There was a significant relationship between hair mercury concentrations in the mothers and children (Fig. 5). Also, the mercury concentration in maternal hair was significantly correlated with the methylmercury concentration in umbilical cord, but no significant association was seen between the child's hair mercury concentration and methylmercury concentration in umbilical cord (Fig. 6). Therefore, the maternal hair mercury concentration could be applied as a proxy for the exposure level at parturition.

Figure 5 Relationship between hair mercury concentrations in 327 mothers and children in Japan. Black (●) and white (○) circles represent subjects in Akita and Tottori, respectively.

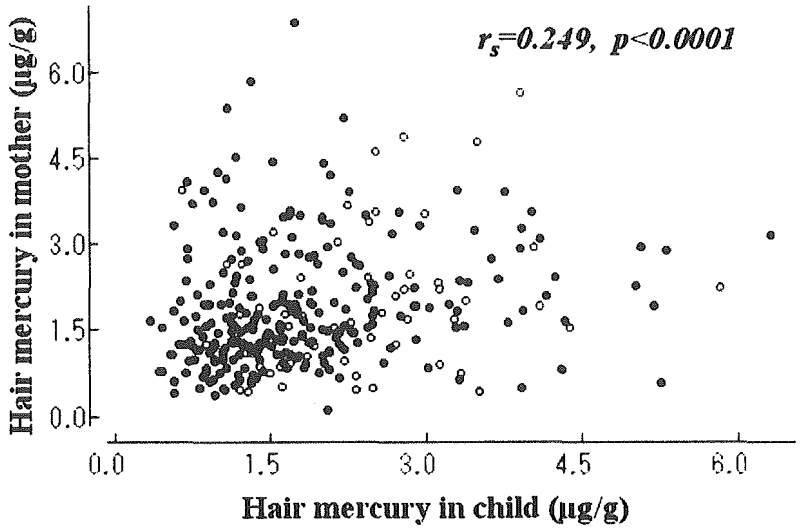
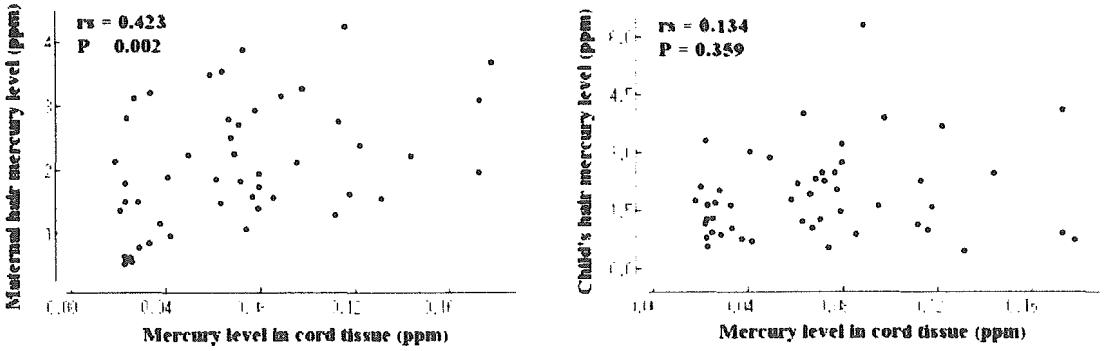


Figure 6 Relationships between the methylmercury level in umbilical cord and hair mercury level in 50 mothers and children in Japan



Possible confounders

Results of the body weight at birth, gestation period, smoking and drinking habits during pregnancy obtained by interview and questionnaire are shown in Table 2. Of

327 children, 21 had low birth weight of less than 2500 g. There was no child with phenylketonuria, maple syrup urine disease, homocystinemia, galactosemia, congenital hypothyroidism, neuroblastoma, or adrenal hyperplasia. According to present and past history of illness, there were one child with spinal progressive muscular atrophy, one with congenital malformation (cleft palate), and one with epilepsy. Therefore, 23 children either with the above disease or with low birth weight (<2500 g) were excluded from the study population.

Table 2 Basic characteristics of 327 mothers and the children

	Mean (or Number, %)	SD	Range
Body weight at birth (g)	3142	436	1568 ~ 4568
Gestation period (weeks)	39.0	1.5	33 ~ 42
Smoking during pregnancy	25, 7.6%		
Drinking during pregnancy	43, 13.1%		
Natural delivery (no Caesar)	290, 88.7%		
Gestosis (edema, anemia, etc)	130, 39.8%		
Febrile convulsion	30, 9.2%		
Otitis media	132, 40.4%		

Table 3 Results of postural sway test in 156 boys and 148 girls

Results of postural sway test	BOYS	GIRLS	Statistical significance (P)
	Mean± SD	Mean± SD	
Without foam			
Transversal sway distance (mm) [eyes open]	5.50±1.97	4.40±1.22	0.0000
Sagittal sway distance (mm) [eyes open]	5.66±2.03	4.76±1.53	0.0000
Sway area (mm^2) [eyes open]	874±597	553±283	0.0000
Sway velocity (mm/s) [eyes open]	16.5± 5.4	13.8± 3.8	0.0000
Transversal sway distance (mm) [eyes close]	6.12±2.04	4.88±1.46	0.0000
Sagittal sway distance (mm) [eyes close]	6.27±1.90	5.16±1.43	0.0000
Sway area (mm^2) [eyes close]	1276±920	757±438	0.0000
Sway velocity (mm/s) [eyes close]	23.0± 8.0	18.7± 5.6	0.0000
With foam			
Transversal sway distance (mm) [eyes open]	6.15±1.74	4.99±1.13	0.0000
Sagittal sway distance (mm) [eyes open]	6.86±2.07	5.97±2.04	0.0003
Sway area (mm^2) [eyes open]	1302±738	883±449	0.0000
Sway velocity (mm/s) [eyes open]	23.8± 7.0	19.4± 5.5	0.0000
Transversal sway distance (mm) [eyes close]	7.68±2.44	6.06±1.56	0.0000
Sagittal sway distance (mm) [eyes close]	7.77±2.65	6.56±1.84	0.0000
Sway area (mm^2) [eyes close]	2082±1514	1391±878	0.0000
Sway velocity (mm/s) [eyes close]	33.5±11.1	27.7± 8.6	0.0000

Table 4 Results of tremor, coordination, and reaction time tests in 156 boys and 148 girls

Other neurobehavioral tests	BOYS	GIRLS	Statistical significance (P)
	Mean ± SD	Mean ± SD	
Tremor test:			
Intensity (m/s^2), right	0.191 ± 0.068	0.168 ± 0.052	0.0013
Center frequency (Hz), right	5.50 ± 0.93	5.64 ± 0.90	0.2633
Intensity (m/s^2), left	0.221 ± 0.088	0.202 ± 0.070	0.0419
Center frequency (Hz), left	5.08 ± 0.94	5.19 ± 0.76	0.2598
Ear-hand coordination test:			
Mean difference in slow rhythm (s), right	-0.069 ± 0.060	-0.078 ± 0.055	0.1681
Mean difference in slow rhythm (s), left	-0.073 ± 0.053	-0.069 ± 0.054	0.5619
Mean difference in fast rhythm (s), right	-0.082 ± 0.049	-0.062 ± 0.055	0.0007
Mean difference in fast rhythm (s), left	-0.084 ± 0.051	-0.063 ± 0.052	0.0010
Reaction time:			
Mean time (s), right	0.352 ± 0.060	0.360 ± 0.057	0.1839
Mean time (s), left	0.372 ± 0.063	0.383 ± 0.058	0.0822
Eye-hand coordination:			
Mean time (ms)	653 ± 81	681 ± 77	0.0014
Variance (SD, ms)	166 ± 39	156 ± 39	0.0248
Minimal time (ms)	267 ± 137	304 ± 147	0.0309
Maximal time (ms)	951 ± 70	957 ± 57	0.4158
Error number	6.4 ± 4.9	3.5 ± 3.4	0.0000

Table 5 Results of neurophysiological tests in 156 boys and 148 girls

Neurophysiological tests	BOYS	GIRLS	Statistical significance (P)
	Mean ± SD	Mean ± SD	
Brainstem auditory evoked potentials:			
Peak I latency (ms) [20Hz]	1.78 ± 0.15	1.75 ± 0.15	0.1393
Peak III latency (ms) [20Hz]	3.95 ± 0.17	3.85 ± 0.16	0.0000
Peak V latency (ms) [20Hz]	5.79 ± 0.21	5.65 ± 0.21	0.0000
Peak I latency (ms) [40Hz]	1.82 ± 0.16	1.80 ± 0.17	0.2812
Peak III latency (ms) [40Hz]	4.05 ± 0.18	3.94 ± 0.17	0.0000
Peak V latency (ms) [40Hz]	5.93 ± 0.20	5.78 ± 0.21	0.0000
ECG:			
Heart rate (/s)	81.1 ± 9.6	83.6 ± 10.8	0.0260
QTc interval (ms)	392.2 ± 15.4	392.4 ± 15.5	0.8958
R-R interval analysis:			
CV_{RR} (%)	6.20 ± 2.28	6.32 ± 2.23	0.5984
C-CV_{HF} (%)	3.93 ± 2.14	4.10 ± 2.14	0.4678
C-CV_{LF} (%)	4.16 ± 1.82	4.40 ± 1.85	0.2407
%LF	52.9 ± 12.3	52.6 ± 12.5	0.8748

Effects of mercury exposure on child neurodevelopment in Japan

In using the multiple regression analysis to control for age and sex, any dose-effect relationships between the hair mercury concentration in either the mothers or children and outcome variables of neurobehavioral or neurophysiological tests were not statistically significant ($p>0.05$). Regarding the postural sway test, all parameters in 156 boys were significantly larger than those in 148 girls (Table 3); some parameters of other neurobehavioral tests in the boys were also significantly larger than those in the girls (Table 4). Similarly, some BAEP latencies in the boys were significantly prolonged as compared to the girls (Table 5).

International comparison between data in Japan and Madeira

In the Madeiran study including 143 mothers and their children, the hair mercury concentration, child's age, BAEP latencies, other neurobehavioral tests, and some possible confounders were surveyed in 1995 (Murata *et al.* 1999a). Other examinations except the BAEP seemed to be affected by language, education, or socioeconomic factors. For the quality assurance, the BAEP was measured by the same manner and examiner. As shown in Table 6, the age in the Madeiran children was similar to that in the Japanese children.

*Table 6 BAEP latencies (mean±SD) in children obtained from Japan and Madeira**

	Japan (n=292)	Madeira (n=143)+
Age	6.90±0.30	6.93±0.30
BAEP:		
I peak (20Hz)	1.77±0.15	1.98±0.23‡
III peak (20Hz)	3.88±0.17	4.15±0.29‡
V peak (20Hz)	5.69±0.21	6.02±0.30‡
I-III interpeak (20Hz)	2.11±0.13	2.17±0.23†
III-V interpeak (20Hz)	1.81±0.15	1.87±0.17†
I peak (40Hz)	1.82±0.17	2.06±0.25‡
III peak (40Hz)	3.97±0.18	4.32±0.35‡
V peak (40Hz)	5.81±0.21	6.28±0.34‡
I-III interpeak (40Hz)	2.15±0.14	2.26±0.28‡
III-V interpeak (40Hz)	1.85±0.13	1.96±0.21‡

* Analysis of covariance was used to control for age and gender.

† $p<0.05$, ‡ $p<0.001$.

Figure 7 Latencies of the BAEP in children obtained from Japan and Madeira. Analysis of covariance was used to control for age and gender.

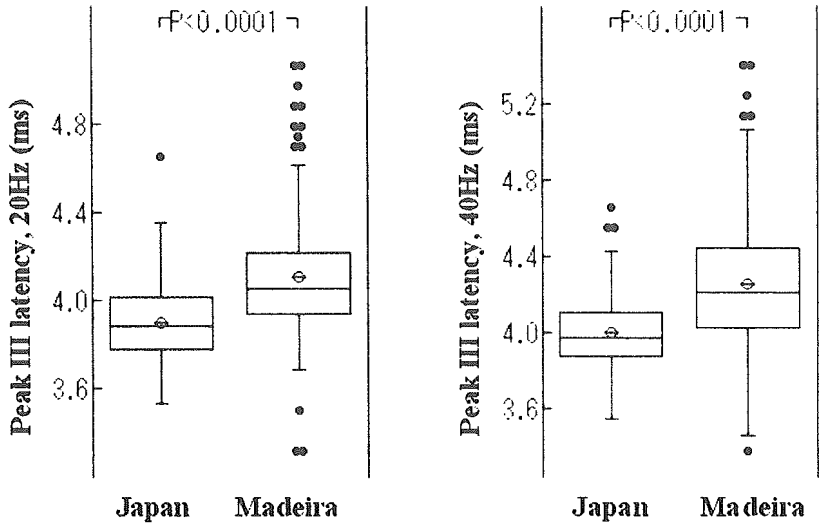
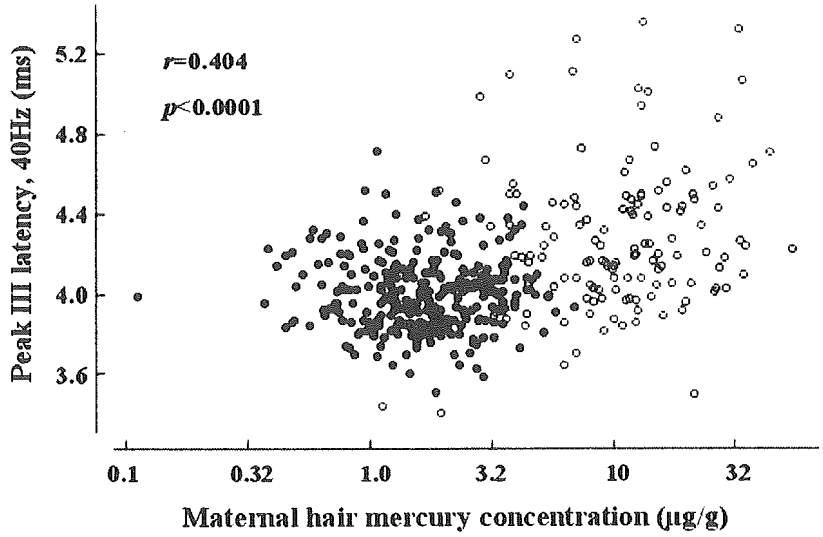


Figure 8 Relationship between maternal hair mercury concentration and peak III latency of the BAEP in children obtained from Japan (●) and Madeira (○). The partial correlation coefficient after adjusting for age, gender and race was 0.110 (p=0.0226).



Medians of hair mercury concentrations were 1.64 (range, 0.11~6.86) μg/g for 292 Japanese mothers and 9.7 (range, 1.12~54.4) μg/g for 143 Madeiran mothers, and the latter was significantly higher than the former. By contrast, means and standard deviations of the BAEP latencies in Japan were significantly smaller than those in Madeira. Fig. 7 exemplifies latencies of the BAEP in Japan and Madeira. Also, significant dose-effect relationships between the hair mercury concentration and BAEP latencies were found in the combined data of Japanese and Madeiran children (Fig. 8); but, current mercury levels in child's hair were not significantly related to any BAEP

latencies (p>0.05), after controlling for age, gender and race.

By using the data of 143 Madeiran children, and combined data of 292 Japanese and 143 Madeiran children, the BMDs and BMDLs were calculated after controlling for age, gender and race, as shown in Table 7. The BMDLs (mean 9.1 μg/g) in the latter became significantly smaller than those (mean 9.9 μg/g) in the former (paired sample t test, p=0.0142).

Table 7 Benchmark dose (BMD, μg/g) and its lower 95% confidence limit (BMDL, μg/g) at benchmark response level of 0.05 according to dose-effect models for BAEP latencies at 20 Hz and 40 Hz in 143 Madeiran children and 292 Japanese children

	Data in Madeira				Combined data of Madeira and Japan			
P_0=0.05	Linear model*		Power model**		Linear model*		Power model**	
	BMD	BMDL	BMD	BMDL	BMD	BMDL	BMD	BMDL
20Hz:								
Peak III latency	20.48	11.03	20.67	11.20	16.17	9.97	16.34	10.12
Peak V latency	15.81	9.46	15.98	9.63	14.10	9.14	14.26	9.30
Interpeak I-III latency	18.20	10.40	18.40	10.57	14.51	9.24	14.69	9.40
40Hz:								
Peak III latency	14.65	9.02	14.83	9.17	11.23	7.84	11.40	7.99
Peak V latency	18.21	10.33	18.39	10.50	16.49	10.37	16.65	10.53
Interpeak I-III latency	15.23	9.31	15.43	9.47	11.72	8.02	11.89	8.16

Maternal hair mercury levels (μg/g) were used as a proxy for exposure biomarker at parturition.

* Linear model: $[BAEP]=b_0+b_1\cdot[dose]+b_2\cdot[age]+b_3\cdot[gender]$ $(+b_4\cdot[race])$.

** Power model: $[BAEP]=b_0+b_1\cdot[dose]^k+b_2\cdot[age]+b_3\cdot[gender]$ $(+b_4\cdot[race])$.

Discussion

None of Japanese children in this study had any clinical mercury-related abnormalities, although it is well known that patients with Minamata disease have neurological signs and symptoms such as paresthesia, constriction of visual field, intention tremor, impairment of hearing and speech, mental disturbances, and unsteady gait (Kurland *et al.* 1959). Also, medians of current mercury exposures were 1.63 μg/g for the mothers and 1.65 μg/g for the 7-year-old children. These values are consistent with previous ones reported by Japanese researchers (Wakisaka *et al.* 1990; Sakamoto *et al.* 1993; Yasutake *et al.* 2003; Iwasaki *et al.* 2003), and do not exceed the safe limit (10 μg/g) of the International Programme on Chemical Safety (1990), as well as the

no-observed-adverse-effect level (NOAEL) and BMDL of methylmercury which have been reported to be 15.3 μg/g calculated from the Seychelles Child Development Study by the Agency for Toxic Substances and Disease Registry (1999) and 12 μg/g calculated from the Faroese birth cohort study by the US Environmental Protection Agency (2001), respectively. There were no regional differences in the mercury level either between fishing and non-fishing areas or between cities and towns (Table 1). In addition, the hair mercury concentration in the children was slightly but significantly correlated with that in the mothers, and no significant difference in the current mercury level was observed between the mother and child. In this way, it is suggested that Japanese children ingest a similar dose per body weight of methylmercury to their parents, independent of residential areas.

In the present study, the maternal hair mercury concentration had close relation to the methylmercury concentration in umbilical cord, although we could not observe in the children; at least, qualitative evidence would be provided that the maternal hair mercury concentration can be used as a proxy for the mercury exposure level at parturition. In another study, the regression (*i.e.*, gradient) of the peak III latency of the BAEP on maternal hair mercury in the Madeiran cross-sectional study was similar to that on maternal hair mercury at birth in the Faroese birth cohort study, and the BMDs and BMDLs, calculated from the former alone were almost similar to those from the combined data of both children (Murata *et al.* 2002). Thus, the current mercury level in maternal hair reflects the mercury exposure level at birth, under the condition that the dietary habit on fish consumption remains unchanged after the pregnancy with the child.

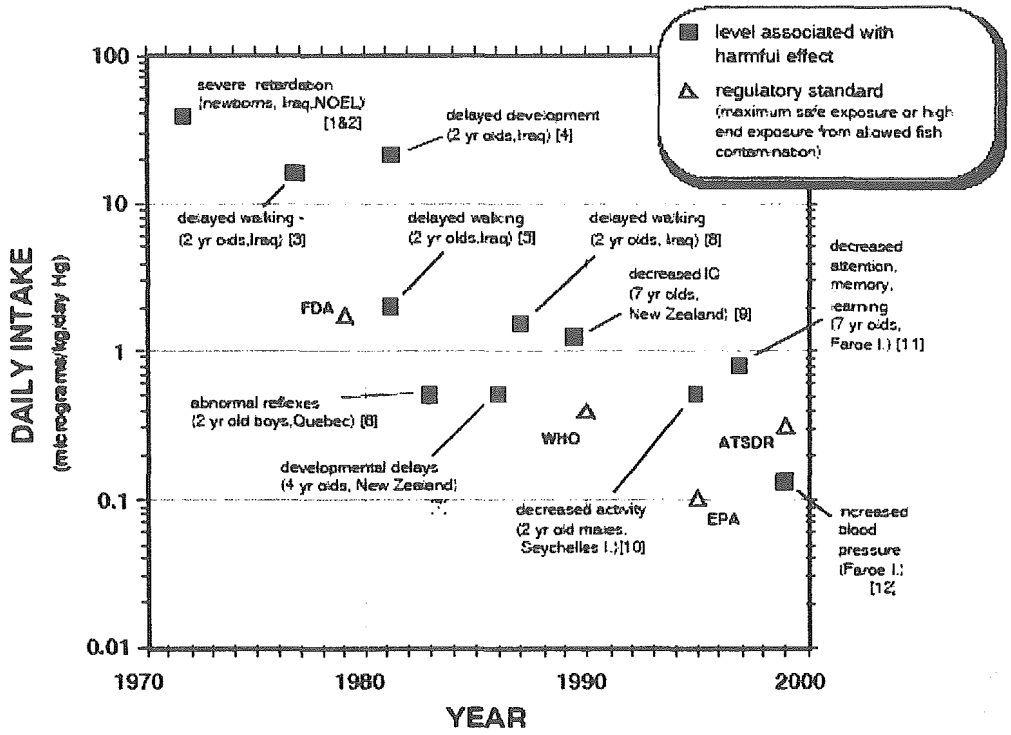
Since we failed to find any dose-effect relations of the outcome variables to mercury exposure in Japanese children, it might infer that Japanese children with mercury levels of less than 7 μg/g had no adverse effects of methylmercury exposure. However, two notes of warning would be struck against the negative finding: (1) The Faroese birth cohort had an enormously wide range of mercury exposure (Grandjean *et al.* 1997), but the range of our exposure biomarker was extremely small. In addition, the former sample number was three times as much as the latter. (2) The effects of possible confounders, such as artificial hair waving (Yamamoto *et al.* 1978; Yasutake *et al.* 2003; Iwasaki *et al.* 2003) and change in dietary habit on fish consumption, were not fully excluded in the present study. At least, mothers with artificial hair waving of this study had about 80% of hair mercury levels in mothers without it. Such exposure misclassification may have underestimated the true effect in risk assessment (Grandjean *et al.* 2002). A larger population that includes higher-level exposures will increase the statistical power. In addition, assessment of prenatal exposure should be improved by

analysis of umbilical cord in the present study or by detailed assessments in prospective studies.

All the BAEP latencies were significantly shorter in the Japanese children than in the Madeiran children (Table 6 and Fig. 7). Although the device used for the BAEP measurement differed between both areas despite the same setting conditions such as bandpass and stimulation, we did not find any differences between pairs of the latencies measured in eight volunteers by using the two different devices. The interpeak I-III and I-V latencies of the BAEP were significantly prolonged in patients with fetal Minamata disease despite the fact that no significant findings were found in patients with acquired Minamata disease (Hamada *et al.* 1982), and there were the significant differences in interpeak III-V and I-V latencies of the BAEP between the Ecuadorian children, exposed to elemental mercury vapors and methylmercury-contaminated food, with blood mercury levels of 20-89 μg/liter and with levels below 20 μg/liter (Counter 2003); the difference in the interpeak between both studies (*i.e.*, I-III and III-V latencies) may imply the differential effects of prenatal and postnatal exposures. Moreover, significant dose-effect associations have been observed between mercury exposure and prolonged latencies of the BAEP in the Faroese birth cohort and Madeiran children (Murata *et al.* 1999a, 1999b). It is therefore suggested that these differences between the Japanese and Madeiran children, would have been attributable mainly to mercury, inasmuch as the mercury exposure level was extremely higher in the latter than in the former.

A BMDL of approximately 9 μg/g in maternal hair for BAEP latencies in the present study is somewhat low as compared to recently calculated BMDLs for other neurological outcome variables in the Faroese children (Budtz-Jørgensen *et al.* 2000) and in a New Zealand population (Crump *et al.* 1998). Using several curve functions, an average BMDL of about 10 μg/g was calculated for crude neurological abnormalities in children exposed in connection with the poisoning incident in Iraq (Cox *et al.* 1989; Crump *et al.* 1995). Higher BMDLs were also reported from a study in the Seychelles, where clear effects on psychological test have not been detected so far (Crump *et al.* 2000). Judging from these reports, as the outcome variable (or endpoint) measured in each study shifts away from the clinical to subclinical adverse effects, the exposure level, at which such an effect emerges, appears to become lower, even as shown in Fig. 9 (Schettler *et al.* 2000). In this respect, the BAEP latencies, as well as neuropsychological tests including Boston Naming Test and California Verbal Learning Test employed in the Faroese birth cohort study (Grandjean *et al.* 1997), are suggested to be one of the most sensitive endpoints to methylmercury exposure.

Figure 9 Declining threshold of harm for mercury (Schettler et al. 2000)



According to the *a priori* hypothesis, the cord-blood mercury concentration is expected to be the best predictor for neurobehavioral decrements in children (Grandjean *et al.* 1992, 1999). Also, it has been demonstrated that the mercury concentration in umbilical cord was significantly correlated with the mercury concentration in cord blood (r_s=0.85, p<0.001), rather than that in maternal hair (r_s=0.77, p<0.001) (Dalgård *et al.* 1994; Akagi *et al.* 1998). Although we obtained only 50 umbilical cords from our Japanese children, it would be possible to reduce exposure imprecision for risk assessment of methylmercury if we could collect more umbilical cords in the same subjects. And, it will enable us to conduct a retrospective cohort study on the effects of prenatal methylmercury exposure on neurodevelopment in Japanese children.

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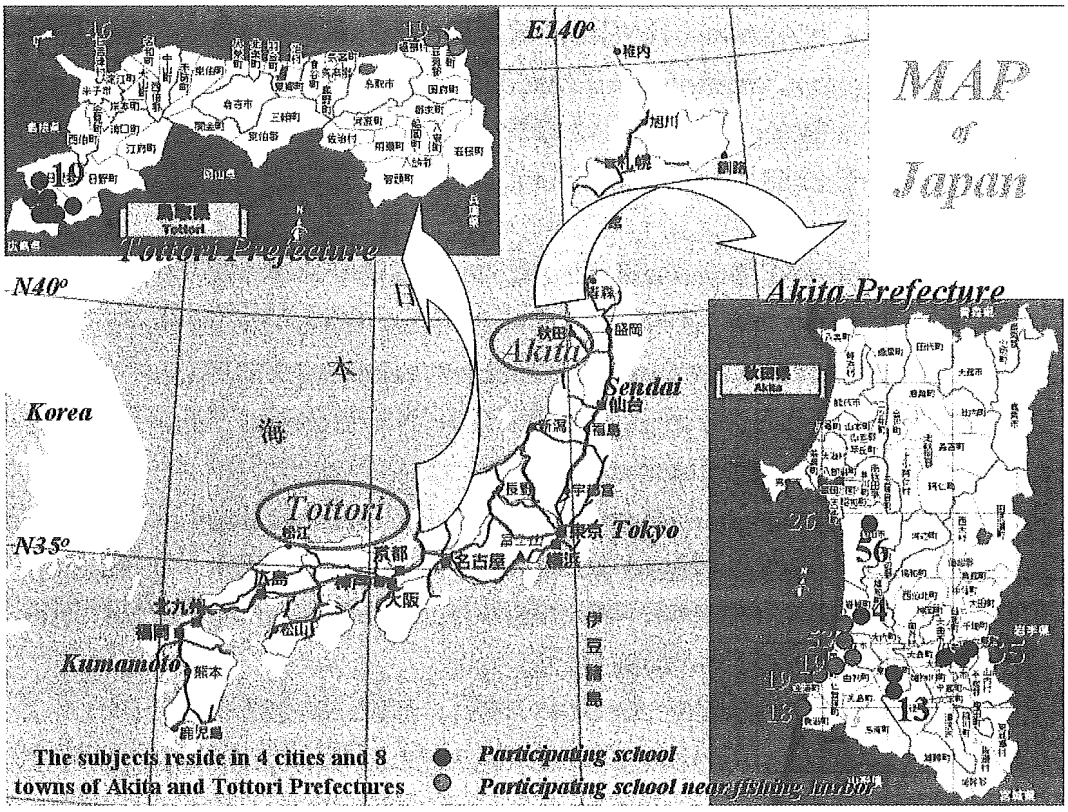
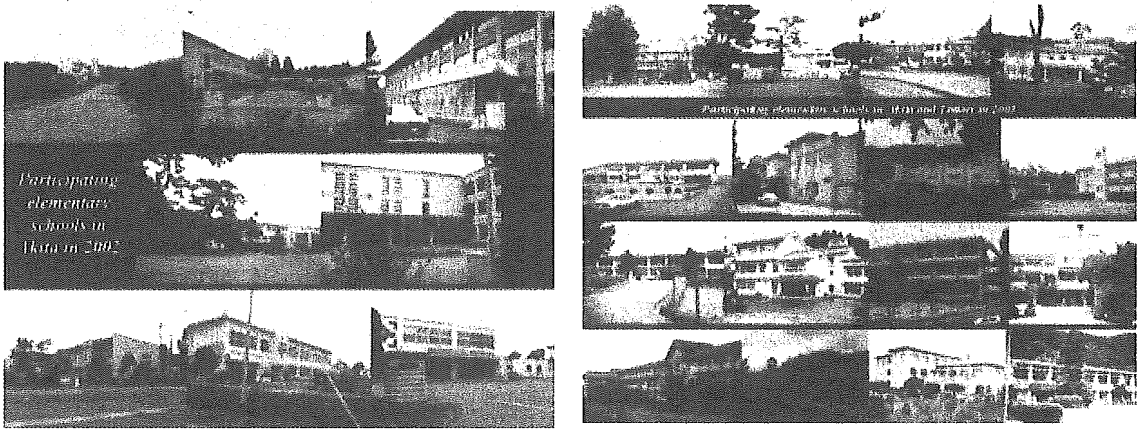
Appendix 1　Map in Japan



Appendix 2　Participating elementary schools in 2002 and 2003



EFFECTS OF PERINATAL EXPOSURE TO ENVIRONMENTALLY PERSISTENT ORGANIC POLLUTANTS AND HEAVY METALS ON NEUROBEHAVIORAL DEVELOPMENT IN JAPANESE CHILDREN: III. MATERNAL SMOKING CONFOUNDS NEONATAL NEUROBEHAVIORAL STATUS

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Introduction

From several epidemiological studies, it has been reported that there are some associations between perinatal exposures to PCBs, dioxins and heavy metals and neurobehavioral defects such as postnatal growth delay and poorer cognitive function. In this study, we designed a prospective cohort study to examine the effects of perinatal exposures to environmentally persistent organic pollutants on neurobehavioral development in Japanese children. This report showed some preliminary data regarding the results of the Neonatal Behavioral Assessment Scales (NBAS), in which we examined the effect of maternal smoking during pregnancy as the confounding factor to the infant neurobehavioral development.

Maternal smoking is well known to have various influences on birth outcome and growth parameters. Children of smoking mothers are often born with a lower birth weight than expected for gestational age. In addition, there is an increased chance of premature labour, intrauterine growth retardation, and perinatal mortality. Recently, the concern on the effects on the neurobehavioral development has been also increased. In studies of the effects of teratogens, NBAS has been recommended and is commonly used as a measure of central nervous system (CNS) development in the neonates. Several investigators have reported that prenatal exposure to tobacco adversely affects CNS development, as measured by the NBAS. Even though, there is no report on this association in Japan.

Methods and Materials

Three hundred forty-four pregnant healthy women attending two obstetrics clinics in Sendai gave their consents to participate in this study according to guidelines established by the ethical committee established by the Tohoku University Graduate School of Medicine. The mean age at the time of delivery was 31.85 (SD 4.69). Women were asked about tobacco use before and during

pregnancy. They were then classified as Nonsmokers, Ex-smokers and Smokers; the women who ceased to smoke prior to or throughout pregnancy were defined as the Ex-smokers. Information was obtained about pregnancy, labour, and delivery conditions from their medical records.

The NBAS was administrated at three days of after birth to 344 (179 boys and 165 girls) infants. They were all singleton and full-term (37 to 42 weeks) infants. In addition, their birth weight was 2500g or more. Examiners of the NBAS were trained and certified to administer the NBAS at the Training Center for NBAS in the Nagasaki University School of Medicine in Japan. Reliability checks were conducted throughout data collection to maintain a 90% level of agreement. Examiners were unaware of the exposure status of the infants including the maternal use of tobacco.

In statistical analysis, comparison between groups of continuous demographic, health, and growth outcomes were performed with ANOVA. For the comparison of categorical variables such as sex and parity were performed using Fisher's exact test. Analyses controlling for maternal age at the time of delivery, sex, Apgar scores, gestational age, birth weight, length and head circumference were performed using a multiple regression analysis.

Results and Discussion

Twelve (3.4%) of 344 mothers smoked an average of 13.0 (SD 6.5) cigarettes per day during pregnancy. Of 332 mothers who did not smoke, 44 were Ex-smokers and 288 were Nonsmokers. There was a significant difference between the infants of smoking and nonsmoking mothers in maternal age at the time of delivery, but no differences in gestational age, Apgar scores, birth weight, length, and head circumference were observed (Table 1).

Table 1 Maternal and infant characteristics

	Maternal smoking during pregnancy		
	No		Yes
	Nonsmoker (n=288)	Ex-smoker (n=44)	Smoker (n=12)
Maternal age*	32.2 (4.3)	30.4 (6.0)	29.1 (4.3)
[1)] Sex (M/F)	145/143	28/16	6/6
[1)] Parity (first/other)	144/144	30/14	6/6
Apgar 1min	8.0 (0.9)	7.9 (0.7)	8.3 (0.5)
Gestational age (weeks)	39.4 (1.4)	39.7 (1.3)	39.2 (1.4)
Birth weight (g)	3018 (378)	3108 (380)	3184 (415)
Birth length (cm)	48.9 (2.2)	49.3 (1.3)	49.0 (2.1)
Head circumference (cm)	33.5 (1.4)	33.4 (1.3)	33.9 (1.4)

ANOVA Mean (SD)

[1)] Fisher's exact test

* $P<0.05$

There were no significant differences among three groups on any of the seven NBAS cluster scores. When the scores of the 28 behavioral items of the NBAS were examined, the infants of Smokers were significantly poorer than those of Ex-smokers and Nonsmoker on the three items, "General tone", "Peak of excitement" and "Cuddliness" (Table 2). These three items remained to be significant after controlling for maternal age at the time of delivery, sex, Apgar scores, gestational age, birth weight, length, and head circumference.

Table 2 NBAS scores

	Maternal smoking during pregnancy		
	No		Yes
	Nonsmoker	Ex-smoker	Smoker
General tone*	4.5 (1.3)	5.2 (1.6)	4.4 (2.1)
(*MOTOR cluster*)	n=236	n=42	n=9
Peak of excitement*	5.1 (1.0)	4.9 (1.0)	4.4 (1.1)
(*RANGE OF STATE cluster*)	n=254	n=43	n=11
Cuddliness*	4.6 (1.3)	4.8 (1.8)	3.6 (1.5)
(*REGULATION OF STATE cluster*)	n=254	n=43	n=11

ANOVA Mean (SD)

* $P<0.05$

Using blinded developmental examinations, and adjusting for other factors, we observed some neurobehavioral defects in infants exposed to tobacco. This result indicates that maternal smoking during the pregnancy is an important risk factor in the infant's neurobehavioral development, and therefore, maternal smoking is confirmed to be a significant confounding factor to consider the effect of perinatal exposures to PCBs, dioxins, and heavy metals on neurobehavioral development. In the literatures, maternal smoking during pregnancy is well known to affect neurobehavioral development, but this is the first report to demonstrate this association in Japanese children. Although this study is not originally designed in order to clarify the risks of maternal smoking, the results might be important to consider the smoking of pregnant women in Japan. Several studies have recently found the reduction in IQ scores in children born to women who smoked during pregnancy. This report represents one of our prospective cohort study to examine the perinatal exposures to environmentally persistent organic pollutants and heavy metals on neurobehavioral development. The long-term effects on child neurocognitive functioning such as IQ should be also clarified in our cohort study.

The present results showed not only that maternal smoking could be a potent confounding factor to examine the effect of perinatal exposures to PCBs, dioxins, and heavy metals to

neurobehavioral development, but also that our NBAS seemed to be enough sensitive to detect a small defects potentially present in the infants exposed to the pollutants.

Acknowledgments

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Invited Review

Behavioral Teratology of Mercury and Its Compounds

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SATOH, H. *Behavioral Teratology of Mercury and Its Compounds.* Tohoku J. Exp. Med., 2003, **201** (1), 1-9 —— Mercury and its compounds have a wide spectrum of toxicities depending upon the chemical forms and modes of exposure. Among the various chemical forms, mercury vapor and methylmercury are well known and established as neurotoxic agents. Since the disasters in Minamata and Iraq, in which fetuses were more susceptible than adults to methylmercury exposure, much attention has been focused on prenatal exposure to mercury and its consequence. Recently postnatal effects of in utero exposure to methylmercury through fish (and marine mammals) consumption by mothers have been concerned and several epidemiological studies have been conducted. Therefore, one of the most seriously concerned issues is the postnatal effects of in utero exposure to methylmercury. Because of these observations in humans, animal experiments have been conducted employing prenatal exposure to low levels of mercury. This paper reviews the animal (rodents) experiments concerning "behavioral teratology" of mercury for better understanding of effects of prenatal exposure to mercury and its compounds in addition to commentary on history and framework of behavioral teratology. ———— mercury; methylmercury; prenatal exposure; postnatal effects; behavioral teratology

Mercury and its compounds have a wide spectrum of toxicities depending upon the chemical forms and mode of exposure (Clarkson 2002). Among its various chemical forms, mercury vapor and alkylmercury compounds, especially methylmercury, are well known as neurotoxic agents. In human subjects repeated exposure to mercury vapor at low concentration caused mercurial erethism, which is characterized by behavioral and personality changes (Hunter 1969). Methylmercury exposure has been repeatedly shown to cause neurotoxicity;

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the typical signs and symptoms are described as Hunter-Russell syndrome (Hunter 1969).

Since the disasters in Minamata (Harada 1978) and Iraq (Bakir et al. 1973), in which fetuses were more susceptible than adults to methylmercury exposure, much attention has been focused on prenatal exposure to mercury and its consequence. Recently postnatal effects of in utero exposure to methylmercury through fish (and marine mammals) consumption by mothers have been concerned (Davidson et al. 1995; Myers et al. 1995b; Grandjean et al. 1997). Several epidemiological studies have been conducted (Kjellstrom et al. 1986; Kjellstrom et al. 1989; Myers et al. 1995a; Grandjean et al. 1997). The source of mercury is naturally occurring and there are populations who depend on fish as main protein source. Therefore, one of the most seriously concerned issues is the postnatal effects of in utero exposure to low levels of methylmercury.

Based on these observations in humans, animal experiments have been conducted employing prenatal exposure to methylmercury at low concentrations. In this paper, the animal (rodents) experiments concerning "behavioral teratology" of mercury are reviewed for better understanding of effects of prenatal exposure to mercury and its compounds.

What is behavioral teratology ?

"Behavioral teratology" is a field of science where postnatal effects of prenatal exposure to any foreign stimulant are investigated. It is considered that the concept of behavioral teratology was first established by Werboff in 1960s (Werboff and Gottleib 1963). He showed behavioral effects on the offspring of the maternal rats that had taken tranquillizers during pregnancy (Werboff 1966). He reviewed earlier and his studies concerning the behavior of the offspring born to mother animals given psychotropic drugs during pregnancy and claimed, "the behavior, functional adaptation of the offspring to its environment, is susceptible to teratogenic effects of drugs" (Werboff and Gottleib 1963). This behavioral teratology has later expanded the harmful agents by including environmental pollutants.

The dawn of behavioral teratology in mercury toxicology

Spyker and colleagues are the pioneers to study the postnatal effects of in utero methylmercury exposure. They revealed impaired swimming ability in offspring mice exposed to methylmercury in utero (Spyker et al. 1972). The control mice were able to swim easily but the treated mice showed "freezing; floating in a vertical position with only head above water" and swimming with legs askew. They also found changes in behaviors in the open field test. These results indicated the important conclusion that motor dysfunction and emotional change are detectabe postnatally. It was noteworthy that the offspring mice did not show any physical retardation or overt neurological signs and were considered to be normal until being examined by the above tests.

Spyker and colleagues (Weiss and Spyker 1974; Spyker 1975a, b) defined "behavioral teratology" as the overlapping area between behavioral toxicology and teratology, the biological study of malformations. This means that the cause of abnormality occurs during pregnancy and the effects become overt after birth over the lifetime of an individual. This was clearly shown by the "Six D's" in behavioral teratology (Spyker 1975a):

Abnormal Development
Behavioral Deviation
Neurological Disorder
Immunological Deficiency
Generalized Debilitation
Premature Death

In spite of prenatal exposure to an environmental stimulant offspring may be born as "normal" at birth. During lactational period, abnormal development may be observed; examples will be shown later. When the off-