※ This report is translated by Food Safety Commission Secretariat, The Cabinet Office, Japan.

Food Safety Risk Assessment Related to Methylmercury in Seafood

August 4, 2005

The Food Safety Commission, Japan
The Contaminant Expert Committee

Methylmercury in Seafood

1. Introduction

To assure safety against methylmercury in seafood, the Ministry of Health, Labour and Welfare collected opinions of Joint Sub-Committees on Animal Origin Foods and Toxicology under the Food Sanitation Committee (Pharmaceutical Affairs and Food Sanitation Council(1))and published "Advice for Pregnant Women on Fish Consumption concerning Mercury Contamination" for pregnant and potentially pregnant women.

Subsequently, after the application of the conventional assessment to a general population was reconfirmed, methylmercury was reassessed in the 61st Joint FAO/WHO Expert Committee on Food Additives (JECFA) in mid-June 2003 with a concern that fetuses and infants might have greater risks, based upon the results of epidemiological studies conducted in the Seychelles and Faroe Islands on effects of prenatal exposure to methylmercury via seafood on child neurodevelopment(the 61st JECFA (2), WHO (3)).

To reassess the above Advice, the Ministry of Health, Labour and Welfare recently requested a food safety risk assessment of "methylmercury in seafood" to the Food Safety Committee in the document No. 07230001 from the Department of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare under the date of July 23, 2004, in accordance with Article 24, Paragraph 3, Food Safety Basic Law (Law No. 48 2003).

The concrete contents of the document requested for not only setting a tolerable intake of methylmercury to reassess the Advice for pregnant women and others concerning intake of methylmercury in seafood, but also discussing a high-risk group that might be subject to the Advice, since the high-risk group is not necessarily the same as those in foreign countries.

2. Summary of methylmercury

(1) Physicochemical properties of methylmercury

In general, methylmercury is a solid crystal at room temperature. There are various compounds with different boiling and melting points, including methylmercury chloride, methylmercury bromide, and methylmercury iodide. In addition, they are usually readily soluble in organic solvents.

(2) Dynamic state in the environment (Mechanisms of methylmercury formation)

Circulation of mercury[a.b.c] on Earth is well understood; released mercury vapour is water soluble (e.g. Hg^{++}) and depositsin soil and water after rainfall. Mercury vapour remains in the atmosphere for 0.4-3 years, while the persistence of water-soluble compounds is for several weeks.

Its migration in soil and water is limited in this manner; therefore, it seems that

deposition of mercury occurs within a small area. Chemical reactions from inorganic mercury to methyl compounds are the first step in the biological accumulation process in the hydrosphere. Methylation reactions are caused non-enzymatically or by bacterial actions (WHO[4]).

Formed methylmercury seems to further accumulate in large predatory fish that humans consume and in marine mammals such as toothed whales via food chain or biological accumulation in the water biosphere.

(3) Methylmercury in seafood

For many people, seafood is considered the major source of methylmercury exposure among foods, and its concentration is generally ≦0.4ppm (mg/kg). However, it often exceeds 5ppm in fish that rank higher in the food chain, and relatively high-levels of methylmercury is contained in aged large predatory fish and toothed whales (the 61st JECFA[2], WHO[3], [4]).

(4) Methylmercury intake from foods

Since no data concerning methylmercury intake are available, the data of total mercury intake are summarized below.

According to the Total Diet Surveillance conducted by the Ministry of Health, Labour and Welfare, mercury intake (total mercury) from foods by Japanese was reported to be 1.1μg/kg bw/week[d] (8.1μg/person/day) in 2003, and an mean of 1.2μg/kg bw/week (8.4μg/person/day) in the past decade between 1994 and 2003 (Figure 2). Among them, 84.2% and 15.8% of mercury is reported to be consumed from seafood and other foods, respectively (2003) (Pharmaceutical Affairs and Food Sanitation Council[1]).

On the other hand, in the 61st JECFA, exposure levels in other foreign countries were reported to be 0.3-1.5μg/kg bw/week (GEMS/Food Diet in 5 regions) and 0.1-2.0μg/kg bw/week (intake surveillance in several countries) (the 61st JECFA[2], WHO[3]).

Meanwhile, seafood are the dominant source of methylmercury exposure; it is estimated that methylmercury accounts for 75-100% of total mercury in seafood (the 61st JECFA[2]). In addition, methylmercury levels in *Auxis rochet*, *Squalus acanthias*, and *Prionacee glauca* captured in the Adriatic Sea in 1999 accounted for 69-100% of total mercury (Storelli et al., 2001 [5]).

(5) Indexes of methylmercury exposure

Methylmercury in foods is absorbed from the gastrointestinal tract at a high rate (95-100%). Vaporized methylmercury is absorbed from the lungs, with an absorption rate of approximately 80%. Methylmercury is also absorbed from the skin, but its absorption rate is unknown (Berlin 1979[6]). Since absorbed methylmercury has a high affinity to SH groups, it is supposed to bind to amino acids such as protein, cysteine, and glutathione (Toxicology Today). Cysteine-methylmercury complexes cross the blood-brain barrier and are transported to the brain by the neutral amino acid transport system. This is supposed to be one of the reasons for the strong toxicity that methylmercury exhibits to the central nervous system.

Erythrocytes contain ≧90% of methylmercury in the blood. Methylmercury is incorporated into hair at a fixed ratio to blood at time of hair formation. The typical ratio of hair to blood levels is 250:1 in the steady state. People who are unexposed to inorganic mercury and have a normal diet, inorganic mercury is ≦10%in both blood and hair, and a large part of the mercury measured as total mercury is reasonably considered as methylmercury (or methylmercury derivatives).

Since methylmercury is excreted into bile after glutathione conjugation, feces are its excretory route. However, a large part of the methylmercury is transformed into the cysteine complex and is reabsorbed in the intestine . In vivo, a small amount reacts to form inorganic compounds, where mechanisms involving intestinal bacteria or active oxygen are possible. Inorganic compounds formed in the intestine stimulate excretion into feces. In addition, absorbed inorganic mercury in the body is mainly excreted from the kidney, and mercury excreted with bile into the intestine is poorly reabsorbed; therefore, it is excreted with feces from the body.

Since blood mercury levels are well-correlated with those of brain and other organs, methylmercury levels in blood and erythrocyte are considered good exposure indexes. In addition, hair methylmercury levels are a good exposure index due to a constant ratio between hair and blood methylmercury levels. In practice, measurement of methylmercury levels is difficult; therefore, measurement results of total mercury are often used as an index of methylmercury exposure, since, as described above, a large part of the mercury in the blood, erythrocytes, or hair is methylmercury, provided that there is no exposure to inorganic mercury, or, in case of hair, no external contamination (Berlin 1979[6]). In fact, hair mercury levels of a population, which seems to reflect the exposure to inorganic mercury due to little or no fish intake, is reported to be in the range of 0.2-0.8μg/g. Therefore, use of total hair mercury levels as an index of methylmercury exposure is unlikely to cause errors in a fish-consuming population with mercury levels that are significantly higher than this range (NRC[7]).

3. Findings of methylmercury toxicity

Findings of toxicity of methylmercury to the living body are summarized in excellent review articles (WHO[4], NRC[7], ATSDR[8], etc.) such as the WHO Environmental Health Criteria (EHC), and effects of toxicity on the central nervous system are known to be the most typical cases. When consumed orally, methylmercury is rapidly absorbed from the intestine and rapidly distributed throughout the body via blood. High intake may cause poisoning like Minamata disease and the cases in Iraq (poisoning caused by intake of seed wheat treated with methylmercury fungicide).

In particular, the central nervous systems of developing fetuses with immature blood-brain barriers are most susceptible to methylmercury. As described above, humans are exposed to methylmercury mainly via seafood; therefore, importance of investigation on methylmercury exposure to pregnant women who reside in ordinary environments and the effects on their fetuses has been suggested. In recent years, tolerable intake has been studied by major international organizations.

(1) Major epidemiological studies on methylmercury (See Table 2)

① The Faroe islands study (cohort study) (Appendix 1)

1,023 pairs of children born between March 1, 1986 and December 31, 1987 (75.1% of the total births in this period) and their mothers were registered as a cohort, and neurobehavioral development tests were conducted at ages of 7 and 14 years of ages. Statistically significant relationships between prenatal methylmercury exposure and several neurophysiological and neuropsychological endpoints were observed.

② The Seychelles child development study (cohort study)(Appendix 2)

As a pilot study, the Revised Denver Development Screening Test (DDSTR) and other tests were conducted on a cohort of 804 pairs of mothers and children born in 1987 and 1989 at 5-109 weeks and 66 months after birth. The results showed significant but unclear mercury effects.

In themain study, neurodevelopmental tests were conducted at 6.5, 19, 29, and 66 months, and 9 years of ages with a cohort of 779 pairs of mothers and children born between 1989 and 1990. No effects of methylmercury exposure on nerves, cognition, and behavior of children were observed at any ages.

③ Epidemiological study in New Zealand (cohort study)

Hair mercury levels of approximately 1,000 mothers who consumed fish ≧3times/week were measured. Mothers were divided into a high mercury level group (73 mothers with ≧6ppm of hair mercury levels; 74 children including one twin sibling) and a control group, and a Denver Development Screening Test （DDST） was conducted with 38 children at 4 years of age. The results showed a statistically significant difference; 17% in the control group and 50% in the high mercury level group showed abnormal or suspicious results(Kjellström et al., 1986[9]).

Subsequently, investigation of the Wechsler Intelligence Scale for Children, Revised Manual (WISC-R) and the Test of Language Development (TOLD) were conducted on 57 pairs of children at ages of 6 and 7 and were compared to 3 control groups: 1)Mothers with 3-6ppm hair mercury level during pregnancy, 2)Mothers with ≦3ppm hair mercury level during pregnancy, who eat fish more than 3 times a week, and 3)Mothers with ≦3ppm hair mercury level during pregnancy, who infrequently eat fish. The comparison results showed lower test scores associated with an average hair mercury level during pregnancy of 13-15ppm; however, contribution of methylmercury exposure was small and ethnic backgrounds of children had a significant impact (Kjellström et al., 1989[10]).

(2) Other major studies on toxicity

Studies on cardiotoxicity

Recently, a cohort study (the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD)) has been conducted in Eastern Finland to identify the risk factors on the cardiovascular system and several papers have been published. The subjects were 3,235 Finnish males of 42, 48, 54, or 60 years of ages at the time of baseline surveillance, of which 2,682 (82.9%) participated. The results are described below.

① 1,833 subjects were selected to investigate the relationship between their hair mercury levels and prevalence of acute myocardial infarction (AMI) or death rates of coronary heart disease (CHD) and cardiovascular disease (CVD) (follow-up period: 2-7 years, the mean of approximately 5 years). After statistically adjusting age, CHD, and fish consumption, "men in the highest tertile (≧2.0μg/g) of hair mercury content had a 2.0-fold (95% confidence interval, 1.2 to 3.1; P=0.005) age- and CHD-adjusted risk of AMI and a 2.9-fold (95% CI, 1.2 to 6.6; P=0.014) adjusted risk of cardiovascular death compared with those with a lower hair mercury content" (Salonen et al., 1995[11]).

② A 4-year follow-up study on atherosclerosis was conducted on 1,014 subjects. After adjusting systolic blood pressure, anti dyslipidemic medication, cigarette pack-years, age, dietary iron intake, dietary intake of vitamin C, plasma fibrinogen, high-density lipoprotein (HDL), maximal oxygen uptake, serum fructosamine, and fatty acid, an ultrasound test was conducted on the carotid artery of each male to examine the intima-media thickness (IMT). Compared with the other groups, the intima-media of the groups with ≧2.81mg/kg of hair mercury level was 32% thicker(Salonen et al., 2000[12]).

③ A follow-up study on risk factors for ischemic heart disease was conducted on 1,871 subjects (an average follow-up time of 13.9 years). After adjusting age at time of the baseline surveillance; serum HDL, low-density lipoprotein (LDL) cholesterol, family history of ischemic heart disease, systolic blood pressure, BMI, maximal oxygen uptake, urinary excretion of nicotine metabolites, serum selenium, serum docosahexaenoic acid (DHA) + docosapentaenoic acid (DPA), and intake of alcohol, saturated fatty acids, fiber, and vitamins C and E; risks of acute coronary event, CVD, and CHD increased 1.6 fold (95% confidence interval, 1.24 to 2.06), 1.68 fold (95% confidence interval, 1.15 to 2.44), and 1.56 fold (95% confidence interval, 0.99 to 2.46), respectively, in the groups with ≧2.03mg/kg of hair mercury levels, compared with the other groups(Virtanen et al., 2005[13]).

In another study, a case-control study was conducted between 684 males aged 70 years and younger who were diagnosed with myocardial infarction and lived in Israel or 8 countries in Europe and 724 males of similar ages who lived in the same region. After adjusting age; center, DHA, body mass index (BMI), smoking, alcohol, high density lipoprotein (HDL), diabetes, hypertension, parental myocardial infarction, α-tocopherol, ß-carotene, and selenium; the odds ratio of myocardial infarction was 2.16 (95% confidence interval: 1.09-4.29) in the group with the highest foot nail mercury levels in 5 groups that were divided by foot nail mercury levels (Gualar et.al., 2002[14]).

On the other hand, there is a surveillance that suggests no relationship. The relationship between foot nail mercury levels and CHD risks was examined in 33,737 healthy males aged between 40 and 75 years, and after 5 years of a follow-up period, 470 CHD cases were recorded. After adjusting age at time of being affected with CHD, smoking and other risk factors, mercury levels did not show any significant association with CHD risks. Comparison between the groups with highest and lowest levels showed a relative risk of 0.97 (95% confidence interval, 0.63 to 1.50) in the highest-level group (Yoshizawa et al., 2002[15]).

4. Benefits of seafood consumption

In a cohort of 7,421 children born between 1991 and 1992 in Britain, fish consumption

by mothers during pregnancy and development of verbal and communication abilities in the children were investigated.

Fish consumption by mothers and children were examined by questionnaire, and cognitive development of the children was examined at the ages of 15 months and 18 months by use of the MacArthur Communicative Development Inventory and DDST, respectively. Cord blood mercury levels of 1,054 children were also examined. The results showed low total mercury levels, demonstrating no relationship with neurologic development.

On the other hand, a relationship was observed between fish consumption by pregnant women or children and developmental scores. For example, the adjusted mean MacArthur Comprehension score for children whose mothers consumed fish ≧4times/week was 72 (95% confidence interval, 71 to 74) compared with 68 (95% confidence interval, 66 to 71) among those whose mothers did not consume fish. The results indicate that a relationship exists between fish consumption by mothers during pregnancy and development of verbal and communication abilities in children; moderate fish consumption seems to have positive effects on children's development(Daniels et al., 2004[16]).

Furthermore, the Committee on Toxic Effects of Methylmercury, the National Research Council (NRC) of the American Academy of Science and Technology reported that cardiovascular diseases, osteoporosis, and cancers can be prevented to some extent by recognizing the nutritional advantages of diets rich in fish, that contains many nutrients, including vitamin D, omega-3(n-3) polyunsaturated fatty acid, proteins, selenium, as well as other nutrients that are low in some diets, and by consuming fish on a regular basis (NRC[7]).

As described above, fish are considered beneficial to the health of pregnant women, children, and adults; therefore, consumption of small fish (sardine, horse mackerel and others), in which biological concentration is unlikely, should be recommended and not restricted.

5. Risk assessment in Japan and international organizations (Table 3)

(1) Ministry of Health, Labour and Welfare

In July 1973, the "Expert Committee on Mercury in Seafood" established by the Ministry of Health and Welfare (the Ministry of Health, Labour and Welfare of today) submitted a report of the 16th JECFA assessment results, which suggested a provisional intake limit of 0.17mg/person/week for an adult weighing 50kg (0.5μg/kg bw/day), based upon result of study of sufferers of Minamata disease and others that concluded the minimum onset dose to be 0.25mg/day, as well as animal experiments (Pharmaceutical Affairs and Food Sanitation Council[17]).

(2) Joint FAO/WHO Expert Committee on Food Additives (JECFA)

①In the 16th JECFA held in April 1972, the provisional tolerable weekly intake of total mercury and methylmercury were set. The provisional tolerable weekly intake was set at 0.3mg/person/week for total mercury and ≦0.2mg/person/week for

methylmercury (in the amount of mercury). For 60 kg body weight, the intake of total mercury and methylmercury will be 0.005, 0.0033mg/kg bw/week (data not shown). JECFA concluded that even if dietary methylmercury levels exceed the provisional tolerable weekly intake of methylmercury of 0.2mg/person/week in a fish-eating population, there is no risk to health, as long as it is for a limited period of time (the 16th JECFA[18]).

② In the 22nd JECFA held in April 1978, the provisional tolerable weekly intake of total mercury and methylmercury, including Environmental Health Criteria and others, were reassessed. As a result, the previous assessment(the provisional tolerable weekly intake: 0.3mg/person/week for total mercury, 0.2mg/person/week for methylmercury) was sustained (the 22nd JECFA[19]).

③ In the 33rd JECFA held in May 1988, reassessment was conducted based on the new data obtained. As a result, JECFA confirmed that the previously recommended provisional tolerable weekly intake of 200μg/person/week (3.3μg/kg bw/week)was appropriate to the general population. Here, a concern was expressed over higher risks of harmful effects of methylmercury on pregnant and lactating women. Necessity of further investigation was suggested, since the obtained data were insufficient to recommend a specific intake of methylmercury to this population.

Finally, JECFA concluded that many countries are making efforts to increase fish consumption, since fish are rich in nutrients and are indispensable for a well-balanced diet. Furthermore, regional or ethnic dietary habits have been formed over centuries and established as part of the culture. Recommendations to change these habits must be based on sufficient discussion and potential relevant issues should not be overlooked. Provided that efforts should be continued to minimize methylmercury exposure caused by industrial pollution to humans, the following recommendations have been made.Since the epidemiological studies on populations that consume methylmercury in fish captured in unpolluted areas are limited, the FAO and WHO recommended further investigation. The purpose of the investigation is to determine the presence of harmful effects (e.g. effects on the central nervous system) on a child by exposure to low-level methylmercury in seafood via the mother's body. Also, significance of trace constituents in fish (e.g. selenium) that alleviate the toxicity of methylmercury should be assessed by all possible means (the 33rd JECFA[20]).

④ In the 53rd JECFA held in June 1999, the previous assessment was supported.
Results of the epidemiological studies on the effects of prenatal exposure on child neurodevelopment in the Seychelles and the Faroe Islands were examined, but risk assessment could not be conducted due to the contradictory results. Reassessment was agreed to take place in 2002, when further study results are obtained.

Possible involvement of the following 3 factors were suggested for the contradictory results: ① Differences in assessment time (age) and type of test(s), ② Other factors (exposure to PCB's in the Faroe Islands), ③ Differences in dietary culture (Pilot Whales are consumed in the Faroe Islands, although less frequently than fish, while fish are consumed almost every day in the Seychelles). In addition, since fish are vital source of nutrients in particular regional and ethnic dietary cultures, it was suggested that their nutritional benefits should be regarded as more important than their concerned harmful effects in considering restrictions on methylmercury levels

in fish and fish consumption (the 53rd JECFA[21]).

⑤In the 61st JECFA held in June 2003, the provisional tolerable weekly intake was set at 1.6μg/kg bw/week based on the result of methylmercury exposure which concluded that neurodevelopment was most susceptible to its health effects, and the intrauterine developmental stage was the most susceptible period to neurodevelopmental toxicity.

The calculation method is as follows: In the 2 subject populations in the Seychelles and the Faroe Islands, the mean value of 14mg/kg in the 2 studies was used as an estimate level of maternal hair mercury that reflected the NOAEL for children. After the hair mercury level was converted to a blood level by use of the hair-blood level exchange ratio (250:1), the methylmercury level in the steady state and the corresponding intake of 1.5μg/kg bw/day in one-compartment model (see below for the relations between intake and exposure index and Table 4 for data set of parameters) were assumed. An uncertainty factor of 6.4 in toxicokinetics ($3.2 = 10^{0.5}$)×(hair-fluctuation range converted to blood (2)) was used and the provisional tolerable weekly intake (PTWI) was calculated as follows: (1.5μg/kg bw/day×7)/6.4＝1.6μg/kg bw/week. In the New Zealand study, the above calculation was not adopted as a basis in calculating tolerable intake, since the hair mercury levels of a child's mother differed significantly from the other data, and inclusion of the data made a substantial difference.

As for cardiotoxicity, one cohort study reported that ≧2mg/kg of hair mercury levels increase the risk of acute myocardial infarction two fold, and in a 4-year follow-up study, association with an increased incidence of atherosclerotic cardiovascular disease was reported. JECFA concluded that the obtained data concerning the cardiotoxicity of methylmercury were inconclusive at that time and pointed out the necessity of further investigation.

JECFA reconfirmed that fish are an important constituent of a nutritionally well-balanced diet and should be properly considered in making a public health decision on setting methylmercury levels (the 61st JECFA[2], WHO[3]).

(3) Environmental Protection Agency (EPA)

In the past, the EPA used the Iraqi poisoning cases to set a reference dose (RfD) of methylmercury. In 2001, the EPA reassessed the endpoints as developmental neurophysiological deficits based on the Faroe Islands study on neurodevelopment of prenatally exposed children, and set 46-79ppb of cord blood mercury levels as the Benchmark Dose Lower Confidence Limit (BMDL) (lower limit of 95% confidence interval) due to its neurophysiological effects on a 7-year-old child. The corresponding maternal intake of 0.857-1.472μg/kg bw/day was calculated using one-compartment model. The reference dose was recalculated using an uncertainty factor of 10. As a result, the previous reference dose was not changed, and remained 0.1μg/kg bw/day (EPA[22]).

(4) Department of Health and Human Services/ Agency for Toxic Substances and Disease Registry (ATSDR)

In 1999, ATSDR calculated 1.3μg/kg bw/day of NOAEL using one-compartment model

and the mean of 15.3ppm in the group with the highest maternal hair mercury levels as NOAEL , based on the Seychelles study on neurodevelopment of prenatally exposed 66-month-old children.

The minimal risk level (MRL) of methylmercury (oral) was determined to be 0.3μg/kg bw/day using this NOAEL and an uncertainty factor of 4.5 (① Variability of toxicokinetics and toxicodynamics in humans (3) + ② Slight effects (1.5) detected in the Faroe study) (ATSDR[8]).

(5) UK/COT (COMMITEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIROMENT)

COT reassessed mercury in seafood in 2004, following the JECFA reassessment of methylmercury in 2003. As a result, COT concluded that a methylmercury intake of 3.3μg/kg bw/week (2000 PTWI) may be used as a guideline to protect against non-developmental adverse effects, and the 2003 JECFA PTWI of 1.6μg/kg bw/week is sufficient to protect against neurodevelopmental effects and should be used in assessing the dietary exposure to methylmercury of pregnant women and who may become pregnant within one year (Pharmaceutical Affairs and Food Sanitation Council[17]).

(6) Food Standards Australia New Zealand (FSANZ)

In March 2004, FSANZ renewed the guideline concerning mercury in fish. FSANZ uses 2 PTWI values since fetuses are more susceptible to methylmercury than adults. 3.3μg/kg bw/week is used for the general population and approximately half of it, 1.6μg/kg bw/week, is used for fetuses (Pharmaceutical Affairs and Food Sanitation Council[1]).

6. Findings from the high-risk group

Since a special investigation on the high-risk group is requested by the Ministry of Health, Labour and Welfare, "Background of the request for discussion about the high-risk group", "Concept of the people subject to the current Japanese Advice", "People subject to dietary intervention in foreign countries and concepts of the subjects", and "Findings from prenatal and children toxicity" are summarized separately (Appendix 3).

Meanwhile, descriptions of assessments in foreign countries concerning a high-risk group except fetuses are as follows:

(1) Findings related to infants and methylmercury transport to breast milk

① Discussion in the Pharmaceutical Affairs and Food Sanitation Council in June 2003

Lactating mothers were not subjected to dietary intervention, since a large part of methylmercury does not migrate to children via breast milk, and there was no clear scientific evidence of high susceptibility in infants as some reviewers suggested.

② Descriptions in reviews

a) US NRC (NRC[7])

It is known from animal experiments that excretion of methylmercury into bile is limited in infant rats and monkeys. For this reason, it takes longer for infants to excrete methylmercury compared with mature animals. It should also be noted that the intestinal flora of infant before weaning might have a lower demethylation function. Based on the assumption that the phenomena observed in these test animals are true for humans, human infants might be especially susceptible to methylmercury.

The breast milk of rats, guinea pigs, and humans has been reported to contain methylmercury. For this reason, breast milk is regarded as one of the maternal excretion routes, and at the same time, as an important exposure route of methylmercury to breast-fed infants. Sixteen percent of total mercury in human breast milk is reported to be methylmercury, which is much lower than that observed in the blood.

b) ATSDR (ATSDR[8])

Findings from animal experiments are described. Basically, the findings are identical to those of NRC.

c) UK COT

(Discussion on pharmacokinetics)

Significant amounts of methylmercury also pass into the breast milk of lactating women, resulting in a decreased mercury half-life of approximately 45 days.

Doherty and Gates reported that the excretion rate of mercury in the suckling rodent is less than 1% of the adult excretion rate. Sundberg et al. reported a low elimination of mercury in suckling mice until lactational day 17. This is probably because biliary secretion and demethylation by microflora (which lead to faecal excretion) do not occur in suckling animals. The role of these processes in suckling human infants is unknown.

The concentration of mercury in breast-milk is approximately 5% of the blood mercury concentration of the mother. Amin-Zaki et al. reported that in women exposed to high levels of methylmercury during the Iraqi poisoning incident, 60% of the mercury in breast-milk was in the form of methylmercury. Therefore it may be estimated that the concentration of methylmercury in the breast-milk is approximately 3% of the total mercury concentration in the blood. For an infant to be exposed to methylmercury at the new JECFA PTWI of 1.6μg/kg bw/week, the mother would have to be exposed to the following methylmercury level:

Methylmercury intake of infant:＝0.23μg/kg bw/day（1.6÷7）

Assuming a daily milk intake of 150ml/kg bw
Concentration of methylmercury in milk: 1.53μg/L (0.23÷150)

Assuming 3% methylmercury transfer from maternal blood to milk
Maternal blood mercury level=51.1μg/L (1.53÷0.03)

Using the pharmacokinetic model employed by JECFA in its 2003 evaluation,

and assuming a maternal body weight of 65kg
Maternal methylmercury intake=1.36μg/kg bw/day (9.5μg/kg bw/week)

$$\left[\frac{51.1\times0.09\times65\times0.014}{0.95\times0.05\times65}\right]$$

(High susceptibility group)

Animal experiments indicate that exposure via breast-milk has less serious consequences to the central nervous system than prenatal exposure.

Data from a 5-year longitudinal study following the Iraq poisoning incident have suggested that some children exposed to methylmercury via breast-milk demonstrated delayed motor development.

The Iraqi poisoning cases concluded that breast-fed infants are at less risk than the fetus, since most of the brain development has already occurred and the effects seen in the breast-feeding infant are different from those seen in infants exposed prenatally and not as severe.

There is no evidence that chronic exposure to methylmercury via breast milk at levels below those observed in the Iraqi incident has any adverse effect on the neurophysiological/psychological development of the child.

There is uncertainty with respect to whether infants and young children are at greater risk of methylmercury toxicity whilst the central nervous system is still developing. The limited data available indicate that this is not the case for children but the possibility of increased sensitivity of infants cannot be discounted. Correlation of intakes by the breast-fed infant and the mother indicates that the methylmercury intake of the breast-fed infant is within the 2003 PTWI of 1.6μg/kg bw/week if the mother's intake is within the 2000 PTWI of 3.3μg/kg bw/week.

(2) Findings related to children

① Discussion in Pharmaceutical Affairs and Food Sanitation Council in June 2003

Reviewers expressed their views that there were no findings related to children.

② Descriptions in reviews

a) UK COT (Pharmaceutical Affairs and Food Sanitation Council[(1)])

There have been few studies of the effects of methylmercury on young children. Methylmercury is excreted by children as efficiently as by adults.

The longitudinal study in the Seychelles has attempted to examine the effects of postnatal exposure to methylmercury. This is complicated by the facts that in the Seychelles, the children exposed to methylmercury postnatally are also exposed prenatally, and the study has been unable to demonstrate any mercury-related deficits in the neurological development of children. However higher postnatal methylmercury exposure had a positive association with test scores. It was suggested that this may be because a higher mercury level indicates a high fish intake and therefore a diet rich in n-3-polyunsaturated fatty acids and vitamin E, which have

beneficial effects and may mask any subtle neurological deficits due to chronic low level exposure to methylmercury.

7. Food safety risk assessment

When a food safety risk assessment of methylmercury in seafood is conducted in Japan, it is desirable to conduct risk assessment specific to the Japanese population, based on epidemiological studies on Japanese dietary habits and food culture that include fish consumption. However, such data are currently unavailable; therefore, an assessment was conducted based upon epidemiological studies in foreign countries, including the Republic of Seychelles and Faroe Islands, for findings available at present.

(1) Hazard identification

Findings from Minamata disease and the Iraqi poisoning cases have been summarized in numerous excellent reviews. The target organ of methylmercury is the central nervous system, and its typical poisoning symptoms include an constricted visual field, hearing disorder, anarthria, and ataxia. In the worst case, a patient falls into a coma and eventually dies. In cases of mild exposure, abnormal sensation or feelings of fatigue develop. The threshold levels of body burden[f], at which these symptoms develop, have been set as follows: 25mg for abnormal sensation, 50mg for ataxia, 90mg for anarthria, 180mg for hearing loss, and ≧200mg for death. WHO(4) has identified that blood mercury level of 200μg/L (equivalent to 50ppm of hair mercury level) results in a 5% risk of neurologic effects such as abnormal sensation in adults.

Developing fetal brains are considered more susceptible, since fetal Minamata disease was reported in spite of no symptoms in pregnant mothers. These harmful effects have been demonstrated in animal experiments using mice, rats, and monkeys. As described above, harmful effects on the nervous system are evident, and more attention should be paid to the susceptibility of the developing nervous system.

Animal and in vitro experiments on immunotoxicity have been reported, but there are no sufficient findings of effects on humans. Although reproductive toxicity has been observed in animal experiments, there are no findings related to humans. As for nephrotoxicity, symptoms of renal disorder develop only when the amount of exposure is sufficient to cause neurotoxicity. Cardiovascular toxicity has been reported in a local study in Finland as well as multi-institutional joint researches in Europe and Israel. However, a study conducted in the U.S. with more subjects reported that no relationship between coronary disease and methylmercury exposure via seafood intake was found. Moreover, in the study of residents of the area where Minamata disease sufferers were found, there was no increase in deaths caused by cardiovascular diseases. Since there are studies with inconsistent resultsas well as a finding that n-3-polyunsaturated fatty acid decreases the risk of coronary disease, it is assumed that toxicity of methylmercury exposure to cardiovascular systems by fish consumption will involve complicated factors; therefore, it is difficult to include coronary diseases in this risk assessment. However, it should be focused continuously in the future.

(2) Dose-response assessment

As described above, harmful effects of methylmercury develop most frequently in the nervous system. In addition, methylmercury migrates to fetuses by crossing the

placenta as well as the blood-brain barrier. For this reason, the developing fetal central nerve is considered most susceptible to the effects.

Recently, in calculating a tolerable intake in the major risk assessments such as EPA, epidemiological studies on the effects of maternal exposure on postnatal infants are emphasized and used as a basis of calculation. In the 61st JECFA, toxicity of methylmercury on the nervous system, kidney, and liver was also described and neurotoxicity was confirmed as the most sensitive endpoint.

Therefore, in this assessment, it is considered appropriate to focus on studies of the postnatal effects of prenatal exposure. There are several such studies, and in particular, apart from the severity of the effects, the results of the Faroe neuropsychological study on 7-year-old children exhibit the effects of methylmercury exposure. The Faroe island study is considered highly reliable since approximately 1,000 study subjects are included and it is a cohort study with registered pregnant mothers. On the contrary, no effect of methylmercury exposure is observed in the Seychelles study, but its reliability is considered high since it is a cohort study including more than 700 subjects.

In both studies described above, hair or blood mercury levels were used as an exposure index, and the intake via oral exposure was not measured. Therefore, a metabolic model should be used to calculate the tolerable intake.

One-compartment model is widely used in JECFA and the EPA for the following reasons: Methylmercury exposure via foods is continuous and relatively stable. Methylmercury is not unevenly distributed to a specific organ in the body. Methylmercury is difficult to metabolize (into inorganic compounds) in the body. Also in this assessment, one-compartment model is considered appropriate as a metabolic model. The newly-assessed parameters of the 61st JECFA is used as a reference.

① Epidemiological study

a) The Faroe Islands study

Benchmark Dose (BMD) analysis was conducted using maternal hair or cord blood mercury levels as an exposure parameter, based upon the results of the 7-year-old children cohort of the Faroe Islands study (Budtz-Jørgensen et al., 2000[23]). The BMD and BMDL calculated using 5 endpoints, which were confirmed in a neuropsychological test to have statistically significant relationships with cord blood mercury levels, as response parameters. They are shown in the following Table summarized by Research Committee of National Academy of Sciences.

The Continuous Performance Test (CPT) reaction time of cord blood mercury levels showed the lowest BMD and BMDL values. The test was conducted for 2 years; however, the test result of the 1st year differed from that of the 2nd year, and thus only the data of the 1st year, of which accuracy control was conducted more severely, were analyzed (Grandjean et al., 1997[24], Budtz-Jørgensen et al., 2000[23]). Taking this into account, the National Research Council of National Academy of Sciences determined that it was appropriate to select the Boston Naming Test that showed the second-lowest BMD and BMDL (NRC, 2000[7]).

In this study, cord blood mercury levels were measured, which was considered a better exposure index for prenatal dose-response relationship analysis. However, hair mercury levels of pregnant mothers were used as the exposure index in the Seychelles study. As described later, to conduct risk assessment using the very few valuable data from the epidemiological studies, it may be favorable to include the Seychelles study. Therefore, hair mercury levels of pregnant women, which are commonly used in the two studies, were considered more appropriate as an exposure index. As for hair mercury levels of pregnant women, the Boston Naming Test showed the lowest BMD (15ppm) and BMDL (10ppm). Here, it was considered appropriate to set the BMDL of 10ppm, the 95% lower confidence bound of BMD, as one of the starting points to calculate the tolerable intake.

Table： Calculation of BMD, an endpoint in the Faroe Islands study

Endpoint	Cord blood mercury level (ppb)		Maternal hair mercury level (ppm)	
	BMD	BMDL	BMD	BMDL
Motor function: Finger Tapping Test	140	79	20	12
Attention: CPT reaction time	72	46	17	10
Visual space: Bender Gestalt Test	242	104	28	15
Verbal: Boston Naming Test	85	58	15	10
Verbal memory: California Verbal Learning Test	246	103	27	14

CPT: Continuous performance test
BMD: Benchmark dose
BMDL: 95% lower confidence bound of benchmark dose
Under the assumption that there are 5% abnormal reactions even in unexposed subjects, BMD was calculated as an amount of exposure that results in 5% risk (BMR=0.05).

b) The Seychelles child development study (cohort study)

Results of the Seychelles child development study showed no effects of methylmercury exposure on the nerve, cognition, and behavior in all the 6.5-, 19-, and 29-month-old children. No effect of methylmercury exposure was observed in 66-month-old and 9-year-old children even in the high exposure group with ≧12ppm of maternal hair mercury levels.

Therefore, 12ppm was determined as a value that corresponds to the NOAEL.

c) Epidemiological study in New Zealand

In the 4-year-old child study, 74 children were subjects of DDST, and 38 children born from mothers with high hair mercury levels (≧6ppm) were actually used and compared with the control group of 36 children. The study included limited data and the conducted tests were no more than screening tests.

In addition, three control subjects (① One whose mother's hair mercury levels during pregnancy was 3-6 ppm, ② One who consumes fish more than 3 times a week, and whose mother's hair mercury levels during pregnancy was ≦3ppm, ③ One who consumes fish at a low frequency, and whose mother's hair mercury levels during pregnancy was ≦3ppm) were allocated to one child born from a mother with high hair mercury levels at 6 years of age. Studies including the Wechsler Intelligence Scale for

Children-Revised, Test of Language Development, and the McCarthy Scales of Children's Abilities were conducted with 57 pairs.

The effects of methylmercury exposure were smaller than the contribution of confounding factors such as social stratifications and ethnic groups (Kjellström et al., 1986[9], Kjellström et al., 1989[10]). In addition, there is data of a child born from a mother whose hair mercury level (86mg/kg) in the cohort was the highest, which was more than fourfold higher than the second-highest hair mercury levels (20mg/kg). Regression analysis was statistically significant when this data was excluded but not when included. Due to the uncertainty of the data it is difficult to say that use of the epidemiological study results in New Zealand is appropriate.

② Metabolic model

In the above study, intake (oral exposure) was not measured, and a metabolic model was used to calculate the tolerable intake. The one-compartment model, which was used for assessments in JECFA and the EPA, is used as the metabolic model. Considering the physical size of Japanese, different body weight values are used as a parameter set, and the newly-assessed parameters of the 61st JECFA is used as the other parameters.

Daily methylmercury intake d (μg/kg bw/day), which becomes C (the blood mercury level) (μg/L) in the steady state, is calculated using the following formula:

Maternal daily methylmercury intake: d (μg/kg bw/day)

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

In this formula, each parameter identical to that of JECFA (the 61st) is used as follows. However, 65kg bw is considered too high for Japanese females even during pregnancy, and thus 60kg, the mean value in the late stage of pregnancy, is used.

b = elimination rate constant (0.014 per day^{-1})
bw = body weight (60kg)
V = blood volume (0.09×60liters)
A = fraction of the dose absorbed (0.95)
f = the absorbed fraction distributed to the blood (0.05)

The elimination rate constant is calculated from the biological half-life ($T_{1/2}$) in the blood: $b=0.693 \div T_{1/2}$

③ Uncertainty

Studies and investigations cannot be free from uncertainty, however precisely they may be planned, or however correctly they may be conducted; therefore, uncertainty should also be taken into consideration in this health effect assessment. In usual risk assessments, animal experiment results are often extrapolated, and in these cases, an uncertainty factor of 10 is applied to animal specific differences, to individual differences in a human group, and to when NOAEL is estimated from LOAEL. As for the rest, an uncertainty factor of up to 10 is also applied to each of the factors such as reliability of experiments/investigations and severity of effects.

All the above epidemiological studies focused on prenatal exposure, which is regarded to have the highest susceptibility, and examined the effects on the most sensitive central nervous system by various test methods. Nearly 700-1,000 subjects were included in each study,which are considered sufficient for studies on humans. In addition,

ethnicity, cultural backgrounds, and natural environments were substantially different between the Faroe Islands and the Seychelles. However, the values corresponding to BMDL and NOAEL obtained in these two regions are 10ppm and 12ppm of comparable maternal hair mercury levels, respectively. Since BMDL is considered closer to NOAEL the uncertainty included in these data is considered low. In addition, there is no necessity to apply the uncertainty factor for animal specific differences in risk assessment using human data. Also, the uncertainty factor for the estimation of NOAEL from LOAEL need not be applied. However, considering the variability among living bodies, the following uncertainties are possible:

・To estimate blood mercury levels from hair mercury levels, the ratio of 250:1 (hair mercury level vs. blood mercury level) has been used in the conventional risk assessments by JECFA and others. This ratio ranges from 140 to 370 as the mean value of each study group, and from 137 to 585 for the individual data. Therefore, considering the fluctuation range of the mean values, there is a possibility that the blood mercury levels estimated from hair mercury levels using 250 will be 0.68 (250/370)-1.79 (250/140) fold of the actual blood mercury level, and 0.43 (250/585)-1.82 (250/137) fold for individuals.

・In a radioactive methylmercury intake experiment with volunteers, biological half-lives were reported to be approximately 70 days for the whole body and 50 days for blood compartment, with the excretion factor of 0.014 (Miettinen et al,1971[25]). The excretion factor calculated from actual fish intake was approximately the same value, 0.0099-0.0165 (Sherlock et al. 1984[26]). In the Iraqi poisoning cases, biological half-lives observed from the variability of hair mercury levels showed a bimodal distribution; mean 65 days in the low-level group (excretion constant factor: 0.0107), mean 119 days in the high-level group (excretion constant factor: 0.0058), and maximum 189 days were reported (Al-Shahristani and Shihab, 1974[27]). However, this maximum value was considered an outlier. Thus, considering that the biological half-life estimated from the change of hair mercury levels indicate the half-life of the whole body or blood compartment, the mean value of change to a longer biological half-life (i.e. lower b (excretion constant factor)) might be 1.70 (119/70) for the whole body or 2.38 fold (119/50) for the blood compartment.

④ Setting tolerable intake

In the Faroe Islands study (cohort study), a statistically significant relationship was observed between fetal methylmercury exposure and several neurobehavioral and neuropsychological endpoints. On the contrary, in the Seychelles child development study, no effect of fetal methylmercury exposure on the nerves, cognition, and behavior of children was observed.

The differences between the above studies are summarized as follows:

- Exposure pattern (In the Faroe Islands, whales with relatively high-levels of mercury are infrequently consumed, while in the Seychelles, fish with low-levels of mercury are frequently consumed.)
- Effect indexes used for the neurologic development (Functional-domain specific tests were used in the Faroe Islands. Comprehensive tests were used in the Seychelles)
- Polychlorinated biphenyl (PCB) exposure (The Faroe Isalnds is positive, while the Seychelles is negative)
- Ethnic group (European ethnic group in the Faroe Islands and African ethnic

group in the Seychelles) (NRC[7])

Considering the Japanese population, particularly from the aspect of exposure patterns (and PCB exposure), the population in the Seychelles is considered closer to the Japanese population. However, test results that confirmed significant relationships cannot be ignored. For this reason, considering the BMDL (Boston Naming Test by the maternal hair mercury levels), one of the neurobehavioral endpoints in the Faroe Islands study, and the NOAEL in the Seychelles child development study, the mean of 11ppm between 10ppm and 12ppm of their maternal hair mercury levels was used to calculate d (the daily maternal mercury intake). The method to calculate the mean value from the results of the two studies was also adopted in the JECFA assessment (2003) (In this case; however, the BMDL of 12ppm (the maternal hair mercury levels by the Boston Naming Test) was calculated using a different method.)

The blood mercury level (44μg/L) is calculated from the maternal hair mercury level (11ppm). In addition, maternal daily methylmercury intake (d μg/kg bw/day) is calculated.

$d = (C \times b \times V) / (A \times f \times bw) = 1.167$μg/kg bw/day

b = elimination rate constant(0.014 per day^{-1})

bw = body weight(60kg)

V = blood volume (0.09 × 60 liters)

A = fraction of the dose absorbed (0.95)

f = the absorbed fraction distributed to the blood (0.05)

In addition, the fluctuation range of the elimination rate constant (i.e. biological half-life) should be considered about d＝1.17μg/kg bw/day, besides the fluctuation range in converting maternal hair mercury levels into blood mercury levels. As described above, given the maximum fluctuation range of 2 (hair mercury level vs. blood mercury level), blood mercury levels estimated from hair mercury levels as well as the amount of intake are reduced by half. Given the maximum fluctuation range of 2 in the biological half-life, the elimination rate constant and the intake should be reduced by half. Considering the uncertainty, 0.29μg/kg bw/day (d=1.17μg/kg bw/day÷4) is the intake from the safety standpoint. Therefore, the tolerable weekly intake (TWI) of methylmercury is 2.0μg/kg bw/week (Hg).

Here, risk assessment using the data from the epidemiological studies with humans was conducted and thus uncertainty of the data is considered small compared with that by extrapolating animal experiments results. Subjects of the epidemiological studies were the most sensitive prenatally exposed children, and the endpoints of the effects were examined by various neurobehavioral, neuropsychological, or neurophysiological tests with the highest sensitivity. In the subject regions, the Seychelles and the Faroe Islands, there were substantial differences in cultural and natural environments including ethnic backgrounds, dietary habits, and languages, but not in the values that corresponded to the NOAEL and BMDL. Since there were only a few large-scale cohort studies, the Committee conducted risk assessment based upon the results of the two studies.

In the Faroe study, cord blood mercury levels, which were closer to the fetus and considered an excellent exposure index in dose-response relationship analysis, were also measured. On the contrary, maternal hair mercury levels during pregnancy were an exposure index in the Seychelles study. To conduct risk assessment using a few

valuable results of the epidemiological studies, the hair mercury level, which is common in the two studies, was used as an exposure index.

In addition, even if cord blood level is a better exposure index, the cord blood mercury level should be converted to maternal blood level to calculate a tolerable intake. Because data was insufficient for the conversion and there was a substantial fluctuation range, it was considered difficult to estimate the representative value or fluctuation range. In theory, constructing a more precise model with fetuses separated in the other compartment was considered to replace the one-compartment model for the calculation, but almost no data have been available to construct such a model. Therefore, the Committee decided to convert hair mercury levels to blood mercury levels.

In the risk assessment conducted this time, the BMDL (the lower value in the 95% confidence interval of BMD) in the Faroe study, and 12ppm (the lowest value in the high exposure group with >12ppm of maternal hair mercury levels) in the Seychelles study were adopted from the safety standpoint.

As for uncertainty factors, the Committee selected the ratio of mercury levels between hair and maternal blood as well as the fluctuation range of the biological half-life as uncertainty factors since toxicokinetic variability has a great effect due to the properties of the adopted model. This reflects that different sensitivities to exposure indexes exhibited by hair mercury levels, different blood levels to the same intake in the steady state, and, moreover, observation methods are considered to be the factors explaining the different sensitivities.

(3) Mercury exposure in Japanese

According to the Total Diet Surveillance conducted by the Ministry of Health, Labour and Welfare, the dietary mercury intake (total mercury) of Japanese is 8.1μg/person/day (1.1μg/kg bw/week in 50kg bw) in 2003, of which 84% is consumed from seafood. The mean of the past 10 years between 1994 and 2003 is reported as 8.4μg/person/day (1.2μg/kg bw/week) (Figure 2). Since the methylmercury level is lower than the total mercury level, methylmercury intake becomes lower than the tolerable weekly intake of 2.0μg/kg bw/week that is calculated herein. However, this is a comparison between the mean values, and there are no data of the actual fluctuation range of intake.

According to a report that analyzed the total mercury of the hair collected from all regions in Japan (Yasutake et al., 2004[28]), the geometric mean of the female hair mercury level was 1.37ppm. More detailed analysis of the distribution of hair mercury levels of females between the age of 15 and 49 demonstrated that ≦1ppm accounts for 26.3% of the population, ≦2ppm for 77.8%, ≦5ppm for 98.3%, and ≦10ppm for 99.9%. This indicates that almost all mercury levels of females were <11ppm, the mean value of BMDL and NOAEL values that were the starting point to calculate a tolerable weekly intake.

(4) High-risk group

Based upon the scientific findings that significant effects of methylmercury are

associated with the developing central nervous system, and fetuses are most susceptible to methylmercury exposure, there is a consensus in foreign countries to include pregnant and potentially pregnant women as subjects to dietary intervention, but inclusion of other types of subjects was different by each country. In Japan, it was considered appropriate to assess the high-risk group as a susceptible and highly-exposed group.

① Fetuses

Since methylmercury not only crosses the blood-brain barrier but also migrates to fetuses via the placenta, the developing central nervous system of fetuses is considered the most susceptible.

In addition, brain mercury levels of rats in the fetal stage until the time of birth are approximately 1.5-2 fold higher than those of mother rats, and erythrocyte mercury levels of the cord blood are an mean of 1.4 fold higher than those of human pregnant mothers, and its ratio to total blood methylmercury levels is reported as 1.9 (Ask et al., 2002[29]). Therefore, prenatal exposure to mercury is considered particularly high.

② Infants

Findings in animal experiments showed limited methylmercury excretion into the bile in infant rats and monkeys. For this reason, it takes longer for infants to excrete methylmercury compared with mature animals. Also, a lower demethylation function of intestinal flora in breast-fed infants is possible. It is assumed that the maturity at birth is lower in lab animals such as rats compared with humans, even so if the findings are still true of humans, higher risks of methylmercury exposure in human infants are possible.

Breast milk is considered the main source of exposure for infants, and there is a report that methylmercury accounts for 16% of total mercury in human breast milk. Meanwhile, in the Iraqi poisoning cases, it was reported that 60% of mercury in breast milk of women exposed to high-level methylmercury was in the form of methylmercury. When the maternal mercury intake is below the provisional tolerable intake (3.3μg/kg bw/week) set prior to the 61st JECFA, infant mercury intake via breast milk is 0.56μg/kg bw/week, well-below the provisional tolerable intake intended for pregnant women in the 61st JECFA.

In rats, postnatal brain mercury levels were reduced to approximately 1/10 of that in the late pregnancy and exposure to infants via lactation was minimal. As for human infants, erythrocyte mercury levels were reduced and the cord blood erythrocyte mercury levels at birth was reduced 0.54 fold in 3-month-old infants. Taking these into account, it is assumed that methylmercury exposure levels in breast-fed infants are reduced compared to that of fetuses.

③ Children

Very few investigations have been conducted on the effects of methylmercury on children. Most information was based upon the Minamata, Niigata, and Iraqi poisoning cases, and the amount of exposure in all these cases was extremely high, and the Iraqi cases were acute exposure. In some countries, children, except infants, were subjected to dietary intervention but no concrete evidence has been indicated. Moreover, there are no data of the concerned harmful effects of methylmercury on adult and child health at present. The British COT reported the same excretion efficiency of

methylmercury in children as in adults, and the same brain damage in children via direct exposure as those in adults, while the Seychelles epidemiological study reported that harmful effects of methylmercury on child neurodevelopment could not be demonstrated due to the complexity that children postnatally exposed to methylmercury were also exposed prenatally.

Based upon these findings, methylmercury exposure is considered to have the most profound effects on fetuses; therefore, it is judged appropriate to identify fetuses to be in the high-risk group.

As for infants and children, according to the findings available at present, the amount of exposure declines in infants, children excrete methylmercury like adults, and the effects on their brains is similar to those on adults' brains. Therefore, it is judged reasonable to regard fetuses to be in the high-risk group.

8. Conclusion

(1) High-risk group

Fetuses

(2) Tolerable weekly intake

Methylmercury: 2.0μg/kg bw/week (Hg)

Basis

The basis was the daily methylmercury intake by pregnant women, which was calculated using the one-compartment model with the mean of 11ppm between the two hair mercury levels, 10ppm and 12ppm, considering the BMDL in the Faroe Islands study and the NOAEL in the Seychelles child development study, respectively. Here, considering the uncertainty (concentration ratio between hair and blood mercury levels, and individual differences in the excretion factor), an uncertainty factor of 4 was applied.

Subjects

Subjects were pregnant and potentially pregnant women, since fetuses were determined to be in the high-risk group.

9. Summary and issues in the future

In risk assessment this time, data from human cohort studies were used. Subjects were prenatally exposed children with the highest susceptibility, and the endpoints were also examined by various neurobehavioral, neuropsychological, and neurophysiological tests with the highest sensitivity.

In the subject regions, the Seychelles and the Faroe Islands, there were substantial differences in cultural and natural environments including ethnic backgrounds, dietary habits, and languages, but not in the values that corresponded to the NOAEL and BMDL. Therefore, the data uncertainty was considered low, and the Committee conducted risk assessment based upon the results of the two studies, and calculated the tolerable weekly intake considering the uncertainty factor associated with model construction.

Several issues were unconsidered in this risk assessment. In particular, confounding effects of other dietary constituents including nutrients were not fully assessed. This is mainly because up to the present, almost no study has been conducted from such a perspective. Risk assessment should be conducted on various dietary contaminants with potential neurologic effects, as represented by PCB's, and on effects of their combined exposure, when sufficient findings worthy of discussion are accumulated, including those from ongoing studies.

It has been reported that hair perms reduces hair mercury levels; however, this factor was not considered. This is because in the two cohort studies, it was not identified whether or not pregnant female subjects had hair perms and this factor was not considered in the analysis. Therefore, it was impossible to include hair perms as uncertainty. Besides, reduced hair mercury level leads to lower estimation of blood mercury levels, resulting in safer assesment. In addition, this might be one of the variable factors of the ratio between hair and maternal blood mercury levels.

Recently, methylmercury exposure in adults has been reported to be a risk factor of coronary artery disease and arterial sclerosis but negative results have also been reported, and thus further investigation is required in the future. For this reason, the factor was not included in the risk assessment; however, depending on the outcomes of future studies in this field, it may be considered in the assessment.

It is evident that methylmercury exposure results from consumption of fish that contain methylmercury; however, nutritional advantages of fish consumption such as n-3-polyunsaturated fatty acids should also be noted. In fact, by avoiding intake of large amounts of fish containing high-levels of methylmercury, one can benefit from fish consumption while reducing methylmercury intake. Methylmercury levels in each fish species were published in documents for Veterinary and Aquatic Food Meeting, Food Sanitation Committee, Pharmaceutical Affairs and Food Sanitation Council in August 17, 2004 (Pharmaceutical Affairs and Food Sanitation Council[1]). Studies on nutritional advantages of fish consumption and on the confounding factors of effects of methylmercury consumed via foods containing fish are required in the future. In addition, construction of databases of mercury in fish based upon detailed and sufficient samples is also required. Needless to say, risk communication is also needed to gain full understanding of the public.

Table 1　Mercury and its compounds

Metalic mercury （Elemental mercury, Mercury vapor）	Hg^0
（Inorganic mercury compounds）	Mercurous mercury: Hg^{+} Mercuric mercury: Hg^{++}
（Organic mercury compounds）	Alkyl mercury Phenyl mercury etc.

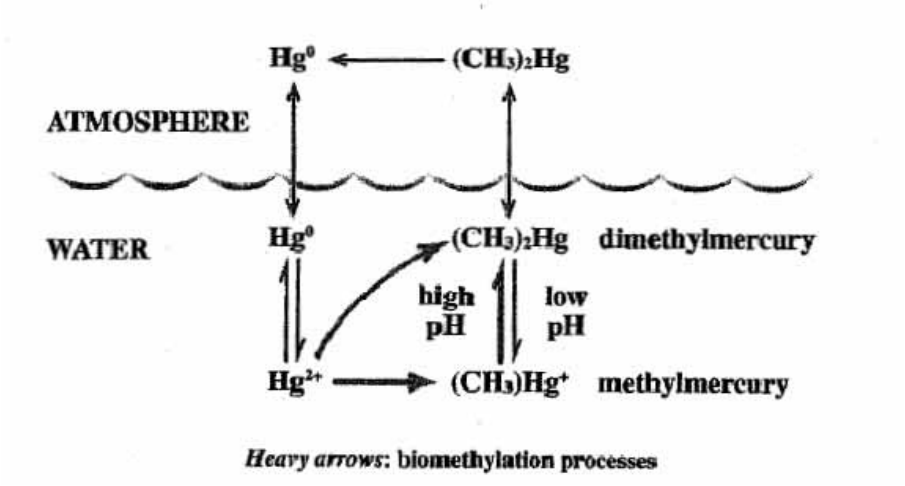

Figure 1　Cycling of mercury compound in the environment

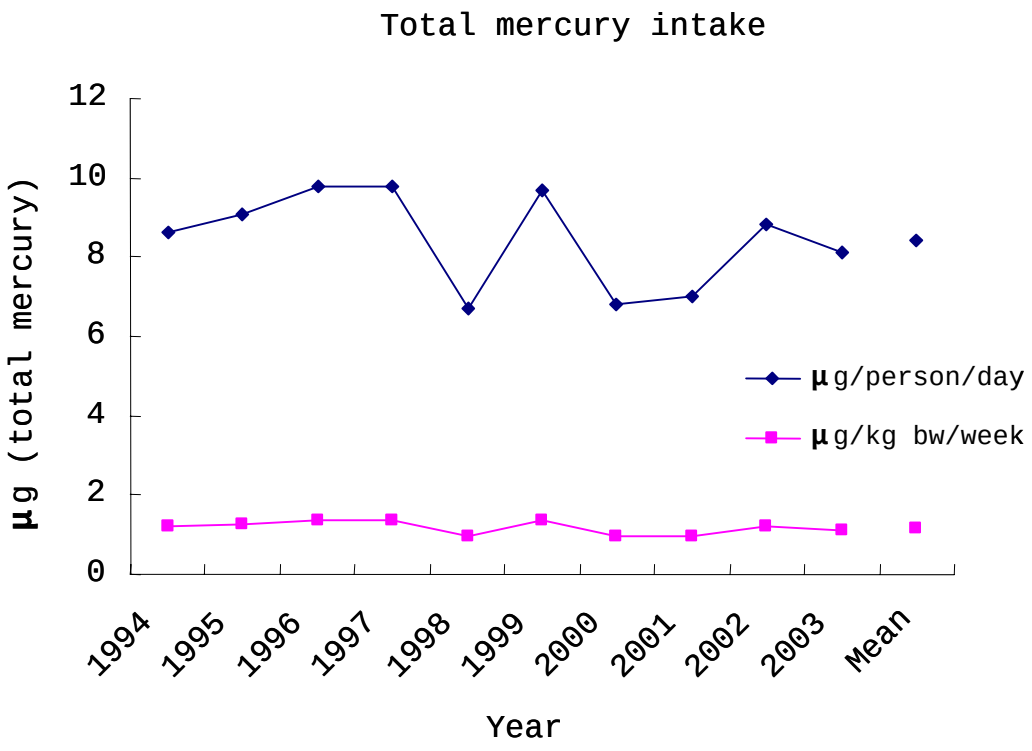

Figure 2　Annual average of total mercury intake (Japan)

Table 2　Summary of main epidemiological studies on methylmercury

	Study name	Summary of the studies					
		Cohort scale	Biomarker	Mercury levels	Age at the assessment	The number of child subjects	Remarks
1	The Faroe Islands study	1,023	Maternal hair mercury levels	Mean: 4.5ppm	1 year old	583	A statistically significant relationship was observed between prenatal methylmercury exposure and several neurophysiological or neuropsychological endpoints. See Appendix 1 for details.
			Cord blood mercury levels	Mean: 24.2ppm	7 years old	923	
					14 years old	883	
2	The pilot study for the Seychelles child development study	804	Maternal hair mercury levels	Median: 6.6ppm	5-109 weeks	789	Significant, but not clear, effects of mercury were observed in the pilot study. In the main study, no effects of methylmercury exposure on child nerves, cognition, and behavior was observed in the child neurodevelopment studies at ages of 6.5, 19, 29, 66 months and 9 years. See Appendix 2 for details.
					66 months	217	
	The main study of the Seychelles child development study	779	Maternal hair mercury levels	Median: 5.9ppm	6.5 months	712-737	
					19 months	738	
					29 months	736	
					66 months	711	
					108 months (9 years)	643	
3	New Zealand	73 (hair mercury levels: >6ppm)/ 935 (high-frequency fish consumers)/ 10,930 (screened subjects)	Maternal hair mercury levels	Mean of the high-risk group: 8.3ppm (High-risk group: ≧6ppm) Analytical value: 6-86ppm (16 samples: >10ppm)	4 years	38 (high-level mercury group) + 36 (low-level mercury group)/74	Neurodevelopment tests with 4-year-old children showed 50% of abnormal or suspicious cases in the high-level mercury group, compared with 17% in the control group, and the difference was statistically significant. Subsequently, neurodevelopment tests with 57 pairs of 6-7-year-old children were conducted and compared with 3 control groups. The comparison results showed lower test scores associated with an average hair mercury level during pregnancy of 13-15ppm, little contribution of methylmercury exposure, and significant effects of ethnic backgrounds of children.
					6 years	57 (high-level mercury group)/237	

Table 3　Methods of methylmercury risk assessment in Japan and abroad

	Institution of the assessment implementation (Yr of implementation)	**Exposure index (main studies)**	Threshold level		Method or ideas to convert the threshold to intake	Intake(dose) (μg/kg bw/day)	Uncertainty factor (UF)	TWI (μg/kg bw/week)	Remarks
			LOAEL	NOAEL/BMDL					
1	The 16th JECFA (1972)	No clear description (Development of Minamata disease etc.(*1))	Blood mercury level: 200μg/L		Birke's formula Erythrocyte level (mg/L) = 1.4×(daily mercury intake mg/person/day)+0.003 (Erythrocyte level is twofold higher than blood mercury levels, and as for blood mercury contents, the abundance ratio of methylmercury between erythrocyte and plasma is 10:1.)	0.3 (mg/person/day)	10	0.3mg/person/day × 7 days/week ÷ 10 (uncertainty factor)=0.2mg/person/week TWI=0.2mg/person/week÷60kg/person= 3.3μg/kg bw/week	The 16th JECFA (15)(p15-16) Kitamura et al. 1976 (28) (P368-369)
			Hair mercury level: 50mg/kg hair		Kojima's formula Hair mercury level=150×(daily methylmercury intake mg/person/day)+1.16	0.3 (mg/person/day)			
2	Ministry of Health, Labour and Welfare (1973)	Development of Minamata disease (Epidemiological study on Minamata disease)			Ideas ① Assuming that the Japanese mean body weight is 50kg, 0.17mg/person/week is obtained using 0.2mg/person/week calculated in the 16th JECFA			(0.17mg/person/week)	Pharmaceutical Affairs and Food Sanitation Council (14) (P5)
			Daily mercury intake: 0.25mg/person/day		② Assuming that 0.25mg/person/day of daily intake is the minimum level for disease development based on a study result on Minamata disease, its 1/10 the level, 0.025mg/person/day, is estimated as NOAEL, which is equal to 0.175mg/person/week.	0.25 (mg/person/day)	10		
				2-year administration tests with monkeys: 30μg/kg/day	③ Results of an experiment with monkeys showed no development at 30μg/kg/day for 2 years; therefore, after converting it for adult humans (50kg), 30μg/kg/day of tolerable intake was calculated using a 50-fold safety ratio.　This is 0.21mg/kg/week.	1.5 (mg/person/day)	50		
3	The 22nd JECFA (1978)	The previous assessment was supported.						3.3	The 22nd JECFA (16) (P26)
4	The 33rd JECFA (1988)	The previous assessment was supported. (Concern of higher risks to pregnant or lactating mothers was pointed out.)						3.3	The 33rd JECFA (17) (P33)
5	The 53rd JECFA (1999)	The previous assessment was supported. (The study results in the Seychelles and Faroes were examined, but risk assessment could not be conducted due to the contradictory results, and							The 53rd JECFA (18) (P93)

		reassessment was planned in 2002 after more study results are obtained.)							
6	The 61st JECFA (2003)	Effects of prenatal exposure on child neurodevelopment (The Seychelles child development study/The Faroe Islands prospective cohort studies)		Maternal hair mercury level: 14mg/kg maternal hair (The threshold level was determined from the mean value of maternal hair mercury levels: 15.3mg/kg (NOAEL in the Seychelles child development study) and 12mg/kg (BMDL in the Faroe Islands study).)	One-compartment model method Daily methylmercury intake (mg/kg bw/day) = (C×b×V)/(A×f×bw) C=56μg/L, b=0.014 day-1, V=5.85L, A=0.95, f=0.05, bw=65kg The hair mercury level is calculated with a hair/blood level conversion factor of 250.	1.5	6.4	1.6	The 61st JECFA (2) (P20-22)
7	EPA (2001)	Effects of prenatal exposure on child neurodevelopment (The Faroe cohort study)		Cord blood mercury levels: 46-79mg/kg cord	One-compartment model method Daily methylmercury intake (mg/kg bw/day) = (C×b×V)/(A×f×bw) C=46-79μg/L, b=0.014 day^{-1}, V=5.85L, A=0.95, f=0.05, bw=67kg	0.857-1.472	10	0.7 (0.1μg/kg bw/day)	EPA (19)(p7)
8	ATSDR (1999)	Effects of prenatal exposure on child neurodevelopment (The Seychelles cohort study)		Maternal hair mercury level: 15.3mg/kg maternal hair	One-compartment model method Daily methylmercury intake (mg/kg bw/day) = (C×b×V)/(A×f×bw) C=61.2μg/L, b=0.014 day-1, V=4.2L, A=0.95, f=0.05, bw=60kg	1.3	4.5	2.0	NRC (6) (APPENDIX A14)
9	COT (2004)	The 61st JECFA assessment was supported. Effects of prenatal exposure on child neurodevelopment (The Seychelles/Faroe cohort studies)						(Protection from harmful effects other than non-developmental toxicity) 3.3 (Protection from prenatal neurodevelopmental effects) 1.6	Pharmaceutical Affairs and Food Sanitation Council (1) (P71)
10	FSANZ (2004)	The 61st JECFA assessment was supported. Effects of prenatal exposure on child neurodevelopment (The Seychelles/Faroe cohort studies)						(Protection of the general population) 3.3 (Protection of fetuses) 1.6	Pharmaceutical Affairs and Food Sanitation Council (1) (P117)

(Note) Parameters in the one-compartment model
C mercury concentration in blood (μg/L)
b elimination rate constant (day^{-1})
V blood volume
A fraction of the dose absorbed (0.95)
f the absorbed fraction distributed to the blood (0.05)
bw body weight

*1 Reference 75
Kitamura et al. (1976), Mercury P368-369, Kodansha Scientific

Table 4 Relationship between exposure indexes (biomarkers) used in the 61st JECFA and intake

(1) Maternal hair mercury levels (H)

↓ Hair mercury levels H (sampling data)

(2) Maternal blood mercury levels (C)

↓ Mean (Hair mercury levels : Blood mercury levels) =140-370 : 1

Individual (Hair mercury levels : Blood mercury levels) =137-585 : 1

- C = (1/R)H ・・・・・・・・・・・・・・・・・・・・・(1)
  - **JECFA**
    - Hair mercury levels : Blood mercury levels
      =250 : 1 ⇒ (1/R) = 1/250
    - **Composite NOEL (BMDL)**
      H = 14 (mg/kg *maternal hair)* ・・・・・・・・(2)

  $C_{(NOEL/BMDL)}$ = 0.056 (mg/L) ((1) and (2))

(3) Maternal mercury intake (daily) (D)

- **Under the assumption of steady state**

$$d = \frac{C \times b \times V}{W \times A \times F} \quad \cdots\cdots\cdots\cdots (3)$$

$$d = \frac{(1/R)\ H \times b \times V}{W \times A \times F} \quad \cdots\cdots\cdots (4)$$

	Factors used in the JECFA
C: mercury concentration in blood (μg/L) b : elimination rate constant (day^{-1}) V : blood volume (liters) W : body weight (kg) A : fraction of the dose absorbed F : the absorbed fraction distributed to the blood R : conversion rate from hair level to blood level	C : 56 (μg/liters) b : 0.014 (day^{-1}) V : 0.09×65 (liters) W : 65 (kg) A : 0.95 F : 0.05 R : 250 **d =** 1.485 = 1.5 **(μg/kg bw/day)**

Appendix 1

Methylmercury in seafood

(1) A major epidemiological study on methylmercury

① The Faroe Islands study (cohort study)

(a) Summary of results

1,023 pairs of children born between March 1, 1986 and December 31, 1987 (75.1% of the total births in this period) and their mothers were registered as a cohort, and neurobehavioral development tests were conducted at ages of 7 and 14 years of ages. Statistically significant relationships between prenatal methylmercury exposure and several neurobehavioral and neuropsychological endpoints were observed.

(b) Background

- (History/culture/ethnic group)

The Faroe Islands are a group of 18 islands of Danish dominion located in the near-middle of the North Atlantic, between Norway and Iceland and at 62 degrees north latitude on the line that connects Denmark and Iceland. Its population is 47,000 and approximately 19,000 of them live in the capital Torshan. As for its culture (language), the Faroe Islands had been isolated from the mainland between the Middle Ages and the early 20th-century. For this reason, they have developed their own unique culture over a long period. In particular, their language (Faroese), like Icelandic, has the same characteristics of old Northern European languages. The whaling in the Faroe Islands is a non-commercial whaling conducted at the community level, and approved by the International Whaling Commission. The average annual catch of pilot whales over the years is 850. Meat and blubber of pilot whales are stored in freezers, or traditionally salted and dried in the open air, or cooked. The mean mercury levels of pilot whales and cods captured around the Faroe Islands in early 1990 were 3.3μg/g(approximately half was methylmercury) and 0.07μg/g (mostly methylmercury), respectively. According to this study, the mean consumption by adults was 12g/day for whale meat and 72g/day for fish meat, and the mean mercury intake was estimated at approximately 36μg/day. In addition, blubber was the main source of PCB intake.

- (Reasons for selecting the study area)

① The hospital and social security systems are the same as those of Northern European communities (Easy generalization of the study groups). ② Relatively little human exchange between the Faroe Islands and Denmark due to the distance (Easy follow-up of cohort groups). ③ Population consists of people exposed to high-level methylmercury via consumpation of whale meat and those who do not consume whale meat (Wide range of methylmercury exposure levels). ④ Use of a common language (Faroese) among island residents (language dependency tests are feasible), and so forth (Murata et al. 2004[1]).

- (Background of conducting the study)

In the mid-1980s, a pilot study on methylmercury was conducted in the Faroe Islands. The median value of blood mercury levels in 53 fertile women (age: 20-50) who lived in out-harbors was 12.1 (range: 2.6-50.1)μg/L, which was 7.6 fold higher than that of women in mainland Denmark (1.6μg/L) (Grandjean et al., 1992[2], Murata et al., 2004[1]). For this reason, between March 1, 1986 and December 31, 1987, a full-fledged study was conducted and led by Prof. Grandjean of the Department of Environmental Medicine, Odense University, and Dr. Weihe, the hospital director of the Faroe Islands.

- (Study institutions)
  - ✓ Department of Environmental Medicine, Institute of Community Health, Odense University, Odense Denmark; later, South Denmark University
  - ✓ Clinic of Occupational Health and Public Health, The Faroese Hospital System, Torshavan, Faroe Island
  - ✓ International joint research：Boston University, Harvard School of Public Health、US Environmental Protection Agency 、 Emory University, University of Copenhagen, the University of Tokyo, Teikyo University, Akita University, US Center For Disease Control and Prevention
  - ✓ Sponsors： Danish Medical Research Council, Danish Health Foundation, Hojaard Foundation、Vestnorden Foundation, Danish Agency for Environmental Protection, US National Institute of Environmental Health Science, European Commission、United Nations University (1994), Nissan Science Foundation（2002-2003）

(c) Sources of exposure

Pilot Whales and fish have been identified as sources of methylmercury exposure in the Faroe Islands. However, detailed data of the methylmercury levels have not been reported. In addition, amalgam in teeth and occupational exposure are not so significant in the Faroe Islands (NIEHS, 1998[3]). The data of the sources of methylmercury exposure in the Faroe Islands described in the report are described below.

According to a hearing investigation in the Faroe Islands, adults consume an average of 72g of fish, 12g of whale meat (muscle), and 7g of fat per day. In the main meals of the Faroe Islands residents, percentages of fish and pilot whales were 44% and 9.5%, respectively (Vestergaard and Zachariassen, 1987: Unpublished). Among fish, cods (mean total mercury level: 0.07μg/g) were consumed the most. The total mercury level of pilot whales was 3.3μg/g (approximately half is methylmercury: Table 1-2) (Grandjean et al., 1993[4], Julshamn et al., 1987 [5]). In addition, based on the hearing investigation and study on mercury levels of pilot whales and cods, the mean total mercury intake of approximately 36μg was calculated (age: ≧14 years) (Weihe et al.,1994[6]).

Muscles, fat, livers, and kidneys of pilot whales were consumed in the Faroe Islands (Julshamn et al., 1987[5]).

Table 1-1 Total mercury, methylmercury, and selenium levels in muscle tissues of pilot whales (Globicephalus Meleanus) caught off the Faroe Islands in 1978.
(Partially recalculated according to the report by Julshamn et al., 1987[5])

Size ("skinn")	Total Hg (mg/kg)	MeHg (mg/kg)	Percentage (MeHg/total Hg)	Se (mg/kg)	Molar ratio (total Hg/Se)	Molar ratio (MeHg/Se)
Fetuses	0.24	0.12	*50*	0.05	1.9	1.0
Fetuses	0.48	0.36	*75*	0.03	6.3	4.7
1	0.21	0.15	*71*	1.5	0.06	0.04
2	0.10	0.06	*60*	0.56	0.07	0.04
3	0.90	0.30	*33*	0.11	3.2	1.1
4	1.2	0.65	*53*	0.42	1.1	0.60
5	1.5	0.75	*49*	0.37	1.6	0.79
5	1.6	0.85	*54*	0.22	2.8	1.5
6	1.6	0.38	*24*	0.33	0.5	0.45
7	2.3	1.3	*57*	0.22	4.1	2.3
7	1.4	0.78	*58*	0.31	1.7	0.99
7	1.2	0.65	*55*	0.32	1.5	0.80
8	1.4	0.85	*61*	0.39	1.4	0.86
8	1.2	0.74	*63*	0.50	0.92	0.58
8	2.8	1.31	*47*	0.64	1.7	0.81
9	2.7	0.91	*33*	0.28	3.9	1.3
10	2.2	0.78	*36*	0.16	5.3	1.9
14	1.8	0.65	*37*	0.40	1.7	0.64
16	2.00	1.72	*86*	0.19	4.1	3.6
Mean*1 (Classification)	1.4	0.68	*49*	0.40	-	-
Mean*2	1.5	0.76	*56*	0.40	2.4	1.3

* 1 : The overall mean was calculated after obtaining mean values for each size classification
*2: The simple mean value

Table 1-2 Mean concentrations of total mercury, methylmercury, and selenium in the blubber, muscle, liver and kidney of mature pilot whales (Globicephalus Meleanus) caught off the Faroe Islands in 1977.
(Julshamn et al., 1987[5])

Tissue	N (Samples)	Concentration (mg/kg fresh weight; mean±SD)		
		Total Hg	MeHg	Se
Blubber	9	0.70±0.28	0.17±0.10(25%)	0.12±0.08
Muscle	10	3.3±1.7	1.6±0.4(48%)	0.25±0.11
Liver	8	280±100	35±10(13%)	172±10
Kidney	6	18±6		1.3±0.8

Table 1-3 Data of Maternal Fish Intake During Pregnancy (Grandjean et al., 1992[2])

No. of fish dinners/wk	Head-count
None	26
1/week	139
2/week	356
3/week	285
4/week	157
5 or more	33
Total	997

Table 1-4 Data of Maternal Pilot Whale Intake During Pregnancy
(Grandjean et al., 1992[2])

No. of whale dinners/wk	Head-count
None	204
1/week	277
2/week	243
3/week	86
4 or more	180
Total	989

Table 1-5 Data of Maternal Fish Intake During Pregnancy When No Pilot Whale Had Been Eaten
(Grandjean et al., 1992[2])

No. of fish dinners/wk	Head-count
None	17
1/week	49
2/week	73
3/week	49
4 or more	16
Total	204

(d) Cohort

➢ (Summary)

The following 4 cohorts were set as the Faroe birth cohorts. As for cohorts 2-4, the main object of the study was not confirming the reproducibility of the results obtained from cohort 1, but analyzing a persistent chemical substance (POPs).

Cohort	Summary of cohorts
Cohort 1	➢ 1,023 pairs of mothers and children born in 1986/1987 (Approximately 75% of the total births in the Faroe Islands) ➢ Study on mercury effects
Cohort 2	➢ 182 pairs of mothers and children born in 1994 ➢ PCB(POPs), Mercury
Cohort 3	➢ 650 pairs of mothers and children born in 1998/1999 ➢ PCB(POPs), Mercury
Cohort 4	➢ 150 pairs of mothers and children born in 2000/2001

➢ (Follow-up)

Neurodevelopmental tests with cohort 1 were conducted at ages of 7 and 14 as shown below.

	Subjects (Dropout rate)	Study period	References
Cohort 1	1,023 (75.1% of the total population of the islands)	Mothers who gave birth between March 1, 1986 and December 31, 1987, and children registered in the Faroe Islands	(2)
➢ **Neurodevelopmental tests at 7 years of age**	923 (Dropout rate 9.7%)	Tests were conducted between 1993 and April-June, 1994 Tests with children who immigrated to mainland Denmark in June, 1994	(7),(8), (9),(10)
➢ **Neurodevelopmental tests at 14 years of age**	883 (Dropout rate 13.7%)	Tests were conducted between 2000 and April-June, 2001 Tests with children who immigrated to mainland Denmark in November, 2000	(11),(12)

(e) Exposure biomarkers used

Biomarkers used in the tests with cohort 1 are indicated below:

	Subjects		References
Cohort 1	Cord blood mercury level	Mean: 24.2μg/L (n=997)	(2)
	Maternal hair mercury level at childbirth	Mean: 4.5μg/g (n=1020)	
	Cord blood selenium level	Mean: 1.4μmol/L (n=1020) (110.5μg/L)	
	Cord blood lead level	Mean: 82nmol/L (n=1015) (17.9μg/L)	
➢ **1-year-old children**	Hair mercury level of 1-year-old children	Geometric mean 1.1μg/g (n=583)	(13)
➢ **7-year-old children**	Hair mercury level of 7-year-old children	Geometric mean 2.99μg/g (n=903)	(7)
	(Maternal hair mercury level at childbirth)	4.27μg/g (n=914)	
	(Cord blood mercury level)	22.9μg/L (n=894)	
➢ **14-year-old children**	Hair mercury level of 14-year-old children	Geometric mean 0.96μg/g (n=839)	(10),(11),(12)

(f) Endpoints (effect indexes)

➢ (Endpoints and reasons for their selection)

Endpoints used in the neurodevelopmental tests with cohort 1 are described below. Endpoints were selected based on the followings:① sensitive to methylmercury exposure, ②reflect the damaged site by methylmercury, ③ generally specific, and ④ applicable to age and culture. In addition, computer-assisted tests were used wherever possible, and at the same time, sufficiently skilled investigators were assigned (Murata et al. 2004[1]).

	Endpoints	Description	References
➢ **7-year-old children**	➢ Neurophysiological tests		
	● Patter reversal visual evoked potentials (VEP)	Evoke delayed conduction by damage and others	(1),(7)
	● Brain stem auditory evoked potentials (BAEP)	Evoke delayed conduction with damage and others	
	● Postural sway	By throwing the gravity center of the body onto a board (stabilometer) placed on a solid floor, the moving distances in back and forth and horizontal directions are measured. Moving distance and area of the gravity center of the body, and Romberg ratio (measurement ratio between eye-opening and eye-closure) are obtained. Moreover, component power spectrum density at frequencies 0-1, 1-2, and 2-4 Hz of swaying gravity center can be calculated by spectrum analysis of deflection data.	
	● Electrocardiographic R-R	Variation factor of R-R interval time on an electrocardiogram is obtained by sequentially measuring one heartbeat time of R-R intervals on an	

interpeak intervals (CV_{RR})	electrocardiogram, and by dividing the calculated standard deviation with its mean value. By spectrum analysis of R-R interval variation in a resting supine position, parasympathetic and sympathetic activities and sympathetic balance can be quantitatively examined.
	A test on micro-motor functions. Subjects are instructed to tap the button as quickly as possible within the given time.
➢ Neuropsychological tests	
● Finger Tapping Test (Neurobehavioral Evaluation System, NES)	A test on micro-motor functions. There is a bright spot that moves up and down with a joy-stick, and moves from left to right over time on the screen. Subjects are asked to move this bright spot as close as possible to the sine curve on the monitor screen.
● Hand–Eye Coordination Test (NES)	
	A test on attention ability. Several pictures of animals are shown successively on the monitor screen, and subjects are asked to press a button at hand quickly when a picture of a cat appears.
● Tactual Performance Test	
● Continuous Performance Test(NES)	
	A test on cognitive functions. Several numbers are auditorily presented to subjects, and subjects are asked to recite them.
	A test on visual analogy. The investigator shows a partially-lacking picture, and subjects are asked to point out the part that is lacking.
● Digit Spans (Wechsler Intelligence Scale for Children, WISC-R)	
	A test on spatial perception. Subject are asked to construct the shape that the investigator presents using several blocks with red and white triangles or quadrangle patterns.
● Similarities(WISC-R)	
	A test on visuomotor ability. Several geometric figures are presented to subjects, and they are asked to correctly depicts them. Upon completion of all the trials, subjects are asked to recall and depict the presented figures.
● Block Designs(WISC-R)	
	A test on memory and learning ability. A list consisting of 12 words is read out, and subjects are asked to recite it (short-term memory). This trial is repeated 5 times. After 20 minutes, the subject is asked to recite the list one more time (long-term memory).
● Bender Gestalt Test	

	● California Verbal Learning Test (Children)	A test on verbal ability. Various pictures depicted on cards are presented to subjects, and subjects are asked to name them.	
	● Boston Naming Test		
	● Nonverbal Analogue of Mood States		
➢ **14-year-old children**	➢ Neurophysiological tests		
	● Patter reversal visual evoked potentials (VEP)		(14), (15)
	● Brainstem auditory evoked potential (BAEP)		
	● Event-related potential(P300)		
	● Coefficient of variation for R-R interpeak intervals (CV_{RR})		
	➢ Neuropsychological test [1]		
	● Finger Tapping Test (Neurobehavioral Evaluation System, NES)		
	● Hand–Eye coordination Test (NES)		
	● Continuous Performance Test (NES)		
	● Digit Spans (Wechsler Intelligence Scale for Children, WISC-R)		
	● Similarities (WISC-R)		
	● Block Designs (WISC-R)		
	● Bender Gestalt Test California Verbal Learning Test(Children)		
	● Boston Naming Test		
	● Nonverbal Analogue of Mood States		
	● Santa Ana Test		

(g) Data analysis method

Multiple linear regression analysis was conducted, considering confounding factors. Structural equation analysis (structural equation model) was conducted at the same time.

A critical level (benchmark dose) was calculated by a benchmark dose method.

[1] 14-year-old neuropsychological test items were prepared based on the lecture by Murata, a reviewer of the 6th Expert Committee on Pollutants.

(h) Results

➢ (The study with 7-year-old children)

① Results of the neuropsychological tests

In multiple regression analysis, increased cord-blood Hg concentration was significantly associated with worse scores on finger tapping (preferred hand, *p=0.05*), continuous performance test in the first year of data collection (false negatives, *p=0.02*; mean reaction time, *p=0.001*), WISC-R digit span (*p=0.05*), Boston Naming Test (no cues, *p=0.0003*; with cues, *p=0.0001*), and the California Verbal Learning Test-Children (short-term reproduction, p=0.02; long-term reproduction, *p=0.05*).

For two end points (WISC-R block design, Bender Gestalt Test errors), associations indicating adverse Hg effects (*p<0.05)* were found when an alternative approach to adjustment for confounders (Peters-Belson method) was applied (*p=0.02, p=0.03*, respectively).

Results were similar when the 15% of the cohort with maternal-hair Hg concentrations greater than 10ppm were excluded from the analyses.

For the tests selected to represent attention (reaction time, *p=0.003*), verbal (Boston naming test, *p=0.02*), and memory (California verbal leaning test, *p=0.004*), the percentages of children with adjusted scores in the lowest quartile increased significantly as cord-blood Hg concentration increased (Grandjean et al., 1997[7], NRC 2000[16]).

In addition, the effects of methylmercury exposure on motor function and verbal ability were statistically significant, even if confounders and effects of PCB exposure were excluded by using structural equation analysis (Budtz-Jørgensen et al., 2000[17]).

② Results of the neurophysiological tests

A statistically significant relationship was shown only between the cord blood mercury level and brainstem auditory evoked potential (40Hz), but no relationship with other biomarkers was demonstrated (Table 3, Grandjean et al., 1997[7]). As for neurophysiological tests, since the electroencephalograph used for the measurement in 1994 was regarded inaccurate, the 7-year-old brainstem auditory evoked potentials measured in 1993 were reanalyzed, and a statistically significant relationship between the cord blood mercury level and maternal blood mercury level at childbirth was shown at III (20Hz, 40Hz) and I-III (40Hz) summit potentials. However, neither of them showed a significant relationship with the hair mercury level of 7-year-old children (Murata et al., 1999[18]).

③ Effects of PCB's

The effects of cofounders in the study with 7-year-old children were statistically analyzed. Fundamental confounders included sex, age, and maternal intelligence, while empirical cofounders included presence of obstetrical/medical diseases, parental education levels, and paternal professions. In addition, districts of residence and PCB (Polychlorinated biphenyls) exposure were considered as other cofounders. Cord tissue levels were used in PCB analysis, and measurement was conducted with 438 children among those who participated in the 7-year-old study. Among them, cord blood PCB levels of 50 children were also measured, which demonstrated a significantly high correlation with the cord tissue PCB levels (correlation factor r=0.90). Three test data, including reaction time, the Boston Naming Test, and the California Verbal Learning Test-long delay, showed significant relationships with the cord tissue PCB levels (significant level of one-sided

5%). However, in the multiple linear regression analysis, using both cord blood mercury and PCB levels as explanatory variables, and indexes of exposure effects (endpoints) as object variables, the cord blood mercury levels showed significant relationships with reaction time and Boston Naming Test results. On the contrary, PCB levels showed no significant relationships with indexes of exposure effects (endpoints) (Grandjean et al., 2001[9], Murata et al., 2002[19]).

④ Effects on blood pressure

A possible relationship between low-level methylmercury exposure and increased blood pressure has been reported (Sorensen et al., 1999[10]).

- (The study with 14-year-old children)

① Neuropsychological tests

Many test results remain unreported. As for continuous reaction time, a statistically significant relationship between cord blood mercury levels and reaction time of 14-year-old children was reported (Grandjean et al., 2002[15]).

② Neurophysiological tests

In the multiple linear regression analysis, which examined the effects of methylmercury exposure on brainstem auditory evoked potentials, statistically significant relationships were indicated between cord blood mercury levels and III/V (20, 40Hz), maternal hair mercury levels and III/I-III (20Hz), and 14-year-old child hair mercury levels and III-V (40Hz) (Murata et al., 2004[12]).

Methylmercury in seafood

(1) A major epidemiological study on methylmercury

② The Seychelles child development study (cohort study)

(a) Summary of results

As a pilot study, the Revised Denver Development Screening Test (DDSTR) and other tests were conducted on a cohort of 804 pairs of mothers and children born in 1987 and 1989 at 5-109 weeks and 66 months after birth. The results showed significant but unclear mercury effects.

In the main study, neurodevelopmental tests were conducted at 6.5, 19, 29, and 66 months, and 9 years of ages with a cohort of 779 pairs of mothers and children born between 1989 and 1990. No effects of methylmercury exposure on nerves, cognition, and behavior of children were observed at any ages.

(b) Background

- (History, culture, ethnic group)

The Republic of Seychelles consists of 115 small and large islands, located out in the West Indian Ocean, northwest of Madagascar Island. The combined area of the islands is 443km^2. The population is approximately 80,000, and around 80% of them live on Mahe Island, the largest island in which the capital Victoria is located. After colonial rule by France (1756) and Britain (1814), they attained independence as a democratic nation in 1976, and established a government organized by a French communist party in the following year; a coalition government by multiple parties was established in 1991.

English, French, and Creole (French origin) are the three national languages used, and Creole is the main home language. Their diet consists of fish, local fruits, vegetables, and imported rice. In many homes, chicken and pork are consumed 1-3 times/week, and beef is seldom consumed due to its high price. As for religions, 90% of them are Roman Catholics and 8% are members of the established Church of England.

Most residents are Creoles (a hybrid race of European and African); however, due to the immigrations of Chinese and Indian merchants in the 19th century, the racial background is complicated.

Tourism and fisheries are the major industries (Shamlaye et al., 1995[26], Oka et al., 2004[28]).

- (Background of conducting the study)

Marsh et al. reported the background of the Seychelles study (necessity of the study) as follows (Marsh et al., (1995)[17]).

First, the Iraqi data demonstrated a dose-response relationship which suggested that the fetal lowest-effect level was indicated by a maximum maternal hair mercury level during pregnancy in the range of 10-15ppm (Marsh et al., 1987[29], Cox et al., 1989[30]). This effect level (tentative) was supported by the studies in Canada and New Zealand,

but the conclusions from these three studies are not definitive because of ① the small numbers of study subjects, ② inability to account for all covariables that may have affected child development, and/or ③ lack of sufficiently sensitive and discriminating measures of outcome (Marsh et al., 1995[17]).

However, after reviewing these studies, the World Health Organization recently concluded that a 5% risk of minimal effects in offspring may be associated with a peak maternal hair mercury level of 10-20ppm (WHO, 1990[30]). A public health concern exists because women of fertile age in fish-consuming countries often exceed this threshold range (Marsh et al., 1995[17]).

➢ (Reasons for selecting the study area)

Marsh et al. reported the following reasons for selecting Seychelles as a candidate area (Marsh et al, 1995[17]):

First, while seeking an appropriate location for a more definitive study on the fetal effects of MeHg, we obtained and analyzed samples of scalp hair from 1,616 people, mostly women of fertile age from fish-eating communities in Canada (Indian and Inuit), Peru, American Samoa, Malta, and the Maldives. Although maximum hair mercury concentrations of 35 to 75ppm were in the right range, studies were abandoned for one or more of the following reasons: excessive alcohol consumption with high prevalence of ① fetal alcohol effects; ② high infant mortality rate; ③ small population size of communities; ④ difficult access and poor communication systems; and ⑤ lack of local collaborators.

Our initial visit to the Republic of the Seychelles was prompted by data on scalp hair mercury concentrations determined by atomic absorption analysis in 36 Seychellois (Matthews, 1983[18]). Matthews reported that mean concentrations varied from <5ppm to 45ppm and that the median concentration was 10ppm with 12% above 20ppm. ① These values straddled the lowest effect level estimated from the Iraqi studies, and contrast with average levels in the USA which fall below 2ppm. ② Our analyses by X-ray fluorescence (XRF) of hair from 90 pregnant Seychellois women were close to those reported by Matthews (1983[18]). In addition, XRF analysis showed that hair concentrations of calcium, copper, iron, zinc, and manganese were all in the normal range for adequately nourished adults.

In addition, Shamlaye et al. (1995[26]) reported that the Seychelles had the following advantages as the study area, : ① Seychellois consume a high quantity and wide variety of ocean fish on a regular basis (over 80% of the population consume fish meals at least once a day. Fish is the main source of protein, and marine mammals do not form part of the diet.), ② the mercury concentration in maternal hair is in the appropriate range to study low-level exposure, ③ Seychelles is 1,000 miles from any continent or large population center, but is served by several international airlines, ④ Communication by telephone, mail and dispatch services is easy and reliable, ⑤ there is no local industry for pollution, ⑥ it is distant from other sources of industrial pollution, and ⑦ Seychellois are a generally healthy population (Maternal tobacco and alcohol use are low).

➢ (Study institutions)

- ✓ The University of Rochester

- ✓ The Seychelles Ministry of Health
- ✓ Cooperation (older than school age)： The Seychelles Ministry of Education)
- ✓ Sponsors： U.S. National Institute of Environmental Health Science, the Seychelles Ministry of Health, and so forth

(c)Sources of exposure

There is a report that approximately > 80% of female inhabitants in the Seychelles consume fish everyday, and its frequency (median) per week during pregnancy is 12 times. According to the report, maternal interviews confirmed a high fish consumption with the median of 12 fish meals per week. Sixteen percent of women consumed 5 to 9 fish meals per week and 75% consumed 10 to 14 fish meals per week during their pregnancy. Only 8% of the study mothers ate fewer than 5 fish meals a week. However, there is no accurate report of methylmercury exposure in the Seychelles, including fish species and amount of intake, therefore no information is available concerning daily exposure to methylmercury via fish (Shamlaye et al., 1995(26)).

As for reference information concerning fish species, mercury levels of each fish species that are commercially important in the fishing industry have been reported (Matthews 1983(18), Table 1). Recently, total mercury and selenium levels of fish species (16 species) that are usually found in the local markets have been reported, and the mean total mercury and selenium levels of all of the species were 0.07mg/kg[1] and 0.29mg/kg, respectively (Robinson et al., 2004(31), Table 2).

[1] The unit is ppm in the original work.

Table 1 Total mercury survey of various species of fish (Matthews et al., 1983[18])

Fish species	Academic name	Mercury range (mg/kg)	Weight range (kg)
1.Yellowfin tuna	*Thunnus albacares*	0.012-0.6	1.6-50.0
2.Skipjack tuna	*Katuswonus pelamis*	0.026-0.448	2.2-5.7
3.Dogtooth tuna	*Gymnosarda unicolor*	0.38-4.4	7.0-40.0
4.Bonito	*Euthynnus affinis*	0.065-1.26	0.9-6.35
5.Bludger(Carangue balo)	*Carangx gymnostethus*	0.025-1.51	0.75-11.45
6.Kingfish	*Acanthocybium solandri*	0.55-1.46	4.8-22.6
7.Becune	*Sphyraena forsteri*	0.26-1.58	0.6-4.7
8.Sailfish	*Istiophorus platypterus*	0.01-0.86	fork length 90-210 cm
9.Bourgeois	*Lutjanus sebae*	0.045-0.69	2.0-13.0
10.Vara Vara	*Lutjanus bohar*	0.135-0.812	0.7-9.1
11.Vielle platte	*Epinephelus flavocaeruleus*	0.13-0.9	4.4-12.7
12.Job	*Aprion viriscens*	0.01-1.035	0.7-8.2

Table 2 Mean total Hg and Se concentrations (mg/kg), with 95% confidence intervals (CI), for the target species (Robinson et al., 2004[31])

Fish species	Academic name	Mean Hg (mg/kg) ±95%CI	Mean Se (mg/kg) ±95%CI
1.Brown spot grouper	*Epinephelus chlorostigma*	0.061±0.009	0.328±0.103
2.Variegated emperor	*Lethrinus variegates*	0.073±0.031	0.513±0.015
3.Pink-earemperror	*Lethrinus lentjan*	0.115±0.017	0.524±0.280
4.Ember parrotfish	*Scarus rubroviolaceus*	<0.01	0.365±0.077
5.Parrot fish	*Hipposcarus harid*	0.032±0.013	0.263±0.178
6.Goatfish	*Parupeneus porphyreus*	0.018±0.008	0.009±0.095
7.Grey Sweetlips	*Plectorhinchus schotaf*	0.142±0.014	0.475±0.204
8.Shoemaker spinefoot	*Siganus sutor*	0.020±0.011	<0.006
9.Streamlined spinefoot	*Siganus argenteus*	<0.01	<0.006
10.Green jobfish	*Aprion virescens*	0.047±0.012	<0.006
11.Red snapper	*Lutjanus bohar*	0.098±0.013	<0.006
12.Carangid[2]	*Carangoides fulvoguttatus*	0.052±0.004	0.261±0.087
13.Rainbow runner	*Elagatis bipinnulata*	<0.01	0.554±0.096
14.Pickhandle barracuda	*Sphyraena jello*	0.360±0.031	0.205±0.032
15.Bonito	*Euthynnus affinis*	0.049±0.017	0.791±0.152
16.Indian mackerel	*Rastrelliger kanagurta*	<0.01	0.407±0.074

[2] Jack fish

(d) Cohort

➢ (Summary)

Cohort 1 is set as a Seychelles birth cohort for the main study that began in 1989 (Marsh et al., 1995[17]).

Cohort 0 is for the pilot study that began in 1987, preceding the main study (Marsh et al., 1995[17], Myers et al., 1995[21]).

Cohort	Summary of the cohorts
Cohort 0 (Pilot study)	804 pairs of mothers and children born in 1987/1988 (15 pairs were excluded based on the standards).
Cohort 1 (Main study)	779 pairs of mothers and children born in 1989/1990 on Mahe Island (50% of the total births, among them, 39 subjects were excluded; inappropriate maternal hair samples(15 subjects), matched with the predetermined criteria for exclusion (18 subjects), twins (6 subjects)).

(Follow-up)

As stated below, the neurodevelopmental studies with cohort 1 (main study) were conducted at 6.5, 19, 29, 66 months and 9 years of age.

Prior to the main study, neurodevelopmental tests with cohort 0 (pilot test) were conducted at 5-109 weeks and 66 months after birth as shown below (Myers et al., 1995[21]).

	Subjects (Dropout rate[3])	Study periods	References
Cohort 0 (Pilot test) Setting	789	1987-1989 registration period of childbearing mothers and children on Mahe Island of the Seychelles.	(17)
➢ **Neurodevelopment tests with 5-109-week-old children**	789 (Dropout rate-%)		(17),(21)
➢ **Neurodevelopment tests with 66-month-old children**	217 (Dropout rate 72.4%)	66 months±3 months	(20)

	Subjects (Dropout rate)	Study periods	References
Cohort 1 setting	740 (Dropout rate-%)	Registration period of one year, between March 1989 and February 1990, of childbearing mothers and children on Mahe Island (Nearly 50% of the total).	(17)
➢ **Neurodevelopment tests with 6.5-month-old children**	712－737 (Dropout rate 0.5-3.5%)	Conducted between September 1989 and August 1990. 6.5 months±2 weeks	(17),(19)

[3] Dropout rates in 789 subjects of the pilot study and 740 subjects of the main study.

➢ **Neurodevelopment tests with 19-month-old children**	738 (Dropout rate 0.2%)	19 months±2 weeks	(6),(17)
➢ **Neurodevelopment tests with 29-month-old children**	736 (Dropout rate 0.5%)	29 months±2 weeks	
➢ **Neurodevelopment tests with 66-month-old children**	711 (Dropout rate 3.9%)	July 1994-October 1995 66 months±6 months	(8),(17)
➢ **Neurodevelopment tests with 108-month-old (9-year-old) children**	643 (Dropout rate 13.1%)		(24)

(e) Mercury exposure indexes used (biomarkers)

Biomarkers used in the study with cohort 0 are indicated below.

	Biomarkers		References
Cohort 0 setting	Maternal hair mercury levels	Median: 6.6(ng/mg)(n=789) Range: 0.6-36.4 Interquartile range: 6.1 Group: 0-3, >3-6, >6-9, >9-12, >12	(21) (20)
➢ **Neurodevelopment tests with 5-109-week-old children**	Maternal hair mercury levels	Ibid.	
➢ **Neurodevelopment tests with 66-month-old children**	Maternal hair mercury levels	Median: 7.1(ng/mg) (n=217) Range: 1.0-36.4 Interquartile range: 6.0 Group: ≦3, 4-6, 7-9, 9-12, >12	(20)

Biomarkers used in the study with cohort 1 are indicated below.

	Biomarkers		References
Cohort 1 setting	Maternal hair mercury levels (during pregnancy)	Median: 5.9(ng/mg)(n=740) Range: 0.5-26.7 Interquartile range: 6.0 Group: 0-3, >3-6, >6-9, >9-12, >12	(19)
➢ **Neurodevelopment tests with 6.5-month-old children**	Ibid.	Ibid.	(19)
➢ **19-month-old children** ➢ **Neurodevelopment tests with 29-month-old children**	Ibid.	Ibid.	(6) (19)
➢ **Neurodevelopment tests with 66-month-old children**	Maternal hair mercury levels (during pregnancy)	Mean (Standard deviation): 6.8(4.5)(ng/mg)(n=711) Range: 0.5-26.7 Group: ≦3(mean 2.0), >3-6(mean 4.5),	(8)

		>6-9(mean 7.4), >9-12(mean 10.3), 12-26.7(mean 15.3)	
	Child hair mercury levels	Mean (Standard deviation): 6.5(3.3)(ng/mg)(n=708) Range: 0.9-25.8 Group: ≦3(mean 2.2), >3-6(mean 4.6), >6-9(mean 7.4), >9-12(mean 10.2), 12-25.8(mean 14.9)	
	Child blood PCB levels	Undetected（n=49）	
➢ **Neurodevelopment tests with 108-month-old (9 year old) children**	Maternal hair mercury levels (during pregnancy)	Mean (standard deviation): 6.9(4.5)(ng/mg)(n=643) group: ≦3, >3-6, >6-9, >9-12, >12	(24)

(f) Endpoints (effect index)

➢ (Endpoints and reasons of their selection)

Tests (endpoints) used in the neurodevelopment tests with cohort 1 (the main study) are indicated below. A test battery was selected that allowed comparison with ① previous studies (Canada, Iraq, and Peru), ② was age-appropriate, ③ tested a wide range of developmental domains, and ④ minimized cultural effects (Marsh et al., 1995[17], Mayers et al., 1994[21]).

In addition, a review of the literature concerning human fetal exposure to low concentrations of methylmercury revealed that adverse effects might occur in one or more of the following eight domains of developmental functioning: ① general cognitive, ② visual-perceptual, ③ speech-language, ④ visual memory, ⑤ visual attention, ⑥ neuromotor-neurological, ⑦ social-emotional, and ⑧ learning-achievement. Tests were then selected to adequately assess each of these domains (Davidson et al., 1995[7]).

Cohort	Study periods	Test (endpoint)	Description	References
Cohort 0 (pilot study)	5-109 weeks after birth	DDST-R: Revised Denver Developmental Screening Test-revised	Screening test to assess the development based on the 4 aspects of ≦ 6-year-old children (personal-social, fine motor adaptive, language, and gross motor). Graded as normal, abnormal, or questionable.	(17),(21)
		General medical/neurologic tests		(17),(21)
	66 months	McCarthy Scales of Children's Abilities	A test on subscales concerning overall measurement of cognitive function, General Cognitive Index (GCI), verbal memory, perception, numerical quantity, and motor function. This test is applicable to 2.5-8.5-year-old children.	(17),(20)

		Preschool Language Scale	A test to measure verbal expression and understanding. Applicable to 1.5-7-year-old children.	
		Woodcock-Johnson Tests of Achievement	A test on reading (characters, words) and numeric ability. Used for tests on learning disorders.	
Cohort 1 (main study)	6.5 months	DDST-R		(19)
		Fagan Infantest:	A test on visual perception memory and visual attention. Nonverbal test. Future perception ability can be predicted. Presumed correlation with IQ measured at about 3 years of age.	(17),(19), (32)
	19 months	Bayley Scale of Infant Development-MDI, PDI	This test is widely used for many studies on child lead exposure in various cultures or fetal life to examine cognitive function. There are 2 subscales (MDI, PDI).	(6),(17)
		Raven Standard Progressive Matrix Test	Major intelligence test for primary caregiver.	(6)
		Home observation for measurement of the environment: home	Assess home environment.	(6)
	29 months	Bayley Scale of Infant Development-IBR	In addition to the tests at 19-month of age, a behavior record of Bayley Scale of Infant Development-IBR is added the to tests at 29-month of age.	(6)
	66 months	McCarthy Scales of Children's Abilities-GCI		(8)
		Preschool Language Scale		(8)
		Woodcock-Johnson Tests of Achievement		(8)
		Bender Gestalt Test	Visual-spatial ability tests.	(8)
		Child Behavior Checklist	A test on child's ability for social adaptive behavior .	(8)
	9 years	(Neuropsychological tests) WISC III: Wechslar Intelligence Scale for Children III full-Scale IQ	Among 13 test items, 5 are used (information, block design, vocabulary, digit span, coding). One of them is a comprehensive clinical method to test child's intellectual ability.	(10),(24)
		Woodcock-Johnson Tests of Achievement, Letter-word recognition, and applied problems subset	(Learning-achievement)	
		California Verbal Learning Test	(Verbal memory)	
		Visual memory subtest of the Wide Range Assessment of Memory and Learning (WARMAL)	(Memory) Aims to assess both memory and learning by asking subjects to draw 4 geometric designs according to memory.	
		Trail making		

		Finger Tapping	(Motor function) A test to measure motion speed by rapidly tapping a button with the index finger.	
		Grooved Pegboard	(Ibid.) A test to assess dexterity of handling according to the length of time to insert a peg into a board.	
		Bruininks-Oseretsky Test of Motor Proficiency	(Ibid.)	
		Boston Naming Test	(Verbal) Test to bring out effective function by asking subjects to name the presented drawings (from common words to complex ones).	
		Beery-Buktenica Developmental Test of visual motor integration and a test of haptic matching	(Hand-eye coordinated movement) Test of copying complex geometric pictures.	
		Connor's Continuous Performance Test	(Continuous response time)	
		Conner's Teacher Rating Scale and Behavior child behavior checklist	(Behavior)	

(g) Data analysis method

Cohort	Test periods	Test items	Covariates[4]	Statistical analysis	References
Cohort 0 (Pilot study)	5-9 weeks after birth	DDST-R	Child's gender Birth weight APGAR score[5] Age at test period Mother's age Alcohol intake/smoking during pregnancy Medical history Socioeconomic factor (The number of persons per room)	Multiple logistic regression analysis	(21)
	66 months	McCarthy Scales of children's abilities and others	Birth weight Mother's age Child's gender Medical history (mother/child) Alcohol intake/smoking during pregnancy Socioeconomic factor (The number of families per room) APGAR score	Multiple liner regression analysis	(20)
Cohort 1 (main study)	6.5 months	DDST-R, Fagan Test	Child's gender Birth weight Order of births Age at pregnancy Medical history (mother/child) Alcohol intake/smoking during pregnancy Intelligence of child-bearer Educational history of parents History of breast-feeding Language used at home Family income	Multiple regression analysis (however, multiple linear regression analysis for continuous outcome, and logistic regression analysis for binary outcome	(19)
	19/27 months	BSID and others	Birth weight Order of births Age at pregnancy Child's gender History of breast-feeding Medical history (mother/child) Intelligence of child-bearer Educational history of parents Alcohol intake/smoking during pregnancy Language used at home Family income HOME (home environment assessment)	Multiple regression analysis (however, multiple linear regression analysis for continuous outcome, and logistic regression analysis for binary outcome	(6)

[4] Covariate： Variable with predicted effects on the results of the study subjects. Covariates might become modifiers of immediate study subjects or of confounder variables and effects (Epidemiological Dictionary (Third edition)).

[5] Measurement of child development after birth

	66 months	McCarthy Scale of Children's abilities-GSI and others	Birth weight Order of births Child's gender History of breast-feeding Audibility Medical history (mother/child) Age at pregnancy Alcohol intake and smoking during pregnancy Intelligence of child-bearer Language used at home Hollingshead Socioeconomic status HOME score	Multiple linear regression analysis	(8)
	9 years	WISC III full-scale IQ and others	Child's gender Investigator The number of family members (family resource scale) The number of parents (family status code) HELPS (Henderson Early Learning Process Scale) Child's age Medical history (child) Mother's age HOME score k-bit (Kaufman brief intelligence test to determine caregiver intelligence) Audibility Mercury levels in child	Linear-regression analysis	(24)

(h) Summary of results

➢ (Pilot study)

- Tests at 5-109 weeks after birth

An association between fetal mercury exposure and development was found when DDST-R scores of questionable and abnormal were combined, a procedure used by previous investigators.

These results should be viewed with caution since the association disappeared when DDST-R scores of questionable were treated in the standard manner as passes (Myers et al., 1995[21]).

- Tests at 66 months after birth

Results of the multiple linear regression analysis indicated that mercury exposure was negatively associated with four endpoints (McCarthy General Cognitive Index and Perceptual Performance subscale and The Preschool Language Scale Total Language and Auditory Comprehension subscale). After normalizing the data by removal of a small number of outliers or highly influential scores, the mercury effects were no longer significant except for auditory comprehension (Myers et al., 1995[20]).

➢ (Main study)

- Tests at 6.5 months after birth

There were three (0.4%) subjects who scored abnormal on the DDST-R and an additional 11 (1.5%) who scored questionable. No analysis was possible because of the small numbers of questionable and abnormal results.

In the Fagan test, the association between fetal mercury exposure and neurodevelopmental endpoints was examined by multiple regression analyses. After adjusting for covariates, no association between the maternal hair mercury level during pregnancy and an adverse neurodevelopmental outcome of the child was identified at 6 1/2 months of age (Myers et al., 1995[19]).

- Tests at 19-29 months after birth

No mercury effect was detected in the BSID scores at both 19 and 29 months.

In the 29-month Bayley infant behavior Record, activity levels (only males) decreased as mercury exposure increased. This is the only endpoint that correlated with prenatal methylmercury exposure (Davidson et al., 1995[6]).

- Tests at 66 months after birth

At 66 months, there was no relationship between harmful effects and methylmercury exposure before and after birth. Some subjects in the highest postnatal methylmercury exposure group produced beneficial results. This relationship remained constant also in the results of the multiple linear regression analysis (Oka et al., 2004[28]).

- Tests at 9 years after birth

Only two endpoints were associated with prenatal MeHg exposure. Increased exposure was associated with decreased performance in the grooved pegboard using the non-dominant hand in males and improved scores in the hyperactivity index of the Conner's teacher rating scale. Covariates affecting child development were appropriately associated with endpoints (Myers et al., 2003[24]).

Appendix 3

Scientific findings from the high-risk group

1. Background of the request for discussion about the high-risk group

(1) The Ministry of Health, Labour and Welfare is planning to reexamine the "Advice for Pregnant Women on Fish Consumption concerning Mercury Contamination (released by the Pharmaceutical Affairs and Food Sanitation Council[(1)] on June 3, 2003: http://www.mhlw.go.jp/shingi/2003/06/s0603-3.html)". For this reason, the Food Safety Committee was requested to conduct a food safety risk assessment of "methylmercury in seafood" by the Ministry of Health, Labour and Welfare on July 23, 2004. More specifically, the Ministry requested to set a tolerable methylmercury intake, and at the same time, to discuss about the high-risk group (the document No. 0723001 from the Department of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare under the date of July 23, 2004).

(2) The current Japanese Advice concerning mercury intake cover "pregnant and potentially pregnant women"; however, the range of people covered by the Advice and those who are subject to the advices in the U.S., Britain, Canada, Australia, and others are not necessarily the same; therefore, discussion about the high-risk group was also requested (the document No. 0723001 from the Department of Food Safety, Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare under the date of July 23, 2004).

2. Concept of the people subject to the current Japanese Advice

The Ministry of Health, Labour and Welfare examined "safety assurance against mercury contained in seafood" in Joint Sub-Committees on Animal Origin Foods and Toxicology under the Food Sanitation Committee, the Pharmaceutical Affairs and Food Sanitation Council, held on June 3, 2003, and published "Advice for Pregnant Women on Fish Consumption concerning Mercury Contamination" of the same date.

On this occasion, the Ministry of Health, Labour and Welfare identified "pregnant and potentially pregnant women" as the high-risk group due to the high fetal susceptibility to mercury exposure. The Ministry of Health, Labour and Welfare described the concept of the subject people in the "Advice for Pregnant Women on Fish Consumption concerning Mercury Contamination (Q & A)" released by the Pharmaceutical Affairs and Food Sanitation Council[(1)] on June 3, 2003: http://www.mhlw.go.jp/topics/2003/06/tp0613-1.html (Reference 1).

3. People subject to dietary intervention in foreign countries and concepts of the subjects

(1) Advices regarding intake of methylmercury in seafood have been published for consumers in the U.S., Britain, Canada, and other countries (Pharmaceutical Affairs and Food Sanitation Council[(1)]). As for the subject people, pregnant and potentially pregnant women are included in common, but other subject people varied by each country (Reference 2).
(2) The concepts (bases) of the subject people in every country are as follows:

① Pregnant women： In every country, pregnant women are subject to dietary intervention based on the scientific findings that methylmercury has critical effects on the developing central nervous system and the most susceptible group results from prenatal exposure.

② Potentially pregnant women： According to the UK COT, there have been no studies of the effects of exposure prior to becoming pregnant. However, because the half-life of methylmercury in the human body is approximately 70 days, steady state concentration is attained in approximately one year and a woman's blood mercury level at the time of becoming pregnant is dependent on the exposure to methylmercury during the preceding year. The Committee therefore agreed that women who may become pregnant within the next year should also be considered as a susceptible population (Pharmaceutical Affairs and Food Sanitation Council[(1)] P68, Morrisette et al., 2004[(2)]). Also in the U.S., the same concept has been suggested (Pharmaceutical Affairs and Food Sanitation Council[(1)] P36, Question 2).

③ Infants： The UK COT states that, the Iraq poisoning incident has suggested that some children exposed to methylmercury via breast-milk demonstrated delayed motor development (Maternal blood mercury levels immediately after the accident: 100-5,000μg/L (estimate)). In addition, the limited data available indicate that this is not the case for children but the possibility of increased sensitivity of infants cannot be discounted. However, there is no statement that identifies infants as the target subjects, for the following reasons: ① There is no evidence that chronic exposure to methylmercury via breast-milk at levels below those observed in the Iraqi incident has any adverse effect on the neurophysiological/psychological development of the child. ② Animal experiments indicate that exposure via breast-milk has less serious consequences to the central nervous system than prenatal exposure. ③ Correlation of intakes by the breast-fed infant and the mother indicates that the methylmercury intake of the breast-fed infant is within the 2003 PTWI of 1.6μg/kg bw/week if the mother's intake is within the 2000 PTWI of 3.3μg/kg bw/week (Pharmaceutical Affairs and Food Sanitation Council[(1)] P68-70, Dellinger 2004[(3)], Julshamn et al., 2004[(4)], Keiding et al., 2003[(5)], Lyketsos 2003[(6)]).

According to the Australia/New Zealand Food Standards, very little mercury from fish is transferred to breast-milk so the risk to the nursing infant is much lower, however, breastfeeding mothers may still wish to follow the advice for pregnant women (Pharmaceutical Affairs and Food Sanitation Council[(1)] P103). No definite evidence has been indicated in the U.S., Ireland, and EU, either, but breastfeeding mothers are subject to dietary intervention from the viewpoint of infant protection (Pharmaceutical Affairs and Food Sanitation Council[(1)] P99-102, P103-146, and P147-150).

④ Children※ (except infants)： In the UK COT, there have been few studies of

※ Although child development after birth varies among individuals, childhood is classified according to ages as follows (Comprehensive Medical Dictionary):

the effects of methylmercury on young children. Most information has come from the poisoning incidents in Minamata, Niigata and Iraq. In all of these cases the exposure were very high, and in Iraq, the exposure was acute. It was suggested that methylmercury is excreted by children as efficiently as by adults, and the study in the Seychelles, the children exposed to methylmercury postnatally are also exposed prenatally, and the study has been unable to demonstrate any mercury-related deficits in the neurological development of children. However, there is uncertainty with respect to whether infants and young children are at greater risk of methylmercury toxicity whilst the central nervous system is still developing, therefore, 16-year-old and younger children are identified as the target subjects (Pharmaceutical Affairs and Food Sanitation Council[1] P88, Lyketsos 2003[6], Landrigan et al., 2003[7], Lapham et al., 1995[8]). On the contrary, children except infants are subject to dietary intervention in every country, although no concrete bases have been indicated.

⑤ Others (adults)： The U.S. EPA states that, for most people, the risk from mercury by eating fish and shellfish is not a health concern (Pharmaceutical Affairs and Food Sanitation Council[1] P38).

In the UK COT, they note that there has been no new information published to indicate that the 2000 PTWI of 3.3μg/kg bw/week is not sufficiently protective of the general population, and consider that a methylmercury intake of 3.3μg/kg bw/week may be used as a guideline to protect against non-developmental adverse effects (Pharmaceutical Affairs and Food Sanitation Council[1] P88, Myers et al., 1995[9]).

Also in Ireland, Australia, and New Zealand, pregnant and potentially pregnant women, breastfeeding mothers, and consumers other than children are similarly identified as the target subjects. In Australia and New Zealand, the tolerable weekly intakes to protect the health of the public (3.3μg/kg bw/week) and fetuses (1.6μg/kg bw/week) have been set, and it is advised that intake should be below these values when consuming Shark (Flaks) or Billfish (Swordfish/Broadbill and Marlin) containing relatively high level of mercury (Pharmaceutical Affairs and Food Sanitation Council[1] P99-150).

4. Findings from prenatal and children toxicity

(1) According to the JECFA, methylmercury is transported to fetuses across the placenta, blood and brain mercury levels of fetuses are generally higher than those of mothers at childbirth, and the amount of methylmercury transported from blood to breast-milk is lower than that from blood to the brain or placenta. Exposure to children via breastfeeding is lower than prenatal exposure (JECFA[10]).

(2) In the comparison study of maternal blood mercury levels and cord blood mercury levels of 119 Swedish females, 0.73μg/L of median maternal blood

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① Newborn (4 weeks after birth), ② Infancy (<1 year), ③ Early childhood (1-6 years), ④ Early school age (6-10 years), ⑤ School age (6-12 years), ⑥ Adolescence (Female: 8 or 10-18 years, Male:10 or 12-20 years), ⑦ Puberty ((Mean) Female: 13 years, Male: 15 years)

mercury level and 1.4μg/L of median cord blood mercury level were reported (ASK et al., 2002[11]).

(3) Seven cases of exposure(age: 22-35, mean: 29.6) were examined by comparing maternal blood levels at childbirth with cord blood levels, and exposed cord blood levels with 3-month-old infant blood levels. Although mercury levels in red blood cells of cord blood were 1.4 fold higher than maternal blood mercury levels during pregnancy, mercury levels in red blood cells of infants decreased during infancy, and mercury levels in red blood cells of 3-month-old infants were 0.54 fold higher than mercury levels in red blood cells of cord blood.

Sakamoto et al. reported that the decrease during infancy could be explained by the small amount of methylmercury migration via breast-milk and rapid growth of infants (approximately 1.9 fold faster) (Sakamoto et al., 2002[12], Table 1).

Table 1 Mercury levels in blood (red blood cells) and breast-milk

Mercury levels in red blood cells			Breast-milk mercury levels (D)	A / B	C/ A
Cord blood (A)	Mother (B)	Infant (3 months old) (C)			
10.6 (13.0) (Geometric mean)	7.1 (8.2)	5.8 (6.9)	0.21 (0.2)	1.4 (1.5)	0.54 (0.53)

(Unit： ng/g:ppb)

Note: The values in parentheses are excerpts from the lecture data of Sakamoto, a reviewer (30, 32).

① Female rats were fed a diet containing 5ppm methylmercury and after their blood levels reached a steady state, they were mated, and the same dose was administered to the mother rats during pregnancy and lactation. Changes of the methylmercury accumulation in each tissue of the mother rats, fetuses, infants, and children were examined. As a result, brain mercury levels of children (during the fetal-birth period) were approximately 1.5-2 fold higher than those of the mother rats, but their brain mercury levels were rapidly reduced to approximately 1/10 during infancy, compared with those during late pregnancy. The same results were reported for the mercury levels in liver and blood (Pan et al., 2004[13]).

② In addition, female rats were given drinking water containing methylmercury chloride (0, 0.5, and 6mg/L) before mating, during pregnancy, and the breastfeeding period between birth and 16 days of age, and brain and blood mercury levels at birth and weaning age (21 days after birth) were measured. Comparison between mercury levels at birth and at weaning age showed 0.48ppm and 0.045ppm in the low-dose group, and 9.8ppm and 0.53ppm in the high-dose group, respectively, demonstrating 1/10-1/20 reduction of brain mercury levels at the weaning age, compared with those at birth. As for the brain weight, the increase was up to 5.5 fold between the birth and weaning age, demonstrating the lowest mercury exposure during this period and a reduction of absolute brain mercury amounts (Newland & Reire, 1999[14]).

(Reference 1)

"Advice for Pregnant Women on Fish Consumption concerning Mercury Contamination" (Q & A) published by the Pharmaceutical Affairs and Food Sanitation Council(1) on June 3, 2003
http://www.mhlw.go.jp/topics/2003/06/tp0613-1.html (Excerpt)

(1) Q5. Only pregnant women are identified as target subjects in the Advice. Does it mean that other people are safe?

(A) Extremely high-level of mercury, especially methylmercury, has been reported to have caused Minamata disease. However, at the levels estimated in the Advice, the concerned health effects are not on ordinary adults but on fetuses with high susceptibility. For this reason, the Advice was drafted with focus on pregnant women. At this time, there are no data that evoke concerns for harmful health effects of mercury on children and adults, except pregnant women; therefore, they are not subject to the Advice.

(2) Q6. Should breastfeeding women be careful about consuming seafood?

(A) As a result of discussion at the Joint Sub-Committees on Animal Origin Foods and Toxicology under the Food Sanitation Committee, the Pharmaceutical Affairs and Food Sanitation Council (hereinafter called "Joint Meeting"), held on June 3, risks during breastfeeding are considered low at present, since risks to health are particularly high during pregnancy but low during breastfeeding due to the reduced mercury migration to breast-milk, compared with maternal blood mercury levels. For this reason, breastfeeding mothers are not subject to the Advice.

Reference 2: Dietary interventions in foreign countries (Pharmaceutical Affairs and Food Sanitation Council[1])

	Country	Pregnant and Potentially pregnant women	Breastfeeding mothers (infants)	Other children	Others	Description of bases	Remarks
(1)	U.S. FDA/EPA	○ 1) Woman who may become pregnant, 2) Pregnant woman.	○ Nursing mothers	○ (parents of) young child	—	—	P33-37 The amount should be reduced in young children
(2)	UK COT	○ 1) Pregnant woman 2) Women who are intending to become pregnant	—	○ Children under 16	—	○	P55
(3)	Canada Health CANADA (FOOD INSPECTION AGENCY	○ Woman of Childbearing age.	—	○ Young children	—	—	P93-97
(4)	Ireland Food Safety Authority of Ireland	○ Woman of Childbearing age, pregnant	○ Breastfeeding woman	○ Young Children	○ Consumers other than the above groups	—	P99-102
(5)	Australia/New Zealand Food standard Agency Food Standard Australia New Zealand	○ Woman Planning (considering) pregnancy, pregnant	○ Breastfeeding woman	○ Young Children	○ Consumers other than the above groups	—	P103-146
(6)	EU European Food Safety Authority	○ Women of childbearing age (in particular, those intending to become pregnant), Pregnant	○ Breastfeeding women	○ Young children	—	—	P147-150

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