Evolution of Our Understanding of Methylmercury as a Health Threat

Chiho Watanabe and Hiroshi Satoh
Department of Environmental Health Sciences, Tohoku University School of Medicine, Sendai, Japan

Methylmercury (MeHg) is recognized as one of the most hazardous environmental pollutants, primarily due to endemic disasters that have occurred repeatedly. A review of the earlier literature on the Minamata outbreak shows how large-scale poisoning occurred and why it could not be prevented. With the repeated occurrences of MeHg poisoning, it gradually became clear that the fetus is much more susceptible to the toxicity of this compound than the adult. Thus, recent epidemiologic studies in several fish-eating populations have focused on the effects of in utero exposure to MeHg. Also, there have been many studies on neurobehavioral effects of in utero exposure to methylmercury in rodents and nonhuman primates. The results of these studies revealed that the effects encompass a wide range of behavioral categories without clear identification of the functional categories distinctly susceptible to MeHg. The overall neurotoxicity of MeHg in humans, nonhuman primates, and rodents appears to have similarities. However, several gaps exist between the human and animal studies. By using the large body of neurotoxicologic data obtained in human populations and filling in such gaps, we can use MeHg as a model agent for developing a specific battery of tests of animal behavior to predict human risks resulting from in utero exposure to other chemicals with unknown neurotoxicity. Approaches developing such a battery are also discussed. — Environ Health Perspect 104(Suppl 2):367–379 (1996)

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Introduction

Methylmercury is recognized as one of the most hazardous environmental pollutants, largely due to endemic disasters such as Minamata disease in Japan and methylmercury poisoning in Iraq, as well as industrial accidents involving methylmercury compounds. A failure to acknowledge and prevent the disease, however, has caused the disasters to be unnecessarily repeated. For example, the second outbreak of Minamata disease (or Niigata Minamata disease) took place in Niigata, Japan, after the outbreak in Minamata. In Iraq, methylmercury poisonings repeatedly occurred from the distribution of wheat seeds drenched with methylmercury.

This paper is divided into three parts. The first part, an extract from the earlier literature, describes how the toxicity of methylmercury or organic mercury was found and recognized. Because methylmercury brought about tragedies many times in different ways, it is important to trace how the tragedies occurred and why they were not prevented. Thus, the early history of poisonings by industrial organic mercury compounds is reviewed and the early stages of the outbreak of Minamata disease are described.

In the second part, experimental studies are reviewed with an emphasis on investigations of behavioral teratology. Methylmercury has been considered an environmental health threat to the nervous systems of developing fetuses since the exploratory work of Spyker et al. in 1972 (1).

In the final part, we present a comparison of human and animal data with regard to behavioral effects of in utero exposure to methylmercury.

Identification of Methylmercury Toxicity

Methylmercury Recognized as an Industrial Toxicant

Organic mercury compounds including methylmercury have been commercially produced since 1930. The use of organic mercury compounds in chemical research, however, goes back to 1863. Organic mercury was first identified as a health hazard in 1866 when two laboratory technicians were poisoned with dimethylmercury (2). A 30-year-old male who had been exposed to dimethylmercury for 3 months "complained of numbness of the hands, deafness, poor vision and sore gums...[He was] unable to stand without support," although no motor palsy was detected. His condition rapidly worsened; he became restless and comatose within a week and died 2 weeks after the onset of symptoms. Another victim was a 23-year-old laboratory technician who had been working in the laboratory for 12 months, although he had handled dimethylmercury for only 2 weeks. He complained of sore gums, salivation, numbness of the feet, hands and tongue, deafness and diminsh of vision. He answered questions only very slowly and with indistinct speech...Three weeks later he had difficulty in swallowing and was unable to speak...[He was] often restless and violent. He remained in a confused state and died of pneumonia 12 months after the onset of symptoms (2).

Most of the signs and symptoms described above resemble those observed in (acute) Minamata disease. Sore gums and salivation were, however, symptoms observed in mercury vapor poisoning. Since dimethylmercury can easily be broken down to produce metallic mercury, it is considered that these symptoms were due to co-existing metallic mercury.

The therapeutic use of diethylmercury against syphilis was tried in Germany in 1887 but was readily abandoned because of extremely high toxicity. Animal experiments showed involvement of the nervous
system. “Incoordination was noticed, especially in rabbits, and motor paralysis was observed in dogs and cats. Tremors, blindness, loss of the sense of smell, deafness, and attacks of wrath on the slightest provocation were observed in many of the dogs” (2). The toxicity of alkylmercury compounds had therefore already been recognized in the 19th century.

Although there had been accidental cases of mercury poisoning and related findings in experimental studies as mentioned above, organic mercury compounds such as aryl and alkyl derivatives continued to be used for seed dressing. “In 1940, Hunter reported four cases of methylmercury poisoning in a factory where functional dusts were manufactured without an enclosed apparatus...[The symptoms were] severe generalized ataxia, dysarthria and constriction of the visual field” (2). The characteristic symptoms of mercury vapor poisoning, with the exception of tremors, were not observed. One of the victims suffered from symptoms (mainly ataxia) for 15 years after exposure had ceased.

“At [patient] necropsy, generalized ataxia was referable to cerebellar cortical atrophy, selectively involving the granule-cell layer of the neocerebellum. The concentric constriction of the visual fields was correlated with bilateral cortical atrophy around the calcarine fissures” (2). This was originally reported in 1954 and later methylmercury poisoning was referred to as Hunter-Russell syndrome. The emergence of a methylmercury poisoning epidemic, Minamata disease, coincided with these years.

Thereafter, cases of organic mercury poisoning were reported in the United Kingdom, the United States, Canada, and Sweden. Since organic mercury compounds were used mainly for seed dressing, most victims were workers in chemical manufacturing plants and farmers and members of their families who accidentally ingested dressed seeds.

From these cases of accidental human exposure, the health hazard of methylmercury and other organic mercury derivatives had been well recognized by the 1950s. Despite this awareness, however, Minamata disease occurred in the same decade, and later methylmercury poisoning surfaced in Iraq.

Minamata Disease

Minamata disease was defined as methylmercury poisoning that occurred among the people living along Minamata Bay in Kyushu, Japan (3). The way in which the victims became exposed to methylmercury was uncommon; they consumed substantial amounts of contaminated fish and shellfish. The source of methylmercury was effluent from a chemical company where mercury was used as a catalyst to produce acetaldehyde. Although methylmercury concentration in the seawater was not high, it was concentrated as it ascended the food chain and thus was in the fish and shellfish that were the staple diet of the villagers. The concentrations of methylmercury in the fish were high enough to cause methylmercury poisoning. Minamata disease is evidently unique in its origin as it involved the bay’s ecosystem.

Minamata disease was first officially reported on 1 May 1956 to the public health authority of Minamata, Kumamoto prefecture (4). During the preceding 10 days, Dr. Hosokawa, the head of the hospital that was affiliated with Chisso (the responsible company), and his colleague experienced two infantile cases of an unknown disease that resulted in death. Since the two infants were sisters and so severe a disease occurred in one family at the same time, the doctors felt that the situation required serious attention and reported it to the public health authority. Moreover, before these two infantile cases, they had dealt with sporadic occurrences of a similar disease (5).

Abnormal gait, dysarthria, ataxia, deafness, and the constriction of the visual field were the main symptoms (6). It was also common to find emotional lability in the form of euphoria or depression. Serious cases displayed states of mental confusion, drowsiness, and stupor. Sometimes, however, the victims were restless and prone to shouting, which often led into coma.

After Dr. Hosokawa’s official report, a committee to study this serious disease consisting of representatives from Minamata City, the affiliated hospital, the municipal hospital, and the Minamata Medical Association was formed. This was called the Kibyuu Taisaku Committee, which literally translates as the antimysterious disease committee. The committee found 30 cases within several months. The epidemic’s first case was reported in December 1953. Then 10 and 11 more patients were confirmed whose onsets began in 1954 and 1955, respectively (Figure 1). The prognosis was poor, and more than 30% of the cases were fatal.

In August 1956, Kumamoto University School of Medicine was asked to join the committee and the Study Group of Kumamoto University was organized. The initial epidemiologic study revealed an entire range of characteristics related to the “mysterious disease” (7). The disease occurred regardless of age. Although family clustering was observed, there was no proof of infectious transfer. Most of the population used wells, but wells were not involved. Ninety percent of the victims’ households were related to fishery, whereas less than 30% were in the control group formed from the households in the neighborhood. More than two thirds of the households consumed fish caught in the bay every day in substantial amounts (sometimes several hundred grams and even up to 1 kg per person in one meal); among the control group, only 6% ate local fish daily and in lesser amounts. The death rate of domestic cats in the victims’ households was also higher than that of the controls; during the period of 1953 to 1956, 50 cats out of 61 died in the victims’ houses whereas only 24 out of 60 died in the control houses.

These epidemiologic findings clearly indicated that substantial fish consumption was the cause of the mysterious disease. These findings also demonstrated that a toxic agent, not a biologic one, in fish was responsible. The study group suggested a ban on the catching and selling of fish from the bay although the local authorities were opposed to this policy.

Having occurred by a unique route of exposure, various extraordinary phenomena and ecological changes preceded the outbreak of Minamata disease; floating dead fish and empty shellfish had been observed a few years before. In one area of the bay, observations of floating fish date back to 1949 (4). Crows were also affected. Cats that were housed in the villagers’ homes showed symptoms similar to those manifested in human victims; the cats showed ataxic gait, slowness, and unsteady
movement. Sometimes they dashed around in a circle and ran hysterically, the latter causing some of them to jump into the sea and drown. There was no evidence that these events had been seriously recognized by the local authorities. Witnesses were later collected in the epidemiologic studies conducted by the study group of Kumamoto University School of Medicine.

Early pathologic examinations (6) of victims suggested that the disease was encephalopathy toxica (toxic encephalopathy). Similar pathologic changes were found in affected cats, birds, and even fish. It is noteworthy that characteristics of the disease were revealed and the study group concluded by the end of 1956 that an unidentified toxic agent in fish was responsible for the disease.

A long period remained, however, before the causal agents could be specified. First, manganese was suspected in November 1956, followed by selenium in April 1957 and thallium in 1958; however, no link between these agents and the disease could be found in feeding experiments. Moreover, clinical and pathologic findings did not support any of these substances as the causal agent (6).

Finally in 1957, organic mercury was first suspected. In pathologic examinations of four cases, lesions in the granular cell layers of the cerebellum were noted (6). Professor Takeuchi, at the Department of Pathology in Kumamoto University consulted pathologic textbooks and found that carbon monoxide and mercury poisonings caused such lesions. A volume that followed the pathologic texts was published in 1958, and a chapter of this volume introduced the study by Hunter and Russell (8). The pathological changes described in the book resembled the findings in the cases of Minamata disease. Since the study group was unable to chemically analyze mercury at that time, Professor Takeuchi and his colleagues tried and succeeded in identifying mercury histologically.

Clinical observations of constriction of the visual field and ataxia also indicated organic mercury poisoning (9). Professor Tokuomi of the Department of Internal Medicine came across a clinical toxicology book (10) in April 1957 that classified symptoms and listed possible agents. Under the item "ataxia," alkylmercury was named with other agents such as atropine, barbiturates, etc. Alkylmercury was also listed as an agent under "restriction of visual fields." At this point, he almost determined the toxic agent responsible, but the conventional wisdom of chemistry did not support this idea; because mercury, especially alkylmercury, was expensive, it did not seem logical that such materials would be discharged. Moreover, it was not understood how alkylmercury could have been synthesized. Thus, Professor Tokuomi abandoned the idea that alkylmercury was a possible agent.

Later, when Professor Takeuchi almost concluded that alkylmercury was the causal agent, Professor Tokuomi once again strongly suspected alkylmercury and decided to reexamine the patients. In addition to making clinical observations, he noted an increase in urinary excretion of mercury, while the administration of British antilewisite (BAL) further revealed increased excretion of mercury in urine (9).

After the establishment of a chemical analysis for mercury, environmental investigations directed by Professor Kitamura of the Department of Public Health also showed elevated mercury concentrations in sediments near the factory waste-water outlet (7). Moreover, organ samples from the victims and affected cats contained high concentrations of mercury.

These results were reported by the Study Group of Kumamoto University in July 1959. Their conclusion was that "Minamata disease occurred by eating contaminated fish and shellfish and organic mercury is most suspected as the causal agent" (6). Though their findings seemed conclusive, arguments about the causal agent continued for several years, partly because the indicated causal agent linked legal responsibility to the company. In addition, the methylmercury synthesis mechanism was not clarified until 1964.

A careful review of the literature resulted in the discovery of a paper published in 1930 that described a type of mercury poisoning different from typical (metallic) mercury vapor poisoning (11). The different type of mercury poisoning was observed among workers in acetaldehyde plants who handled mercury-containing sludge. They did not have stomatitis, which is commonly observed in mercury vapor poisoning. The author had even suspected that mercury in its organic form could have been the cause of the poisoning. Therefore, this different type of mercury poisoning had already been described before the observation of Hunter (12). Moreover, the formation of organic mercury as a by-product in the production of acetaldehyde using mercury was also suspected. Somehow, this literature was not found by the study group.

It took a long time to reach the conclusion that the organic mercury ingested by fish and shellfish was the cause of Minamata disease. However, it was rapidly concluded from the epidemiologic study that the disease was caused by an unidentified toxic agent and that fish and shellfish were involved. Here lie the strength and limits of epidemiology: it is not difficult to recognize a risk factor but it is difficult to specify the causal agent. Considering the state of analytical chemistry at that time, it was more difficult to identify the toxic agent than it is now. It is regrettable that the local authorities did not prohibit fishing in the bay in the early stages of the epidemic of Minamata disease. The conclusion to be drawn after reviewing the events that occurred at the onset of Minamata disease is that epidemiology is able to provide enough evidence to prevent the spread of an unknown disease, even though the specific agent involved has not been determined.

Fetal Minamata Disease

Fetal Minamata disease was first detected in 1958 by Professor Kitamura and his colleagues in the Minamata Bay area (13). They found nine infants who manifested a severe disease resembling cerebral palsy during their epidemiologic investigation. The incidence of the cerebral-palsy-like disease was extremely high among infants who were born in and after 1955. Of 188 births in the area during 1955 to 1958, 13 cases were found. The incidence rate was calculated at 6.9%. Later, three more cases surfaced involving mental retardation and minimal neurologic symptoms. By 1974, 40 cases were confirmed as fetal Minamata disease.

Examination of these children revealed the following signs and symptoms in high incidence: mental retardation, cerebellar ataxia, primitive reflex, and dysarthria in all children (17/17), seizure in 82%, and pyramidal signs in 75%. Sensory disturbance, constriction of the visual fields, and hearing impairment could not be examined because of the serious conditions of the patients.

It was a tradition in Japan to preserve a part of the umbilical cord that remained on a baby after birth which later fell off. Methylmercury concentrations in the cords of the victims were high, and exposure to mercury was thus confirmed.

The mothers of these children had seemed healthy at the time their children were confirmed to have fetal Minamata disease. However, 11 mothers out of 15
showed slight symptoms of Minamata disease in 1962. Later the mothers developed further symptoms, and in 1974, 57% of these mothers experienced constriction of the visual field, one of the typical symptoms of Minamata disease.

From the experience in Minamata, birth control (actually abortion) was advised by the local government to women of childbearing age who lived in the polluted area and who had mercury concentrations of 50 ppm or higher. Only one case of fetal Minamata disease was confirmed in Niigata by 1974 (4).

Methylmercury Poisoning in Iraq

Since organic mercury compounds have been used as seed dressings, poisoning by eating dressed seeds (mainly wheat) have occurred repeatedly (14). In Iraq, three epidemic poisonings were reported: one in 1955 to 1956, another in 1959 to 1960, and the third and largest outbreak in 1971 to 1972 (15). These outbreaks were caused by the distribution of seed grain treated with alkylmercury compounds. Rural people consumed the grain to make homemade bread. The total number of official victims was 6530 including 459 deaths. Symptoms were paresthesia or malaise followed by ataxia, visual field constriction, and hearing impairment.

In the investigation of the tragedy, dose–effect and dose–response relationships were established. Since there was possibly a background incidence, a hockey-stick model, which is composed of a horizontal line and a sloped line, fitted well. In addition, a relationship between mercury concentrations in the hair and blood was also established. Since mercury concentration in hair strands recapitulates the history of methylmercury exposure, analysis of hair mercury provided abundant information about the course of exposure.

Fetal Exposure to Methylmercury in Iraq

In the Iraqi outbreak (15–17), babies with in utero exposure to methylmercury were investigated for physical and mental development. The mothers were interviewed as well. Exposure was estimated by the peak mercury concentration in a single hair strand from each mother.

A scoring system of examination results was adopted in the investigation. Although individual scores exhibited variability, a dose–response relationship was found. Statistical analysis suggested greater effects in boys than in girls.

The data were statistically analyzed in detail to establish a dose–response relationship between the effect and the hair mercury concentration (18). Both logit and hockey-stick models were fitted to the data. From these analyses, the estimated lowest effect level (ELEL) was proposed as a threshold for human populations.

Recent Epidemiological Studies

Since fish-eating populations are exposed to the threat of methylmercury, effects of in utero exposure to methylmercury have been studied (Table 1). In New Zealand, a group with high fish consumption (more than 3 times per week during pregnancy) was identified and the risk of in utero methylmercury exposure was evaluated (23). When the children were 4 years of age, they were tested with the Denver Developmental Screening Test. Children born to mothers with hair mercury levels higher than 6 ppm had a higher prevalence of abnormal results. More comprehensive examinations were given at 6 years of age. At this age, children with

| Study                  | Functions/domains examined                                                                 | References |
|------------------------|-------------------------------------------------------------------------------------------|------------|
| New Zealand            |                                                                                           | (19)       |
| 1983 Survey subjects: 4 years old; n=31 matched pairs (mean pregnancy maternal hair Hg of the exposed group > 6 mg/kg) | Gross motor, fine motor, language, personal-social functions with DDST, abnormal or questionable score increased |
|                        |                                                                                           |            |
| 1985 Survey subjects: 6 years old; n=46 matched pairs (hair Hg > 10 mg/kg n=15; 6-10 mg/kg n=31) | Visual discrimination Sensory tests (touch, tactile, thermosensitivity) Poorer performance in WISC-R or in Test of Language Development |
| Canada                 |                                                                                           | (20)       |
|Subjects: 12–30 months old, n=234 (maximum gestational hair Hg; mean = 6 mg/kg) | Neurologic examinations Abnormal tendon reflex (only in boys) DDST——no effect. |
| Faroe Islands          |                                                                                           | (21)       |
|Subjects: 7 years old (Pilot study showed maternal hair Hg median 4.5 mg/kg; n=1023) | Vigilance-attention (frontal lobes)* Manual motor coordination (cerebral motor system) Mood Tactile processing and memory (parietal lobes) (Bender Gestalt test) Nonspecific brain damage Short-term memory (left temporal lobe) Attention and tracking (frontal lobes) WISC-R Block Designs (cortically right frontal and parietal; subcortically basal ganglia and white matter) Naming (left temporal) Reasoning and cognitive flexibility (frontal lobe) |
| Seychelles Islands     |                                                                                           | (22)       |
|Subjects: 6, 18, 26, and 66 months old | Global-cognitive (human, monkey)* Visual-perceptual (monkey) Speech-language (human) Visual memory (monkey) Visual attention (monkey) Neuromotor/neurologic (human) Social–emotional (monkey) Learning-achievement (rat) |

Abbreviations: DDST, Denver Developmental Screening Test; WISC-R, Wechsler Intelligence Scale for Children-Revised. *Brain area whose function is associated with the performance of the behavioral task. *Data from the species provided the rationale for including the behavioral task.
methylmercury exposure performed worse than children with less exposure, but the variance explained by methylmercury exposure was small.

Currently possible neurobehavioral outcomes of prenatal methylmercury exposure are being evaluated in large-scale prospective studies on human fish-eating populations. In the Seychelles (22), children up to 5 years old are being studied in terms of development of cognitive functions and more specific effects. Test items were selected based on the preceding reports on behavioral consequences of prenatal low-level methylmercury exposure in human as well as in nonhuman primates. In the Faroe Islands (21), a cohort of 7-year-old children being studied. Test items were chosen so as to cover a wide variety of behaviors, but at the same time, maximize the specificity of the evaluated functions. Results of these studies are yet to come but are expected to reveal possible neurobehavioral consequences of perinatal methylmercury exposure.

**Table 2.** Effects of prenatal methyl mercury exposure on the development of reflexive behavior.

| Animal (strain) | Dose(s), mgHg/kg (route) | Period of administrations | Behaviors examined | Age(s) at examination, days | Findings | References |
|----------------|--------------------------|---------------------------|--------------------|-----------------------------|----------|-----------|
| Mice (JCL-ICR) | 0.4 or 4 daily (gi)      | GD 15 to weaning          | Reflexes, cliff avoidance | 4–21                        | No change | (24)      |
| Mice (CFW)     | 6.0 × 1 (sc)             | GD 9                      | Righting reflex and walking | 1, 3, and 8                 | Tendency of retardation but not statistically significant | (25)      |
| Rats (SD)      | 4.3 ×4 (sc)              | GD 0, 3, 7, and 11        | Observation of reflexes and behavior | Birth to 21, daily | No change | (26)      |
| Rats (Charles River) | 0.08, 0.4, or 2.0 × 10 (gi) | GD 6–15                  | Righting reflexes | Birth to 28, daily | No change | (27)      |
| Rats (Holtzman) | 2 ppm daily (diet)       | GD 0 throughout experimental period | Righting reflexes by dropping | 7–17 | Retardation | (28)      |
| Rats           | 0.2, or 6 × 4 (gavage)   | GD 6–9                    | Negative geotaxis | 7–10 | No change | (29)      |

Abbreviations: gi, gastric intubation; sc, subcutaneous injection; diet, food containing methylmercury compounds; GD, day(s) of gestation.

**Table 3.** Effects of prenatal methyl mercury exposure on swimming ability.

| Animal (strain) | Dose(s), mgHg/kg (route) | Period of administrations | Age(s) at examination, days | Findings | References |
|----------------|--------------------------|---------------------------|-----------------------------|----------|-----------|
| Mice (129/SvSi) | 5.4 × 1 (ip)             | GD 7 or 9                 | 31                          | Impairment | (1)      |
| Mice (JCL-ICR) | 0.4 or 4 daily (gi)      | GD 15 to weaning          | 7, 10, and 14               | Retardation in 10-day-old females | (24)      |
| Rats (SD)      | 4.3 ×4 (sc)              | GD 0, 3, 7, and 11        | 21                          | No change | (26)      |
| Rats (Holtzman) | 2 ppm daily (diet)       | GD 0 throughout experimental period | 7–17 | Retardation | (28)      |
| Rats           | 0.025, 0.05, 0.5, or 5.0 × 4 (gavage) | GD 6–9 | 14 | Impaired in 0.5 and 5.0 mg/kg groups | (30)      |

Abbreviations: ip, intraperitoneal injection; sc, subcutaneous injection; gi, gastric intubation; diet, food containing methylmercury compounds; GD, day(s) of gestation.

**Neurobehavioral Profile of Prenatal Methylmercury Toxicity in Experimental Animals**

Past experiences have shown that fetuses are much more vulnerable to methylmercury exposure than adults. In the Minamata disease epidemic and in the methylmercury poisoning in Iraq, infants were affected by in utero exposure. Methylmercury readily crosses the placental barrier and is transported to the developing nervous system. Embryos and fetuses have been considered much more susceptible to methylmercury than adults. In Minamata, mothers with minimal symptoms, such as numbness of the extremities and perioral region, gave birth to severely affected infants. Moreover, pathologic changes observed in the patients of fetal Minamata disease were much more destructive, presumably because the architecture of the nervous system in the fetuses was under development during the in utero exposure to methylmercury. In the Iraqi tragedies, perinatal exposure cases were observed. How in utero exposure to methylmercury affects postpartum life is of interest and importance in terms of susceptibility. In this section, therefore, the focus is on the neurobehavioral consequences of in utero exposure to methylmercury. Because most of the regulatory agencies required behavioral tests in rodents, rodent and primate studies are described separately.

**Studies in Rodents**

Since the pioneering study of Spyker et al. (1), a considerable number of investigations on the effects of in utero methylmercury exposure have been reported. Tables 2 through 11 summarize the results of these studies according to the test procedures employed and according to functional categories (as proposed by Rees et al. (49)). These tables are an expansion of the compilation by Shimai and Satoh (50).

**Motor Development and Functions (Tables 2 and 3).** The reflexes of rats were not affected except in the results of Olson.
and Bousch (28). In mice, no change or slight retardation was observed. The latter was partly counteracted by co-administration of selenium. Retardation of development of swimming ability was an important result shown by Spyker et al. (1) and was observed in most of the studies.

**Cognitive Functions (Tables 4–6).** Most investigations showed impairment in mice and in rats in learning a maze or water escape.

**Sensory Functions (Table 7).** Most of the behavioral studies in this functional domain have been done on visual functions of primates and a few have been done on rodents. Elsner (40) trained rats to press a lever with predetermined ranges of force and time. The impaired performance of methylmercury-exposed rats was considered to be a result of deficit in tactile-kinesic systems.

**Motivation and Arousal Behavior (Tables 8–10).** In mice, spontaneous activities were decreased; the results were inconsistent in rats. Selenium supplement partly counteracted the hypoactive effects of methylmercury (25). In the open-field tests, two investigations employing an identical strain of mice showed comparable results: longer latency, decreased urination, and increased backing. In rats, however, no change was observed, although increased locomotion was found when challenged with amphetamine. Increased susceptibility was observed in two studies although inducing methods were different.

**Social Functions (Table 11).** While three studies found slight or no effects on ultrasonic vocalization, Elsner et al. (48), using highly sophisticated devices, observed significant differences between the treated and control animals. Rats exposed to methylmercury were found to be more aggressive than vehicle control in dyadic encounters (51).

**Studies in Rodents: Methylmercury as a Model Agent**

In the last decade, several attempts have been made to evaluate various behavioral tests as end points of prenatal neurotoxic insults (29,30,52,53). Because methylmercury was known as a typical behavioral teratogen, it was included in these attempts as a model agent. Because various aspects of behavioral functions were examined in each of these studies, results of these studies will be compared in terms of the toxicity profile of methylmercury.

In the Collaborative Behavioral Teratology Study (CBTS) involving six laboratories (29), 0, 2, or 6 mg/kg body weight (bw) of methylmercury were given daily to pregnant rats at gestation days 6 to 9 (Tables 1,6,8,10). The offspring were evaluated with a test battery covering wide aspects of behavioral functions. Auditory startle habituation was most consistently affected among the laboratories. Vorhees (52) employed the same dose regimen used in the CBTS and evaluated their own test battery, the Cincinnati test battery, which emphasized reflex ontogeny. Generally, the result coincided with those of the CBTS. Vorhees (52) also recommended that

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Table 4. Effects of prenatal methylmercury exposure on avoidance learning.

| Animal (strain) | Doses (mgHg/kg (route)) | Period of administrations | Behaviors examined | Age(s) at examination, days | Findings | References |
|----------------|-------------------------|---------------------------|-------------------|-----------------------------|----------|------------|
| Mice (CFW)    | 5.0 x 1 (gi)            | GD 8                      | Passive avoidance | 56                          | Rapid extinction | (31)       |
| Mice (CFW)    | 2.5, or 5 x 1 (gi)      | GD 8                      | Two-way avoidance | 56                          | Impaired learning in 3 and 5 groups | (31)       |
| Mice (CFW)    | 4.6 or 13.5 x 1 (sc)    | GD 9                      | Taste aversion    | 24                          | Rapid extinction | (32)       |
| Mice (JCL-ICR)| 0.4 or 4 daily (gi)     | GD 15 to weaning          | Two-way avoidance | 224–252                     | Impaired learning in all groups | (24)       |
| Rats (SD)     | 8.0 x 1 (iv)            | GD 4                      | Lever press avoidance | 110–140                  | Impaired learning | (33)       |
| Rats (Long-Evans) | 4.0 or 6.4 x 1 (gi) | GD 8 or 15                | Two-way avoidance | 63                          | Impaired learning | (34)       |
| Rats          | 0.0, 0.025, 0.05, 0.5, or 5 x 4 (gavage) | GD 6–9                  | Passive avoidance | 90                          | No change | (30)       |
| Rats (SD)     | 6.4 x 1 (gi)            | GD 15                     | "Step-down" passive avoidance | 60                  | Rapid extinction | (35)       |

Abbreviations: iv, intravenous injection; sc, subcutaneous injection; gi, gastric intubation; GD, day(s) of gestation.

Table 5. Effects of prenatal methylmercury exposure on maze and water escape learning.

| Animal (strain) | Doses (mgHg/kg (route)) | Period of administrations | Apparatus or behaviors examined | Age(s) at examination, days | Findings | References |
|----------------|-------------------------|---------------------------|-------------------------------|-----------------------------|----------|------------|
| Mice (CFW)    | 1.2, 3, 5, or 10 x 1 (gi) | GD 8                      | Water escape                  | 56                          | No change | (31)       |
| Mice (JCL-ICR)| 0.4, or 4/day (gi)      | GD 15 to weaning          | Water T-maze                  | 42 and 140–147              | Shorter latency in 4 mgHg/kg group | (24)       |
| Rats (Holtzman) | 2.5 daily (drinking water) | GD 0 to weaning          | Water T-maze                  | 30                          | Impaired learning | (35)       |
| Rats (Holtzman) | 2 ppm daily (diet)     | GD 0 throughout experimental period | Symmetrical maze             | 60                          | Deficit in marlin meat-fed group | (28)       |

Abbreviations: gi, gastric intubation; diet, food containing methylmercury compounds; GD, day(s) of gestation.
Table 6. Effects of prenatal methylmercury exposure on operant learning.

| Animal (strain) | Dose(s), mgHg/kg (route) | Period of administrations | Behaviors examined | Age(s) at examination, days | Findings | References |
|-----------------|--------------------------|---------------------------|--------------------|-----------------------------|----------|------------|
| Mice (CFW)      | 5.0 x 1 (gi)             | GD 8                      | Lever press conditioned suppression | 56              | No change | (37)       |
| Rats (SD)       | 1.2, 2.6, or 4.7 x 3 (gi) | GD 0.7, and 14            | Lever press Amphetamine challenge to RI performance | 180             | Low drug sensitivity in 2.6 and 4.7 groups | (37)       |
| Rats (Wister)   | 0.04 or 1.6 x 4 (gi)     | GD 6–9                    | Lever press multi DRH-TO | 90              | Deficits in both groups | (38)       |
| Rats (Wister)   | 0.004, 0.008, or 0.04 x 4 (gi) | GD 6–9 | Lever press multi-DRH-TO | 120             | Deficits in 0.008 and 0.04 groups | (39)       |
| Rats (SD)       | 10.0 x 1 (iv)            | GD 4                      | Lever press CRF | 110–140         | Slower learning and extinction | (32)       |
| Rats (Long-Evans)| 6.4 x 1 (gi)            | GD 8 or 15                | Lever press Amphetamine challenge to DRL performance | 150             | Low drug sensitivity | (34)       |
| Rats            | 0, 2, or 6 x 4 (gavage)  | GD 6–9                    | Discrimination and reversal |                | In 6 mg/kg group, correct response decreased | (29)       |
| Rats            | 0, 0.025, 0.05, 0.5, or 5.0 x 4 (gavage) | GD 6–9 | Visual discrimination and reversal | ?              | Affected 5 mg/kg group | (30)       |
| Rats            | 0, 0.025, 0.05, 0.5, or 5.0 x 4 (gavage) | GD 6–9 | Spatial alternation | 75–145          | Affected 0.05 mg/kg and larger doses | (30)       |

Abbreviations: iv, intravenous injection; gi, gastric intubation; GD, day(s) of gestation; RI, random interval; DRH-TO, differential reinforcement of high rates–time out; CRF, continuous reinforcement; DRL, differential reinforcement of low rates.

Table 7. Effects of prenatal methylmercury exposure on sensory functions.

| Animal (strain) | Dose(s), mgHg/kg (route) | Period of administrations | Behaviors examined | Age(s) at examination, days | Findings | Reference |
|-----------------|--------------------------|---------------------------|--------------------|-----------------------------|----------|-----------|
| Rats            | 0, 1.5, or 5.0 mg/l (in water) | 2 weeks before conception to lactation | Lever press with required force | 300       | Increased failure in successful performance | (40) |

Table 8. Effects of prenatal methylmercury exposure on spontaneous activities.

| Animal (strain) | Dose(s), mgHg/kg (route) | Period of administrations | Age(s) at examination, days | Findings | References |
|-----------------|--------------------------|---------------------------|-----------------------------|----------|------------|
| Mice (129/SvSI)| 5.1, 6.8, or 10.2 x 1 (sc) | GD 10                    | 24, 44, and 64              | Decrease in 6.8 and 10.2 treated groups | (41) |
| Mice (C3H/HeN) | 16.0 x 1 (gi)            | GD 13,14,15,16, or 17    | 3–8 weeks (every week)      | Decreased | (42)       |
| Rats (SD)       | 8 x 1 (iv)               | GD 4                      | 110–140                     | Decrease in a plain enclosure No change in enriched environment | (33) |
| Rats (Long-Evans)| 4 or 6.4 x 1 (gi)        | GD 8 or 15                | 4, 8, 15, and 22            | Increase in both groups | (43)       |
| Rats (SD)       | 6.4 x 1 (gi)             | GD 8                      | 15, 22, 40, and 60          | No change | (44)       |
| Rats            | 0, 2, or 6 x 4 (gavage)  | GD 6–9                    | 21–120                      | Only males affected | (29)       |
| Rats            | 0, 0.025, 0.05, 0.5, or 5.0 x 4 (gavage) | GD 6–9 | 70–190 | 5 mg/kg group affected in two types of activity monitor | (30) |
| Rats (SD)       | 0, 2, or 6 x 3 (gi)      | GD 6–9                    | 60                           | Hypoactivity, Se-supplemented diet (1.3 ppm) partly antagonized | (45) |

Abbreviations: sc, subcutaneous injection; iv, intravenous injection; gi, gastric intubation; diet, food containing methylmercury compounds; GD, day(s) of gestation.
Table 9. Effects of prenatal methylmercury exposure on the open field test results.

| Animal (strain) | Dose(s), mgHg/kg (route) | Period of administrations | Apparatus and period of observation | Age(s) at examination, days | Findings | References |
|-----------------|--------------------------|----------------------------|------------------------------------|-----------------------------|----------|------------|
| Mice (129/Sv/SJ) | 5.4 × 1 (ip) | GD 7 or 9 | 50 × 50 cm², 2 min | 30 and 31 | Longer latency; increased backing; Decreased defecation and urination | (1) |
| Mice (CFW) | 1, 2, 3, 5, or 10 × 1 (gi) | GD 8 | 75 × 75 cm², 5 min | 56 | No change | (31) |
| Mice (129/Sv/SJ) | 5.1, 6.8, or 10.2 × 1 (sc) | GD 10 | 63 × 63 cm², 2 min | 23 | Longer latency; Decreased grooming; Decreased urination | (41) |
| | 3.4 × 3 (sc) | GD 10–12 | | 33 | Longer latency; Decreased rearing; Increased backing | |
| Mice (JCL/ICR) | 0.4 or 4.0 daily (gi) | GD 15 to weaning | 50 × 50 cm², 2 min | 21 | Increased activity and rearing in 4.0 group | (24) |
| | | | | | Increased rearing in 4.0 group | |
| | | | | | No change | (224–252) |
| Rats (SD) | 6.4 × 1 (gi) | GD 8 | | 15 and 22 | Apomorphine-induced stereotyped sniffing elicited only in the treated group | (44) |
| | | | | | Increased activity and rearing in 4.0 group | |
| | | | | | No change | |
| | | | | | Apomorphine-induced stereotyped sniffing potentiated | |
| Rats (SD) | 6.4 × 1 (gi) | GD 15 | 60 × 60 cm², 15 min | 14, 21, and 60 | No change | (35) |
| | | | | | Amphetamine-induced locomotion increased | |

Abbreviations: ip, intraperitoneal injection; sc, subcutaneous injection; gi, gastric intubation; GD, day(s) of gestation.

Table 10. Effects of prenatal methylmercury exposure on susceptibility to convulsions and seizures.

| Animal (strain) | Dose(s), mgHg/kg (route) | Period of administrations | Age(s) at examination, days | Findings | References |
|-----------------|--------------------------|----------------------------|-----------------------------|----------|------------|
| Mice (129/Sv/SJ) | 5.1, 6.8, or 10.2 × 1 (sc) | GD 10 | 70 | Increased susceptibility to flurothyl-induced convulsion | (41) |
| Mice (HUS Sabra) | 6.4 × 1 (sc) | GD 12 | 28–31 | Increased susceptibility to audiogenic seizure | (46) |
| Rats | 0, 2, or 6 × 4 (gavage) | GD 6–9 | 18–19, 57–58 | Startle facilitation in auditory startle habituation | (29) |
| Rats | 0, 0.025, 0.05, 0.5, or 5.0 × 4 (gavage) | GD 6–9 | 60, 120, 180, and 210 | Startle facilitation observed in males of 0.5 mg/kg and more in auditory startle habituation | (30) |

Abbreviations: iv, intravenous injection; gi, gastric intubation; GD, day(s) of gestation.

Table 11. Effects of prenatal methylmercury exposure on ultrasonic vocalization.

| Animal (strain) | Dose(s), mgHg/kg (route) | Period of administrations | Age(s) at examination, days | Findings | References |
|-----------------|--------------------------|----------------------------|-----------------------------|----------|------------|
| Rats (SD) | 0.1, 6, 3.2, or 4.8 × 1 (gi) | GD 7 | 5, 7, 9, and 11 | Slight effects; stronger effects by ages and order stimuli | (47) |
| Rats (SD) | 6.4 × 1 (gi) | GD 15 | 4, 8, and 12 | No change | (35) |
| Rats | 0, 0.025, 0.05, 0.5, or 5.0 (gavage) | UV during sexual behavior | 240 | No change | (30) |
| Rats (Wistar) | 0, 0.18–0.27, or 0.59–0.86 (mgHg/kg/day) drinking water | Two weeks before pairing and continued during experiment | 5, 7, 9, 11, 13, and 15 | Developmental delay; Reduction in number of cells; Shortening of basal-interval and call duration; Flattening and shift of frequency distribution | (48) |

Abbreviations: iv, intravenous injection; gi, gastric intubation; GD, day(s) of gestation; UV, ultrasonic vocalization.
evaluation of swimming ontogeny and BIEL maze learning should be included because of their sensitivity to methylmercury exposure. Collaborative studies were also done by Elsner and his colleagues (30, 53). In the first trial (53), female rats were given methylmercury in drinking water at concentrations of 0, 1.5, or 5 mg/l from 2 weeks before pairing until weaning. Among the various test items examined, a discrete trial spatial alternation task was shown to be the most sensitive, both in terms of effective dose 50% (ED50) and of no toxic effect level (NTEL). In the second trial (30), a wider range of doses was employed to include lower exposure levels. Thus, rat dams were administered 0.025, 0.05, 0.5, or 5.0 mg/kg/day of methylmercury during gestational days 6 to 9. Among the behavioral tests, the discrete trial spatial alternation task was found, as it was in the first trial, to be the most sensitive, with effects detectable in the 0.05 mg/kg/day group. It should be noted that differences in performance in a visual discrimination task, another rather demanding operant task, could only be detected at doses of 5.0 mg/kg/day, the largest dose employed.

**Studies in Nonhuman Primates**

**Motor Functions.** Contrary to the rodent studies, little work has been done in this category in primates (Table 12).

**Cognitive Functions.** Gunderson et al. (57) reported that exposed infant monkeys paid less visual attention to novel stimuli. The result was interpreted as a deficit in visual recognition memory. Object permanence development in infants (55) and delayed alternation in adult monkeys (56), both assumed to be tests of spatial memory, were examined with one cohort of monkeys. Of these two paradigm tests, only the object permanence development was impaired by methylmercury. Thus monkey studies so far did not show any persistent cognitive deficits caused by in utero methylmercury exposure.

**Sensory Functions.** Taking advantage of the similarity between the visual system of monkeys and humans, Rice and Gilbert (59) examined visual effects of prenatal and postnatal exposure to methylmercury. Spatial vision was affected in both the studies, but temporal vision was impaired only by exposures that had started before birth.

**Social Functions.** By observation and coding of elements of behavior in monkeys, Burbacher et al. (60) found less frequent social behaviors among the exposed groups.

What Do These Results Tell as a Whole?
The experimental studies have shown that some of the test items detected some behavioral alterations caused by prenatal exposure to methylmercury, at least when high doses (but not high enough to cause severe maternal toxicity or fetotoxicity) were given to the animals. In this sense, a proper combination of these tests would have been successful in detecting some effects of prenatal methylmercury exposure, although a given single item might not have produced a positive result.

Some behavioral tests were shown to be particularly sensitive to prenatal methylmercury exposure. Among others, the spatial alternation test (30), the tactile-kinesthetic test (40), and the DRH task (39) showed deviations from control at very low dose levels. At these doses, other simple tests such as those included in a functional observation battery, would fail to show any changes. It is unknown, however, whether such differential sensitivities among the tests reflected the nature of the behavioral tasks per se or reflected the nature of the effect of methylmercury. Evaluation of these tasks against other agents may show that the latter was the case. On the other hand, more mechanistic analyses of these behaviors might reveal the inherent sensitivities of these tests, which would support the former explanation. It should also be noted that the reproducibility of these test results must be demonstrated; e.g., Elsner (40) could not reproduce the deviation in the spatial alternation task (30) obtained in their first trial, and no laboratory has published rodent behavioral studies that showed effects from the same low level of exposure as was demonstrated in the DRH studies (39).

Thus, it is not clear from these tables, which cover a broad spectrum of behavioral functions, whether there are any functional categories particularly vulnerable to prenatal methylmercury exposure. It may be that these results simply indicate that the behavioral consequences of prenatal

| Table 12. Behavioral effects of in utero exposure to methylmercury in nonhuman primates. |
|-----------------------------------------------|---------------|----------------|----------------|----------------|----------------|
| Behavior examined                           | Species       | Dose(s)         | Period of administrations | Age(s) at examination, days | Major findings          | References |
| Cognitive function                          |               |                |                            |                            |                            |           |
| Spatial memory                              | Macaca        | 0, 50, 70, 90 µg/kg/day | 150-750 days before and during pregnancy | Infants | Retardation in development (55) |
| Object permanence development               | fascicularis  |                |                            |                            |                            |           |
| Spatial delayed alternation                  | Macaca        | 0, 50, 70, 90 µg/kg/day | 150-750 days before and during pregnancy | 7-9 years | No effect (56) |
| fascicularis                                |                |                |                            |                            |                            |           |
| Visual recognition memory                   | Macaca        | (50, 70 µg/kg/day) | 52 days                    | Infants and juveniles | Reduced attention to novel stimuli (57) |
| fascicularis                                |                |                |                            |                            |                            |           |
| Discrimination reversal performance and fixed interval | Macaca      | 10, 25, 50 µg/kg/day | Before conception, throughout the experiment |                    | No effect (56) |
| fascicularis                                |                |                |                            |                            |                            |           |
| Sensory function                            | Macaca        | 10, 25, 50 mgHg/kg/day | From pregnancy to 4-4.5 years |                    | Both spatial and temporat vision impaired (59) |
| Spatial and temporal visual function        | fascicularis  |                |                            |                            |                            |           |
| Social function                             | Macaca        | (50 µg/kg)      | 150-750 days before and during pregnancy | From 2 weeks to 8 months | Social behavior reduced, passive nonsocial behavior increased (60) |
| Social behavior                             | fascicularis  |                |                            |                            |                            |           |

* Same cohort of monkeys was used.
methylmercury exposure are widespread among various behavioral functions. Also, the fact that most of the tests (except for some tests with primates) were apical rather than specific to one functional category might obscure any profile that might be present. For example, the effects observed in some learning evaluations, such as Biel maze (52) or the DRH operant task (39), might result from motor incapacity rather than from learning. Likewise the change in audiogenic startle habituation might result from either ototoxic effect or a learning deficit (61, 62).

These facts may not be problematic if one does not intend to characterize the neurotoxicologic profile but only intends to detect some adverse effects of methylmercury. It is clear, however, that for such a characterization, one must seek another set of test items that is more specific to each functional category.

Comparison of Human and Animal Data on Neurobehavioral Effects of Prenatal Methylmercury Exposure

Gaps between Animals and Humans

Burbacher et al. (63) thoroughly reviewed the literature dealing with neuropathologic and/or neurobehavioral effects of prenatal methylmercury exposure in humans, nonhuman primates, and rodents. They concluded that neurotoxicity of methylmercury in terms of behavioral and pathologic effects had remarkable similarities among humans, (nonhuman) primates, and small mammals at high levels of exposure (i.e., brain mercury levels of 12–20 ppm) and that at moderate or low levels of exposure, neurobehavioral effects were regarded as similar when functional categories (e.g., motor, sensory, cognitive, etc.) rather than specific end points were compared. To be exact, they observed that at least two of three species shared such effects as “early reflex behaviors, motor coordination, visual functioning, and complex performance” (63) as a result of prenatal methylmercury exposure. It should be noted that these shared responses covered a broad range of behavioral categories.

Despite the conclusion reached by Burbacher et al. (63), there seem to be some gaps between human and animal studies dealing with neurobehavioral consequences of prenatal methylmercury exposures. The first gap is the level of specificity. As discussed above, epidemiologic studies of fish-eating human populations have looked, and are continuing to look, into behavioral end points in a more specific way than experimental studies in animals have. For example, the New Zealand study (19) suggested some functional domains such as fine-motor functions and language skills were vulnerable to methylmercury, although no clear-cut profile had been delineated. The Seychelles study (22) has adopted test items that are more or less connected with specific functional domains. The Faroe Islands study (21) is the most explicit in this regard because it systematically chose behavioral end points that are related to focal brain pathology. In comparison, most of the rodent studies usually adopted rather apical tests. On the other hand, what the behavioral changes demonstrated in rodents imply regarding human behavior might not always be clear-cut. For example, what kind of behavioral deficit in humans does the impaired DRH performance in rats predict? How well does an effect on spatial memory in rodents predict the effect on spatial memory in humans? These are issues that need to be answered to establish a test battery that aims at predicting the neurotoxicologic profile of an agent, which, in turn, can be extrapolated to human behavior.

The second gap relates to the periods of testing. In the above-mentioned human studies, developmental profiles of infants and children, including such higher functions as cognition or learning, were examined. Such emphasis on behavioral evaluation in the developmental period is partly because any follow-up studies extended into adulthood will be extremely difficult to conduct in natural human populations where socioeconomic factors exert significant impacts on behavioral development and where relocations of the subjects are not uncommon. On the other hand, in rodent experiments behaviors were usually examined in the adults; this is especially true for examining complex behaviors including schedule-controlled operant behaviors. Thus, developmental profiles of such complex behaviors have been rarely obtained except for the quantitative analyses of the development of ultrasonic vocalization (48) or of auditory startle habituation (29). In monkeys as described above, effects of in utero exposure on spatial memory were apparent in infants but not in adults (55, 56), suggesting a reversible nature of the effect on this function. It should be noted, however, that the test techniques employed in the two studies were not identical or even similar, and, as the authors have acknowledged (56), they might evaluate different functions.

The third gap refers to the difference in the types and periods of exposures. In most of the rodent studies, methylmercury was administered several times between 5 and 15 days of gestation. Since the concern regarding human populations is related to exposure derived from fish consumption, lower level exposures with longer durations (including both preconception as well as neonatal periods) should be evaluated, although neonatal treatment might confound the results by affecting the dams’ behavior. Also, it may be important to examine differential susceptibility to methylmercury among different stages of both the gestational as well as neonatal periods.

Development of Specific Test Batteries

Methylmercury has acquired a unique status among hazardous chemicals in our environment in that a) existence of developmental neurotoxicity is apparent in both humans and animals, b) a relatively large body of neurologic and behavioral data is available in both humans and animals when compared to most other chemicals, and c) the ongoing large-scale epidemiologic studies are evaluating behavioral functions in more specific ways than routine neurologic or psychologic batteries. Thus, by taking into account current and expected future findings, as well as the human–animal gaps described above, methylmercury may now serve as a model agent for developing a more specific test battery of animal behavior that could be used to predict possible human hazards resulting from prenatal exposure to other chemicals.

To develop such a battery, it is essential to have a choice of behavioral domains or categories and a choice of specific behavioral items for each domain. For the domains or categories, the choices adopted by Rees et al. (49), the National Center for Toxicological Research (64), or the Faroe Islands study (21) are useful as guidelines. In the remaining part of this paper, we will focus on the second step of the procedure.

To choose specific behavioral items for a given domain, there are two possible approaches. The first is an approach in which a behavior of an animal that is functionally or operationally analogous to human behavior of concern will be chosen as the test item. For example, the results of the discrete trial spatial alternation task used by Elsner et al. (30) were compared
to the attention disorder (and minimal brain dysfunction) seen in human children. Testing the tactile-kinesthetic system of rats was suggested by human studies that showed a relationship between attention deficit disorder and poor development of the tactile-kinesthetic system in children. If such a functional analogy could be validated with some appropriate experiments, this type of approach could provide a means to directly predict human behavior on the basis of rodent behavior. A problem with this approach, however, is that an apparent similarity between behaviors exhibited by different species does not always guarantee the same underlying neurologic mechanisms. Thus, extensive validation is required in this regard.

As an alternative approach, one can examine a particular behavior with a known neurological mechanism that is related to human behavior of concern. Stanton and Spear argue for this approach, suggesting that such neural comparability became known not only for sensory functions but also for a number of behavioral functions that might be examined in a psychologic evaluation. If so, this can be a powerful approach, especially when a prediction of the neurotoxicologic characteristics of a given agent in a human population is needed. Although it seems that lesion studies or pharmacologic studies may provide valuable information in this regard, few attempts at developing test batteries for neurobehavioral toxicity seem to have fully used such information.

Recently, a behavioral test battery that may be used for evaluating several aspects of central nervous system function in primates has been developed. The battery includes several test items that are chosen by the functional analogy of certain behaviors between humans and monkeys and others because of suspected correlation between the task and the integrity of certain brain areas. Thus, the choice of the test items used both of the above approaches. The battery was tested against acute effects of several reference compounds to demonstrate differential sensitivity of each task to different compounds. Although much work has to be done to validate each item and demonstrate the sensitivity of this battery, especially for chronic effects, this approach seems worthy of extensive pursuit. It is hoped to have a similar specific battery applicable to rodents, because it is much easier to run parallel experiments of neurochemical and neuropsychologic examinations with rodents than it is with primates. Although some human behavioral functions exist that cannot be assessed in rodents, e.g., color vision or language skills, rodents can accomplish highly complex behaviors such as 24-arm radial mazes, or repeated acquisition of correct sequences. These complex behaviors may be used for examining specific functional domains, such as those evaluated in the monkey battery.

Finally it should be pointed out that there are some basic items that have been dropped in most of the behavioral studies. The first one is the determination of the internal dose. Lack of an appropriate measure of the internal dose, e.g., brain Hg concentration, makes the significance of certain behavioral findings (regardless of whether they are positive or negative) somewhat ambiguous. In the case of in utero exposure, the dose should be determined not only at the time of testing but also during the prenatal period. The second is potential influences of the subjects' genetic background. Although the influence of genetic background on kinetics (excretion and distribution) of methylmercury has been evaluated, influences on behavioral effects seem to have scarcely been examined. In Iraq, individual differences were recognized in terms of the neurologic susceptibility of infants to prenatal methylmercury exposure as previously described. Individual differences were also a factor of consideration in choosing test items in the Faroe Islands study. Genetic background must be one of the determinants of such individual differences, and thus, requires further consideration. In general, systematic study of genetic influences on behavior is best conducted with rodents. In this respect again, a specific battery with rodents, if properly developed, would be of great value.

### Table 13. Behavioral functions and items examined in the National Center for Toxico logical Research Operant Test Battery.

| Behavioral function | Behavioral item |
|---------------------|----------------|
| Motivation (to respond for reinforcement) | Progressive ratio task |
| Learning Position and color discrimination | Incremental repeated acquisition task (hippocampus*) |
| Time estimation | Conditioned position responding task (prefrontal cortex) |
| Short-term memory and attention | Temporal response differentiation task |
|                    | Delayed matching-to-sample task (hippocampus) |

*Brain region that is thought to be related with the task is in parentheses.

### References

1. Spyker JM, Sparber SB, Goldberg AM. Subtle consequences of methylmercury exposure: behavioral deviations in offspring of treated mothers. Science 177:621-623 (1972).
2. Hunter, D. Mercury. In: The Disease of Occupations. 5th ed. London: English Universities Press, 1969:288-332.
3. Arima S, ed. Minamata Disease—Studies during These 20 Years and Problems Remaining for Today [in Japanese]. Tokyo: Seirinsha, 1979.
4. Harada M. Introduction—1: History of the study on Minamata disease and problems remaining for today [in Japanese]. In: Minamata Disease—Studies during These 20 Years and Problems Remaining for Today (Arima S, ed). Tokyo: Seirinsha, 1979:3-26.
5. Hosokawa H, Noda K, Misumi H, Kakita T, Kojima T. Investigation on the mysterious disease in Minamata [in Japanese]. In: Minamata Disease—Studies during These 20 Years and Problems Remaining for Today (Arima S, ed). Tokyo: Seirinsha, 1979:253-258.
6. Takeuchi T. Introduction—2: Advancement of the pathological study on Minamata disease—investigation of unknown disease and revelation of causal agent, organic mercury [in Japanese]. In: Minamata Disease—Studies during These 20 Years and Problems Remaining for Today (Arima S, ed). Tokyo: Seirinsha, 1979:27-48.
7. Kitamura S. "Epidemiology" of Minamata disease [in Japanese]. In: Minamata Disease—Studies during These 20 Years and Problems Remaining for Today (Arima S, ed). Tokyo: Seirinsha, 1979:81-94.
8. Hunter D, Russell DS. Focal cerebral and cerebellar atrophy in a human subject due to organic mercury compounds. J Neurol Neurosurg Psychiatry 17:235-241 (1954).
9. Tokuomi H. Clinical investigation of Minamata disease [in...
10. Von Oettingen WF. Poisoning: A Guide to Clinical Diagnosis and Treatment. New York: Paul B. Hoeber, 1952.

11. Zanger H. Erfahrungen über Quicksilvergiftungen [in German]. Archiv für Gewerbepathologie und Gewerbehygiene. 1:593-560 (1930).

12. Hunter D. Poisoning by methyl mercury compounds. Quart J Med 9:193–213 (1940).

13. Harada M. Congenital (or fetal) Minamata disease [in Japanese]. In: Minamata Disease—Studies during These 20 Years and Problems Remaining for Today (Arima S, ed). Tokyo: Seirinsha, 1979;345–363.

14. Dö R. Introduction—3: Review on the studies on organic mercury poisoning and discussions from the view point of social medicine [in Japanese]. In: Minamata Disease—Studies during These 20 Years and Problems Remaining for Today (Arima S, ed). Tokyo: Seirinsha, 1979;49–77.

15. WHO. Effects on man. In: Environmental Health Criteria 101: Methylmercury. Geneva: World Health Organization, 1990;68–99.

16. Marsh DO, Myers GJ, Clarkson TW Amin-Zaki L, Tikriti S, Majeed MA, Dabbagh AR. Dose-response relationship for human fetal exposure to methylmercury. Clin Toxicol 21:1311–1318 (1981).

17. Marsh DO, Clarkson TW, Cox C Myers GJ, Amin-Zaki L, Tikriti S. Fetal methylmercury poisoning: relationship between concentration in single strands of maternal hair and child effects. Arch Neurol 44:1017–1022 (1987).

18. Cox C, Clarkson TW, Marsh DO, Amin-Zaki L, Tikriti S, Myers GJ. Dose–response analysis of infants prenatally exposed to methylmercury: an application of a single compartment model to single-strand hair analysis. Environ Res 49:318–332 (1989).

19. Kjellstrom TP, Kennedy P, Wallis P, Mantell C. Physical and Mental Development of Children with Prenatal Exposure to Mercury from Fish. Stage 1: Preliminary Tests at Age 4. Solna: National Swedish Environmental Board, 1986.

20. McKeown-Eyssen GE, Ruedy J, Neims A. Methyl mercury exposure in northern Quebec: II. Neurologic findings in children. Am J Epidemiol 118:470–479 (1983).

21. White RF, Debes F, Dahl R, Grandjean P. Development and field testing of neuropsychological test battery to assess the effects of methylmercury exposure in the Faroe Islands. In: Proceedings of the International Symposium on Assessment of Environmental Pollution and Health Effects from Methylmercury, 8–9 October 1993, Kumamoto, Japan. Minamata, Japan: National Institute for Minamata Disease, 1994;127–140.

22. Davidson PW. Measuring neurodevelopmental outcomes of young children following prenatal dietary methylmercury exposure. In: Proceedings of the International Symposium on Assessment of Environmental Pollution and Health Effects from Methylmercury, 8–9 October 1993, Kumamoto, Japan. Minamata, Japan: National Institute for Minamata Disease, 1994;106–111.

23. Kjellström T. Effects of methylmercury exposure in utero: studies in New Zealand and proposal for future studies. In: Proceedings of the International Symposium on Epidemiological Studies on Environmental Pollution and Health Effects of Methylmercury, 2 October 1992, Kumamoto, Japan. Minamata, Japan: National Institute for Minamata Disease, 1993;67–78.

24. Tanimura TE, Ema E, Kihara T. Effects of combined treatment with methylmercury and polychlorinated biphenyls (PCBs) on the development of mouse offspring. In: Neural and Behavioral Teratology (Persaud TVN, ed). Lancaster, PA: MTP Press, 1980;163–198.

25. Satoh HN, Yasuda N, Shimai S. Development of reflexes in neonatal mice prenatally exposed to methylmercury and selenite. Toxicol Lett 25:199–203 (1985).

26. Mottet NK. Effects of chronic low-dose exposure of rat fetuses to methylmercury hydroxide. Teratology 10:173–189 (1974).

27. Sobotka TJ, Cook MP, Brodie RE. Effects of perinatal exposure to methylmercury on functional brain development and neurochemistry. Biol Psychiatry 8:307–320 (1974).

28. Olson K, Boush GM. Decreased learning capacity in rats exposed prenatally and postnatally to low doses of mercury. Bull Environ Contam Toxicol 13:73–79 (1975).

29. Buelke-Sam J, Kimmel CA, Adams J, Nelson CJ, Vorhees CV, Wright DC, St Omer V, Karol BA, Butter RE, Geyer MA, Holson JF, Kuchler C, Wayner MJ. Collaborative Behavioral Teratology Study: results. Neurobehav Toxicol Teratol 7:591–624 (1985).

30. Elsner J, Hodel B, Suter KE, Oelke D, Ulbrich B, Schreier G, Cuomo V, Cagiano R, Rosengren LE, Karlsson JE, Haglid KG. Detection limits of different approaches in behavioral teratology, and correlation of effects with neurochemical parameters. Neurotoxicol Teratol 10:155–167 (1988).

31. Hughes JA, Annau Z. Prenatal behavioral effects in mice after perinatal exposure to methylmercury. Pharmacol Biochem Behav 43:385–391 (1991).

32. Shimai SH, Satoh H, Yasuda N. Taste aversion learning and perinatal methylmercury exposure in mice. Ind Health 22:41–44 (1984).

33. Scharlock RL, Brown WJ, Kark RP, Menon NK. Perinatal methylmercury intoxication: behavioral effects in rats. Dev Psychobiol 14:313–318 (1981).

34. Eccles CU, Annau Z. Prenatal methyl mercury exposure: II. Alterations in learning and psychotropic drug sensitivity in adult offspring. Neurobehav Toxicol Teratol 4:377–382 (1982).

35. Cagiano R, De-Salvia MA, Renta G, Tortella E, Braghirodi D, Parenti C, Zanoli P, Baraldi M, Annau Z, Cuomo V. Evidence that exposure to methyl mercury during gestation induces behavioral and neurochemical changes in offspring of rats. Neurotoxicol Teratol 12:23–8 (1990).

36. Zenick H. Behavioral and biochemical consequences in methyl mercury chloride toxicity. Pharmacol Biochem Behav 2:709–713 (1974).

37. Hughes JA, Sparber SB. d-Amphetamine unmasks postnatal consequences of exposure to methylmercury in utero: methods for studying behavioral teratogenesis. Pharmacol Biochem Behav 8:365–375 (1978).

38. Mutsch HR, Bornhausen M, Kriegel H, Greim H. Methylmercury chloride induces learning deficits in prenatally treated rats. Arch Toxicol 40:103–108 (1978).

39. Bornhausen M, Mutsch HR, Greim H. Operant behavior performance changes in rats after prenatal methylmercury exposure. Toxicol Appl Pharmacol 56:305–310 (1980).

40. Elsner J. Tactile-kinesthetic system of rats as an animal model for minimal brain dysfunction. Arch Toxicol 65:465–473 (1991).

41. Su MQ, Okita GT. Behavioral effects on the progeny of mice treated with methylmercury. Toxicol Appl Pharmacol 38:195–205 (1976).

42. Inouye MK, Murao K, Kajiwara Y. Behavioral and neuropathological effects of perinatal methylmercury exposure in mice. Neurobehav Toxicol Teratol 7:227–232 (1985).

43. Eccles CU, Annau Z. Prenatal methyl mercury exposure: I. Alterations in neonatal activity. Neurobehav Toxicol Teratol 4:371–376 (1982).

44. Cuomo VL, Ambrosi Z, Annau, Cagiano R, Brunello N, Racgni G. Behavioural and neurochemical changes in offspring of rats exposed to methyl mercury during gestation. Neurobehav Toxicol Teratol 6:249–254 (1984).

45. Fredriksson A, Gardlund AT, Bergman K, Oskarsson A, Ohlin B, Danilson B, Archer T. Effects of maternal dietary supplementation with selenium on the postnatal development of rat offspring exposed to methyl mercury in utero. Pharmacol Toxicol 72:377–382 (1993).

46. Menashi MA, Ormay A, Yanai J. Transplacental effects of methylmercury chloride in mice with specific emphasis on audiogenic seizure response. Dev Neurosci 5:216–221 (1982).
METHYLMERCUry AS A HEALTH THREAT

47. Adams J, Miller DR, Nelson CJ. Ultrasonic vocalizations as diagnostic tools in studies of developmental toxicity. Neurobehav Toxicol Teratol 5:29–37 (1983).

48. Elsner J, D Suter, S Alder. Microanalysis of ultrasound vocalizations of young rats: assessment of the behavioral teratogenicity of methylmercury. Neurotoxicol Teratol 12:7–14 (1990).

49. Rees DC, Francis EZ, Kimmel CA. Qualitative and quantitative comparability of human and animal developmental neurotoxicants: a workshop summary. Neurotoxicology 11:257–269 (1990).

50. Shimai S, Satoh H. Behavioral teratology of methylmercury. J Toxicol Sci 10:199–216 (1985).

51. Royalty J, Taylor GT, Korol BA. The effects of prenatal exposure to methylmercury on aggressive behavior in the rat. Neurotoxicol Teratol 9:87–93 (1987).

52. Vorhees CV. Comparison of the Collaborative Behavioral Teratology Study and Cincinnati Behavioral Teratology test batteries. Neurobehav Toxicol Teratol 7:625–633 (1985).

53. Elsner J, Suter KE, Ulbrich B, Schreiner G. Testing strategies in behavioral teratology: IV. Review and general conclusions. Neurobehav Toxicol Teratol 8:585–590 (1986).

54. Buelte-Sam J. Practical considerations in establishing reliable and sensitive neurobehavioral test methods. Zentralbl Bakteriol Mikrobiol Hyg B 185:4–9 (1987).

55. Burbacher TM, Grant KS, Mottet NK. Retarded object permanence development in methylmercury exposed Macaca fascicularis infants. Dev Psychol 22:771–776 (1986).

56. Gilbert SG, Burbacher TM, Rice DC. Effects of in utero methylmercury exposure on a spatial delayed alternation task in monkeys. Toxicol Appl Pharmacol 123:130–136 (1993).

57. Gunderson VM, Grant-Webster KS, Burbacher TM, Mottet NK. Visual recognition memory deficits in methylmercury-exposed Macaca fascicularis infants. Neurotoxicol Teratol. 10:373–379 (1988).

58. Rice DC. Effects of pre plus postnatal exposure to methyl mercury in the monkey on fixed interval and discrimination reversal performance. Neurotoxicology 13:443–452 (1992).

59. Rice DC, Gilbert SG. Effects of developmental exposure to methyl mercury on spatial and temporal visual function in monkeys. Toxicol Appl Pharmacol 102:151–163 (1990).

60. Burbacher TM, Sackett GP, Mottet NK. Methylmercury effects on the social behavior of Macaca fascicularis infants. Neurotoxicol Teratol 12:65–71 (1990).

61. Stanton ME, Spear LP. Workshop on the qualitative and quantitative comparability of human and animal developmental neurotoxicity, Work Group I report: comparability of measures of developmental neurotoxicity in humans and laboratory animals. Neurotoxicol Teratol 12:261–267 (1990).

62. Adams J. Methods in behavioral teratology. In: Handbook of Behavioral Teratology (Riley EP, Vorhees CV, eds). New York:Plenum Press, 1986;67–100.

63. Burbacher TM, Rodier PM, Weiss B. Methylmercury developmental neurotoxicity: a comparison of effects in humans and animals. Neurotoxicol Teratol 12:191–202 (1990).

64. Paule MG. Analysis of brain function using a battery of schedule-controlled operant behaviors. In: Neurobehavioral Toxicology: Analysis and Interpretation (Weiss B, O'Donoghue JD, eds). New York:Raven Press, 1994;331–338.

65. Wenk GL, Olton DS. Lesion analysis. In: Neurobehavioral Toxicology (Annan Z, ed). Baltimore:Johns Hopkins University Press, 1986;268–276.

66. Paule GM, Allen RR, Bailey JR, Scaller AC, Ali SF, Brown RM, Slukker W Jr. Chronic marijuana smoke exposure in the rhesus monkey II: Effects on progressive ratio and conditioned position responding. J Pharmacol Exp Ther 260:210–222 (1992).

67. Miller DB, Eckerman DA. Learning and memory measures. In: Neurobehavioral Toxicology (Annan Z, ed). Baltimore:Johns Hopkins University Press, 1986;94–152.

68. Cohn J, Cox C, Cory-Slechta DA. The effects of lead exposure on learning in a multiple repeated acquisition and performance schedule. Neurotoxicology 14:329–346 (1993).

69. Inoue M, Kajiwara Y, Hirayama K. Dose- and sex-dependent alterations in mercury distribution in fetal mice following methylmercury exposure. J Toxicol Environ Health 19:425–435 (1986).