

Toxicity and Risk Assessment of Trace Elements

Nutritional Factors May Modify the Toxic Action of Methyl Mercury in Fish-Eating Populations^{1,2}

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ABSTRACT The historical record of clinical cases of methyl mercury poisoning dates back to the 19th century when the first chemical synthesis occurred. The potent fungicidal properties of both methyl and the closely related ethyl mercury compound were subsequently discovered, which led to widespread agricultural application for prevention of fungal infection in seed grain. Several catastrophic outbreaks of poisoning occurred in the mid-20th century when the treated seed grain was mistakenly used to prepare homemade bread. The largest outbreak took place in rural Iraq in the early 1970s. Human poisonings also occurred in Japan due to the release of methyl mercury into bodies of fresh and ocean water. The most infamous outbreak occurred in the area of Minamata Bay: methyl mercury, which was unwittingly discharged into the ocean water, avidly accumulated in the aquatic food chain to such an extent that people who consumed fish were severely poisoned. Today, human exposure to methyl mercury occurs from consumption of fish and sea mammals. Inorganic mercury that is present in aquatic sediments is methylated by microorganisms and accumulates in the aquatic food chain. Although no cases of clinical poisoning have been reported, a number of epidemiological studies have been carried out that raise the possibility of prenatal damage. Previous studies (especially the Iraq outbreak) indicate that the prenatal stage of the life cycle is the most vulnerable. However, ongoing epidemiological studies of heavy fish consumers of the Seychelles Islands in the Indian Ocean do not reveal adverse effects. To the contrary, the results of some developmental tests that were conducted on prenatally exposed children indicate beneficial outcomes that correlate with mercury levels during pregnancy. This article discusses the potential role of micronutrients in fish as a plausible explanation for these findings. *J. Nutr.* 133: 1539S–1543S, 2003.

KEY WORDS: • *methyl mercury* • *micronutrients* • *fish*

Donald Hunter (1) in his book *Diseases of Occupations* provides a detailed history of human exposure to methyl mercury and its toxic consequences. Methyl mercury compounds were first synthesized in the 1860s in a chemistry laboratory in London. The technicians involved in the synthesis became

poisoned and died. This event was such a shock to the chemistry profession that this form of mercury was given little attention for the remainder of the century.

Methyl mercury as a fungicide

However, in the early 20th century, the potent fungicidal properties of both methyl and ethyl mercury compounds were discovered. This led to the wide application of methyl and ethyl mercury as seed dressing. Cereal grains such as wheat are highly susceptible to a fungal infection when planted in soil. The resulting disease, which is commonly referred to as “blunt disease,” can cause wide devastation and substantial reduction in the crop yield. The short-chain alkyl (methyl and ethyl) mercury compounds kill the fungus while the seed is in the soil and are not accumulated into the growing plant. Thus, the cereal crop that is grown from the treated grain is not contaminated with mercury.

Few, if any, cases of poisoning were reported in Western countries despite the widespread agricultural applications. Cases of poisoning did occur during manufacture as reported by Hunter et al. (2). The signs and symptoms of poisoning indicate that the central nervous system is the prime target. The first symptom is paresthesia (numbness or tingling sensation) in the extremities and circumorally. In severe cases, there is rapid progression to

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ataxic gait, dysarthria, constriction of the visual fields and hearing loss. A morphological examination of the brain from a person who died many years after being poisoned revealed that damage was restricted to specific anatomical areas such as the cerebellum and visual cortex. The nerve cells were destroyed. The destruction was so selective that the granule cells in the cerebellum were almost completely destroyed, whereas the neighboring Purkinje cells were relatively unaffected. However, in a fatal case of dimethyl poisoning, destruction of both granule and Purkinje cells was reported (3).

Several massive outbreaks of poisoning were reported from developing countries in the 1950s–1970s period. The signs and symptoms were identical to those reported after occupational exposure to methyl mercury, except in the case of ethyl mercury, kidney damage was also manifested. The largest outbreak of methyl mercury poisoning took place in rural Iraq in the winter of 1971–1972 (4). Cereal grain (mainly wheat that was intended for planting) had been treated with a methyl mercury fungicide. The warning labels on the grain sacks, such as the skull and cross-bones sign, had always been effective in Western countries, but this and other warning signs proved ineffective in this rural Arab population. All of the seed grain was not planted; substantial amounts were used to prepare homemade bread, which is the staple foodstuff in rural Iraq.

Methyl mercury poisoning is characterized by a long latent period between exposure and the appearance of signs and symptoms. The longest reported period is ~5 mo (3). In Iraq, the contaminated bread was eaten daily for weeks or months. In some cases, what would prove to be a lethal dose was consumed before signs of poisoning appeared. The mechanisms that underlie the latent period are still not understood, although various possibilities have been discussed (5).

Studies of the Iraq outbreak produced the first quantitative data on the relationships between the ingested dose and blood and hair levels on the one hand and the prevalence of signs and symptoms of poisoning on the other (4). It was found that the first neurological symptoms appear at hair levels in the range of 50–100 ppm with corresponding blood levels from 200 to 400 ppb in cases of adult poisoning.

Severe cases of prenatal poisoning were also observed in this outbreak in confirmation of observations that were made in the Japanese outbreaks (see *Methyl mercury as an industrial byproduct*). In addition, a milder form of prenatal poisoning was identified that was characterized by a history of delayed achievement of developmental milestones and abnormal reflexes (6). Moreover, it was possible to demonstrate a quantitative relationship between maximum maternal hair levels during pregnancy and the prevalence of abnormal development in prenatally exposed infants. The data indicated that adverse effects on development might occur at maternal hair levels as low as 10 ppm.

These data indicate that the developing brain in the prenatal period is considerably more sensitive to the toxic effects of methyl mercury than the mature adult brain. Examination of autopsy brains from prenatally exposed infants in Iraq revealed a different pattern of brain damage. The cellular developmental processes were inhibited, such as neuronal cell division and migration, which resulted in widespread deranged cytoarchitecture of the brain. These observations were made on severe cases of prenatal poisoning. The changes in brain morphology that underlie the milder cases of prenatal poisoning are unknown.

Methyl mercury as an industrial byproduct

Industrial applications of inorganic compounds of mercury as catalysts in the synthesis of acetaldehyde led to the inadvertent production of methyl mercury compounds. This discovery was

made as a result of an outbreak of methyl mercury poisoning in fishermen's families and other fish consumers living close to an ocean bay on the coast of Japan. The neurological signs and symptoms were given the name "Minamata disease" after the name of the ocean bay. It took many years before methyl mercury was identified as the causal agent. A visiting physician from Scotland was the first to suggest the real cause; he had seen the earlier cases of occupational poisoning and recognized the symptoms. Eventually, the methyl mercury source was traced to the discharge of waste from a chemical factory. Perhaps one reason for the long delay in identifying the cause was that it was difficult to believe that an industrial byproduct discharged into a large ocean bay could be bioaccumulated to such an extent in that it fatally poisoned those consuming the fish. Indeed, that the avid bioaccumulation of methyl mercury in the aquatic food chain attains its highest concentrations in long-lived predatory fish is an important lesson from the Minamata outbreak.

The outbreak in Minamata and a subsequent similar one in Niigata, Japan provide the first suggestive evidence that the developing brain of the fetus is especially sensitive to methyl mercury that is ingested by the mother. For example, a few cases were reported where the offspring suffered severe brain damage, whereas the mother was only mildly affected. This conclusion was confirmed in the Iraq outbreak.

Methyl mercury as a natural product

In the 1960s Swedish investigators discovered high levels of methyl mercury in the feathers of fish-eating birds [for a review see ref. (7)]. These birds were migratory: they spent part of the year in North Africa and part in Sweden. The feathers grown in Sweden had the high methyl mercury levels. The source was soon identified as freshwater fish, mainly northern pike. The source of the methyl mercury was a mystery because no methyl mercury compounds were discharged to Swedish waters. Eventually, the source of the methyl mercury was traced to aquatic sediments where microorganisms such as methanogenic bacteria methylated inorganic mercury. The source of inorganic mercury could be natural or the result of industrial discharge. One of the main sources at that time was chloralkali plants that used liquid mercury as an electrode in the electrolysis of sodium chloride.

These initial Swedish discoveries led to worldwide surveys. Methyl mercury was detected in virtually all species of fish, both ocean and freshwater, with the highest levels found in fish at the top of the predatory food chain. Levels in the edible tissues of fish-eating sea mammals were among the highest ever recorded. The global mobility of mercury is such that high levels were found in areas remote from industrial sites of release such as in the Northern Arctic.

Studies on populations known to have high fish-consumption levels revealed levels in blood or hair that exceeded the lowest risk levels determined from the Iraqi poisoning outbreak. **Figure 1** compares estimates of ranges of hair levels in several fish-eating populations with risk levels. These ranges do not overlap risk levels for adult exposure but substantially overlap the 10-ppm hair level as the lowest effect level for prenatal exposure.

The Seychelles study

In view of the uncertainties in extrapolating risk estimates from the Iraq outbreak, a study was started in the Seychelles Islands in the Indian Ocean. Women of childbearing age were reported to have a range of hair levels of mercury that extended above the minimum prenatal risk levels in Iraq (8). Early ob-

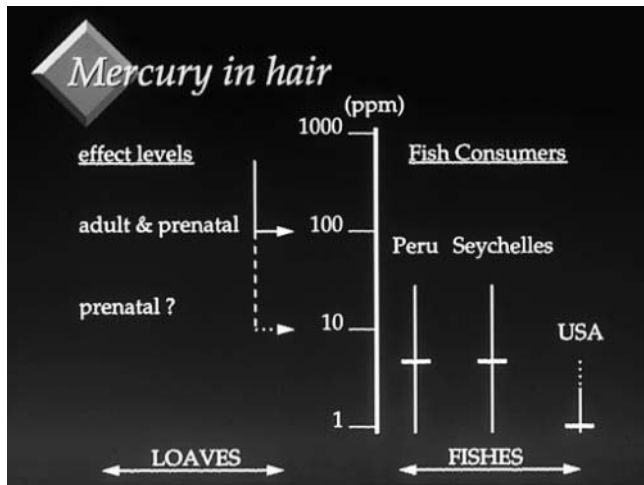


FIGURE 1 Hair levels of mercury associated with risk of adverse effects of methyl mercury from adult or prenatal exposures (*loaves*) as compared to levels in hair found in populations of high fish consumers (*fishes*). The risk levels were estimated from studies of an outbreak of poisoning from loaves of homemade bread contaminated with a methyl mercury fungicide.

ervation indicated a high level of consumption of ocean fish. The population appeared to be free of other environmental contaminants such as lead and polychlorinated biphenyls. A birth rate of ~1,500/y would ensure the establishment of a cohort of infant-mother pairs of sufficient size to provide a powerful test of the Iraqi risk predictions.

In fact, two cohorts were established, each of >700 infant-mother pairs. The first cohort was established as a pilot study to obtain background levels of mercury and to test the feasibility of sample collection, storage and transport, to test the measures to be used for child development and neuropsychological performance and to explore environmental factors (covariates) that might affect the test scores. The main cohort was established 2 y later. This involved the full battery of tests and covariates. Also, in the main cohort, great care was taken to test the children at the same age. The children were examined at various ages in both the pilot and main cohorts.

The average hair levels of mercury during pregnancy were used as the measure of prenatal exposure to methyl mercury. The centimeter of hair next to the scalp was used as the measure of postnatal exposure. Multiple linear regression analysis was used to test for any correlation of end-point measures with pre- or postnatal mercury levels. Several covariates were included in the analysis.

The earliest results from the pilot cohort suggested that adverse developmental effects might be occurring at maternal hair levels in the range predicted from Iraq, which were roughly in the 10–20-ppm range. In examinations at later ages in the pilot cohort, some test scores also indicated adverse correlations with mercury. However, the statistical significance of the regression relationship was heavily dependent on a few points.

Examinations of children in the main cohort revealed no evidence of adverse effects from either pre- or postnatal exposure to methyl mercury. A surprising finding in the results of the examination of children at 66 mo of age was that several test scores improved as either pre- or postnatal mercury levels increased. An example is given in **Figure 2**. The scatter of points is typical of child-performance measures of this type, because there are considerable individual differences in the test scores. Nevertheless, linear regression analysis reveals statistically significant beneficial correlations.

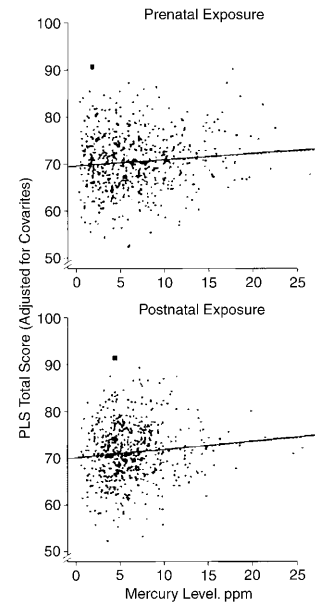


FIGURE 2 The preschool language score (PLS) adjusted for covariates is plotted against the maternal hair levels (prenatal exposure). The points, denoted by small squares, are outliers defined as being three standard deviations from the regression line. Two regression lines were drawn, one for all the data points (*solid line*) and one with outliers deleted (*broken line*). The slopes of the two lines do not differ significantly. These regression analyses indicate a statistically significant improvement of PLS as either prenatal or postnatal mercury levels increase. [Adapted from Davidson et al. (9) Copyrighted 1998, American Medical Association].

Altogether, four different tests exhibited a beneficial correlation with either pre- or postnatal mercury levels. The most dramatic improvement in test scores was seen in a measure of errors made by the children in drawing objects. The Bender-Gestalt error scores showed a 45% reduction over the range of postnatal mercury levels (1–25 ppm).

Nutritional studies

Observations made at an early stage of the Seychelles study indicated that hair levels of mercury correlated with fish consumption. This is not a surprising finding, given that ocean fish are the only known source of methyl mercury. This finding is in line with many other observations of fish-eating populations (10). Thus, the possibility arises that the mercury levels used in the regression analyses are surrogates for fish consumption. It is the latter that might be responsible for the apparent beneficial correlations with mercury.

A new cohort has now been established that includes nutritional measures with mercury measures to see whether nutrients derived from fish can explain our results. We are especially interested in the following nutrients.

Docosahexaenoic acid

A considerable literature based on both animal experiments and human studies indicates that long-chain polyunsaturated fatty acids (LCPUFA)⁴ are essential for normal brain development (11,12). Linoleic and linolenic acids (C18 chain length) are the essential polyunsaturated fatty acids, because they are the precursors of the LCPUFA (C20 and C22), which

⁴ Abbreviations used: DHA docosahexaenoic acid; LCPUFA, long-chain polyunsaturated fatty acid.

are produced by chain elongation and desaturation. However, fetal and neonatal tissues cannot perform these reactions with sufficient capacity to provide the growing brain with its requirements for LCPUFA (13). Especially important are arachidonic 20:4(n-6) acid and docosahexaenoic 22:6(n-3) acid (DHA), which are needed as structural components of neuronal membranes (14). The major dietary source of arachidonic acid is red meat (15), which is not widely consumed in the Seychelles (16). Dietary fish, however, can provide a rich source of the other preformed LCPUFA (DHA) for both the mother and the developing infant.

The demand for LCPUFA is highest in the last trimester of pregnancy when brain growth is at its greatest. DHA is especially important and rises from 6% of total fatty acids in the maternal plasma to 18% in the fetal brain (14). We propose to use cord-blood DHA as our primary measure of prenatal exposure to LCPUFA and breast milk as our primary measure of postnatal exposure. There are only a few reports of assessment of neurodevelopmental effects of postnatal exposure to LCPUFA. In general, global tests of infant development in some studies of DHA supplementation have shown enhancement. For example, Willats et al. (17) reported enhanced problem-solving skills at 20 mo of age following dietary supplementation with LCPUFA between birth and 4 mo of age. It has also been reported (12) that preterm infants have normal development of the visual system only if they receive an adequate supply of LCPUFA in breast or formula milk. This may also be the case in normal-term infants (18). The higher concentration of LCPUFA, which exists mainly as DHA in the brains of breastfed infants, may be one explanation for the observation that breastfed infants score better on visual and developmental tests than infants fed formula diets (19).

In the Seychelles, 94.4% of the mothers are still breastfeeding at 1 mo postpartum. According to a French study (20), the amount of LCPUFA decreased from birth to 30 d postpartum and then remained unchanged. We propose, therefore, to use DHA measured in breast milk and/or calculated from formula feed at 1 mo as our indicator of postnatal exposure to LCPUFA.

Iodine

Iodine is crucial for the developing brain. Iodine deficiency disorders include a range of intellectual impairments; the most serious complication is congenital hypothyroidism (21). Clinical symptoms of iodine deficiency disorders do not appear to be problematic in the Seychelles. Nevertheless, in confirmation of an earlier study (22), a recent report associates mild hypothyroidism in pregnancy with impaired neuropsychological development (23). Although hypothyroidism is usually an autoimmune disorder in developed countries, Haddow et al. (23) note that iodine deficiency should be considered as a causative factor for hypothyroidism in less-developed countries. It was also demonstrated that the sustained and largely irreversible damage to the neuropsychological system of the developing fetus is likely to occur by the end of the second trimester of pregnancy (24,25). Although certain seafoods are a rich source of iodine, a wide range of levels may be expected in the Seychelles with the lowest levels in low fish consumers. We therefore plan to monitor maternal thyroid function during the first and second trimesters by measuring serum thyroid-stimulating hormone levels and using the average value as our primary exposure variable to prenatal iodine.

Iron

Iron deficiency is associated with adverse effects on behavioral and psychomotor development in infants and

children (26). Iron uptake into brain is maximal during the period of rapid brain growth in the rat (27). Although the true impact of childhood iron deficiency or suboptimal iron status on cognitive development remains unclear, human studies demonstrate that iron-deficient children have alterations in attention span, lower intelligence scores and some degree of perceptual disturbance (28). There are strong reasons to believe that the relationship of iron to cognition is developmentally linked and is largely, but not wholly, a postnatal phenomenon. We therefore propose to estimate iron status at delivery using multiple criteria (29) from measures in cord blood as our prenatal exposure variable. Measures of iron in breast milk and/or formula feed, appropriately adjusted for differences in bioavailability between the two sources, will be used as a measure of early postnatal exposure. Subsequent postnatal exposure will be estimated from blood samples collected at 19 and 29 mo of age.

Choline

The National Academy of Sciences U.S.A. in 1998 identified choline as a required nutrient for humans and recommended a daily intake. Pregnancy and lactation are periods when maternal reserves are depleted. At these times, the availability of choline for normal brain development is critical. For example, supplementation of rat pups with choline in wk 2 of life results in lifelong memory enhancement (30). The effect of choline appears to be on the hippocampus, which is the memory center of the brain that influences the rate of birth and death of nerve cells in this center.

Of special interest is the finding that choline may protect against neurotoxicity. Guo-Ross et al. (31) found that administration of choline during the prenatal period protects against the postnatal toxicity of an *N*-methyl-D-aspartate receptor antagonist. Studies on protection against the neurotoxic effects of prenatal exposure to methyl mercury have not yet been reported, but these new findings do raise this possibility.

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