Human Developmental Neurotoxicology

edited by
David C. Bellinger
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Preface

*Know anything? I don't even suspect anything!*

—Yogi Berra, when, following a particularly dismal performance on a test, a teacher asked, “Yogi, don’t you know anything?”

*As we know, There are known knowns. There are things we know we know. We also know There are known unknowns. That is to say We know there are some things We do not know. But there are also unknown unknowns, The ones we don’t know We don’t know.*

—Donald H. Rumsfeld, U.S. Secretary of Defense, news briefing, Feb. 12, 2002

Those who investigate the impacts of chemical exposures on children’s neurodevelopment are not quite in the same dire epistemological straits as Yogi Berra. We at least suspect quite a lot of things. Rumsfeld’s musings, although resembling a Zen koan in their obtuseness, are, nevertheless, more apt. Some questions have answers that most (although rarely all) observers would endorse. For example, if asked, “Is children’s neurodevelopment adversely affected at levels of lead exposure that are not high enough to cause an overt encephalopathy?” most investigators would probably answer affirmatively. In Rumsfeld’s system for classifying knowledge, this would be a “known known.” An increase in the specificity with which a question is phrased will, however, often result in responses that are more variable, both in content and the respondent’s level of certainty, suggesting that the issue in question would be better classified as a “known unknown.”

“What is the functional form of the dose-response relationship?”, “To what extent do the expressions of nervous system toxicity depend on the age at which exposure occurs?” and “What is the natural history of lead-associated neurodevelopmental deficits under different degrees of postnatal environmental enrichment?” are examples of such questions. And then there are the “unknown unknowns.” These bear on issues that we won’t even think to ask questions about until we learn enough to appreciate the existence of a mystery that, heretofore, lay unrecognized.

These are not issues solely of academic interest. In the past two decades, children’s abilities to process information, to reason, to learn, and to achieve a positive psychosocial adjustment have emerged as critical endpoints in risk assessments of chemical exposures. It is recognized that children with even subtle impairments of these skills, who cannot function near the peak of their potential, will not fare as well as those who do in a technological marketplace that places high value on analytic and communication skills and the ability to adapt quickly and effectively to shifting demands and opportunities. It is
no longer adequate to make public health decisions based on risk assessments that use as critical endpoints frank neurological disease or mental retardation (to say nothing of endpoints such as the LD50—the dose at which 50% of animals in the “exposed” group die). Exposure standards will be insufficiently protective if based on using approaches that do not acknowledge the potential importance, both to the individual and to society, of “sub-clinical” effects, that is adverse impacts that are not severe enough to meet diagnostic criteria for a disease or that do not correspond to a pattern that is sufficiently common to have been canonized as a “diagnosis.”

The increasing recognition of “subtle” effects of neurotoxicant exposures and their acceptance as worthy of concern has resulted in a steady evolution over the past 30 years in the study methodologies applied in human developmental neurotoxicology studies. Case series describing the severe neurological deficits of children who developed Congenital Minamata Disease after prenatal exposure to methyl mercury or who presented with a fulminant lead encephalopathy were sufficient to convince us of the serious neurotoxicities caused by high dose exposure to these metals. Studies that were more analytic, using a case-control design, became necessary when the goal was to determine whether the prevalence of clinically-defined childhood morbidities, for example diagnoses such as learning disabilities or attention deficit hyperactivity disorder, differed between children who were considered “exposed” or “unexposed” to some chemical. The effect measures applied in case-control studies were typically the odds ratio and relative risk, expressing the extent to which the morbidity was more likely to be present among children in the “exposed” than the “unexposed” group. When concern began to shift to possible sub-clinical impairments, it was necessary to mount cohort studies, in which participants were selected on the basis of exposure status rather than outcome status, and outcomes were often represented dimensionally rather than as diagnoses that were noted as being simply present or absent. Prospective studies were recognized as preferable to cross-sectional studies because they permitted an investigator to establish the temporal precedence of exposure vis a vis outcome, to characterize the natural history of exposure-outcome associations, and to identify age-dependent variations in susceptibility. In contrast to the odds ratio or relative risk statistics calculated in case-control studies, the effect measures calculated in cohort studies were more often the rate of change in the dimensional outcome per unit increase in an exposure index, providing new options for modeling dose-response and dose-effect relationships and developing points of departure for risk assessments.

Other recent trends in the evolution of human developmental neurotoxicology research are notable. At the same time that the endpoints considered important to society were broadened to include sub-clinical as well as clinical impairments within a particular health domain, the range of health domains of interest as possible targets of neurotoxicant exposures has also broadened. For a variety of reasons, neurotoxicological studies traditionally focused on cognitive morbidities, defined rather narrowly and often consisting solely of IQ, as the critical endpoints. Beginning in the 1980s, however, attention was drawn in the general pediatric literature to what was called “the new morbidity,” referring to behavioral disorders and maladaptive psychosocial function. A concern with such disorders is increasingly reflected in neurotoxicological studies, with diagnoses such as juvenile delinquency, attention deficit hyperactivity disorder, and autism spectrum disorder serving as the endpoints of interest. Another trend is increasing sophistication of the methods used to address the critical issue of confounding bias by characterizing more accurately and comprehensively the panoply of factors that, apart from the chemical exposure of interest, can affect a child’s health. Determining how a chemical exposure fits into the complex web of influences on child development, its effects perhaps being exacerbated by some of these other influences and mitigated by others, also
became an important part of the evaluation of study hypotheses. These trends resulted in studies that have become increasingly complex and multidisciplinary, requiring, at least, the collaboration of toxicologists, developmental psychologists, epidemiologists, biostatisticians, and analytical chemists. The contributions of psychiatrists, sociologists and cultural anthropologists will be increasingly important as the range of endpoints of interest continues to broaden and the child development models used to evaluate chemical effects become richer in depth and complexity.

The purpose of this book is to describe the state-of-the-art in the design, conduct, and interpretation of human developmental neurotoxicology studies. Authors were asked to do more, however, than to describe current knowledge and methods in their fields of research. They were asked to address the question, “How can we do better?” and encouraged to identify the advances that need to be made in order to allow investigators to clarify the “known unknowns” and to identify the “unknown unknowns” that will be the foci of future research.

Chapters in section one focus on specific environmental chemical exposures, including mercury, PCBs, lead, and solvents. Although the first three are among the chemicals that have been most intensively studied, many knowledge gaps remain, continuing to inspire debate and to render the risk assessment process contentious. Because of the current world-wide concern about mercury toxicity, two chapters are devoted to this metal. Although there is some overlap in the topics covered, the chapters provide complementary perspectives on the conduct and interpretation of mercury studies as well as on their public health implications.

The chapters in section two focus on the developmental neurotoxicities associated with intentional exposures to chemicals or chemical mixtures. Some of these are medications administered therapeutically, such as anti-epileptic and chemotherapeutic drugs, while others are drugs used recreationally, including tobacco, alcohol, and cocaine. The potential adverse effects of exposures to recreational drugs generally receive much more attention than do the potential adverse effects of exposures to therapeutic drugs, perhaps due, at least in part, to differences in the risk-benefit calculus appropriate to these two classes of exposures. Exposure to therapeutic drugs occurs as a result of a decision that the avoidance of a health risk associated with a medical condition outweighs the risks associated with their use, which might be substantial (i.e., “side effects”). In contrast, use of recreational drugs, particularly during pregnancy, would not be expected to provide any health benefit to the fetus or child, which could make the risk of even subtle “side effects” on a child enough to tip the balance in favor of avoiding the exposure.

Chapters in section three focus on critical issues in the assessment of exposure and outcome. Separate chapters focus on the special considerations germane to the assessment of exposures to accumulative chemicals, such as many metals, and to the assessment of exposures to chemicals with relatively short biological residence times, such as organophosphate pesticides. Another chapter focuses on special issues that pertain to characterizing voluntary exposures, such as marijuana and cocaine. The chapters focusing on outcome assessment address considerations in assembling, modifying, and validating a battery of tests, the emerging role for neuroimaging modalities in assessing neurotoxicity, and the special challenges faced in studying the contributions of childhood neurotoxicant exposures to the development of adult neurologic disease.

Chapters in section four provide perspectives on several aspects of the analysis and interpretation of developmental neurotoxicity studies. These include the strategies used to identify potential confounding variables to include in regression models and the potential utility of analytic strategies such as structural equation modeling in characterizing the relationships among exposures, outcomes, confounders, and mediators. Another chapter
shows how our understanding of exposure-outcome associations can be enriched by taking into account the broader social environment within a child that is developing and building multi-level models of complex inter-relationships. Two other chapters address issues pertaining to study interpretation, one focusing on how consideration of the experimental animal literature on a chemical’s developmental neurotoxicity can inform both the choice of methods used in human studies, and the inferences drawn about behavioral mechanisms and, ultimately, about causality. A chapter that focuses on the effects of chemical exposures on thyroid hormone signaling pathways illustrates the daunting distance we have to go to bridge the yawning chasm that separates the observations made in epidemiologic studies of developmental neurotoxicity and an understanding of the biological mechanisms generating those observations.

The chapters in section five place the contributions of developmental neurotoxicity research in a broader context. Two chapters provide the perspectives of groups who could be characterized as “consumers” of such studies, namely those who use the data clinically to manage patients exposed to neurotoxic chemicals, and those who use the data as the basis for formulating public policy. They help us to understand ways in which this research can be designed, conducted, analyzed in ways that will make the data more useful to those who apply the findings. The final chapters alert us to the hazards that can arise in doing research that threatens industrial interests or that enriches investigators who place themselves in positions of conflict of interest. These caveats remind us of the quite profound effects that developmental neurotoxicity research have on people’s health and livelihoods, and the special responsibilities we assume in undertaking such work.

David C. Bellinger
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Methylmercury: A Model Neurotoxicant and Risk Assessment Dilemma

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MERCURY AS AN ENVIRONMENTAL POISON

The New York Times of August 26, 1991, carried a headline on its first page that reverberates with debates that are even more strident today: “Despite Era of Concern for the Earth, Mercury is Re-emerging as a Peril.” It pointed to the deepening concern over contamination by mercury of aquatic life and how it had aroused governmental agencies charged with environmental protection and public health to take preventive and regulatory action. It described the origins of the catastrophe in Minamata, Japan, a fishing village whose inhabitants suffered an epidemic of methylmercury poisoning from contaminated fish. It told of the conflicts faced by sport and subsistence fishermen about the safety of consuming their catch, and of the confusion and wariness on the part of fish consumers.

The intensity of the debate has not waned. Instead, it has even widened in scope to include forms of mercury other than the methyl form found in aquatic species. It now includes the vaccine preservative Thimerosal, indicted as a risk factor for autism because it is 50% ethylmercury which, like methylmercury, is a potent neurotoxicant. It also includes dental amalgams, an argument carried on for 150 years but becoming more forceful because of the availability of alternatives. Advocacy groups and individuals have raised alarms because chewing releases mercury vapor from fillings that then is inhaled.

Why is mercury the focus of such intense discussion at this time? It is not just the evidence of its potency as a toxicant, which Hunter (1), in his treatise on occupational medicine, described in his review of the “ancient metals.” It more likely is a symptom of our distrust of the information made available to us in the realm of food and chemical safety. We have been deceived or misled about chemical hazards on so many occasions at the same time we’ve received reassurances that our concerns are unfounded that we raise a shield of skepticism. We often have seen how commercial or political interests distort the truth, and have witnessed so many disastrous episodes that it requires a suspension of hardened cynicism to believe in reassurances.

Methylmercury is the principal subject addressed here, but in the environment it is entangled with other forms of mercury and other neurotoxic entities. Of all mercury species, methylmercury, however, is the one with the broadest implications for public
health. It is found primarily in a major food source, fish, so it affects populations worldwide. It became a model for behavioral teratology as a definitive example of an environmental neurotoxicant, and it contains lessons useful to the study of other agents with the potential to interfere with neurobehavioral development (2). Methylmercury also illustrates the problems posed to regulatory agencies and their need to translate into exposure standards questions such as the shape of dose-response functions and the inherent limitations of epidemiology. In most instances, the translation is a challenge because most of the toxicity information takes the form of high-dose experiments in animals and, except for mass poisonings, low-level environmental exposures in humans, requiring a succession of tentative extrapolations to harmonize these disparities. Like other agents discussed in this volume, methylmercury exemplifies how an appropriate definition of low-level exposure is a constantly moving target.

From some perspectives, discussions of neurotoxicity attributable to environmental chemicals involve a marginal problem compared, say, to the serious disabilities of the fetal alcohol syndrome (FAS). Comparability is not the issue, however. Even FAS can be seen as a minor problem compared to the lives erased by guns, child abuse, and auto accidents. It is the inexorable accumulation of many different risks that is so appalling. Exposure to environmental toxicants, although incurring far less dramatic effects than FAS, is more widely distributed in the population, and their effects, because they are so insidious, are easily overlooked. A small effect on IQ, such as an effect size of 0.33 (one-fifth of a standard deviation or 3 IQ points, producing a mean score of 97 rather than 100) assigns 3.2% of the population to the category “retarded” rather than 2.3%.

Figure 1 depicts such a condition. It is the kind of situation that animates how we view the neurobehavioral risks of lead and PCBs as well. That is, their adverse effects are

![IQ Distribution Charts](image)

**Figure 1** Upper chart shows an IQ distribution with a mean of 100 and SD of 15. The dark area represents the 2.3% of the population below 70. The light area represents those with IQs below 100 and above 70. The lower chart depicts an IQ distribution with a mean of 97. Here, 3.2% of the population falls below 70. IQ of 100 is shown on both charts as the vertical line.
expressed primarily as shifts in the distribution of population scores rather than, as with clinical assessments, deficits in individuals. This is the core issue for methylmercury, too.

ANTICIPATION OF THE CURRENT DEBATE

In 1968, we held the first of over 20 Rochester Conferences on Environmental Toxicity (3). By that time, Swedish scientists had alerted the world that the catastrophe of methylmercury poisoning at Minamata, Japan, represented not a local problem but rather one with global dimensions. They had demonstrated that environmental discharges of all forms of mercury (metallic, inorganic divalent, phenylmercury) can all be converted by natural processes in aquatic settings to the highly toxic methylmercury form (4). They had been alerted to the existence of a methylmercury problem in Sweden’s waters by a decline in sea bird populations, in species such as ospreys, that depended on fish for sustenance. Their findings recapitulated the questions that had been posed by Rachel Carson (5) about pesticides when she observed diminished bird populations. By analyzing the mercury content of bird feathers in museums and comparing them to contemporary levels, they demonstrated the sharp rises coincident with the introduction of organic mercury fungicides into Swedish agriculture. (Feathers, like growing hair, reflect methylmercury concentrations in the blood). They also showed how the process of bioconcentration could magnify toxic exposures: pike in Swedish waterways bore flesh levels of methylmercury as much as 3000 times greater than the ambient water concentration due to increases at successive trophic levels as predators consumed contaminated prey.

At the 1968 meeting, Robert Risebrough, of the University of California, Berkeley, asked the following question:

Why should mercury be a problem in the northern countries rather than in the more temperate areas?

Fredrik Berglund of the National Institute of Public Health, Sweden, replied:

I think one reason that this problem does not exist in the United States with mercury is that the levels are not known...I feel, personally, that the problem also exists here as it does in other parts of the world but it is not recognized.

Recognition soon came, with the discovery, in 1970, of methylmercury contamination in the Great Lakes, stemming mainly from the discharge of metallic mercury by chlor-alkali plants. These plants use large pools of liquid mercury as electrodes to convert brine into chlorine and sodium hydroxide. The current methylmercury agenda, with its emphasis on prenatal exposure, was set the following year with a mass chemical poisoning in Iraq (6). This history parallels the concurrent development of neurobehavioral toxicology as a science.

MINAMATA AND IRAQ

Two mass chemical disasters awakened us to the threats posed to developing brains by methylmercury. One occurred in Japan, the other in Iraq. Progressive stages in the evolution of neurobehavioral toxicology can be traced by how those two mass poisonings helped spawn a discipline that pulled toxicology from its traditional reliance on lethality and pathology towards criteria such as IQ scores and performance on behavioral tests.
What the Japanese later called Minamata Disease arose from methylmercury contamination of Minamata Bay, which adjoins a fishing village in the southernmost Japanese island of Kyushu. A change in catalyst use in the production of acetaldehyde by the Chisso factory on the shores of the bay resulted in the discharge of mercury into the bay, where it contaminated the fish and shellfish consumed by the villagers, especially fishermen and their families. The tragedy of Minamata has been told many times and in many ways, but perhaps none as effectively as in the photo essay by Eugene and Aileen Smith (7). It depicted with one unnerving episode the price Japan had paid for industrialization carried out with little concern for its environmental consequences.

Minamata disease, after a lengthy period of puzzlement about its etiology, was eventually discovered to be due to methylmercury exposure. Its signs, in adults, had been known for decades because of accidents. They are listed in Table 1.

The victims of that tragedy extend beyond the group whose manifestations were easily identified. A much larger group most surely bears scars that are not as easily or directly ascribed to methylmercury because their legacy came in the form of functional deficits that required close study with appropriate tests and comparisons at a time when quantitative measures of exposure were not available.

The drama of that episode, however, has tended to blur an important environmental lesson. Like some other environmental contaminants, methylmercury delivered its toxicological message insidiously. Figure 2 charts the rising incidence of Minamata Disease over a three-years period. Cases appeared sporadically. Dr. Hajime Hosokawa, a physician employed by Chisso, courageously proposed that a link existed between the factory and the continuing appearance of cases. His hypothesis was dismissed by Chisso management, but he based his argument on an almost intuitive epidemiology and, perhaps, drew from a famous experiment with cats described by Eto et al. (8). By 1958, almost no cat could be found in the Minamata district. Even as far back as 1952, they had begun to display convulsions, ataxia, and what the inhabitants termed “dancing disease.” Because of this history, a cat experiment was designed to test the conclusion, declared by a study group from Kumamoto University, that organic mercury was responsible for the outbreak. Ten cats were fed food mixed with industrial waste produced in the Chisso plant. The results were not made public, but Eto et al. (8) found the specimens and conducted pathological and chemical assays. Typical lesions of methylmercury poisoning were observed in the central nervous system tissue, and were associated with markedly elevated mercury levels (9).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Signs and Symptoms of Methylmercury Poisoning in Adults</th>
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<tbody>
<tr>
<td>Sensory</td>
<td>Paresthesia (numbness and tingling)</td>
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<tr>
<td></td>
<td>Pain in limbs</td>
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<td></td>
<td>Visual disturbances (field constriction)</td>
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<td></td>
<td>Hearing disturbances</td>
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<td></td>
<td>Asterognosis (discrimination by touch)</td>
</tr>
<tr>
<td>Motor</td>
<td>Disturbances of gait</td>
</tr>
<tr>
<td></td>
<td>Weakness, leg unsteadiness, falling</td>
</tr>
<tr>
<td></td>
<td>Thick, slurred speed (dysarthria)</td>
</tr>
<tr>
<td></td>
<td>Tremor</td>
</tr>
<tr>
<td>Other</td>
<td>Headaches, rashes, “mental disturbance”</td>
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Figure 2 reflects a property of methylmercury toxicity that helped obscure the connection between fish and shellfish consumption and Minamata disease: the period of silent toxicity between exposure and onset of effect. Although 95% of ingested methylmercury is absorbed, delayed toxicity, or displacement in time between exposure and its consequences, makes it difficult to discern a relationship, a gap that can deceive investigators about etiology or lead to disasters that could have been averted or diminished if acted on earlier. This property of methylmercury is discussed later.

Minamata also provided the first suggestions of fetal vulnerability. Pregnant women who evinced no signs of poisoning themselves gave birth to offspring with severe neurological deficits who later received the diagnosis of Fetal or Congenital Minamata Disease. These prenatally-exposed children were characterized by severe neurological impairments that included ataxia, neuromotor disability, seizures, hearing loss, microcephaly and cognitive impairment (10). Some of these individuals, now in their 50s, are maintained in a special unit attached to the Minamata Disease Research Institute, which was formed and is supported by the Japanese government.

In a bizarre repetition of Minamata about 10 years later, another Japanese town, Niigata, also experienced an epidemic of methylmercury poisoning (11). Again, it arose from a factory that manufactured acetaldehyde and that, in the process, discharged inorganic and methylmercury into a waterway, in this instance, the Agano river. The authors report that 690 adults have been certified as victims of methylmercury poisoning. They also report that even many of those exposed prenatally, whose mothers’ hair levels may have exceeded 50 ppm, a level now considered excessive, show little evidence of adverse effects. These measurements have not been verified by current analytical methods, however.

The second mass poisoning, and the one best documented, occurred in Iraq in the winter of 1971–72. A severe drought in the summer of 1971 impelled the Iraqi government to purchase 80,000 tons of seed wheat for planting in 1972. It also requested that the wheat be treated with a methylmercury fungicide, a request made in error. Hungry farmers in rural Iraq, rather than planting the wheat, baked it into bread. During a three-month period that winter, as many as 5000 victims may have died with as many as 50,000 suffering severe illness.
Because Thomas W. Clarkson at the University of Rochester had recently reported on a sulfhydryl-binding resin that accelerated the clearance of methylmercury from the body, he was contacted by the Iraqi Ministry of Health to secure the resin. The ultimate outcome of that request was establishment of a laboratory in Baghdad and a survey of the exposed population in the countryside (12). It led to the use of hair as a biomarker of exposure history and, of supreme importance, a clear demonstration of the exquisite sensitivity of the fetal brain. Children whose mothers had been pregnant during the outbreak were examined at about 30 months of age to assess developmental milestones such as walking, a difficult task because of undocumented birth dates coupled with the need to conduct examinations in the field. Nevertheless, those data suggested that maternal body burdens in the range of those seen in fish-consuming populations might pose a risk to neurobehavioral development (13) and eventually stimulated several prospective studies in such populations. Interpretation of the results of those studies provides the basis for the current debates about the neurodevelopmental risks of methylmercury.

Minamata and Iraq aroused enormous anxieties about their implications for child development (14). Both episodes identified the human fetal brain as the most vulnerable target of methylmercury neurotoxicity, an expectation confirmed by studies in animal models (15,16). Such models have provided the main basis for exploring the mechanisms underlying the pathological features observed in human brains. These were described by Choi et al. (17), who examined the brains of two full term newborn human infants in Iraq who were exposed to methylmercury early in pregnancy due to maternal ingestion of methylmercury–contaminated bread. The brains contained high levels of mercury. Disturbed development was marked by incomplete or abnormal migration of neurons to the cerebellar and cerebral cortices, and deranged cortical organization. Numerous heterotopic neurons were seen in the white matter of cerebrum and cerebellum. The characteristic laminar pattern of the cerebral cortex was distorted in many regions. Such a pattern of damage is unlike the focal neuronal damage observed in poisoned adults and children exposed postnatally.

Studies in mice by Sager et al. (18) and Rodier et al. (19) indicate that mitosis is blocked by methylmercury exposure during metaphase. The mechanism appears to be disruption of microtubules in the mitotic spindle. Mitotic arrest can prove devastating to the developing brain, especially before birth, when the bulk of neurons are formed. Choi (20), also in mice, noted abnormal patterns of neuronal migration and cortical cytoarchitecture, another feature of methylmercury neuropathology.

**THE CURRENT SCENE**

In practice, the only source of methylmercury exposure is food from aquatic and marine environments. Because these represent crucial food sources, and a dominant one for protein in some populations, methylmercury ascended to a problem of global dimensions. Although several smaller epidemiological studies have been undertaken (21–27), two large, longitudinal cohort studies occupy the center of the risk debate. One, undertaken by investigators from the University of Rochester, was situated in the Republic of the Seychelles, an island group in the Indian Ocean. Another, conducted by investigators from the University of Southern Denmark, in Odense, was situated in the Faroe Islands in the North Atlantic.

Both research groups chose to study the relationship between prenatal exposure to methylmercury and a variety of neurodevelopmental measures. In the Seychelles project, methylmercury concentration in maternal hair was chosen as the biomarker, a choice
governed by data demonstrating a close correlation between blood levels and levels in growing hair and by ease of storage for transportation and later assays. Because scalp hair grows about 1.1 cm per month, a 12 cm hair strand can recapitulate a one-year exposure history, a vital factor in tracing the outbreak of poisoning in Iraq (6). McDowell et al. (28) point out another advantage of hair assays; namely, because they are noninvasive, they elicit much better response rates from population subgroups such as children that usually resist blood sampling. The hair:blood ratio roughly averages 250:1, but with substantial variation (29,30) and higher ratios in children.

The question of how hair levels relate to brain levels will be discussed later. Based on the autopsy material from the Seychelles (31), maternal hair and infant brain levels are closely correlated.

The Faroes investigators selected cord blood, which reflects exposure during the later stages of pregnancy, as their main index of exposure, but additional analyses showed a high correlation with hair levels. Maternal hair levels averaged 6.9 ppm in the Seychelles and 4.27 in the Faroes. In the U.S., recent NHANES data showed a geometric mean of 0.12 ppm in children, and 0.20 ppm in women (32). Frequent fish consumers, compared with non-consumers, showed geometric mean hair mercury levels for women of 0.38 versus 0.11 ppm and levels for children of 0.16 versus 0.08 ppm. In the Seychelles, mothers reported eating a mean of 12 fish meals weekly. In the Faroes, adults reported mean daily consumption of 72 grams of fish, 12 grams of whale muscle, and 7 grams of whale blubber. The differences in consumption patterns are significant; the methylmercury source in the Seychelles is ocean fish, while in the Faroes it is primarily pilot whales. The differences create an interpretive dilemma, as discussed later, because these whales are heavily contaminated with other neurotoxicants such as PCBs and a variety of other organohalogens.

The Seychelles cohort, consisting of 779 mother-infant pairs enrolled in 1989–1990, has so far been assessed at 6.5, 19, 29, 66, and 107 months of age by a variety of neuropsychological tests appropriate for the cohort’s stage of development (33). For example, at 19 months of age, the main assay consisted of the Bayley Scales of Infant Development. At 107 months of age, the investigators were able to deploy a broad variety of instruments ranging from those assessing motor function (e.g., grooved pegboard) to those assessing cognitive function (e.g., Boston Naming Test) to those assessing conduct (e.g., Child Behavior Checklist). From all of these assessments, based on multiple regression analyses that included potential confounders, only a handful of scattered endpoints revealed any correlation with mercury exposure indices. And, of these, some showed a positive relationship between exposure level and performance, but the relationship depended on age.

The Faroes cohort was also assessed with a battery of tests that included neurophysiological (e.g., visual and auditory evoked potentials) as well as neuropsychological (e.g., motor function, cognitive function, subjective state) instruments. The study was initiated with the collection of maternal hair and cord blood levels from 1022 singleton births. At about 7 years of age, 917 of the children were recruited to undergo testing (34). Multiple regression analyses, adjusted for confounders, comprised the main statistical technique. Neither the neurophysiological endpoints nor clinical examinations showed a relationship with methylmercury exposure. In contrast to the Seychelles results, analyses of the neuropsychological indices demonstrated significant negative associations with prenatal methylmercury measures in all five domains chosen for assessment: motor, attention, visuospatial, language, and memory.

The different conclusions derived from the Seychelles and Faroes studies remain puzzling but, also, contentious. A National Academy of Sciences committee, charged with
establishing a risk assessment of methylmercury in seafood, chose the Faroes data as its basis because it offered a clear outcome (35). Specifically, it relied upon the Boston Naming Test as the index measure. It then applied Benchmark Dose (BMD) modeling to derive an exposure level associated with a specified risk value. For such calculations, a Benchmark Response (BMR) is chosen; for example, a value of 5%, which means an increased risk of an abnormal response of 5%. A BMD is then calculated by fitting a mathematical model to the dose-response function, and, on the basis of that function, deriving the dose of the agent that would be predicted to produce, for a BMR of 5%, an excess risk of that magnitude. Because of statistical uncertainty about the central estimate BMD, a lower 95% confidence limit (BMDL) for the BMD is also computed.

The committee determined a BMDL of 58 ppb in cord blood (equal approximately to 12 ppm in hair) as the appropriate exposure level from which to derive risk values. The value of 58 ppb was extrapolated to an equivalent intake of 1.1 μg/kg of methylmercury per day. In setting exposure standards, it is common to divide a value such as the BMDL by one or more uncertainty factors to provide a margin of protection. Dividing that value by uncertainty factors summing to about 10 yields a Reference Dose (RfD) (equivalent to an acceptable daily intake) of 0.1 μg/kg per day. The EPA defines a RfD as an exposure level that is without adverse effects over a 70 years lifespan. This RfD translates into a weekly consumption of one 198 g (7 oz) can of tuna for an adult.

BMD models have also been applied to the Seychelles data set (36). As the authors note, such models can prove useful even when a statistically significant dose-related trend is absent. On the basis of an increase of 0.1 in the probability of an adverse response (the BMR), maternal hair BMDLs calculated from the Seychelles data ranged from about 19 to about 30 ppm. These values are not markedly different from the Faroes BMDL, nor from BMDLs based upon a New Zealand cohort (23,24) or the data from Iraq (37).

The Faroes BMDL values can roughly be translated into brain levels. In nonhuman primates, the blood-brain ratio is approximately 3–10:1 (38–41). In the Seychelles cohort, comparing maternal blood with infant brains secured at autopsy yielded ratios between 5.1 and 6.7 (31). Based on these values, we would expect brain levels at the BMDL to roughly approximate 300 ppb.

Infant brains from the Seychelles were examined by Lapham et al. (42). They found no evidence of neuropathology, assessed by criteria similar to those of Choi et al. (17); these brains all exhibited levels of mercury less than 300 ppb, however, while discernible anatomical abnormalities (by conventional pathological techniques) are seen in experimental animals at levels of 1000 ppb and above (43).

**OTHER COMPLICATIONS**

The underlying question for the risk assessment of methylmercury is converting exposure magnitude into risk, which is then translated into the form of the dose-response function. Response takes the form of scores on one or more neuropsychological tests. Dose takes the form of methylmercury in blood or hair. Methylmercury is a biomarker of seafood consumption. (The term seafood is used generically to refer to fish, crustaceans, sea mammals, and other forms of aquatic life eaten by humans and coming from both marine and freshwater sources). In general, those who consume more of these foods exhibit higher levels of methylmercury. Investigators pursuing the question of methylmercury risks, like toxicologists in general, traditionally assume a monotonic dose-response function; that is, that higher exposure levels correspond to greater adverse effects. The dose-response relationship for any particular agent and endpoint may assume a different shape due either
to the nature of the biological response or because of interactions or confounding with other factors (cf., 44). Instead, it might assume a U-shaped function, as suggested in some data from the Seychelles project (45), especially in populations where fish rather than other aquatic species comprise major portions of the diet. Adverse developmental outcomes, that is, could be associated with both low and high levels of methylmercury, and optimal outcomes might be associated with intermediate values, as described below.

U-shaped functions are common in nutrition. For example, manganese is required by neonates for normal skeletal and inner ear development and as components of critical enzymes (46). Elevated manganese exposures in neonatal rats, however, can also induce neurochemical and behavioral abnormalities (47). Vitamins share the same kind of U-shaped dose-response properties and, like manganese, reflect different endpoints in the two arms of the U. In the methylmercury example, lower levels might reflect diminished intake of important nutrients such polyunsaturated long chain fatty acids (PUFAs). The n-3 (omega-3) fatty acids, such as docosahexanoic acid (DHA), are abundant in fish. Higher levels of methylmercury would overcome such beneficial properties and induce toxicity. This phenomenon may underlie the finding of an inverse relationship between maternal hair mercury levels and scores of male offspring at 66 and 107 months of age on the Conners Rating Scales, which are used as measures of ADHD (45). These results do not contradict the recognized threats to brain development posed by higher levels of methylmercury exposure; they merely suggest that the neurotoxic risks associated with low levels of exposure may be concealed by the benefits of fish consumption for which methylmercury serves as an exposure marker.

Choline levels are also high in fish. Zeisel (48) reviewed the evidence suggesting an essential role for choline in human neurodevelopment and cognitive functioning. Studies in rats have demonstrated that supplementation with choline during embryonic development or immediately following birth can result in improved memory performance, which is maintained throughout life (49). Whether supplementation or deprivation later in postnatal development could affect complex cognitive, sensory, motor or behavioral functions is unclear. Common developmental disorders including ADHD, dyslexia, dyspraxia and autistic spectrum disorders (ASD) may also involve functional deficiencies or imbalances in n-3 and n-6 fatty acids (50). But the Seychellois diet is high in these fatty acids, and the levels of PUFAs in blood in Seychellois women is six times higher than in U.S. women.

THE RISK EQUATION AND EFFECT MODIFICATION

Few epidemiological studies examining exposure to environmental neurotoxicants can without ambiguity dissociate themselves from the multitude of other toxic risks encountered during development nor, in some cases, such as pesticides, from the question of potential public health benefits. For some environmental contaminants, such as dioxin and lead, no benefits are attached to exposure. For pesticides, it could be argued that exposure must be weighed against the benefits of pest control and efficient agricultural production (51). For seafood, the dilemma is how to construct a risk assessment equation across several dimensions. We are faced with three axioms:

1. All fish, crustaceans, and sea mammals contain methylmercury in variable concentrations.
2. Fish, crustaceans, and sea mammals also contain nutrients, again in variable concentrations, such as n-3 fatty acids, choline, and iodide, that are essential for brain development.
3. Many fish, crustaceans, and sea mammals are also contaminated with a variety of toxic contaminants other than methylmercury; for example, cadmium, PCBs, polybrominated diethyl ethers (PBDEs) dioxins, DDT and its metabolites, other organochlorine pesticides, and a variety of polycyclic aromatic hydrocarbons such as benzo[a]pyrene. Disentangling the individual contributions of such contaminants, all of which can exert adverse effects on brain function and development, is a daunting, if not impossible challenge, especially because of the potential for additive or synergistic combinations. The mixture problem, a legacy of the extent to which we have thoughtlessly contaminated the environment, is hardly confined to seafood, and pervades every facet of current toxicology.

Achieving an appropriate balance of risks and benefits for seafood presents an unusual dilemma that needs further exploration. The third axiom above noted that other common contaminants, as discussed in other chapters in this book, offer hazards of their own. Methylmercury is found primarily in muscle tissue. Organohalogenes are concentrated in fatty tissue. Fish that are low in methylmercury and high in n-3 fatty acids, such as salmon and herring, because of their high fat content, also tend to contain higher concentrations of the organohalogenes. The consumer, as a result, is compelled to choose between toxicants in selecting different varieties of seafood. Farm-raised salmon, for example, may be contaminated by levels of PCBs exceeding those recommended by agencies such as the U.S. EPA (52). These authors also noted wide regional differences in the degree of contamination. Fish raised in farms in Scotland and the Faroes contained the highest concentrations of PCBs, while those raised in Chile contained the least.

The high lipid solubility of PCBs and other organohalogenes presents a special problem for brain development. The fetal and neonatal brain is 60% lipid, and it consumes a high proportion (70%) of the energy drawn from the mother. Its energy consumption is one reason it is so vulnerable. Fetal and infant brain development are highly dependent on the availability of two long-chain polyunsaturated fatty acids, docosahexanocic acid (DHA) and arachidonic acid (AA). Seafoods provide these nutrients in relative abundance compared to terrestrial sources (53). These authors argue, in fact, that the evolution of the hominid brain depended on the availability of these fatty acids, which were provided in fish consumed by littoral communities.

The organohalogen connection, for which PCB levels provide an exposure index, creates interpretive complications. Fangstrom et al. (54), in their analyses of PCBs in pregnant Faroese women, observed high concentrations of PCBs in serum, “possibly the highest so far reported in a population” and attributed these values primarily to the consumption of pilot whale blubber. Some neurotoxic effects of PCB exposure were uncovered in the 7 years assessment (34). The later stratified analyses conducted by Grandjean et al. (55) suggested that neurotoxicity stemming from PCB exposure may be detectable only at the higher levels of methylmercury, which suggests an additive or more complex model. Whether joint PCB and methylmercury exposure can be disentangled by statistical methods is unclear because we do not understand the mechanisms of interaction, or, indeed, if interaction is the proper term for this situation (56).

A study of sport fish consumers residing in a community on the shore of Lake Ontario, designed primarily to explore the influence of prenatal PCB exposure on neurobehavioral development, has also unearthed a complex PCB–methylmercury relationship (57). It is, in essence, a mirror-image of Grandjean et al. (55). In this cohort, in offspring exhibiting the higher PCB levels, methylmercury concentrations in maternal
hair provided a significant predictor of performance on the McCarthy Scales of Children’s Abilities.

In a somewhat parallel analysis of PCB-methylmercury interactions, Grandjean et al. (58) reported that breast feeding in the Faroes is associated with diminished growth, an outcome attributed to both methylmercury and PCBs. Put another way, the benefits of breast feeding for neuropsychological development and immune function in a community such as the Faroes have to be balanced against the risk of growth retardation.

These PCB-methylmercury interactions can be viewed from the perspective of effect modification as well as from a mechanistic perspective. Bellinger (59) defines the term to describe a phenomenon in which the effects of one variable, such as dose, depend upon the levels of another variable, such as socioeconomic status. Bellinger pointed to the latter as a model, because of his finding that an effect of lead on IQ scores could be seen in children from low SES but not in high SES families. Analogously, in the Ontario and Faroe cohorts, an effect of PCBs (or methylmercury) in combination with methylmercury (or PCBs) depended on the level of the other agent.

INORGANIC MERCURY

The risk assessment challenge of methylmercury in seafood is also compounded by simultaneous exposure to inorganic mercury from sources other than food. In the form of mercury vapor (Hg\(^\text{vapor}\)), elemental mercury is a potent neurotoxicant. The complications for methylmercury risk assessment due to concurrent exposure to the vapor species arise from two sources. One is the potential developmental neurotoxicity of mercury vapor itself. The other is the possibility that the vapor and organic species share common mechanisms of toxicity. Because assays of mercury in blood often do not distinguish inorganic from organic species, much of the data in the literature cannot be used to compare their individual contributions to selected endpoints.

Although mercury vapor is a venerable and potent adult neurotoxicant, its hazards for nervous system development are virtually unknown. The fetus may be exposed to both the methyl and vapor forms. While methylmercury is consumed in the diet, exposures to the vapor form may occur in the workplace, as a result of certain religious practices employing metallic mercury (60), through the use of mercury-containing cosmetics (61), or, the most common source, by mercury vapor emitted by dental amalgam restorations and inhaled by pregnant women. Both the organic and vapor forms of mercury are then transmitted to the fetus. Chewing is known to produce vapor, which is then inhaled by the mother and carried to the fetus. Information about the joint effects of these two mercury species is scant, but, on the basis of mechanistic considerations and sketchy animal data, warrants careful consideration.

Berlin (62) argues forcefully that inorganic mercury could pose a threat to fetal brain development. He notes, based on in vitro experiments, that toxic effects are discernible at concentrations of 1–10 ng/g in tissue, which lie in the range measured in infants and fetuses from amalgam-bearing mothers. Further, he argues, anatomical and behavioral disorders are seen in rats and in monkeys exposed developmentally at fetal brain concentrations of 10–200 ng/g, which fall below the concentrations seen to produce such effects with methylmercury. In addition, he points to epidemiological studies indicating that occupational exposure to mercury vapor is associated with elevated risks of mental retardation and infants labelled as small for gestational age.
Inorganic mercury levels in fetal tissue are significantly correlated with the number of maternal amalgam surfaces (63). And Bjornberg et al. (64) report that inorganic Hg in cord blood increased significantly with increasing number of maternal dental amalgam fillings. Not only are billions of individuals world-wide exposed to the emission of mercury vapor from amalgam restorations, but some pregnant women suffer an increase in dental problems such as caries and gingivitis, and become candidates for dental restorations (65). In some communities, dental care, including restorations, is promoted as part of prenatal care.

Like methylmercury, inhaled mercury vapor is transported from mother to fetus, across the placenta, in much the same manner in which it crosses from the blood into the brain (66,67). Furthermore, we also know that maternal vapor exposure in humans is correlated with mercury blood levels in the fetus (68). Ionic mercury neither enters the brain nor penetrates the fetus.

One source of concern about coexposure arises from morphological findings in primates exposed to inhaled mercury vapor (summarized in 69). Prenatal exposure resulted in brain growth retardation, sulcal abnormalities and increases in the number of heterotopic neurons in the cerebrum. In particular, the subpially localized heterotopic neurons with their disoriented apical dendrites indicated arrested cell migration. Similar changes of subpially localized cells have been noted in humans after exposure to methylmercury (17).

Consequently, both empirical and mechanistic reasons suggest that combined exposure to methylmercury and inhaled mercury vapor might together produce effects more severe than those seen from exposure to either agent alone. These are based on the following considerations: (1) both agents produce prenatal damage, (2) both produce behavioral changes in animals at levels that are not overtly toxic, (3) arrested neuronal migration is detectable after both agents, and (4) both agents result in the release in the brain of the same toxic species of mercury, namely, mercuric mercury.

Further, as with methylmercury, infants and children seem significantly more vulnerable than adults to mercury vapor toxicity. The syndrome of acrodynia (“painful limbs”), also known as “Pink Disease” because of the erythema and prolonged episodes of irritability and crying, began to make its appearance in medical journals early in this century (70). Its etiology was ascribed mainly to infectious diseases. Only in 1947 was it discovered to be a manifestation of mercury toxicity, when Warkany and Hubbard (71), at the University of Cincinnati, detected high levels of mercury in the urine of children with Pink Disease. The sources consisted of teething powders, which contained calomel (mercurous chloride), and some anti-helminthics. When calomel was withdrawn from teething powders in England, Pink Disease reports fell sharply except for instances probably arising from other exposure sources. Although inorganic mercury compounds were responsible for the early outbreaks, almost all of the contemporary reports of Pink Disease in the medical literature point to mercury vapor as the exposure source (72).

Despite the fact that inhaled mercury vapor distributes to the fetal brain in humans (63) and monkeys (73), no investigations of subclinical developmental changes in children, like those conducted for in utero exposure to lead and methylmercury (74), have been published.

A study of pregnant women in Sweden (75) found that 28% of the mercury in blood during early pregnancy proved to be inorganic while the rest was comprised of methylmercury. The inorganic component was mostly contributed by dental amalgams. And, as noted earlier, Bjornberg et al. (64), also showed a close relationship between number of maternal amalgams and inorganic mercury in cord blood, but a lower proportion of total mercury, perhaps because of changes in dental care. These values
should be viewed against the figures derived from the 1999–2000 NHANES survey, which indicated that about 8% of women had blood mercury concentrations higher than the U.S. EPA recommended RfD of 5.8 μg/L (76), which assumes that almost all the mercury in blood is contributed by the methyl form.

In a more recent assessment, Mahaffey et al. (32), also based on the 1999–2000 NHANES survey, used measures of both total and inorganic mercury in blood to calculate the organic (methyl) component. They noted that, as the organic proportion rose with an increasing number of fish meals, the inorganic proportion dwindled, but that is to be expected if the vapor exposure remains fairly constant. This argument is not a valid dismissal of the potential contribution of mercury vapor to mercury-associated developmental neurotoxicity. In fact, it could be argued that, as organic mercury intake increases, the potential neurotoxicity of the vapor component becomes increasingly important. In fact, if animal data provide any guidance, the combined exposure may even be synergistic (77).

One complication arising from these possibilities is that measures of cord blood mercury often measure only total mercury (27,30,78). Although total mercury is closely related to dietary intake of methylmercury, it is conceivable that the inorganic component may itself be correlated with later neuropsychological measures. Hair levels of mercury do not reflect vapor exposure, so that for most purposes it may be the preferred index.

Speciation is also crucial for measuring transfer of mercury to the infant from breast milk. Oskarsson et al. (79) measured mercury concentrations in breast milk, blood, and hair samples, collected six weeks after delivery, from 30 Swedish women. Milk samples showed an average of 51% of total mercury in the form of inorganic mercury. In blood, similar to the findings of Vahter et al. (75), an average of only 26% was present in the inorganic form. Both total and inorganic mercury concentrations in blood and milk were significantly correlated with the number of amalgam restorations. Estimated methylmercury intake from fish and concentrations in breast milk were not related. The potential confounding of different mercury species both in the measurement of endpoints and in biomarker assays warrants precautions such as measuring mercury in maternal and infant urine; urinary mercury provides an index of vapor exposure and is correlated with the number of amalgam restorations.

**DELAYED TOXICITY**

Latent, or delayed, toxicity applies to the phenomenon in which detectable expressions of adverse effects are displaced in time from an acute exposure or after exposure has ceased (80). It represents the core issue, not just for methylmercury, but for all neurodevelopmental toxicants. Cognitive difficulties, retarded language acquisition, and attention deficit disorders, for example, are identified only at a stage of maturation when the organism is expected to be functioning appropriately for that stage. The main challenge for developmental neurotoxicology is how to connect such manifestations to exposures occurring earlier in life.

One impediment to securing such an anchor is the documented but rather limited plasticity of the developing brain. The prevailing doctrine used to be that young brains can recover even from the type of severe damage that disables adult brains. One source of this belief was the experiments of Margaret Kennard, who, in the 1930s and 1940s, compared the effects of brain lesions in infant monkeys with presumably similar lesions in adult monkeys and found far less functional disturbance in the infants than in the adults.
Examined more carefully, however, the doctrine lacked sound support. Kennard, in fact, later reported that deficits in motor function not observed during infancy began to emerge as the lesioned monkeys matured (81). Similar findings were reported after pyramidal tract lesions. In rat studies, aberrant social behaviors in rats subjected to amygdala lesions during infancy appeared only when they reached puberty.

The classical experiments of Goldman (82) provided compelling evidence for the phenomenon that has been called, “growing into” the lesion. Monkeys subjected to removal of dorsolateral cortex when infants did not differ from controls on a delayed alternation task at 15 months of age. At 2 years of age, the lesioned monkeys committed significantly more errors than the control monkeys even though they had practiced the task 9 mo earlier.

One way to account for such observations is to presume that the behavioral deficits appear only at the time when the damaged region would typically begin to subserve the function being tested. Another way to interpret the phenomenon is to view it as the structure becoming “committed” to a specific range of functions as the organism matures.

Prenatal exposure to relatively modest MeHg concentrations (e.g., <10 ppm in maternal hair) may influence only those higher order cognitive functions that develop with maturity. As a result, deficits may not begin to appear until the brain has developed to a stage at which it has “grown into” the lesions caused by earlier exposure. Johnson and Almli (83) describe the phenomenon as follows: “One of the most perplexing results found when brain damage is sustained during infancy is that many behavioral deficits do not become manifest until after considerable time has elapsed…Goldman…has suggested that delayed effects following brain damage sustained at an early age may be related to the degree of functional maturity of the brain region-behavior relationship under study…With maturation, as that brain region would normally become committed to the behavior in question, deficits in behavior of the brain-damaged animal become manifest.” The temporal patterning sequence seen by Gogtay et al. (84) in their MRI mapping study of cortical development reveals that higher-order association cortices mature only after lower-order somatosensory and visual cortices, the functions of which they integrate, are developed. Changes are taking place even into the late teens, so that earlier damage might not emerge, if the “growing into the lesion” phenomenon holds in this instance, until that stage and with tests based on complex cognitive function.

The experimental data are supported by observations in humans. Lenneberg (85), on the basis of his experience with brain-injured children offered the following comment: “One may say that the child with a perinatal cerebral injury only gradually ‘grows into his symptoms,’ and that both lesions and symptoms have their own ramified consequences, often affecting distant structures years after the primary injury.”

These venerable lesion studies are reviewed here to illustrate the principle that the brain is a dynamic entity and that it changes through the lifespan in often dramatic ways that are reflected in behavior. This principle needs to guide why the conclusions we have reached on the basis of our current information about methylmercury should also remain in a dynamic state, so to speak. Our appreciation of how the consequences of exposure may vary through the lifespan remains fairly primitive.

Two animal studies are illuminating examples of this principle. Spyker (15) administered 4 or 8 mg/kg methylmercury to pregnant mice on gestational day 7, 9, or 12. She then maintained them for a lifetime. Most of the offspring displayed no overt signs of toxicity at weaning. At about 30 days of age, the treated offspring showed deviant behavior in open-field testing and in swimming behavior. As they matured, and especially as they approached senescence, they began to exhibit a multitude of disabilities: obesity,
kyphosis, muscular atrophy, difficulty in righting themselves, and an assortment of less obvious behavioral deficits. They also died sooner than control mice.

Two studies in rats, Newland and Rasmussen (86) and Newland et al. (87), also demonstrated interactions with aging following developmental exposure to methylmercury. As they aged, the treated subjects began to display deviations from control performance on complex behavioral tasks. These were not grossly aberrant performances; the deficits occurred earlier during senescence (86), or were expressed as slower acquisition (87).

Evidence from nonhuman primates also indicates that effects of low exposures to MeHg during postnatal development may not emerge until later in life. Such delayed neurotoxicity, appearing years after cessation of exposure, was suggested in reports from Minamata, and the publications cited above provide further evidence for such a phenomenon. These primate studies provide even more impressive evidence for delayed toxicity. In one study, Rice (88) exposed one group of monkeys to methylmercury beginning during gestation and continuing until 4 years of age. In tests of auditory function conducted at 11 and 19 years of age, the monkeys exhibited deficits in pure-tone thresholds, especially at the later age. Another group of monkeys received low daily doses of methylmercury from birth to 7 years of age. After a six-years hiatus, at 13 years of age, they began to display mild signs of somatosensory dysfunction in their home cages (40) consisting mainly of impaired dexterity and clumsiness in handling items of food. These observations were validated by tests of vibration sensitivity (89). The monkeys dosed during gestation and until 4 years of age also showed somatosensory deficits (90).

Figure 3 depicts the situation graphically and compares it to the Minamata outbreak. There, latency periods as long as 15 years have been reported and described as “Minamata disease of late onset” (91).

![Delayed onset in primates](image)

Delayed onset in primates*

- Start dosing
- 50 µg Hg/kg/day
- End dosing
- Onset effects
- Years of age

Late onset of Minamata cases**

- End of acute Minamata outbreak
- Reports of “new cases”
- 1960 - 1975

Figure 3  The late onset of methylmercury poisoning in nonhuman primates and in humans after exposure to methylmercury in Minamata.*Based on Rice (1996) in primates and **Igata (1991) in humans.
These primate experiments, although revealing sensory and motor deficits resulting from developmental exposures, failed to demonstrate the kind of cognitive performance deficits assayed by certain forms of schedule-controlled operant behavior (92,93). Newland et al. (94), relying on another form of operant behavior, did demonstrate deficits associated with prenatal exposure. In this situation, the exposed animals showed retarded adaptation to shifts in reinforcement contingencies. Different monkey species as well as different dosing schedules may underlie the different findings.

POSTNATAL EXPOSURES IN HUMANS

The primary question addressed by human developmental studies up to now is whether prenatal exposure to methylmercury is associated with adverse effects on behavioral function during childhood. The long-term impact of postnatal dietary exposure has largely been overlooked. Exposure doesn’t cease at birth, however. In populations in which fish comprises a significant part of the diet, it is consumed over a lifetime. We remain largely ignorant of the joint contribution to neurobehavioral outcomes of prenatal and postnatal exposure. The two largest epidemiological studies, one in the Faroe Islands (34), the other in the Seychelles (33), have provided data on pre-adolescent ages. A recent reanalysis of the Seychelles data (95) suggests the possibility that postnatal exposure may incur subtle adverse effects that only become apparent as children approach adolescence. Nonlinear multiple regression analyses of scores on the Child Behavior Checklist at 107 months of age showed a significant adverse association, on the Thought Problems subscale, with contemporary hair levels of methylmercury. Figure 4 shows a partial residual plot for depicting the association (p = 0.002). No effects are seen below approximately 8 ppm, but there is an increasing trend (adverse effect) above that level.

![Figure 4](image_url)

**Figure 4** Plot showing the relationship between scores on the Thought Problems subscale of the Child Behavior Checklist and postnatal methylmercury exposure as measured by hair levels at 107 months of age. The analysis was based on the application of semiparametric additive models. Above about 10 ppm the data points are fewer and the confidence intervals widen. The rug plot (vertical marks) along the bottom illustrates the distribution of postnatal exposure in this cohort. **Source:** From Ref. 95.
These results, indicating effects of postnatal exposure, to some extent parallel those of Murata et al. (96) with the Faroe Islands cohort on brainstem auditory evoked responses at 14 years of age. When tested at age 7 years, (34,97), the cohort exhibited delayed latencies associated with elevated prenatal exposures based on cord blood. The newer data, based on age 14 years, showed increased delays associated, as well, with current exposures as measured by the children’s hair levels of mercury.

Subtle adverse consequences emerging during later childhood and adolescence, as also seen in experimental animals, should come as no surprise. Adolescence, like the perinatal period, is a tumultuous stage of development during which profound changes are occurring in brain function (84). Adams and colleagues (98) noted that “in addition to the dramatic neuroendocrinological and physical changes associated with adolescence…it is clear that the brain of the adolescent also undergoes striking transformations…Several brain regions undergo prominent remodeling…These regions include the prefrontal cortex…These data suggest that the adolescent period of brain development should be a time of particular vulnerability to insult…However, there has been surprisingly little investigation in either humans or animals of the vulnerability of the adolescent brain to developmental perturbation. Moreover, adverse impacts on neurocognitive and behavioral functions during such periods are likely to have protracted consequences for latter success.” This question deserves to be urgently pursued in methylmercury research.

These recent data are especially relevant because, in fish-consuming populations, prenatal and postnatal exposures are entwined. However, prenatal and postnatal exposures seem to result in different patterns of brain damage, and, as a result, tend to produce different phenotypes. Prenatal exposure disrupts cell migration and differentiation throughout the developing brain in mice (19) and in humans (20). Such extensive damage would be expected to induce widespread impairment manifested as deficits in cognitive function, sensory function, motor function, information processing and language, memory, attention, and social communication. The syndrome of what Japanese investigators labeled Congenital Minamata Disease, which was marked overtly by neurological signs, included such global effects.

In contrast, postnatal exposure, at least in adults, appears to be predominantly focal with most damage centered in areas deep in the sulci such as the calcarine fissure and the folds of the cerebellum in both nonhuman primates (99) and humans (9). It is this pattern of damage that accounts for the signs and symptoms associated with methylmercury poisoning (Table 1) before the sensitivity of the developing brain was appreciated.

**Aging**

The emphasis on early development has tended to obscure a larger issue: the consequences of lifetime exposure. The overwhelmingly predominant source of methylmercury is fish and other aquatic species. Fish consumption takes place through a lifetime and, during advanced age, the brain, as seen in animal studies, may begin to reflect the consequences of cumulative exposure. In fact, in its investigation of the catastrophe at Minamata, Japan, the Kumamoto University committee made the following statement (100):

“…the problem about the relationship between a small amount of methylmercury pollution for a long period and its accumulation in the brain still remains obscure…Subclinical Minamata disease was sometimes revealed by detectable symptoms during the aging process…The subclinical Minamata disease…could be called a delayed type of Minamata disease in aged people.”

A more quantitative assessment of this finding can be found in Kinjo et al. (101). They compared over one thousand Minamata disease patients aged 40 or over with
controls matched by age and sex. A questionnaire interview surveyed subjective complaints and activities of daily living (ADL). The patients had significantly higher rates of all complaints than the controls, particularly in the categories of sensory disturbances such as paresthesias and motor difficulties such as tremor and weakness. Differences between patients and controls increased with age, and ADL disability in MD patients was aggravated by aging.

The observations in Minamata emphasize how crucial it is, to more fully understand the risks stemming from developmental exposures, to study outcomes not only during the relatively early phases of the life cycle, but throughout the lifetime (87,102). The risks are ambiguous because early, low-level exposures may produce undetected, latent (103), or “silent” damage (104,105) that will be manifested only when functional capacities are challenged by other conditions, such as aging (106,107), drugs (108,109), or complex behavioral situations (86,87).

The effects ascribed to aging underscore the principle that neurotoxicity may be manifested differently at different stages of the life cycle (88,92). Certainly, older humans handle drugs and toxicants differently from younger adults who handle them differently from children and neonates. Furthermore, the age question deserves a greater emphasis than it has received in the past. It is important to ask how such exposures might affect “aging” processes themselves (110). The pioneering experiments of Spyker (15) and Spyker et al. (111), the primate studies of Rice (90,92), and the aging models offered by Weiss and Simon (107) and Weiss et al. (106) show how these issues become closely intertwined when addressing the long-term consequences of exposures to toxic agents during early development.

UNRESOLVED ISSUES

Despite the flood of research unleashed by the disasters in Minamata and Iraq, perplexing questions about the risks to neurobehavioral development posed by methylmercury persist. One stimulus for such questions is the source of exposure. Fish is an essential part of the diet for many populations, so exposure is inescapable. Even in populations where other protein sources are readily available, such as ours, fish remains an important food. Whatever risks are associated with fish consumption need to be weighed against its nutritional properties, some of which were discussed earlier. Moreover, methylmercury is hardly the only contaminant to be considered in evaluating the potential risks of fish consumption. Our grasp of how such risks combine is relatively primitive (56).

Second, we must recognize that the selection of endpoints for assessing methylmercury neurotoxicity reveals incompatibilities between epidemiological and laboratory research. The human studies, in part because of their reliance on standardized tests—perhaps inescapably—have featured cognitive function. Animal studies indicate deficits in motor and sensory function to be the clearest outcomes of exposure, in parallel with poisonings in adult humans. The appropriate endpoints need to be pursued more intensively in developmentally-exposed humans.

Finally, the issue that has been least adequately addressed is how to formulate risks based on lifetime exposure. We are especially deficient in our understanding of the combined effects of prenatal and postnatal exposure. Development is a continuous process, although sometimes treated as though it is separated into a series of individual journeys. Delayed or latent effects, and the contribution of aging, with its accompanying decline in compensatory processes, have not been incorporated into the risk equation.
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