spring become more mature, various kinds of behavioral examinations are possible and behavioral deviation and neurological disorder may be found. With aging, immunological deficiency and generalized debilitation may be observed and finally the individual may die earlier than expected indicates as premature death. Every item contains "D," thus they are called six D's.

As the framework of a behavioral teratology study, observations such as above were proposed by Spyker. Behavioral teratology tries to find effects of prenatal exposure not only at the early stage of life but also over the lifetime after development and aging.

Current framework of behavioral teratology study: Eight D's

However, six D's do not include very important item for the life, reproduction. Later Tanimura (Tanimura 1980) proposed another D, *Reproductive Debility*. As the issues concerning endocrine disrupting chemicals are being argued, reproductive debility must be seriously concerned. He also emphasized *Birth Defects* that are found at birth because of his expertise, anatomy. Thus, current framework of behavioral teratology study can be shown as Eight D's (Fig. 1), although all the D's in the framework are not examined by the actual studies.

Subtle consequences: Important observation items in the investigation

As emphasized by Spyker and colleagues, subtle consequences are the important findings of behavioral teratology (Spyker et al. 1972; Zbinden 1981). Subtle consequences may mean, 1. so slight as to be difficult to detect or 2. not immediately obvious. Therefore, birth defect that is obvious at once may not be considered by actual studies on behavioral teratology. Abnormal development, behavioral deviation and neurological disorder are items usually examined in behavioral teratology studies.

Immunological deficiency, generalized debilitation or premature death is not usually considered, probably because immunological deficiency may not be directly detected by the behavioral methods. As for generalized

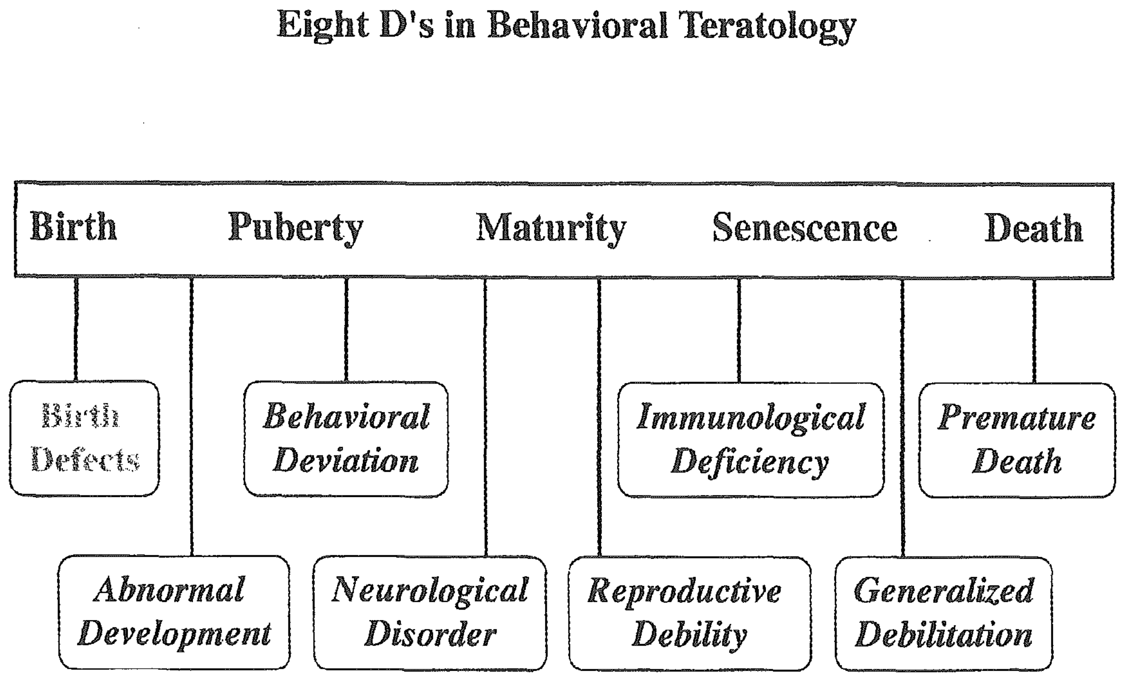

Fig. 1.

debilitation and premature death, previous experiments did not continue such long. Scientists are forced into publishing their studies quickly; they are unable to wait until experimental animals age enough to evaluate debilitation or to observe their death. Generalized debilitation and premature death are two important items, because an individual develops its abilities depending upon its age and accelerated aging may deteriorate the abilities. If deteriorating earlier than normal aging, the individual may not feel totally happy. Therefore, evaluating the possible effect by animal is important.

Findings in behavioral teratology studies

A considerable number of investigations on the effects of in utero methylmercury exposure using animal experimental models have been reported so far (see reviews by Shimai and Satoh 1985; Watanabe and Satoh 1996). The following were observed and reported; development of reflexive behavior during lactational period, swimming ability before and after weaning, passive and active avoidance learning, maze and water escape learning, operant learning, sensory function, spontaneous activity, open field test, susceptibility to convulsion and seizure, and ultrasonic vocalization. Most of the reports revealed retardation, impairment or change, although some failed.

The observed effects above are classified as follows according to the functional category (Table 1, Shimai and Satoh 1985; Watanabe and Satoh 1996). Sensory functions are difficult to examine in animal experiments (Evans et al. 1975; Rice and Gilbert 1995). Most of the experiments examining this functional category were done with primates on visual functions. Although Elsner (1991) trained rats to press a lever with predetermined forces and found impaired performance in methylmercury exposed rats. They had been given methylmercury during the period between 2 weeks before conception and lactation at the concentrations of 1.5 or 5.0 mg Hg/liter in drinking water. It is also interesting that they were examined at 300 days old.

Each function is important for an individual animal to survive. Further, most of these functions develop with age and deteriorate later in the lifetime. It is important that these functions develop under controlled conditions and in the right order of time. If a function does not develop at appropriate time, the individual may have difficult time to survive. Therefore, in behavioral teratology abnormality in ontogeny or ontogenesis must be considered as well as deficit of functions.

Examples of investigations

1. Development of righting reflex and walking activity

As mentioned above, behavioral teratology study has tendency to examine the offspring animal in early development stage. Satoh and colleagues (Satoh et al. 1985) examined the effects on development of righting reflex and walking activity by prenatal exposure. Preg-

TABLE 1. *Functional categories of behavioral teratology*

Functions	Behavior and response
Motor development and functions	Reflexive behavior; Swimming ability
Cognitive functions	Maze, Avoidance, or Operant learning
Motivation and arousal behavior	Spontaneous or Open field activity; Susceptibility to convulsion and seizure
Social functions	Ultra sonic vocalization
Sensory functions	

nant mice were injected with methylmercury at the dose of 6.0 mg Hg/kg on gestational day 9. They were allowed to litter and offspring mice were examined postnatal days 1, 3 and 8 for development of righting reflex and walking activity. The average righting reflex scores of methylmercury treated offspring were lower than the control on postnatal days 1 and 3. On postnatal day 8, however, no difference was found between the two groups. The methylmercury treated offspring showed similar ontogenic pattern (score increases with age of days) to the control, though the scores postnatal days on 1 and 3 were lower. Therefore methylmercury treatment makes the progress slightly being retarded. Similar findings were observed for the score of walking activity.

2. Avoidance learning

With young adult offspring, two-way avoidance can be conducted: An animal has to move from a compartment of a shuttle box to another compartment to avoid electric shock. The animal is warned by sound before electric shock is applied. The learning ability is evaluated how many trials are necessary to avoid electric shock (moving to the safe compartment before electric shock by hearing the sound). On gestational day 8 or 15, methylmercury (4.0 or 6.4 mg Hg/kg) was given to maternal rats by gastric intubations (Eccles and Annau 1982). When the offspring were 63 days old, acquisition was evaluated in a shuttle box. The offspring born to the treated mother needed more trials to learn avoidance. The difference was more distinct among the groups whose mother were given methylmercury on gestational day 15 than those given on gestational day 8. The control offspring needed 50 trials to acquire avoidance and the offspring born to the methylmercury treated mothers needed more than 200 trials. There is no difference between the two doses. Among the offspring given methylmercury on gestational day 8, the effect was not distinguished from the control.

The offspring were also examined later for reacquisition. After acquisition was established the same procedure is repeated without electric shock and thus offspring soon learn that electric shock does not come and they do not move and stay in the same compartment. This is the extinction training. Then again electric shock is applied and reacquisition is evaluated. Learning deficit was clearly shown among the offspring groups with prenatal methylmercury exposure in a dose-dependent manner. The control needed approximately 40 trials for reacquisition, while offspring treated with methylmercury on gestational day 15 with 4.0 or 6.4 mg Hg/kg of methylmercury needed 100 or 150 trials, respectively.

3. Maze learning test with methylmercury exposure originated from marlin or tuna meat

In these experiments described above mostly methylmercury compounds were given. In one experiment (Olson and Boush 1975), however, they gave marlin meat containing methylmercury to maternal rats and found deficit of the offspring in a maze test. Maternal rats were given one of the three diets from gestational day 0 throughout the experiment. Thus, first the maternal rats were exposed and later after weaning offspring rats were exposed. The diets were 1) rat chow for the control, 2) marlin meat + rat chow to adjust methylmercury concentration at 2 mg Hg/kg diet and 3) tuna meat + rat chow + methylmercury hydroxide to adjust methylmercury concentration at 2 mg Hg/kg diet. Offspring rats of 60 day old were examined in symmetrical mazes. It is interesting that offspring given marlin meat diet showed more errors than did the control, while offspring rats given tuna meat did not show more errors. Does methylmercury from different sources affect differently, or tuna meat possibly contained protective agent? No difference between selenium concentrations in both fish meats was reported.

4. Differential reinforcement of high rate; the lowest dose that produced prenatal methylmercury effects experimentally

The doses of methylmercury were various among experiments. They range roughly from the tenth of mg to ten mg Hg/kg body weights of maternal animals. Dosing regimens were single or repeated injections or gastric intubations. Only a few experiments employed dosing through diet (or drinking water).

The lowest dose that produced postnatal effects of prenatal methylmercury exposure in the animal experiment was, however, much smaller (Musch et al. 1978; Bornhausen et al. 1980). The offspring showed deficit in operant learning. In their experiment using the lowest dose (Bornhausen et al. 1980) maternal rats were given methylmercury chloride during gestational day 6-9 by gastric intubations. The doses were 0.004, 0.008 or 0.04 mg Hg/kg per day. At 4 month old, offspring rats were examined by an operant test called differential reinforcement of high rate (DRH). In this test schedule a rat is required to press a small lever at a predetermined number of times within a predetermined time interval to obtain a small pellet of food. Thus, DRH2/1 means two lever presses within one second. No difference was found among the four different dose groups (0, 0.004, 0.008 or 0.04 mg Hg/kg) for the success rate of the DRH2/1 test. But as for the DRH4/2 and 8/4, which require more lever presses, performance decreased in a dose-dependent manner. It is noteworthy that the total dose given to a mother rat is 0.16 mg Hg/kg at the highest dose group. This experiment has shown the lowest amount of methylmercury to produce behavioral changes ever.

5. Postnatal effects of mercury vapor exposure during gestation

Experiments investigating postnatal effects of in utero exposure to mercury vapor are scarce. One (Danielsson et al. 1993) of the few studies is as follows: Maternal rats were exposed to mercury vapor at 1.8 mg Hg^0 for 1 hour or 4 hours during gestational day 11-14 and 17-20. Spatial learning of offspring rats was tested in a radial arm maze. This test is to time the latency to obtain all the food pellets in distal ends of the radial arms. Reentry to the arm of which pellet was already taken was counted as an error. Offspring rats with in utero mercury vapor exposure needed longer time to get the pellets and made more errors. This result indicates neurobehavioral effects of prenatal exposure to mercury vapor in utero.

6. Interaction of methylmercury and mercury vapor co-exposure

It is likely that people in real life are exposed to various kinds of pollutants. As for mercury, people may be exposed to methylmercury by fish consumption and to mercury vapor with dental amalgam. Therefore, an experiment where animals were exposed to both mercury vapor and methylmercury was done (Fredriksson et al. 1996). The test procedure is similar to the previous one. Maternal rats were exposed to mercury vapor at 1.8 mg Hg^0 for 1.5 hour/day during gestational day 14-19. They were also given methylmercury chloride at a daily dose of 2 mg Hg/kg during gestational day 6-9. Co-exposure to mercury vapor and methylmercury caused longer time and more errors in the radial maze test comparing with single exposure to either methylmercury or mercury vapor.

7. Postnatal effects of maternal stress and methylmercury exposure during pregnancy

Whether postnatal development and behavior are affected by interaction with maternal stress was also examined (Colomina et al. 1997). Pregnant mice were exposed to methylmercury (1.6 mg Hg/kg/day) during gestational day 15-18. Stress was given as immobilization in a cylinder, 2 hours/day. However, no significant interaction on developmental landmarks or neurobehavioral development of offspring mice

TABLE 2. *Factors that may influence the effects of prenatal mercury exposure*

Species, strain and sex of experimental animals
Time of exposure during pregnancy
Heat exposure during pregnancy
Maternal behavior, Maternal care and Fostering
Nutrition, e.g. Selenium and Poly unsaturated fatty acids
Environment after birth, e.g. Enriched environment
Behavioral examinations employed
Age at examination
Miscellaneous

during lactational period was observed.

Factors that may influence the postnatal behavioral effects of prenatal mercury exposure

Retardation of development of reflexes was partly counteracted by co-administration of selenium (Satoh et al. 1985). Moreover, offspring mice born to the dams fed with selenium deficiency diet and given methylmercury injections during gestation were more severely affected than the offspring of groups of selenium deficiency alone or methylmercury administration alone (Satoh et al. 1997). Maternal heat exposure before methylmercury administration during gestation did not enhance the postnatal effects of methylmercury, though heat exposure showed interactions (Yin et al. 1997). Since in the studies of behavioral teratology, the main goal is to elucidate postnatal effects of in utero exposure that does not cause overt maternal, fetal or neonatal toxicities, the doses become necessarily low. This also means the effect is more easily influenced by other factors and agents in the environment. Therefore, recognition of these factors and agents are important to evaluate experimental results. In Table 2 factors to be considered are listed.

Most behavioral evaluations were done during lactational periods or at several tens days of age. Few examined the effects at the elderly, though aging is an important factor to evaluate full spectrum of methylmercury toxicity.

Conclusions

This review shows that behavioral teratology reveals subtle consequences of in utero exposure to mercury vapor and methylmercury; namely, behavioral teratology is sensitive to detect postnatal effects of prenatal exposure to mercury. However, some experiments, which were not described here, failed to detect the effects. In these experiments the doses used were similar to those above mentioned. Therefore, behavioral teratology is not always sensitive. For example, the behavioral effects may be masked by the age of examination.

Another problem of behavioral teratology is the underlying mechanism(s) of behavioral changes have not been fully investigated. What is the mechanism of behavioral changes? It may be alteration(s) in 1) developmental and ontogenetic, 2) physiological and psychological, 3) neurochemical and pharmacological function(s), or 4) histopathological changes. Further studies including investigation into mechanisms are necessary with the considerations on possible "interactions" such as selenium status and other environmental exposure and with the efforts expanding of investigation period over the lifetime of animals as indicated by the framework, namely, "Eight D's."

References

Bakir, F., Damluji, S.F., Amin-Zaki, L., Murtadha, M., Khalidi, A., Al-Rawi, N.Y., Tikriti, S., Dahahir, H.I., Clarkson, T.W., Smith, J.C. & Doherty, R.A. (1973) Methylmercury poisoning in Iraq. *Science*, **181**, 230-241.

Bornhausen, M., Musch, H.R. & Greim, H. (1980) Operant behavior performance changes in rats after prenatal methylmercury exposure. *Toxicol. Appl. Pharmacol.*, **56**, 305-310.

Clarkson, T.W. (2002) The three modern faces of mercury. *Environ. Health Perspec.*, **110**, Suppl. 1, 11-23.

Colomina, M.T., Albina, M.L., Domingo, J.L. & Corbella, J. (1997) Influence of maternal stress on the effects of prenatal exposure to methylmercury and arsenic on postnatal development and behavior in mice: a preliminary evaluation. *Physiol. Behav.*, **61**, 455-459.

Danielsson, B.R., Fredriksson, A., Dahlgren, L., Gardlund, A.T., Olsson, L., Dencker, L. & Archer, T. (1993) Behavioural effects of prenatal metallic mercury inhalation exposure in rats. *Neurotoxicol. Teratol.*, **15**, 391-396.

Davidson, P.W., Myers, G.J., Cox, C., Shamlaye, C.F., Marsh, D.O., Tanner, M.A., Berlin, M., Sloane-Reeves, J., Cernichiari, E. & Choisy, O. (1995) Longitudinal neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from maternal fish ingestion: outcomes at 19 and 29 months. *Neurotoxicology*, **16**, 677-688.

Eccles, C.U. & Annau, Z. (1982) Prenatal methyl mercury exposure: II. Alterations in learning and psychotropic drug sensitivity in adult offspring. *Neurobehav. Toxicol. Teratol.*, **4**, 377-382.

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

Evans, H.L., Laties, V.G. & Weiss, B. (1975) Behavioral effects of mercury and methylmercury. *Fed. Proc.*, **34**, 1858-1867.

Fredriksson, A., Dencker, L., Archer, T. & Danielsson, B.R. (1996) Prenatal coexposure to metallic mercury vapour and methylmercury produce interactive behavioural changes in adult rats. *Neurotoxicol. Teratol.*, **18**, 129-134.

Grandjean, P., Weihe, P., White, R.F., Debes, F., Araki, S., Yokoyama, K., Murata, K., Sorensen, N., Dahl, R. & Jorgensen, P.J. (1997) Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol. Teratol.*, **19**, 417-428.

Harada, M. (1978) Congenital Minamata disease: intrauterine methylmercury poisoning. *Teratology*, **18**, 285-288.

Hunter, D. (1969) Mercury. In: *The Diseases of Occupations*, The English Universities Press Ltd., London, pp. 288-332.

Kjellstrom, T., Kennedy, P., Wallis, S. & Mantell, C. (1986) Physical and mental development of children with prenatal exposure to mercury from fish. Stage 1: Preliminary test at age 4. Report (3080), National Swedish Environmental Protection Board, Solna, Sweden.

Kjellstrom, T., Kennedy, P., Wallis, S., & Mantell, C. (1989) Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2: Interviews and psychological tests at age 6. Report (3642), National Swedish Environmental Protection Board, Solna, Sweden.

Musch, H.R., Bornhausen, M., Kriegel, H. & Greim, H. (1978) Methylmercury chloride induces learning deficits in prenatally treated rats. *Arch. Toxicol.*, **40**, 103-108.

Myers, G.J., Davidson, P.W., Cox, C., Shamlaye, C.F., Tanner, M.A., Marsh, D.O., Cernichiari, E., Lapham, L.W., Berlin, M. & Clarkson, T.W. (1995a) Summary of the Seychelles child development study on the relationship of fetal methylmercury exposure to neurodevelopment. *Neurotoxicology*, **16**, 711-716.

Myers, G.J., Marsh, D.O., Davidson, P.W., Cox, C., Shamlaye, C.F., Tanner, M., Choi, A., Cernichiari, E., Choisy, O. & Clarkson, T.W. (1995b) Main neurodevelopmental study of Seychellois children following in utero exposure to methylmercury from a maternal fish diet: outcome at six months. *Neurotoxicology*, **16**, 653-664.

Olson, K. & Boush, G.M. (1975) Decreased learning capacity in rats exposed prenatally and postnatally to low doses of mercury. *Bull. Environ. Contam. Toxicol.*, **13**, 73-79.

Rice, D.C. & Gilbert, S.G. (1995) Effects of developmental methylmercury exposure or lifetime lead exposure on vibration sensitivity function in monkeys. *Toxicol. Appl. Pharmacol.*, **134**, 161-169.

Satoh, H., Watanabe, C., Yin, K. & Inaba, H. (1997) Postnatal effects of in utero exposure to methylmercury; with emphases on co-exposure to heat or selenium deficiency. *Proceedings of NIMD Forum '97*, Minamata, pp. 113-120.

Satoh, H., Yasuda, N. & Shimai, S. (1985) Development of reflexes in neonatal mice prenatally exposed to methylmercury and selenite. *Toxicol. Letters.*, **25**, 199-203.

Shimai, S. & Satoh, H. (1985) Behavioral teratology of methylmercury. *J. Toxicol. Sci.*, **10**, 199-216.

Spyker, J.M. (1975a) Assessing the impact of low level chemicals on development: behavioral and latent effects. *Federation Proceedings*, **34**, 1835-1844.

Spyker, J.M. (1975b) Behavioral Teratology and Toxicology. In: *Behavioral Toxicology*, edited by B. Weiss & V.G. Laties, Plenum Press, New York, pp. 311-349.

Spyker, J.M., Sparber, S.B. & Goldberg, A.M. (1972) Subtle consequences of methylmercury exposure: behavioral deviations in offspring of treated mothers. *Science*, **177**, 621-623.

Tanimura, T. (1980) Introductry remarks on behavioral teratology. *Cong. Anom.*, **20**, 301-318. (in Japanese)

Watanabe, C. & Satoh, H. (1996) Evolution of our understanding of methylmercury as a health threat. *Environ. Health Perspect.*, **104**, Suppl. 2, 367-379.

Weiss, B. & Spyker, J.M. (1974) The susceptibility of the fetus and child to chemical pollutants. Behavioral implications of prenatal and early postnatal exposure to chemical pollutants. *Pediatrics*, **53**, 851-859.

Werboff, J. (1966) Tranquillizers in pregnancy and behavioural effects on the offspring. *Nature*, **209**, 110.

Werboff, J. & Gottleib, J.S. (1963) Drugs in pregnancy: Behavioral Teratology. *Obstet. Gynecol. Survey*, **18**, 420-423.

Yin, K., Watanabe, C., Inaba, H. & Satoh, H. (1997) Growth and behavioral changes in mice prenatally exposed to methylmercury and heat. *Neurotoxicol. Teratol.*, **19**, 1-7.

Zbinden, G. (1981) Experimental methods in behavioral teratology. *Archi. Toxicol.*, **48**, 69-88.

Tohoku J. Exp. Med., 2003, **200**, 67-73

Estimation of Daily Mercury Intake from Seafood in Japanese Women: Akita Cross-Sectional Study

YOKO IWASAKI, MINESHI SAKAMOTO,[1] KUNIHIKO NAKAI,[2] TOMOKO OKA,[2] MIWAKO DAKEISHI,[3] TOYOTO IWATA,[3] HIROSHI SATOH[2] and KATSUYUKI MURATA[3]

Environmental Health Department, Ministry of the Environment, Tokyo 100-8975,
[1]*Department of Epidemiology, National Institute for Minamata Disease, Minamata 867-0008,*
[2]*Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai 980-8575, and*
[3]*Environmental Health Sciences, Department of Social Medicine, Akita University School of Medicine, Akita 010-8543*

IWASAKI, Y., SAKAMOTO, M., NAKAI, K., OKA, T., DAKEISHI, M., IWATA, T., SATOH, H. and MURATA, K. *Estimation of Daily Mercury Intake from Seafood in Japanese Women: Akita Cross-Sectional Study.* Tohoku J. Exp. Med., 2003, **200** (2), 67-73 —— We estimated daily mercury intakes from seafood in 154 mothers residing in several cities and towns in Akita, Japan, to address the relationships between the reference dose (*RfD* of 0.1 μg/kg body weight per day, derived by US EPA) and daily mercury intakes, combined with hair mercury levels. The frequency and volume of seafood ingested by them were examined using a food frequency questionnaire (FFQ) with 25 kinds of full-scale pictures including fish and shellfish items. Hair mercury concentrations in the mothers were also determined. The geometric means in the mothers were 15.3 (2.65-48.4) μg/day for daily mercury intakes from seafood, calculated on the basis of the references on mercury contents, and 1.73 (0.49-5.82) μg/g for hair mercury concentrations. The daily mercury intake was significantly correlated with hair mercury concentrations (Spearman rank correlation coefficient $r_s=0.335$, $p<0.001$). No significant differences in mercury intakes were found either between mothers residing in fishing and non-fishing areas or between those in cities and towns ($p>0.05$). Assuming the methylmercury content rate of 75% in seafood mercury and body weight of 55 kg, the mothers were estimated to ingest methylmercury of 0.21 μg/kg body weight per day. It is suggested that daily mercury intakes, calculated by the FFQ, reflect hair mercury levels, and there is no interregional difference in the daily mercury intake unless any special circumstance exists. Daily methylmercury intake in more than 90% of Japanese women may exceed the *RfD*, and it

Received April 28, 2003; revision accepted for publication June 9, 2003.
Address for reprints: Katsuyuki Murata, Environmental Health Sciences, Department of Social Medicine, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan.
e-mail: winestem@med.akita-u.ac.jp

therefore seems to be far from the present state of Japanese dietary lives. ———— daily mercury intake; seafood; food frequency questionnaire; reference dose; methylmercury

Humans are mainly exposed to methylmercury from seafood and freshwater fish, and poisoning incidents have also involved grain treated with this substance as fungicide (Kurland et al. 1959; Bakir et al. 1973). After the first epidemic in Minamata, Japan, where many fishermen and their families developed methylmercury poisoning in the 1950s, the disease was dubbed Minamata disease (Igata 1993). The cause was fish contaminated by drainage, containing methylmercury, from a chemical production plant. But, the main problem today is the widespread occurrence of methylmercury in fish and marine mammals, thus exposing human populations with a high fish intake and especially those who consume from high levels of the food chains. Since the risk to children from dietary methylmercury exposure is unclear, current advisories on fish consumption issued by national and state authorities aim mainly at pregnant women or women of reproductive age groups (United Nations Environment Programme Chemicals 2002).

Risk assessment of methylmercury exposure is an important public-health consideration as a basis for preventive efforts (National Research Council 2000). From data on the Iraqi poisoning episode, Cox et al. (1989) estimated the maternal hair mercury concentration of approximately 10 μg/g as a safe level for prenatal mercury exposure. Taking into account uncertainty factors, the US Environmental Protection Agency (1997) then proposed a safe level (reference dose, *RfD*) for methylmercury exposure at 0.1 μg/kg body weight per day. However, since little information has been developed concerning the daily mercury intake in Japan (Shishido and Suzuki 1974), the significance of the *RfD* remains unclear. For this reason, we estimated the daily mercury intake from seafood in Japanese women by using a food frequency questionnaire (FFQ), to address the relationships between the *RfD* and daily mercury intakes, combined with hair mercury concentrations.

Materials and Methods

This research was carried out as a part of the Akita cross-sectional study on the effects of prenatal methylmercury exposure on child neurodevelopment. Prior to this, the study protocol was approved by the ethical review committee at the Akita University School of Medicine. The nature of the procedures used in the present study was explained to the parents at eight elementary schools, and mothers and the 7-year-old children were invited for this study during the period of July-September in 2002. The children at 7 years of age were chosen in accordance with the preceding study on the risk assessment of methylmercury exposure (Murata et al. 1999). The participating subjects, from whom informed consent was obtained, were 154 mothers at 35.7±4.2 (range, 25~48) years of age, and the children, residing in two cities and three towns of Akita Prefecture, Japan. Four of the eight schools, which the children went to, were located in near the fishing harbor. Hair samples were obtained, by cutting strands of hair close to the scalp, from the occipital area in all mothers and children. The hair length was generally about 10 cm, ranged from 1 to 30 cm. And, total mercury in aliquots of dried hair samples (15 to 20 mg), cut into small pieces (<2 mm) with scissors after being washed well with detergent and rinsed two times with acetone, was determined by the cold vapor atomic absorption spectrophotometry method at the National Institute for Minamata Disease (Akagi and Nishimura 1991).

Detailed survey on the frequency and volume of seafood ingested in a year was conducted by a trained interviewer at the schools or civic centers where examinations on child neurodevelopment were also done, showing 25 kinds of full-scale pictures including fish, shellfish and seaweed items (e.g., tuna, swordfish, skipjack tuna, codfish, flatfish, mackerel, sardine, sea bream, whale, salmon, eel, crab, prawn, octopus, squid, oyster, sea urchin, fish paste, shellfish, seaweed, and etc.) to each mother, based upon the FFQ (Nakai et al. 2003), i.e., a modified version of Date et al. (1996). Then, the total mercury intake from seafood (μg/year) was estimated on the basis of the previous references on mercury concentrations in seafood (Yamamoto et al. 1990; Nakagawa et al. 1997), and daily mercury intake was calculated dividing by 365 days. Also, questionnaires on hair-dyed and artificial hair waving were collected from the mothers.

The significance of the relationships between daily mercury intake and hair mercury concentrations was analyzed by the Spearman rank correlation coefficient (r_s). The Wilcoxon signed rank test was used to evaluate the difference in the hair mercury concentration between the mother and child. The two-way analysis of variance by the SS model of type II was available for evaluation of the interregional differences (i.e., between cities and towns, and between fishing and non-fishing areas). All analyses were performed using the Statistical Package for the Biosciences (SPBS V9.5) (Murata and Yano 2002).

RESULTS

The geometric mean of daily mercury intakes, calculated from the 154 mothers, was 15.3 (range, 2.65～48.4) μg/day. The geometric means of hair mercury levels were 1.73 (range, 0.49～5.82) μg/g in the mothers and 1.64 (range, 0.45～6.32) μg/g in the children, but there was no significant difference in the hair mercury concentration between the mother and child ($p>0.05$). The daily mercury intake was significantly correlated with hair mercury concentrations in the mothers and children (Fig. 1). Also, maternal hair mercury was significantly correlated with child's hair mercury ($r_s=0.291$, $p<0.001$). On the other hand, no significant differences in the daily mercury intake or hair mercury concentrations were found either between mothers residing in cities and towns or between those in fishing and non-fishing areas (Table 1 and Fig. 1). There were no significant correlations between their ages and either the daily mercury intake or hair mercury concentrations ($r_s=-0.011$～0.013, $p>0.05$).

As the average and standard deviation (S.D.) values of body weight were 54.6 and 9.55 kg in 16 353 women aged 30-44 years, residing in Akita Prefecture (2002's data of the Akita Prefectural Center of Health Care), body weight of 55 kg for mothers was used to convert daily ingested dose (μg/day) to that per body weight (μg/kg body weight per day). Assuming the methylmercury content rate of 75% in seafood mercury (Notification of the Environmental Hygiene Bureau, Ministry of Health and Welfare on July 23rd, 1973), the mothers were speculated to ingest methylmercury of 0.21 μg/kg body weight per day (geometric mean), as shown in Table 2.

A significant difference in the hair mercury level was seen between the 47 mothers with artificial hair waving (mean$\pm$S.D., 1.70$\pm$0.95 μg/g) and 107 mothers without (2.10$\pm$0.98 μg/g); this difference was statistically significant ($p=0.011$) when controlling for daily mercury intake. On the other hand, the hair mercury level did not differ significantly between the 120 mothers with dyed hair (1.95$\pm$1.03 μg/g) and 34 mothers without (2.07$\pm$0.78 μg/g).

DISCUSSION

This study showed that the daily dietary

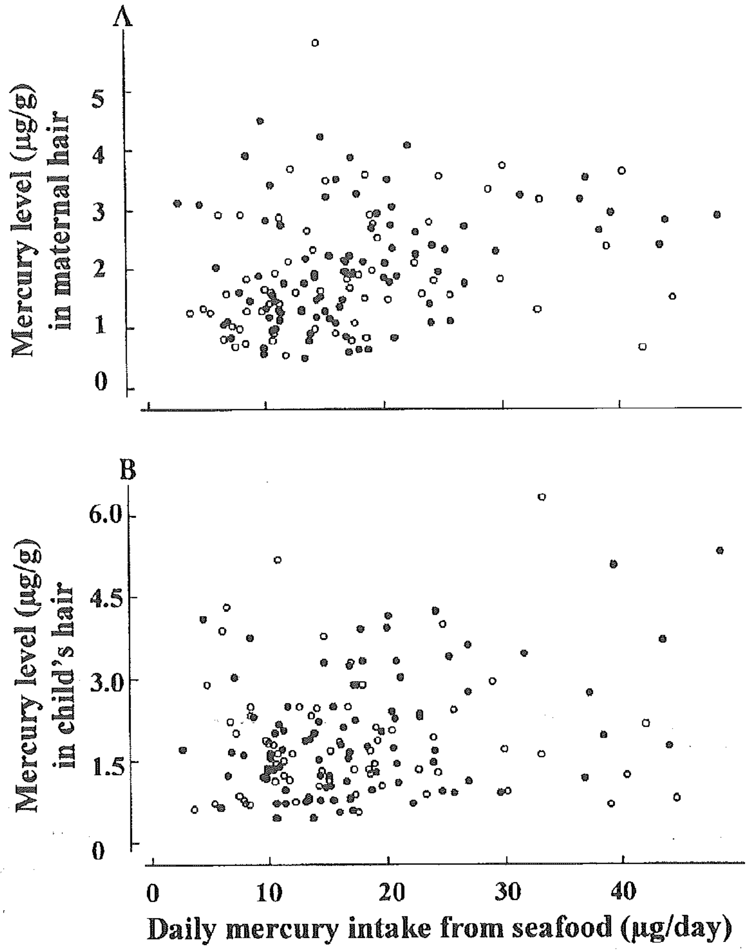

Fig. 1. Relationships between the daily mercury intake and hair mercury concentrations in 154 mothers and the children in Akita, Japan. r_s, ○ and ● indicate the Spearman rank correlation coefficient, and subjects residing in fishing and non-fishing areas, respectively.
A: Mercury level ($\mu g/g$) in maternal hair, $r_s = 0.335$, $p < 0.001$.
B: Mercury level ($\mu g/g$) in child's hair, $r_s = 0.161$, $p < 0.047$.

TABLE 1. *Residence-specific mercury concentrations and daily mercury intakes in 154 mothers and the children in Akita, Japan (number of samples, mean ± S.D.)*

	Non-fishing areas	Fishing areas
Mother's hair (μg/g)[a]		
Cities	56, 1.92±0.90	26, 1.90±1.22
Towns	35, 2.17±1.07	37, 1.95±0.87
Child's hair (μg/g)[b]		
Cities	56, 1.99±1.21	26, 1.76±0.98
Towns	35, 1.90±0.94	37, 1.96±1.24
Mother's daily intake (μg/day)[c]		
Cities	56, 19.0±9.9	26, 17.0±10.4
Towns	35, 15.9±7.0	37, 17.0± 9.0

[a] $F = 0.567$ ($p = 0.638$); [b] $F = 0.263$ ($p = 0.852$); [c] $F = 0.875$ ($p = 0.455$).

TABLE 2. *Distribution of daily methylmercury intakes (μg/kg body weight per day), estimated from 154 mothers in Akita, Japan, under the assumption that body weight of mother was 55 kg, and methylmercury-mercury ratio in seafood was 0.75*

Daily intake	Number of mothers	Proportion (%)
≦0.1	13	8.4
≦0.2	55	35.7
≦0.3	50	32.5
≦0.4	19	12.3
≦0.5	6	3.9
>0.5	11	7.2

intake of mercury (median 15.9 μg/day), examined by one interviewer using the FFQ, was significantly associated with hair mercury concentrations, especially in the mothers (median 1.8 μg/g). Shishido and Suzuki (1974) measured the contents of total and organic mercury from dishes of five daily diets, and the daily intakes ranged from 3.3 to 70.9 μg for total mercury and from 2.7 to 70.0 μg for organic mercury. According to the Faroe Islands Prospective Study, an average total mercury intake per person over age 14 could be calculated to be about 36 μg/day based on the data from the questionnaire study and mercury concentrations in whale and cod, and maternal hair mercury concentrations in the cohort showed a median of 4.5 μg/g (Grandjean et al. 1992; Weihe and Grandjean 1994). Concerning an interference of recall bias (that is, an inevitable problem on the FFQ), another study of the FFQ with 122 food items has reported that the correlation coefficients between nutrients estimated by the first and second tests conducted at an interval of one week (i.e., reproducibility) ranged from 0.64 for vegetable protein to 0.78 for calcium (Date et al. 1996). Therefore, this method with the FFQ is suggested to provide a useful approach for estimating daily mercury intake.

In the mothers of the present study, the daily methylmercury intake was calculated to be 0.21 μg/kg body weight per day, under the assumption that the methylmercury content was 75% of seafood mercury. Probably, children would also ingest a similar level of methylmercury, because there was no significant difference in the hair mercury level between the mother and child. The US Environmental Protection Agency (1997) described that the dose of 1.1 μg/kg body weight per day was the total daily quantity of methylmercury that was ingested by a 60 kg individual to maintain a hair concentration of 11 μg/g. Despite the different calculation process, this closely resembles our outcome in the relation of daily methylmercury intake to hair mercury concentration. If the above assumption is correct, daily methylmercury intake in more than 90% of Japanese women and children may exceed the *RfD* of 0.1 μg/kg body weight per day (US Environmental Protection Agency 1997; National Research Council 2000). For that reason, the *RfD* does not seem to be suitable for Japanese dietary lives, especially with respect to the achievement of the object. For the establishment of a reference dose of the daily methylmercury intake for Japanese, risk assessment of methylmercury exposure should be carried out in a Japanese cohort, like the Seychelles Child Development Study (Davidson et al. 1998) and the Faroe Islands Prospective Study (Grandjean et al. 1997). Moreover, additional study is required to determine the methylmercury concentration contained in each of fish, shellfish and seaweed directly.

Hair mercury concentrations in the mothers of Akita were below the safe limit (10 μg/g) of the International Programme on Chemical Safety (1990), and the mothers with artificial hair waving had lower hair mercury concentrations than did those without. Our study population consisted of 82 mothers in cities and 72 mothers in towns. Also, 666 137 persons resided in cities and 517 870 resided in towns in Akita Prefecture in March 2001, which were almost similar to our study population with regard of the residential distribution. Yasutake et al. (2003) examined total mercury levels of 1666 female hair samples collected from five districts of Japan, presenting that the geometric mean was 1.43 μg/g, and that the geometric mean in each district varied from 1.23 to 2.50 μg/g. Similar mercury levels in Japan have been reported by some researchers (Wakisaka et al. 1990; Sakamoto et al. 1993). In this way, although these hair mercury levels may have been somewhat underestimated due to artificial hair waving (Yamamoto and Suzuki 1978; Yasutake et al. 2003), our data appear to reflect current mean values in Japanese women.

Any significant difference in the daily mercury intake or maternal hair mercury level was not observed either between fishing and non-fishing areas or between cities and towns in Akita Prefecture (Table 1), although hair mercury levels in Chiba were significantly elevated when compared to those in Minamata, Kumamoto, Wakayama and Tottori (Yasutake et al. 2003); while, the authors did not describe any reason. In the time of the epidemic outbreak of methylmercury poisoning in Minamata, fishermen used to barter fish and shellfish for rice in the territorial society, independent of monetary economy. However, according to the rumor that intake of fish caught from Minamata Bay caused a peculiar disease, most of people except fishermen and their families did hardly eat fish contaminated by methylmercury, and patients with Minamata disease increased mainly in fishing villages (Doi 1994). In contrast, we can purchase all types of commodities including fish and shellfish everywhere because of infiltration of monetary economy and development of the distribution system. Thus, the absence of interregional differences in the daily mercury intake may have been attributable to the equalization of dietary lives.

In conclusion, the daily intake of dietary methylmercury from seafood in Japanese women was estimated to be between 0.09 and 0.53 μg/kg body weight per day (i.e., 5 and 95 percentiles). The actual intake may exceed the above estimate, because the current content rate of methylmercury in seafood mercury is suggested to be more than 0.75 (Kehrig et al. 1998). Anyway, since they are changeable through various circumstances, a continuous monitoring of methylmercury is necessary.

Acknowledgments

We thank Prof. Philippe Grandjean for his valuable suggestions, and Dr. Satoshi Terui and the Akita Prefectural Education Board for their cooperation of collecting the study population. This study was supported by a grant from Japan Ministry of the Environment.

References

Akagi, H. & Nishimura, H. (1991) Speciation of mercury in the environment. In: *Advances in Mercury Toxicology*, edited by T. Suzuki, N. Imura & T.W. Clarkson, Plenum Press, New York, pp. 53-76.

Bakir, F., Damluji, S.F., Amin-Zaki, L., Murtadha, M., Khalidi, A., Al-Rawi, N.Y., Tikriti, S., Dhahir, H.I., Clarkson, T.W., Smith, J.C. & Doherty, R.A. (1973) Methylmercury poisoning in Iraq. *Science*, **181**, 230-241.

Cox, C., Clarkson, T.W., Marsh, D.O., Amin-Zaki, L., Tikriti, S. & Myers, G.G. (1989) Dose-response analysis of infants prenatally exposed to methyl mercury: An application of a single compartment model to single-strand hair analysis. *Environ. Res.*, **49**, 318-332.

Date, C., Yamaguchi, M. & Tanaka, H. (1996) Development of a food frequency questionnaire in Japan. *J. Epidemiol.*, **6**, S131-S136.

Davidson, P.W., Myers, G.J., Cox, C., Axtell, C., Shamlaye C., Sloane-Reeves, J., Cernichiari, E., Needham, L., Choi, A., Wang, Y., Berlin, M. & Clarkson, T.W. (1998) Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study. *JAMA*, **280**, 701-707.

Doi, R. (1994) Minamata disease. In: *Toxicology Today: from Toxicology to Biomedical Sciences*, edited by H. Satoh, Kinpodo Publisher, Kyoto, pp. 93-108.

Grandjean, P., Weihe, P., Jørgensen, P.J., Clarkson, T., Cernichiari, E. & Viderø, T. (1992) Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. *Arch. Environ. Health*, **47**, 185-195.

Grandjean, P., Weihe, P., White, R.F., Debes, F., Araki, S., Yokoyama, K., Murata, K., Sørensen, N., Dahl, R. & Jørgensen, P.J. (1997) Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol. Teratol.*, **19**, 417-428.

Igata, A. (1993) Epidemiological and clinical features of Minamata disease. *Environ. Res.*, **63**, 157-169.

International Programme on Chemical Safety (1990) *Methylmercury (Environmental Health Criteria 101).* World Health Organisation, Geneva.

Kehrig, H.A., Malm, O., Akagi, H., Guimaraes, J.R. & Torres, J.P. (1998) Methylmercury in fish and hair samples from the Balbina Feservoir, Brazilian Amazon. *Environ. Res.*, **77**, 84-90.

Kurland, L.T., Faro, S.N. & Siedler, H. (1959) Minamata disease: the outbreak of a neurologic disorder in Minamata, Japan, and its relationship to the ingestion of seafood contaminated by mercuric compounds. *World Neurol.*, **1**, 370-395.

Murata, K., Weihe, P., Renzoni, A., Debes, F., Vasconcelos, R., Zino, R., Araki, S., Jørgensen, P.J., White, R.F. & Grandjean, P. (1999) Delayed evoked potentials in children exposed to methylmercury from seafood. *Neurotoxicol. Teratol.*, **21**, 343-348.

Murata, K. & Yano, E. (2002) *Medical Statistics for Evidence-Based Medicine with SPBS User's Guide.* Nankodo Publisher, Tokyo.

Nakagawa, R., Yumita, Y. & Hiromoto, M. (1997) Total mercury intake from fish and shellfish. *Chemosphere*, **35**, 2909-2913.

Nakai, K., Sakamoto, M., Murata, K., Oka, T., Suzuki, K., Okamura, K., Hosokawa, T., Mori, Y. & Satoh, H. (2003) Effects of perinatal exposures to methylmercury and environmentally persistent organic pollutants on neurobehavioral development in Japanese children: a protocol for the prospective cohort study. In: *Proceedings of NIMD FORUM 2002: The Study of Fetal Methylmercury Exposure and Children Development.* National Institute for Minamata Disease, Minamata, pp. 51-58.

National Research Council (2000) *Toxicological Effects of Methylmercury.* National Academy Press, Washington, DC.

Sakamoto, M., Nakano, A., Akagi, H., Kitano, T. & Futatsuka, M. (1993) Difference by sex and age of mercury concentration in red blood cells. *Jpn. J. Hyg.*, **48**, 911-919.

Shishido, S. & Suzuki, T. (1974) Estimation of daily intake of inorganic or organic mercury via diet. *Tohoku J. Exp. Med.*, **114**, 369-377.

United Nations Environment Programme Chemicals (2002) *Global Mercury Assessment.* UNEP Chemicals, Geneva.

US Environmental Protection Agency (1997) *Mercury Study for Congress. Volume V: Health Effects of Mercury and Mercury Compounds. EPA-452/R-97-007.* EPA, Triangle Prak.

Wakisaka, I., Yanagihashi, T., Sato, M. & Nakano, A. (1990) Factors contributing to the difference of hair mercury concentrations between the sexes. *Jpn. J. Hyg.*, **45**, 654-664.

Weihe, P. & Grandjean, P. (1994) Sources and magnitude of mercury exposure in the Faroe Islands: overall design of the cohort study. In: *Proceedings of the International Symposium on Assessment of Environmental Pollution and Health Effects from Methylmercury.* National Institute for Minamata Disease, Minamata, pp. 112-126.

Yamamoto, I., Matsuda K. & Sato, C. (1990) *Contents of Heavy Metals in Seafood along the Coast of Hokkaido.* Hokkaido Institute of Public Health, Sapporo.

Yamamoto, R. & Suzuki, T. (1978) Effects of artificial hair-waving on hair mercury values. *Int. Arch. Occup. Environ. Health*, **42**, 1-9.

Yasutake, A., Matsumoto, M., Yamaguchi, M. & Hachiya, N. (2003) Current hair mercury levels in Japanese: survey in five districts. *Tohoku J. Exp. Med.*, **199**, 161-169.

トピックス

メチル水銀の基準摂取量のゆくえ

村田勝敬[1]、嶽石美和子[2]、佐藤　洋[3]

米国環境保護庁（U.S. Environmental Protection Agency, EPA）は、メチル水銀に感受性の高い小集団（特に、妊娠中に曝露を受けた胎児）の健康への影響を防止する目的で、メチル水銀の基準摂取量（毎日摂取しても人体に影響を及ぼさないとされる量、RfD）を 0.1 μg/kg/日と 1995 年に定めた。現在、EPA はこの RfD の改訂作業を行っている。日本ではどのようにして算出されたのかよく理解されないまま、この数値が一人歩きしているように思われるので、算出の経緯とどのような意味を持つのかについて解説する。

リスク評価のための集団

水俣病で代表されるメチル水銀中毒は、日本では工場排水を、またイラクでは食品汚染を介して集団発生した。日本の水俣病研究では、発生当時の曝露評価が行われていなかったので、メチル水銀の人体影響に関する量－反応関係を検討することができなかった。これに対し、イラクのメチル水銀中毒禍は、発生直後より米国の研究者が曝露および影響評価を行っていた。1980 年代半ばには、幾つかのメチル水銀に関する前向き疫学調査が研究の途についたばかりであった。このため、EPA は RfD を決定するに当たり、イラクの研究を採用したのである。

1)　秋田大学医学部環境保健学教授

2)　同大学院生

3)　東北大学大学院医学系環境保健医学教授

イラクの研究では、妊娠中にメチル水銀で処理された小麦から作ったパンを摂取した女性から生まれた子どもの神経発達異常（18 ヶ月児の歩行および 24 ヶ月児の言語の遅れ）、脳性麻痺、深部腱反射異常等が二人の神経内科医によって調べられた。また、母親の頭髪はＸ線蛍光分光光度計で分析され、水銀濃度は 1 ～674 μg/g (ppm) であった。これらの母子 81 組のデータを用いて、メチル水銀曝露による量－反応関係が検討された。

毛髪－血中濃度比率の算出

頭髪の水銀濃度はその毛が生成されるときの血中メチル水銀濃度を反映する。頭髪の長さは 1 月に 1 cm伸びるとされ、またイラク女性の髪は非常に長かったので、妊娠期間中の水銀濃度を遡って推定することが可能であった。頭髪水銀濃度と同時期の血中水銀濃度の関係については多数の報告があり、250：1（頭髪水銀 μg/g：血中水銀 μg/ml）が採用されている。これにより、妊娠中の母体の血中濃度は、頭髪水銀濃度から算出される。

臨界濃度の決定

小児に神経発達の影響が現れ始める濃度（臨界濃度）は、Marsh らが報告した種々の神経影響を全て考慮した発症率と妊娠期間中の母親の頭髪水銀濃度から、ベンチマークドース（BMD）法

（図）で推定された。**EPA** が実際に使用した量－反応関係は、$P(d)=P_0+(1-P_0)(1-\exp[-\beta \cdot d^k])$ で表され、d は曝露量、P_0 はバックグラウンド反応率（=0.12468）、β は勾配（=$9.47 \cdot 10^{-3}$）、k は形状母数（=1.000）であった。ここで算出された **BMD** は頭髪水銀濃度で 11 μg/g であり、換算式により血中水銀濃度 44 μg/ℓとなった。

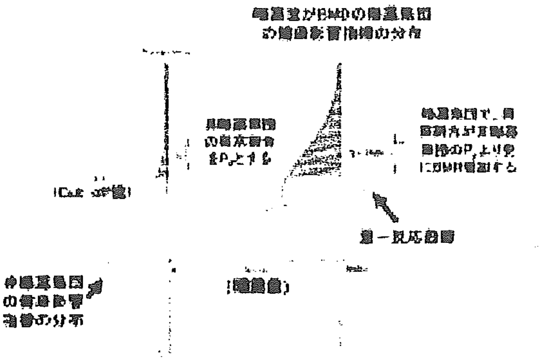

図　BMD の基本的考え方－ベンチマークドース（BMD）は、バックグラウンド反応率（P_0）を超えて一定量の異常増加（これをベンチマークレスポンスと呼ぶ，BMR）をもたらす時の曝露レベルである

RfD の算出

ある血中濃度（C、上述値 44 μg/ℓ）に対応する一日当たりのメチル水銀の食事摂取量（**d**、μg/日）は下式で示される。

$$d = C \cdot b \cdot V / (A \cdot f)$$

ここで用いられた記号は、吸収率（**A**）、体内総水銀の循環血液中に存在する割合（**f**）、排泄定数（**b**）、体内総血液量（**V**）である。この式の意味は $d \cdot A \cdot f = C \cdot b \cdot V$ と変形すると理解しやすい。すなわち、右辺はメチル水銀が血中に存在する量（**C・b**）のうち１日に排泄される量を表す。左辺は１日当たりのメチル水銀摂取量のうち体内に取り込まれる量（**d・A**）が血液中に存在する量を示し、排泄量と維持（摂取）量は平衡状態（血液以外のところにあるメチル水銀量は変化しないと仮定）であれば等しくなる。これらの数値は、以下の実験・研究により決定された。

(1) 吸収率Ａ　放射性同位元素でラベルした硝酸メチル水銀を水に溶かし、健常ボランティア３名に与えた後の体内への吸収率は 95%以上であった。別の研究者による同様の実験でも確認され、これより吸収率は 0.95 と定められた。

(2) 体内水銀の血中に存在する比率 f　ヒト体内に吸収されたメチル水銀が循環血液中に存在する割合に関して、幾つかの研究報告がある。ある研究者は、メチル水銀に汚染された鮪を食べた成人男子５名の結果に基づき、吸収量の 0.059 が総血液の中に存在すると推定した。また、別の研究者は ^{203}Hg-メチル水銀を含む魚を食べた男性９名と女性６名で、曝露後数日で血液１ℓにつき総負荷量の約 10%が現れ、その後 100 日以上経過して約５%になったと報告した。このようにして、血中に存在するメチル水銀割合 **f** として 0.05 が採用され、WHO も同じ値を用いている。

(3) 排泄定数 b　メチル水銀の半減期については、４つの研究で毛髪あるいは血中のメチル水銀濃度の測定から 35～189 日と推定され、その平均は 0.014 であった。また、魚に含まれる 43～233 μg/日の水銀を３ヵ月間摂取した 20 名のボランティアから得られた平均も 0.014 であった。以上より **b** 値として 0.014 /日が用いられた。

(4) 体内総血液量Ｖ　血液量は通常体重の７%であるといわれているが、妊娠中は血液量が 20%から 30%増えることがあり、体重当たりの血液量は約 8.5～９%になる。イラク女性の体重に関するデータはなかったので、妊娠中の体重を 58 kg、血液量を９%と仮定すると、血液量は 5.22ℓとなる。計算では **V** 値として５ℓが採用された。

以上より、血中水銀濃度 44 μg/ℓを生じる一日当たりのメチル水銀の食事摂取量 **d** を計算することができるが、さらに体重 60 kg (**bw**、上の 58kg を四捨五入）の人が摂取したと仮定すると、$d = C \cdot b \cdot V / (A \cdot f \cdot bw)$ となる。この式に前述の数値を当てはめると、$d = 44\mu g/\ell \cdot 0.014/日 \cdot 5\ell / (0.95 \cdot 0.05 \cdot 60kg)$ であり、血中 44 μg/ℓまたは毛髪中 11 μg/g のメチル水銀濃度を維持する一

日当たりの食事摂取量として 1.1 μg/kg/日が導出される。

RfD は、さらに不確実係数（UF）と修飾係数（MF）を加味し、以下の式で算出される。

RfD ＝d／（UF・MF）
＝(1.1 μg/kg/日)/(10・1)
＝0.1 μg/kg/日

UF の 10 は、①2世代間の生殖要因（母体血から胎児血へのメチル水銀の透過率や胎内での神経毒性影響など）の不確実性、②ヒト集団に内在するバラツキ（特に、メチル水銀の幅広い生物学的半減期や毛髪－血中濃度比率に起因するバラツキ）、③長期曝露からの後遺症に関するデータの欠如を考慮しての結果である。また、MF には 1 を使用している。

おわりに

現在、米国国立科学アカデミー（National Academy of Sciences, NAS）が選択したフェロー諸島前向き研究の7歳児の結果による BMD（および RfD）の見直し作業とともに、14 歳児における神経影響の解析結果の評価がおこなわれている。このフェロー研究では、母親の出産時の頭髪水銀濃度および臍帯血水銀濃度が測定されており、より直接的な出生前後の“胎児”の血中水銀濃度を測定していることが最大の強みである。

また、追跡調査としての 14 歳児データは、出生後のメチル水銀曝露の影響を評価することもできる。入手可能なデータの中で、メチル水銀に最も感受性の高い影響指標として、胎児期曝露による小児神経発達影響が NAS の委員会で是認されている。しかし、免疫・心血管系への低濃度曝露影響も示唆されており、何を影響指標とするかが今後の検討課題となりそうである。

RfD は 0.1 μg/kg/日と算出されたが、上述の UF は専門家（NAS）による判断、公衆衛生の目標、EPA の規制勧告等に影響される政策的な係数と考えるべきである。かかる意味で、UF に含まれるヒト集団に内在するバラツキ（b、V、A、f）や胎児の曝露濃度がたとえ正確に算出できるようになっても、米国においては UF＞1 である可能性が高い。

一方、メチル水銀の RfD の改訂は、公衆衛生とともに環境保全に大いに関連する。米国の州機関が水質基準の確立や水銀の大気・水への放出規制の設定に RfD を使用するだろうし、既に 40 州で淡水産魚介類摂取に係わる勧告が出されている。

このように RfD の改訂は、産業界における排出方法やリサイクルの選択肢だけでなく、魚市場や人々の食物選択に影響を及ぼす可能性がある。それゆえ、一層の科学的根拠に基づく RfD の改訂がおこなわれることを EPA に望むとともに、わが国でも、国民の理解の得られる同様の基準値を設定することが緊急の課題であると考えられる。

文献は “US EPA: Mercury Study for Congress. Volume V: Health Effects of Mercury and Mercury Compounds. EPA-452/R-97-007 (1997)” に大半が記載されていますので、関心ある方は http://www.epa.gov/oar/mercury.html からダウンロードして下さい。また、関連情報として、日衛誌 57 巻 564-570 頁 (2002)をご参照下さい。

Tohoku J. Exp. Med., 2004, **202**, 227-237

The Tohoku Study of Child Development: A Cohort Study of Effects of Perinatal Exposures to Methylmercury and Environmentally Persistent Organic Pollutants on Neurobehavioral Development in Japanese Children

KUNIHIKO NAKAI, KEITA SUZUKI, TOMOKO OKA, KATSUYUKI MURATA,[1] MINESHI SAKAMOTO,[2] KUNIHIRO OKAMURA,[3] TORU HOSOKAWA,[4] TAKEO SAKAI,[5] TOMOYUKI NAKAMURA,[6] YOSHINORI SAITO,[6] NAOYUKI KUROKAWA, SATOMI KAMEO and HIROSHI SATOH

Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai 980-8575,
[1]Division of Environmental Health Science, Akita University School of Medicine, Akita 010-8543,
[2]National Institute for Minamata Disease, Minamata 867-0008,
[3]Department of Obstetrics, Tohoku University Graduate School of Medicine, Sendai 980-8575,
[4]Department of Human Development, Faculty of Education, Tohoku University, Sendai 980-8576,
[5]Miyagi Children's Hospital, Sendai 989-3126, and
[6]Miyagi Prefectural Institute of Public Health and Environment, Sendai 983-8666

NAKAI, K., SUZUKI, K., OKA, T., MURATA, K., SAKAMOTO, M., OKAMURA, K., HOSOKAWA, T., SAKAI, T., NAKAMURA, T., SAITO, Y., KUROKAWA, N., KAMEO, S. and SATOH, H. *The Tohoku Study of Child Development: A Cohort Study of Effects of Perinatal Exposures to Methylmercury and Environmentally Persistent Organic Pollutants on Neurobehavioral Development in Japanese Children.* Tohoku J. Exp. Med., 2004, **202** (3), 227-237 ── Several birth cohort studies have shown adverse effects of perinatal exposures to methylmercury (MeHg) and environmentally persistent organic pollutants (POPs). These chemicals are ingested mainly through fish consumption, but little is known about the hazardous effects in Japanese, whose fish consumption is high. The present study, the Tohoku Study of Child Development, was designed to examine the effects of perinatal exposures to MeHg, polychlorinated biphenyls (PCB), dioxins, pesticides, and other chemicals in Japanese children. Six

Received January 8, 2004; revision accepted for publication February 13, 2004.
Address for reprints : Kunihiko Nakai, V.M.D., Ph.D., Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine, 2-1 Seiryomachi, Aoba-ku, Sendai 980-8575, Japan.
e-mail: nakaik@mail.tains.tohoku.ac.jp

Some results from this study were presented at the NIMD Forum 2003 held at Niigata, Japan, on November 20, 2003.

hundred eighty-seven pregnant women were participated in this study with their written informed consent. Maternal peripheral blood, cord blood, cord tissue, placenta, and breast milk samples were collected for chemical analysis. Maternal hair was also taken for MeHg analysis. Infants born at full term were assessed by neurobehavioral tests: the Brazelton Neonatal Behavioral Assessment Scale at three days old, the Kyoto Scale of Psychological Development and the Bayley Scales of Infant Development at 7 and 18 months old, and the Fagan Test of Infant Intelligence at 7 months old. The children will be continuously followed up to ages 6-7 years. Maternal food intake frequency, maternal IQ, socioeconomic status, and home environment were assessed as covariates. The results of this cohort study will allow us to evaluate associations between the neurobehavioral development of children and perinatal exposures to MeHg and environmentally POPs in Japan. ——— cohort; development; dioxin; methylmercury; polychlorinated biphenyls

The neurobehavioral effects of prenatal exposures to methylmercury (MeHg) and environmentally persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), dioxins, and pesticides are of great concern worldwide (Nakai and Satoh 2002). It was shown that prenatal MeHg exposure causes the delay of development of cognitive functions in Faroe Islands (Grandjean et al. 1997), Madeira Islands (Murata et al. 1999), and New Zealand (Kjellstorm et al. 1986), although studies conducted in the Seychelles showed the absence of toxic effects of prenatal exposures to MeHg (Davidson et al. 1998). Several epidemiological studies have also shown the evidence of the adverse effects of perinatal PCB exposure on neurodevelopment. Cohort studies in North Carolina (Rogan et al. 1986), Michigan (Jacobson et al. 1985, 1990), NewYork (Darvill et al. 2000; Stewart et al. 2000), The Netherlands (Patandin et al. 1999; Vreugdenhil et al. 2002), Germany (Winneke et al. 1998; Walkowiak et al. 2001), and Faroe Islands (Grandjean et al. 2001) demonstrated negative associations between perinatal PCB exposure and cognitive functions in children.

MeHg and POPs constitute a group of persistent environmental chemicals. Due to their hydrophobic nature and resistance towards metabolism, they are found in every level of the food chain. Consequently, these chemicals accumulate in humans mostly through the consumption of food, particularly that of fish and shellfish origins. Indeed, the consumption of fish and shellfish is the major route of dioxin exposure (>80% of all food sources) in Japan (Ministry of Health, Labour and Welfare 2002). From the nutritional perspective, fish is usually recommended for pregnant women because it is rich in some nutrients such as n-3 polyunsaturated fatty acids (PUFA) essential for the perinatal growth of the brain. Therefore, from the perspective of risk assessment, the above health hazard issues are particularly of importance in fish-eating populations.

In this report we present a protocol of our cohort study, the Tohoku Study of Child Development, on the effects of perinatal exposures to MeHg and POPs on neurobehavioral development among Japanese children. We hypothesize that the prenatal/postnatal exposures to the above chemicals delay or disturb the normal growth and neurobehavioral development of children. Exposure assessment includes measurements of multiple chemicals that may potentially affect the child development. Health risk of children was mainly evaluated by neurobehavioral tests. In studies designed to examine neurobehavioral development, multiple confounding factors including food intake habit, home environment,

TABLE 1. *Inclusion criteria for the Tohoku study*

Mother
1. Absence of thyroid dysfunction, mental and psychological diseases, hepatitis, immune deficiency, malignant tumor, diabetes mellitus requiring antidiabetic agents, and any other severe diseases that may affect the normal growth of fetus
2. No severe preeclampsia and severe gestational diabetes mellitus
3. No in vitro fertilization
4. Japanese as the mother tongue
5. Written consent

Infant
1. Absence of congenital anomalies or severe diseases
2. Singleton birth at term from 36 to 42 weeks of gestation
3. Body weight of more than 2400 g, and when the term was 36 weeks of gestation, body weight of more than 2500 g

socioeconomic status, and others must be considered. These issues that must be considered in a study design are reported.

Study design

Recruitment of cohort. Healthy pregnant women were recruited with their informed consent at obstetrical wards of two hospitals in Sendai. To establish an optimal study population, only infants born at term (36 to 42 weeks of gestation) without congenital anomalies or diseases are included. Pregnancy and delivery should have been completed without overt signs of serious illness or complications. The inclusion criteria are shown in Table 1. The study protocol was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine.

Sample collections. The hair samples were collected from the mothers after delivery. Most epidemiological studies on MeHg exposure have used mercury concentration in hair to estimate the body burden (WHO 1990). Since hair growth rate is independent of gender or racial differences (Cernichiari et al. 1995), by assuming a constant rate of hair growth equal to 1.1 cm per month (Cox et al. 1989), it is possible to generate a profile of MeHg exposure based on the mercury concentrations in serial segments of scalp hair. The hair samples were cut next to the scalp, in the nape area, with stainless steel scissors. The samples were placed in a plastic bag and kept in a desiccator until analysis.

Since most commercially available plastic and glass materials are possibly contaminated with a significant amount of chemicals such as POPs, all glassware used for sample collection and storage was treated by heating at 400°C in a chemically clean chamber to exclude the possible contamination with PCBs and dioxins. All other materials were confirmed to be clean before use.

Blood samples were collected from mothers at 28 weeks of pregnancy. For blood collection, a vacuum system heparin tube confirmed to be without contamination was used to collect peripheral blood (30 ml), and centrifuged within 4 hours for 20 minutes at 3000 rpm; plasma and whole blood were stored at –80°C until analysis.

A blood sample (more than 50 ml) from the umbilical cord was collected into a bottle using heparin as the anticoagulant after the delivery. Placenta and cord tissues were also collected after the delivery. Since the placenta is a large organ, which is a heterogeneous mixture of placental cells and decidual tissues containing maternal and fetal blood, representative samples of placenta were obtained as follows: the placenta was divided into 20-30 pieces that were randomly separated