smokers. Smokers reported that they smoked an average of 13.0 ($SD = 6.5$, range 2–20) cigarettes per day during pregnancy.

Maternal and Infant Characteristics

Table 1 shows the maternal and infant characteristics. Mothers of the smoking group were younger than in the other two groups ($F_{2,341} = 6.40$, $p < .01$). Infants' characteristics did not differ among the three groups of mothers.

TABLE 1
MATERNAL AND INFANT CHARACTERISTICS

	Maternal Smoking During Pregnancy						F	η^2
	No				Yes			
	Nonsmokers ($n = 288$)		Exsmokers ($n = 44$)		Smokers ($n = 12$)			
	M	SD	M	SD	M	SD		
Maternal Prenancy								
Age†	32.2	4.3	30.4	6.1	29.1	4.3	6.40	.04
Alcohol use (no/yes)[a]	225/63		30/14		4/8			
Delivery type (spontaneous/Cesarean)[a]	233/55		40/14		8/4			
Parity (first/other)[a]	144/144		40/14		6/6			
Infant								
Sex (M/F)[a]	145/143		28/16		6/6			
Gestational age, wk.	39.4	1.4	39.7	1.3	39.2	0.7	1.34	.008
Birth weight, gm	3018.0	380.0	3108.0	380.0	3184.0	415.4	1.44	.008
Birth length, cm	48.9	2.2	49.3	1.8	49.0	2.3	0.59	.003
Head circumference, cm	33.5	1.4	33.4	1.3	33.9	1.5	0.87	.005
Apgar, 1 min.	8.0	0.9	7.9	0.7	8.3	0.5	1.50	.009

[a]Fisher's exact test. †$p < .01$ by analysis of variance.

Neonatal Behavioral Assessment Scale Performance

Table 2 shows the unadjusted mean Neonatal Behavioral Assessment Scale scores for the three groups. There were no significant differences among groups on the seven clusters. The 29 behavioral items were analyzed next. On the inanimate auditory item, the infants of Exsmokers showed a higher mean score than the Nonsmokers ($F_{2,285} = 3.42$, $p < .05$). For the general tone item, significant differences among groups were found ($F_{2,305} = 3.14$, $p < .05$). For the peak of excitement, the infants of Smokers had a lower mean score than the other two groups ($F_{2,305} = 3.51$, $p < .01$).

Table 3 shows the adjusted mean Neonatal Behavioral Assessment Scale scores of the three groups. There were no significant differences for any of the seven clusters. Among the behavioral items, general tone was still statistically significant with covariates controlled ($F_{2,290} = 3.33$, $p < .05$). Peak of excitement, cuddliness, and inanimate auditory orientation were not significant after adjustment for covariates.

TABLE 2
NEONATAL BEHAVIORAL ASSESSMENT SCALE UNADJUSTED MEAN SCORES OF THREE GROUPS

Cluster and Behavioral Item	Nonsmokers ($n=288$)		Exsmokers ($n=44$)		Smokers ($n=12$)		F	η^2
	M	SD	M	SD	M	SD		
Habituation	6.57	1.38	6.65	1.33	6.36	1.29	0.20	.001
Response decrement to light	5.82	1.94	5.77	1.94	5.00	2.19	0.98	.007
Response decrement to rattle	7.04	1.51	7.16	1.25	6.91	1.36	0.57	.005
Response decrement to bell	7.82	2.12	8.09	2.33	8.13	1.97	0.08	.001
Response decrement to foot stimulation	6.25	2.16	6.14	1.96	6.00	1.50	0.11	.001
Orientation	4.57	1.13	4.91	1.18	4.40	1.42	1.94	.01
Animate visual	4.85	1.34	4.62	1.45	4.00	0.93	0.75	.005
Animate visual and auditory	5.05	1.32	5.30	1.28	4.42	1.13	1.56	.01
Inanimate visual	3.90	1.50	4.33	1.73	4.04	1.17	1.66	.02
Inanimate visual and auditory	5.23	1.55	5.58	1.59	4.88	1.36	1.21	.009
Animate auditory	4.33	1.73	4.48	1.88	4.50	2.33	0.16	.001
Inanimate auditory*	4.50	1.33	5.09	1.63	4.44	2.13	3.42	.02
Alertness	4.30	1.28	4.74	1.67	4.33	1.50	1.99	.01
Motor	4.63	0.65	4.59	0.69	4.55	0.89	0.18	.001
General tone*	5.06	0.99	4.87	1.03	4.36	1.12	3.14	.02
Motor maturity	4.16	1.14	4.28	1.06	4.82	1.25	1.95	.01
Pull to sit	4.91	1.21	4.75	1.25	4.64	1.69	0.28	.002
Defensive movements	4.76	1.64	4.70	1.60	4.82	1.99	0.04	.000
Activity	4.29	0.70	4.28	0.77	4.09	0.70	0.42	.003
Range of State	3.88	0.73	3.84	0.77	3.77	0.95	0.13	.001
Peak of excitement*	3.20	1.12	3.32	0.93	2.36	1.03	3.51	.02
Rapidity of build-up	4.14	1.51	4.04	1.74	4.09	1.45	0.07	.000
Irritability	4.34	1.63	4.31	1.89	5.00	1.49	0.92	.006
Lability of state	3.83	0.80	3.79	0.95	3.82	1.33	0.04	.000
Regulation of State	4.23	1.16	4.48	1.10	4.29	1.92	0.91	.006
Cuddliness	4.64	1.33	4.72	1.75	3.64	1.50	2.85	.02
Consolability	4.44	1.51	4.95	1.49	4.13	2.95	2.01	.01
Self-quieting	4.13	2.56	4.36	2.48	4.36	3.35	0.21	.001
Hand to mouth	3.66	2.33	3.91	2.45	4.36	2.87	0.67	.004
Autonomic Stability	5.69	1.00	5.92	1.02	6.09	0.88	0.65	.004
Tremulousness	5.31	1.82	5.66	2.05	6.27	1.67	1.96	.01
Startles	6.91	1.48	7.09	1.49	7.36	1.03	0.72	.01
Lability of skin color	4.86	0.93	5.02	0.85	4.64	0.92	0.24	.002
Reflex	2.65	1.85	2.89	2.13	3.18	2.44	0.65	.004

*$p < .05$ by analysis of variance.

DISCUSSION

Several studies have found negative associations between maternal smoking and neonatal muscle tone. Law, *et al.* (2003) examined the association of maternal smoking during pregnancy with infant neurobehavioral status, using the NICU Network Neurobehavioral Scale at 48 hours after birth. They stated that infants of their smoking group showed more signs of

TABLE 3

NEONATAL BEHAVIORAL ASSESSMENT SCALE ADJUSTED MEAN SCORES OF THREE GROUPS

Cluster and Behavioral Item	Nonsmokers ($n=288$)		Exsmokers ($n=44$)		Smokers ($n=12$)		F	η^2
	M	SD	M	SD	M	SD		
Habituation	6.41	0.13	6.50	0.22	6.63	0.40	0.55	.004
Response decrement to light	5.53	0.18	5.29	0.31	5.27	0.57	0.23	.002
Response decrement to rattle	7.00	0.21	7.19	0.36	7.51	0.66	0.94	.009
Response decrement to bell	7.75	0.18	7.98	0.29	8.05	0.54	0.71	.005
Response decrement to foot stimulation	6.11	0.22	6.08	0.36	6.00	0.72	0.05	.001
Orientation	4.57	0.11	4.93	0.18	4.49	0.36	2.76	.02
Animate visual	4.40	0.13	4.66	0.21	4.08	0.44	1.33	.01
Animate visual and auditory	4.91	0.13	5.24	0.22	4.57	0.48	2.19	.02
Inanimate visual	3.80	0.19	4.33	0.31	4.04	0.66	1.71	.02
Inanimate visual and auditory	5.35	0.17	5.66	0.27	4.85	0.56	1.64	.01
Animate auditory	4.32	0.17	4.48	0.27	4.78	0.56	0.65	.005
Inanimate auditory	4.62	0.14	5.14	0.23	4.57	0.46	3.08	.02
Alertness	4.34	0.15	4.77	0.23	4.39	0.47	2.53	.02
Motor	4.66	0.06	4.57	0.10	4.58	0.19	0.32	.002
General tone*	5.11	0.10	4.88	0.16	4.45	0.30	3.33	.02
Motor maturity	4.24	0.10	4.33	0.16	4.75	0.31	1.34	.009
Pull to sit	4.79	0.12	4.68	0.20	4.62	0.37	0.21	.001
Defensive movements	4.85	0.16	4.70	0.27	4.96	0.50	0.04	.001
Activity level	4.27	0.07	4.24	0.12	4.06	0.22	0.49	.003
Range of State	3.88	0.07	3.80	0.12	3.91	0.23	0.03	.001
Peak of excitement	3.26	0.11	3.35	0.18	2.58	0.33	2.24	.02
Rapidity of build-up	4.14	0.15	3.84	0.25	4.02	0.48	0.19	.002
Irritability	4.45	0.17	4.28	0.20	5.32	0.54	1.23	.008
Lability of state	3.69	0.09	3.72	0.14	3.86	0.27	0.24	.002
Regulation of State	4.37	0.12	4.61	0.20	4.44	0.37	0.40	.003
Cuddliness	4.73	0.14	4.78	0.23	3.78	0.43	2.85	.02
Consolability	4.68	0.17	5.17	0.29	4.41	0.58	1.65	.01
Self-quieting	4.08	0.26	4.42	0.44	4.31	0.81	0.13	.001
Hand to mouth	3.91	0.24	4.06	0.39	4.58	0.74	0.46	.003
Autonomic Stability	5.72	0.10	5.85	0.16	5.91	0.31	0.33	.002
Tremulousness	5.53	0.17	5.75	0.28	5.75	0.51	0.31	.002
Startles	6.79	0.15	6.77	0.24	7.20	0.46	0.54	.004
Lability of skin color	6.79	0.15	6.77	0.24	7.20	0.46	0.11	.001
Reflex	3.09	0.18	3.40	0.31	3.37	0.57	0.49	.003

*$p < .05$ by analysis of covariance.

stress or abstinence and were hypertonic and excitable. Fried, *et al.* (1987) examined the association of infant neurological status with maternal cigarette smoking, marijuana use, and alcohol use. They assessed the neurological status by Prechtl Neurological Examination at 9 and 30 days after birth and found that prenatal cigarette exposure was associated with hypertonicity and increased nervous system excitation, particularly at 30 days. Dempsey, *et al.*

(2000) assessed neonatal neurobehavioral status with a neurological examination and reported an association of neonatal hypertonicity with maternal smoking. Our observation of the association between maternal smoking during pregnancy and a lower score for general tone may be in line with these studies. Infants' muscle tone is thought to indicate the maturity of the central nervous system (Prechtl, 1977).

Many reports have described the association of maternal smoking with birth weight (Andres & Day, 2000; Matsubara, *et al.*, 2000; Weitzman, *et al.*, 2002). However, no association of maternal smoking and birth weight was noted here. In this cohort study were included only infants who were full-term (36–42 weeks of gestation) and had a birth weight of over 2500 gm. Although selection bias is possible given to exclusion of lower-birth weight infants, there were 22 infants who were excluded due to lower birth weight and shorter gestational age, none of whose mothers had been smoking during pregnancy. Therefore, this type of selection bias would not be expected. Moreover, Law, *et al.* (2003) reported a negative association of maternal smoking with neurobehavioral status, including muscle tone, without finding one with birth weight. In addition, the nicotine in cigarettes is thought to act as a neuroteratogen that interferes with fetal development (Slotkin, 1998). This suggests that maternal smoking adversely affects neurobehavioral development at smoking doses which do not affect growth parameters such as birth weight; however, these results suggested that maternal smoking directly affects infant neurobehavioral status.

Although Japan Ministry of Health, Labour and Welfare reported that the prevalence of women who smoked during pregnancy was 10.0% in 2000, the prevalence of smokers in this study was small (3.4%). Therefore, the interpretation of the findings may require some consideration. It was possible that the association of neonatal neurobehavioral status with maternal smoking was not examined sufficiently given the small number of smokers. The reason for this small number of smoking mothers is not known, but there are several possibilities. First, it is possible that the smokers were originally few. The present study was done at big hospitals in an urban area. Women who were interested in health participated in our cohort study. Moreover, guidance for smoking cessation is provided pregnant women in the hospitals where this study was conducted. Second, the data on smoking habits were collected by a self-report questionnaire. Objective measurements such as salivary, serum, or urine cotinine levels were not available. Although several studies have yielded modest correlations between self-reported smoking and biomarkers of cigarette exposure (Bennie, Mchugh, Macpherson, Borland, & Moir Malik, 2004; Fendrich, Mackesy-Amiti, Johnson, Hubbell, & Wislar, 2005; Yano, 2005), misclassification of the mothers' cigarette use cannot be ruled out. Ernhart, Morrow-Tlucak, Sokol, and Martier (1988) reported that mothers report more drinking in pregnancy retrospectively than when interviewed during pregnancy. It is possible that these smoking mothers did not describe their actual smoking habits because of hospital provided guidance for smoking cessation. However, since this study is a longitudinal cohort study to monitor the growth of these children, re-administering the questionnaire on maternal smoking habits during pregnancy will be done so re-analysis of the classification of the mothers' cigarette use can be done in the near future.

Several studies have linked reductions of motor function, general cognitive function, or psychiatric disturbances such as attention deficit hyperactivity disorder in childhood, to maternal smoking during pregnancy (Fried, *et al.*, 1998; Trasti, Vik, Jacobsen, & Bakketeig, 1999; Day, Richardson, Goldschmidt, & Cornelius, 2000). This present study is a prospective cohort study, so evaluation of the long-term effects of maternal smoking during pregnancy on child development from diverse angles will be continuous. The clinical significance of a negative change in muscle tone in newborns is poorly understood, but it may be linked to motor, cognitive, or behavioral problems in later childhood. It is important to investigate the association between infant muscle tone status and cognitive or behavioral outcomes during childhood.

Finally, there was an association of a lower score for muscle tone with maternal smoking, but interpretation may require some considerations, given the small number of smokers which may be a limitation of this study. Since this is the first report in which an association between maternal smoking and neurobehavioral status in Japanese infants has been noted, continued examination of the association of maternal smoking during child development from diverse angles is important. Outcomes of this study might be important in considering the effects of smoking by pregnant women in Japan.

REFERENCES

ANDRES, R. L., & DAY, M. C. (2000) Perinatal complications associated with maternal tobacco use. *Seminars in Fetal & Neonatal Medicine*, 5, 231-241.

BENNIE, V., MCHUGH, S., MACPHERSON, L., BORLAND, B., MOIR, K., & MALIK, K. (2004) The validation of self-reported smoking status by analyzing cotinine levels in stimulated and unstimulated saliva, serum and urine. *Oral Diseases*, 10, 287-293.

BRAZELTON, T. B. (1973) *Neonatal Behavioral Assessment Scale*. London: Spastics International Medical Publ.

BRAZELTON, T. B., & NUGENT, J. K. (1995) *Neonatal Behavioral Assessment Scale*. (3rd ed.) London: Mac Keith Press. (Distr. by Cambridge Univer. Press)

DAY, N. L., RICHARDSON, G. A., GOLDSCHMIDT, L., & CORNELIUS, M. D. (2000) Effects of prenatal tobacco exposure on preschoolers' behavior. *Journal of Developmental and Behavioral Pediatrics*, 21, 180-188.

DEMPSEY, D. A., HAJNAL, B. L., PARTRIDGE, J. C., JACOBSON, S. N., GOOD, W., JONES, R. T., & FERRIERO, D. M. (2000) Tone abnormalities are associated with maternal cigarette smoking during pregnancy in *in utero* cocaine-exposed infants. *Pediatrics*, 106, 79-85.

ERNHART, C. B., MORROW-TLUCAK, M., SOKOL, R. J., & MARTIER, S. (1988) Underreporting of alcohol use in pregnancy. *Alcoholism: Clinical and Experimental Research*, 12, 506-511.

Espy, K. A., Riese, M. L., & Francis, D. J. (1997) Neurobehavior in preterm neonates exposed to cocaine, alcohol, and tobacco. *Infant Behavior and Development*, 20, 297-309.

Fendrich, M., Mackesy-Amiti, M. E., Johnson, T. P., Hubbell, A., & Wislar, J. S. (2005) Tobacco-reporting validity in an epidemiological drug-use survey. *Addictive Behaviors*, 30, 175-181.

Fried, P. A., Watkinson, B., Dillon, R. F., & Dulberg, C. S. (1987) Neonatal neurological status in a low-risk population after prenatal exposure to cigarettes, marijuana, and alcohol. *Journal of Developmental and Behavioral Pediatrics*, 8, 318-326.

Fried, P. A., Watkinson, B., & Gray, R. (1998) Differential effects on cognitive functioning in 9- to 12-year olds prenatally exposed to cigarettes and marihuana. *Neurotoxicology and Teratology*, 20, 293-306.

Jacobson, S. W., Fein, G. G., Jacobson, J. L., Schwartz, P. M., & Dowler, J. K. (1984) Neonatal correlates of prenatal exposure to smoking, caffeine, and alcohol. *Infant Behavior and Development*, 7, 253-265.

Law, K. L., Stroud, L. R., LaGasse, L. L., Niaura, R., Liu, J., & Lester, B. M. (2003) Smoking during pregnancy and newborn neurobehavior. *Pediatrics*, 111, 1318-1323.

Lester, B. M., Als, H., & Brazelton, T. B. (1982) Regional obstetric anesthesia and newborn behavior: a reanalysis toward synergistic effects. *Child Development*, 53, 687-692.

Maternal and Child Health Division, Equal Employment, Children and Families Bureau, Ministry of Health, Labour and Welfare. (2000) [*Infants and children: physical growth survey*]. Tokyo: Author. [in Japanese]

Matsubara, F., Kida, M., Tamakoshi, A., Wakai, K., Kawamura, T., & Ohno, Y. (2000) Maternal active and passive smoking and fetal growth: a prospective study in Nagoya, Japan. *Journal of Epidemiology*, 10, 335-343.

Mattson, S. N., Calarco, K. E., Chambers, C. D., & Jones, K. L. (2002) Interaction of maternal smoking and other in-pregnancy exposures: analytic considerations. *Neurotoxicology and Teratology*, 24(3), 359-367.

Nakai, K., Suzuki, K., Oka, T., Murata, K., Sakamoto, M., Okamura, K., Hosokawa, T., Sakai, T., Nakamura, T., Saito, Y., Kurokawa, N., Kameo, S., & Satoh, H. (2004) The Tohoku Study of Child Development: a cohort study of effects of perinatal exposures to methylmercury and environmentally persistent organic pollutants on neurobehavioral development in Japanese children. *Tohoku Journal of Experimental Medicine*, 202, 227-237.

Oyamade, U. J., Cole, O. J., Johnson, A. A., Knight, E. M., Westney, O. E., Laryea, H., Hill, G., Cannon, E., Fomufod, A., Westney, L. S., Jones, S., & Edwards, C. H. (1994) Prenatal substance abuse and pregnancy outcomes among African American women. *Journal of Nutrition*, 124, 994S-999S.

Prechtl, H. F. R. (1977) *The neurological examination of the full term newborn infant.* (2nd ed.) London: Heinemann Medical Spastics International Medical Publ.

Richardson, G. A., Day, N. L., & Taylor, P. M. (1989) The effect of prenatal alcohol, marijuana, and tobacco exposure on neonatal behavior. *Infant Behavior and Development*, 12, 199-209.

Saxton, D. W. (1978) The behavior of infants whose mothers smoke in pregnancy. *Early Human Development*, 2, 363-369.

Slotkin, T. A. (1998) Fetal nicotine or cocaine exposure: which one is worse? *Journal of Pharmacology and Experimental Therapeutics*, 285, 931-945.

Trasti, N., Vik, T., Jacobsen, G., & Bakketeig, L. S. (1999) Smoking in pregnancy and children's mental and motor development at age 1 and 5 years. *Early Human Development*, 55, 137-147.

Weitzman, M., Byrd, R. S., Aligne, C. A., & Moss, M. (2002) The effects of tobacco exposure on children's behavioral and cognitive functioning: implications for clinical and public health policy and future research. *Neurotoxicology and Teratology*, 24, 397-406.

Yano, E. (2005) Japanese spousal smoking study revisited: how a tobacco industry funded paper reached erroneous conclusions. *Tobacco Control*, 14, 227-233.

Accepted June 22, 2006.

EFFECT OF PERINATAL EXPOSURE TO ENVIRONMENTALLY PERSISTENT ORGANIC POLLUTANTS AND HEAVY METALS ON NEUROBEHAVIORAL DEVELOPMENT IN JAPANESE CHILDREN: PCBS EXPOSURE AND NEONATAL NEUROBEHAVIORAL STATUS

Suzuki K[1 2 6], Nakai K[1], Nakamura T[1], Hosokawa T[2], Okamura K[3], Sakai T[4], Kurokawa N[1], Kameo S[1], Murata K[5], and Satoh H[1]

[1]Environmental Health Sciences, Tohoku University Graduate School of Medicine, Japan; [2]Human Development and Disabilities, Tohoku University Graduate School of Education, Japan; [3]Obstetrics, Tohoku University Graduate School of Medicine, Japan; [4]Miyagi Children's Hospital, Japan; [5]Environmetal Health Sciences, Akita University School of Medicine, Japan; [6]Research Fellow of the Japan Society for the Promotion of Science, Japan

Introduction

The neurobehavioral effects of perinatal exposures to methylmercury (MeHg) and environmentally persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs), dioxins and pesticides are of great concern world wide. Several epidemiological studies have reported some associations of perinatal exposure to MeHg or PCBs with neurobehavioral deficit, such as postnatal growth delay and poorer cognitive functions[1]. These chemicals accumulate in human body mostly through the consumption of food, especially fish and shellfish. On the other hand, since fish is rich in nutrients such as polyunsaturated fatty acid (PUFA) essential for normal brain development of fetus, fish has been usually recommended for pregnant women. Therefore, from perspective of risk assessment, these health hazard issues are important for fish eating populations.

We have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposure to MeHg, PCBs, and dioxins on neurobehavioral development in Japanese children[2]. We registered 599 mother-infant pairs from January 2001 to September, 2003 in an urban area of Tohoku district, Japan. Samples such as maternal peripheral blood, cord blood, breast milk and maternal hair were collected for chemical determination. For the assessment of neurobehavioral development, Brazelton Neonatal Behavioral Assessment Scale (NBAS) was administered when children were three days old, and other tests including Bayley scales of infant development second edition and Kaufman assessment battery for children were performed with growth of children.

In the present study, we report the preliminary results on the association of NBAS with total PCB concentration in cord blood and maternal fish intake.

Materials and Methods

Participants were 163 mother-infant pairs whose variables including PCBs concentration in cord blood, NBAS, and other covariates were available. Mean maternal age at the delivery was 31.9 (SD4.2). Infants consisted of 87 boys and 76 girls, and they were all singleton and full-term (36-42 weeks) gestation without congenital anomalies or diseases. Birth weight was 2400g or more. Information was obtained about pregnancy, delivery conditions and infant characteristics from medical record.

PCBs concentration was measured from whole of cord blood collected immediately after delivery. All 209 congeners were analyzed using HR-GC/MS (Metocean environmental Inc, Shizuoka, Japan). Total PCB concentration represented the sum of the all measured congeners, expressed as ng/g-fat.

Maternal fish intake was estimated using the semi-quantitative food frequency questionnaire (FFQ) for 122 individual foods and recipes[3] and 13 additional items regarding fish and shellfish. The FFQ was administered at four days after delivery. Trained investigators showed mothers a real size photograph of each food, then, mothers answered the frequency and the amount of intake per meal.

For other variables of chemical analysis, hair mercury (hair Hg) concentration was analyzed from maternal hair samples taken at two days after delivery. Total hair Hg concentration was measured by cold vapor atomic absorption[4] at National Institutes of Minamata Disease (Minamata, Japan). Thyroid hormones including thyroid-stimulation hormone (TSH), total thyroxine (T4), triiodothyromine (T3), free T4 and free T3, were measured from plasma of cord blood by SRL, Inc. (Tokyo, Japan).

NBAS was administered on three days after delivery. Examiners of the NBAS were trained and certified at the training center for NBAS in Nagasaki University School of Medicine, Japan. Reliability check was conducted throughout the data collection to maintain a 90% level of agreement.

In the statistical analysis, a stepwise multiple regression analyses were performed for adjustment of covariates. The potential covariates were as follows; maternal age at delivery, maternal alcohol drinking during pregnancy, maternal smoking habit, maternal total energy intake, delivery type, parity, gestational age, gender, birth weight, apgar score, TSH and T3 concentration in cord blood, maternal hair Hg concentration, and NBAS examiners. The significance level was set at 5%.

Results and Discussion

The mean total PCB concentration in cord blood was 65.3 ng/g-fat (SD 43.4), and the mean maternal total fish intake was 2.6 kg/year (SD 1.6). Table 1 shows results of multiple regression analyses. A positive association was found between total PCB concentration and the score of the regulation of state cluster. A negative association was found between total fish intake and the score of the habituation cluster.

Early studies reported the negative associations of prenatal PCBs exposure with neurobehavioral development. We hypothesized the negative associations between total PCBs in cord blood and the NBAS. On the other hand, because fish is rich in nutrients such as PUFA essential for brain development, we also hypothesized the beneficial effects of maternal fish intake on the neonatal neurobehavioral status. Our findings are not in line with our hypotheses and the findings from early studies. We do not know clearly the reason for these findings, there are several possibilities. First, it is possible that the level of PCB exposure in our study was too low to detect the effects of PCB exposure on neonatal neurobehavioral status. We supposed that the level of PCB exposure in our study might be lower than those of early studies[5, 6, 7], although it is difficult to compare PCB levels among studies for different analytical methods. Second, since this report is based on the preliminary results, the sample size too small to detect the effects of low level exposure to PCB. The entire participant of our cohort study is 599 mother-infant pairs, and we collected not only samples of cord blood, but also maternal peripheral blood and breast milk. The measurements of chemicals including PCBs are now on going, and the further study will reveal the associations of neurobehavioral development with perinatal PCBs exposure and fish intake.

Table 1. The results of multiple regression analyses

	Habituation	Orientation	Motor	Range of state	Regulation of state	Autonomic stability	Reflex
Total PCBs (ng/g-fat)[1]	0.47	0.34	0.28	-0.04	0.81*	0.16	-1.39
Total fish intake (kg/year)[1]	-0.87*	-0.00	0.20	-0.34	0.73	-0.23	-0.28

(Beta value) * $p < .05$

[1]Log translations, $Log_{10}X$, were used on the value of total PCB concentration and total fish intake.

Acknowledgements

We thank all families who participated in the study. The study protocol was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine. This research was funded by Japan Ministry of Health, Labour and Welfare, Research on Risk of Chemical Substances.

References

1. Nakai K, Satoh H. *Tohoku J Exp Med* 2002; 196: 89-98.
2. Nakai K, Suzuki K, Oka T, Murata K, Sakamoto M, Okamura K, Hosokawa T, Sakai T, Nakamura T, Saito Y, Kurokawa N, Kameo S, Satoh H. *Tohoku J Exp Med* 2004; 202: 227-237.
3. Date C, Yamaguchi M, Tanaka H. *J Epidemiol* 1996; 6: S131-S136.
4. Akagi H, Nishimura H. In: *Advances in Mercury Toxicology*, Suzuki T, Nobumasa I, Clarkson T.W. (ed.), Plenum, New York, 1991: 53-76.
5. Huisman M, Koopma-Esseboom C, Lanting C, van der Paauw C.G, Tuinstra L.G.M.Th, Fiedler V, Weisglas-Kuperus N, Sauer P.J.J, Boersma E.R, Touwen B.C.L. *Early Human Dev* 1995; 43: 165-176.
6. WinnekeG, Bucholski A, Heinzow B, Kramer U, Schmidt E, Walkowiak J, Wiener J, Steingruber H. *Toxicol Lett* 1998; 102-103: 423-428
7. Stewart P, Reihman J, Lonky E, Darvill T, Pagano J. *Nurotoxicol and Teratol* 2000; 22: 21-29.

ELSEVIER

Available online at www.sciencedirect.com
SCIENCE @ DIRECT®

Int. J. Hyg. Environ.-Health 209 (2006) 337–344

International Journal of Hygiene and Environmental Health

www.elsevier.de/ijheh

Fish consumption and time to pregnancy in Japanese women

Chikako Arakawa[a], Jun Yoshinaga[a,*], Kunihiro Okamura[b], Kunihiko Nakai[c], Hiroshi Satoh[c]

[a]*Institute for Environmental Studies, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[b]*Department of Obstetrics, Graduate School of Medicine, Tohoku University, Japan*
[c]*Department of Environmental Health Sciences, Graduate School of Medicine, Tohoku University, Japan*

Received 30 July 2005; received in revised form 3 February 2006; accepted 17 February 2006

Abstract

The work described in this paper examined the possible relationship between fish consumption and human fecundity among Japanese women using hair mercury level and time to pregnancy (TTP) as indicators of fish consumption and fecundity. We hypothesized that hair mercury concentrations reflect the level of fish consumption and, consequently, can also be taken to indicate the level of intake of organochlorine compounds (OCs) such as dioxins and polychlorinated biphenyls (PCBs) for which fish are the primary source, and which can disrupt normal human reproductive processes. TTP was obtained by a self-administered questionnaire from women who had been delivered of a baby at either of two hospitals in Sendai, Japan, during the period of January 2002–March 2004. Total mercury concentration in their hair (0–3 cm from the scalp) was determined by cold vapor atomic absorption spectrometry.

Of the 298 women approached, 193 (65%) reported their TTP. The subjects were classified into two groups according to their TTP: group 1, 0–12 months TTP; and Group 2, >12 months TTP. A step-down procedure backward binominal logistic regression analysis was performed by using age, BMI, parity, frequency of intercourse, life-style parameters (smoking, drinking and dietary habits) and hair mercury level of the female subjects and their partners as independent variables. Two separate analyses were performed by including/excluding information on the partners of the subjects.

The analyses did not extract hair mercury concentration as significant indicating that fish consumption did not prolong TTP, which was not consistent with the results of our previous study, i.e., in that study frequent fish eaters showed prolonged TTP. Possible reasons of the negative result are discussed and it is suggested that the hair mercury levels in the present study were not an appropriate indicator of fish consumption of the subjects or of their exposure to OCs. Further study on the relationship between fish consumption (and, ultimately, of OCs intake) and fecundity with more appropriate indicators of fish consumption and/or fish-mediated pollutants intake are warranted to characterize the health risk posed by fish consumption.

Keywords: Time to pregnancy; Fecundity; Hair mercury concentration; Fish consumption; Japanese women

*Corresponding author. Present address: Kashiwanoha 5-1-5, Kashiwa, Chiba 277-8563, Japan. Tel./fax: 81 4 7136 4716.
E-mail address: junyosh@k.u-tokyo.ac.jp (J. Yoshinaga).

1438-4639/$ - see front matter
doi:10.1016/j.ijheh.2006.02.004

Introduction

Fish has been the major source of protein for Japanese people since ancient times and its health-giving properties have recently been highlighted by the health authorities in many countries. Japanese people consumed, on average, 94 g of fish and shellfish per person daily in 2001 (Ministry of Health, Labor and Welfare, Japan, 2003). It is important, however, to evaluate the health risks posed by fish consumption because it is well known that fish can be a major source of hazardous chemicals such as polychlorinated biphenyls (PCBs), dioxins, and methylmercury.

We are interested in the possible effects of exposure to environmental chemicals on human fecundity. Time to pregnancy (TTP) has attracted interest in several countries as a measure of human fecundity (Juul et al., 1999). However, until recently it has not been used in reproductive health research in Japan. In our previous study on the applicability of TTP to Japanese subjects, we found a statistical association between frequent fish eating and prolonged TTP (Arakawa et al., 2003). We hypothesized that the prolonged TTP was due to excessive exposure (through fish consumption) to chemicals, particularly to organochlorine compounds (OCs) that can disrupt normal human reproductive processes. Our previous results were consistent with those of Buck et al. (2000), but not with the results of several other studies (Buck et al., 1997, 1999; Axmon et al., 2002). The inconsistency may have resulted from the semi-quantitative estimation of fish consumption, such as fish eating frequency, used in those studies. Moreover, in our previous study, the number of subjects was limited ($n = 92$). It was necessary, therefore, to re-evaluate our findings using a larger population and with a more quantitative indicator of fish consumption.

As a quantitative indicator of fish consumption by subjects from a fish-eating population without occupational mercury exposure, the total mercury concentration in hair has been used (Yamaguchi et al., 1971; Iwasaki et al., 2003). In the work reported here, we investigated the possible relationship between fish consumption and human fecundity in Japanese women using mercury concentration in hair as a quantitative indicator of fish consumption.

Materials and methods

Study design and sampling

We approached the subjects in a prospective cohort on child development and environmental exposure to chemicals (Nakai et al., 2004). Women who had been delivered of a baby at either of two hospitals in Sendai, Japan during the period of January 2002–March 2004, were asked to participate in our study. Only women who had given written consent after having the purpose and design of our study explained to them were included in the investigation.

Hair samples were obtained from the subjects on the second day postpartum. A lock of hair was cut with stainless steel scissors as close to the scalp as possible. Samples were placed in plastic bags such that the scalp end of the hair was identifiable in each case and they were kept in desiccators until analysis.

On the third day postpartum subjects were asked to complete questionnaires that had been developed in our preliminary study (Arakawa et al., 2003). The questionnaire included questions on biological attributes and life style parameters (dietary habits, smoking, drinking, the intake of caffeinated beverages, etc.) of both the woman before pregnancy and of her partner, and on TTP (How long did you try to become pregnant after cessation of contraception?). Dietary habits were revealed by questions about the frequency of consumption per week, and beverage consumption by the number of cups per week.

The Ethical Committee of Tohoku University approved this study.

Analytical methods

Clippings from the scalp end of the lock of hair, 3 cm in length, were taken and subjected to mercury analysis. Determination of total mercury concentration was carried out by cold vapor atomic absorption spectrometry (Akagi and Nishimura, 1991) with minor modifications. Analytical accuracy was ensured by analyzing the Human Hair Reference Material NIES CRM No. 13 from the National Institute of Environmental Studies (Lot #650, Tsukuba, Japan). Details of the mercury analysis are given elsewhere (Nakai et al., 2004).

Statistical methods

The subjects were classified into two groups according to the reported TTP: group 1 (TTP 0 – 12 months) and group 2 (TTP >12 months). Biological attributes and life-style parameters of both the subject and her partner were compared between the two groups by chi-square tests and by t-tests. The body mass indices (BMI) of the subjects were calculated from the reported weights and heights before pregnancy. Caffeine consumption was calculated from the cups-per-week-consumption and the caffeine contents of the beverages (Bunker and McWilliams, 1979, Wilcox et al., 1988, Stanton and Gray, 1995). Correlations between fish consumption and total mercury concentrations in hair were analyzed by the

Spearman rank correlation analysis. A step-down procedure backward binominal logistic regression analysis was employed to extract statistically significant independent variable(s) that contributed to classify the subjects into groups 1 and 2. Independent variables used in these analyses were biological attributes (age, parity, and BMI), frequency of intercourse, total mercury concentration in hair, and life style parameters (smoking, alcohol intake, caffeine intake, frequencies of milk and meat consumption) of both the female subject and her partner. Since there was a significantly positive correlation between the subject and her partner for age and some of the life-style parameters, it was not considered appropriate to include both of these variables for the subject and her partner as independent variables. Therefore, two separate binominal logistic regression analyses were carried out: one by using age and life-style parameters of the female subject only, and another by combining those variables for the subject and her partner (e.g., sum of the ages of the subject and her partner). Cox regression was also performed using the same variables as those of the logistic regression analysis, but TTP as continuous variable. Statistical analysis was performed using SPSS for Windows version 11.5.

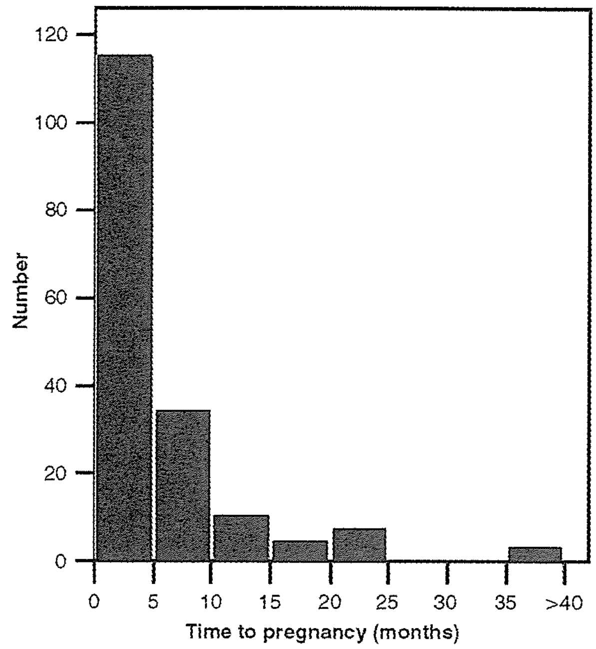

Fig. 1. Distribution of time to pregnancy ($n = 180$).

Results

Of the 298 women approached, 193 (65%) reported their TTP. We excluded subjects who got pregnant in spite of contraception and who had had infertility treatment from the data analysis (four and nine women, respectively) because their TTP may not have represented actual fecundity. Thus, 180 women were eligible for statistical analysis. Fig. 1 shows the TTP distribution of the 180 subjects. Comparison of demographic parameters for subjects who reported TTP and those who did not was given in Table 1. This table showed that there were significant differences in some parameters between the two groups.

The geometric mean of the total mercury concentrations in hair was 2.01 µg/g (range 0.42–9.35 µg/g, $n = 177$). There was a significant association between frequency of fish consumption and total mercury concentrations in hair ($r = 0.19$, $p = 0.01$, Fig. 2).

Table 2 compares biological attributes and life style parameters of both the subject and her partner for the two groups defined according to TTP. Meat consumption of partner was the only variable that was significantly different between the groups. Approximately, 60% of the subjects and > 80% of their partners consumed fish more than 3 meals/week for total subjects (Table 1) and for those reported TTP (Table 2). Fig. 3 shows total mercury concentration in the hair of subjects divided by reported TTP. The geometric means of mercury concentrations in hair were 2.01 µg/g for group 1 and 1.97 µg/g for group 2, and the difference was not statistically significant.

Alcohol consumption was the only variable that was extracted for prolonged TTP by a step-down procedure backward binominal regression analysis when age and lifestyle parameters of the female subject only were included (Table 3). When summed variables were used, prolonged TTP was associated with decreasing parity and increasing sum of age of the subject and her partner (Table 4). Sum of age and parity were the variables with marginal insignificance ($p = 0.06$) found in Cox regression analysis for the summed variables. Total mercury concentration in hair was not selected as significant in either of the analyses. When hair mercury concentration was replaced with frequency of fish consumption in the logistic regression analyses, the frequency was not selected as significant either.

Discussion

The response rate to the question of TTP asked of the present subjects (65%) was slightly lower than that of our previous study (75% response rate). This may have resulted from the fact that information on TTP was sought at 2–3 gestational months in our previous study and at postpartum in this study. However, the distribution of the reported TTP for our subjects (Fig. 1) was

Table 1. Comparison of characteristics of the participants who reported/did not report their TTP

		TTP		Total	p value
		Not reported	Reported		
Number		118	180	298	
Age (years)					
Subject		31.9	30.6	31.4	0.14
Partner		33.7	31.9	32.9	0.05
BMI (kg/m^2)		21.2	20.6	20.8	0.08
Number of births (times)		1.9	1.5	1.7	<0.001
Hair mercury level (μg/g)		2.02	2.01	2.01	0.94
Frequency of intercourse (%)					
<1/week		51	41	44	0.19
1/week		30	40	37	
>2/week		19	19	19	
Smoking (%)					
Subject		25	16	19	0.05
Partner		62	54	57	0.20
Milk (glass/week)					
Subject		4.4	5.3	4.9	0.09
Partner		2.6	4.4	3.7	<0.001
Caffeine (mg/week)					
Subject		1114	1332	1247	0.04
Partner		1188	1337	1280	0.17
Alcohol (g/week)					
Subject		75.7	69.7	72.0	0.75
Partner		183	170	175	0.77
Fish consumption (%)					
Subject	<1/week	2.9	1.7	2.1	0.65
	1–2/week	41	39	40	
	3–5/week	42	49	47	
	>5/week	14	11	12	
Partner	<1/week	2.9	1.7	2.1	0.34
	1–2/week	21	17	19	
	3–5/week	57	62	60	
	>5/week	19	19	19	
Meat consumption (%)					
Subject	<1/week	3.9	4.0	3.9	0.98
	1–2/week	38	36	37	
	3–5/week	44	46	46	
	>5/week	14	14	14	
Partner	<1/week	1.0	0.0	0.4	0.40
	1–2/week	20	15	17	
	3–5/week	54	55	55	
	>5/week	25	30	28	

similar to those reported previously (e.g., Curtis et al., 1999) as well as to the distribution obtained in our previous study on Japanese subjects (Arakawa et al., 2003). The present result again shows that the TTP questionnaire is applicable to Japanese women in spite of possible differences in their sexual practices compared to those of Western women, e.g., in their preferred methods of contraception.

The geometric mean of the total mercury concentrations (2.01 μg/g) was close to those obtained in a recent

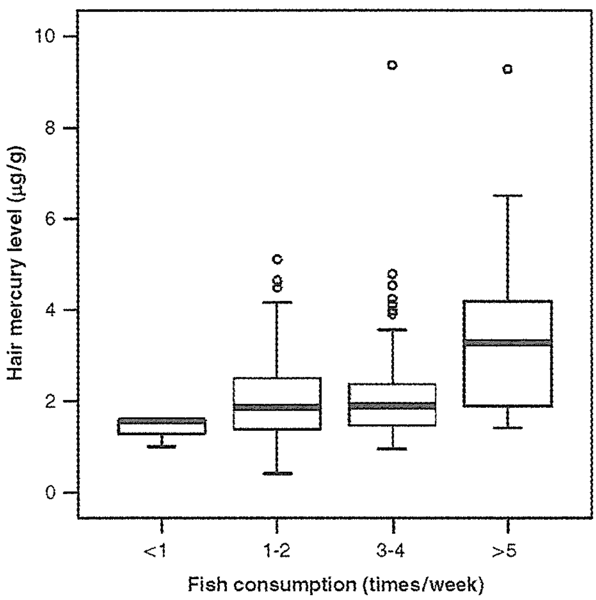

Fig. 2. Relationship between frequency of fish consumption (meals per week) and hair mercury level.

study in which geometric mean values of 1.23–2.50 μg/g were reported for mercury concentrations in the hair of persons from 5 cities in Japan (total number of samples 1666) (Yasutake et al., 2003). This result indicated that fish consumption of our subjects was similar to that of the average Japanese.

There were significant differences in some parameters between the subjects who reported TTP and those who did not (Table 1). Subjects who did not report TTP were older and had a higher number of previous births. The difference in parity could be explained by the age difference: there was a positive correlation between age and parity for the subjects ($r = 0.33$, $p < 0.001$), and the slope of the regression line indicated that the differences in parity were consistent with age difference. Older women who already have children probably do not memorize TTP because they are likely to pay less attention to getting pregnant. In other words, women who were more anxious about and paid more attention to getting pregnant were the subjects of our analysis. Whether this bias has a bearing on our research objectives should be carefully considered.

In our multivariate analyses, parity and age were significant variables that contributed to prolonged TTP when partners' effects were taken into consideration (Table 4). This result was consistent with those of other studies that reported associations between parity and TTP (e.g., Howe et al., 1985; Olsen, 1991), age of female subject and/or her partner and TTP (e.g., Baird and Wilcox, 1985, Howe et al., 1985; Olsen, 1991; Spinelli et al., 1997). Inconsistent results for the logistic regression analysis were obtained when age and life-style parameters for the subject only were included although the reason for the inconsistency was not clear. The reason why alcohol intake was a significant variable for the classification of our subjects into the two groups (Table 3) was also not clear. The results of logistic regression analysis were virtually the same when Cox regression analysis was employed, though statistical significance was not obtained ($p = 0.06$). However, the important finding was that hair mercury concentration was not selected as significant in either of logistic regression analysis and Cox regression analysis.

It is well known that some OCs, such as dioxins and PCBs, can disrupt normal human endocrine functions (Longnecker et al., 1997; Watanabe et al., 1999), and that these compounds concentrate along food chains (IPCS, 1992; Rolff et al., 1993) and thus fish can contain OCs at high concentrations. Therefore, fish consumption is the predominant route of the intake of OCs for the general population with fish-eating habits (Svensson et al., 1991; Tsutsumi et al., 2001). In addition to this, the total mercury concentration in hair has been considered a quantitative indicator of fish consumption (Yamaguchi et al., 1971; Iwasaki et al., 2003). Thus, we employed hair mercury level as a marker of fish consumption, and of the exposure level to OCs, to relate to TTP, based on the assumption that there is a strong correlation between the concentrations of OCs and mercury in fishes, and consequently, in the tissues and body fluids of humans who consume fish.

The result of this study, however, was not consistent with our previous finding that frequent fish eaters showed prolonged TTP (Arakawa et al., 2003). The reasons for this inconsistency may partly be attributable to the fact that we employed total mercury concentration in the hair segment 0–3 cm from the scalp. The hair mercury level represents fish consumption in the last trimester of the subjects' gestation: clearly this does not correspond to the period of TTP. It is possible that the amount of fish consumption changed after pregnancy. This may be the reason for the low correlation between fish-eating frequency and hair mercury level (Fig. 2), which, though statistically significant, was weaker than those recorded in previous studies (Bjornberg et al., 2005). We should, perhaps, have employed samples of hair of > 10 cm from the scalp.

Another possible reason for the negative result of the present study was that hair mercury levels are not correlated with the intake of the chemicals in fish that can affect fecundity. We assumed that the concentrations of OCs and mercury in marine foods would correlate with each other because of their co-biomagnification. However, Bjerregaard and Hansen (2000) reported that the concentrations of OCs and mercury did not correlate in the blood of women who consumed marine food. Therefore, it is clear that we should employ direct indicators that reflect the level of exposure to OCs

Table 2. Comparison of characteristics of the participants between group 1 (TTP: 0–12 months) and group 2 (TTP: >12 months)

		Time to pregnancy		Total	p value
		0–12	>12		
Number		160	20	180	
Age (years)					
Subject		30.6	31.0	30.6	0.70
Partner		31.7	33.7	31.9	0.10
BMI (kg/m^2)		20.7	20.5	20.6	0.83
Number of births (times)		1.5	1.4	1.5	0.23
Hair mercury level (μg/g)		2.01	1.97	2.01	0.82
Frequency of intercourse (%)					
<1/week		40	50	41	0.24
1/week		40	45	40	
>2/week		21	5.0	19	
Smoking (%)					
Subject		15	18	16	0.41
Partner		51	64	54	0.10
Milk (glass/week)					
Subject		5.5	3.4	5.3	0.05
Partner		4.5	3.9	4.4	0.62
Caffeine (mg/week)					
Subject		1314	1469	1332	0.48
Partner		1313	1469	1337	0.30
Alcohol (g/week)					
Subject		59.1	155	69.7	0.12
Partner		169	182	170	0.79
Fish consumption (%)					
Subject	<1/week	1.9	0.0	1.7	0.94
	1–2/week	39	40	39	
	3–5/week	49	50	49	
	>5/week	11	10	11	
Partner	<1/week	1.9	0.0	1.7	0.29
	1–2/week	16	30	17	
	3–5/week	61	65	62	
	>5/week	21	5.0	19	
Meat consumption (%)					
Subject	<1/week	4.5	0.0	4.0	0.68
	1–2/week	36	35	36	
	3–5/week	45	55	46	
	>5/week	15	10	14	
Partner	<1/week	0.0	0.0	0.0	0.03
	1–2/week	14	25	15	
	3–5/week	53	70	55	
	>5/week	33	5.0	30	

to relate to TTP. To date, we know of only one such study: Axmon et al. (2004) investigated the relationship between serum/plasma 2,2′4,4′,5,5′-hexachlorobiphenyl (CB-153) concentrations, a biomarker of total PCB exposure, and TTP. They did not find any association between blood CB-153 concentrations and TTP. They estimated the blood concentrations of CB-153 for the subjects when the index pregnancies took place, namely, 1–36 years previously (median: 20 years), from the CB-153 concentrations in blood that had been drawn from

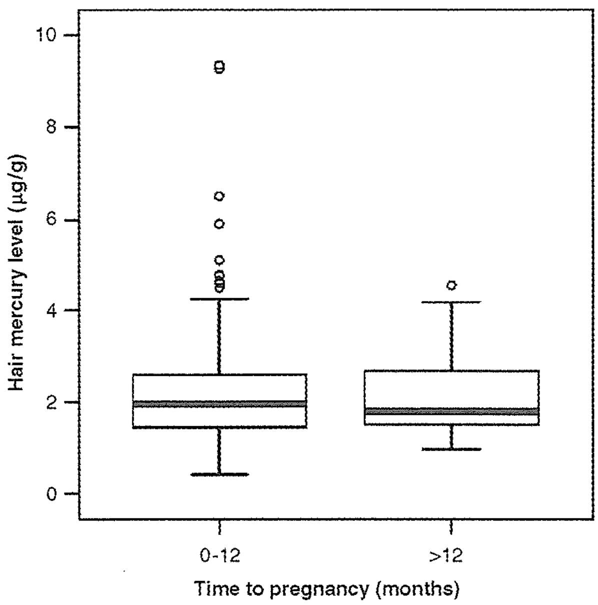

Fig. 3. Comparison of hair mercury level between group 1 (TTP: 0–12 months) and group 2 (TTP > 12 months).

Table 3. Odds ratio of risk factors

	Odds ratio	95% CI	*p* value
Alcohol	1.61	1.05–2.49	0.03

The variable "age" "milk" "alcohol" "caffeine" "meat" "smoking" of the female subjects only were used.

Table 4. Odds ratio of risk factors

	Odds ratio	95% CI	*p* value
Parity	0.44	0.20–0.97	0.04
Age	3.10	1.60–6.04	<0.001

The variables "age" "milk" "alcohol" "caffeine" "meat" "smoking" were generated by summing data for the female subject and her partner because there was a positive correlation between them for these variables.

the subjects in 2000. It is very probable that the blood CB-153 concentrations estimated after so many years would have been subject to gross errors, and this was possibly a major reason why these investigators failed to find any association between blood CB-153 concentrations and TTP.

It may be worth pointing out that the present result indicates a lack of association between TTP and methylmercury exposure in Japanese. In other words, methylmercury exposure at the present level does not affect human fecundity. This may be valuable information in the hazard identification of methylmercury.

In summary, we could not obtain reproducible results showing a positive relationship between fish consumption and TTP in this cohort study using hair mercury levels as an indicator of fish consumption, although such a relationship had been indicated in our earlier study. We will extend our survey by including direct indicators that can reflect exposure level to OCs during the duration of the TTP to characterize the health risk posed by fish consumption, which is important for Japanese and other fish-eating populations.

Acknowledgments

We thank all parturient women for their participation in this study. We also thank the staff of Tohoku Study of Child Development for their support.

References

Akagi, H., Nishimura, H., 1991. In: Suzuki, T., Nobumasa, I., Clarkson, T.W. (Eds.), Advances in Mercury Toxicology. Plenum Press, New York, pp. 53–76.

Arakawa, C., Yoshinaga, J., Mizumoto, Y., Abe, M., 2003. Preliminary study on the measurement of human fecundity – applicability of time to pregnancy to Japanese subjects. Nippon Koshu Eisei Zasshi 50, 414–419 (In Japanese with English Abstract).

Axmon, A., Rylander, L., Stromberg, U., Hagmar, L., 2002. Female fertility in relation to the consumption of fish contaminated with persistent organochlorine compounds. Scand. J. Work Environ. Health 28, 124–132.

Axmon, A., Rylander, L., Stromberg, U., Jonsson, B., Nilsson-Ehle, P., Hagmar, L., 2004. Polychlorinated biphenyls in serum and time to pregnancy. Environ. Res. 96, 186–195.

Baird, D.D., Wilcox, A.J., 1985. Cigarette smoking associated with delayed conception. J. Am. Med. Assoc. 253, 2979–2983.

Bjerregaard, P., Hansen, J.C., 2000. Organochlorines and heavy metals in pregnant women from the Disko Bay area in Greenland. Sci. Total Environ. 245, 195–202.

Bjornberg, K.A., Vahter, M., Grawe, K.P., Berglund, M., 2005. Methyl mercury exposure in Swedish women with high fish consumption. Sci. Total Environ. 341, 45–52.

Buck, G.M., Sever, L.E., Mendola, P., Zielezny, M., Vena, J.E., 1997. Consumption of contaminated sport fish from Lake Ontario and time-to-pregnancy. New York State Angler Cohort. Am. J. Epidemiol. 146, 949–954.

Buck, G.M., Mendola, P., Vena, J.E., Sever, L.E., Kostyniak, P., Greizerstein, H., Olson, J., Stephen, F.D., 1999. Paternal Lake Ontario fish consumption and risk of conception delay, New York State Angler Cohort. Environ. Res. 80, S13–S18.

Buck, G.M., Vena, J.E., Schisterman, E.F., Dmochowski, J., Mendola, P., Sever, L.E., Fitzgerald, E., Kostyniak, P., Greizerstein, H., Olson, J., 2000. Parental consumption of contaminated sport fish from Lake Ontario and predicted fecundability. Epidemiology 11, 388–393.

Bunker, M.L., McWilliams, M., 1979. Caffeine content of common beverages. J. Am. Diet Assoc. 74, 28–32.

Curtis, K.M., Savitz, D.A., Weinberg, C.R., Arbuckle, T.E., 1999. The effect of pesticide exposure on time to pregnancy. Epidemiology 10, 112–117.

Howe, G., Westhoff, C., Vessey, M., Yeates, D., 1985. Effects of age, cigarette smoking, and other factors on fertility: findings in a large prospective study. Br. Med. J. 290, 1697–1700.

IPCS, 1992. Polychlorinated Biphenyls and Terphenyls, second ed. World Health Organization, International Programme on Chemical Safety, Geneva (Environmental Health Criteria 140).

Iwasaki, Y., Sakamoto, M., Naka, K., Oka, T., Dakeishi, M., Iwata, T., Satoh, H., Murata, K., 2003. Estimation of daily mercury intake from seafood in Japanese women: Akita cross-sectional study. Tohoku J. Exp. Med. 200, 67–73.

Juul, S., Karmaus, W., Olsen, J., The European Infertility Subfecundity Study Group, 1999. Regional differences in waiting time to pregnancy: pregnancy-based surveys from Denmark, France, Germany, Italy and Sweden. Hum. Reprod. 14, 1250–1254.

Longnecker, M.P., Rogan, W.J., Lucier, G., 1997. The human health effects of DDT (dichlorodiphenyltrichloroethane) and PCBS (polychlorinated biphenyls) and an overview of organochlorines in public health. Annu. Rev. Public Health 18, 211–244.

Ministry of Health, Labor and Welfare, 2003. The National Nutrition Survey in Japan, 2001. Dai-ichi Shuppan, Tokyo.

Nakai, K., Suzuki, K., Oka, T., Murata, K., Sakamoto, M., Okamura, K., Hosokawa, T., Sakai, T., Nakamura, T., Saito, Y., Kurokawa, N., Kameo, S., Satoh, H., 2004. The Tohoku study of child development: a cohort study of effects of perinatal exposures to methylmercury and environmentally persistent organic pollutants on neurobehavioral development in Japanese children. Tohoku J. Exp. Med. 202, 227–237.

Olsen, J., 1991. Cigarette smoking, tea and coffee drinking, and subfecundity. Am. J. Epidemiol. 133, 734–739.

Rolff, C., Broman, D., Näf, C., Zebühr, Y., 1993. Potential biomagnification of PCDD/Fs – new possibilities for quantitative assessment using stable isotope trophic position. Chemosphere 27, 461–468.

Stanton, C.K., Gray, R.H., 1995. Effects of caffeine consumption on delayed conception. Am. J. Epidemiol. 15, 1322–1329.

Spinelli, A., Figa-Talamanca, I., Osborn, J., 1997. Time to pregnancy and occupation in a group of Italian women. Int. J. Epidemiol. 26, 601–609.

Svensson, B.G., Nilsson, A., Hansson, M., Rappe, C., Akesson, B., Skerfving, S., 1991. Exposure to dioxins and dibenzofurans through the consumption of fish. N. Engl. J. Med. 324, 8–12.

Tsutsumi, T., Yanagi, T., Nakamura, M., Kono, Y., Uchibe, H., Iida, T., Hori, T., Nakagawa, R., Tobiishi, K., Matsuda, R., Sasaki, K., Toyoda, M., 2001. Update of daily intake of PCDDs, PCDFs, and dioxin-like PCBs from food in Japan. Chemosphere 45, 1129–1137.

Watanabe, S., Kitamura, K., Nagahashi, M., 1999. Effects of dioxins on human health: a review. J. Epidemiol. 9, 1–13.

Wilcox, A., Weinberg, C., Baird, D., 1988. Caffeinated beverages and decreased fertility. Lancet 2, 1453–1456.

Yamaguchi, S., Matsumoto, H., Matsuo, S., Kaku, S., Hoshide, M., 1971. Relationship between mercury content of hair and amount of fish consumed. HSMHA Health Rep. 86, 904–909.

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

CONCENTRATIONS OF DIOXINS AND PCBS IN CORD BLOOD IN JAPANESE CHILDREN FROM THE TOHOKU STUDY OF CHILD DEVELOPMENT

Nakamura T[1,3], Nakai K[1], Suzuki K[1], Koizumi A[2], Shamoto H[2], Yamauchi M[2], Matsumura T[2], Saito Y[3], Kameo S[1], Satoh H[1]

[1]Department of Environmental Sciences, Tohoku University Graduate School of Medicine, Sendai, Japan; [2]Environmental Risk Research Center, Institute of General Science for Environment, METOCEAN Environment Inc., Shizuoka, Japan; [3]Miyagi Prefectural Institute of Public Health and Environment, Sendai, Japan

Introduction

From several cohort researches, it has been reported that the perinatal exposure to the methylmercury and persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), has been associated with neurobehavioral development on postnatal growth[1]. We have started a prospective cohort study to examine the effects of perinatal exposures to POPs on neurobehavioral development in Japanese children (The Tohoku Study of Child Development, TSCD)[2]. We registered 599 mother-infant pairs in this cohort study.

Measurement of PCBs in previous epidemiological researches was performed by homologue or limited isomer, but congener-specific analysis was not done. Furthermore, dioxins measurement in cord blood was not reported in many papers since this measurement required high sensitivity and a large amount of samples.

In the present study, we established a measurement procedure to simultaneously determine dioxins and all congener-specific PCBs by high resolution gas chromatograph mass spectrometer (HRGS/HRMS) using isotopic dilution method from the same sample. Then, we determined the concentration of dioxins and PCBs in whole cord blood in Japanese children.

Materials and Methods

PCDDs, PCDFs, dioxin-like PCBs and total PCBs in whole cord blood were analyzed by HRGC/HRMS. After a whole cord blood (about 20 ml) was weighed and added with the clean-up spike containing ^{13}C-labeled standard mixture of PCDDs, PCDFs, dioxin-like PCBs and PCBs, and then crude lipid in sample was extracted. This extract dissolved in *n*-hexane was purified on a multi-layer silica gel column. The purified solution was divided into two aliquots of 80 % for determination of PCDDs, PCDFs and dioxin-like PCBs (aliquot A) and 20 % for determination of congener-specific PCBs (aliquot B). The aliquot B was concentrated without further purification and measured by HRGC/HRMS. The aliquot A was fractionated on active carbon-dispersed silica gel column to PCDDs, PCDFs, *non-ortho* PCBs fraction and *mono-ortho* PCBs fraction, and measured for each fraction by

HRGC/HRMS. HRGC/HRMS analysis was conducted on a 6890 series GC (Agilent Technology, USA) equipped with Autospec-Ultima (Micromass, UK). Measurements of PCDDs , PCDFs and *non-ortho* PCBs were used with SCLV injection system (SGE, Australia).

Results and Discussion

The distribution of total PCBs concentration in whole cord blood was shown in Figure 1. The mean total PCBs concentration was 65.3ng/g-fat (SD 43.4, median 55.6, minimum 12.1, maximum 238.1, n = 163). The mean total TEQ was 13.1pg-TEQ/g-fat (SD 7.7, median 10.9, minimum 3.5, maximum 44.5, n = 84). The correlation between total PCBs and TEQ was shown in Figure 2. This correlation coefficient was 0.93 ($p < 0.01$, n = 84).

Although there are few reports on TEQ in whole cord blood in Japan, a governmental report prepared by Morita[3] is very suggestive. In that report, the TEQ values that were based on the sum of PCDDs and PCDFs were similar or slightly lower than that of our study. Fukata et al.[4] reported the PCBs concentration in cord serum in Japanese, in which the median was 63.0ng/g-fat (n=32). Our results of total PCB concentration were same level as compared with their results.

It was shown that the correlation between TEQ and total PCBs in cord blood was absolutely high in Fig 2. This suggests that it would be possible for measurement of TEQ or total PCBs to evaluate each other value. In addition, using both TEQ and total PCBs should be avoided in a multiple liner regression analysis because of multicollinearity. Consequently, measurement of both TEQ and PCBs in the same sample should be reconsidered.

The sources of dioxins and PCBs were different. In Japan, dioxins were released unintentionally into environment as an impurity of the agricultural chemicals. On the other hand, PCBs is the chemical substance which had been used intentionally for insulation oil, thermal catalyst and coating compound. As the reasons why high correlation was observed between dioxins and total PCBs in the cord blood, it would be suggested that the behavior of both chemicals in the ecosystem and the mechanism which pollutes the human body through the bioaccumulation by the food chain is similar.

Acknowledgments

We thank all the families who participated in this study. The study protocol was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine. This research was funded by Japan Ministry of Health, Labour and Welfare, Research on Risk of Chemical Substances.