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## BRAIN DEVELOPMENT AND METHYLMERCURY: UNDERESTIMATION OF NEUROTOXICITY

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### Abstract

Methylmercury is now recognized as an important developmental neurotoxicant, though this insight developed slowly over many decades. Developmental neurotoxicity was first reported in a Swedish case report in 1952, and from a serious outbreak in Minamata, Japan a few years later. While the infant suffered congenital poisoning, the mother was barely harmed, thus reflecting a unique vulnerability of the developing nervous system. Nonetheless, exposure limits for this environmental chemical were based solely on adult toxicity until 50 years after the first report on developmental neurotoxicity. Even current evidence is affected by uncertainty, most importantly by imprecision of the exposure assessment in epidemiological studies. Detailed calculations suggest that the relative imprecision may be as much as 50%, or greater, thereby substantially biasing the results toward the null. In addition, as methylmercury exposure usually originates from fish and seafood that also contains essential nutrients, so-called negative confounding may occur. Thus, the beneficial effects of the nutrients may appear to dampen the toxicity, unless proper adjustment is included in the analysis to reveal the true extent of adverse effects. These problems delayed the recognition of low-level methylmercury neurotoxicity. However, such problems are not unique, and many other industrial compounds are thought to cause developmental neurotoxicity, mostly with less epidemiological support than methylmercury. The experience obtained with methylmercury should therefore be taken into account when evaluating the evidence for other substances suspected of being neurotoxic.

### Keywords

Epidemiology; methylmercury compounds; neurotoxicity syndromes; prenatal exposure delayed effects

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By end of the third week of gestation in humans, the fetal brain has already begun its formation and any interruption during this early period can result in severe abnormalities of the brain and spinal cord.<sup>1</sup> During the third trimester, pathways for nervous system functions are being formed, thus making the brain particularly vulnerable to transplacental transfer of neurotoxic chemicals at this time. This vulnerability has been amply documented by the effects of toxic metal compounds, such as methylmercury.<sup>2</sup> The outcome of developmental neurotoxicity may not be immediately apparent in the infant, but deficits will become evident later on as long-standing or irreversible dysfunctions.<sup>3</sup>

The outcome of developmental neurotoxicity may not be immediately apparent in the infant, but deficits will become evident later on as long-standing or irreversible dysfunctions. More generally, the delayed recognition of developmental neurotoxicity due to MeHg heralds some limitations in scientific documentation that may lead to deficient prevention of neurotoxic exposures.

Methylmercury neurotoxicity was first demonstrated in adults, with extensive evidence accumulated from poisoning episodes.<sup>4</sup> However, adverse effects proved to be difficult to diagnose due to the latency period of several weeks to months between exposure and development of clinical symptoms.<sup>5</sup> Thus, in regard to routine health examinations of exposed workers, Ahlmark stated,<sup>6</sup> “Such symptoms [of methylmercury poisoning] scarcely differ from those generally found in neurasthenics when they think that they have been exposed to toxic risks.” Not surprisingly, therefore, the vulnerability of the developing human brain to methylmercury was only discovered later on, as children in fishing populations experienced lasting adverse effects on brain function due to MeHg crossing the placental barrier.<sup>7</sup> The delayed recognition of developmental neurotoxicity due to MeHg is of more general concern, as it heralds some limitations in scientific documentation that may lead to deficient prevention of neurotoxic exposures.

The evolution of insights into methylmercury neurotoxicity demonstrates the challenges in documenting neurodevelopmental deficits due to prenatal neurotoxicant exposures.<sup>3</sup> First, the decrements may not be detectable until several years after the causative exposure. Second, early adverse effects may be nonspecific and difficult to document, although even slight deviations from optimal brain development are likely to be considered adverse and unwanted. As a further reason for delayed recognition, MeHg exposure mainly originates from fish and seafood, which contain essential nutrients that may provide a beneficial effect on brain development. Thus, the opposite effects of mercury and the nutrients need to be addressed, so that properly adjusted measures can be generated for each component’s effects on the relevant outcomes, so that neither is being underestimated.<sup>8</sup>

MeHg exposure mainly originates from fish and seafood, which contain essential nutrients that may provide a beneficial effect on brain development. Thus, the opposite effects of mercury and the nutrients need to be addressed, so that properly adjusted measures can be generated for each component’s effects on the relevant outcomes, so that neither is being underestimated.

Individual and community studies have contributed clear evidence indicating that maternal consumption of MeHg can have serious and irreversible effects on the physical and mental development of children, even if the mother exhibits no outward symptoms. While heavy exposures constitute a clear hazard with adverse neurological signs and test deficits, the effects of more common medium and low-level exposures are less pronounced and more difficult to document and quantify. As apparently conflicting evidence was available, the U.S. White House in 1998 convened an international workshop with 30 invited experts, who were asked to critically examine the scientific evidence. The experts chose to emphasize a variety of possible uncertainties and possible confounders. The conclusions stated,<sup>9</sup> “Even when dietary stresses and coexposures to other chemicals could plausibly enhance or alter risk, it was still deemed that there are inadequate data on this subject to draw meaningful conclusions at this time.” As the results of epidemiological studies in this field may easily be underdetermined, the question emerges how low a dose, if any, is safe especially for that of a pregnant mother and her unborn child. An assessment of the uncertainties in such research and their interpretation therefore becomes crucial.

## INITIAL OBSERVATIONS OF CONGENITAL METHYLMERCURY POISONING

The year 1952 was the first time when a case of congenital methylmercury poisoning was described.<sup>10</sup> A Swedish family had inadvertently used flour made from a methylmercury-treated seed grain. Since weaning at nine months, the infant ate the porridge made with this flour, as did the pregnant mother, who was herself asymptomatic. She delivered the second child who at first also appeared healthy. Soon after, both infants were found to be mentally retarded and severely deficient in motor development. Furthermore, their condition was virtually unchanged two years later. Although the doses received by the mother and her two children are not known, this case report suggested that the nervous system could be much more vulnerable to methylmercury toxicity during early development, including the fetal stage. Possibly due to incomplete differential diagnosis and consideration of genetic causes, this case report has been generally ignored, although it most likely represents the first evidence of developmental neurotoxicity by methylmercury.

Shortly after the Swedish case was reported, a considerably larger scale case of the long-term exposure to MeHg in Minamata, Japan began to unravel, now due to the ingestion of contaminated fish; infants were evidently poisoned in their mother's womb.<sup>11</sup> Many children born from 1955 and later had neurodevelopmental disturbances.<sup>12</sup> Children under 9 years of age appeared to be particularly numerous among the Minamata patients. In some cases, the effects of MeHg exposure *in utero* resulted in offspring born with congenital methylmercury poisoning<sup>13</sup> manifested as severe neurological deficits, though the mothers appeared unaffected or suffered only mild symptoms.<sup>14, 15</sup>

Most of these children were not immediately diagnosed, as the spastic paresis-like syndrome common in these children was less distinctive than the clinical picture of the adult poisoning cases, where tunnel vision was especially characteristic. The early signs in an infant with congenital poisoning (i.e., mental retardation, movement problems, seizures, primitive reflexes, and speech difficulty) could be easily mistaken for some other pediatric disease, and mild stages could be simply overlooked. Thus, diagnosis was usually made only later on, when milestone achievement had clearly failed.

Neuropathology data from detailed autopsies were supplemented by histological, histochemical, and chemical examinations. It became clear that the adult disease was associated with localized lesions in certain brain areas (such as the calcarine, postcentral, precentral, and temporal transverse cortices and deep structures of the cerebellar hemispheres of the brain). Whereas, methylmercury poisoning in children showed more widely distributed damage on the brain. However, infants and children who had become poisoned before birth from their mother's diet showed a completely diffuse pattern of damage with disruption of normal structures.<sup>16, 17</sup>

These findings strongly supported that early developmental exposure could cause a much more serious disease. As stated by a visionary pathologist already in 1977,<sup>18</sup> "It may thus be supposed that the fetal brain is more fragile and susceptible to toxic agents, since it is immature and still undergoing development. Clearly, prevention of Minamata disease, especially congenital cases, is a first requirement, and the greatest care should be taken by pregnant women since the fetus has a higher sensitivity."

The Minamata study is one of two extensive community incidents. The other incident occurred about a decade later in Iraq, though the MeHg exposure was not from the eating fish, but contaminated bread from grain seed that had been treated with a methylmercury fungicide. The analysis of data from mother and infant pairs after the outbreak based on MeHg maternal hair levels showed significant relationships between the mother's exposure and the child's neurological and physical development based on appropriate milestones.<sup>19, 20</sup>

In 1978, Iraqi pediatrician, Laman Amin-Zaki, collaborated with U.S. colleagues to investigate the effects of methylmercury exposure in 49 children. Although the exposed children were examined via crude neurological tests at various ages, development of language and motor function of children exposed prenatally was found to be delayed.<sup>21</sup> A later report described the use of advanced analytical technology to determine mercury concentration profiles in single hair strands, so that the researchers could get a calendar record of the methylmercury exposure during the entire duration of the pregnancy.<sup>22</sup> These results suggested that the nervous system during early development in utero had a fivefold greater vulnerability to methylmercury based on the delayed achievement of developmental milestones; the researchers also concluded that an increased risk of developmental toxicity occurred at a maternal hair mercury concentration above 10 and up to 20 µg/g.<sup>23</sup>

## MAJOR PROSPECTIVE COHORT STUDIES

So far, three major longitudinal prospective cohort studies launched in the 1970s and 1980s have examined methylmercury-exposed children in New Zealand,<sup>24</sup> the Faroe Islands,<sup>25</sup> and the Seychelles<sup>26</sup>. The New Zealand study effectively adjusted for the beneficial effects of seafood nutrients by selecting pregnant women with a high fish intake and comparing the children of mothers with a high mercury exposure with those with a low. In the Faroes, mercury contamination of fish is limited, and most of the exposure comes from ingestion of pilot whale meat as pemmican or steaks. The results from these two populations suggested a link between prenatal MeHg exposure from the mothers' seafood consumption and neurobehavioral shortfalls in the children. The deficits in attention, visuospatial function, language and verbal memory appeared to conflict with those reported in the Seychelles. However, further review of the results identified several uncertainties, and statistical analysis indicated wide confidence limits so that the two studies did not mutually disagree.<sup>27</sup> The main evidence used for U.S. and international guidelines has stemmed from these three cohorts.<sup>28</sup> Attempts to combine the findings from the three studies have been difficult, as different methods were used for exposure assessment and outcome measures. Even when the same type of neurobehavioral test was used, differences in administration and culture-dependence limits the extent to which the data can be merged. Nonetheless, the Faroes data suggested that the most sensitive brain functions showed a delay in development of 1.5-2 months at age 7 years associated with each doubling of the prenatal MeHg exposure. This delay corresponded to about 10% of the standard deviation for these tests, which would correspond to about 1.5 IQ points. This finding agrees well with the difference of 2 IQ points between the two groups of New Zealand children, where the high-level exposure was approximately twice that of the controls. Although limited follow-up is available from a more recent birth cohort from the Seychelles, the associations between fetal exposures and early child development from fish consumption showed that the benefits from long-chain polyunsaturated fatty acids were obscured if the adverse effects of MeHg were not taken into account; likewise, confounding of the adverse effects of MeHg by the beneficial effects of essential fatty acids was also documented, thus demonstrating that this confounding must be considered when evaluating data in longitudinal observational studies of seafood contaminants and nutrients.<sup>29</sup>

## MORE RECENT STUDIES

Similarly, more recent studies (as outlined here and in Table 1) have shown mixed results with some indication that eating fish and marine mammals may lead to neurobehavioral or developmental abnormalities. The first North American study of Cree Indian infants had limited documentation of the exposure, but some support nonetheless emerged that subtle adverse effects may be associated with developmental exposure to MeHg.<sup>30</sup> Similar findings were reported from populations in the Amazon basin.<sup>31</sup> One study from Eastern Europe

illustrated that cord and maternal blood mercury concentrations at relatively low seafood consumption are associated with delayed psychomotor development in first-year infants. Though the cohort study showed a narrow range of exposure and did not allow for assessment of exact dose-response relationships, the study indicated an increased risk for delayed neurodevelopment in infants posed by fairly low ranges of MeHg exposure.<sup>32</sup>

Additionally, two studies conducted in the United States one in New York City and another in Boston examining fish/seafood consumption present a similar dilemma as illustrated in Table 2. The regression analyses used to evaluate the data from the NYC study indicated no significant association between cord blood or maternal blood total mercury, though there was an inverse association between the log cord mercury and the Bayley Scales of Infant Development psychomotor score at 36 months and with Performance, Verbal and Full IQ scores on the Wechsler Preschool and Primary Scale of Intelligence, Revised (WPPSI-R) at 48 months after controlling for confounders such as fish/seafood consumption.<sup>33</sup> The Boston study, whereas, suggests that interventions in early life to reduce exposure to low levels of mercury such as the nutrient benefits like docosahexaenoic acid from eating fish/seafood, should be weighed against the potential harm Hg in fish may cause.<sup>34</sup>

Most recently, a Japanese study reported on the neurobehavioral effects on prenatal exposure to methylmercury. The relationship between the Neonatal Behavioral Assessment Scale clusters and the exposure markers and maternal seafood intake was assessed by multiple regression analysis to adjust for possible confounders. While prenatal exposure to MeHg adversely affects neonatal neurobehavioral function, maternal seafood intake during gestation seems to have benefits. When concomitant polychlorinated biphenyl (PCB) exposure and maternal seafood intake were adjusted for, prenatal exposure to MeHg, even in low doses, appeared to impair neurobehavioral function in the neonates.<sup>35</sup> These studies therefore support the findings from the Faroes and New Zealand, as well as the most recent Seychelles data.

## EXPOSURE ASSESSMENT

Methylmercury exposure depends on frequency of fish intake, the size of each meal, and the particular species. The highest concentrations of methylmercury occur in predatory fish and marine mammals because of the biomagnification through aquatic food chains.<sup>36</sup> However, the contamination also varies much within individual species,<sup>37</sup> thus rendering dietary questionnaires particularly imprecise for assessment of methylmercury exposure.

Mercury concentrations of (maternal) hair and of cord blood are the most commonly used exposure biomarkers for assessment of prenatal exposure to MeHg. Although both may be affected by exposures to inorganic or elemental mercury, they generally reflect the methyl species. The blood concentration provides an estimate of the recent exposure, as methylmercury in blood has a half-life of 45–60 days. The cord-blood concentration at parturition therefore reflects the exposure during the third trimester. Given the average hair growth rate of 1 cm per month, a hair sample of 8 cm taken at parturition will mainly reflect the exposure during the first two trimesters. In addition, the hair–mercury concentration can reflect the calendar of exposure events along the hair shaft.<sup>38</sup> Routine quality assurance usually focuses on the laboratory performance as such, and atomic absorption analyses can general be carried out with a relative imprecision better than 5%. To assess the validity of the analytical quality, biomarker data of prenatal methylmercury exposure, i.e., mercury concentrations in cord blood, cord tissue, and maternal hair, were compared with questionnaire information on dietary exposure.<sup>39</sup> Although these parameters correlated well with one another, substantial scattering was apparent. Because at least three exposure parameters were available, factor analysis and structural equation modeling could be applied



to determine the total imprecision of each biomarker. For the cord-blood parameter, the total imprecision was 25-30%, and almost twice as much for maternal hair. Thus, the total imprecision of these biomarkers much exceeded the normal laboratory variability, although it was less than the imprecision associated with dietary questionnaire data.<sup>39</sup>

This issue has not been readily appreciated. One commenter suggested that the Faroese would be exposed to bolus doses of MeHg when whale hunters (and their wives, presumably) gorged themselves with whale meat.<sup>40</sup> Although this depiction is misleading, as whale meat is either frozen or cured for long-term usage, any variable exposure could further augment the imprecision of the exposure assessment, which was probably not what the author had in mind. To examine such concerns in the Faroes study, mercury concentrations were measured in two sets of hair samples, one 9-cm sample reflecting the whole pregnancy period, the other consisting of the most proximal 2 cm. Cases that showed more than a small disagreement between the two, thus suggesting variable exposure during the pregnancy period were then excluded from the regression analyses. This exclusion caused the mercury effect to increase,<sup>41</sup> thereby confirming the anticipation that imprecision would cause a bias toward the null.<sup>39</sup>

## OUTCOME ASSESSMENT AND CLINICAL TESTING

The validity of outcome variables depends on their sensitivity to the exposure under study and the associated specificity, i.e., lack of sensitivity to the influence of other factors, including confounders. The choice of effect parameters must both be feasible and appropriate for the age of the children, and for the setting of the study. Tests that depend only minimally on cooperation of the subject have the advantage of being less likely to be affected by motivation. Tests of higher-order neuropsychological functioning may only be possible when a child has reached school age or beyond. As most neurobehavioral tests have been developed and standardized in the US and Europe, they may be of uncertain validity in other cultures and when translated to a language not previously used in standardization efforts. In addition, many tests require special skills of the examiner. All of these issues need to be considered when evaluating the study findings.

Most studies of MeHg neurotoxicity employed a battery of neurobehavioral tests, some of which appeared to be more sensitive to mercury neurotoxicity than others. Simple comparisons of regression coefficients may provide suggestions for the most sensitive parameter, at least within the confines of a particular study. To facilitate such comparisons, the regression coefficient may be expressed as a proportion of the standard deviation of the test result, or as a delay in mental development calculated from the regression coefficient for age.

## REGULATORY ASSESSMENTS OF METHYLMERCURY NEUROTOXICITY

Baker's<sup>4</sup> dose-response data were used for the first risk assessment of methylmercury by an expert committee under the World Health Organization and the U.N. Food and Agriculture Organization in 1978.<sup>42</sup> This first international evaluation of methylmercury toxicity recommended a provisional tolerable weekly intake of 200  $\mu\text{g}$  (or 3.3  $\mu\text{g}/\text{kg}$  body weight). Although the experts realized that "clinical data from Japan indicate that the fetus is more sensitive than the mother," they refrained from recommending any special protection. The Swedish report from 1952 was mentioned only in passing and did not attract special attention. In 1990, when the developmental neurotoxicity from the Iraqi incident was reviewed by the International Programme on Chemical Safety,<sup>23</sup> early signs of fetal neurotoxicity were deemed to occur when maternal hair-mercury concentrations exceed 10–20  $\mu\text{g}/\text{g}$ . Increased vulnerability of the unborn child was considered only from data on neurological abnormalities and delayed milestone development.<sup>20-22</sup> These international

expert committees found insufficient data on developmental neurotoxicity and based their evaluations primarily on the more detailed adult toxicity data. These conclusions formed the basis for risk assessment for the next 25 years<sup>43</sup> and for the current safety limits used by the US Food and Drug Administration, for example.

In the most recent assessment, the experts decided to disregard the New Zealand study and base its considerations on the two other large prospective studies. The experts decided to weigh in the benefits of fish consumption, there by allowing a greater MeHg exposure in order not to cause decreases in fish intake<sup>43</sup> or cause undue panic and abstention from fish consumption in local populations. As later research showed that the Seychelles data, and to a lesser extent the Faroes data, had been affected by negative confounding from fish nutrients, this decision effectively counted in the benefits from nutrients twice.

## DISCUSSION

The neurotoxic effects of MeHg on the fetus have been well documented based on the finding of several cohorts conducted in a number of fish eating communities worldwide.<sup>4, 14, 15, 28, 33, 35, 44-46</sup> Maternal consumption of MeHg from fish and seafood (or seed grain that has been treated with MeHg fungicide) can have serious and irreversible effects on the neurobehavioral development of children even in the absence of symptoms in the mother. Although evidence was first published in 1952 that methylmercury was a developmental neurotoxicant, international consensus and regulation only was reached 50 years later. However, the impact of uncertainties (like under- and overestimation) has not yet been considered by the committees that set standards (Figure 1).

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The existence of confounding can distort the true association between an exposure and a toxic effect outcome if the confounding variable is not controlled either in the study design or the analysis phase. The main concern was that known or unknown confounders could have caused the MeHg-associated effects seen in the Faroes and New Zealand. Perhaps co-exposure to PCBs, another marine pollutant, could have caused the neurotoxicity observed. However, adjustment of the Faroes data for PCB exposure did not eliminate the MeHg-associated effects.<sup>47</sup> In fact, if these effects were to be explained by another pollutant (or other confounder, whether chemical, social or genetic), this parameter would need to be more closely associated with the cord-blood Hg concentration than with the maternal hair-Hg to cause the greater effect estimates for the cord-blood level than for the hair level. The confounder would also have to become a better risk indicator when mothers with variable MeHg exposure were excluded. It is difficult to imagine a confounder that would satisfy these requirements.

Confounding is often assumed to occur in the same direction as the toxicant exposure, but the relationship between the benefits and risks associated with fish and seafood consumption represents so-called negative confounding: the exposure to methylmercury occurs from fish and seafood (the confounder) which are also associated with beneficial nutrients, thereby counteracting the mercury toxicity as illustrated in Figure 1.<sup>8</sup> Although both MeHg and nutrients may affect the same epidemiological outcomes (in opposite directions), most studies addressing one of them have ignored the potential negative confounding by the other. Substantial underestimation of the effects of mercury toxicity and fish benefits occurs from the lack of confounder adjustment and imprecision of the exposure parameters. In the Faroes, adjustment for maternal fish intake during pregnancy only resulted in fairly small increases in the calculated MeHg effects.<sup>48</sup> The limited effect was due to the poor

correlation between fish intake and the MeHg exposure biomarkers, which were mainly affected by whale meat intake. However, in the most recent Seychelles cohort, adjustment for fish intake resulted in the MeHg effects becoming statistically significant.<sup>26</sup>

In evaluating the evidence on MeHg neurotoxicity, researchers optimistically assumed that the exposure biomarkers were precise.<sup>9</sup> Exposure imprecision and thus misclassification will generally be non-directional, thereby leading to an underestimation of dose-effect relationships.<sup>49</sup> This problem may be exaggerated by potential confounders that are correlated with the exposure. In a regression analysis, inclusion of such variables may then further add to the bias toward the null hypothesis,<sup>50</sup> even in cases where the potential confounder has no independent effect on the outcome.

The Faroes data suggest that hair-mercury as an exposure biomarker may have a relative imprecision of about 50%. Other studies may include similar levels of imprecision and associated underestimation of the MeHg effects. For example, prenatal MeHg exposure in the Seychelles was characterized from the mercury content of maternal hair collected six months after parturition. A small study documented that the Hg content correlated significantly with Hg in brain tissue obtained at autopsy of deceased cohort children.<sup>51</sup> However, the substantial scattering suggests the existence of a sizable degree of imprecision.

All of these issues are crucial in regard to dose-response relationships and calculation of exposure limits, but they have not been considered yet in risk assessments from regulatory agencies or international organizations. Taking into account imprecision and negative confounding would likely cut the lowest exposure limit – the one used by the U.S.EPA – by 50% or more.<sup>38</sup>

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MeHg is not unique as a developmental neurotoxicant. Due to the relative ease in measuring mercury concentrations by atomic absorption and the relative wealth of epidemiological data, this substance has illustrated some key concerns and their impacts on our current appreciation of the public health impacts of this neurotoxicant. However, the neurotoxic universe probably includes a substantial number of industrial chemicals.<sup>3</sup> The factors illustrated by the MeHg research probably play a major role in underestimating the public health significance of developmental neurotoxicity of these chemicals.

## CONCLUSION

The evidence that methylmercury is a developmental neurotoxicant developed only slowly within a time frame of about 50 years, despite shocking documentation of debilitating congenital poisonings from Minamata, Japan, and other locations. Although the mother of the poisoned infant appeared to escape unscathed, the unique vulnerability of the developing human nervous system was not accepted by scientific committees for several decades. The desire for indisputable proof was the main obstacle, along with uncertainty, most importantly imprecision of the exposure assessment. A surprising relative imprecision of 50% has been documented for a commonly used exposure biomarker, and such imprecision generally causes bias of the results toward the null. Further, methylmercury comes from fish and seafood, a main source of certain essential nutrients that are important for brain development. A high degree of association between toxic MeHg and the essential nutrients results in negative confounding. For this reason, the beneficial effects of the nutrients may appear to dampen the toxicity, and only recently appropriate adjustment has been included in the analysis and revealed the true extent of the MeHg neurotoxicity. These problems are not unique to MeHg. Many other industrial compounds are thought to cause developmental



neurotoxicity, but the documentation is blurred by similar problems. The experience obtained with methylmercury should therefore be taken into account when evaluating the evidence for other substances suspected of being neurotoxic.

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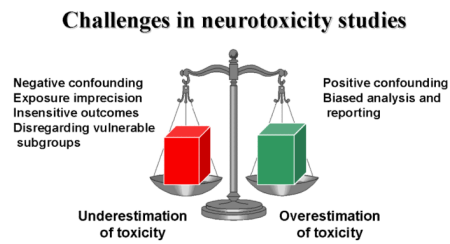
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## ABBREVIATIONS

<b>MeHg</b>	methylmercury
<b>Hg</b>	mercury
<b>PCB</b>	polychlorinated biphenyl
<b>IQ</b>	intelligence quotient



**Figure 1.**

Factors that can contribute to overestimation and underestimation of the toxicity caused by environmental chemicals, as illustrated by methylmercury neurotoxicity.

**Table 1**

## Time Course of Insights into Methylmercury Toxicity and Related Interventions

Year	Event
1866	First published record of fatal occupational methylmercury poisoning
1940–1954	Poisoning cases in workers at fungicide production plants
1952	First report on developmental neurotoxicity in two infants
1956	Discovery of a disease of unknown origin in Minamata, Japan
1955–1972	Poisoning epidemics from use of methylmercury-treated seed grain for cooking in Iraq, Guatemala, Pakistan, Sweden, and the USA
1973	Dose–response relationship described in poisoned adults in Iraq
1978	Exposure limit of 3.3 µg/kg per week based on toxicity in adults; Cree children in Canada assessed at low MeHg exposure
1986	First report on adverse effects in children related to maternal fish intake during pregnancy (New Zealand)
1997	Population study shows adverse effects in children from methylmercury in maternal seafood intake (Faroe Islands)
1998	White House expert workshop identifies uncertainties in evidence
2000	National Research Council (U.S.) supports exposure limit of 0.1 µg/kg per day
2003	Updated international exposure limit of 1.6 µg/kg per week
2004	European expert committee recommends that exposures be “minimized”
2009	International agreement on controlling mercury pollution



Table 2

Cross-sectional studies of neurodevelopmental effects in children exposed to methylmercury (studies with more than 100 subjects examined)

Country (reference)	Number (age range)	Main source of exposure	Exposure biomarker	Average exposure* (range)	Outcome measures	Results (ns = not significant)
Canada (McKeown-Eyssen et al., 1983)	234 children (12-30 months)	freshwater fish	maternal hair during pregnancy	$m_g = 6 \mu\text{g/g}$ (0-24)	Growth parameters Neurological examination	Abnormalities of tendon reflexes (boys only, no dose-response)
Peru (Marsh et al., 1995)	131 infants	marine fish	maternal hair during pregnancy	$m_g = 7 \mu\text{g/g}$ (1-29)	Growth parameters Developmental milestones	ns
Madeira (Murata et al., 1999)	153 children (7 years)	marine fish	child hair maternal hair	$m_g = 3.8 \mu\text{g/g}$ $m_g = 9.6 \mu\text{g/g}$	Neurological examination Neuropsychological tests Brainstem auditory evoked potentials Visual evoked potentials	ns ns I-III (and I-V) interpeak latency correlated with maternal Hg NI45 correlated with maternal Hg
Brazil (Grandjean et al., 1999)	420 children (7-12 years)	freshwater fish (gold mining area)	child hair maternal hair	$m_g = 11.0 \mu\text{g/g}$ $m_g = 11.6 \mu\text{g/g}$	Finger-tapping Santa Ana dexterity test WTSC-R Digit span Stanford-Binet Copying Recall Bead memory	ns $\beta = -5.58$ $p=0.001$ ns $\beta = -3.40$ $p=0.003$ $\beta = -1.23$ $p=0.02$ ns
French Guiana (Cordier et al., 2002)	248/290 children neurological ex. (6m-6 years) 206/243 children neuropsych. tests (5-12 years)	freshwater fish (gold mining area)	child hair maternal hair	$m_g = 10.2 \mu\text{g/g}$ $m_g = 12.7 \mu\text{g/g}$	Neurological examination Finger tapping Stanford-Binet test Blocks Copying Bead memory McCarthy Digit spans forward Leg coordination	Increased tendon reflexes ns ns $\beta = -2.98$ $p<0.001$ ns ns $\beta = -3.72$ $p=0.006$

Country (reference)	Number (age range)	Main source of exposure	Exposure biomarker	Average exposure* (range)	Outcome measures	Results (ns = not significant)
USA (NYC) (Lederman et al., 2008)	329/738 pregnant women	fish/seafood	cord and maternal blood	m = 7.8 µg/L	Neurological examination Bayley Scales Wechsler Revised(WPPSI-R)	$\beta = -4.2, p < 0.001$ $\beta = -3.8, p < 0.001$
USA (Boston, MA) (Oken et al., 2008)	341/1579	fish/seafood	maternal hair, blood	m = 3.8 ng/g	PPVT WRA VMA	ns ns
Japan (Suzuki et al., 2010)	599/1500 pregnant women	fish/seafood	maternal hair, blood	m = 2.22 µg/g (hair); 52.4 ng/g (cord blood)	Neurobehavioral assessment	ns

\* mg, arithmetic mean; µg, geometric mean; WISC-R, Wechsler Intelligence Scale for Children-Revised WPPSI, Wechsler Preschool and Primary Scale of Intelligence, Revised; PPVT, Peabody Picture Vocabulary Test; WRAVMA, Wide-Range Assessment of Visual Motor Abilities