RESIDUES AND TRACE ELEMENTS

Maternal Body Burden of Organochlorine Pesticides and Dioxins

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To investigate the body burden of organochlorine pesticides and dioxins in Japanese women, 125 milk samples were collected from 41 mothers in 1994, 42 in 1995, and 42 in 1996. Of the 125 samples, 82 were from primipara mothers (first delivery) and 43 were from multipara mothers (second or later delivery). By using capillary gas chromatography with electron capture detection, β-HCH and *p,p′*-DDE were detected as the major chlorine pesticides in human milk. Average levels of β-HCH and *p,p′*-DDE were 475 and 368 ng/g lipid, respectively, in primipara breast milk, 314 and 259 ng/g lipid in multipara breast milk, and 420 and 330 ng/g lipid in total breast milk. Dieldrin, heptachor epoxide, oxychlordane, *trans*-chlordane, and *cis*-chlordane were detected at lower average levels of 3, 4, 34, 41, and 5 ng/g lipid, respectively. By using high-resolution gas chromatography with mass spectrometric detection, dioxins were detected in all samples. Average levels of total polychlorinated dibenzo-*p*-dioxin (PCDD), total polychlorinated dibenzofuran (PCDF), total PCDD + PCDF, total coplanar polychlorinatedbiphenyl (CoPCB), and total dioxin were 10.0, 7.8, 17.7, 9.9, and 27.5 TEQ (toxic equivalent) pg/g lipid, respectively, in primipara breast milk; 7.0, 5.8, 12.8, 7.3, and 20.1 TEQ pg/g lipid in multipara breast milk; and 8.9, 7.1, 16.1, 8.9, and 25.0 TEQ pg/g lipid in total breast milk. In primipara breast milk, significant correlations were found among levels of β-HCH, *p,p′*-DDE, total PCDD-TEQ, total PCDF-TEQ, total CoPCB-TEQ, and total TEQ except for less correlation between *p,p′*-DDE and total PCDF-TEQ. Levels of these analytes also significantly increased depending on mother's age, except for total Co-PCB-TEQ. For the correlation with food habit, the only positive correlation was between total PCDF-TEQs and fish intake.

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Although human milk is immunologically beneficial for infants, it often contains lipophilic pollutants. From the 1970s to the 1980s, organochlorine pesticides and polychlorinated biphenyls (PCBs) in human milk were monitored many times in European countries, the United States, Canada, and Japan (1–4). Results showed that pollutant levels in developed countries had gradually decreased since the banning of PCBs and persistent pesticides (5, 6). On the other hand, polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have emerged as unintended toxic pollutants (7, 8).

The toxicities of PCDDs and PCDFs have been studied intensively (9, 10), and their hazardous effects on humans have been pointed out (11). Until recently, risk assessment of these chemicals has been made on the basis of acceptable or tolerable daily intake (ADI or TDI, mg or pg/kg body weight; 12), and when the ratio of the estimated dose of a chemical to ADI or TDI is <1.0, the chemical has been considered safe.

Recently, *p,p′*-DDT and its metabolite *p,p′*-DDE were reported as endocrine disrupters (13, 14). It is also well known that dioxins affect biosynthesis and biodecomposition of hormones (15). Whether *p,p′*-DDT, *p,p′*-DDE, and dioxins in mother's milk have adverse effects on breast-fed babies has not been established. We analyzed human milk from 125 mothers to investigate the exposure of breast-fed babies to organochlorine pesticides and dioxins and to determine the background body burden of organochlorine pesticides and dioxins in modern Japanese women.

Experimental

Materials

(**a**) *Samples.*—Milk samples (50–100 mL each) were collected from 41 mothers (27 first deliveries or primiparas and 14 second or later deliveries or multiparas) ranging in age from 24 to 38 years in 1994, 42 mothers (28 primiparas and 14 multiparas) ranging in age from 21 to 36 years in 1995, and 42 mothers (27 primiparas and 15 multiparas) ranging in age from 21 to 40 years in 1996 at Kyushu University Hospital ca 3 months after delivery. The mothers were also living in Fukuoka. All samples were stored in a refrigerator (–20°C) until analysis.

(**b**) *Solvents.*—Hexane, acetone, ethyl acetate, diethyl ether, ethanol, petroleum ether, acetonitrile, and nonane. All

Table 1. Elution profile and recovery of organochlorine pesticides by Florisil column chromatography[a]

Analyte	Fraction 1, %	Fraction 2, %	Total, %	LOD, ng/mL	LOQ, ng/g lipid
α-HCH	0.0	73.2	73.2	0.2	2
β-HCH	0.0	83.5	83.5	0.3	3
γ-HCH	0.0	81.9	81.9	0.2	2
δ-HCH	0.0	91.7	91.7	0.3	3
Heptachor	100.5	0.0	100.5	0.2	2
Heptachlor epoxide	0.0	73.1	73.1	0.2	2
Oxychlordane	0.0	79.0	79.0	0.2	2
trans-Chlordane	0.0	71.6	71.6	0.1	1
cis-Chlordane	16.8	66.4	83.2	0.1	1
trans-Nonachlor	118.4	0.0	118.4	0.2	2
cis-Nonachlor	0.0	81.3	81.3	0.2	2
Aldrin	49.4	0.0	49.4	0.3	3
Dieldrin	0.0	67.7	67.7	0.3	3
Endrin	0.0	83.6	83.6	0.3	3
p,p'-DDE	93.1	0.0	93.1	0.3	3
p,p'-DDD	95.1	0.0	95.1	0.3	3
o,p'-DDT	0.0	82.7	82.7	0.3	3
p,p'-DDT	0.0	75.7	75.7	0.3	3

[a] Experiments were done in quadruplicate.

solvents were PR grade guaranteed for purity by gas chromatography with electron capture detection (GC–ECD) after 300-fold concentration. They were purchased from Wako Pure Chemical Co. Ltd., Tokyo, Japan.

(c) *Analytical standards.*—α-HCH, β-HCH, γ-HCH, δ-HCH, dieldrin, aldrin, endrin, heptachlor, heptachlor epoxide, *trans*-chlordane, *cis*-chlordane, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, *o,p'*-DDT, oxychlordane, *trans*-nonachlor, and *cis*-nonachlor were purchased from Wako Pure Chemical.

Standard solutions of regular and ^{13}C-labeled PCDD, PCDF, and coplanar polychlorinatedbiphenyl (CoPCB) were purchased from Wellington Laboratories, Ontario, Canada. PCDDs, PCDFs, and CoPCBs are listed as follows:

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (PCDD), 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (HxCDD), 1,2,3,6,7,8-HxCDD, 1,2,3,4,7,8,9-heptachlorodibenzo-*p*-dioxin (HpCDD), 1,2,3,4,6,7,8-HpCDD, octachlorodibenzo-*p*-dioxin (OCDD), 2,3,7,8-tetrachlorodibenzofuran (TCDF), 1,2,3,7,8-pentachlrodibenzofuran (PCDF), 2,3,4,7,8-PCDF, 1,2,3,4,7,8-hexachlorodibenzofuran (HxCDF), 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,4,7,8,9-heptachlorodibenzofuran (HpCDF), 1,2,3,4,6,7,8-HpCDF, octachlorodibenzofuran (OCDF), 3,4,3',4'-tetrachlorobiphenyl (TCB), 3,4,5,3',4'-pentachlorobiphenyl (PeCB), 3,4,5,3',4',5'-hexachlorobiphenyl (HCB).

(d) *Standard solutions.*—Two sets of standard solutions were used for accurate calibration of GC peaks. (*1*) *Solution A.*—100 ng/mL each of α-HCH; β-HCH; γ-HCH; δ-HCH; *p,p'*-DDE; *p,p'*-DDD; and *o,p'*-DDT; *trans*-chlordane; *cis*-chlordane; oxychlordane; *trans*-nonachlor; and *cis*-nonachlor. (*2*) *Solution B.*—100 ng/mL each of heptachlor, heptachlor epoxide, aldrin, dieldrin, endrin, and *p,p'*-DDT.

(e) *Sodium sulfate anhydrate.*—PR grade, Wako Pure Chemical.

(f) *Absorbents.*—(*1*) *Florisil.*—100 mesh, PR grade (Floridin, Quincy, FL), washed with diethyl ether–hexane (1 + 1, v/v) in a Soxhlet apparatus for 8 h; activated at 130°C for 16 h prior to use. (*2*) *Silica gel–silver nitrate.*—Prepared by mixing 45 g silica gel 60 (70–230 mesh, Merck, Darmstadt, Germany) with 5 g silver nitrate dissolved in a small amount of water and evaporating the water.

(g) *10% Potassium oxalate solution.*—Prepared by dissolving reagent-grade potassium oxalate (Wako Pure Chemical) in distilled-in-glass, hexane-washed water.

(h) *5% Sodium chloride solution.*—Prepared from reagent-grade NaCl (Wako Pure Chemical) and washed with PR-grade hexane.

(i) *Sulfuric acid.*—PR grade, Wako Pure Chemical.

(j) *Water.*—Distilled in glass and washed with hexane.

(k) *Internal standard.*—Carbophenothion (Riedel-de Haen, Hannover, Germany).

Apparatus

(a) *GC system.*—Hewlett-Packard 5890 gas chromatograph equipped with ^{63}Ni ECD. Operating conditions: column temperature held at 60°C for 1 min and then raised to 140°C at 10°C/min, to 210°C at 1°C/min, and then to 260°C at 3°C/min; injector port, 250°C; detector, 300°C; carrier gas, helium; column pressure, 10 psi; makeup gas, nitrogen at 50 mL/min; injection mode, splitless for 1 min.

(b) *Quantitation column.*—SPB-5 (Supelco, Bellefonte, PA) capillary column, 0.32 mm id × 30 m and 0.25 μm film thickness.

(c) *Confirmation column.*—MP65HT (Quadrex, New Haven, CT) capillary column, 0.25 mm id × 30 m and 0.1 μm film thickness. Column temperature held at 80°C for 1 min and then raised to 160°C at 25°C/min, held for 5 min, and then raised to 210°C at 3°C/min; injector port, 210°C; detector, 300°C; carrier gas, helium; column pressure, 10 psi; makeup gas, nitrogen; at 50 mL/min; injection mode, splitless for 1 min.

(d) *GC with mass spectrometer detection (GC–MSD).*—Hewlett-Packard 5890 Series II (Wilmington, DE) gas chromatograph with a Finnigan Mat 90 (San Jose, CA) MSD.

(e) *Quantitation column for GC–MSD.*—SP2331 (Supelco) capillary column, 0.32 mm id × 60 m and 0.2 μm film thickness.

Dietary Habit Questionnaire

Mothers answered a questionnaire to provide background information, including body weights of mothers and babies, breast-feeding practices, delivery records, and dietary habits.

Sample Preparation for Analysis of Organochlorine Pesticides

(a) *Sample extraction.*—Milk sample (15 g) was weighed into a 200 mL separatory funnel (A), and then 2 mL 10% potassium oxalate solution and 20 mL ethanol were added. The mixture was shaken vigorously for 1 min. Then, 20 mL diethyl ether–petroleum ether (1 + 1, v/v) was added, and the mixture was shaken for 5 min. The ether phase was transferred into another separatory funnel (B), containing 20 mL 5% NaCl. The aqueous phase was extracted twice with 10 mL diethyl ether–petroleum ether (1 + 1, v/v). The ether phase was transferred into the second separatory funnel (B) and shaken for 5 min. The ether phase was separated from the aqueous phase, dried, concentrated, and weighed as lipid.

(b) *Defatting.*—A small amount of lipid (ca 0.2 g) was dissolved in 10 mL hexane, and the solution was transferred into a 100 mL separatory funnel. Hexane-saturated acetonitrile (20 mL) was added, and the mixture was shaken for 5 min. After partition, the acetonitrile layer was removed and transferred to a round vessel. The hexane layer was extracted twice with 20 mL hexane-saturated acetonitrile. The collected acetonitrile layer was evaporated under vacuum at <40°C without being allowed to dry. The remaining solvent was evaporated at ambient conditions prior to column chromatography.

(c) *Cleanup.*—Cleanup by Florisil column chromatography was performed according to the method reported by Kuwahara et al. (16). Activated Florisil (5 g) in a 50 mL beaker was slurried with 30 mL hexane and transferred to a column. When the Florisil had settled, ca 1 g anhydrous granular sodium sulfate was layered on it. When the level of the liquid reached the top of granular sodium sulfate, the inside walls of the column were rinsed with 30 mL hexane. Then, a defatted milk sample equivalent to 0.2 g lipid weight was loaded onto the column top and drained at the rate of 1 drop/s without allowing the column top to dry. The column was eluted with 30 mL hexane (fraction 1), followed by 80 mL diethyl ether–hexane (15 + 85, v/v; fraction 2). Several drops of nonane were added to each eluate as a keeper, and the eluate was concentrated under vacuum conditions at <30°C to a suitable volume. The concentrate was transferred to a 10 mL glass tube with hexane. The solvent in the glass tube was evaporated completely under a gentle N_2 flow, and residues were redissolved in hexane at a concentration of 0.1 mg lipid weight equivalent/mL and analyzed by capillary GC–ECD. Prior to injection of sample into the gas chromatograph, carbophenothion was added to each sample at 20 ng/mL as an internal standard.

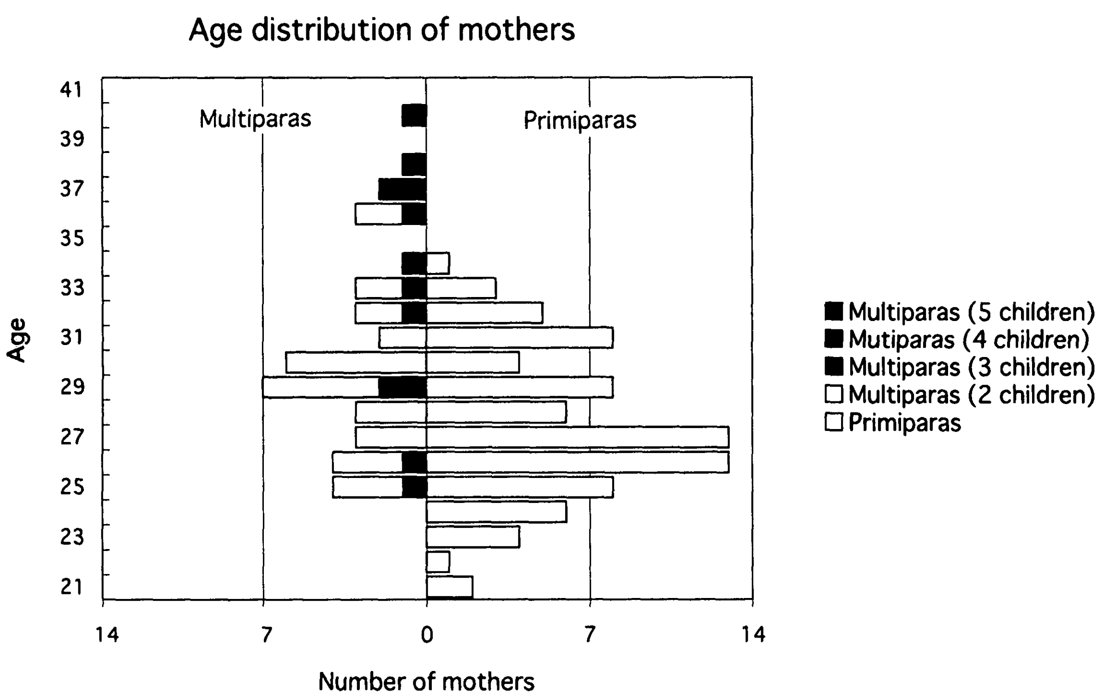

Figure 1. Distribution of 125 breast milk samples according to age of mother.

Table 2. Organochlorine pesticide concentrations (ng/g, lipid) and dioxin TEQs (pg/g, lipid) in primipara breast milk[a]

Analyte	No. of samples with analyte level > LOQ	Amount found			
		Minimum	Maximum	Average	Median
β-HCH	82	39	1360	475	379
Dieldrin	28	ND[b]	27	3	<3
p,p'-DDE	82	67	1330	368	302
p,p'-DDT	69	ND	51	15	13
Total DDT	82	80	1350	383	336
Heptachlor epoxide	53	ND	23	4	4
Oxychlordane	80	ND	427	36	22
trans-Nonachlor	82	6	116	44	38
cis-Nonachlor	57	ND	17	6	6
Total PCDD-TEQ	82	4.5	19.0	10.0	9.8
Total PCDF-TEQ	82	2.7	16.3	7.8	7.4
Total PCDD/PCDF-TEQ	82	7.2	33.0	17.7	17.1
Total CoPCB-TEQ	82	2.7	29.1	9.9	9.6
Total TEQ (PCDD/PCDF/CoPCB)	82	9.9	48.5	27.5	26.5

[a] *N* = 82; age: minimum 21, maximum 34, average 28.1, median 27; fat, %: minimum 0.98, maximum 8.18, average 3.95, median 3.87.
[b] ND, below LOQ: 1–3 ng/g for dieldrin, 2–3 ng/g for *p,p'*-DDT, 1–2 ng/g for heptachlor epoxide, 1–2 ng/g for oxychlordane, and 1–2 ng/g for *cis*-nonachlor.

(**d**) *Identification and quantitation.*—Two microliters each from fraction 1 and fraction 2 samples was injected. Each sample GC peak was identified by accurate coincidence with the retention time of an authentic standard (± 0.020 min). Identified peaks were checked by using another capillary column (MP65HT). In addition, quantitation was performed by comparing the peak height of each sample peak with that of each standard. To accurately quantitate existing chemicals other than dieldrin and endrin, we added 1 mL sulfuric acid to the GC hexane samples, centrifuged the mixture, and then injected 2 μL of the supernant. We used cow's milk to determine recoveries by this method. Elution profiles of organochlorine pesticides into fractions 1 and 2, recoveries, limits of detection (LODs), and limits of quantitation (LOQs) are shown in Table 1. LODs were determined at a signal-to-noise ratio of 3 on the chromatogram of standard samples.

Sample Preparation and Quantitation for Analysis of Dioxins

To 15 g milk, 20 ^{13}C-labeled internal standard dioxins were added at amounts ranging from 100 pg each for PCDD, OCDD, and PCDFs to 500 pg for CoPCB and OCDD. Lipids

Table 3. Organochlorine pesticide concentrations (ng/g, lipid) and dioxin TEQs (pg/g, lipid) in multipara breast milk[a]

Analyte	No. of samples with analyte level > LOQ	Amount found			
		Minimum	Maximum	Average	Median
β-HCH	42	ND[b]	935	314	261
Dieldrin	18	ND	25	3	<3
p,p'-DDE	43	32	1320	259	206
p,p'-DDT	36	ND	45	15	13
Total DDT	43	52	1320	274	206
Heptachlor epoxide	24	ND	13	3	2
Oxychlordane	37	ND	325	29	15
trans-Nonachlor	42	ND	143	35	32
cis-Nonachlor	24	ND	12	4	4
Total PCDD-TEQ	43	1.6	15.3	7.0	6.3
Total PCDF-TEQ	43	1.3	20.5	5.8	4.8
Total PCDD/PCDF-TEQ	43	2.9	23.8	12.8	11.4
Total CoPCB-TEQ	43	1.2	14.4	7.3	6.8
Total TEQ (PCDD/PCDF/CoPCB)	43	4.2	36.0	20.1	18.9

[a] *N* = 43; age: minimum 25, maximum 40, average 30.4, median 30.0; fat, %: minimum 1.38, maximum 8.48, average 4.29, median 4.40.
[b] ND, below LOQ: 1–3 ng/g for dieldrin, 2–3 ng/g for *p,p'*-DDT, 1–2 ng/g for heptachlor epoxide, 1–2 ng/g for oxychlordane, and 1–2 ng/g for *cis*-nonachlor.

Table 4. Organochlorine pesticide concentrations (ng/g, lipid) and dioxin TEQs (pg/g, lipid) in total breast milk[a]

Analyte	No. of samples with analyte level > LOQ	Amount found			
		Minimum	Maximum	Average	Median
β-HCH	124	ND[b]	1360	420	333
Dieldrin	46	ND	27	3	<3
p,p'-DDE	125	32	1330	330	265
p,p'-DDT	105	ND	50	15	13
Total DDT	125	52	1350	345	285
Heptachlor epoxide	77	ND	23	4	3
Oxychlordane	117	ND	427	34	19
trans-Nonachlor	124	ND	143	41	36
cis-Nonachlor	81	ND	17	5	5
Total PCDD-TEQ	125	1.6	19.0	8.9	9.1
Total PCDF-TEQ	125	1.3	20.5	7.1	6.7
Total PCDD/PCDF-TEQ	125	2.9	33.0	16.1	16.0
Total CoPCB-TEQ	125	1.2	29.1	8.9	8.6
Total TEQ (PCDD/PCDF/CoPCB)	125	4.2	48.5	25.0	24.2

[a] $N = 125$; age: minimum 21, maximum 40, average 29.2, median 28.0; fat, %: minimum 0.98, maximum 8.48, average 4.06, median 3.96.
[b] ND, below LOQ: 1–3 ng/g for dieldrin, 2–3 ng/g for *p,p'*-DDT, 1–2 ng/g for heptachlor epoxide, 1–2 ng/g for oxychlordane, 1–2 ng/g for *cis*-nonachlor.

were extracted, and 0.3–0.5 g of the extracts was dissolved in hexane and cleaned up by liquid–liquid partitioning with sulfuric acid, chromatography with silica gel–silver, and chromatography with activated carbon. The PCDD, PCDF, and CoPCB fraction was isolated and concentrated. The residue was dissolved in 10 μL nonane and spiked with internal standards. One microliter of the solution was injected to the GC–MSD system (Iida et al., to be published).

Table 5. Comparison of primapara and multipara breast milk from mothers ranging in age from 25 to 34 years

Parameter	Primiparas ($N = 69$)	Multiparas ($N = 37$)
Age of mother	28	29
Fat (%)	3.96	4.26
β-HCH (ng/g lipid)	531[a]	316[a]
Dieldrin (ng/g lipid)	3	3
p,p'-DDE (ng/g lipid)	381[a]	234[a]
p,p'-DDT (ng/g lipid)	16	16
Total DDT (ng/g lipid)	396[a]	250[a]
Heptachlor epoxide (ng/g lipid)	5[b]	3[b]
Oxychlordane (ng/g lipid)	37	32
trans-Nonachlor (ng/g lipid)	45	35
cis-Nonachlor (ng/g lipid)	6[b]	4[b]
Total PCDD-TEQ (pg/g lipid)	10.2[a]	7.0[a]
Total PCDF-TEQ (pg/g lipid)	8.0[a]	6.0[a]
Total PCDD/PCDF-TEQ (pg/g lipid)	18.2[a]	13.1[a]
Total CoPCB-TEQ (pg/g lipid)	10.1[a]	7.3[a]
Total TEQ (PCDD/PCDF/CoPCB) (pg/g lipid)	28.3[a]	20.4[a]

[a] Significant difference at $p < 0.01$.
[b] Significant difference at $p < 0.05$.

Results and Discussion

Concentrations of Organochlorine Pesticides and Dioxins in Milk

We investigated milk samples collected in 1994–1996 from 125 mothers (82 primiparas and 43 multiparas) ranging in age from 21 to 40 years at 2 or 3 months after delivery to determine concentrations of organochlorine pesticides and dioxins. Figure 1 shows the age distribution and delivery records of the mothers. Tables 2–4 show analyte levels in milk samples. In this study, *p,p'*-DDD and *o,p'*-DDT were not detected in the milk samples. Therefore, total DDT is the sum of *p,p'*-DDE and *p,p'*-DDT. β-HCH and *p,p'*-DDE were detected in almost every milk sample. All analytes except for dioxins (or TEQ values; TEQ is the abbreviation of toxic equivalent and is obtained by multiplying a dioxin concentration with its toxic equivalency factor [TEF] which the World Health Organization [WHO] proposed) showed normal logarithmic distribution.

The average levels of β-HCH and *p,p'*-DDE were 475 and 368 ng/g lipid, respectively, in primipara breast milk, 314 and 259 ng/g lipid in multipara breast milk, and 420 and 330 ng/g lipid in total breast milk. Lower levels of oxychlordane and *trans*-nonachlor were measured in most samples at detection rates of 94 and 99%, respectively, for total breast milk. Average levels of oxychlordane and *trans*-nonachlor were 36 and 44 ng/g lipid, respectively, in primipara breast milk, 29 and 35 ng/g lipid in multipara breast milk, and 34 and 41 ng/g lipid in total breast milk. *p,p'*-DDT was detected in 84% of total breast milk samples at an average level of 15 ng/g. Trace levels of dieldrin, heptachlor epoxide, and *cis*-nonachlor were detected in 37, 62, and 65% of total breast milk samples, at average levels were 3, 4, and 6 ng/g lipid, respectively, in primipara breast milk; 3, 3, and 4 ng/g lipid in multipara breast milk; and 3, 4, and 5 ng/g lipid in total breast milk.

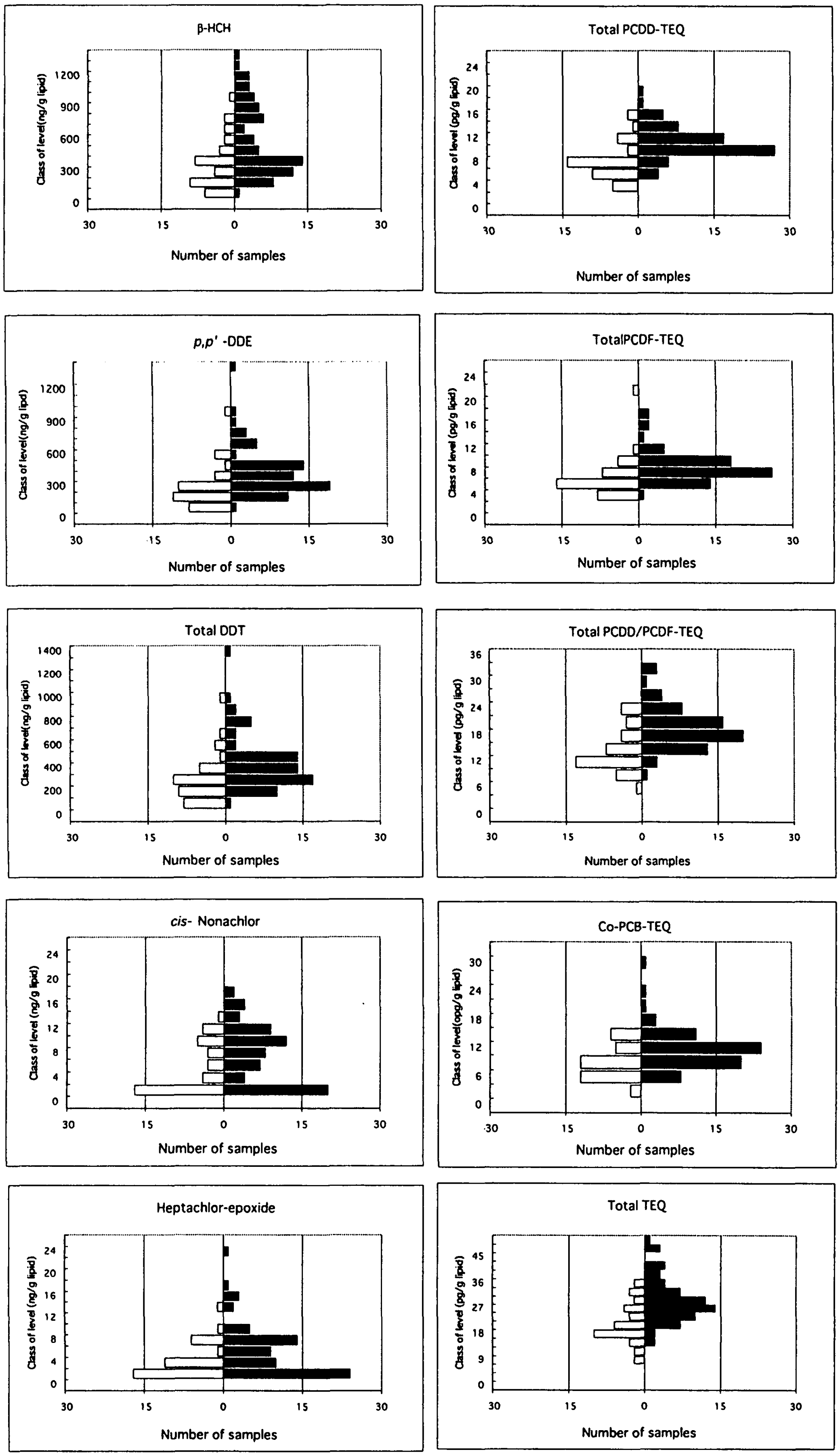

Figure 2. Distribution of 106 breast milk samples from mothers ranging in age from 25 to 34 years according to presence of various analytes. Legend: closed bar, primipara; open bar, multipara.

Table 6. Correlations among age, lipid content, analyte levels(based on lipid) in the primipara breast milk and dietary habits (fish and meat intake) of the mothers

Parameter	Age	Fat, %	β-HCH	Dieldrin	*p,p'*-DDE	*p,p'*-DDT	Total DDT	Heptachlor epoxide	Oxychlordane	*trans*-Nonachlor	*cis*-Nonachlor	Total PCDD-TEQ	Total PCDF-TEQ	Total PCDD/PCDF-TEQ	Total Co-PCB-TEQ	Total TEQ	Fish intake (frc/week)	Meat intake (frc/week)
Age	1																	
Fat (%)	0.080	1																
β-HCH	0.556[a]	0.078	1															
Dieldrin	–0.122	–0.146	0.104	1														
p,p'-DDE	0.411[a]	–0.097	0.538[a]	0.215	1													
p,p'-DDT	0.390[a]	–0.022	0.460[a]	0.260	0.480[a]	1												
Total DDT	0.423[a]	–0.102	0.555[a]	0.219	0.997[a]	0.533[a]	1											
Heptachlor epoxide	–0.056	0.000	–0.056	0.263	–0.047	0.128	–0.042											
Oxychlordane	–0.073	–0.186	0.017	0.137	–0.009	0.219	0.014	0.111	1									
trans-Nonachlor	0.168	–0.009	0.195	–0.087	0.305[a]	0.201	0.316[a]	0.309[b]	0.291[a]	1								
cis-Nonachlor	0.094	–0.094	0.203	0.056	0.318[b]	0.361[a]	0.335[b]	0.415[a]	0.147	0.246	1							
Total PCDD-TEQ	0.226[b]	–0.184	0.325[a]	0.06	0.226[b]	0.171	0.240[b]	0.045	0.181	0.068	0.087	1						
Total PCDF-TEQ	0.233[b]	–0.198	0.301[a]	–0.092	0.188	0.161	0.198	0.025	0.124	0.185	0.168	0.634[a]	1					
Total PCDD/PCDF-TEQ	0.254[b]	–0.213	0.347[a]	–0.026	0.228[a]	0.184	0.242[b]	0.040	0.170	0.141	0.143	0.905[a]	0.903[a]	1				
Total Co-PCB-TEQ	0.172	–0.107	0.314[a]	0.125	0.388[a]	0.454[a]	0.405[a]	–0.045	0.133	0.146	0.442[a]	0.481[a]	0.442[a]	0.510[a]	1			
Total TEQ	0.249[b]	–0.190	0.381[a]	0.054	0.345[a]	0.343[a]	0.362[a]	0.000	0.176	0.165	0.326[b]	0.820[a]	0.798[a]	0.895[a]	0.840[a]	1		
Fish intake (frc/week)	0.142	0.031	0.132	0.104	–0.052	-0.036	-0.049	–0.26	0.146	0.002	–0.079	0.001	0.277[b]	0.146	0.069	0.124	1	
Meat intake (frc/week)	–0.110	–0.139	–0.067	–0.061	–0.003	0.077	0.003	0.011	0.049	0.074	–0.313	0.171	0.17	0.186	–0.081	0.07	-0.052	1

[a] Significant at $p < 0.01$.
[b] Significant at $p < 0.05$.

The dioxins PCDDs, PCDFs, and CoPCBs were detected in every milk sample. Average levels of total PCDDs, total PCDFs, total PCDD + PCDF, total CoPCB, and total dioxin in primipara breast milk were 10.0, 7.8, 17.7, 9.9, and 27.5 TEQ pg/g lipid, respectively. Average levels in multipara breast milk were 7.0, 5.8, 12.8, 7.3, and 20.1 TEQ pg/g lipid, and those in total breast milk were 8.9, 7.1, 16.1, 8.9, and 25.0 TEQ pg/g lipid, respectively.

We examined the difference in the average levels of milk pollutants in 69 primipara and 37 multipara breast milk samples from mothers ranging in age from 25 to 34 years. Table 5 shows significant differences between the average levels of analyte in primipara and multipara breast milk for *p,p'*-DDE, total *p,p'*-DDT, β-HCH, total PCDD-TEQ, total PCDF-TEQ, total PCDD/PCDF-TEQ, total CoPCB-TEQ, and total TEQ at $p < 0.01$, and for heptachlor epoxide and *cis*-nonachlor at $p < 0.05$. (PCDD-TEQ, PCDF-TEQ, PCDD/PCDF-TEQ, and CoPCB-TEQ are values obtained by multiplying each concentration with each toxic equivalency factor which WHO proposed.) Figure 2 shows the distributions of primipara and multipara breast milks for each analyte.

Correlation Among Analyte Level in Primipara Breast Milk and Mother's Age and Dietary Habits

Table 6 shows significant correlation among β-HCH, *p,p'*-DDE, total DDT, total CoPCB-TEQ, and total TEQ ($p < 0.01$). β-HCH especially correlated with every dioxin TEQ. *p,p'*-DDE and total DDT correlated with every analyte except dieldrin, heptachlor epoxide, oxychlordane, and total PCDF-TEQ. Dieldrin had very little correlation with all other analytes. *trans*-Nonachlor and *cis*-nonachlor are accessory ingredients of technical chlordane (17), which was once used as a termite-exterminating agent for houses in Japan, and heptachlor epoxide is a biotransformation product of chlordane. These 3 analytes showed some significant correlations with other analytes ($p < 0.01$ or $p < 0.05$), but they correlated little with any of the dioxin TEQs except for the significant correlations found between *cis*-nonachlor and total CoPCB-TEQ and between *cis*-nonachlor and total TEQ. Another biotransformation product, oxychlordane, correlated only with *trans*-nonachlor ($p < 0.01$).

Overall, most dioxin TEQs correlated with β-HCH, *p,p'*-DDE, and total DDT ($p < 0.01$ or $p < 0.05$).

Significant correlations also were found between mother's age and the levels of β-HCH, *p,p'*-DDE, and dioxin TEQs except CoPCB-TEQ. This finding suggests that these pollutants are easily accumulated in the human body, probably through the food chain, as has already been reported by many researchers.

b-HCH and *p,p'*-DDE had been banned in Japan in the 1970s. In addition, PCDD, PCDF, and CoPCB were once produced as by-products of synthetic pesticides and PCBs and diffused into the environment (7, 8), polluting food stuff (18). However, the correlations among β-HCH, *p,p'*-DDE, and dioxin TEQs observed in this study should be specific to Japan and may not hold for other countries.

It was reported that 96.3% of human exposure to organochlorine pesticides and dioxins is due to ingested food (19). In addition, we also reported that most lipophilic-pollutant intake is due to fish and meat, especially with egg, milk, and dairy processed food (20).

We distributed a questionnaire to the milk donors when milk was collected. Donors answered questions about dietary habit, such as intake frequency per week of fish and meat. Positive correlation was found only between intake frequency of fish and total PCDF-TEQ. By using the recent dioxin data reported by the Ministry of Health and Welfare in Japan (21), the median levels of dioxins in 2 fish commodities (horse mackerel, 3; conger eel, 3; flat fish, 4; bastard halibut, 2; mackerel, 1; Japanese sea bass, 1; sea breams, 6; atka mackerel, 1) and 21 meat commodities (domestic beef, 4; imported beef, 3; domestic pork, 4; imported pork, 3; domestic chicken, 4; imported chicken, 2; unknown, 1) could be calculated. They were 0.080 and 0.009 TEQ pg/g for total PCDD-TEQ in fish and meat, respectively; 0.189 and 0.017 TEQ pg/g for total PCDF; and 0.475 and 0.021 TEQ pg/g for total CoPCB-TEQ, and 0.843 and 0.107 TEQ pg/g for total TEQ, respectively. Assuming that the amounts of fish and meat intake are almost the same, it is possible that fish intake might influence dioxin levels in breast milk more than can meat intake. For β-HCH and *p,p'*-DDE, no direct correlation with fish or meat intake was observed, suggesting that more attention should be paid not only to main dishes but also to other foods processed from fish and meat. No correlation was found between fish intake frequency and the level of CoPCB-TEQ, although amounts of CoPCB-TEQ in fish were high. Further study of the metabolism of CoPCB-TEQ in the human body might be needed.

A study of 2 less polluted milk samples indicated that one mother consumed only small amounts of meat or fish (β-HCH, ND; *p,p'*-DDE, 55.9 ng/g lipid; dioxins, 9.2 TEQ pg/g lipid), and the other never consumed meat or fish (β-HCH, 19.2 ng/g lipid; *p,p'*-DDE, 65.9 ng/g lipid; dioxins, 4.2 TEQ pg/g lipid). Both mothers were multiparas (3 and 4 children) and consumed unhulled rice periodically. Delivery frequency should be a strong factor that could reduce the maternal body burden. Dietary fibers also would inhibit assimilation of pollutants, although to a small extent, by adsorbing pollutants and shortening the transit time in the gastrointestinal tract (22).

Time Trends of Average Levels of Organochlorine Pesticides and Dioxins in Milk

According to our laboratory annual reports from 1973 to 1982 (23), the average levels in milk of β-HCH, total DDT, and dieldrin decreased drastically since around 1975 when they were banned. In this study, we found 1/18 (17.4 ng/g milk), 1/8 (14.0 ng/g milk), and 1/40 (0.2 ng/g milk) of the levels of these compounds found in 1971 (312 ng/g milk for β-HCH; 119 ng/g milk for total DDT; 7 ng/g milk for dieldrin). Dieldrin, the TDI of which is 0.1 μg/kg body weight, decreased so much that the levels found in the present study were below LOQ by GC–ECD. No data exist on time trends for dioxins in Japan. According to a recent preliminary report (24), dioxins (TEQs) in Japanese maternal milk have been decreasing gradually since 1973, as have β-HCH, total DDT, and dieldrin. Our data on total PCDD-TEQ, total PCDF-TEQ, total PCDD/PCDF-TEQ, total

CoPCB-TEQ, and total dioxin TEQ in this study were comparable with the data in that preliminary report.

Recently, Nagayama et al. (25) found negative correlations between total DDT (mostly *p,p'*-DDE) in the same milk samples and thyroxins (T3 and T4) in the blood of babies who had been nursed for about 1 year ($p < 0.001$) and between total DDT (mostly *p,p'*-DDE) and the ratio of lymphocyte subpopulations (CD4/CD8) in the blood of the babies ($p < 0.021$; 25). Therefore, further research is needed to ascertain whether these chemicals in human milk have adverse effects on infant health, by carefully comparing chemical data with biological data, such as development of sexual organs and intelligence in babies. Also, countermeasures to reduce the level of endocrine disrupters in food and environment should be taken throughout the world.

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