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Assessing pre/post-weaning neurobehavioral development for perinatal exposure to low doses of methylmercury

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ABSTRACT

Fetuses and neonates are known to be high-risk groups for Methylmercury (MeHg) exposure. MeHg can be transferred to the fetus through the placenta and to newborn offspring through breast milk. The aim of the present study was to investigate the neurotoxic effects of low doses of MeHg (1 and 5 μg/mL in drinking water) administration, from gestational day 1 to postnatal day (PND) 21, on the neurobehavioral development of rats. The results showed that the no-observed-effect level of MeHg is somewhere in the range of 1–4 μg/mL. Neurobehavioral development analysis revealed a delayed appearance of cliff drop and negative geotaxis reflexes in the 5 μg/mL MeHg exposure group. Developmental exposure to MeHg affected locomotor activity functions for the females, but not for the males, implying that the female pups were more vulnerable than the male pups. All pups exposed to 5 μg/mL of MeHg showed a significant deficit in motor coordination in the rotarod test compared with controls, and the highest accumulated concentrations of Hg were found in the cerebellum, followed by the hippocampus and cerebral cortex, indicating that the cerebellum is a possible target for MeHg toxicity. We demonstrated adverse effects of developmental exposure to MeHg associated with tissue concentrations very close to the current human body burden of this persistent and bioaccumulative compound.

Introduction

Methylmercury (MeHg) is an important environmental toxicant that causes neurological and developmental impairment in both humans and animals. Fetuses and neonates are known to be high-risk groups for MeHg exposure. MeHg can be transferred to the fetus through the placenta and to offspring through breast milk (Sakamoto et al., 2007; Montgomery et al., 2008; Nyland et al., 2011). Due to the recycling of mercury from the environment, it is common to ingest small quantities of mercury due to consumption of contaminated freshwater fish and seafood (Grandjean et al., 2001; Knobeloch et al., 2007; Ulrich et al., 2007; Chumchal et al., 2010). Of the different routes of exposure, most humans are exposed to Hg by ingestion of food and/or water contaminated with MeHg. According to Cheng et al. (2009) there is a wide variation in hair Hg concentrations between the fathers, mothers and children of the same household, which is probably related to the quantity, frequency and type of fish consumed. High levels of MeHg are found often in large predatory freshwater and saltwater fish, such as northern pike, salmon, swordfish, tuna, and shark (Simmonds et al., 2002). Accumulation of Hg in tissues of these fish is related primarily to the predatory nature and the longevity of these

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fish in contaminated waters. For example, previous studies estimated that as many as 500,000 babies born yearly in the U.S. have cognitive deficits that may be linked to perinatal MeHg exposure (Trasande et al., 2006; Montgomery et al., 2008; Dórea et al., 2011; Gao et al., 2007) and have found that there is a neurodevelopmental risk for males from perinatal MeHg exposure resulting from fish consumption. Therefore it is important to obtain more information regarding the neurotoxic effects of perinatal low dose MeHg exposure, particularly at dosages that approximate human consumption, on the development of offspring.

A number of experimental studies have been conducted in mice and rats, in order to evaluate the potential noxious effects of developmental exposure to MeHg on developing and adult organisms. The results indicate that MeHg can cause persistent disturbances in spontaneous motor behavior and dysfunction in learning and memory in adult mice and rats (Goulet et al., 2003; Grotto et al., 2011). However, the effects of MeHg on neurobehavioral development are not well defined. Therefore, the specific aims of this study were to investigate whether exposure to low doses of MeHg can induce developmental neurotoxic effects. The MeHg doses (1 and 5 μg/mL in drinking water) were chosen based on data showing that 5 μg/mL of MeHg did not induce any typical poisoning symptoms or histopathological findings in adult rats, even if they continued ingesting it for over 2 years (Eto et al., 1997; Yasutake et al., 1997). Therefore, we assumed that the dose level was moderate for adolescent rats in this experiment (Sakamoto et al., 2002). The mothers continued to be given the diet after parturition, and thereby their offspring were exposed to MeHg through breast milk until weaning. This study was designed mainly to determine the changes in MeHg levels along with pre/post-weaning neurobehavioral development of male and female pups. We also want to demonstrate adverse effects of developmental exposure to MeHg associated with tissue concentrations very close to the current human body burden of this persistent and bioaccumulative compound. The GD 1 to PND 21 period of administration was chosen because it spans from the formation of the first central nervous system areas to weaning (PND 21), when indirect exposure to the compounds through the mother ends (Rice and Barone, 2000). Furthermore, this prolonged developmental exposure is constant and maternally-mediated, like that occurring in human infants.

1. Materials and methods

1.1. Animals and treatments

All experimental procedures involving animals were performed in compliance with the National Institute of Minamata Disease (NIMD) on the care and use of laboratory animals. Wistar rats (9 females and 9 males, 10 weeks old) were supplied from Central Institute for Experimental Animals (CLEA) Japan. Amphimixis was allowed after 3 days of acclimatization. Females were inspected daily for the presence of the vaginal plug (gestational day, GD 0). On GD 1, the males were removed and 9 females were randomly divided in three treatment groups including 0 (control), 1 and 5 μg/mL MeHg. MeHg dissolved at concentrations of 1 and 5 μg/mL, respectively, was administered daily to rats in their drinking water, from GD 1 to postnatal day (PND) 21. On PND 22, the litters were culled to 10–12 pups and weaned.

On PND 40, two male and two female pups randomly selected from each litter of each group were euthanized for Hg analysis. The pups for Hg analysis were perfused *via* the ascending aorta with phosphate buffer after the blood sample was collected. All brains were immediately removed and dissected over ice-cold glass slides to remove the cerebellum, cerebral cortex and hippocampus. Liver and kidney samples were also collected and washed repeatedly in ice-cold physiological saline for Hg analysis. The tissues for Hg analysis were frozen at –20°C until assay.

1.2. Assessment of pre-weaning neurobehavioral development

Every 2 days, from PND 3 to 21, six pups randomly selected from each litter of each group were used for postnatal assessment of neurobehavioral development. The following reflexes were scored (Branchi et al., 2002): *Righting reflex*: pup was placed on its back on a flat surface and the time to turn over with all four paws was recorded, with a cut-off time of 2 sec. *Cliff avoidance*: pup was positioned on the edge of a bench, with its forepaws and nose just over the edge. The time of withdrawal of head and both forefeet was recorded, with a cut-off time of 2 sec. *Negative geotaxis*: pup was placed on a 45° angle slope with its head downwards, and the percent success rate and the time necessary to turn around 180° were recorded, with a cut-off time of 5 sec.

1.3. Assessment of post-weaning neurobehavioral development

1.3.1. Motor coordination

For the evaluation of coordination and balance, the rotarod test was performed with three trials per day for two consecutive days from PND 34–35. The apparatus (Natsume, Tokyo, Japan) consisted of a bar, 8 cm in diameter and 10 cm long, which rotated at 15 r/min. The duration time, that is, the time from when the pup was mounted on the rod until it fell off, was recorded in seconds. The individual performance was cut off at 60 sec (Sakamoto et al., 2002). All pups were tested.

1.3.2. Locomotor activity

The locomotor activity was assessed using an open field on PND 36. Briefly, each pup was moved from its home cage to the center square (5 cm × 5 cm) of the open field (15 cm × 15 cm), and covered with a Plexiglas box (15 cm × 15 cm × 15 cm) for 5 min. The number of line crossings and central square entries was scored. All pups were tested.

1.3.3. Spatial learning

To assess spatial learning and memory, all pups were tested in the Y-maze on PND 39. Each pup was placed at the end of one arm (arm A) facing the center of the maze, and allowed to move freely within the maze for a period of 3 min. The total number of arms entered and the order of arm entries were recorded. The total number of arms entered provides an indication of locomotor activity, and the order of arm entries provides a measure of spontaneous alternation behavior and thus working memory (Podhorna and Brown, 2002).

1.4. Hg analysis

Total mercury concentrations were determined according to the oxygen combustion-gold amalgamation method using mercury analyzer MA 2000 (Nippon Instruments, Tokyo, Japan).

1.5. Statistical analysis

The χ^2-test and repeated measure ANOVA were used for the statistical analysis of data from the reflex development and rotarod rotating tests. Student's *t*-test was used for data on locomotor activity, learning and memory and Hg concentrations.

2. Results

2.1. Effects on somatic development

Low dose MeHg did not significantly affect pregnancy duration, proportion of successful deliveries, litter size, or sex ratio. The relative anogenital distance, measured at PND 1, was also not affected by MeHg ($p > 0.05$, data not shown). Pup body weight analysis during the period from PND 1 to 33 revealed that there were no significant differences between exposed groups and controls ($p > 0.05$, data not shown).

2.2. Behavioral effects

2.2.1. Effects on reflex maturation

The righting reflex did not differ among groups (Fig. 1). However, the cliff avoidance and negative geotaxis reflexes were delayed significantly in 5 μg/mL MeHg-exposed offspring relative to controls ($p < 0.05$; χ^2-test).

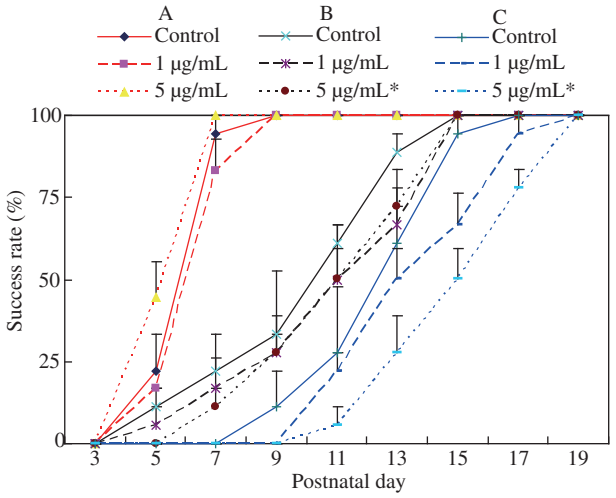

Fig. 1 – Reflex development of pups perinatally exposed to MeHg or vehicle. A: righting reflex; B: cliff avoidance test; C: Negative geotaxis reflexes. Data are represented as mean ± SEM (n = 18 pups/group). * $p < 0.05$ *vs.* control group by χ^2-test.

2.2.2. Effects on motor coordination, locomotor activity and learning and memory

There were no differences between control and 1 μg/mL MeHg-treated offspring in coordination and balance on the rotarod ($p > 0.05$, Table 1). However, both male and female offspring given 5 μg/mL of MeHg had significantly ($p < 0.01$ by χ^2-test) lower success rate compared to the controls. For the locomotor activity test, increased frequency of line crossing and central square entries were observed in MeHg-treated rats (Table 1). However, the number of line crossings and central square entries were significantly different only between the high dose of MeHg-treated female offspring and corresponding controls ($p < 0.05$, by *t*-test). There were no differences among groups of either gender in the learning and memory test on the Y-maze ($p > 0.05$ by *t*-test, Table 1).

2.3. Tissue levels of Hg

Total mercury (T-Hg) levels in different tissues of offspring are shown in Table 2, in which it is shown that T-Hg levels in the blood, liver, kidney, cerebellum and hippocampus of 5 μg/mL MeHg-treated offspring significantly increased compared with those in the controls ($p < 0.01$, by *t*-test). However, the Hg levels in the cerebral cortex did not differ significantly between the exposed groups and controls ($p > 0.05$).

3. Discussion

The present investigation showed that perinatal exposure to the lower dose of MeHg (1 μg/mL) did not produce any behavioral changes compared to the controls. However, the 5 μg/mL dose of MeHg could induce a delayed appearance of the cliff drop and negative geotaxis reflexes (Fig. 1), impairment of motor coordination functions and alteration of female locomotor activity (Table 1), when administered during brain growth. Supporting our data, Goulet et al. (2003) have found that horizontal exploration was reduced and working memory in the modified T maze was impaired in female offspring, following perinatal treatment with MeHg (4, 6, or 8 μg/mL of MeHg in drinking water). Sakamoto et al. (2002) reported that developmental exposure to 5 μg/mL of MeHg could cause a significant deficit in motor coordination in the rotarod test and a learning disability in the passive avoidance response test. Franco et al. (2006) have found that the exposure of lactating mice to MeHg (10 mg/L in drinking water) causes significant impairments in motor performance in the offspring. Roegge et al. (2004) have found that MeHg (0.5 μg/mL in dams' drinking water) did not cause any impairments on the parallel bars or rotating rod tasks of offspring. Our study also showed that perinatal exposure to 0.5 μg/mL of MeHg did not result in any behavioral differences compared to the control (data not shown). This indicates that 1 μg/mL MeHg is the highest dose tested in this type of experimental set up that not give rise to neurobehavioral derangements. Taken together, these findings indicate that the no-observed-effect level (NOEL) is somewhere in the range of 1–4 μg/mL.

Of interest is that some epidemiological studies suggested that boys would be more susceptible than girls to the

Table 1 – Effects on motor coordination, locomotor activity and learning and memory of offspring developmental exposure to MeHg or vehicle from GD 1 to PND 21.

Behavioral effects		Motor coordination [a]	Locomotor activity (frequency) [b]		Learning and memory (Y-maze) [c]	
		Success rate (%)	Line crossing	Central square entries	Total entries (number)	Correct arm entries (%)
Male	Control	88.9 ± 11.3	56.3 ± 4.5	2.2 ± 0.4	18.5 ± 1.1	67.6 ± 3.0
	1 μg/mL	72.2 ± 14.2	71.6 ± 5.5	2.8 ± 0.6	20.8 ± 1.3	68.2 ± 3.3
	5 μg/mL	38.9 ± 11.2*	67.9 ± 5.7	2.6 ± 0.4	17.5 ± 1.3	73.1 ± 2.6
Female	Control	70.0 ± 6.9	67.2 ± 4.0	2.4 ± 0.4	19.1 ± 1.4	69.2 ± 3.3
	1 μg/mL	83.3 ± 0	68.2 ± 6.4	2.4 ± 0.4	22.4 ± 1.8	73.8 ± 2.2
	5 μg/mL	44.4 ± 5.0*	83.6 ± 4.8*	3.8 ± 0.5*	21.8 ± 1.4	71.9 ± 3.1
Male + female	Control	77.5 ± 3.2	61.6 ± 3.1	2.3 ± 0.3	18.8 ± 0.9	68.4 ± 2.2
	1 μg/mL	78.0 ± 7.3	69.9 ± 4.2	2.6 ± 0.3	21.6 ± 1.1*	71.0 ± 2.0
	5 μg/mL	41.7 ± 7.3*	76.5 ± 4.0**	3.1 ± 0.4	19.4 ± 1.0	72.5 ± 2.0

Data are represented as mean ± SEM (n = 18–20 male or/and 16–18 female pups/group). *$p < 0.05$, **$p < 0.01$ vs. control.
GD: gestational day; PND: postnatal day; Methylmercury: MeHg.
[a] Percentages of offspring reaching the criterion of 60 sec on rotarod rotating at 15 rpm with six trials repeated.
[b] The number of line crossing and central square entries of offspring in the open field for five minutes.
[c] Total entries and percentage correct arm entries made in the Y-maze.

neuropathological effects of MeHg developmental exposure (Marsh et al., 1987; Goulet et al., 2003), contrary to our findings. In this study, no significant differences were found in any treated male rats in locomotor activities, while among female pups exposed to 5 μg/mL of MeHg an increased frequency of line crossing and central square entries was observed (Table 1). Our finding is similar with several other animal experiments; Vitalone et al. (2008) have found that with developmental exposure to low doses of MeHg, no significant differences were found in treated male rats in negative geotaxis, while among female pups a delay in achieving reflex maturation was observed. In the outbreak of MeHg poisoning in Japan and Iraq, it was proven that there was not only a prenatal transfer of MeHg through the placenta into the fetus, but also a postnatal exposure of the neonates *via* the breast milk of the dams (Fujita and Takabatake, 1977; Amin-Zaki et al., 1981). Some studies suggested that infants reared on breast milk for a long period might be at increased risk (Nunes and Ferreira, 2000). Goulet et al. (2003) found that MeHg exerted an effect on the performance of females, but not of males, on two of the four measurements. All treated females exhibited less locomotion than control mice when the open field was new, but not in the following four sessions when the environment was becoming increasingly familiar. The mechanisms for these effects are unclear. However, in this study for the 5 μg/mL MeHg-exposed group, the accumulations of mercury in all tissues of female pups including cerebellum, cerebral cortex and hippocampus were higher than those of male pups (Table 2). This provided some possible explanation as to why female pups were more vulnerable than male pups in this study.

The 5 μg/mL MeHg-exposed pups showed significant impairment both in motor coordination (both males and females) and in locomotor activity (females only, Table 1), although Y maze performance showed no apparent abnormalities. The results were similar to other studies. Many studies have found that for rats' developmental exposure to MeHg the motor coordination was affected, but not spatial learning and memory performance (Fredriksson et al., 1996; Sakamoto et al., 2002). Locomotor activity in the open field is difficult to interpret because its precise neurobehavioral significance is not well understood (Goulet et al., 2003). Lesion studies suggest that the nucleus accumbens mediates both locomotor activity and exploration, whereas limbic structures

Table 2 – T-Hg levels in the tissues of rat offspring (ng/g wet weight, mean ± S.E.M).

		Blood	Liver	Kidney	Cerebellum	Cerebral cortex	Hippocampus
Male	Control	98.3 ± 22.2	319.7 ± 18.0	382.3 ± 8.7	313.8 ± 9.4	228.6 ± 47.9	294.3 ± 8.0
	1 μg/mL	145.0 ± 29.1	410.8 ± 78.4	698.1 ± 55.5**	545.8 ± 175.1	352.6 ± 72.1	422.5 ± 31.8*
	5 μg/mL	240.2 ± 17.9**	532.7 ± 81.4*	1355.3 ± 125.4**	492.2 ± 38.7*	409.0 ± 69.3	479.5 ± 23.8**
Female	Control	139.5 ± 23.7	330.0 ± 20.2	462.8 ± 52.8	380.8 ± 25.9	427.0 ± 13.7	309.8 ± 12.3
	1 μg/mL	189.8 ± 4.2	417.0 ± 57.6*	882.0 ± 124.0	415.0 ± 71.1	396.5 ± 38.8	433.5 ± 22.6**
	5 μg/mL	279.5 ± 3.8**	538.2 ± 46.7*	1690.0 ± 325.2*	509.5 ± 28.2*	416.3 ± 96.1	505.0 ± 44.3*
Male + female	Control	118.9 ± 17.2	324.8 ± 12.3	422.6 ± 30.0	347.3 ± 19.4	327.8 ± 49.6	302.1 ± 7.4
	1 μg/mL	167.4 ± 16.6*	413.9 ± 43.6	790.1 ± 73.3**	480.4 ± 89.4	374.6 ± 37.9	428.0 ± 17.6**
	5 μg/mL	259.8 ± 12.0**	535.4 ± 42.0**	1522.7 ± 172.9**	500.8 ± 21.7**	412.7 ± 53.0	492.2 ± 23.2**

n = 3 male or/and 3 female pups/group.
*$p < 0.05$, **$p < 0.01$ *vs.* control by *t*-test.

(amygdala, hippocampal formation, and prelimbic frontal cortex) would modulate the behavioral response to novelty rather than locomotor activity itself (Burns et al., 1996). Damage to the cerebral cortex was correlated with moderate Hg brain doses, whereas damage to the basal ganglia and hippocampus was correlated with high Hg brain doses (Burbacher et al., 1990). In the present experiment, Hg levels in different brain regions of adolescent pups were very low (Table 2), thus, damage to the cerebral cortex was likely during the lactation periods, but nothing can be assumed about basal ganglia and hippocampus. The Y-maze test may reflect the recent memory ability, and multiple brain regions including the hippocampus and amygdala may be associated with this memory function.

The cerebellum has an important role in mediating certain aspects of motor function, including balance and coordination. There is clear evidence that the cerebellum is a possible target for MeHg toxicity (Roegge and Schantz, 2006). Autopsy studies in MeHg-exposed humans found the cerebellum to have the highest levels of total mercury in the brain (Lapham et al., 1995; Pedersen et al., 1999), and cerebellar damage is often reported following MeHg exposure in humans. A number of studies on rodents have reported delayed or abnormal development of the cerebellum after developmental treatment with low doses of MeHg (Goulet et al., 2003). In this study, the motor impairments observed are consistent with other studies in which rodents were exposed to moderate to high doses of MeHg (Franco et al., 2006; Bellum et al., 2007). In the rotarod task, pups were trained to maintain balance on a rod as rotation accelerates. Although all groups exhibited improvement in agility over sequential days of training, a small proportion of pups in the 5 μg/mL MeHg-exposed group, 38.9% for males and 44.4% for females, reached the criterion, while a majority of pups in the control group (88.9% for males, 70.0% for females) did so (Table 1). These data could reflect an overall decrease in coordination and/or impaired motor learning. In either case, these deficits likely result from mercury-induced dysfunction in the cerebellum.

In the present study, when comparing levels of T-Hg in different brain regions of pups developmentally exposed to 5 μg/mL of MeHg, the highest concentrations were found in the cerebellum, followed by the hippocampus and cerebral cortex (Table 2), which confirms that the cerebellum is one of the targets of mercury exposure (Roegge and Schantz, 2006). Significantly high T-Hg levels were also found in blood, liver and kidney tissues when compared with corresponding controls, which clearly indicated that internal exposure to MeHg continued after puberty. Hg levels in all tissues of females perinatally exposed to 5 μg/mL of MeHg were higher than those in males. One factor that could contribute to this pattern of results in early postnatal development is that males excrete much higher levels of Hg in urine than females (Yasutake and Hirayama, 1988). Another factor is females' lower capacity to maintain glutathione levels in the brain after treatment with MeHg (Goulet et al., 2003). It is noteworthy that average Hg levels in the cerebellum, cerebral cortex and hippocampus of 5 μg/mL of MeHg-exposed pups were only 1.7, 1.4 and 1.7 times the average levels of Hg found in human brain tissues (287.6 ng/g, Lewandowski et al., 2003).

In conclusion, the results of this study suggest that developmental MeHg exposure causes serious impairment in neurological development and motor coordination functions in developing rats. This study also indicates that NOEL for these effects is in the range of 1–4 μg/mL of MeHg in drinking water. The accumulations of mercury in all tissues of female pups were higher than those of male pups, implying that the female pups were more vulnerable than the male pups. We demonstrated adverse effects of developmental exposure to MeHg associated with tissue concentrations very close to the current human body burden of this persistent and bioaccumulative compound.

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REFERENCES

Amin-Zaki, L., Majeed, M.A., Greenwood, M.R., Elhassani, S.B., Clarkson, T.W., Doherty, R.A., 1981. Methylmercury poisoning in the Iraqi suckling infant: a longitudinal study over five years. J. Appl. Toxicol. 1 (4), 210–214.

Bellum, S., Thuett, K.A., Grajeda, R., Abbott, L.C., 2007. Coordination deficits induced in young adult mice treated with methylmercury. Int. J. Toxicol. 26 (2), 115–121.

Branchi, I., Alleva, E., Costa, L.G., 2002. Effects of perinatal exposure to a polybrominated diphenyl ether (PBDE 99) on mouse neurobehavioural development. Neurobehav. Toxicol. 23 (3), 375–384.

Burbacher, T.M., Rodier, P.M., Weiss, B., 1990. Methylmercury developmental neurotoxicity: a comparison of effects in humans and animals. Neurotoxicol. Teratol. 12 (3), 191–202.

Burns, L.H., Annett, L., Kelley, A.E., Everitt, B.J., Robbins, T.W., 1996. Effects of lesions to amygdala, ventral subiculum, medial prefrontal cortex, and nucleus accumbens on the reaction to novelty: implication for limbic–striatal interactions. Behav. Neurosci. 110 (1), 60–73.

Cheng, J.P., Gao, L.L., Zhao, W.C., Liu, X.J., Sakamoto, M., Wang, W.H., 2009. Mercury levels in fisherman and their household members in Zhoushan, China: impact of public health. Sci. Total Environ. 407 (8), 2625–2630.

Chumchal, M.M., Drenner, R.W., Cross, D.R., Hambright, K.D., 2010. Factors influencing mercury accumulation in three species of forage fish from Caddo Lake, Texas, USA. J. Environ. Sci. 22 (8), 1158–1163.

Dórea, J.G., Bezerra, V.L.V.A., Fajon, V., Horvat, M., 2011. Speciation of methyl- and ethyl-mercury in hair of breastfed infants acutely exposed to thimerosal-containing vaccines. Clin. Chim. Acta 412 (17-18), 1563–1566.

Eto, K., Yasutake, A., Miyamoto, K.I., Tokunaga, H., Otsuka, Y., 1997. Chronic effects of methylmercury in rats. II. Pathological aspects. Tohoku J. Exp. Med. 182 (3), 197–205.

Franco, J.L., Teixeira, A., Meotti, F.C., Ribas, C.M., Stringari, J., Pomblum, S.C.G., et al., 2006. Cerebellar thiol status and motor deficit after lactational exposure to methylmercury. Environ. Res. 102 (1), 22–28.

Fredriksson, A., Dencker, L., Archer, T., Danielsson, B.R.G., 1996. Prenatal coexposure to metallic mercury vapour and methylmercury produce interactive behavioural changes in adult rats. Neurotoxicol. Teratol. 18 (2), 129–134.

Fujita, M., Takabatake, E., 1977. Mercury levels in human maternal and neonatal blood, hair and milk. Bull. Environ. Contam. Toxicol. 18 (2), 205–209.

Gao, Y., Yan, C.H., Tian, Y., Wang, Y., Xie, H.F., Zhou, X., et al., 2007. Prenatal exposure to mercury and neurobehavioral development of neonates in Zhoushan City, China. Environ. Res. 105 (3), 390–399.

Goulet, S., Doré, F.Y., Mirault, M.E., 2003. Neurobehavioral changes in mice chronically exposed to methylmercury during fetal and early postnatal development. Neurotoxicol. Teratol. 25 (3), 335–347.

Grandjean, P., Weihe, P., Burse, V.W., Needham, L.L., Storr-Hansen, E., Heinzow, B., et al., 2001. Neurobehavioral deficits associated with PCB in 7-year-old children prenatally exposed to seafood neurotoxicants. Neurotoxicol. Teratol. 23 (4), 305–317.

Grotto, D., Vicentini, J., Angeli, J.P.F., Latorraca, E.F., Monteiro, P.A.P., Barcelos, G.R.M., et al., 2011. Evaluation of protective effects of fish oil against oxidative damage in rats exposed to methylmercury. Ecotoxicol. Environ. Saf. 74 (3), 487–493.

Knobeloch, L., Gliori, G., Anderson, H., 2007. Assessment of methylmercury exposure in Wisconsin. Environ. Res. 103 (2), 205–210.

Lapham, L.W., Cernichiari, E., Cox, C., Myers, G.J., Baggs, R.B., Brewer, R., et al., 1995. An analysis of autopsy brain tissue from infants prenatally exposed to methylmercury. Neurotoxicology 16 (4), 689–704.

Lewandowski, T.A., Ponce, R.A., Charleston, J.S., Hong, S., Faustman, E.M., 2003. Effect of methylmercury on midbrain cell proliferation during organogenesis: potential cross-species differences and implications for risk assessment. Toxicol. Sci. 75 (1), 124–133.

Marsh, D.O.T., Clarkson, T.W., Cox, C., Myers, G.J., Amin-Zaki, L., Al-Tikriti, S., 1987. Fetal methylmercury poisoning. Relationship between concentration in single strands of maternal hair and child effects. Arch. Neurol. 44 (10), 1017–1022.

Montgomery, K.S., Mackey, J., Thuett, K., Ginestra, S., Bizon, J.L., Abbott, L.C., 2008. Chronic, low-dose prenatal exposure to methylmercury impairs motor and mnemonic function in adult C57/B6 mice. Behav. Brain Res. 191 (1), 55–61.

Nunes, J., Ferreira, S., 2000. Possible mercury intoxication of newborns via breast milk. Front. Fetal Health 2 (3), 18–20.

Nyland, J.F., Wang, S.B., Shirley, D.L., Santos, E.O., Ventura, A.M., de Souza, J.M., et al., 2011. Fetal and maternal immune responses to methylmercury exposure: a cross-sectional study. Environ. Res. 111 (4), 584–589.

Pedersen, M.B., Hansen, J.C., Mulvad, G., Pedersen, H.S., Gregersen, M., Danscher, G., 1999. Mercury accumulations in brains from populations exposed to high and low dietary levels of methyl mercury. Concentration, chemical form and distribution of mercury in brain samples from autopsies. Int. J. Circumpolar Health 58 (2), 96–107.

Podhorna, J., Brown, R.E., 2002. Strain differences in activity and emotionality do not account for differences in learning and memory performance between C57BL/6 and DBA/2 mice. Genes Brain Behav. 1 (2), 96–110.

Rice, D., Barone Jr., S., 2000. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ. Health Perspect. 108 (Suppl. 3), 511–533.

Roegge, C.S., Schantz, S.L., 2006. Motor function following developmental exposure to PCBS and/or MEHG. Neurotoxicol. Teratol. 28 (2), 260–277.

Roegge, C.S., Wang, V.C., Powers, B.E., Klintsova, A.Y., Villareal, S., Greenough, W.T., et al., 2004. Motor impairment in rats exposed to PCBs and methylmercury during early development. Toxicol. Sci. 77 (2), 315–324.

Sakamoto, M., Kakita, A., Wakabayashi, K., Takahashi, H., Nakano, A., Akagi, H., 2002. Evaluation of changes in methylmercury accumulation in the developing rat brain and its effects: a study with consecutive and moderate dose exposure throughout gestation and lactation periods. Brain Res. 949 (1-2), 51–59.

Sakamoto, M., Kaneoka, T., Murata, K., Nakai, K., Satoh, H., Akagi, H., 2007. Correlations between mercury concentrations in umbilical cord tissue and other biomarkers of fetal exposure to methylmercury in the Japanese population. Environ. Res. 103 (1), 106–111.

Simmonds, M.P., Haraguchi, K., Endo, T., Cipriano, F., Palumbi, S.R., Troisi, G.M., 2002. Human health significance of organochlorine and mercury contaminants in Japanese whale meat. J. Toxic. Environ. Health Part A Curr. Iss. 65 (17), 1211–1235.

Trasande, L., Schechter, C., Haynes, K.A., Landrigan, P.J., 2006. Applying cost analyses to drive policy that protects children: mercury as a case study. Ann. N. Y. Acad. Sci. 1076, 911–923.

Ulrich, S.M., Ilyushchenko, M.A., Tanton, T.W., Uskov, G.A., 2007. Mercury contamination in the vicinity of a derelict chlor-alkali plant. Part II. Contamination of the aquatic and terrestrial food chain and potential risks to the local population. Sci. Total Environ. 381 (1-3), 290–306.

Vitalone, A., Catalani, A., Chiodi, V., Cinque, C., Fattori, V., Goldoni, M., et al., 2008. Neurobehavioral assessment of rats exposed to low doses of PCB126 and methyl mercury during development. Environ. Toxicol. Pharmacol. 25 (1), 103–113.

Yasutake, A., Hirayama, K., 1988. Sex and strain differences of susceptibility to methylmercury toxicity in mice. Toxicology 51 (1), 47–55.

Yasutake, A., Nakano, A., Yiyamoto, K., Eto, K., 1997. Chronic effects of methylmercury in rats. I. Biochemical aspects. Tohoku J. Exp. Med. 182 (3), 185–196.