diet	alteration in response patterns under FI schedule; increased responding (5 mg/kg); no effect of Se diet on MeHg-induced behavioral alterations	Reed & Newland (2007)
Rat, Long-Evans	Se diet 3 weeks before MeHg exposure; MeHg exposure 2.5 weeks before mating to PND 16	0.5 or 5 mg/kg MeHg alone or in combination with 0.06 or 0.6 mg/kg Se diet	no impairment in acquisition of DRH schedule; perseveration of high-rate operant behavior; no effect of Se diet on MeHg-induced behavioral alterations	Newland et al. (2013)
Mouse, ARE-hPAP Tg	GD 7 to PND 7	0.5 mg/kg	↓ exploratory activity; no impairment in spatial learning or motor coordination; ↑ immobility time on forced swimming test and tail suspension test	Onishchenko et al. (2007)
Mouse, C57BL/6	GD 2 to PND 21	4, 6 or 8 mg/kg	↓ locomotor activity in novel open field (females); no impairment in motor coordination or reference memory; ↓ working memory on modified T-maze (6 and 8 mg/kg, females)	Goulet et al. (2003)
Rat, Wistar	GD 7 to PND 21	0.5 mg/kg MeHg alone or in combination with 100 mg/kg PCB126 diet	delayed negative geotaxis (females); no impairment in spatial memory, object discrimination, motor coordination, passive avoidance or anxiety; hyperactivity in females (MeHg and PCB126 combination)	Vitalone et al. (2008, 2010)
Rat, Wistar	GD 1 to PND 21	1, 5 or 10 mg/kg	impairment in motor coordination (5 mg/kg); no impairment in spatial memory	Fujimura et al. (2012)
Rat, Wistar	GD 1 to PND 21	10 mg/kg MeHg alone or in combination with 10 mg/kg PFOA	↑ locomotor activity; no impairment in motor coordination or spatial learning; delayed negative geotaxis; blocked MeHg-induced ↑ in locomotor activity (MeHg and PFOA combination)	Cheng et al. (2013)
Mouse, B6C3F1 x CBA/J	4 weeks before breeding to PND 13 or throughout lifetime	1 or 3 mg/kg	altered hind-limb splay distance; ↓ acquisition of choice behavior; altered wheel running (3 mg/kg, lifetime)	Weiss et al. (2005)
Rat, Long-Evans	Diet 3 weeks before MeHg exposure; MeHg exposure 2 weeks before breeding to PND 16	0.5 or 5 mg/kg MeHg alone or in combination with fish oil or coconut oil diet	no impairment of developmental milestones; ↑ response rates on large FR schedules (perseveration); ↓ acquisition of DRL; ↑ breakpoint on PR schedule; no effect of fish oil or coconut oil diet	Paletz et al. (2006)
Rat, Long-Evans	Se diet 3 weeks before MeHg exposure; MeHg exposure 2.5 weeks before mating to PND 16	0.5 or 5 mg/kg MeHg alone or in combination with 0.06 or 0.6 mg/kg Se diet	↑ error rate and perseverative responding on spatial discrimination reversal procedure; no effect of Se diet	Reed et al. (2006)
Rat, Long-Evans	Diet 3 weeks before MeHg exposure; MeHg exposure 2 weeks before breeding to PND 16	0.5 or 5 mg/kg MeHg alone or in combination with fish oil or coconut oil diet	↑ error rate and perseverative responding during the first reversal on both spatial and visual discrimination reversal procedures; no effect of fish oil or coconut oil diet	Paletz et al. (2007)
Rat, Long-Evans	Se diet 3 weeks before MeHg exposure; MeHg	0.5 or 5 mg/kg MeHg alone or in combination	↑ breakpoint on PR schedule; enhanced reinforcer efficacy (5 mg/kg MeHg, 0.6 mg/kg Se)	Reed et al. (2008)

Animal model	Exposure time	Dose	Behavioral effects in offspring	References
	exposure 2.5 weeks before mating to PND 16	with 0.06 or 0.6 mg/kg Se diet		
Rat, Long-Evans	4 weeks before mating to PND 16	0.5 or 6.4 mg/kg	↓ acquisition of choice between concurrently available reinforcement schedules	Newland et al. (2004)

PND postnatal day; *GD* gestational day; *FI* fixed interval schedule of reinforcement; *DRH* differential reinforcement of high-rate behavior; *FR* fixed ratio schedule of reinforcement; *DRL* differential reinforcement of low-rate behavior; *PR* progressive ratio schedule of reinforcement