クについて関連性を明らかにできるものと期待
される。これまで海外で報告されてきた調査事
例を参考にしつつ，今後とも調査研究を進めて
いきたい。

文　献

1) Nakai K, Satoh H : Developmental neurotoxicity following prenatal exposures to methylmercury and PCBs in humans from epidemiological studies. Tohoku J Exp Med, 196 : 89-98, 2002.

2) Walkowiak J, Wiener JA, Fastabend A, Heinzow B, Kramer U, Schmidt E, Steingruber HJ, Wundram S, Winneke G : Environmental exposure to polychlorinated biphenyls and quality of the home environment : effects on psychodevelopment in early childhood. Lancet, 358 : 1602-7, 2001.

3) Davidson PW, Myers GJ, Cox C, Axtell C, Shamlaye C, Sloane-Reeves J, Cernichiari E, Needham L, Choi A, Wang Y, Berlin M, Clarkson TW : Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment : outcomes at 66 months of age in the Seychelles Child Development Study. JAMA, 280 : 701-7, 1998.

4) Sakamoto M, Kubota M, Liu XJ, Murata K, Nakai K, Satoh H : Maternal and fetal mercury and n-3 polyunsaturated fatty acids as a risk and benefit of fish consumption to fetus. Environ Sci Technol, 38 : 3860-3, 2004.

5) 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 : 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 : 227-37, 2004.

6) 仲井邦彦，佐藤　洋：内分泌攪乱物質の健康影響に関する疫学研究から－周産期曝露の影響を中心として－．最新医学, 57 : 229-35, 2002.

7) 岡　知子，鈴木恵太，仲井邦彦，細川　徹，佐藤　洋：Bayley式乳幼児発達検査第2版の日本国内での実施の試み．医学のあゆみ, 212 : 259-62, 2005.

8) Fagan JF, Detterman DK : The Fagan test of infant intelligence : A technical summary. J Appl Dev Psychol, 13 : 173-93, 1992.

9) 鈴木恵太，岡　知子，仲井邦彦，細川　徹，佐藤　洋：新奇選好を応用した乳幼児の視覚認知検査．医学のあゆみ, 212 : 253-7, 2005.

10) 岩田豊人，村田勝敬：環境有害因子に曝露された小児の神経運動機能の評価．医学のあゆみ, 212 : 104-7, 2005.

11) Konishi Y, Kuwabara K, Hori S : Continuous surveillance of organochlorine compounds in human breast milk from 1972 to 1998 in Osaka, Japan. Arch Environ Contam Toxicol, 40 : 571-8, 2001.

12) Fukata H, Omori M, Osada H, Todaka E, Mori C : Necessity to measure PCBs and organochlorine pesticide concentrations in human umbilical cords for fetal exposure assessment. Environ Health Perspect, 113 : 297-303, 2005.

13) Darvill T, Lonky E, Reihman J, Stewart P, Pagano J : Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. Neurotoxicol, 21 : 1029-38, 2000.

14) Longnecker MP, Gladen BC, Patterson DGJ, Rogan WJ : Polychlorinated biphenyl (PCB) exposure in relation to thyroid hormone levels in neonates. Epidemiology, 11 : 249-54, 2000.

15) Jacobson JL, Jacobson SW : Intellectual impairment in children exposed to polychlorinated biphenyls in utero. New Engl J Med, 335 : 783-9, 1996.

16) Jacobson SW, Fein GG, Jacobson JL, Schwartz PM, Dowler JK : Prenatal exposure to an environmental toxin : A test of the multiple effects model. Dev Psychol, 20 : 523-32, 1984.

17) Jacobson SW, Jacobson JL, Fein GG : Environmental toxins and infant development. In : Theory and research on behavioral pediatrics, vol. 3 (Fitzgerald HE, Lester BM, Yogman MW, eds) . New York : Plenum Press, p96-146, 1986.

18) Jacobson SW, Fein GG, Jacobson JL, Schwartz PM, Dowler JK : The effect of intrauterine PCB exposure on visual recognition memory. Child Dev, 56 : 853-60, 1985.

19) Jacobson JL, Jacobson SW, Humphrey HE : Effects of in utero exposure to polychlorinated biphenyls and related contaminants on cognitive functioning in young children. J Pediatr, 116 : 38-45, 1990.

20) Rogan WJ, Gladen BC, Mckinney JD, Carreras N, Harady P, Thullen J, Tinglestad J, Tully M : Neonatal effects of transplacental exposure to PCBs and DDE. J Pediatr, 109 : 335-41, 1986.

21) Gladen B, Rogan W, Hardy P, Thullen J, Tingelstad J : Development after exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethane transplacentally and through human milk. J Pedatr, 113 : 991-5, 1988.

22) Rogan WJ, Gladen BC : PCBs, DDE, and child development at 18 and 24 months. Ann Epidemiol, 1 : 407-13, 1991.

23) Gladen B, Rogan W : Effects of perinatal polychlorinated biphenyls and dichlorodiphenyl dichloroethane on later development. J Pediatr, 119 : 58-63, 1991.

24) Stewart P, Reihman J, Lonky E, Darvill T, Pagano J : Prenatal PCB exposure and neonatal behavioral assessment scale (NBAS) performance. Neurotoxicol Teratol, 22 : 21-9, 2000.

25) Stewart P, Fitzgerald S, Reihman J, Gump B, Lonky E, Darvill T, Pagano J, Hauser P : Prenatal PCB exposure, the corpus callosum, and response inhibition. Environ Health Perspect, 111 : 1670-7, 2003.

26) Huisman M, Koopman-Esseboom C, Fidler V, Hadders-Algra M, van der Paauw CG, Tuinstra LG, Weisglas-Kuperus N, Sauer PJ, Touwen BC, Boersma ER : Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. Early Human Develop, 41 : 111-27, 1995.

27) Koopman-Esseboom C, Weisglas-Kuperus N, Ridder M, van der Paauw CG, Tuinstra LG, Sauer PJJ : Effects of polychlorinated biphenyl/dioxin exposure and feeding type on infant's mental and psychomotor development. Pediatr, 97 : 700-6, 1996.

28) Huisman M, Koopman-Esseboom C, Lanting CI, van der Paauw CG, Tuinstra LG, Fidler V, Weisglas-Kuperus N, Sauer PJ, Boersma ER, Touwen BC : Neurological condition in 18-month-old children perinatally exposed to polychlorinated biphenyls and dioxins. Early Human Develop, 43 : 165-76, 1995.

25

29) Lanting CI, Patandin S, Fidler V, Weisglas-Kuperus N, Sauer PJ, Boersma ER, Touwen BC : Neurological condition in 42-month-old children in relation to pre- and postnatal exposure to polychlorinated biphenyls and dioxins. Early Human Develop, 50 : 283-92, 1998.

30) Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N : Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. J Pediatr, 134 : 33-41, 1999.

31) Vreugdenhil HJ, Mulder PG, Emmen HH, Weisglas-Kuperus N : Effects of perinatal exposure to PCBs on neuropsychological functions in the Rotterdam cohort at 9 years of age. Neuropsychology, 18 : 185-93, 2004.

32) Vreugdenhil HJ, Van Zanten GA, Brocaar MP, Mulder PG, Weisglas-Kuperus N : Prenatal exposure to polychlorinated biphenyls and breastfeeding : opposing effects on auditory P300 latencies in 9-year-old Dutch children. Dev Med Child Neurol, 46 : 398-405, 2004.

33) Winneke G, Bucholski A, Heinzow B, Kramer U, Schmidt E, Walkowiak J, Wiener JA, Steingruber HJ : Developmental neurotoxicity of polychlorinated biphenyls (PCBS) : cognitive and psychomotor functions in 7-month old children. Toxicol Lett, 102-103 : 423-8, 1998.

34) Steuerwald U, Weihe P, Jorgensen PJ, Bjerve K, Brock J, Heinzow B, Budtz-Jorgensen E, Grandjean P : Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. J Pediatr, 136 : 599-605, 2000.

35) Rogan WJ, Gladen BC, Mckinney JD, Carreras N, Harady P, Thullen J, Tinglestad J, Tully M : Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethane (DDE) in human milk : effects of maternal factors and previous lactation. Am J Public Health, 76 : 172-7, 1986.

36) Jacobson JL, Janisse J, Banerjee M, Jester J, Jacobson SW, Ager JW : A benchmark dose analysis of prenatal exposure to polychlorinated biphenyls. Environ Health Perspect, 110 : 393-8, 2002.

37) Muckle G, Ayotte P, Dewailly EE, Jacobson SW, Jacobson JL : Prenatal exposure of the northern Quebec Inuit infants to environmental contaminants. Environ Health Perspect, 109 : 1291-9, 2001.

26

THE BIOLOGICAL MONITORING PROGRAM OF PERSISTENT ORGANIC POLLUTANTS IN JAPAN: 1. CONCENTRATIONS OF ORGANOCHLORINE PESTICIDES IN BREAST MILK, CORD BLOOD AND MATERNAL BLOOD

Nakai K [1], Nakamura T [1,2], Suzuki K [1,3], Shibata Y [4], Murata K [5], Satoh H [1]

[1]Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai, 980-8575 Japan; [2]Miyagi Prefectural Institute of Public Health and Environment, Sendai, 983-8666 Japan; [3]Department of Human Development and Disabilities, Tohoku University Graduate School of Education, Sendai, 980-8576; [4]National Institute for Environmental Studies, Tsukuba, 305-8506 Japan; [5]Department of Environmental Health Sciences, Akita University School of Medicine, Akita, 010-8543 Japan.

Abstract

Persistent organic pollutants (POPs) are ubiquitous environmental contaminants that accumulate in lipid-rich body tissues. Although POPs are thought to be hazardous to health, the overall epidemiological data are as yet insufficient to draw any conclusions. Thus, exposure monitoring and epidemiological examination of the Japanese population are of importance to determine the health risks due to POPs exposure. The Ministry of the Environment of Japan (MOE) has been conducting systematic monitoring of POPs according to the Stockholm Convention. We provided some biological samples for the POPs biological monitoring project, and reanalyzed the report of the MOE. In this presentation, we summarize the data of organochlorine pesticides in human pair samples of breast milk, cord blood and maternal blood. We also analyzed the associations of pesticide concentrations with TSH and thyroid hormones in maternal and cord blood, since disruption of the hypothalamus-pituitary-thyroid axis is a hypothetical mechanism for POPs-induced adverse effects.

Introduction

Persistent organic pollutants (POPs) are ubiquitous environmental contaminants that accumulate in lipid-rich body tissues. Their lipophilicity and intrinsic resistance to biological degradation processes are responsible for bioaccumulation and biomagnification in the food chain, and consequently they can be found in humans at considerable concentrations. Although these concentrations are usually decreasing and almost at the background level, longer term exposure may cause potential risks to human health.

In humans, some POPs have been claimed to possess endocrine-disrupting potency. DDE exposure is related to TSH and estradiol levels among middle-aged and elderly men.[1] There is a significant negative association between the serum HCB concentration and total T4 in cord blood.[2] These findings suggest that exposure to POPs may affect the hypothalamus-pituitary-thyroid and the hypothalamus-pituitary-gonadal axes. Reproductive defects may be associated in part with exposure to hormonally active environmental chemicals during fetal and childhood development.[3] A growing number of reports have demonstrated the association between adverse effects in children and exposure to POPs at low doses over a longer period. Human perinatal exposure to PCBs has been also shown to be associated with several adverse effects.[4] However, little information is available regarding the delayed neurobehavioral development in infants following exposure to DDE.[5,6] Perinatal exposure to HCB was also shown to be associated with poor social competence in children.[7] Although the overall epidemiological data are not yet sufficient to allow us to draw firm conclusions, exposure monitoring and epidemiological examination of the Japanese population are important for risk assessment.

In Japan, the Ministry of the Environment (MOE) has been conducting systematic monitoring of chemicals over a 30-year period. The MOE initiated refined environmental monitoring including POPs in FY2002 according to the Stockholm Convention.[8,9] Recently, the MOE also added biological monitoring of human samples. Since information on blood levels of POPs in Japan is very limited, this monitoring of all the POPs covered by the convention will contribute to the future effectiveness evaluation. We have been collaborating with the MOE's POPs monitoring project by providing biological samples from our prospective birth cohort study, The Tohoku Study of Child Development (TSCD). We reanalyzed the results from the MOE's monitoring project, and summarize the data of organochlorine pesticides in human pair samples of breast milk, cord blood and maternal blood in this presentation.[10] We also analyzed the associations of pesticide concentrations with TSH and thyroid hormones in maternal and cord blood, since the disruption of the hypothalamus-pituitary-

thyroid axis is a hypothetic mechanism for POPs-induced adverse effects.

Materials and Methods

Biological samples analyzed were randomly selected from the participants in the TSCD, and provided anonymously to the MOE. The TSCD study protocol was previously reported.[3] Briefly, maternal peripheral blood was collected using heparin as the anticoagulant agent in the morning when the pregnancy was at 28 weeks. The cord blood was collected immediately after delivery. These whole blood samples were frozen at −80°C until the chemical analysis. Breast milk was collected one month after delivery, and then frozen similarly.

Chemical determination was performed with high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) by IDEA Consultants, Inc. (Tokyo, Japan) as part of the MOE project as described in another report in this book. TSH, total T4 and total T3 were measured from the plasma of cord and maternal blood by SRL, Inc. (Tokyo, Japan). The statistical analyses were performed using JMP ver. 5.1.2. When the levels of data were not normally distributed, the data were log-transformed for statistical analysis.

The TSCD was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine, and all mothers provided signed informed consent.

Table 1 Pesticide concentrations if breast milk, cord blood and maternal blood (pg/g-fat)

Chemicals	Breast milk Median (Min-Max)	Cord blood Median (Min-Max)	Maternal blood Median (Min-Max)
Aldrin	nd (nd)	nd (nd)	nd (nd)
cis-Chlordane	460 (200-3100)	440 (210-1460)	620 (220-2060)
trans-Chlordane	180 (80-1400)	330 (120-770)	190 (130-490)
Oxychlordane	11700 (2700-46800)	4940 (1280-17530)	5520 (1540-17270)
cis-Nonachlor	3400 (860-10570)	960 (280-2780)	1680 (470-4860)
trans-Nonachlor	22480 (6620-100950)	6690 (1690-26260)	12830 (3620-52370)
o,p'-DDD	nd (nd-510)	nd (nd-100)	nd (nd-100)
p,p'-DDD	300 (100-14510)	120 (nd-590)	240 (60-430)
o,p-DDE	380 (180-950)	250 (90-600)	340 (170-730)
p,p'-DDE	143300 (31700-331500)	68180 (12330-385690)	93270 (17280-271390)
o,p-DDT	1220 (550-4170)	450 (190-1420)	700 (200-2130)
p,p'-DDT	7620 (2310-19390)	2450 (560-7330)	3950 (1080-10070)
Dieldrin	4290 (2100-17480)	3140 (1370-13580)	3280 (1440-9810)
Endrin	nd (nd-490)	nd (nd)	nd (nd)
Heptachlor	nd (nd-370)	nd (nd-170)	nd (nd)
trans-Heptachlorepoxide	nd (nd)	nd (nd)	nd (nd)
cis-Heptachlorepoxide	4480 (1800-24140)	2490 (670-12720)	2680 (730-12520)
HCB	16380 (6870-37260)	16700 (6440-39980)	13810 (5560-39580)
α-HCH	290 (150-1570)	310 (130-1910)	220 (120-580)
β-HCH	49010 (11500-213990)	29030 (4860-90490)	29350 (4750-196100)
γ-HCH	220 (50-2340)	340 (150-5080)	220 (100-2180)
δ-HCH	nd (nd-310)	nd (nd-140)	nd (nd)
Mirex	740 (170-1880)	410 (120-1380)	1110 (280-2890)
Parlar-26	1880 (760-7040)	660 (230-3020)	960 (300-2550)
Parlar-40	20 (nd-100)	nd (nd-180)	nd (nd-70)
Parlar-41	230 (nd-560)	nd (nd-240)	110 (nd-220)
Parlar-44	230 (60-640)	nd (nd-380)	70 (nd-200)
Parlar-50	3150 (1280-12490)	850 (280-4140)	1440 (480-4220)
Parlar-62	230 (nd-820)	nd (nd-510)	40 (nd-360)

n=68 for breast milk and cord blood, n=49 for maternal blood.

Table 2. Correlation of pesticide concentrations among breast milk, cord blood and maternal blood

	Breast milk						
	c-CHL	t-CHL	OxyCHL	c-Nonachlor	t-Nonachlor	p,p'-DDE	p,p'-DDT
Cord blood	0.543**	0.191	0.831**	0.836**	0.871**	0.837**	0.796**
Maternal blood	0.729**	0.291	0.943**	0.954**	0.959**	0.920**	0.878**
	Breast milk						
	Dieldrin	c-HCE	HCB	β-HCH	Mirex	Parlar-26	Parlar-50
Cord blood	0.821**	0.800**	0.879**	0.800**	0.673**	0.789**	0.778**
Maternal blood	0.819**	0.928**	0.921**	0.844**	0.894**	0.904**	0.917**

Pearson's r after log-transformed. n=68 for breast milk and cord blood, and n=49 for breast milk and maternal blood.

** $p < 0.001$

Results and Discussion

Concentrations of pesticides in breast milk, cord blood and maternal blood are shown in Table 1. The highest values were observed for p,p'-DDE in all three materials. Since the use of DDT was prohibited in 1971 in Japan, this finding indicates the nature of intrinsic resistance to biological degradation of DDT/DDE. Mirex and toxaphene were measurable in most samples. Since neither chemical has ever been used in Japan, the route and the source of contamination are not fully understood.

High correlations of most chemicals among the three materials were observed as shown in Table 2. These findings indicated the usefulness of breast milk as a monitoring material for human exposure. Breast milk is rich in fat and therefore lipophilic chemicals such as POPs are accumulated, and it can be easily obtained from lactating women. In breast milk, most chemicals also correlate with each other (data not shown).

Since the working hypothesis on POPs-induced adverse effects is the disruption of the hypothalamus-pituitary-thyroid axis, associations of pesticides with TSH, T4 and T3 in maternal blood and cord blood were analyzed as shown in Table 3. It was confirmed that dioxins, PCBs and DDT/DDE were associated with TSH and thyroid hormones. In addition, other minor pesticides such as HCB, nonachlor, and toxaphenes were also associated with TSH, T4 and T3. Since there were clear multicollinearities among the chemicals, the causal relationships between the pesticide exposures and the levels of TSH and thyroid hormone remain to be clarified.

POPs exhibit bioaccumulation and biomagnification in the food chain, and therefore human exposure is thought to be mainly through the consumption of fish. However, maternal fish intake did not correlate with the concentrations of any of the chemicals (data not shown).

Further monitoring assessments and epidemiological examinations will make it possible to understand the exposure characteristics and biological effects of POPs on humans.

References

1. Rylander L, Wallin E, Jonssson BA, Stridsberg M, Erfurth EM, Hagmar L. *Chemosphere* 2006; 65:375.
2. Sala M, Sunyer J, Herrero C, To-Figueras J, Grimalt J. *Occup Environ Med* 2001; 58:172.
3. Toppari J, Larsen JC, Christiansen P, Giwercman A, Grandjean P, Guillette LJJ, Jegou B, Jensen TK, Jouannet P, Keiding N, Leffers H, McLachlan JA, Meyer O, Muller J, Rajpert-De Meyts E, Scheike T, Sharpe R, Sumpter J, Skakkebaek NE. *Env Health Perspect Suppl* 1996; 104:741.
4. Nakai K, Satoh H. *Tohoku J Exp Med* 2002; 196:89.
5. Ribas-Fito N, Cardo E, Sala M, Eulalia de Muga M, Mazon C, Verdu A, Kogevinas M, Grimalt JO, Sunyer J. *Pediatr* 2003; 111:e580.
6. Torres-Sachez L, Rothenberg SJ, Schnaas L, Cebrian ME, Osorio E, del Carmen Hernandez M, Garcia-Hernandez RM,

del Rio-Garcia C, Wolff MS, Lopez-Carrillo L. *Env Health Perspect* 2007; 115:435.
7. Ribas-Fito N, Torrent M, Carrizo D, Julvez J, Grimalt JO, Sunyer J. *Env Health Perspect* 2007; 115:447.
8. Yoshida Y, Enomoto Y, Kamiya K. *Organohalogen Compounds* 66 (2004) 3504.
9. Ministry of the Environment, Japan. Environmental Monitoring Report on Persistent Organic Pollutants (POPs) in Japan 2002-2004, www.env.go.jp/en/chemi/pops/rep2002-2004.pdf 2006.
10. Ministry of the Environment, Japan. http://www.env.go.jp/press/press.php?serial=7896

Table 3.   Relationship of POPs with maternal age, gestational age and concentrations of TSH and thyroid hormones

	Maternal age	Gestational age	TSH and thyroid hormones					
			Maternal blood			Cord blood		
			TSH	T4	T3	TSH	T4	T3
Breast milk (n=68, except for n=67 for maternal TSH/T4/T3)								
TEQ	ns	ns	0.232§	ns	0.225§	ns	-0.224§	ns
PCB	ns	ns	0.205§	0.231§	0.257*	ns	ns	ns
trans-Nonachlor	ns	ns	ns	ns	0.281*	ns	-0.238§	ns
p,p'-DDE	ns	ns	ns	ns	ns	ns	ns	ns
p,p'-DDT	ns	ns	ns	ns	ns	ns	ns	ns
Dieldrin	0.229§	-0.265*	ns	*ns	0.235§	ns	ns	ns
cis-Heptachlorepoxide	ns	ns	ns	ns	ns	0.243*	-0.212§	ns
HCB	ns	ns	ns	ns	ns	ns	-0.228§	ns
β-HCH	ns	ns	ns	ns	ns	ns	ns	ns
Mirex	0.422**	-0.232§	0.234§	ns	ns	0.233§	ns	ns
Parlar-26	ns	ns	ns	0.291*	0.395**	ns	ns	ns
Parlar-50	ns	ns	ns	0.287*	0.381**	ns	ns	ns
Maternal blood (n=49, except for n=48 for maternal TSH/T4/T3)								
TEQ	ns	ns	0.264§	0.272§	0.375**	ns	ns	ns
PCB	ns	ns	0.277§	0.287*	0.402**	ns	ns	ns
trans-Nonachlor	ns	ns	0.274§	ns	0.429**	ns	-0.243§	ns
p,p'-DDE	ns	ns	0.242§	ns	0.252§	ns	ns	-0.251§
p,p'-DDT	ns	ns	ns	0.299*	0.426**	ns	-0.279§	ns
Dieldrin	0.351*	ns	ns	ns	0.247§	ns	ns	ns
cis-Heptachlorepoxide	ns	ns	ns	ns	ns	ns	ns	ns
HCB	ns	ns	0.251§	0.310*	0.391**	ns	ns	ns
β-HCH	ns	ns	ns	ns	0.267§	ns	ns	ns
Mirex	0.391**	-0.251§	0.342*	ns	0.324*	0.284*	ns	ns
Parlar-26	ns	ns	ns	0.267§	0.468**	0.261§	ns	ns
Parlar-50	ns	ns	ns	0.296*	0.500**	0.269§	ns	ns
Cord blood (n=68, except for n=67 for maternal TSH/T4/T3)								
TEQ	ns	ns	0.203§	ns	ns	ns	-0.270*	ns
PCB	ns	ns	0.239§	ns	0.240§	ns	ns	ns
trans-Nonachlor	ns	ns	0.238§	ns	0.244*	ns	-0.217§	ns
p,p'-DDE	ns	ns	ns	ns	ns	ns	-0.208§	ns
p,p'-DDT	ns	ns	0.206§	ns	ns	ns	-0.238§	ns
Dieldrin	ns	-0.237§	ns	ns	ns	ns	-0.302*	-0.260*
cis-Heptachlorepoxide	ns	-0.278*	ns	ns	ns	0.262*	-0.335**	-0.208§
HCB	ns	ns	ns	ns	ns	0.202§	-0.293*	ns
β-HCH	0.206§	ns	ns	ns	ns	ns	ns	ns
Mirex	0.294*	-0.239§	0.234§	ns	ns	ns	ns	ns
Parlar-26	ns	-0.237§	ns	ns	0.300*	ns	ns	ns
Parlar-50	ns	-0.220§	ns	ns	0.324**	ns	ns	ns

Pearson's r after log-transformed. §p<0.1, * p<0.05, ** p<0.01

THE BIOLOGICAL MONITORING PROGRAM OF PERSISTENT ORGANIC POLLUTANTS IN JAPAN: 2. CONCENTRATIONS OF DIOXINS AND POLYCHLORINATED BIPHENYLS IN BREAST MILK, CORD BLOOD AND MATERNAL BLOOD

Nakamura T[1,2], Nakai K[2], Suzuki K[2,3], Kameo S[2], Suzuki S[1], Saito Y[1], Shibata Y[4], Hosokawa T[3], Okamura K[5], Sakai T[6], Satoh H[2]

[1]Miyagi Prefectural Institute of Public Health and Environment, Sendai, 983-8666 Japan; [2]Department of Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai, 980-8575 Japan; [3]Department of Human Development and Disabilities, Tohoku University Graduate School of Education, Sendai, 980-8576 Japan; [4]National Institute for Environmental Studies, Tsukuba, 305-8506 Japan; [5]Department of Obstetrics, Tohoku University Graduate School of Medicine, Sendai, 980-8575 Japan; [6]Miyagi Children's Hospital, Sendai, 989-3216 Japan.

Abstract

Persistent organic pollutants (POPs) such as dioxins and polychlorinated biphenyls (PCBs) are bioaccumulative chemical toxins that are resistant to degradation. POPs are thought of as hazardous contaminants. The Ministry of the Environment of Japan (MOE) has been conducting environmental monitoring of POPs since FY2002 on the basis of the Stockholm Convention on POPs. Since we provided some biological samples for the POPs biological monitoring project, we reanalyzed the report from the MOE. In this presentation, we summarize the data on dioxins and PCBs in human pair samples of breast milk, cord blood and maternal blood. We also analyze the associations of the concentrations of these compounds with thyroid-stimulating hormone (TSH) and thyroid hormones in maternal and cord blood, since disruption of the hypothalamus-pituitary-thyroid axis is a hypothetical mechanism for dioxin- and PCB-induced adverse effects. Concentrations of dioxins and PCBs in each biological sample were at levels similar to those in previous reports on Japanese, and high correlations among the three biological samples were observed. Furthermore, single regression analysis showed a statistically significant correlation of dioxins and PCBs with TSH and thyroid hormones such as total thyroxine (T4) and triiodothyromine (T3).

Introduction

POPs such as polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and PCBs are bioaccumulative chemical toxins that are resistant to degradation. Generally, the main source of exposure to POPs for the general population is thought to be food because the physicochemical attributes of POPs such as lipophilicity and persistence cause bioaccumulation in the food chain, and consequently they can be found in humans at considerable concentrations. Although these concentrations tend to decrease and to be at the background level in industrialized nations, long-term exposure may cause potential risks to human health.

In humans, POPs have been claimed to possess endocrine-disrupting potency. Dioxins, expressed as toxic equivalent (TEQ) levels, were correlated significantly with lower T3 and T4 levels in maternal blood, and with higher blood concentrations in breast-fed infants[1]. There was a significant negative association between dioxin concentrations in breast milk and total T4 in the blood of breast-fed infants[2]. On the other hand, higher environmental background exposure to dioxins significantly increased the T4 concentration in the blood of infants[3]. These findings suggest that exposure to dioxins may affect the hypothalamus-pituitary-thyroid axis. A growing number of reports have demonstrated the association between adverse effects in children and exposure to POPs at low doses over a longer period. In particular, human perinatal exposure to PCBs has been shown to be associated with immunological changes[4], neural and developmental changes[5,6], lower psychomotor development[7,8], defects of short-term memory and spatial learning ability[9] and lower cognitive development[10]. Therefore, monitoring and epidemiological verification of exposure to POPs are necessary to assess the health risks to the Japanese population.

In Japan, the MOE has been conducting the POPs monitoring project[11] since FY2002 for the monitoring of chemicals in each of the environmental media and to obtain data that can contribute to effective evaluations in the Stockholm Convention on POPs. Recently the biological monitoring of human samples was added. We have been collaborating with the POPs biological monitoring project of the MOE by providing biological samples

from our prospective birth cohort study, The Tohoku Study of Child Development (TSCD)[12]. We reanalyzed the results and summarized the data of dioxins and PCBs in human pair samples of breast milk, cord blood and maternal blood[13]. We also analyzed the associations of the concentrations of these compounds with TSH and thyroid hormones in maternal and cord blood, since the disruption of the hypothalamus-pituitary-thyroid axis is a hypothetical mechanism for dioxin- and PCB-induced adverse effects.

Materials and Methods

The biological samples analyzed were randomly selected from the participants in the TSCD, and provided anonymously to the MOE. These samples were measured by IDEA Consultants, Inc. (Tokyo, Japan) as part of the MOE project. This study protocol was previously reported[12]. Briefly, the maternal peripheral blood was collected using heparin as the anticoagulant agent in the morning when the pregnancy was at 28 weeks. The cord blood was collected immediately after delivery. These whole blood samples were frozen at –80°C until the chemical analysis. The breast milk was collected one month after delivery, and then frozen similarly. The TSCD was approved by the Medical Ethics Committee of the Tohoku University Graduate School of Medicine, and all mothers provided signed informed consent.

Chemical analysis was conducted following the methods in the environmental monitoring report on persistent organic pollutants (POPs) in Japan 2002-2004[14]. Briefly, the biological samples were spiked with ^{13}C-labeled POPs as internal standards before extraction. The samples were extracted with liquid-liquid extraction and then extracts were purified by multilayer silica gel column chromatography. Active carbon dispersed silica gel column chromatography was further used for purification of PCDD/Fs and dioxin-like PCBs (DL-PCBs). For the other POPs, extracts were purified by Florisil column chromatography except for silica gel column chromatography for toxaphene. Congener-specific determination of the compounds was performed by high resolution gas chromatograph-high resolution mass spectrometry (HRGC-HRMS) or negative ion chemical ionization mass spectrometry (GC-NICIMS) for toxaphene by isotope dilution quantification. Although control samples were analyzed for every 9-sample batch, they did not contain significant amounts of the target compound. TSH, total T4, and total T3 were measured from the plasma of cord and maternal blood by SRL, Inc. (Tokyo, Japan). The statistical analyses were performed using JMP ver. 5.1.2..

Results and Discussion

Concentrations of TEQ and total PCBs in breast milk, cord blood and maternal blood are shown in Table 1. TEQ was calculated by the WHO (1998) toxic equivalency factor[15] (TEF) assuming that the amount of congeners below the determination limit was zero. These data were roughly in agreement with previous studies[16-21]. In these biological samples, TEQ and PCB levels in breast milk were higher than in cord blood and maternal blood. Concentrations of TEQ and total PCBs among the three biological samples showed high correlations (Figure 1). Therefore, to predict the concentrations of dioxins and PCBs for the purpose of biological monitoring, it might be useful to measure the concentrations in breast milk. The homologue pattern of PCBs in breast milk was similar in composition to those of cord blood and maternal blood. The predominant homologues in the biological samples were HxCBs, followed by HpCBs, PeCBs and TeCBs.

The correlations between TEQ and PCBs in breast milk, cord blood and maternal blood were very high (Table 2). It was found that the contribution ratio of DL-PCBs to total TEQ was about 40% and the percentage of the DL-PCB concentration in total PCBs was 10% by the congener-specific analysis of PCBs in these biological samples. Because of the high correlation between TEQ and PCBs, the levels of exposure to dioxins for the population could be estimated from the results of PCB measurements. We may be able to simplify the monitoring method by eliminating the determination of dioxins from the analytical procedures.

The working hypothesis is that dioxins and PCBs cause adverse effects via disruption of thyroid hormone regulation and metabolism. Indeed, as shown in Table 3, there were significant correlations of PCBs with T3, and T4 in maternal blood ($p < 0.05$). Similarly, there were correlations of TEQ and PCBs with several thyroid function indicators in breast milk and cord blood ($p < 0.05$). Although the exact mechanisms by which dioxins and PCBs affect the levels of TSH and thyroid hormones are not fully understood, these results suggest that exposure to dioxins and PCBs could cause hormonal disturbance of thyroid function.

Table 1. Concentrations of TEQ (pg-TEQ/g-fat) and PCBs (ng/g-fat) in breast milk, cord blood and maternal blood

Compound names	Breast milk Median (Min - Max)	Cord blood Median (Min - Max)	Maternal blood Median (Min - Max)
Dioxins			
PCDD/Fs-TEQ	9.9 (2.0-25)	5.4 (0.28-16)	8.6 (2.8-26)
DL-PCBs-TEQ	6.8 (2.1-21)	2.9 (0.74-7.3)	4.8 (1.4-11)
Total TEQ	17 (4.2-45)	8.3 (1.1-22)	13 (4.8-33)
PCBs			
Total PCBs	102 (31-274)	40 (12-128)	76 (20-163)

n=68 for breast milk and cord blood, n=49 for maternal blood.
TEQ was calculated by the WHO (1998) TEF assuming that the amount of congeners below the determination limit was zero.

References

1. Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LG, Brouwer A, Sauer PJ. *Pediatr Res.* 1994; 36:468.
2. Nagayama J, Okamura K, Iida T, Hirakawa H, Matsueda T, Tsuji H, Hasegawa M, Sato K, Ma HY, Yanagawa T, Igarashi H, Fukushige J, Watanabe T. *Chemosphere* 1998; 37: 1789.
3. Pluim HJ, de Vijlder JJ, Olie K, Kok JH, Vulsma T, van Tijn DA, van der Slikke JW, Koppe JG. *Environ Health Perspect* 1993; 101:504.
4. Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, Hooijkaas H. *Environ Health Perspect* 2000; 108:1203.
5. Jacobson JL, Jacobson SW. *Neurotoxicology* 1997; 18:415.
6. Stewart P, Reihman J, Lonky E, Darvill T, Pagano J. *Neurotoxicol Teratol* 2000; 22:21.
7. Weisglas-Kuperus N. *Chemosphere* 1998; 37:1845.
8. Walkowiak J, Wiener JA, Fastabend A, Heinzow B, Kramer U, Schmidt E, Steingruber HJ, Wundram S, Winneke G. *Lancet* 2001; 358:1602.
9. Jacobson JL, Jacobson SW, Humphrey HE. *J Pediatr* 1990; 116:38
10. Stewart PW, Reihman J, Lonky EI, Darvill TJ, Pagano J. *Neurotoxicol Teratol* 2003; 25:11.
11. Yoshida Y, Enomoto Y, Kamiya K. *Organohalogen Compounds* 2002; 66:3504.
12. 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.
13. Ministry of the Environment, Japan. http://www.env.go.jp/press/press.php?serial=7896
14. Ministry of the Environment, Japan. http://www.env.go.jp/en/chemi/pops/rep2002-2004.pdf
15. Van den Berg M, Birnbaum L, Bosveld AT, Brunstrom B, Cook P, Feeley M, Giesy JP, Hanberg A, Hasegawa R, Kennedy SW, Kubiak T, Larsen JC, van Leeuwen FX, Liem AK, Nolt C, Peterson RE, Poellinger L, Safe S, Schrenk D, Tillitt D, Tysklind M, Younes M, Waern F, Zacharewski T. *Environ Health Perspect* 1998; 106:775.
16. Kunisue T, Muraoka M, Ohtake M, Sudaryanto A, Minh NH, Ueno D, Higaki Y, Ochi M, Tsydenova O, Kamikawa S, Tonegi T, Nakamura Y, Shimomura H, Nagayama J, Tanabe S. *Chemosphere* 2006; 64:1601.
17. Uehara R, Peng G, Nakamura Y, Matsuura N, Kondo N, Tada H. *Chemosphere* 2006; 62:1135.
18. Suzuki G, Nakano M, Nakano S. *Biosci Biotechnol Biochem* 2005; 69:1836
19. Fukata H, Omori M, Osada H, Todaka E, Mori C. *Environ Health Perspect* 2005; 113:297.
20. Iida T, Hirakawa H, Matsueda T, Takenaka S, Nagayama J. *Chemosphere* 1999; 38:3497.
21. Morita M. A research report of Health and Labour Sciences Research Grants, Ministry of Health Labour and Welfare, 1998 (in Japanese).
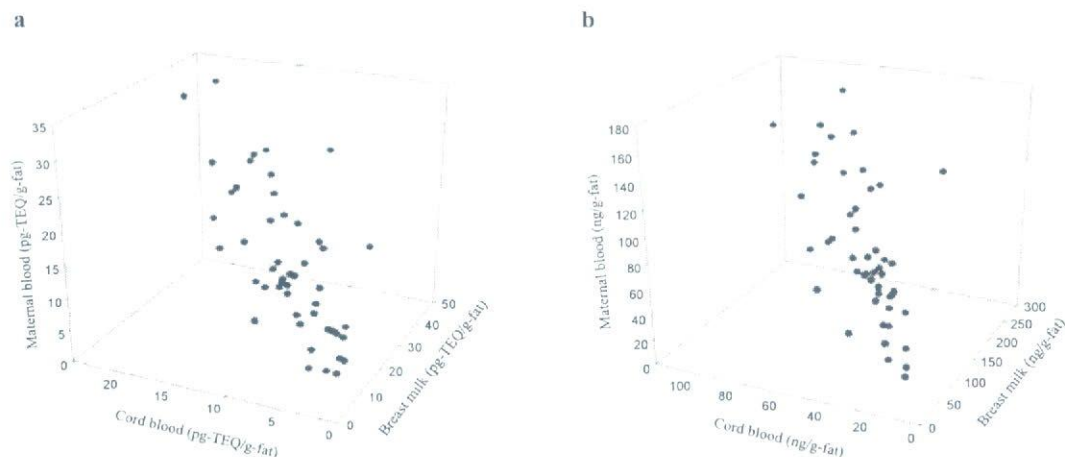
a
b

Fig. 1 Relationship of TEQ (a) and PCBs (b) among pair samples of breast milk, cord blood and maternal blood.

Table 2. Pearson correlation coefficients of TEQ and PCBs in breast milk with those of cord blood and maternal blood

	Breast milk		Cord blood		Maternal blood	
	TEQ	PCBs	TEQ	PCBs	TEQ	PCBs
Breast milk						
TEQ	-					
PCBs	0.901	-				
Cord blood						
TEQ	0.763	0.633	-			
PCBs	0.716	0.808	0.826	-		
Maternal blood						
TEQ	0.938	0.854	0.794	0.756	-	
PCBs	0.841	0.927	0.699	0.843	0.892	-

Pearson's r ($p < 0.001$) after log-transformed. n=49 for breast milk, cord blood and maternal blood.

Table 3. Pearson correlation coefficients of TEQ and PCBs with TSH, total T4 and total T3 in breast milk, cord blood and maternal blood

	Maternal blood			Cord blood		
	TSH	T4	T3	TSH	T4	T3
Breast milk (n=68, except for n=67 for maternal TSH/T4/T3)						
TEQ	0.232	0.158	0.225	0.146	-0.224	-0.138
PCBs	0.205	0.231	0.257*	0.137	-0.163	-0.075
Cord blood (n=68, except for n=67 for maternal TSH/T4/T3)						
TEQ	0.203	0.038	0.150	0.071	-0.270*	-0.051
PCBs	0.239	0.131	0.240	0.024	-0.144	0.085
Maternal blood (n=49, except for n=48 for maternal TSH/T4/T3)						
TEQ	0.264	0.271	0.375*	0.145	-0.238	-0.094
PCBs	0.278	0.287*	0.402*	0.185	-0.150	-0.038

n=68 for breast milk and cord blood, n=49 for maternal blood.
Pearson's r after log-transformed. * p<0.05

ASSOCIATIONS OF NEONATAL NEUROBEHAVIORAL STATUS WITH CORD BLOOD PCB, MATERNAL HAIR MERCURY, AND MATERNAL FISH INTAKE IN THE TOHOKU STUDY OF CHILD DEVELOPMENT

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

[1] Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai, 980-8575, Japan; [2] Human Development and Disabilities, Tohoku University Graduate School of Education, Sendai, 980-8576, Japan; [3] Department of Obstetrics, Tohoku University Graduate School of Medicine, Sendai, 980-8575, Japan; [4] Miyagi Children's Hospital, Sendai, 989-3126, Japan; [5] Department of Environmental Health Sciences, Akita University School of Medicine, 010-8543, Japan

Abstract

We have been performing a prospective cohort study, the Tohoku Study of Child Development (TSCD), to examine the effects of perinatal exposures to environmentally persistent organic pollutants and heavy metals on neurobehavioral development of offspring. In the present study, we examined the associations of the Neonatal Behavioral Assessment Scale (NBAS) with the total PCB concentrations in cord blood, maternal hair mercury (hair Hg), and maternal fish intake. Multiple regression analyses indicated some significant associations of the NBAS clusters with hair Hg, but there were no significant associations between total PCBs and any cluster of NBAS. These findings suggest that prenatal methylmercury exposure adversely affects neonatal neurobehavioral status.

Introduction

Several epidemiological studies have indicated some associations of perinatal exposure to polychlorinated biphenyls (PCBs) and methylmercury (MeHg) with developmental deficits such as postnatal growth delay and poor cognitive functions. A common form of perinatal exposure is maternal fish intake; however, fish also contain some nutritive factors such as n-3 polyunsaturated fatty acids (n-3 PUFA) essential for normal brain development in the fetus and infant. From the 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 PCBs and MeHg on neurobehavioral development in Japanese children[1]. Previously[2], we reported some preliminary data about the associations of neonatal neurobehavioral status with total PCBs in cord blood and maternal fish intake. Since additional data on PCBs in cord blood have recently become available, in the present study we reexamined the associations of neonatal

neurobehavioral status with total PCBs in cord blood, maternal hair mercury (hair Hg), and maternal fish intake.

Materials and Methods

The subjects were 392 mother-infant pairs whose variables including the PCB concentration in cord blood, the NBAS, and other covariates were available. The mean maternal age at delivery was 31.9 (SD4.2) years. The infants consisted of 203 boys and 189 girls, and they were all singletons from full-term (36-42 weeks) gestation without congenital anomalies or diseases. Birth weight was 2400g or more. Information was obtained about pregnancy, delivery and infant characteristics from medical records.

The PCB concentration was measured from whole cord blood collected immediately after delivery. All 209 congeners were analyzed using HR-GC/MS (IDEA Consultants, Inc, Tokyo, Japan). The total PCB concentration represented the sum of the all measured congeners, expressed as ng/g-fat.

The hair Hg concentration was analyzed from maternal hair samples taken two days after delivery. The total hair Hg concentration was measured by cold vapor atomic absorption[4] at the National Institute for Minamata Disease (Minamata, Japan).

Maternal fish intake was estimated using a semiquantitative food frequency questionnaire (FFQ) for 122 individual foods and recipes[3] and 13 additional items regarding fish and shellfish. The FFQ was administered four days after delivery. Trained investigators showed life-size photographs of each food to the mothers, after which they were asked to answer questions about the frequency and the amount of intake per meal.

Thyroid hormones, including thyroid-stimulating hormone (TSH), total thyroxine (T4), triiodothyromine (T3), free T4 and free T3, were measured from plasma of cord blood by SRL, Inc. (Tokyo, Japan).

The NBAS was administered 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 checks were conducted throughout the data collection to maintain a 90% level of agreement.

In the statistical analysis, 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, sex, birth weight, Apgar score 1 min after delivery, TSH, T4 and T3 concentrations in cord blood, and the NBAS examiners. The significance level was set at 5%.

Results and Discussion

The mean total PCB concentration in cord blood was 55.9 ng/g-fat (SD 35.3) (median 48.4), the mean total hair Hg level was 2.2 μg/g (SD 1.1) (median 2.0), and total fish intake was 25.9 kg/year (SD 17.8) (median 22.7). Table 1 shows the results of multiple regression

analyses. The total PCBs in cord blood and the total fish intake were not significantly associated with any seven clusters of the NBAS. The total hair Hg was negatively associated with the motor score, and positively associated with the range of state and the reflex scores. For the motor and the range of state, higher scores mean more optimal behavioral status, and for the reflex, lower scores mean more optimal status because the reflex score indicates the number of unusual reflexes. Thus, the results suggested that prenatal MeHg exposure adversely affected neonatal neurobehavioral status. In early studies, an adverse effect of prenatal MeHg exposure on neurodevelopment was found in the Faroe Islands[5] and Boston[6], but not in the Seychelles[7]. Our findings are in line with the former two, although the types of examination were different.

Regarding the effects of PCB exposure, early studies demonstrated adverse effects of prenatal PCB exposure on neurodevelopment[8,9]. However, our findings do not agree with those studies. Several possibilities may account for this discrepancy. First, the levels of PCB exposure in Japanese pregnant women have decreased during the past several decades[10]. It is plausible that the level of PCB exposure in our cohort was too low to induce adverse effects on neonatal neurobehavioral status. Second, we used the value of the total PCB concentration as the exposure value. In the Oswego study, highly chlorinated PCBs (Cl7-9) were strongly associated with lower scores of the NBAS, but the total PCB level was not[7]. Therefore, we examined the associations of the NBAS with highly chlorinated PCBs, but found no significance (data not shown). Third, levels of toxicants such as PCB and MeHg and nutritive factors, including n-3 PUFA, vary among different fish types. The Japanese diet relies heavily on steamed rice, fish and vegetables. Indeed, the Japanese eat great amounts of fish, and they also eat many kinds of fish. This food lifestyle may contribute to the differences in the consequences of cohort studies. Further studies will require consideration of the potential risks of fish intake in the context of potential benefits. Since the TSCD study is a prospective cohort study, we will readdress these health issues when the children become older.

Acknowledgments

Table 1. Results of multiple regression analyses

	Total PCBs (ng/g fat)[1]		Total hair Hg (µg/g)[1]		Total fish intake (kg/year)[1]		R^2 of the model
	Standardized beta	F	Standardized beta	F	Standardized beta	F	
Habituation	0.10	0.15	0.37	3.48	-0.31	1.14	0.12
Orientation	0.08	0.21	0.06	0.10	0.17	0.65	0.26
Motor	0.01	0.01	-0.28	5.60*	0.18	2.10	0.16
Range of state	-0.02	0.02	0.38	6.85**	-0.08	0.28	0.11
Regulation of state	0.22	1.27	-0.29	1.70	0.27	1.39	0.08
Autonomic stability	0.02	0.02	0.19	1.07	-0.23	1.38	0.12
Reflex	-0.26	0.72	0.74	4.52*	-0.11	0.09	0.17

$* p < 0.05 \quad ** p < 0.01$

[1] Log translations, $Log_{10} X$, were used on values of total PCBs, hair Hg, and total fish intake.

References

1. 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.
2. Suzuki K, Nakai K, Nakamura T, Hosokawa T, Okamura K, Sakai T, Kurokawa N, Kameo S, Murata K, and Satoh H. *Organohalogen Compounds*. 2006; 68: 1201-1204.
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. (ed.), Plenum, New York, 1991: 53-76.
5. Grandjean P, Weihe P, White R, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R, and Jorgensen P. *Neurotoxicol Teratol.* 1997; 19: 417-428.
6. Oken E, Wright R, Kleinman K, Bellinger D, Amarasiriwardena C. Hu H, Rich-Edwards J, and Gillman M. *Environ Health Perspect.* 2005; 113: 1376-1380.
7. Myers G, Davidson P, Cox C, Shamlaye C, Palumbo D, Cernichiari E, Sloane-Reeves J, Wilding G, Kost J, Huang L, and Clarkson T. *Lancet*. 2003; 361: 1686-1692.
8. Stewart P, Reihman J, Lonkey E, Darvill T and Pagano J. *Nurotoxicol and Teratol.* 2000; 22: 21-29.
9. Vreugdenhil H, Van Zanten G, Mulder P, Weisglas-Kuperus N. *Dev Med Child Neurol.* 2004; 46; 398-405.
10. Konishi Y, Kuwabara K, and Hori S. *Arch Environ. Toxicol.* 2001; 40: 571-578.