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Are Neuropathological Conditions Relevant to Ethylmercury Exposure?

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Abstract Mercury and mercurial compounds are among the environmentally ubiquitous substances most toxic to both wildlife and humans. Once released into the environment from both natural and anthropogenic sources, mercury exists mainly as three different molecular species: elemental, inorganic, and organic. Potential health risks have been reported from exposure to all forms; however, of particular concern for human exposure relate to the potent neurotoxic effects of methylmercury (MeHg), especially for the developing nervous system. The general population is primarily exposed to MeHg by seafood consumption. In addition, some pharmaceuticals, including vaccines, have been, and some continue to be, a ubiquitous source of exposure to mercurials. A significant controversy has been whether the vaccine preservative ethylmercury thiosalicylate, commonly known as thimerosal, could cause the development of autism. In this review, we have discussed the hypothesis that exposure to thimerosal during childhood may be a primary cause of autism. The conclusion is that there are no reliable data indicating that administration of vaccines containing thimerosal is a primary cause of autism. However, one cannot rule out the possibility that the individual gene profile and/or gene–environment interactions may play a role in modulating the response to acquired risk by modifying the individual susceptibility.

Keywords Mercury · Ethylmercury · Thimerosal · Autism · Neuropathology

Introduction to Mercurial Compounds

Mercurial compounds are major environmental pollutants. Natural sources of mercury (Hg) are volcanoes, oceanic sediments, crust degasification, and forest fires; whereas anthropogenic sources include mining, chloroalkali manufacturing, and combustion of fossil fuels. There are three different Hg molecular species: elemental (Hg^0), inorganic (Hg^{2+}), and organic (MeHg). Organic mercury originates from inorganic mercury in rivers, lakes, and oceans, likely as the result of the methylating activity of sulfate-reducing bacteria. In the aquatic food chain MeHg is bioaccumulated and biomagnified. The highest levels are found in predator fish, which are the main source of human exposure to MeHg (Aschner et al., in press).

The toxic effects of inorganic mercury were known since antiquity (Clarkson 1972) whereas the devastating consequences of exposure to organic mercury compounds were discovered much later. At the beginning of the 20th century, MeHg found an important application in agriculture as a fungicide, consequently its manufacturing increased, resulting in occupational exposure. In 1938 neurological symptoms were reported among factory workers, providing the first accurate description of signs and symptoms of MeHg poisoning (Hunter et al. 1940).

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The dramatic neurotoxic effects of MeHg became tragically apparent in the 1950s and early 1960s when two mass health disasters occurred in Japan, first in Minamata, then in Niigata. Hg was released in the environment by an acetaldehyde manufacturing plant, entered the aquatic food chain and accumulated as MeHg in fish and shellfish. In the region of Minamata Bay, and the syndrome caused by MeHg poisoning was called Minamata disease (Tsubaki and Takahashi 1986). Another severe accident took place in the early 1970s in Iraq, when seeds treated with MeHg for agriculture purposes were used for baking bread. This resulted in the poisoning of thousands of people, with 459-hospital mortalities (Bakir et al. 1973). The description of symptoms and the pathology was in agreement with previous findings reported during the poisoning in Minamata. The incidents in Japan and Iraq revealed the particularly devastating neurotoxic effects of MeHg on the developing nervous system. Lately, also exposure to much lower level of MeHg from dietary sources was shown to have unfavorable neurodevelopmental effects as reported by prospective studies of populations in the Faroe Islands and New Zealand (Kjellstrom et al. 1989; Grandjean et al. 1997). Recent studies point to the critical role that maternal diet can play in the onset of MeHg developmental neurotoxicity (Davidson et al. 2008).

Although the general population is primarily exposed to MeHg by seafood consumption, some pharmaceuticals, including vaccines, have been a source of exposure to mercurials. Of particular concern is the vaccine preservative ethylmercury thiosalicylate, thimerosal, which has been linked to autism. A discussion on its use and potential health effect, specifically as they relate to extrapolations from studies with MeHg follows below.

Ethylmercury—General Properties and Poisoning

Preceding its usage as a vaccine preservative, ethylmercury compounds, in the form of diethylmercury were used in the treatment of syphilis as early as the 1880s. Later on, in the 20th century, the fungicidal properties of the short-chain alkyl mercury compounds were fully recognized, leading to commercialization of agricultural applications containing ethylmercury. A variety of organic mercury compounds were subsequently used to prevent seed-borne diseases of cereal (Jalili and Abbasi 1961; Al-Damluji 1962). Ethylmercury fungicides were effectively and safely used for decades, nonetheless, several poisoning outbreaks have occurred in developing countries. Two outbreaks occurred in rural Iraq in 1956 and 1960 upon misuse of the fungicide ethylmercury toluene sulfonilamide (Jalili and Abbasi 1961). Having missed the planting season, the ethylmercury containing grains were used by the farmers' families for baking bread. Hundreds of cases of severe poisoning with fatal outcomes ensued. Ethylmercury poisonings have also been reported in China as recently as the 1970s after farmers consumed rice treated with ethylmercury chloride (Zhang 1984).

Several ethylmercury poisonings in rural Iraq in the 1950s provide a basis for comparisons between the toxicity of the two organomercurial species (Jalili and Abbasi 1961). Risk assessments on the effects of these compounds on the nervous system were carried out under the assumption that the dose–effect and dose–response relationships for MeHg are similar to ethylmercury. It was on this basis that thimerosal was removed from vaccines commonly given to infants and children (American Academy of Pediatrics (AAP) and US PHS 1999). Since then, as discussed below, it has been shown that the kinetics of tissues disposition and metabolism differ from those for MeHg.

Use of Thimerosal in Vaccines

Ethylmercury thiosalicylate, also known under the trade names thimerosal and merthiolate (American Academy of Pediatrics (AAP) 1999), was introduced as a preservative in vaccines in the 1930s, after a series of studies in several animal species and humans provided evidence for its safety and effectiveness (Powell and Jamieson 1931). Thimerosal in concentrations of 0.001% (1 part in 100,000) to 0.01% (1 part in 10,000) has been shown to be effective in clearing a broad spectrum of pathogens. A vaccine containing 0.01% thimerosal as a preservative contains 50 μg of thimerosal per 0.5 ml dose or approximately 25 μg of mercury per 0.5 ml dose (US Food and Drug Administration 2007). After approximately 70 years of safe practice and a long record of effectiveness in preventing bacterial and fungal contamination of vaccines with only minor local reactions at the site of injection. In Sweden and Denmark already in late 1980s concerns were raised about vaccines containing thimerosal, which decreased and eventually disappeared by 1993. In 2001 the use of thimerosal as a potential toxic hazard to infants was also questioned in the US (Ball et al. 2001). Though it is still used in developing countries, where advantages of multiple use vials outweigh thimerosal's putative toxicity (World Health Organization (WHO) 2002), it was removed from the US market in 2001.

The absence of thimerosal in vaccines has not modified the occurrence of autism in Denmark (Madsen et al. 2003) and similar conclusions were also reached by two other independent studies (Heron and Golding 2004; Parker et al. 2004). Review of the epidemiological studies of autism and thimerosal exposure is beyond the scope of this manuscript.

However, several studies, the last as recently as 2007 (Hviid et al. 2003; Parker et al. 2004; Thompson et al. 2007), have failed to support a causal association between early exposure to mercury from thimerosal-containing vaccines and immune globulins and deficits in neuropsychological functioning in children between the ages of 7 and 10 years. Notably, a study of prenatal mercury exposure due to a high-seafood diet contaminated by mercury in pregnant mothers in the Seychelles Islands also did not find in utero mercury exposure differences in the rate of autistic offspring (Myers et al. 2003).

What's Flawed in Extrapolating the Risk Assessment of Thimerosal by Direct Comparison with MeHg

The World Health Organization (1996), the US Environmental Protection Agency (EPA) (2007), the US Agency for Toxic Substances and Disease Registry (1999), and the US Food and Drug Administration (2007) have assessed the risk associated with MeHg in diet and have published recommendations for safe exposures to this metal. These exposure levels range from 0.1 μg/kg body weight/day (EPA) to 0.47 μg/kg body weight/day (WHO) (US Food and Drug Administration 2007).

An infant generally receives three doses of DTP vaccine or a total of 75 μg of ethylmercury during the first 14 weeks of life (US Food and Drug Administration 2007). If the hepatitis B vaccine is added to the immunization schedule during the first 14 weeks of life, the maximum exposure to ethylmercury is 112.5 μg. If *Haemophilus influenzae* type b conjugate (Hib) vaccine is added during the same time, the total ethylmercury dose reaches 187.5 μg. Thus, some infants receiving vaccines according to the recommended schedule will receive doses of mercury exceeding the cut-off levels established by regulatory agencies. However, as mentioned above, and as will be illustrated in detail below, the application of MeHg risk assessment guidelines to thimerosal (or ethylmercury) exposure assumes that ethylmercury and MeHg are identically distributed in the body, specifically in the central nervous system (CNS), reaching equal concentrations at sensitive sites and exerting similar toxic sequelae. This assumption is invalid as it is refuted by existing scientific evidence.

Most human exposures to ethylmercury are in the form of thimerosal, and tissue disposition patterns of mercury in experimental animals after equivalent doses of ethylmercury chloride or thimerosal are the same (Suzuki et al. 1973). Accordingly, it appears that the thiosalicylic acid anion attached to ethylmercury in the thimerosal plays no role in influencing the fate of ethylmercury in the body. Thus, thimerosal rapidly dissociates to release ethylmercury (Reader and Lines 1983; Tan and Parkin 2000), which is the active species of concern.

Conclusions on the toxicity of ethylmercury (thimerosal) are predominantly drawn from analogies to the organomercurial, MeHg. While the scientific literature supports the concept that MeHg is a potent developmental neurotoxin, the assertion that thimerosal leads to developmental disorders in children is hypothetical and unsubstantiated, resting on indirect and incomplete information, primarily from analogies with MeHg. This approach is not surprising, as until recently there was sparse information on the disposition of ethylmercury as compared to MeHg. However, results from the few studies that have provided a direct comparison between these compounds (Clarkson et al. 2003; Magos 2003; Clarkson and Magos 2006; Magos and Clarkson 2006) have established that extrapolation of ethylmercury's disposition and toxic potential from the MeHg literature is flawed, as distinct differences exist with respect to the pharmacokinetic behavior of the two organomercurials. Simply stated, MeHg is not a suitable reference for evaluating ethylmercury toxicity.

Key observations to substantiate this statement include the following: (1) mercury clears from the body much faster after the administration of ethylmercury than after the administration of MeHg; (2) the brain-to-blood mercury concentration ratio established for MeHg will overestimate mercury in the brain after exposure to ethylmercury; and (3) because ethylmercury decomposes much faster than MeHg, the risk of brain damage is less for ethylmercury than for MeHg. The following discussion provides detailed support for my expressed opinion that the scientific literature does not substantiate to a reasonable degree of scientific certainty that thimerosal, a vaccine preservative, has caused developmental disorders in some children.

Mercury Clears from the Body Much Faster After the Administration of Ethylmercury Than After the Administration of MeHg

While ethylmercury accumulates in the brain, distinct differences in the pharmacokinetics of ethylmercury and MeHg exist. Magos et al. (1985) examined the disposition of ethylmercury versus MeHg in rats administered the respective chloride salts. Rats were treated with 8 mg/kg of methylmercuric or ethylmercuric chloride or 9.6 mg/kg of ethylmercuric chloride. The study concluded that at equimolar doses, MeHg exposure resulted in higher brain levels of the organic species than did treatment with ethylmercury (significance of the mercury species, namely organic versus inorganic, will be addressed below). In a second study, Burbacher et al. (2005) reported on the disposition of

mercury in infant monkeys intramuscularly injected with ethylmercury in the form of thimerosal in comparison with a second group of monkeys orally dosed with a MeHg compound. The study intended to mimic the immunization schedule in human neonates. Corroborating Magos's et al. (1985) conclusions in the rat experimental model, this study (Burbacher et al. 2005) also reported that levels of organic mercury were lower in the brains of infant monkeys exposed to thimerosal compared to those exposed orally to MeHg. The brain half-times (defined as the time it takes for the brain level of mercury to decrease by half) also differed. The clearance half-times for organic mercury in the brain were 58 days on average after oral MeHg exposure versus 14 days after injection of ethylmercury. Simply stated, this means that organic mercury in the form of MeHg will persist in the brain for much longer periods of time than its relative organomercurial, ethylmercury. If the data from infant monkeys predict half-times in brain as well as they do for whole blood (see below), then, one would expect most of the organic mercury in an infant to be cleared from brain tissue during the 2-month interval between repeated immunizations (Clarkson and Magos 2006). Importantly, the mere presence of ethylmercury in the brain does not establish causality.

Observations by Pichichero et al. (2002) on levels of mercury in samples of stool and urine indicate that substantial excretion of mercury is taking place via the fecal route upon the administration of ethylmercury. Urinary excretion of ethylmercury appeared to be negligible. Thus, ethylmercury appears to behave like MeHg with fecal excretion accounting for most of the elimination from the body. The absorption rate and initial distribution volume of total mercury are also reported to be generally similar after ethylmercury injections and oral MeHg exposure (Burbacher et al. 2005). In other words, peak total blood mercury levels after a single exposure to either ethylmercury or MeHg are very similar, implying that the organic mercury compounds behave similarly in the early hours after exposure.

This is also consistent with results reported by Stajich et al. (2000) where blood levels in pre- and full-term infants 48–72 h after receiving 12.5 μg mercury from a single hepatitis B vaccine were measured. The average total mercury concentration in samples of whole blood was 7.36 and 2.24 μg Hg/l for pre- and full-term infants, respectively. Average body weights for pre- and full-term infants were 748 and 3,588 g, respectively. If one assumes that the volume of the blood compartment of infants at this developmental stage approximates 9% (90 ml/kg) and 8% (80 ml/kg) body weight for pre-term and full-term babies, respectively (Diem and Lentner 1970), then their blood volumes approximate 72 and 287 ml, translating to a mercury content of 0.53 and 0.64 μg Hg, respectively (Clarkson and Magos 2006). These amounts of mercury in the blood compartment correspond to 4.2 and 5.1% of the injected dose, overlapping with the 5% for the initial deposition of MeHg into the blood compartment of adults (Clarkson and Magos 2006). These estimates can only be approximate, since earlier measurements were not taken in the study, and it is likely that levels of mercury were higher closer to the injection time. However, in the absence of any other data, it is reasonable to assume that the initial distribution of ethylmercury to the blood compartment does not differ greatly from that of MeHg.

Pichichero et al. (2002) evaluated two groups of 20 infants, at 2 and 6 months. The infants received a normal pediatric schedule of vaccines containing thimerosal as a preservative. In addition, the authors measured levels of mercury in two control groups receiving thimerosal-free vaccines. One consisted of 11 infants 2 months of age and the other of 10 infants who were 6 months of age. Mercury was measured several weeks after the last vaccination. The highest recorded blood level was 5.1 μg Hg/l in a 2-month-old, representing peak mercury levels in these infants approximately 5 days after the last vaccination. Most blood levels were below 2 μg Hg/l (Pichichero et al. 2002). The authors estimated the expected blood levels based on the dose and made the assumptions that the clearance from blood followed first-order kinetics (i.e., a single half-time), that 5% of the dose was initially distributed to the blood compartment, and that the volume of the blood compartment was 8% of the body weight. The calculated half-time was approximately 7 days. Magos and associates (Magos 2003) used allometric considerations and also concluded that a half-time of 7 days in infants is what would be expected from the published adult half-times. A third study in infant monkeys (Burbacher et al. 2005) concluded that terminal half-time of mercury is 8.6 days. These short half-times differ from the available data on the half-time clearance of MeHg, establishing that virtually all of the mercury derived from a single injection of thimerosal (ethylmercury) would be cleared from the infant's blood in a 2-month period between consecutive vaccinations. This would not be the case with exposure to MeHg, which in infant monkeys had a blood half-time of approximately 19 days (Burbacher et al. 2005).

As pointed out by Burbacher et al. (2005), there is a significant difference in blood half-times between MeHg and ethylmercury in infant monkeys. This is associated with a remarkable accumulation of blood mercury during repeated exposure to MeHg. Although the initial blood mercury concentration (at 2 days after the first dose; see above) did not differ between the two groups, the peak blood mercury concentration in the MeHg-exposed infant monkeys rose to a level nearly three times higher than in the thimerosal monkeys after the fourth dose. In addition,

the blood clearance of total mercury is 5.4-fold higher after intramuscular thimerosal than after oral MeHg exposure, implying that mercury is cleared at a much faster rate in infant monkeys dosed with thimerosal versus MeHg.

There are additional significant differences in the pharmacokinetic behavior between MeHg and ethylmercury. The kinetics of clearance of total mercury in the blood compartment is quite different for the two species (Burbacher et al. 2005). The one-compartment model best described blood concentrations after MeHg exposure, while a two-compartment model best described blood concentrations after ethylmercury exposure. Thus, ethylmercury will be cleared from the blood much faster compared to MeHg. These findings mitigate the idea that thimerosal will persist longer in the blood of vaccinated infants and establish that for an equivalent level of exposure; the area under the curve of total blood mercury concentrations in human infants receiving repeated intramuscular injections of thimerosal-containing vaccines will be significantly lower than that in those exposed to MeHg via the oral route.

The Brain-to-Blood Mercury Concentration Ratio Established for MeHg Will Overestimate Mercury in the Brain After Exposure to Ethylmercury

Caution should be drawn in using whole blood mercury levels as a biomarker for absorbed dose of MeHg or ethylmercury, and by extrapolation their concentration in the brain. While in general, there exists a high correlation between concentrations of total mercury in whole blood and corresponding concentrations in autopsy brain samples, such correlations occur only under steady-state conditions when blood and brain levels are no longer changing (Cernichiari et al. 1995). However, whole blood is not a good predictor of brain mercury levels under non-steady-state conditions when levels of mercury in both the blood and brain continuously change, reflecting different half-times of clearance from the two compartments. The large difference in the blood mercury compared to the brain half-time for the thimerosal-exposed monkeys (6.9 vs. 24 days, respectively) (Burbacher et al. 2005) indicates that blood mercury is not a good indicator of risk of adverse effects on the brain, particularly under conditions of rapidly changing blood levels, such as those observed after vaccinations (Clarkson and Magos 2006).

As mentioned above, Burbacher et al. (2005) established that there is a significant difference in brain half-times between MeHg and ethylmercury in infant monkeys. If the data from infant monkeys predict half-times in brain as well as they do for whole blood (see above), then, most of the organic mercury would be expected to clear from brain during the 2-month interval between vaccinations. This would not be true for the inorganic species, as it was noted that a much higher proportion of inorganic mercury is found in the brains of thimerosal treated infant monkeys than in the brains of MeHg exposed monkeys (up to 71 vs. 10%), with absolute inorganic mercury concentrations in the brains of the thimerosal-exposed monkeys reaching levels twice as high as in the MeHg-treated monkeys. These findings are consistent with the dealkylation of ethylmercury to the inorganic mercury species, pointing out that the process is much more extensive for ethylmercury than for MeHg. However, the significance of this process is unknown and the concept that it can be equated with neurotoxicity is unsubstantiated by the scientific literature.

Long-term exposure to MeHg results in accumulation of inorganic mercury in brains of adults. This inorganic species appears to be associated with selenium and is assumed to be present as an insoluble inert form (World Health Organization (WHO) 1990). However, the role of inorganic mercury freshly deposited in infant brains on repeated exposure to vaccines remains unknown, and the assertion that it is more toxic than organic forms of mercury is not scientifically proven. Previous reports have actually suggested that the dealkylation of mercury may represent a detoxification process that helps to protect the CNS by increasing the rate of mercury elimination from the brain (Magos 2003) and more rapidly reducing its mercury burden.

Because Ethylmercury Decomposes Much Faster Than MeHg, the Risk of Brain Damage is Less for Ethylmercury Than for MeHg

The idea that the inorganic species of mercury is the damaging species is not new. It has been proposed that latency period associated with MeHg exposure might be due to the slow production and accumulation of the divalent inorganic mercury in the brain over periods of months (Vahter et al. 1994). However, as reported by Weiss et al. (2002) one would expect the buildup of inorganic mercury to be faster at higher levels of MeHg exposure, resulting in a shorter latency period. This is contrary to evidence published in the literature (Magos et al. 1985; Weiss et al. 2002). Thus, there is no evidence in the literature to substantiate that inorganic mercury retained in the CNS plays any role in the neurotoxicity of organomercurials, including ethylmercury, nor is there evidence corroborating a putative role for inorganic mercury derived from demethylation of MeHg in the development of neurological effects during the chronic latent phase of exposure.

Additional studies (Charleston et al. 1994; Vahter et al. 1994, 1995; Charleston et al. 1995, 1996) in adult *M. fascicularis* monkeys addressed the pharmacokinetics of MeHg demethylation in the brain. Higher inorganic mercury concentrations were noted in the brains of the monkeys 6 months after MeHg exposure had ended, whereas organic mercury had cleared from the brain. The estimated half-time of organic mercury of 37 days in the brain of these adult monkeys was consistent across various brain regions and was analogous to the brain half-time of MeHg in the infant monkeys reported by (Burbacher et al. 2005). The estimated half-time of inorganic mercury in the brain in the same adult monkeys varied greatly across brain regions, corresponding to 227–540 days. The concentrations of inorganic mercury also varied significantly across brain regions, in some areas remaining unchanged (thalamus) while doubling in others (pituitary) 6 months after exposure to MeHg had ceased (Vahter et al. 1994, 1995). Stereologic and autometallographic studies indicated that inorganic mercury persisted in the monkeys' brains and it was associated with a significant increase in the number of microglia and a decline in the number of astrocytes. It is noteworthy that these effects were noted 6 months after cessation of chronic exposure to MeHg (Charleston et al. 1994, 1995, 1996) and that the effects in the adult macaques were associated with brain inorganic mercury levels approximately five times higher than those observed by Burbacher et al. (2005) in the infant monkeys vaccinated with ethylmercury. At this point, no data is available on the longer-term effects (>6 months) of inorganic mercury on brain structure or function, and it has yet to be established whether changes in the number of microglial and astrocytic cells in the developing brain are inherent to exposures to the lower levels of inorganic mercury associated with vaccinations.

There are additional data that raise serious doubt that inorganic mercury is the proximate species of MeHg-induced brain damage. For example, recent studies (Bland and Rand 2006; Tamm et al. 2008) identified Notch as a potential target for MeHg toxicity in the developing nervous system. The Notch receptor pathway is a highly conserved signaling mechanism that influences cell fate decisions, proliferation, migration, and neurite outgrowth during neural development (Bland and Rand 2006).These authors demonstrated a concentration- and time-dependent increase in Notch receptor activity with MeHg exposure in three distinct fruit fly (*Drosophila*) cell lines. Exposure to MeHg resulted in a 4–5.5-fold increase in Notch signaling while inorganic mercury (in the form of $HgCl_2$) was significantly less active in inducing Notch activity, suggesting a mechanism specific to organic species of mercury. In rodent neural stem cells, which are highly sensitive to MeHg toxicity (Tamm et al. 2006) low MeHg levels (2.5–10 nM) activated Notch signaling, as assessed by the increased activity in a specific Notch-reporter assay and by the increased cleavage of the Notch intracellular domain (Tamm et al. 2006). While several studies have shown equipotent toxicity for methyl- and ethylmercury in tissue culture experiments, in general, inorganic mercury has proven to be less toxic than MeHg in both vertebrate and invertebrate neuronal model systems. For example, MeHg is 6–40 times more toxic than inorganic mercury in PC12 pheochromocytoma cells, as measured by viability (Parran et al. 2001, 2003). While inorganic mercury and MeHg show nearly equivalent ability to kill cells (as measured by lethal $dose_{50}$, referred to as LD_{50} values, which correspond to the concentrations required to kill 50% of the cells) for viability in a mosquito derived cell line, MeHg is approximately 20 times more effective than inorganic mercury in inhibition of proliferation in these cells (Braeckman et al. 1997). In addition, MeHg is 10 times more potent than inorganic mercury in depressing nerve fiber growth in chick dorsal root ganglia explants (Miura et al. 2000). Altogether, these studies refute the notion that inorganic mercury in a number of model systems ranging from invertebrate animals to mammalian systems is the proximate source of damage associated with exposure to methyl- and likely ethylmercury.

Finally, there appears to be little concordance between the cellular damage inflicted by MeHg and the distribution of inorganic mercury. Damage to the cerebellum was observed only in MeHg-treated adult rodents and these animals had much lower levels of inorganic mercury in the brain than animals comparably treated with ethylmercury (Magos et al. 1985; Magos 2003). Moreover, the results did not indicate the presence of inorganic mercury deposits in the area where MeHg inflicted cerebellar damage, areas such as the granular layer. These findings are consistent with findings in the rat experimental model (Magos et al. 1985) where no damage was associated with repeated ethylmercury exposures despite the presence of inorganic mercury (see below), supporting the hypothesis that inorganic mercury is not directly involved in triggering tissue damage.

Given identical doses of MeHg and ethylmercury, more total mercury was deposited in the brain of mice (Harry et al. 2004), rats (Magos et al. 1985), and monkeys (Burbacher et al. 2005) after the administration of MeHg than after ethylmercury. In all animal models the concentration of inorganic mercury was much lower in the brains of MeHg-treated animals than in those treated with the same dose of ethylmercury. Notably, gastric gavage of rats with five daily doses of 8.0 mg Hg/kg methyl- or ethylmercuric chloride MeHg led to severe brain damage and degeneration (10 days after the last dose), while rats dosed with an identical dose of ethylmercury were completely

normal (Magos et al. 1985). The same authors reported that in order to elicit some brain damage in the rats they had to increase the dose of ethylmercury to the borderline of a lethal dose (Magos et al. 1985). Considering the repeated daily dosing, the insufficient time to allow for substantial clearance of mercury and the use of exceedingly high doses, these results further support that MeHg is not a suitable reference for evaluating ethylmercury toxicity.

A study by Hornig et al. (2004) noted the effects of thimerosal on an autoimmune disease sensitive strain of mice (SJL/J). The authors reported growth delay and behavioral changes, such as reduced locomotion and exaggerated response to novelty. Brain tissue examination revealed densely packed, hyperchromic hippocampal neurons with altered glutamate receptors and transporters. Other strains of mice, C57Bl/6J and BALN/cJ, did not show these effects at the same dosing schedule.

Notably, Hornig et al. (2004) tried to mimic the 1-year dosing schedule of infants with a 10-day schedule in mouse pups. While the time intervals between vaccinations of infants assure nearly complete clearance from brain and blood of the organic species, even a 7–8 times higher clearance rate in mouse pups (Hornig et al. 2004) would not prevent significant accumulation in mice. Thus, the model is not suitable for comparisons with vaccinated infants, as it will lead to blood and brain mercury burdens far in excess of those expected in humans. Notably, a more recent study assessing immune system function as an important factor influencing vulnerability of the developing nervous system to thimerosal in which SJL/J mice were injected with thimerosal, with and without combined HiB and DTP vaccines failed to indicate pervasive developmental neurotoxicity following vaccine-level thimerosal injections in these mice, and provided no support for the hypothesis that thimerosal exposure contributes to the etiology of neurodevelopmental disorders.

Additional Considerations on Comparisons Between Ethyl- And MeHg

Case reports of victims of MeHg and ethylmercury poisoning provide further support for the differential effects of MeHg and ethylmercury. A patient who ingested 83 mg/kg thimerosal (41 mg Hg/kg) in a suicide attempt had 14,000 mg/l blood mercury and developed anuria, coma, polyneuropathy, and respiratory failure. He had a complete recovery with no permanent brain damage (Pfab et al. 1996). A MeHg-exposed worker had 1840 mg Hg/l in blood and presented with similarly severe intoxication, but remained ataxic, dysarthric and with constricted visual fields (Magos 1998). Death has also been reported in two boys who ate meat from a butchered hog that had been fed seed treated with ethylmercuric chloride (Cinca et al. 1980). However, given the delay between mercury consumption and the onset of symptoms, the amount of organic mercury ingested in these cases is difficult to ascertain. As mentioned earlier, large-scale poisonings have also occurred in Iraq in 1956 and 1960 (Bakir et al. 1973, 1976). Thirty-one pregnant women were victims of poisoning; 14 women died from ingesting wheat flour from seeds treated with ethylmercury *p*-toluene sulfonanilide (Bakir et al. 1973, 1976). Infants were born with blood mercury concentrations of 2,500 μg/l and suffered severe brain damage. These blood mercury levels are orders of magnitude greater than those expected in a vaccinated infant (5.1 μg Hg/l was the highest concentration in a 2-month-old infant upon vaccination) (Pichichero et al. 2002). Additional reports of acute toxicity associated with ethylmercury exposure included the administration of immune globulin (gamma globulin) and hepatitis B immune globulin (Lowell et al. 1996), choramphenicol formulated with 1000 times the proper dose of thimerosal as a preservative (Axton 1972), thimerosal ear irrigation in a child with tympanostomy tubes (Rohyans et al. 1984) and thimerosal treatment of omphaloceles in infants (Fagan et al. 1977). The total doses of thimerosal administered in these reports of acute toxicity ranged from ~3 mg/kg to several hundred mg/kg, compared to 50 μg of thimerosal per 0.5 ml dose or approximately 25 μg of ethylmercury per 0.5 ml dose in a vaccinated child. Thus, these acute clinical cases of accidental and intentional poisonings with high doses of thimerosal serve no value in evaluating health risks (cardiac, neurological, or otherwise) associated with recommended immunization protocols.

Epidemiological studies from Canada (McKeown-Eyssen and Ruedy 1983) and New Zealand (Kjellstrom et al. 1986, 1989) have suggested that exposures to MeHg below 10 ppm might cause subtle neurodevelopmental abnormalities in prenatally exposed children. This finding was corroborated in a study conducted in the Faeroe Islands (Grandjean et al. 1997). Notably, several epidemiological studies question the association between MeHg and neurodevelopmental effects. For example, data from Peru (Marsh et al. 1995) and from the Seychelles Child Development Study (SCDS) have not confirmed subtle neurodevelopmental abnormalities in prenatally exposed children (Davidson et al. 1995, 1998; Myers et al. 1995, 2009). The potential role of additional factors, including exposure to polychlorinated biphenyls in the Faroe Islands cohort (Longnecker et al. 2003). The potential role of additional factors, including exposure to polychlorinated biphenyls in the Faroe Islands cohort, as well as aspects related to samples and data analyses (Longnecker et al. 2003) have been considered as possible reasons for the apparent discrepancy. Possible beneficial

associations between maternal and child hair MeHg levels and several endpoints of child development have been reported. Using the Avon Longitudinal Study of Parents and Children (ALSPAC) to assess the possible benefits and hazards to a child's development of different levels of maternal seafood intake during pregnancy Hibbeln et al. (2007) reported that maternal seafood intake during pregnancy of less than 340 g per week was associated with increased risk of their children being in the lowest quartile for verbal intelligence quotient (IQ). Low maternal seafood intake was also associated with increased risk of suboptimum outcomes for social behavior, fine motor, communication, and social development scores. For each outcome measure, the lower the intake of seafood during pregnancy, the higher the risk of suboptimal developmental outcome. Thus, maternal seafood consumption of less than 340 g per week in pregnancy did not protect children from adverse outcomes. Though mercury consumption was not assessed in this study, it is reasonable to assume that greater fish consumption was paralleled by increased MeHg intake. The beneficial effects of fish consumption corroborate those in the SCDS, questioning the rationale for FDA's fish advisories to limit seafood consumption and suggest that the consequence of these advisories may be detrimental with opposite effects to those originally intended.

Conclusions

Methodologically sound and rigorous epidemiological studies have largely failed in finding a significant correlation between thimerosal-containing vaccines and autism. However, efforts to reduce the exposure of infants, children, and pregnant women to any form of mercury from various sources should continue.

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