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Review

Methylmercury toxicity and functional programming☆

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Abstract

Purpose: Adverse health effects of developmental toxicants may induce abnormal functional programming that leads to lasting functional deficits. This notion is considered from epidemiological evidence using developmental methylmercury neurotoxicity as an example.
Most important findings: Accumulating evidence indicates that adverse effects may occur even at low-level methylmercury exposures from seafood and freshwater fish. Neurobehavioral outcomes are usually non-specific, and imprecise exposure assessment results in a bias toward the null. Essential nutrients may promote the development of certain brain functions, thereby causing confounding bias. The functional deficits caused by prenatal methylmercury exposure appear to be permanent, and their extent may depend on the joint effect of toxicants and nutrients.
Principal conclusions: The lasting functional changes caused by neurodevelopmental methylmercury toxicity fit into the pattern of functional programming, with effects opposite to those linked to beneficial stimuli.

Keywords: Adolescent child food contamination; Methylmercury compounds; Neuropsychological tests; Neurotoxicity syndromes; Prenatal exposure delayed effects

1. Introduction

Fetal and early postnatal development constitutes the most vulnerable stage of human life, in regard to adverse effects of environmental toxicants [1]. While much attention has been paid to malformations, low birth weight, and other adverse pregnancy outcomes, recent research has revealed that more subtle effects during early development may also lead to functional deficits and increased disease risks later in life [1,2]. These developmental toxicity considerations parallel the "fetal programming" issues explored in experimental and epidemiological studies. Both emphasize the timing of exposure as crucial, and both can result in health outcomes that occur after a substantial delay [3]. While much attention has been paid to the beneficial effects caused by essential nutrients, toxicants warrant attention due to their effects in the opposite direction. Fig. 1 illustrates how these two sets of factors may affect the same outcomes during development and growth.

Adverse effects on the nervous system are of particular concern, because neurodevelopmental disorders affect 5–10% of babies born worldwide [4]; they include dyslexia, mental retardation, attention deficit/hyperactivity disorder, cerebral palsy, autism, and subclinical decrements in brain function. Although their etiology is mostly unknown, a small number of industrial chemicals – lead, methylmercury, polychlorinated biphenyls, arsenic, certain pesticides and toluene – are generally recognized possible causes of such disorders [5]. Research on these substances shows that exposures during early development can cause brain injury at dose levels much lower than those affecting adult brain functions [5]. If a developmental process in the brain is halted or inhibited, there is little chance for repair, and a small change may have substantial consequences [6–8]. The lasting damage may therefore be considered an effect on functional programming.

The present paper reviews current evidence on developmental methylmercury neurotoxicity in regard to such functional misprogramming and its long-term consequences, mainly building upon a recent review [9]. Evidence from poisoning outbreaks first demonstrated the severe and widespread damage that may occur to the brain when exposed to methylmercury during

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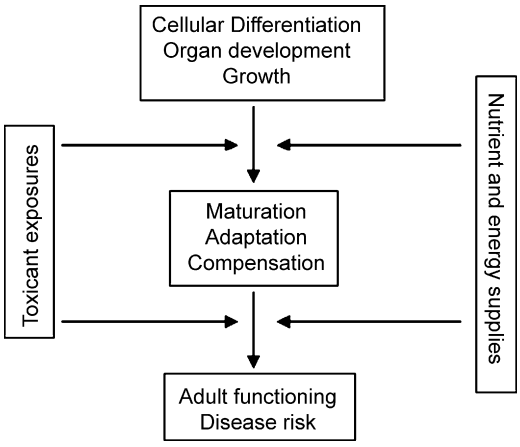

Fig. 1. Impact of toxicant exposures and nutrients on functional programming.

early development. In Minamata, Japan, it was noted that the pregnant mother could appear in good health, while her child would be born with serious congenital methylmercury poisoning. Although only crude dose–response relationships were obtained, the incidents demonstrated the serious consequences of excess exposures to this neurotoxicant and documented that the developing brain is a highly sensitive target.

Recent risk assessments for methylmercury have been published by national and international bodies (e.g., the (U.S.) National Research Council [10], the U.S. Environmental Protection Agency [11], and the Joint FAO/WHO Expert Committee on Food Additives [12]). These reports concluded that the developing brain is the main target for methylmercury toxicity, and they emphasized three prospective epidemiological studies as the main basis for deriving an exposure limit, i.e., studies conducted in New Zealand, the Faroe Islands, and the Seychelles. A brief review of these studies will illustrate the strengths and weaknesses of the evidence currently available (Table 1).

2. Major prospective studies of developmental methylmercury neurotoxicity

2.1. New Zealand

From a group of 11,000 expectant mothers, hair-mercury concentrations were determined for the 1000 mothers, who had consumed at least three fish/seafood meals per week during pregnancy [13]. Seventy-three of these mothers were highly exposed, with a hair-mercury result above 6 μg/g. These women were likely to have eaten highly contaminated shark meat. At the first follow-up at age 4 years, 31 high-exposure children and 31 reference children with lower exposure were matched for potential confounders (i.e., mother's ethnic group, age, child's birthplace and birth date). The high-exposure group showed lower scores on the Denver Developmental Screening test [13].

A follow-up of the highly exposed children took place at age 6 years, now with three control groups with lower prenatal mercury exposure at the same seafood intake [14]. During pregnancy, mothers in two of these matched control groups had high fish consumption and average hair-mercury concentrations of 3–6 μg/g and 0–3 μg/g, respectively. A total of 61 of the highly exposed children were available for examinations. Stepwise robust multiple regression analysis showed that the full and the performance IQ of the Wechsler Intelligence Scale for Children (WISC-R), the McCarthy scales for children's abilities (both perceptual and motor scores), and the Test of Oral Language Development (a standardized test used in child development studies in New Zealand) were most strongly associated with the maternal hair-mercury concentration [14]. A reanalysis of this study [15] replicated the association between high maternal mercury exposure and reduced test performance. The statistical significance was very much influenced by one outlier, and additional associations became statistically significant when this subject was excluded. However, an important strength of this study is that adjustment for high fish intake was included in the study design.

2.2. Faroe islands

The Faroe Islands are located in the North Atlantic between Norway, Shetland and Iceland. In this fishing community, excess exposure to methylmercury is mainly due to the traditional habit of eating meat from the pilot whale. Ingestion of whale blubber causes exposure to lipophilic contaminants, notably polychlorinated biphenyls (PCBs). Two birth cohorts have been studied. The first consisted of 1022 children born during a 21-month period in 1986–1987 [16]. Prenatal methylmercury exposure was determined from mercury concentrations in cord blood and maternal hair. At age 7 years, 917 of the cohort children were examined. The physical examination included sensory function assessment and functional neurological examination

Table 1
Main characteristics of three prospective studies of methylmercury exposed children [9]

Attribute	New Zealand	Faroes	Seychelles
Source of exposure	Shark and ocean fish	Whale, ocean fish and shellfish	Ocean fish
Exposure assessment	Maternal hair at parturition	Cord blood and maternal hair	Maternal hair ≥6 months after parturition
Concomitant exposures	Lead in house paint and air	PCBs in whale blubber	Pesticide use in tropics
Language	English (and Pacific languages)	Faroese (and Danish)	Creole (and English and French)
Socioeconomic setting	Industrialized western	Industrialized Scandinavian	Middle-income developing
Family-setting	Urban, mixed cultures	Traditional	Mainly matriarchal
Outcome tests	Omnibus	Domain-related and neurophysiological	Omnibus and domain-related
Clinical examiners	Clinical specialists	Clinical specialists	Nurse/student

with emphasis on motor coordination and perceptual-motor performance. Main emphasis was placed on detailed neurophysiological and neuropsychological function tests. A repeat examination was carried out at age 14 years, again with a high participation rate, and a clinical test battery similar to the one previously applied.

The main finding at the 7-year follow-up was that decrements in attention, language, verbal memory, and, to a lesser extent, in motor speed and visuospatial function, were associated with prenatal methylmercury exposure; and that the cord-blood mercury concentration was the best risk indicator [16]. These findings were robust in the full Faroes data set in analyses controlled for age, sex and confounders, and they persisted after exclusion of high-exposure subjects. Support for these findings was seen in some of the neurological tests, but particularly in delays in brainstem auditory evoked potentials [17]. Likewise, prenatal methylmercury exposure was associated with a decrease in the normal heart rate variability and a tendency toward increased blood pressure [18]. At the 14-year follow-up, mercury-associated delays in peak III of the brainstem auditory evoked potentials remained [19], as did the decreased heart rate variability [20]. Both functions involve brainstem nuclei, and some association may be anticipated, but the correlations became weaker when adjusted for mercury exposure. The neuropsychological outcomes at age 14 showed associations with the prenatal exposure data similar to those at age 7, although the explained variance had decreased at this age [21].

A subsequent study in the Faroes included 182 singleton term births. These children were first examined by the Neurological Optimality Score (NOS) at age 2 weeks; the NOS decreased significantly at higher cord-blood mercury concentrations [22]. Phospholipid fatty acid concentrations were measured in both maternal and cord blood and were associated with prolonged gestation, but they did not seem to affect the NOS. In addition, because methylmercury exposure in the Faroes primarily originates from consumption of whale meat, the results of these studies are unlikely to be seriously confounded by nutrients in fish.

2.3. Seychelles

Two birth cohorts were formed in the Seychelles, an archipelago in the Indian Ocean, both groups involving about 800 children (i.e., about 50% of all children born during the recruitment period) [23]. Recruitment was about 6 months after birth, at which time a maternal hair sample was obtained for mercury analysis. The hair segment that represented the pregnancy period was identified from the assumption that hair grows 1.1 cm per month. The first cohort was later considered a pilot study due to incomplete information on covariates.

A subset of 217 children from the pilot cohort was evaluated at 66 months [24]. Maternal hair mercury was negatively associated with four outcomes: the McCarthy General Cognitive Index and Perceptual Performance subscale; and the Preschool Language Scale Total Language and Auditory Comprehension subscale. When statistically determined outliers were removed from the analyses, statistical significance of the association remained only for auditory comprehension.

The main Seychelles study included evaluation of the children at 6.5, 19, 29 and 66 months of age, and again at 8 years. No association with maternal hair mercury was found for most endpoints in these children [25]. At 29 months there was an association between mercury exposure and decreased activity level in boys only, who also showed a possible mercury-associated delay in age for walking, but the latter was not significant when adjusted for confounders. The most detailed examination was then carried out at age 8 years using tests thought to be similar to those applied in New Zealand and the Faroes. In calculating possible effects of prenatal methylmercury exposure, the regression equations included adjustment for postnatal exposure. No clear association between deficits and maternal hair-mercury concentrations was evident [25]. Mercury exposure in this population comes from ocean fish only, but adjustment for beneficial effects of fish consumption was not attempted.

2.4. Overall findings

Despite the apparent differences between the three studies of mercury-exposed populations, the results do not necessarily disagree. For example, when the hair-mercury concentration is taken as the exposure biomarker in both the Faroes and the Seychelles studies, and the Boston Naming Test is used as the outcome parameter, the confounder-adjusted regression coefficient from the two studies does not differ to a statistically significant extent [26]. This test provided the most consistent evidence of adverse effects in the Faroes study [16,21]. Further, some differences would be anticipated, because the studies used different methods for assessment of exposures and outcomes, and because the epidemiological settings are different.

Supporting evidence comes from several cross-sectional studies, where study populations include subjects with high-level methylmercury exposures [9]. However, due to the remote locations (e.g., in the Arctic or the Amazon basin), less sophisticated outcome parameters were chosen, also taking into account possible differences in culture, language, and school education. The numbers of children examined ranged from tens to a few hundred.

Despite the differences in cultural settings and other limitations, several findings of these studies appear to be concordant [9]. The results tend to confirm that attention, motor coordination and speed, and visuospatial function are sensitive targets of methylmercury toxicity. The vulnerability of language and verbal memory was not evaluated in the cross-sectional studies but is apparent from the prospective New Zealand and Faroes data.

Several cross-sectional studies emphasized functional testing to provide links to neuropathological evidence on brain regions affected by methylmercury exposure at different developmental stages. Other studies included intelligence tests, which are less appropriate for assessment of domain-related dysfunctions, but may be more easily interpreted, e.g., in terms of economic losses caused by the adverse effects.

The external validity of all of the studies depends on the reliability of the exposure assessment, the sensitivity of the outcome variables, and the possible significance of confounders and other bias. These issues are reviewed below, as they also relate to epidemiological evidence in this field in general.

3. Critical evaluation of evidence on developmental neurotoxicity

3.1. Exposure assessment precision

The purpose of the ideal exposure assessment is to provide a correct measure of the amount of the toxicant that reached the target tissue during the sensitive time period. Mercury concentrations in blood, hair, and other specimens are often applied as exposure biomarkers, but must be considered proxy variables, which are always imprecise to some extent. Exposure misclassification is likely to cause underestimation of the true effect of the exposure [27].

In prospective studies, samples for mercury analysis have included maternal hair, cord blood, and cord tissue [9]. In addition, maternal dietary questionnaires have been used to obtain information on the origin and approximate magnitude of the methylmercury exposure. Cross-sectional studies rely on surrogate measures of past exposures. Because the developing brain is considered the main target of methylmercury toxicity, evaluation of these studies must assume that exposures measured at the time of the postnatal examination represent causative exposures at the time of the greatest vulnerability of the nervous system. Irregular exposures and the added impacts of postnatal exposure will complicate the evaluation. Exposure variability is likely to introduce error in the exposure assessment, and such misclassification would lead to underestimation of the dose–response relationship. In agreement with this prediction, exclusion of Faroese subjects with variable exposures during gestation, as assessed by variability of maternal hair-mercury concentrations, tended to increase the associations between the mercury exposure and the deficits [28].

Statistically, the "true" exposure can be estimated if at least three exposure indicators are available [27,29]. Such calculations have recently shown that the coefficient of variation for the hair-mercury imprecision is about 50%, thus making this biomarker about twice as imprecise as the blood concentration [27,29]. Variations in hair growth rates, hair color, hair treatment and external contamination may add to the larger imprecision of mercury in hair as an indicator of individual exposure levels [28]. As a general problem in epidemiology, the greater the imprecision, the greater the impact on the regression coefficient for the exposure biomarker. At the same time, adjustment for confounders with better precision will cause additional bias toward the null hypothesis [30].

The same considerations relate to nutrient intake, although they can be more reliably assessed from questionnaires, since nutrient concentrations in food items vary less than contaminants. However, the degree of imprecision is seldom known, and sensitivity analyses should therefore cover a range of imprecision levels [31].

3.2. Outcome parameter sensitivity and specificity

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). Tests that depend only minimally on cooperation of the subject have the advantage of being less likely to be affected by motivation. Many advanced neuropsychological tests are only possible when a child has reached school age, thus at a substantial time interval after the causative exposure.

Neurological tests are useful as crude clinical measures that may also identify children who suffer from disorders of other origin. The results available [9] suggest that prenatal methylmercury exposure may adversely affect motor coordination and perceptual motor performance, but the clinical tests used are too crude to provide solid evidence of dose-related abnormalities.

Developmental tests may be useful for studies of small children and may be less dependent on differences in culture than tests feasible for older children, but they may be of limited sensitivity to subtle changes. Nonetheless, some exposure-related changes have been reported [13,24].

Neuropsychological tests may be sensitive to the test situation, such as the use of an interpreter, changes in temperature, and other aspects that may be important when a test is used for the first time in a particular culture. Traditionally, studies in this field have included standard intelligence test batteries, because of the wealth of information available on such tests and the known implications of deficient performance [14,25]. Although intelligence tests may not be the most appropriate and sensitive for methylmercury toxicity, a shift in IQ levels was documented in the New Zealand study [13]. The average WISC-R full-scale IQ for the study population ($n = 237$) was 93, but in the group with maternal hair mercury above 6 μg/g ($n = 61$) the average was 90. Because the average exposure in the latter group was about four-fold higher than in the study population as a whole, this difference corresponds to a loss of 1.5 IQ points for a doubling of the exposure. Another way of presenting these shifts in IQ is to estimate the increased number of subjects with very low IQ as methylmercury exposure increases. In New Zealand, an IQ below 70 (=mental retardation) was twice as common (increase from 5 to 10%) in the highest hair mercury group (>10 μg/g) compared to the group with hair mercury below 6 μg/g [32]. For the IQ range 71–85 the increase was from 20 to 25%

Other tests may be preferable as more specific reflections of functional domains (e.g., attention, motor speed, verbal memory) that may be interpreted in regard to the location of neuropathological lesions in poisoning cases. Results from such tests suggest that developmental methylmercury exposure is related to widespread, possibly diffuse, damage. However, these tests may not be independent of culture and language, and care must be taken when comparing the results from different populations. Comparison with IQ tests may involve the regression coefficients for the domain-related tests expressed as a proportion of the standard deviation of the test results [33]. The most sensitive outcome parameters show decrements of about 10% of the standard deviation for each doubling of the prenatal methylmercury exposure level. Had an IQ scale been used, with

a standard deviation of 15 IQ points, a doubling in the exposure could have caused a deficit of about 1.5 IQ points. These findings agree with the New Zealand data.

Neurophysiological tests provide a sensitive and objective evaluation of brain dysfunction that is less prone to be affected by motivation or socioeconomic confounding. Brainstem auditory evoked potentials record the transmission of electrical signals within the brain. The latency of peak III was significantly increased at higher intrauterine exposure to mercury [16,17,19]. In addition, in 14-year-olds, prolonged latencies of peak V were linked to the current mercury exposure, not the prenatal exposure [19]. If this parameter is primarily affected by postnatal exposure, it may be useful to separate effects occurring as a result of exposures at different stages of development.

Some neurobehavioral tests may reflect beneficial effects of *n*-3 polyunsaturated fatty acids from seafood [34], in some cases perhaps a balance between nutrient and toxicant effects. For example, contrast sensitivity in the Faroese mercury-exposed children was much better than anticipated [35], and visual evoked potential latencies were shorter [16]. However, the associations with maternal dietary parameters were weak, possibly due their imprecise reflection of fatty acid intakes. Functional outcomes sensitive to mercury may also be sensitive to nutrients in seafood. Thus, the maternal fish dinner frequency during pregnancy was a significant predictor of improved motor function, and adjustment for this factor resulted in an increase of the mercury regression coefficient [21]. Nutrients and mercury therefore had opposite effects.

Although the developing brain is considered the critical target organ in regard to methylmercury, recent evidence has suggested that such exposure may also promote or predispose to the development of heart disease [36–39]. Heart rate variability, a well-documented risk factor for cardiovascular disease and mortality, decreases at higher prenatal methylmercury exposures [18,20]. An association with increased blood pressure was apparent at age 7 years, and the decreased variability was linked to delays in evoked potential latencies. This evidence suggests a possible contribution by neurotoxicity to the pathogenesis of cardiovascular disease. Low birth weight may be a risk factor for increased blood pressure [3], but this parameter was of no significance in the Faroes, except that the mercury effect appeared stronger in children with lower birth weight [18]. Although yet preliminary, this evidence suggests that improved understanding of the fetal origins of cardiovascular disease would need to consider toxicants as well as nutrients.

4. Discussion

The epidemiologic evidence of methylmercury neurotoxicity is fairly consistent. There is no dispute about the very serious prenatal effects that occurred in Minamata at maternal hair-mercury concentrations thought to be in the range of 10–100 μg/g [9–12,40]. It would seem intuitively logical that less severe effects may occur at the exposure ranges examined in the more recent studies. The Faroes study showed that each doubling in prenatal mercury exposure corresponded to a delay of 1 or 2 months in mental development at age 7 years [16]. Because rapid development occurs at that age, such delays may be important. Also, even small shifts in a measure of central tendency may be associated with large changes in the tails of the distribution. Such developmental delays are likely to be permanent, as shown by the experience with lead neurotoxicity [1], where effects seemed to persist into adulthood, perhaps even becoming more pronounced with time.

Certain weaknesses of the available evidence need to be interpreted prudently. Among major concerns raised in regard to possible overestimation of methylmercury toxicity were: (a) association of mercury intake with exposure to other neurotoxic pollutant(s); (b) other types of residual confounding; and (c) inadequate adjustment for multiple comparisons [41]. Subsequent studies have not revealed any major causes of overestimation of mercury neurotoxicity [9]. For example, the Faroese are exposed to polychlorinated biphenyls (PCBs), which occur in whale blubber. Detailed analyses of the Faroes data failed to show any important impact of PCB exposure on the neurotoxicity outcomes [29,42], and this contaminant would be unlikely to affect outcomes at, e.g., New Zealand.

The potential for overestimation of a toxic effect seems to be less than the risk of underestimation. Thus, exposure imprecision is often ignored and generally results in bias toward the null [27,29,30]. Further, fish intake has recently been shown to cause confounding in the opposite direction of mercury toxicity, thereby attenuating the apparent impact of the exposure [21,31]. A major nutrient in fish, *n*-3 long-chain fatty acids may prolong the gestation period and cause an increase in birth weight, with associated developmental benefits [43]. By examining only one of the two sets of influences in Fig. 1, confounding bias can occur and cause underestimation of the true magnitude of their impact.

The molecular mechanism for opposite effects of methylmercury and essential nutrients is not known. Quite possibly, they may affect different molecular targets that impact on the same clinical outcomes, whether birth weight or neurobehavioral performance. Substantial experimental evidence is available to support the notion that methylmercury causes cellular toxicity in the nervous system [10,11]. In combination with parallel observations in experimental studies, the associations observed in epidemiological studies are therefore likely causal. Because adverse effects may interfere with physiological functions that are otherwise linked to fetal programming processes, a separation of toxic effects from deranged programming would seem difficult to document, and it certainly cannot be resolved from epidemiological studies. The experience on methylmercury therefore illustrates that developmental neurotoxicity may be profitably considered from the perspective of functional programming. Fig. 1 then provides a useful, although simplified epidemiological framework that includes consideration of covariates that may confound the assessment of both toxicant effects and nutrient effects.

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