Chapter-22

HEALTH EFFECTS AND RISK ASSESSMENTS

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INTRODUCTION

Detailed risk assessments for methylmercury have been recently published by national and international bodies (e.g., the (U.S.) National Research Council (NRC, 2000), the U.S. Environmental Protection Agency (U.S.EPA, 2001), and the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2003). These reports concluded that the developing brain is the main target for methylmercury toxicity, and they emphasized the prospective epidemiological studies as the main basis for deriving an exposure limit. The same conclusion was reached in UNEP's global assessment (UNEP, 2002).

Evidence from poisoning outbreaks in Japan and Iraq have clearly demonstrated the severe and widespread damage that may occur to the brain when exposed to methylmercury during 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. However, the confirmation of methylmercury as the etiologic agent came

late, and case-related exposure information was therefore difficult to obtain. After the Iraqi poisoning episode, which happened during a famine, exposure information was gleaned from segmental analysis of long hair strands from the mothers, while assuming a constant hair growth rate. Although these studies do not provide detailed dose-response relationships, they demonstrated the serious consequences of excess exposures to this neurotoxicant and documented that the developing brain is a highly sensitive target.

This paper reviews the human evidence with particular emphasis on recent epidemiological data on neurobehavioral effects, and it discusses the uncertainties involved in assessing human health risks based on observational studies. Three major prospective cohort studies have been conducted in New Zealand, the Faroe Islands, and the Seychelles. Cross-sectional studies of neurobehavioral function will also be considered, as will the recent evidence of methylmercury-associated cardiovascular disease.

New Zealand

A group of 11,000 mothers, who gave birth to children in 1978, was initially screened, and the hair-mercury concentrations were determined for the 1000 mothers, who had consumed 3 fish meals per week during pregnancy. Seventy-three mothers had a hair mercury result above 6 μg/g, thereby constituting a high-exposure group. 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 (Kjellstrom et al., 1986).

A follow-up of the original cohort was carried out at age 6 years, now with three control groups with lower prenatal mercury exposure (Kjellstrom et al., 1989). During pregnancy, mothers in two of these control groups had high fish consumption and average hair mercury concentrations of 3–6 μg/g and 0–3 μg/g, respectively. Matching parameters were maternal ethnic group, age, smoking habits, residence, and sex of the child. At this time, 61 of the high exposure children were available for examinations. Lead levels in cord blood and garden soil were tested to assess potential confounding, but there was no association between lead and methylmercury exposure.

Results of the psychological performance tests correlated well. Stepwise robust multiple regression analysis showed that the full (and performance) Wechsler Intelligence Scale for Children (WISC-R), the McCarthy scales for

children's abilities (perceptual and motoric) 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 (Kjellstrom et al., 1989). The proportion of the variance in test results due to hair mercury above 6 μg/g was about 2 %, which was similar to the influence of social class and home language, two of the main confounders accounted for in the analysis. Robust regression analysis reduced the impact of one extreme outlier (with maternal hair-mercury above 80 μg/g). A reanalysis of the full database of this study (Crump et al., 1998) replicated the association between high maternal mercury exposure and reduced test performance, but the statistical significance was very much influenced by the extreme outlier. However, when this subject was excluded additional associations became statistically significant.

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). The first birth cohort consisted of 1,022 children born during a 21-month period in 1986-1987 (Grandjean et al., 1997). Prenatal methylmercury exposure was determined from mercury concentrations in cord blood and maternal hair; both spanned a range of about 1000-fold. Cohort members were invited for detailed examination at school age (7 years), and a total of 917 of eligible children (90.3%) participated. The physical examination included a sensory function assessment and a functional neurological examination with emphasis on motor coordination and perceptual-motor performance. Main emphasis was placed on detailed neurophysiological and neuropsychological function tests that had been selected as sensitive indicators of abnormalities thought to be caused by methylmercury. A repeat examination was carried out at age 14 years, again with a high participation rate, with a clinical test battery similar to the one previously applied.

The main finding at the 7-year follow-up in the Faroes 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; the cord-blood mercury concentration was the best risk indicator (Grandjean et al., 1997). 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 (Murata et al., 1999). Likewise, prenatal methylmercury exposure was associated with a decrease in the normal heart rate variability and a tendency of increased blood pressure (Sørensen et al., 1999).

Data on the 14-year follow-up are currently being processed, but two recent publications describe the neurophysiological outcomes that are unlikely to be affected by socioeconomic confounders (Murata et al., 2004; Grandjean, 2004). Mercury-associated delays in peak III of the brainstem auditory evoked potentials remained at age 14 years, as did the decreased heart rate variability. Both functions involve brainstem nuclei, and the results showed significant correlations that became weaker when adjusted for mercury exposure. This finding suggests that brainstem toxicity may be an important component of developmental methylmercury neurotoxicity.

Delays in brainstem auditory evoked potentials peak V (i.e., the signal elicited by the transmission of the electrical signal to the midbrain) were associated only with the current methylmercury exposure (Figure 1) (Murata

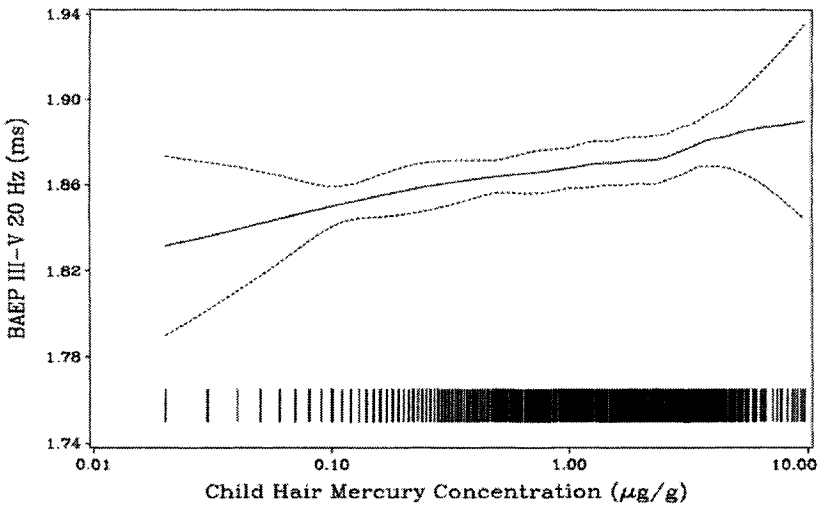

Figure 1. Latency of peak V of the brainstem auditory evoked potentials recorded in 859 Faroese children at 14 years and adjusted for sex and age (Murata et al., 2004). The association is estimated in a generalized additive model analysis, using the current hair-mercury concentration as an indicator of current exposure. The broken lines indicate the point-wise 95% confidence interval for the dose-response relationship. Each vertical line above the horizontal axis represents one observation at the exposure level indicated.

et al., 2004). The average hair-mercury concentration in the adolescents examined was about 1 μg/g (i.e., close to the current Reference Dose (RfD) established by the U.S.EPA).

This observation suggests that the vulnerability of the brain may extend into the teenage period, and that even exposures similar to the RfD may cause adverse effects.

Another prospective study (Cohort 2) included 182 singleton term births. These children were first examined by the Neurological Optimality Score (NOS) at age two weeks. Detailed information was obtained on exposures both to methylmercury and to lipophilic pollutants. The NOS showed significant decreases at higher cord-blood mercury concentrations, while PCB was not important (Steuerwald et al., 2000).

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) (Shamlaye et al., 1995). For exposure assessment, a hair sample was obtained from the mother, in many cases six months after birth. The hair segment that represented the pregnancy period was identified from the assumption that hair grows 1.1 cm per month. The authors noted that the first pilot study was not as well-controlled as the main or longitudinal study: there were fewer covariates, medical records were not reviewed as carefully, there was less information on socio-economic status.

A subset of 217 children from the pilot cohort was evaluated at 66 months (Myers et al., 1995). 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 and observations considered to be influential 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 (Myers et al., 1995). 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.

Table 1. Main differences between three of the prospective studies of methyl-mercury-exposed children.

Attribute	New Zealand	Faroes	Seychelles
Source of exposure	Shark and other large ocean fish	Whale, ocean fish and shellfish	Ocean fish
Exposure assessment	Maternal hair at parturition	Cord blood and maternal hair	Maternal hair ≤ 6 mo after parturition
Concomitant exposures	Lead in house paint and air	PCBs (whale blubber)	Pesticide use in tropics
Language	English (and Pacific languages)	Faroese (and Danish)	Creole (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

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 association between deficits and maternal hair-mercury concentrations was evident (Myers et al., 2003). Despite the apparent differences between the three studies of mercury-exposed populations, they may not necessarily be in disagreement. 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 (Keiding et al., 2003). 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. The New Zealand study population may be most similar to continental Europe and North America, even though about half of the high-exposure mothers were of Pacific Island descent. Their diet is high in ocean fish, and very few of them smoke, but otherwise their exposures in daily life would not be very different from other New Zealand women or, indeed, women from other Western societies.

New data will soon appear from Japan, where a prospective study has just initiated (Nakai et al., 2004), as has a cross-sectional study that includes analysis of preserved umbilical cords as a retrospective measure of the children's prenatal exposure levels (Murata et al., 2004). Further, the hair samples collected for the New Zealand cohort of 11,000 women may be used for potential follow-up studies (Kjellstrom, personal communication).

Cross-sectional studies

The neurotoxicity of methylmercury should be judged on the basis of the overall strength of the total evidence available. Several cross-sectional studies (Table 2) supplement the prospective data. As with the prospective studies, researchers have chosen populations that include representation of high-level exposures to methylmercury. However, due to the remote settings (e.g., in the Arctic or the Amazon basin), less sophisticated parameters had to be chosen, also taking into account the possible differences in culture, language, and school education. Some studies focus on the impact of methylmercury from freshwater fish, e.g., in the Amazonian region, where fish contamination is increased by pollution from gold-mining activities. In the Arctic, the traditional diet includes marine mammals and other species high in the food chain that accumulate biomagnified methylmercury. The numbers of children examined have ranged from tens to a few hundred. Selection bias, especially when studying older children, is possible; usually no information was reported about children who were unavailable for the study. Most likely such selection would result in healthier children being examined, thereby potentially obscuring exposure-related effects.

The studies also differ in regard to the exposure intervals covered. Exposure levels range from an average of about 5 μg/g maternal hair (Cree Indians in Northern Québec, and two subgroups in the Amazon basis) up to 13 μg/g in French Guiana, and even higher in Northern Greenland.

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..

Despite the differences in cultural settings and other limitations, several findings of these studies appear to be concordant (Table 2). 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.

Exposure Assessment

The purpose of an appropriate exposure assessment is to provide a correct measure of exposure in terms of the amount that has reached the toxicological target during the relevant time period. Because the validity

depends on the degree to which the exposure biomarkers reflect the "true" exposure, they can be considered only proxy variables, which are always imprecise to some – usually unknown – extent. This issue is important, because exposure misclassification is likely to cause underestimation of the true effect of the exposure.

In prospective studies, samples for mercury analysis have included maternal hair, cord blood, and cord tissue. 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 fetal exposure, because the children were enrolled several years after birth. In the populations studied (Table 2), the diet probably changed little over time due to the preponderant role of fish among available resources. In addition, fish contamination by mercury most likely remained fairly stable in the years following birth. Such stability was evidenced by the study in Peru, in which peak and average concentrations of mercury in maternal hair during pregnancy were very similar and in French Guiana where hair mercury levels measured five years apart in different villages were remarkably constant.

Most studies in Table 2 used maternal hair collected at time of child's examination. In two studies, the child's own exposure (blood or hair measurement) was used instead due to incomplete data on maternal exposure levels, and because a good correlation was documented between the child's and the mother's exposure levels (Grandjean et al., 1999).

This association is to be expected when older children are examined in communities where they are usually sharing the meals with their parents at home. If the child's current exposure does not even provide a correct ranking between individuals, it could introduce exposure misclassification and thus underestimate the risk. However, if the child's exposure is lower than the maternal level, an exaggeration of the risk at a certain exposure level may ensue.

A hair sample may provide a calendar of mercury exposure. Although hair growth rates are known to vary, a 9-cm hair sample obtained at parturition or shortly thereafter would be thought to represent the average mercury exposure during the whole pregnancy.

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)
Canada (McKeown-Eyssen at al., 1983)	234 children (12-30 months)	freshwater fish	maternal hair during pregnancy	m_a=6 μg/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 μg/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 μg/g m_g= 9.6 μg/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 N145 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 μg/g m_g= 11.6 μg/g	Finger-tapping Santa Ana dexterity test WISC-R Digit span Stanford-Binet Copying Recall Bead memory	ns β= -5.58 p=0.001 ns β= -3.40 p=0.003 β= -1.23 p=0.02 ns
French Guiana (Cordier et al., 2002)	248/290 children neurological ex. (6m-6 years) 206/243 children neuropsy. tests (5-12 years)	freshwater fish (gold mining area)	child hair maternal hair	m_g= 10.2 μg/g m_g= 12.7 μg/g	Neurological examination Finger tapping Stanford-Binet test Blocks Copying Bead memory McCarthy Digit spans forward Leg coordination	Increased tendon reflexes ns ns β= -2.98 p<0.001 ns ns β= -3.72 p=0.006

* m_a, arithmetric mean; m_g, geometric mean; ns = not significant

In New Zealand, monthly (10-mm segment) exposure levels varied by a factor of 2, and on average the peak monthly exposure was about 50% higher than the 9-month average (Kjellstrom, 1989). In the Faroes, similar variations were recorded, with coefficients of variation mostly being below 25% (Grandjean et al., 2003). Comparison of hair segments from the Faroes and the Seychelles showed similar short-term variations (Lanzirotti et al., 2002).

Mercury analyses should always be supported by detailed quality control procedures (e.g., blind comparison with other experienced laboratories and use of certified samples) as was first done in the New Zealand study (Kjellstrom et al., 1989). Until recently, the biomarker imprecision was thought to be appropriately reflected by such laboratory quality data, although the low levels of imprecision (usually about 5% or less) could not explain why associations between mercury concentrations in hair and blood often showed wide scattering. An additional consideration is that the hair mercury concentration is subject to variability, e.g., due to hair type, hair color, external contamination, and leaching due to permanent hair treatments (Grandjean et al., 2002). In international comparisons, three main types of hair structure are recognized (i.e., African, Caucasian, and Oriental), but good data for calibration with blood concentrations exist only for the latter two hair types.

When calculating an exposure level from the hair mercury concentration, an average hair-to-blood ratio of 250 has been generally used (U.S.EPA, 2001). This ratio is appropriate for Caucasian and Oriental hair (Grandjean et al., 2002), but is known to vary between individuals. The 95th percentile differs from the median by a factor between 2 and 3. It also depends on the concentration level, and it changes with age (Budtz-Jørgensen et al., 2004a). For example, in 7-year-old Caucasian children (with finer hair than adults), the ratio is about 370 (i.e., about 50% higher than the ratio of 250 attained by age 14 years).

The possible impact of irregular exposures also needs to be considered. Thus, "bolus" exposures might be more toxic than steady exposures at an average level, but the dose eventually reaching the fetal brain from a maternal seafood meal would be unlikely to represent a steep peak. Nonetheless, 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 subjects with variable exposures during gestation tended to increase the associations between the mercury exposure and the deficits (Grandjean et al., 2003).

The "true" exposure can be estimated if at least three exposure indicators

are available. Such calculations have recently shown that the coefficient of variation for the hair-mercury imprecision is about 50% and thus maiking this biomarker about twice as imprecise as the blood concentration (Budtz-Jørgensen et al., 2004a). 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 (Budtz-Jørgensen et al., 2003b).

Outcome Variables

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 at the same time 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. The more advanced neuropsychological tests are only possible when a child has reached school age. However, such tests may be of uncertain validity, if they have not previously been applied in the same culture. 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 employed a battery of neurobehavioral tests, some of which appeared to be more sensitive to mercury neurotoxicity than others. Simple comparison 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.

Benchmark dose levels may also be used as a basis for comparison. Thus, the most sensitive neurological, neuropsychological, and neurophysiological effects parameters all exhibit benchmark dose levels of 5-10 μg/g hair. Despite the great variability of the study settings and the outcome variables, a substantial degree of concordance exists and that the combined evidence is quite convincing in regard to the dose-response relationship.

Neurological tests

All prospective studies and several cross-sectional studies included at least one neurological examination (e.g., McKeown - Eyssen et al., 1983;

Marsh et al., 1995; Steuerwald et al., 2000; Cordier et al., 2002). Unfortunately, the protocols of examination differ between studies: in the Canadian and French Guiana studies, evaluation of sensory functions, cranial signs, muscle tone, stretch reflexes and coordination were conducted. Both studies reported abnormal tendon reflexes in association with an increased maternal hair concentration. However, these signs were mild and isolated, and the reproducibility of the assessment in the French Guiana study was reported to be poor (Cordier et al., 2002). In the study in Peru (Marsh et al., 1995), essential details of the neurological evaluation (items, age of the children examined) were not presented, thus precluding evaluation of the results; frequencies of abnormal signs were reported to be independent of maternal mercury concentrations. In Madeira and the first Faroes cohort, the neurological examination emphasized motor coordination and perceptual motor performance (Grandjean et al., 1997; Murata et al., 1999); children who failed the most difficult of the 19 functional neurological tasks tended to have slightly higher exposures than children who performed well.

In summary, the clinical neurological tests provide limited evidence linking low-dose methylmercury exposures to detectable abnormalities. The absence of clear, positive findings most probably reflects the lack of sensitivity of this type of examination within this range of exposures. One weakness is that the performance on a clinical test is rated by the examiner, thereby introducing a potential subjective aspect. In addition, scoring is usually a simple pass-fail or pass-questionable-fail, thereby limiting the sensitivity.

Developmental tests

Among the prospective studies, the New Zealand children were examined at 4 years of age using the Denver Developmental Screen Test (DDST). It consists of four major function sectors: gross motor, fine motor, language and personal-social, where possible scores are abnormal, questionable, or normal. The prevalence of developmental delay was 52% for children in the high-exposure group and 17% for controls; the delays most frequently affected fine motor and language sectors. The Bayley Scales of Infant Development (BSID) was used in the Seychelles, but no mercury-associated deficits were reported. Developmental tests may be useful for such studies in small children, they may be less dependent on differences in culture than are tests appropriate for older children, but they may be of limited sensitivity to subtle changes.

Neuropsychological tests

While likely to be more sensitive in revealing early neurotoxic changes, neuropsychological tests require that the administration is standardized, and they may show examiner-dependence. Further, they may be sensitive to details in 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. In New Zealand, two psychologists tested the same children with shortened version of the test battery and documented a remarkable agreement (Kjellstrom et al., 1989). In the Faroes and several other studies, each test was administered to all children by the same examiner, thus limiting the possible impact of examiner-related differences.

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. The New Zealand study applied the Wechsler Intelligence Scale for Children (WISC) and McCarthy Scale of Children's Abilities at age 6. Likewise, the Seychelles examinations included the McCarthy Scales. These intelligence tests may not be the most appropriate and sensitive for methylmercury toxicity. Still, significant effects were found on WISC and McCarthy in the New Zealand study. The New Zealand study also used the "Test of Oral Language Development" (a standard test widely used in New Zealand school programs), and this test appeared to be the one most affected by methylmercury exposure.

In the Faroes, the approach taken was to emphasize tests that reflected functional domains (e.g., attention, motor speed, verbal memory). The functions chosen were those that were most likely to be affected by developmental methylmercury exposure, as judged from location of neuropathological lesions in poisoning cases and as illustrated by studies of other developmental neurotoxicants, especially lead. The Boston Naming test appeared to be the most sensitive outcome. Similar tests were included when the Seychelles cohort was examined at age 8 years. However, application of the Boston Naming test in a different culture may not be unproblematic. For example, the sequence of stimuli may not necessarily represent an increasing degree of difficulty (e.g., if pictures of an igloo or an acorn are not as familiar to children in a tropical developing country as they are to northern children from the Faroes, or the United States, where the test was developed). In addition, each stimulus was meant to have one correct answer, but since Seychellois is a mixed language, some stimuli in the Boston Naming test are known to have as many as three correct answers.

Thus, even if the same test material and instructions (after translation) are

used, the validity of the tests will differ.

A variety of neuropsychological tests, including WISC subtests were used under different circumstances in the cross-sectional studies in Madeira, Greenland, Brazil and French Guiana. In the first two and in an Indian group studied in Brazil, all tests were administered with the use of an interpreter, thereby making the test results less reliable. Likewise, the Continuous Performance test used in the Seychelles was not supervised by an examiner, thereby allowing for possible untoward variability that would be less likely if the examiner was present. Results on such computer-assisted tests will also be affected by the prior computer experience of the child.

Due to the type of populations studied, the researchers attempted to avoid culture-biased and language-dependent tests, thereby precluding evaluation of important domains. The functions evaluated therefore focused on motor speed and motor coordination, visuospatial organization, attention, and short-term memory. Several tests were common to these studies and also overlap with the prospective studies (e.g., Finger Tapping, and Stanford-Binet Bead Memory). At increased exposure levels, reduced scores were evidenced on the Santa Ana dexterity test in Brazil and the McCarthy Leg coordination test in French Guyana. In these two studies, scores on the Stanford-Binet Copying test (that measures visuospatial organization) were negatively associated with mercury exposure with similar regression coefficients in the two studies. Several types of errors occurred in these tests, and the French Guiana study pointed more specifically at rotation errors among younger children (5-6 years old). Such errors would suggest possible insult in the parietal lobes of the brain resulting in developmental delay in the learning to place objects in space (Sullivan et al., 1999). Whether or not this type of test would appear sensitive in other populations living in other cultural environments needs to be established.

Neurophysiological testing

As an objective evaluation of brain dysfunction that is probably less sensitive to motivation or socioeconomic confounding, neurophysiological tests have been applied in several studies. Their applicability requires advanced instrumentation and depends on skilled examiners. An outcome that has previously been found to be sensitive to lead exposure is brainstem auditory evoked potentials. They are recorded using surface electrodes placed on the skull while the child listens to a stimulus in one ear. The transmission of the electrical signals within the brain is then recorded as peaks that represent the acoustic nerve, an intermediate connection in the

pons, and the midbrain. The latency of peak III was significantly increased at higher intrauterine exposure to mercury. Parallel associations were found in 7-year-old children in the Faroes and in Madeira, and this observation was replicated in the Faroese cohort when examined at 14 years. A smaller study from Ecuador also reported delays in peak III at higher exposure levels. In addition, prolonged latencies of peak V among the 14 year-olds were linked to the current mercury exposure only (Figure 1). As a parameter primarily affected by postnatal exposure, this particular outcome seems to differ from the majority of functions sensitive to methylmercury during fetal development.

Confounding Variables

Three major reasons for confounding have been noted as to why a mercury effect might have been overestimated: (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 (NIEHS, 1998). The best protection against confounding problems is to select a study setting where such concerns are unlikely and, if relevant, may be adjusted for appropriately. Thus, a homogeneous society with limited differences in socioeconomic and cultural factors should be preferred. The existence of residual confounding can never be fully excluded. On the other hand, "phantom" covariates should not be invoked to explain away biologically plausible associations between methylmercury exposure and neurobehavioral deficits. In addition, most attention is usually paid to confounders that affect the outcomes in the same direction as the exposure under study, but confounders may also have the opposite effect of attenuating the apparent impact of the exposure. Thus the potential for overestimation of a toxic effect should not be raised without also paying attention to the risk of underestimation.

Standard multiple regression methods are often used for controlling for confounding effects. Even in the absence of confounding, adjustment for such established predictors as sex, age, and maternal intelligence, should be included to obtain a more precise estimate of the mercury effect. In general, the prudent approach is usually to include all covariates that may be potential confounders. However, in situations where the exposure is measured with some degree of imprecision (as is the case here), this approach may result in biased estimates. Inclusion of such covariates, which are associated with the exposure but without any explanatory power in regard to the effect, will then increase the underestimation of the effect of the exposure of interest (Budtz-

Jørgensen et al., 2003).

As a main concern in regard to confounding, socioeconomic conditions vary substantially between the study settings. Although mercury neurotoxicity was reported in almost all studies, differences within each study could be important. New Zealand and the Faroes represent relatively wealthy, industrialized populations, where socioeconomic differences are thought to be limited, but most of the other studies were carried out in developing countries, where basic sanitary problems are common, and where nutritional deficiencies may occur, both of which may be difficult to adjust for. In the Seychelles, stunting still occurs in a small percentage of children (WRI, 2003). In New Zealand, ethnic differences appeared to play a role, but the analysis was based on matching of the children in the different exposure groups for ethnicity. An additional factor of possible interest is consanguinity, which is more frequent in island populations and other isolated communities. However, to cause confounding, the degree of consanguinity would have to be associated with mercury exposure and at the same time result in neurobehavioral deficits. Both assumptions seem unlikely, but documentation from an individual study would be a major undertaking.

The family structure and home environment are documented as important determinants of childhood development. Within the populations studied, circumstances may vary. For example, only about 25% of the births in the Seychelles are nuptial, while an additional 50% are recognized by a father, but about 25% of children have no known father (MISD, 1998). Accordingly, children of the Seychelles cohort were said to be accompanied by a "care-giver", often a relative, with whom the child was living (Myers et al., 2003). The variable family structure, which may be difficult to adjust for in statistical analyses, contrasts with the more uniform circumstances of most other studies with a traditional and stable family structure. In the New Zealand study, low social class and non-English home language reduced, as anticipated, the score on some tests and more than 6 months of breastfeeding increased the score on some tests (Kjellstrom et al., 1989). These variables were accounted for in the multiple regression analysis.

Among other known developmental neurotoxicants, none is as prevalent as ethanol. Most studies reported that maternal alcohol use during pregnancy was minimal, but in some cases it may be difficult to assess because of the importance of home-brewed beverages (e.g., in the Seychelles) (Perdrix et al., 1999).

In particular the Faroese are exposed to polychlorinated biphenyls (PCBs), in this case from eating whale blubber. Detailed analyses of the Faroes data failed to show any important impact of PCB exposure on the

neurotoxicity outcomes (Budtz-Jørgensen et al., 1999; Grandjean et al., 2002; Steuerwald et al., 2000). The relative importance of PCB and mercury was assessed in structural equation analysis taking into account imprecision in both variables. Inclusion of PCB exposure attenuated the mercury effect somewhat, but mercury remained statistically significant, while PCB was far from that (Budtz-Jørgensen et al., 2002). In New Zealand and the Seychelles, the ocean fish consumed is unlikely to be contaminated by PCB, and the same would be the case with freshwater fish in the Amazon Basin.

A reverse effect may occur if subjects who do not eat mercury-containing fish instead consume fruits and vegetables that contain pesticides. Such exposures are more likely in tropical developing countries. The neurotoxicity of many pesticides could then potentially cause neurodevelopmental effects in children with low-level mercury exposures, thus blurring the dose-response relationship. Although pesticide use might be a cofactor in the Seychelles, no information is available to evaluate this possibility, and this issue has apparently not been considered in the epidemiological studies. Nonetheless, the United Nations Food and Agriculture Organization (1997) helped remove large amounts of obsolete pesticides (such as malathion) from the Seychelles. Given the neurotoxic potential of these substances, and the likelihood of their use in tropical countries, exposure potentials need to be considered.

An additional consideration is natural toxins, such as cyanides present in cassava grown in the tropics (Dora, 2002). If non-fish eaters consume more cassava than those with a high fish intake, then cyanide exposure could potentially cause a confounding bias toward the null hypothesis, as has been suggested by Dora in regard to evidence from Brazil. This concern may also relate to the Seychelles, where clusters of so-called tropical myeloneuropathies have occurred as a possible effect of cyanide intoxication from cassava consumption (Roman et al., 1985).

Certain essential nutrients in fish and seafood may provide beneficial effects on brain development, thereby possibly counteracting adverse effects of the contaminants. This possibility has often been mentioned in regard to ocean fish (Myers et al., 2003). Perhaps, if ocean fish contains higher concentrations of essential n-3 fatty acids than do freshwater fish, then this difference could perhaps explain why the mercury dose-response relationship appears to be steeper in populations that rely on river fish. Although high intake of these fatty acids may cause an increase in birth weight, no effect on early neurobehavioral development was found in the Faroes (Steuerwald et al., 2000).

Selenium concentrations in ocean fish from New Zealand do not depend on fish size, while mercury concentrations increased linearly with size

(Kjellstrom, 2000). Although selenium has been considered to potentially provide protection against mercury effects, cord-blood selenium concentrations in the Faroes did not impact on mercury-associated deficits. It therefore seems likely that essential nutrients would counteract no more than limited aspects of mercury neurotoxicity.

Cardiovascular Effects

Although the developing brain is considered the critical target organ in regard to methylmercury, recent evidence has suggested that mercury from fish and seafood may promote or predispose to the development of heart disease. This evidence is yet inconclusive, but deserves attention, because it suggests that a narrow definition of subpopulations at risk, i.e., pregnant women, might leave out other vulnerable groups.

The first studies of methylmercury-associated cardiovascular disease were carried out in Finland. One important study showed that the intima-media thickness of the carotid arteries in apparent association with the degree of mercury exposure from fish (Salonen et al., 2000). A possible mechanism may be induction of lipid peroxidation. In this regard, the interesting observation was also made that, while essential fatty acids from fish may prevent cardiovascular mortality, this beneficial effect may be cancelled or overwhelmed by concomitant exposure to methylmercury (Rissanen et al., 2000). The increased risk seems to occur at hair-mercury concentrations above 2 μg/g, i.e., only twice the level corresponding to the U.S.EPA Reference Dose. More recent information tends to support these findings. A large multi-center study from Europe showed an increased risk of cardiovascular disease associated with toenail mercury concentrations (Guallar et al., 2002). However, this finding was mainly due to a particularly strong risk observed in one of the centres. In a U.S. study of health care workers, only a minimal risk was seen, but after exclusion of the dentists with high toenail-mercury concentrations likely due to amalgam exposures, the risk was similar to the one observed in the European study (Yoshizawa et al., 2002).

The possible interaction between toxic methylmercury and beneficial polyunsaturated fatty acids is of particular relevance in northern populations. Early evidence suggested that Inuits in Greenland had a low cardiovascular morbidity and mortality that was linked to their high intake of n-3 long-chain polyunsaturated fatty acids. Recent evidence indicates that this notion may be erroneous (Bjerregaard et al., 2003). The potential significance of methylmercury in this regard is therefore highly relevant.

Risk Assessment and Exposure Limits

In deriving exposure limits from epidemiological data, regulatory authorities have increasingly relied upon the use of benchmark dose estimates (Budtz-Jørgensen et al., 2001). According to usual default settings, an exposure at the benchmark dose (BMD) results in an increased frequency of a pathological outcome from 5% to 10%. The benchmark dose level (BMDL) is then the point of departure that represents the lower 95% confidence limit of the BMD. When using a linear dose scale, outliers with high exposure levels may become highly influential. The BMDLs from the New Zealand data were 17-24 μg/g, but when the child with the highest mercury level was omitted, they decreased to 7.4-10 μg/g (Crump et al., 1998).

JECFA (2003) considered the BMDL an exposure that is "without appreciable adverse effects in the offspring". This interpretation may be true under some circumstances, but in large epidemiological studies, where the confidence interval is relatively narrow, the BMDL will be closer to the BMD. For example, the results from the Faroes show that exclusion of the subjects with a maternal hair-mercury concentration above 10 μg/g (a cut-off level lower than the BMDL used by JECFA) barely altered the regression coefficients and the *P*-values (Grandjean et al., 1997). The BMDL is therefore not a no-adverse-effect level (NOAEL), but rather a lowest-observed-effect level (LOEL). This consideration is likely to affect the choice of uncertainty factors, especially in regard to brain function, where even small decrements may be of substantial social and economic impact.

JECFA (2003) used BMDLs based on the maternal hair-mercury concentration. In contrast to the NRC (2000), JECFA decided to exclude the New Zealand study and therefore arrived at a higher overall average BMDL. For the Faroes study, the BMDL chosen by JECFA was 12 μg/g maternal hair (i.e., an average for the linear dose-response curve for several different functions and not the most sensitive brain function, as preferred by NRC).

The problem of choosing the most sensitive function may be resolved by using a structural equation model for deriving integrated BMD and BMDL values (Budtz-Jørgensen et al., 2003b; Budtz-Jørgensen et al., 2004b). This calculation includes all exposure information, confounders, and cognitive outcomes, and also takes into regard effects of measurement uncertainty. Using this advanced statistical approach, the overall BMDL is calculated at 6 μg/g maternal hair (or 43 μg/L cord blood). Thus, by incorporating the complete data set in the assessment, the resulting hair-based BMDL is only half the size of the BMDL chosen by JECFA (2003).

This finding is in agreement with the general finding that measurement

uncertainty (in the exposure or the response) leads to overestimation of the benchmark results (Budtz-Jørgensen et al., 2003a; Budtz-Jørgensen et al., 2004b). Thus, although the above calculations are based on the Faroes study only, it is likely that such refinements of the BMDL calculations using data from other studies would result in a similar, if not greater, decrease in the BMDL results.

In calculating an exposure limit from a BMDL, an uncertainty factor is usually applied to take into account sources of variation in individual susceptibility as well as insufficiencies in the data base (e.g., concerning effects on target organs other than the developing nervous system). The NRC (2000) and U.S. EPA (2001) chose a total uncertainty factor of 10. However, JECFA (2003) concluded that "the two study samples represent diverse populations", and that "no uncertainty factor is needed to account for variation in vulnerability among subgroups". This decision is also based on the assumption that the most sensitive effects are the average neurobehavioral outcomes in the two studies, on which the overall average BMDL was based. However, JECFA had included results from a study that did not identify statistically significant decrements, thus hardly representing a vulnerable population.

JECFA included only an uncertainty factor of 3.2 to account for the total human inter-individual variability for dose reconstruction (converting maternal blood concentration to a steady-state dietary intake). Although in accordance with default calculations, it omits the consideration of toxicodynamic sources of variation as well as insufficiencies in the data base. Along with an uncertainty factor of 2 for conversion of hair-mercury concentrations to intake levels, the total uncertainty factor used was 6.4.

The choice of uncertainty factors explains in part the difference in the recommended exposure limits (Table 3). Another decision is which studies to include. Most important perhaps, the adjusted BMDL (see above) will result in lower exposure limits than those arrived at in the risk assessments carried out so far.

Table 3. Calculated exposure limits for methylmercury.

	NRC (2000)	**JECFA (2003)**
Number of studies	One (three)	Two
Exposure biomarker	Cord blood (hair)	Hair
BMDL selected	58 μg/L cord blood	14 μg/g hair
Uncertainty factor	10	3.2 and 2
Exposure limit	0.1 μg/kg per day	1.6 μg/kg per week

Public Health Relevance

Recent discussions on methylmercury have erroneously pictured the situation as a conflict between a negative and a positive study. This misleading characteristic may be related to disagreements between regulatory agencies and has been exploited by vested interest groups. In epidemiology, the term non-positive is often used in regard to studies that were unable to detect a particular effect. Also, no matter how positive a study is, all observational studies have weaknesses, and a prudent judgment should be based on the total amount of evidence available, no on single studies, whether positive or not. The present chapter has demonstrated that the scientific evidence on methylmercury neurotoxicity is fairly consistent, and that adverse effects are likely to occur even at low-level exposures. 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 (Tsubaki and Irukayama, 1977; NRC, 2000; UNEP, 2002). It would seem intuitively logical that less severe effects may occur at the exposure ranges found in the more recent studies. Because of the global significance of methylmercury contamination of food, these scientific findings need to be expressed in terms that may facilitate an evaluation of their public health significance.

The Faroes study showed that each doubling in prenatal mercury exposure corresponded to a delay of one or two months in mental development at age 7 years (Grandjean et al., 1997). 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, at least in part, but the long-term implications are unknown. The experience with lead neurotoxicity suggests that such effects are likely to remain and that they may even become more apparent with time.

A shift in IQ levels was documented in the New Zealand study (Kjellstrom et al., 1989). The average WISC-R full-scale IQ for the study population (n= 237) was 93. In the group with maternal hair mercury above 6 μg/g (n=61) the average was 90. The average exposure in the latter group would be about 4-fold higher than in the study population as a whole. 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 (Kjellstrom, 2000). For the IQ range 71-85 the increase was from 20 to 25%, but due to

the small number of children, this result was not statistically significant.

Another approach was used in the Faroes in the absence of formal IQ tests. The regression coefficients were expressed as a proportion of the standard deviation of the test results (Grandjean et al., 1999). The most sensitive outcome parameters show a decrement of about 10% of the standard deviation at 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 are in full agreement with the New Zealand data.

Each of these estimations is associated with some degree of uncertainty. Some scientific uncertainties are bound to remain, although new prospective cohort studies on methylmercury neurotoxicity are starting to provide new evidence. However, the documentation is not going to expand substantially or otherwise provide much clearer guidance for regulatory agencies. The experience with lead research (Bellinger and Needleman, 2001) has amply illustrated that apparent disagreement is likely to occur between studies carried out by different methods in different settings. In the absence of complete coherence, decisions on preventive efforts should be justified by all available evidence, taking into account its various uncertainties and inconsistencies. Potential costs and other societal consequences of policy decisions – including decisions to do nothing - also deserve fair consideration. However, these issues should be addressed separately from the discussion of toxicological and epidemiological concerns. Otherwise, the erroneous impression will be generated that disagreements on preventive measures are solely due to uncertainties in epidemiologic evidence.

CONCLUSIONS

As previously documented (e.g. with lead pollution), a full coherence among all available evidence should not be anticipated. Decisions on preventive efforts should therefore be justified by the scientific database at large. Still, the current evidence on adverse health effects of methylmercury reveals a substantial consistency, also in regard to low-level dietary exposures.

Calculated exposure limits for methylmercury published by national and international bodies differ only little, although the approaches were not the same. However, none of the reports took into account the impact of measurement imprecision, and benchmark results used by the committees are therefore biased toward higher values. The NRC and the U.S. EPA used a

larger total uncertainty factor that may have compensated for this problem. While the focus was on protecting the fetus against adverse effects, recent evidence on postnatal neurotoxicity suggests that the protection also include adolescence. Emerging evidence that cardiovascular disease risks in adults may be associated with methylmercury exposure suggests that this protection could prudently be extended to the population at large.

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PART-V:
REGIONAL CASE STUDIES