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Neurobehavioural and Molecular Changes Induced by Methylmercury Exposure During Development

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There is an increasing body of evidence on the possible environmental influence on neurodevelopmental and neurodegenerative disorders. Both experimental and epidemiological studies have demonstrated the distinctive susceptibility of the developing brain to environmental factors such as lead, mercury and polychlorinated biphenyls at levels of exposure that have no detectable effects in adults. Methylmercury (MeHg) has long been known to affect neurodevelopment in both humans and experimental animals. Neurobehavioural effects reported include altered motoric function and memory and learning disabilities. In addition, there is evidence from recent experimental neurodevelopmental studies that MeHg can induce depression-like behaviour. Several mechanisms have been suggested from *in vivo*- and *in vitro*-studies, such as effects on neurotransmitter systems, induction of oxidative stress and disruption of microtubules and intracellular calcium homeostasis. Recent *in vitro* data show that very low levels of MeHg can inhibit neuronal differentiation of neural stem cells. This review summarises what is currently known about the neurodevelopmental effects of MeHg and consider the strength of different experimental approaches to study the effects of environmentally relevant exposure *in vivo* and *in vitro*.

Keywords: Methylmercury; Neuro-ontogeny; Neurites; Development; Neurochemistry; Neuropathology; Neurobehaviour; Cell death; Oxidative stress; Calcium; Microtubules

INTRODUCTION

Neurodevelopmental disorders are estimated to affect 3-8% of the infants born each year in the USA, and in total about 12% of all American children under the age of 18 years (for review see Hass, 2006). The sensitivity of the developing central nervous system to toxic insults has caused concern about potential neurotoxic effects of substances that children and fetuses are exposed to (Grandjean and Landrigan, 2006).

Methylmercury (MeHg) is an example of a food contaminant that clearly can be toxic to human neurodevelopment, although uncertainties remain regarding the safety reference dose for the toxic effects. Due to increased awareness and regulations, environmental MeHg contamination caused

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by human activities has decreased considerably. However, there are substantial amounts left from past emissions in the soil and sediments of many places, delaying the return to natural environmental levels. MeHg is formed by microbial methylation of inorganic mercury in sediments and soil, and is bioaccumulated and biomagnified in the aquatic food chain. Consumption of contaminated fish, shellfish and sea mammals is the main source of human exposure to MeHg (EPA-US, 1990).

Dietary MeHg is nearly completely absorbed in the gastrointestinal tract (Clarkson, 1972). It quickly enters the bloodstream and is distributed to various organs including all areas of the brain, where approximately 10% of the ingested MeHg is deposited (Clarkson, 2002). MeHg crosses the placenta, with higher levels in fetal cord blood compared to maternal blood (Vahter *et al.*, 2000). The exposure risk decreases after birth, however even the limited excretion of MeHg in milk makes breast feeding a potential exposure source for infants (Grandjean *et al.*, 1994; Oskarsson *et al.*, 1996). In addition to having a more sensitive nervous system infants are also believed to have lower excretion capacity for MeHg (Nordenhäll *et al.*, 1998), further increasing their vulnerability to this substance.

Health consequences of high exposure to MeHg became apparent after poisoning episodes in the 1950-70s in Japan and Iraq when thousands of people were intoxicated by food that was heavily contaminated by MeHg (for review, see NRC 2000). MeHg exposure mainly damaged the nervous system, and the most severely affected patients died. The fetal brain was found to have higher vulnerability to MeHg than the adult brain; neurodevelopmental effects were observed at exposure levels where the mothers displayed little or no overt signs of toxicity.

Following these disasters, several epidemiological studies have been conducted in fish eating communities around the world to test for neurodevelopmental effects of lower levels of MeHg (for review, see Spurgeon, 2006). Two large scale longitudinal studies have been performed in two island populations with high intake of fish and other seafood. In the Faroe Islands associations were found between cord blood mercury levels at birth and adverse effects on attention, language and memory when children were examined at seven years of age (Grandjean *et al.*, 1997). Delayed latencies of brainstem auditory evoked potentials were also found to be associated with cord blood and maternal hair mercury levels (Murata *et al.*, 1999). A follow-up analysis of the same cohort at age 14 years yielded similar results with regard to prenatal mercury exposure and neurobehavioral function (Debes *et al.*, 2006), as well as the latencies of brainstem auditory evoked potentials (Murata *et al.*, 2004).

In contrast, no significant adverse effects were found in a study of the Seychelle Islands population (Myers *et al.*, 2003; Davidson *et al.*, 2006). There are no obvious explanations for the disparities between the studies, and the results have been interpreted differently by different authorities, leading to diverse estimations of the threshold dose for the neurodevelopmental effects of MeHg.

In the attempt to understand the neurodevelopmental effects of MeHg and its impact on neural function, a conspicuous number of experimental studies, including functional, biochemical and molecular analyses, have been performed over the years. Here we review the current knowledge concerning the developmental neurotoxicity of MeHg and the underlying molecular processes. Table I shows a brief summary of functional effects and suggested molecular mechanisms.

NEURODEVELOPMENTAL EFFECTS OF MeHg IN EXPERIMENTAL ANIMALS

The developmental neurotoxicity of oral exposure to MeHg has been extensively studied in animal models (Burbacher *et al.*, 1990a; Gilbert and Grant-Webster, 1995; Watanabe and Sathoh, 1996). Consistent with the effects observed in humans, the consequences of intrauterine MeHg exposure in experimental animals range from increased rates of intrauterine death, delayed developmental growth, and disorganization of brain architecture to more subtle effects and developmental delays depending on the dose and time of exposure during gestation.

MeHg kinetics is susceptible to species-related differences and this should be borne in mind when relating results from experimental animal studies with the real life human situation. The absorption rates for dietary MeHg are similar in humans and experimental animals (Clarkson, 1972), and blood organic mercury is mostly bound to red blood cells.

Nonetheless, the red cells-to-plasma ratio for this metal differs among animal species (Clarkson, 1972), as well as brain-to-blood distribution ratios. Rat blood binds much more mercury than the blood of many other species, including other rodents or humans. For this reason, the dosing regimen for rats must be about 10-fold higher than that of primates (including humans) to achieve similar brain mercury levels (Newland and Rasmussen, 2000). The excretion rate of MeHg also varies between different species (Clarkson, 1972; Vahter *et al.*, 1994). Because of these metabolic differences it is more appropriate to base interspecies comparisons of MeHg dose-effect relationships on the target-organ dose, rather than on the administered dose.

Neuropathological Effects in Experimental Animals

The neuropathological changes associated with high level developmental MeHg exposure show a certain degree of similarities across species (Burbacher *et al.*, 1990a). Like humans, non-human primates and small mammals display reduced brain size, damage to the cortex and basal ganglia, gliosis, loss of cells and sparing of the diencephalon in response to high brain Hg levels (12-20 µg/g). An interspecies comparison of the histopathological changes produced by moderate (3-11 µg/g) or low (<3 µg/g) brain Hg concentrations is not possible, because of the lack of human data on immature organisms (Burbacher *et al.*, 1990a). However, consistent experimental evidence supports that brain structural alterations can occur in the presence of Hg concentrations well below the 12 µg/g threshold. To date, early studies on prenatal exposure of mice have indicated ultrastructural alterations of Purkinje cells and granule neurons in the cerebellum of adult animals following a single injection of 8 mg MeHg/kg on gestational day 9 (GD9) (Chang *et al.*, 1977). Moreover, Peckham and Choi (1988) described an irregular distribution of neurons within the cerebral cortex of offspring at postnatal day 10 (PND10) (brain Hg levels: 1.35 µg/g) after daily maternal administration of 2 mg MeHg/kg throughout the gestational period. Postnatal exposure of mice to MeHg caused reduced dendritic arborisation of Purkinje cells (3 x 5 mg MeHg/kg, PND 3-5; Choi *et al.*, 1981) and decreased thicknesses of the cerebellar molecular and internal granular layers (1 x 4 mg/kg on PND2 resulting in 1.8 µg Hg/g tissue on PND3; Sager *et al.*, 1984) in cerebellum. More recently, chronic pre- + post-natal exposure to MeHg (4 ppm MeHg, in the drinking water, GD0-PND30) was found to affect several morphometric indices of cerebellar cortex development, including reduced widths of the molecular and external granular layers. In these pups the mean Hg brain level was 4 µg/g on PND7 and decreased to 0.7, 0.45 and 0.25 µg/g on PND14, PND21 and PND30, respectively, despite the continuous exposure to Hg through lactation (Markowski *et al.*, 1998).

In rats, the distribution pattern of neuronal damage observed in fetuses at fullterm/neonates after prenatal exposure seems to be different from that elicited

Table I Neurodevelopmental effects of methylmercury and possible underlying mechanisms

Functional effect	LOAEL (infant or neonatal Hg brain levels)	Suggested molecular mechanisms
Neuropathological damage	Humans: <12 µg/g Rats: 4.5 µg/g	Oxidative stress and cell death Altered calcium homeostasis Cytoskeletal disruption Impaired neurite outgrowth and neuronal differentiation
Neurobehavioural deficits	Humans: 0.5-1.0 µg/g Rats: 3.5 µg/g	Impairments in neurotransmission and signal transduction Endocrine disruption Deficient neuronal excitability

LOAEL = Lowest Observed Adverse Effect Level

by postnatal or adult exposure (Wakabayashi *et al.*, 1995; Kakita *et al.*, 2000; Sakamoto *et al.*, 2004). For example, a 10-day-long treatment with 10 mg MeHg /kg body weight/day caused minimal damage to the hippocampus and brainstem of newborn rats (exposure after PND2), widespread lesions throughout the CNS of young developing rats (exposure after PND15) and extensive neuronal damage in the cerebellum and spinal dorsal nerve roots of adult rats (exposure after PND60) (Wakabayashi *et al.*, 1995). Noteworthy, in rats synaptogenesis begins 10-14 days after birth and, at this developmental stage, the degree of maturation of the rat cerebral cortex corresponds to that of the full-term human newborn. Consecutive trans-placental exposure to lower levels of MeHg (1 mg/kg body weight/day), resulting in offspring brain Hg concentrations of 11-12 μg/g, elicited astrocytosis and neuronal degeneration in the brainstem and to a lower extent in the limbic system (including the hippocampus and the amygdala) of 1-3 day-old neonates (Kakita *et al.*, 2000). At 6 months of age MeHg-exposed rats still displayed significantly fewer neurons in the amygdala and hippocampus than controls (Kakita *et al.*, 2000). In another study, focal cerebellar dysplasia, including heterotopic location of Purkinje cells and granule cells, has been reported in weanling and pubertal rats after consecutive and moderate dose exposure throughout gestation and lactation (Sakamoto *et al.*, 2002). At birth, the Hg level was 4.5 μg/g in the pup brain. The latter rapidly declined to about 1 μg/g during the suckling period, thus confirming the limited MeHg transfer from mother to offspring *via* secreted milk as compared to the placenta (Sakamoto *et al.*, 2002). Recently, Roegge *et al.* (2006) failed to demonstrate changes in the cerebellum of weanling and adult rats after perinatal exposure to very low levels of MeHg (total maternal intake: 1.20 mg beginning 4 weeks prior to breeding and ending on PND16).

Neurobehavioural Effects in Experimental Animals

Experimental animal studies support the human epidemiological data that developmental exposure to MeHg can have long-term effects including behavioural disturbances at a young age. In addition an increased risk for neurodegenerative disorders later in life has been suggested (Weiss *et al.*, 2002; Landrigan *et al.*, 2005).

Considering the findings in children affected by exposure to MeHg, most of the animal studies have aimed to investigate motor and cognitive impairments following developmental exposure. Here we review mainly studies on the effects of maternally mediated exposure. Various effects have been reported depending on the dose, exposure conditions, strain and gender. As observed in epidemiological studies of human infants and children (McKeown-Eyssen *et al.*, 1983; Grandjean *et al.*, 1998), several experimental studies in rodents (Rossi *et al.*, 1997; Gimenez-Llort *et al.*, 2001; Onishchenko *et al.*, 2007) have reported greater developmental effects in males than in females. The mechanisms underlying the gender-dependent differences may be due to various factors (Vahter *et al.*, 2006) and need to be further clarified.

Different animal strains may have different susceptibility to neurotoxicants, including MeHg. One example of the importance of the genetic background is a study of different mouse strains showing that locomotor activity was affected by repeated prenatal MeHg-exposure (3 mg/kg, GD12-14) in C57BL/6Cr and BALB/c, but not in C57BL/6J mice (Kim *et al.*, 2000).

A study performed by Gimenez-Llort *et al.* (2001) showed that both male and female MeHg-treated rat offspring (GD7-PND7 0.5 mg/kg) had increased activity during the initial exploration period when they were tested at the age of 14 days, while 21-day-old males displayed decreased exploratory activity. In contrast, single exposure to 8 mg/kg of MeHg GD8 or GD15 did not cause detectable changes in locomotor activity of male rats tested at PND 40 or PND 60 (Baraldi *et al.*, 2002; Carratù *et al.*, 2006).

The outcome of prenatal damage may not necessarily be apparent until a moment when a neurodevelopmental defect is unmasked or precipitated by a subsequent insult. Particularly, subtle toxicant-induced behavioural effects become more evident when pharmacological challenges are applied. Young MeHg-exposed rats were found to be more sensitive to the effects of dopamine receptor agonist apomorphine on locomotor activity but less sensitive to selective D_2 receptor agonist U-91356A than control rats (Gimenez-Llort *et al.*, 2001; Daré *et*

al., 2003). Developmental exposure to MeHg also caused increased sensitivity to behavioural effects of amphetamine such as hyperactivity, stereotypic and self-injurious behaviour in rats and mice (Cagiano *et al.*, 1990; Rasmussen and Newland, 2001; Wagner *et al.*, 2006).

The cerebellum, a brain structure responsible for balance and motor coordination in mammals, is known to be one of the main targets for MeHg toxicity (Philbert *et al.*, 2000). Therefore, motor coordination is one of the important parameters of behavioural evaluation of MeHg-induced neurotoxic effects. A deficit in rotarod performance was found after postnatal lactational exposure to MeHg (Franco *et al.*, 2006) as well as after both prenatal and postnatal treatment with MeHg (Sakamoto *et al.*, 2002). According to data from the other studies, only prenatal MeHg-exposure did not cause coordination impairments in young animals (Doré *et al.*, 2001; Carratù *et al.*, 2006). These data suggest that despite of the fact that offspring are exposed to MeHg *in utero* at a greater extent than during the suckling period of postnatal life, motor coordination in rodents appears to be more affected if the exposure occurs postnatally. High susceptibility of the cerebellar system to postnatal toxic insults can be due to the fact that the rodent CNS develops over a long time period extending from the embryonic phase through several weeks into the postnatal period (Rice and Barone, 2000). In particular, dominating neuronal populations in cerebellum (granule cells and Purkinje cells) are produced and/or maturate during first weeks of postnatal life (Ferguson, 1996). Therefore, developmental processes, such as proliferation, differentiation, migration, synaptogenesis etc. that are affected by exposure to toxic agents during this period, may result in altered motor function in later life.

Several studies have reported learning disabilities in the passive avoidance test performed in young and adult rats after developmental exposure to MeHg (Cagiano *et al.*, 1990; Zanoli *et al.*, 1994; Kakita *et al.*, 2000; Baraldi *et al.*, 2002; Sakamoto *et al.*, 2002). Doré *et al.* (2001) also demonstrated an impairment of reference and working memory in the radial maze, as well as a deficit in spatial alternation training in the T-maze in female mice after *in utero* exposure to MeHg. Similarly, working and reference memory deficits were observed in male mice when monitoring learning behaviour in the home cage (Onishchenko *et al.*, 2007). Widholm *et al.* (2004) showed that developmental exposure of rats to MeHg could lead to alterations in associative ability or attention in a series of spatial alternation tasks, where animals had to alternate operant responses to two spatial locations from trial to trial under different conditions.

When it comes to spatial learning in the water maze test, reports have been contradictory, showing either normal (Fredriksson *et al.*, 1996; Rossi *et al.*, 1997; Kakita *et al.*, 2000; Onishchenko *et al.*, 2007) or impaired cognitive function in MeHg-exposed animals (Kim *et al.*, 2000; Baraldi *et al.*, 2002; Daré *et al.*, 2003).

Baraldi *et al.* (2002) and Carratù *et al.* (2006) reported that in the novel object recognition test young rats prenatally exposed to MeHg spent the same time exploring familiar and new objects while control animals showed higher exploration preference for an unknown object. This deficit might reflect either failure of recognition, or disruption of the instinctive propensity to explore novelty (Mamby, 2001). Interestingly, several studies reported decreased exploratory activity in MeHg-exposed animals under conditions of normal motor function (Rossi *et al.*, 1997; Carratù *et al.*, 2006; Onishchenko *et al.*, 2007). These observations taken together with data on impaired reference memory suggest that both motivational and cognitive alterations can contribute to novel object recognition failure found in MeHg-exposed animals. Alterations following developmental exposure of rodents to MeHg seem to be complex, and besides learning and motor disorders can include changes in emotional behaviour. In particular, a predisposition to depressive-like behaviour was persistently found in MeHg-exposed male mice tested at different ages (Onishchenko *et al.*, 2007).

Learning and memory disabilities have also been reported in non-human primates (Burbacher *et al.*, 1986; Gunderson *et al.*, 1988; Gilbert *et al.*, 1996). Additionally, losses in visual and auditory functioning (Rice and Gilbert, 1990; Rice, 1998; Burbacher *et al.*, 2005) and altered social behaviour (Burbacher *et al.*, 1990b) have been documented in MeHg-prenatally exposed monkeys.

The neurochemical basis of MeHg-induced behavioural alterations may be referred to distur-

bances in a number of neurotransmitter systems, initially occurring during exposure and followed by long-lasting changes in brain functioning.

Neurochemical, Neuroendocrine and Electrophysiological Changes in Experimental Animals

Neurotransmission

Control of dopamine (DA) levels and of DA-receptor interactions are essential for brain function. The dopaminergic system is implicated in a number of processes such as attention, cognition, motor, and reward-related behaviours. In humans, dysfunctions of this neurotransmission system are associated with several neurological, neuroendocrine and psychiatric disorders, including Parkinson's and Huntington's diseases, schizophrenia, mood disorders, deficits in attention, motor control and perception (see reviews by Bozzi and Borrelli, 2006; Kienast and Heinz, 2006). Manifestations of central dopaminergic dysfunction differ depending on the site (*e.g.*, dorsal and ventral striatum, cortical regions) of their neurobiological correlate (Kienast and Heinz, 2006).

Direct as well as indirect evidence has been provided with respect to an involvement of the dopaminergic system in MeHg neurodevelopmental toxicity. For example, a study in rat offspring following *in utero* exposure to 1 mg MeHg /kg body weight/day has shown delayed alterations of a number of brain dopaminergic parameters including DA levels, DA turnover, and synaptosomal DA uptake, whose onset occurred approximately at the time of weaning (Bartolome *et al.*, 1984). Differently, other investigators did not report changes in the offspring regional brain levels of DA in weanling (Castoldi *et al.*, 2006) or adult (Lindström *et al.*, 1991) rats after long-term maternal exposure. Transient effects on DA receptor number associated with behavioural dysfunctions have been observed in rat pups following a single high-dose MeHg treatment during the late stage of gestation (Cagiano *et al.*, 1990).

The important role of striatal dopaminergic neurotransmission in locomotor control is well known. As mentioned, behavioural changes suggestive of altered dopaminergic neurotransmission have been observed following repeated perinatal exposure (GD7-PND7) to low doses of MeHg (0.5 mg/kg/day) in pre-pubertal as well as in adult rats (Rossi *et al.*, 1997; Gimenez-Llort *et al.*, 2001; Daré *et al.*, 2003). In particular, an altered motor response to apomorphine, a D_1, D_2 and D_3 DA receptor agonist, was found in male rats tested at PND21, a time period crucial for the ontogeny of DA receptors in the rat brain. The biochemical correlate of such neurobehavioral change was a significant reduction in D_2 receptor binding in the caudate putamen (Daré *et al.*, 2003). Changes in locomotor activity were still present at 6 months of age, in a gender-specific fashion (male gender only) (Rossi *et al.*, 1997). Noteworthy, this protocol of treatment resulted in higher total Hg levels in cerebellum (about 1.3 μg/g) compared to in the rest of the brain (0.5 μg/g) at the end of treatment (PND7).

With respect to the adrenergic system, a selective increase in rat cerebellum noradrenaline levels has been found in adult rats after *in utero* + lactational + direct dietary exposure to MeHg (Lindström *et al.*, 1991), but not in prepubertal rats after prenatal exposure only (Bartolome *et al.*, 1984). The latter authors, using the same regimen of exposure, have also reported a MeHg-induced disruption of the normal ontogeny of adrenergic receptor sites during the first month of postnatal life (Bartolome *et al.*, 1987).

The enzyme monoamine oxidase (MAO), whose predominant form in the human brain is MAO-B (80-95%), catalyzes the degradation of monoamine neurotransmitters and it is important for serotonergic neuronal development and neurobehaviour (Whitaker-Azmitia *et al.*, 1994). Developmental exposure to MeHg has been shown to result in gender- and brain-area dependent changes in cerebral MAO activity in the rat (Beyrouty *et al.*, 2006; Castoldi *et al.*, 2006). MeHg (1 mg/kg body weight/day, GD7-PND7) was found to selectively reduce the MAO-B activity in the cerebellum of PND21 male rats only, without affecting it in other male brain areas, or in the female offspring's brain (Castoldi *et al.*, 2006). MeHg also affected the regional content of DA and serotonin metabolites, homovanillic acid (HVA) in hippocampus and 5-hydroxy-indole-3-acetic acid (5-HIAA) levels in cerebellum, respectively (Castoldi *et al.*, 2006).

Concerning whole MAO (MAO-A + MAO-B) activity, Beyrouty *et al.* (2006) reported that in rats, continuous maternal exposure to MeHg for 4 weeks before conception until GD20 significantly

decreased the PND41 offspring's brain MAO activity in a gender- (females only) and brain area- (brainstem only) dependent fashion. Concomitantly, the female offspring displayed an increased auditory startle response amplitude, possibly in accordance with the delayed brainstem auditory evoked potential latencies observed in 14-year-old Faroese children exposed to MeHg (Murata *et al.*, 2004). Notably, the startle test allows assessment of simple sensorimotor neuronal circuitry in the lower brainstem, which involves monoamine neurotransmitters (Beyrouty *et al.*, 2006).

The cholinergic system is essential for normal brain development as a modulator of neuronal proliferation, migration and differentiation processes (Hohmann and Berger-Sweeney, 1998). The cholinergic muscarinic receptors (MRs), in particular, are involved in several CNS functions, including learning and memory (Levine *et al.*, 2001).

In rats, a single high dose (8 mg/kg b.w.) of MeHg on GD15 was found to transiently reduce the density of cholinergic muscarinic receptors in the offspring's cerebral cortex (Zanoli *et al.*, 1994). Muscarinic receptors represent a target for MeHg also after moderate and consecutive perinatal exposure. In this respect, oral treatment of rat dams with 1 mg, but not 0.5 mg MeHg/kg/day, from GD7 to PND7, has been found to cause a delayed (PND21) enhancement of the number of cortical and cerebellar muscarinic receptors both in pups and in their dams: this increase was more marked in dams than in pups, in accordance with the higher Hg levels present in the adult brain (7-9 μg/g *vs* 1.5-1.7 μg/g in offspring). Delayed receptor up-regulation may compensate for early stage effects such as MeHg-induced inhibition of brain acetylcholine synthesis and consequent reduction of brain acetylcholine levels and direct competitive antagonism of MeHg at these receptors (Coccini *et al.*, 2000; 2006).

In addition to the monoaminergic and cholinergic systems, MeHg exposure has been shown to target other neurotransmission systems, at least after a single high-dose exposure in an advanced stage of gestation, including temporary changes in the GABA benzodiazepine receptor system (Guidetti *et al.*, 1992) and delayed dysfunctions in the opiatergic system (Zanoli *et al.*, 1997). Long-lasting changes in the glutamatergic system have been reported (Cagiano *et al.*, 1990) and cognitive deficits elicited by prenatal MeHg exposure in adult rats have been linked to specific changes in gene expression of hippocampal *N*-methyl-D-aspartate (NMDA) receptors, more specifically increased mRNA levels of the NR-2B subunit (Baraldi *et al.*, 2002). In addition, MK-801, an NMDA receptor antagonist, has been found to provide a certain degree of neuroprotection in developing rats orally treated for 7 consecutive days (PND16 - PND22) with 10 mg/kg MeHg (Miyamoto *et al.*, 2001). Glutamate-mediated excitotoxicity has been proposed as one of the possible mechanisms underlying MeHg neurotoxic effects and this may apply to the developing organisms too. Organic Hg inhibits glutamate uptake and increases excitatory amino acid efflux from astrocytes, thus resulting in increased extracellular glutamate concentrations and excitotoxic damage to the neighbouring cells (Aschner *et al.*, 2000).

In addition to the studies reviewed here, there is also evidence from *in vitro* studies of brain synaptosomes and brain tissue slices showing effects of MeHg on different neurotransmitter systems (*e.g.*, Rajanna and Hobson, 1985; Farina *et al.*, 2003).

Protein Kinase C (PKC)

PKC consists of a large family of related isoenzymes (α, ε, β, γ, etc.) which respond to the second messengers calcium, diacylglycerol and arachidonic acid. PKC-mediated phosphorylation of brain proteins is implicated in neuronal growth and differentiation, signal transduction, modulation of ion channel activity, synaptic plasticity and associate learning. It has been demonstrated that gestational MeHg exposure (1 or 2 mg /kg bw day, GD6-GD15) can modify the ontogeny of specific PKC isoenzymes and regional PKC activity during postnatal development (PND1-85) in rat brain (Haykal-Coates *et al.*, 1998). In particular, the higher dose (devoid of overt maternal or fetal toxicity) caused a persistent decrease in regional PKCα and PKCε immunoreactivity, with the cortex, hippocampus and cerebellum being the most affected areas. The α and ε isoforms are expressed early during postnatal development and correlate with growth and proliferation within specific regions (Haykal-Coates *et al.*, 1998).

Trophic Factors

Several *in vitro* studies have documented the ability of MeHg to interfere with the physiological functions of trophic factors (see below for details). *In vivo* studies addressing this issue during brain developmental are very limited. However, persistent alterations in nerve growth factor (NGF) levels have been observed in the hippocampus (increase) and septum (decrease) of weanling and adult rats, possibly resulting from an interrupted retrograde transport of NGF from the hippocampus to the basal forebrain due to microtubule depolymerization (Lärkfors *et al.*, 1991).

S100B is a calcium binding protein produced and secreted by astrocytes, which can be trophic (enhancing neuronal survival and glial proliferation) or neurotoxic (pro-apoptotic) depending on its concentration. Reversible changes in hippocampal tissue content of S100B along with glial fibrillary acidic protein have been described in neonates of MeHg-treated pregnant rats (Vicente *et al.*, 2004a). These regional specific changes in S100B might be related to the MeHg-induced cognitive and epileptogenic disorders and support an involvement of glial cells in MeHg neurotoxicity.

Hormones

Several chemical pollutants, *e.g.*, dioxins, polychlorinated biphenyls (PCBs), and heavy metals including mercury, can disrupt endocrine functions in animals. Hormones (gonadal steroids, thyroid hormones and glucocorticoids) are implicated in the physiological development and function of the CNS (see Schantz and Widholm, 2001, for review). For this reason, it has been suggested that some of the neurotoxic effects elicited by chemical exposure during development arise from endocrine disruption. Recent evidence indicates that maternal exposure to low doses of MeHg (5 mg/kg chow) from GD0 to PND10 can disrupt thyroid hormone metabolism in newborn mice, which may lead to an excessive and noxious increase in T3 concentrations in the developing brain (Mori *et al.*, 2006). Another study of the same group failed to demonstrate changes in serum T4 levels in murine fetuses. Nonetheless, some effects similar to those observed as a response to hypothyroidism were detected in fetal brains (Watanabe *et al.*, 1999b). Congenital hypothyroidism produces marked neurological deficits and results in delayed myelinogenesis, alterations in cell migration, neuronal differentiation and neurotransmitter function.

Neuronal Excitability

Altered electrophysiological characteristics of developing rat cortical neurons have been described after chronic MeHg chloride treatment (Vilagi *et al.*, 2000). In particular, a slight decrease in the membrane potential and in the amplitude of spikes together with an enhanced excitability has been reported in somatosensory cortical slices obtained from 4-week-old rats developmentally exposed to very low doses of MeHg (maternal ingestion of 0.375 mg/kg/day throughout gestation and lactation, Vilagi *et al.*, 2000). The same type of maternal treatment also enhanced epileptogenicity in PND28 rat offspring (Szasz *et al.*, 1999).

Antioxidant Systems

The formation of reactive oxygen species (ROS) plays a significant role in the onset of MeHg neurotoxicity, as shown by *in vitro* and *in vivo* studies (Clarkson, 1972; Sager *et al.*, 1984; Sarafian and Verity, 1991; Fredriksson *et al.*, 1996; Atchison and Hare, 1994; Dare *et al.*, 2000; Usuki *et al.*, 2001). Neuronal cells from rats with decreased antioxidants defenses are more susceptible to MeHg toxicity (Ahlbom *et al.*, 2000). A recent report by Vicente *et al.* (2004b) has shown that the hippocampal antioxidant system is seriously compromised in neonates born to MeHg-exposed rat dams. In particular, low activities of glutathione peroxidase, catalase and superoxide dismutase (SOD) were evident at birth. SOD activity was still reduced on PND30. At this age, protein oxidation and a decrease of total antioxidant reactivity were also observed (Vicente *et al.*, 2004b).

Glutathione (GSH) is involved in free radical scavenging, xenobiotic detoxification and maintenance of protein thiol redox status. In prenatal studies in mice, short-term MeHg exposure (GD12-14) has been associated with increased GSH levels and lipid peroxidation in the fetal brain (Watanabe *et al.*, 1999a). Using the same experimental conditions, these investigators also reported changes in the activities of selenoenzymes in the fetal mouse brain, including a dose-dependent inhibition of glutathione peroxidase (Watanabe *et al.*, 1999b).

Alterations in glutathione redox status have also been observed in mouse fetal brain after 10 ppm MeHg exposure (in the drinking water) throughout gestation (Thompson *et al.*, 2000).

MECHANISTIC STUDIES: *IN VITRO* MODELS

Many questions remain regarding the mechanisms underlying the neurotoxic effects of MeHg. Several molecular targets have been implicated in both *in vitro* and *in vivo* studies and no single mechanism is likely to be explanatory. One biochemical characteristic of MeHg that is believed to be important, and which is shared by other mercurial compounds, is the high affinity for protein sulfhydryl (SH) groups (Hughes, 1957). This type of interaction could alter the structure of a large number of proteins and lead to disruptive effects on various intracellular functions. As discussed in the previous section, several neurotransmitter signalling systems are affected by MeHg, which could explain the behavioural consequences in animals and humans. Furthermore, induction of oxidative stress has been reported after MeHg exposure, as well as impairment of the cells' calcium homeostasis (Sarafian and Verity, 1991; Limke *et al.*, 2004a). Both these processes are potentially relevant for MeHg-induced cell death in the developing nervous system, and immature neurons may be particularly sensitive to these alterations (Mundy and Freudenrich, 2000).

Oxidative Stress

The brain is particularly susceptible to the harmful effects of oxidative stress, a condition caused by an imbalance between the generation of free radicals and the cell's protective antioxidant system (Duchen, 2000). Free radicals have one or more unpaired electrons and thus the ability to react with crucial cell components such as proteins, DNA and lipids. Both enzymes like catalase, peroxidase and superoxide dismutase (SOD), and non-enzymatic compounds, including GSH, are involved in the cellular defense against free radicals.

MeHg can affect several functions of the mitochondria (for review, see Limke *et al.*, 2004a) and interference of the mitochondrial electron transport system can cause overproduction of oxygen radicals, or ROS. ROS formation and oxidative stress in the brain as a result of MeHg exposure has been observed both *in vivo* and in several *in vitro*-models: Increased lipid peroxidation, superoxide and hydrogen peroxide amounts have been detected in brain tissue from mice injected with methylmercuric chloride or MeHg, along with impaired SOD, glutathione reductase and glutathione peroxidase activities, as well as decreased GSH levels (Yee and Choi, 1994; Stringari *et al.*, 2006). As mentioned, injection of pregnant female rats with MeHg also resulted in decreased SOD activity in the hippocampus of the exposed offspring (Vicente *et al.*, 2004b). Similar results, and in some cases also an attenuated effect on the mitochondrial membrane potential, have been reported for a number of *in vitro*-models including primary cultures of cerebellar granule cells (Sarafian and Verity, 1991; Mundy and Freudenrich, 2000; Kaur *et al.*, 2006), astrocytes (Shanker *et al.*, 2004; Kaur *et al.*, 2006) and various cell lines of relevance for the nervous system (Daré *et al.*, 2001b; Belletti *et al.*, 2002; Gatti *et al.*, 2004; Garg and Chang, 2006; Johansson *et al.*, 2006).

MeHg's high affinity for thiol compounds makes GSH a target for binding, leading to a decrease in the levels of GSH that can be used in the cell's oxidant defence system. This conjugation with GSH is suggested to be a major pathway for MeHg efflux (Fujiyama *et al.*, 1994), indicating that the intracellular GSH levels might determine the elimination capacity, and accordingly the resistance, of a cell to MeHg toxicity. Thiol resins have been used to treat MeHg -poisoned humans, leading to reduced half-life of MeHg in blood (Clarkson *et al.*, 1981). Addition of antioxidants have been found to reduce the ROS production caused by MeHg (Shanker and Aschner, 2003; Shanker *et al.*, 2005; Kaur *et al.*, 2006; Johansson *et al.*, 2006) and to protect against MeHg induced cell death both *in vivo* (Usuki *et al.*, 2001) and *in vitro* (Park *et al.*, 1996; Daré *et al.*, 2000; Belletti *et al.*, 2002; Gatti *et al.*, 2004; Johansson *et al.*, 2006).

Calcium Homeostasis

Calcium signalling is essential for many cell functions and the cytosolic levels are controlled by a complex system of membrane channels and pumps, both in the cell membrane and in organelles like the endoplasmatic reticulum and mitochondria

(Berridge *et al.*, 2000). Sustained elevated Ca^{2+} levels have been found in various cell types after MeHg exposure, and protective effects of Ca^{2+}-chelators or Ca^{2+}-channel blockers have been reported *in vitro* and *in vivo* (Sarafian, 1993; Atchison and Hare, 1994; Sakamoto *et al.*, 1996; Graff *et al.*, 1997; Marty and Atchison, 1997; 1998; Sirois and Atchison, 2000; Gasso *et al.*, 2001). The MeHg-induced increase is a multi-phase process that has been studied in several cell types, and is probably the result of initial mobilization of Ca^{2+} from intracellular stores, followed by entry of extracellular Ca^{2+} through voltage-gated channels in the plasma membrane (for review, see Limke *et al.*, 2004a). Ca^{2+} overload, or alterations of the intracellular Ca^{2+} compartmentalization, can trigger either apoptotic or necrotic cell death (for review, see Orrenius *et al.*, 2003). It leads to activation of multiple enzymes, including PKC, phospholipases, cell death-promoting proteases such as calpains, and DNA cleaving endonucleases. Due to the massive concentration gradient of Ca^{2+} across the cell membrane, restoration of normal intracellular levels after an increase is a highly energy-demanding process, which by itself can be deleterious for the cell (Limke *et al.*, 2004a). MeHg can also cause cell cycle alterations resulting in inhibition of cell proliferation, and a study of primary rat embryonic midbrain neuroepithelial cells implicated calcium in this process (Faustman *et al.*, 2002). Moreover, indirect effects of the increase in intracellular Ca^{2+} could be involved in some other cellular effects of MeHg, including inhibition of cell migration (for review, see Komuro and Rakic, 1998). Elevated intracellular Ca^{2+} concentrations might also aggravate the damage caused by free radicals, and uncontrolled Ca^{2+} release from the mitochondria has been associated with oxidative stress.

Microtubule Function

A functioning cytoskeleton is essential for many cellular processes during the development of the nervous system, including cell survival, proliferation, differentiation and migration, all of which have been reported to be affected by MeHg - Inhibition of cell division has been observed both *in vivo* (Sager *et al.*, 1984) and *in vitro* (Ponce *et al.*, 1994; Miura *et al.*, 1999; Faustman *et al.*, 2002), although not in all studies (Lewandowski *et al.*, 2003). Impaired migration of cerebellar granule cells has been found in MeHg-exposed organotypic cultures and is suggested to lead to apoptotic cell death (Kunimoto and Suzuki, 1997). MeHg-induced effects on differentiation and formation of axons and dendrites are discussed below.

One of the targets for MeHg-binding that has received the most attention is the SH-groups of tubulin (Vogel *et al.*, 1985). These groups are accessible both on the surface and the end of microtubules. The reported consequences of this interaction *in vitro* include depolymerization of existing microtubules (Sager *et al.*, 1983; Vogel *et al.*, 1985; Graff *et al.*, 1997; Castoldi *et al.*, 2000) as well as inhibition of microtubule assembly (Sager *et al.*, 1983; Vogel *et al.*, 1985). *In vitro* data indicate that immature microtubules in developing neurons are more sensitive to MeHg than more mature forms (Graff *et al.*, 1997). The cytoskeletal breakdown precedes MeHg-induced apoptosis in cerebellar granule cells (Castoldi *et al.*, 2000; 2001). The cell cycle arrest observed in several MeHg exposed neuronal *in vitro* models could be a result of a destroyed mitotic spindle, and has been suggested to be an important event in the development of apoptosis (Ponce *et al.*, 1994; Miura *et al.*, 1999). In addition to the experimental studies mentioned, neuropathological findings (*e.g.*, reduced brain size and inhibition of neuronal migration) in postmortem brains of infants exposed *in utero* to MeHg during the Iraqi outbreak, are also in concert with a disruption of microtubule function (Choi *et al.*, 1978).

Differentiation and Neurite Outgrowth

Cell differentiation and formation of neurite processes constitutes crucial steps in the development of CNS, and toxic interference could potentially lead to neurodevelopmental effects. MeHg disrupts NGF-induced differentiation of the rat pheochromocytoma PC12 cell line at doses that do not cause overt cytotoxicity (Parran *et al.*, 2001). Similarly, recent data has shown that low doses of MeHg can inhibit spontaneous neuronal differentiation of neural stem cells *in vitro* (Tamm *et al.*, 2006). Remarkably, these effects were found using doses comparable to those reported in cord blood from the general Swedish population.

Several signalling pathways have been implicated

in neuronal differentiation. For example, NGF-induced differentiation of PC12 cells involves activation of both extracellular signal-regulated kinases (ERK1/2) and c-jun N-terminal kinases (JNKs) (Waetzig and Herdegen, 2003). Neurite elongation, branching and maintenance are dependent on cytoskeletal proteins as well as their various modification factors. Directed growth of neurites is regulated by so called guidance cues that can promote or repel axonal growth cone advancement and/or regulate dendritic development. Remodelling of the cytoskeleton is the key intracellular response, enabling these processes (Kalil and Dent, 2005). The guidance cues include ephrins, netrins, slits and semaphorins (Dickson, 2002). Neurotrophins, such as NGF and BNDF (brain-derived neurotrophic factor), also regulate neuronal shape and induce neurite outgrowth, although their roles in axon guidance *in vivo* have not yet been fully established (Gallo and Letourneau, 2004). Cell adhesion molecules (CAMs) play a key role in brain development, including neurite elongation and synaptogenesis, and NCAM, N-cadherin and L1 are all indicated to promote axonal growth (Skaper, 2005). Recent data indicate that some of the molecules that guide axons can also influence dendritic growth (McAllister, 2002; Whitford *et al.*, 2002). Disruption of this intricate system of attractants and repellents could cause neurodevelopmental damage. Sublethal concentrations of MeHg are reported to interfere with axonal morphogenesis in cultured embryonic chick (E8) forebrain neurons

Table II Cell death pathways induced by MeHg in different *in vitro* models

Model	Exposure dose	Cell death pathway	References
Cerebellar granule cells (primary culture, rat)	0.1-1.5 μM 1 μM 0.5-10 μM 1 μM 30 nM	Caspase-independent Calpain activation Translocation of AIF	Daré *et al.*, 2000; 2001a; Castoldi *et al.*, 2000; Fonfria *et al.*, 2002; Sakaue *et al.*, 2005
D384 cells (human astrocytoma cell line)	1 μM	Caspase-independent Lysosomal disruption Decreased mitochondrial membrane potential	Daré *et al.*, 2001b
HT22 cells (mouse hippocampal cell line)	4 μM	Caspase-independent Calpain activation Lysosomal disruption Decreased mitochondrial membrane potential	Tofighi *et al.*, 2006
C17.2 cells (mouse neural progenitor cell line)	0.5-1 μM	Bax activation, Cytochrome *c* release Caspase activation Calpain activation	Tamm *et al.*, 2006
Embryonic cortical neural stem cells (primary culture, rat)	0.05 μM	Bax activation, Cytochrome *c* release Caspase activation Calpain activation	Tamm *et al.*, 2006
SH-SY5Y cells (human neuroblastoma cell line)	0.1-100 μM	Decreased mitochondrial dehydrogenase activity Caspase activation	Toimela and Tahti, 2004
U-373MG cells (human glioblastoma cell line)	10 -100 μM	Decreased mitochondrial dehydrogenase activity Caspase activation	Toimela and Tahti, 2004

AIF = Apoptosis-Inducing Factor

(Heidemann *et al.*, 2001).

Although the molecular mechanisms behind the negative effects of MeHg on differentiation are currently unclear, some pathways have been indicated: As mentioned, the negative effect of MeHg on tubulin polymerization is a plausible underlying factor, but MeHg has also been reported to alter the mRNA and protein expression of ephrin and several ephrin receptors in differentiated P19 embyonal carcinoma cells (Wilson *et al.*, 2005). MeHgCl exposure in rats (PND3-13) is reported to affect expression and polysialylation of the neural cell adhesion molecule NCAM180 in the brain (Dey *et al.*, 1999). In addition, MeHg is suggested to decrease the NGF-induced autophosphorylation of the TrkA receptor in PC12 cells (Parran *et al.*, 2003). Under normal conditions, binding of NGF to TrkA results in autophosphorylation followed by a cascade of intracellular signalling events involved in the regulation of survival and differentiation (Fujita *et al.*, 1989). MeHg can also induce activation of Notch signalling in a *Drosophila*-model (Bland and Rand, 2006), and constitutive expression of the Notch-protein is reported to inhibit neurite outgrowth in PC12 cells (Levy *et al.*, 2002).

Cell Death

Depending on cell type and the exposure conditions (dose and duration) MeHg can induce apoptotic or necrotic cell death (Kunimoto, 1994; Nagashima *et al.*, 1996; Castoldi *et al.*, 2000; Daré *et al.*, 2000; 2001a; Tamm *et al.*, 2006). In addition to *in vitro* results, apoptosis has also been detected in the cerebellum of MeHg intoxicated rats (Nagashima *et al.*, 1996; Nagashima, 1997). Apoptosis is an energy-demanding, regulated process characterized by activation of signalling pathways leading to specific cleavage of proteins and DNA, condensation of the nucleus, cell shrinkage and fragmentation into so called apoptotic bodies that can be phagocytized (for review, see Orrenius *et al.*, 2003). Unlike the apoptotic process, during which plasma membrane integrity is maintained, necrosis involves swelling of the cell and its organelles resulting in cell lysis and risk of inflammation due to leakage of the intracellular content. Different apoptotic pathways activated by MeHg are summarized in Table II.

Oxidative stress can cause apoptosis (Chandra *et al.*, 2000) and as already discussed antioxidants can protect from MeHg-induced cell death. Transcriptional profiling of MeHg-exposed pheochromocytoma PC12 cells showed altered expression of genes associated with oxidative stress after 6 hours and increases in genes related to cell cycling and apoptosis after 24 hours (Wilke *et al.*, 2003).

Lysosomes are sensitive to oxidative stress (Ollinger and Brunk, 1995) and disruption of the lysosomal membranes followed by apoptotic cell morphology and DNA fragmentation was observed in MeHg treated astrocytoma D384 cells (Daré *et al.*, 2001b). These data point to involvement of lysosomal proteases as executor factors in MeHg-induced apoptosis in this model.

Traditionally, one distinguishes between the intrinsic or mitochondrial apoptotic pathway, characterised by release of mitochondrial factors such as cytochrome *c*, and the extrinsic pathway, triggered by ligand binding to members of the death receptor family (Gupta, 2001). In two neural stem cell models (the murine-derived multipotent C17.2 cell line and primary cortical cultures from E15 rat embryos), MeHg induced apoptosis *via* the mitochondrial pathway, as shown by Bax activation, cytochrome *c* translocation, and caspase activation (Tamm *et al.*, 2006). In addition, concomitant activation of the calcium-regulated protease calpain was observed, and full protection from cell death was achieved when pre-treating the cells with inhibitors against both caspases and calpains. Calpain activation is also detected in cerebellar granule cells exposed to MeHg and can be reduced by antioxidants (Daré *et al.*, 2000; Sakaue *et al.*, 2005). In contrast to the neural stem cells, the neuroblastoma SH-SY5Y and glioblastoma U-373MG cell lines (Toimela and Tahti, 2004; Tamm *et al.*, 2006), MeHg-induced apoptosis seems to be caspase independent in cerebellar granule cells, D384 astrocytoma cells and HT22 hippocampal cells (Castoldi *et al.*, 2000; Daré *et al.*, 2000; 2001a,b; Fonfria *et al.*, 2002; Tofighi *et al.*, 2006).

As already described in more detail, several neurotransmitter systems have been implicated in MeHg toxicity in experimental animal studies. Some *in vitro* studies also suggest involvement of neurotransmission in MeHg-induced cell death. For example, activation of muscarinic M3 receptors is reported to contribute to the elevated intracellular Ca^{2+} levels in cerebellar granule cells (Limke *et al.*,

2004b). Also, similar to the neuroprotective effects in MeHg-exposed rats (Miyamoto *et al.*, 2001; Juarez *et al.*, 2005), NMDA-receptor antagonists can block the toxic effects also in cerebellar neuron cultures (Park *et al.*, 1996).

CONCLUSIONS

The devastating effects of high exposure to MeHg on human neurodevelopment became evident after the disasters in Japan and Iraq. When it comes to exposure levels measured in populations with a diet rich in fish the picture is more complex, although several epidemiological studies have reported associations with negative outcomes.

Studies in non-human primates and rodents, as well as observations of individuals exposed early in life during the Minamata bay's accident, suggest that aging can exacerbate the neurotoxic effects of MeHg (Rice, 1996; Newland and Rasmussen, 2000; Weiss *et al.*, 2002; Landrigan *et al.*, 2005). Since the aging process reduces the number of neural cells, the brain may gradually lose its capability to compensate for cell loss or cell function impairments occurring during development. This could explain the observed delayed symptoms of neurotoxicity. Mercury exposure has also been suggested as a risk factor for the development of Alzheimer's disease, as high levels of this metal have been reported in blood and hair of patients (Hock *et al.*, 1998).

Neurodevelopmental effects of MeHg have been the focus of many experimental studies using laboratory animals. Different exposure protocols have been used trying to mimic the human exposure situation or to study effects on specific phases of neurodevelopment. Apart from species differences in MeHg kinetics, comparisons to humans are also complicated by discrepancies in the developmental process: Several steps that occur prenatally in human neurodevelopment take place after birth in rodents when the transfer of MeHg from the mother is significantly reduced compared to *in utero*.

Tissue doses allow for better inter-species comparisons. In the studies reviewed here neonatal animals exposed prenatally had mercury levels around 3-12 μg per g brain tissue and effects on neurotransmission and/or neurobehaviour were reported at brain levels in the order of 10^{-1} μg/g at the age of testing. Behavioural effects found in experimental animals included a wide range of changes, *i.e.*, motor deficit, different sensitivity to pharmacological agents, alterations of different types of learning and memory, as well as depression-like behaviour.

The data on brain mercury levels in children are very limited. Developmental MeHg exposure resulting in brain Hg doses between 3 and 11 μg/g tissue has been associated to mental deficiency, abnormal reflexes and muscle tone, and retarded motor development in humans. Delayed psychomotor development of children has been shown to result from brain Hg doses below 3 μg/g (Burbacher *et al.*, 1990a). Total Hg levels reported in asymptomatic neonates from the Seychelles varied between 0.026 to 0.295 μg/g (Lapham *et al.*, 1995). In two full-term babies who died from *in utero* exposure to MeHg during the Iraqi outbreak, brain total Hg levels ranged between >1 and 13.7 μg/g, well above the levels of 0.1 to 0.4 μg/g measured in normal infants in the area of Minamata, Japan (Choi *et al.*, 1978). Recently, based on these data, Lewandowski *et al.* (2003) proposed a human *in vivo* lowest observed adverse effect level (LOAEL) in the range of 0.5-1.0 μg/g. Thus, the current experimental animal studies of prenatal or prenatal + postnatal exposure to MeHg have mostly used exposure regimes that result in neonate brain levels categorised as moderate (Burbacher *et al.*, 1990a) and exceeding the suggested human LOAEL. However, at the age when the animals are analysed for neurodevelopmental effects the levels have decreased and are approximately in the same range as reported in normal infants from populations with a high intake of fish (Choi *et al.*, 1978; Lapham *et al.*, 1995).

In spite of their obvious disparities from the *in vivo* situation, *in vitro* systems can be relevant models to study neurodevelopmental toxicity, especially when it comes to mechanistic studies. Concentrations comparable to the current developmental exposure (*via* cord blood) of the general population in many countries inhibit spontaneous neuronal differentiation of embryonic cortical neural stem cells (Tamm *et al.*, 2006). Clarification of the underlying molecular processes in this cell type is highly relevant from a neurodevelopmental perspective. No single *in vitro* experimental model is of course sufficient to represent the complexity of the nervous system,

as exemplified by the variations in sensitivity and in the molecular responses to MeHg insult. For example, activation of different cell death pathways has been observed in different cell types. Although it is impossible to mimic the human exposure situation *in vitro*, actual target doses should optimally be measured to give an indication of the sensitivity of different models.

Despite regulations that have substantially decreased the environmental mercury contamination, MeHg will remain a pollutant of global concern for many years to come due to its persistent and bioaccumulating nature. Further research is needed on the risk for neurodevelopmental effects at environmentally relevant exposure, taking into account possible interactive effects between MeHg and other potential food contaminants, *e.g.*, polychlorinated biphenyls (PCBs). Since fish is a nutrious and crucial part of many people's diet it is important to keep controlling mercury levels in fish and to give correct and balanced information to the public, in particular to women of child bearing age. This is underscored by recent studies linking developmental mercury exposure to additional negative health effects including increased risk for preterm delivery (Xue *et al.*, 2007) and cardiovascular problems (Grandjean *et al.*, 2004).

The current studies point to defined systems in selected brain regions as specific targets for MeHg, which may explain some of the effects observed after developmental MeHg exposure (*e.g.*, altered dopaminergic transmission might cause the motoric effects). Several characteristics of MeHg toxicity, including induction of oxidative stress, microtubule dysfunction and some effects on neurotransmission, have been observed in both *in vivo* and *in vitro* models. Despite this knowledge, a better understanding of the molecular and cellular effects of MeHg is still required to identify reliable biomarkers that could potentially be used for risk assessment purposes. For elucidation of the potential neurodevelopmental effects of environmentally relevant doses of MeHg, it is essential to study meaningful and sensitive endpoints both *in vivo* and *in vitro*. The evaluation of subtle behavioural changes and alterations in the differentiation potential of neural stem cells seem to be promising strategies to screen and detect developmental neurotoxic effects.

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