EFFECTS OF PRENATAL EXPOSURE TO DIOXINS, PCBs AND ORGANOCHLORINE PESTICIDES ON INDUCTION OF CONGENITAL HYPOTHYROIDISM WITH SPECIAL REFERENCE TO LIPID CONTENTS

Junya Nagayama [1], Hitoshi Kohno [2], Tatsuya Kunisue [3], Hiroshi Shimomura [4], Shinsuke Tanabe [3]

[1] Laboratory of Environmental Molecular Epidemiology, School of Health Sciences, Faculty of Medicine, Kyushu University, Fukuoka 812-8582, Japan; [2] Department of Endocrinology and Metabolism, Fukuoka Children's Hospital, Fukuoka 810-0063, Japan; [3] Center for Marine Environmental Studies, Ehime University, Matsuyama 790-8577, Japan; [4] Shimomura OBGY Clinic, Fukuoka 811-1254

Introduction

In general, risk evaluation of toxic compounds such as dioxins, PCBs, organochlorine compounds and so forth for life as well as human beings has been done based upon their contamination levels on lipid weight basis. However, lipid extraction from objects to be examined is considered quite difficult and such an example is shown in Table 1, in which lipid contents of three blood samples from three different persons are cross-checked by three representative analytical institutions in Japan. As indicated in this table, lipid contents determined by S.T. were much higher than those determined by T.R. and E.U.. Accordingly, if the concentrations of some chemicals, for example HCH and HCB in Table 2, are almost the same on wet weight basis in these three institutions, their concentrations determined by S.T. become the lowest on lipid weight basis. This kind of change in concentrations of toxic compounds is very important because usually their effects and risk evaluation have been done based upon their concentrations on lipid weight basis. In this study, the effects of prenatal exposure to PCDDs, PCDFs and Co-PCBs, which were so-called dioxins, PCBs and organochlorine pesticides such as HCHs, DDT, dieldrin, heptachlor and chlordane on the induction of congenital hypothyroidism, that is, cretinism are investigated based upon their contamination levels on both wet and lipid weight bases, and efficacy of lipid extraction is also discussed from the viewpoint of effect and risk evaluation of toxic compound.

Table 1. Comparison of extractable lipid contents in three human blood samples determined by three different institutions

Institution	Lipid Content (%) of Human Blood Sample		
	A	B	C
T.R.	0.13	0.08	0.11
S.T.	0.46	0.43	0.62
E.U.	0.16	0.09	0.21

Table 2. Comparison of contamination levels of organochlorine pesticides on wet and lipid weight bases in human blood sample C determined by three different institutions

	Blood Sample C					
	Wet Weight (ng/g)			Lipid Weight (ng/g lipid)		
Compound	T	S	E	T (0.11)	S (0.62)	E (0.21)
HCH	0.33	0.440	0.41	310	71	200
DDT	0.24	0.806	1.7	222	134	850
Chlordane*	0.019	0.138	0.146	17	21.9	69.4
HCB	0.078	0.071	0.084	71	11	40

Figure in parenthesis indicates the lipid content (%)
*: trans-Nonachlor + cis-Nonachlor

Materials and Methods

Positive cases of the mass-screening for congenital hypothyroidism in 2001 to 2004 in Fukuoka visited Fukuoka Children's Hospital, Fukuoka, Japan for the minute examination of cretinism. During these four years, total number of the positive cases was 65 (male: 39, female: 26), in which the organochlorine compounds in their mother's milk were determined in 34 cases (male: 22, female: 12). In the 34 positive neonates, 22 neonates (male: 13, female: 9) were congenital hypothyroidism, 4 neonates (male: 3, female: 1) hyperthyrotopinemia and 8 neonates (male: 6, female: 2) transient hyperthyrotropinemia. Serum concentrations of thyrotropin (TSH), free triiodothyronine (f-T_3) and free thyroxine (f-T_4) in the blood sampled at 5 to 20 days after birth were determined by the electrochemiluminescence immunoassay methods using commercially available kits [1]. Normal neonates, which mean negative cases of the mass-screening for the cretinism, were born in Shimomura OBGY Clinic, Fukuoka, Japan and their total number was 108 (male: 58, female: 50). In 108 normal neonates, 103 samples of their mother's milk were analyzed for the organochlorine compounds.

Contamination levels of the organochlorine pesticides and PCBs in the breast milk samples which were collected within 4 weeks after childbirth were quantified by ECD-GC (Hewlett Packard series 6890) using DB-1 fused silica capillary column (30 m length) [2]. Concentrations of dioxins were also measured by HRGC-HRMS (Agilent 6890 series and JEOL JMS-700D) with a resolving power >3000 for mono-ortho PCBs and >10,000 for non-ortho PCBs and PCDD/DFs [3]. Toxic equivalent quantities (TEQs) were estimated based on human/mammal toxic equivalency factors (TEFs) proposed by the World Health Organization (WHO) [4].

Statistical significance was evaluated by the two-tailed student's T test and less than 5% of *p*-value was considered significant.

Results and Discussion

Contamination levels in the breast milk of dioxins, PCBs and DDT in the group of lipid content less than 1.0 % (low lipid group) were significantly lower than those in the group of lipid content more than 1.6% (high lipid group) on wet weight basis, as shown in Table 3. On the contrary, on lipid weight basis their concentrations in the low lipid group were significantly higher than those in the high lipid group (Table 3). This kind of changes in their concentrations between on wet and on lipid weight bases was also observed in other compounds such as PCDDs, PCDFs, Co-PCBs, HCH, chlordane, dieldrin and HCE in the same breast milk samples. Relationships between the lipid contents (%)

Table 3. Contamination levels of dioxins, PCBs and DDT on wet and lipid weight bases in the breast milk of Japanese mother by lipid content

	Concentration					
	Wet Weight (ng/g)			Lipid Weight (ng/g lipid)		
Lipid (%)	Dioxins	PCBs	DDT	Dioxins	PCBs	DDT
1.0 >	-,*** 0.212 ± 0.108	-,*** 1.13 ± 0.60	-,** 2.29 ± 1.67	**,*** 28.5 ± 13.0	**,** 152 ± 78	-,* 306 ± 223
1.0 ~ 1.6	** 0.250 ± 0.115	*** 1.15 ± 0.49	- 3.16 ± 2.35	- 19.2 ± 8.5	- 88 ± 40	- 243 ± 182
1.6 <	0.347 ± 0.170	1.87 ± 0.93	4.37 ± 3.38	16.1 ± 8.1	87 ± 44	206 ± 166

Dioxins: pg-TEQ/g and pg-TEQ/g lipid
*: $0.01 < p < 0.05$, **: $0.001 < p < 0.01$, ***: $p < 0.001$

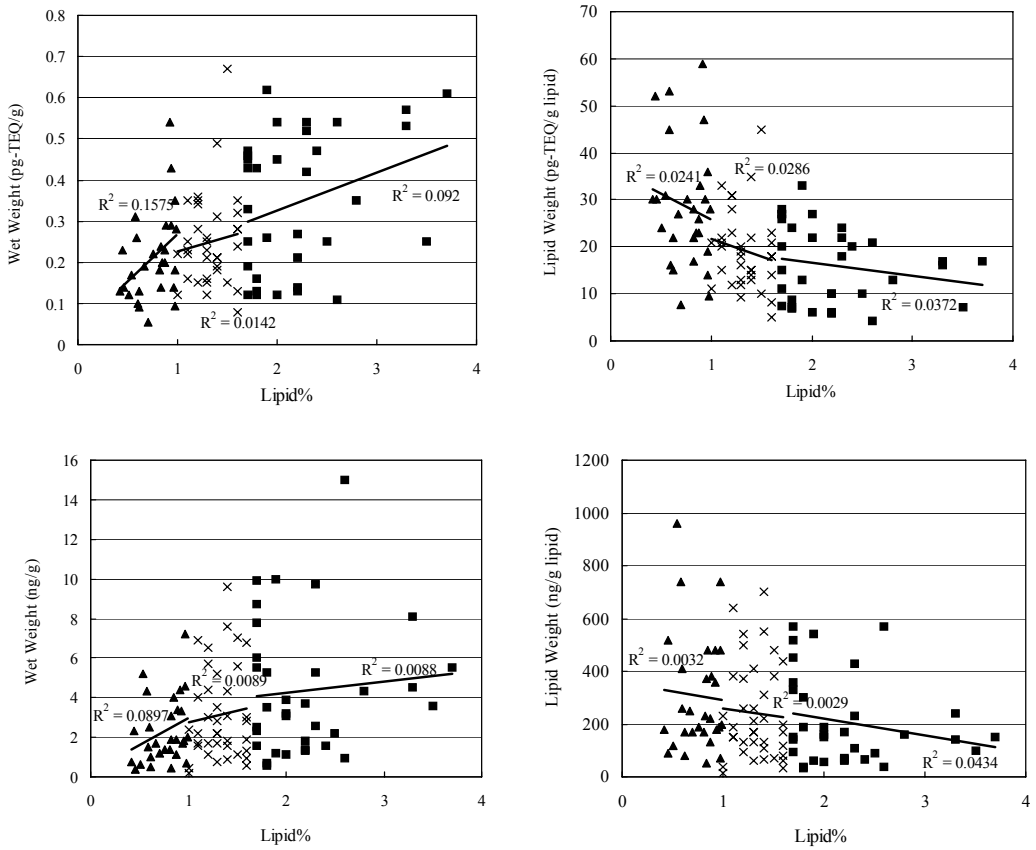

Fig. 1. Relationships the lipid contents (%) and the concentrations of dioxins (upper) and DDT (lower) on wet and lipid weight bases
Lipid content (%): ▲;1.0 > , ×;1.0 ~ 1.6, ■ ; >1.6

extracted from the breast milk samples and the concentrations of dioxins and DDT on both wet and lipid weight bases are shown in Fig. 1. These figures clearly indicate the changes in their concentrations mentioned above.
These results may indicate that due to poor lipid extraction contamination levels of dioxins, PCBs and DDT in the low lipid group become relatively high compared to those in the high lipid group.
Accordingly, we expected such things may happen in case of the induction of cretinism and examined the effects of dioxins, PCBs and organochlorine pesticides on both wet and lipid weight bases, respectively.
As indicated in Table 4, on wet weight basis their concentrations except HCH were significantly higher in the cretinism group than in the normal healthy group, as reported before [5]. However, on lipid weight basis we could not find any significant difference in their contamination levels between the two groups (Table 4).
Taking the difficulties in the extraction of the lipid from samples analyzed into account, we should use the concentrations of the toxic compounds on wet weight basis rather than on lipid weight basis for their effects and risk evaluation. Anyway, for more precise evaluation in exposure to toxic compounds, we have to establish the international standardization or global standard method for lipid extraction from any samples examined, as soon as possible.

Table 4. Contamination levels of dioxins, PCBs and organochlorine pesticides on wet and lipid weight bases in the breast milk of Japanese mothers in normal healthy and cretinism groups

	Concentration			
	Wet Weight (ng/g)		Lipid Weight (ng/g lipid)	
Compound	Normal	Certinism	Normal (1.5)	Certinism (3.0)
Dioxins	0.28 ± 0.15	0.62 ± 0.44**	20.9 ± 11.0	21.1 ± 11.7
PCDDs	0.11 ± 0.06	0.22 ± 0.18*	7.9 ± 4.6	7.5 ± 4.9
PCDFs	0.06 ± 0.03	0.16 ± 0.12**	4.8 ± 2.5	5.3 ± 3.5
Co-PCBs	0.11 ± 0.06	0.25 ± 0.18**	8.2 ± 4.5	8.4 ± 4.7
PCBs	1.5 ± 1.3	2.9 ± 2.1**	113 ± 82	92 ± 42
Pesticides				
DDT	3.7 ± 3.7	7.7 ± 6.2**	266 ± 228	259 ± 174
HCH	1.3 ± 1.2	3.0 ± 4.2	90 ± 74	94 ± 104
Chlordane	0.8 ± 0.7	1.9 ± 1.7**	57 ± 59	64 ± 55
HCB	0.2 ± 0.2	0.3 ± 0.2**	12 ± 11	12 ± 5

Figure in parenthesis indicates the mean lipid content (%)
Dioxins, PCDDs, PCDFs and Co-PCBs: pg-TEQ/g and pg-TEQ/g lipid
*: $0.01 < p < 0.05$, **: $0.001 < p < 0.01$, ***: $p < 0.001$

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