Balance of Intake and Excretion of 20 Congeners of Polychlorinated Dibenzo-*p*-dioxin, Polychlorinated Dibenzofuran and Coplanar Polychlorinated Biphenyl in Healthy Japanese Men

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Daily intake from meals, and excretion from feces and sebum of 20 congeners of polychlorinated dibenzo-*p*-dioxin (PCDD), polychlorinated dibenzofuran (PCDF) and coplanar polychlorinated biphenyl (Co-PCB) were analyzed in healthy Japanese men who are considered to be a standard for the Japanese. Daily intake of these congeners was 84.2 ± 12.5 pg toxic equivalent (TEQ) and daily excretion from feces and sebum were 18.3 ± 5.9 and 23. 8 ± 5.6 pg TEQ, respectively, indicating 22% the daily intake of dioxins from meals is excreted from feces and 29% from sebum. 1,2,3,7,8-Pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD), 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF) and 3,3′,4,4′,5-pentachlorobiphenyl (3,3′, 4,4′,5-PeCB) were most abundant in TEQ, in meals, feces, sebum and blood. Co-PCBs were most efficiently excreted among three congener groups in TEQ, especially from sebum. Octachlorodibenzo-*p*-dioxin (OCDD), 2,3,7,8-tetrachlorodibenzofuran (2,3,7,8-TCDF) and 3,3′,4,4′-tetrachlorobiphenyl (3,3′,4,4′-TCB) were well absorbed from intestine and excreted at more than 100% of intake, mainly from the sebum. In contrast, 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (1,2,3,4,7,8-HxCDD) and 1,2,3,7,8,9-hexachlorodibenzofuran (1,2,3,7,8,9-HxCDF) were excreted at relatively low levels from sebum, although well absorbed from the intestine, and the excretion ratios were lowest among the 20 congeners. It was suggested that 80.5–85.2% TEQ of each congener-group might be absorbed from the intestine, after taking bile excretion into consideration.

Key words —— dioxin, intake, excretion, feces, sebum, man

INTRODUCTION

It is well known that dioxin and its congeners easily accumulate in the human body and they are excreted slowly from feces, sebum and breast milk. The metabolism of these compounds has been well studied in experimental animals,[1–3] however, human data is limited. The half-life of 2,3,7,8-tetradibenzo-*p*-dioxin (2,3,7,8-TCDD) has been estimated to be 8.7 years (8.0–9.5 years) in a study of 343 Vietnam War veterans, using blood samples from 1982–1992,[4] and other studies on Germans indicated 5.8 years[5] and 7.2 years.[6] As for the half-lives of congeners, they were found to be 15.7 years for 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (1,2,3,7,8-PeCDD), 8.4 years for 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (1,2,3,4,7,8-HxCDD), 3.7 years for 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin (1,2,3,4,6,7,8-HpCDD), and 6.7 years for octachlorodibenzo-*p*-dioxin (OCDD)[5,6] and 33 years for 2,3,4,7,8-pentachlorodibenzofuran (2,3,4,7,8-PeCDF).[7]

The major source of dioxin congeners is from meals in daily life, and the Japanese daily intake has been reported to be around 2.4 pg toxic equivalent (TEQ) /kg b.w./day based on the food analyses, under the Total Diet Study by the Ministry of Health and Welfare, Japan.[8] Concerning excretion, the concentrations of congeners in milk from women[9–11] and sebum of men and women without accidental exposure,[12] and excretion into feces in infant[10,13,14] are

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available. Urinary excretion was estimated to be less than 25–35% of daily excretion in feces.[15] In rhesus monkeys, the urinary elimination was reported to be 28% of that in feces.[16]

Further, daily intake from meals and daily excretion from feces have been studied in German people who had been with and without occupational exposure, and it has been suggested that the level of daily excretion from feces exceeded that of daily intake in both cases.[7,17] However, it has been demonstrated that sebum plays an important role in excretion in addition to feces.[12,18] Thus, in this study, we clarified levels of daily intake from meals and daily excretion from feces and sebum in men, by analyzing the levels of 20 congeners of polychlorinated dibenzo-*p*-dioxin (PCDD), polychlorinated dibenzofuran (PCDF) and coplanar polychlorinated biphenyl (Co-PCB). We also analyzed blood levels of dioxins to clarify body burden.

MATERIALS AND METHODS

Chemicals —— PCDDs, PCDFs and Co-PCBs, and $^{13}C_{12}$-PCDDs, $^{13}C_{12}$-PCDFs and $^{13}C_{12}$-Co-PCBs were purchased from the Cambridge Isotope Laboratories (Massachusetts, U.S.A.). All solvents used were of dioxin-analysis grade.

Samples Subjected to Analyses of Dioxins ——

Food: Three volunteers aged 30 (A), 23 (B) and 28 (C) ate daily meals composed of the same menu for nine days. Duplicates of three meals per day were pooled on experimental days 3, 6 and 9, weighing about 1.5 kg each, and mixed in a food mixer separately. The mixture was dispensed into 10 one-liter Teflon tubes, and the mixer was washed with 50 ml of ultra-pure water and 5 ml each added to the tubes. 30 pg of $^{13}C_{12}$-TCDD/F–HpCDD/F, 60 pg of $^{13}C_{12}$-OCDD, 100 pg of $^{13}C_{12}$-TCB–HxCB were added to each tube and then subjected to dioxin extraction.

Feces: Feces was collected from the three volunteers everyday, on experimental days 3–9. Each day, the feces of each volunteer were collected in a one-liter Teflon tube weighing about 200 g, and homogenized with 1/10th the volume of ultra-pure water and kept at 4°C until use. The samples were subjected to dioxin extraction within 3 days, after addition of $^{13}C_{12}$-isomers as described above.

Blood: Fifty g of blood was collected from volunteers A and B on experimental day 4 and from volunteer C on day 5. The blood was transferred to a 250 ml-Teflon centrifuge-tube, and $^{13}C_{12}$-isomers were added as described above and subjected to dioxin extraction within the same day.

Sebum: Every morning on experimental days 3–9, the three volunteers wiped their faces with 1 g of the cotton that was washed with toluene, hexane, acetone and ethanol and then soaked in 50% ethanol/ultra-pure water. The cotton swabs containing 7 days of sebum, kept at 4°C, were pooled into a tube and then $^{13}C_{12}$-isomers were added to each tube as described above and subjected to dioxin extraction within the same day.

Extraction of Lipid/Dioxins ——

Diet: Dioxins were extracted by the method of Toyoda *et al.*[8] In brief, 10 samples in Teflon tubes, prepared as described above, had 200 ml of 2 N NaOH and 150 ml of methanol added to make a final concentration of 0.8 N, and were then shaken for 30 min and stood overnight at room temperature. To this mixture, 100 ml hexane was added and shaken gently for 10 min. The upper hexane layer was transferred to a Teflon container, and the lower layer was extracted twice with 100 ml of hexane. The pooled hexane fraction was washed three times with 1/2 volume of 2% NaCl solution. The upper hexane layer was passed through a column containing 20 g of Na_2SO_4, and to the lower layer another 100 ml of hexane was added. After gently shaking, the upper layer obtained was passed through the same Na_2SO_4 column and the pooled hexane fraction obtained was concentrated to about 2 ml by an evaporator, and subjected to a cleanup process.

Feces: Dioxins were extracted by the method of Iida *et al.*[19] In brief, the samples prepared as described above were shaken after addition of 200 ml of chloroform/methanol (CM, 2 : 1 v/v) for 30 min, and passed through a quartz filter. The residue on the filter was transferred into a Teflon container and extracted three times with 200 ml CM and 5-min sonication. Enough ultra-pure water was added to separate the chloroform layer.

Blood: A lipid fraction that contained dioxins was extracted by the $(NH_4)_2SO_4$-hexane method, from 50 g whole blood samples.[20]

Sebum: A lipid fraction was obtained according to the method by Iida *et al.*[12] In brief, to the preparation obtained as described above, 30 ml of acetone/hexane (2 : 1, v/v) was added, subjected to sonication and centrifuged at 2000 rpm for 10 min. The supernatant obtained was transferred into a centrifuge tube and this process was repeated twice more. The pooled supernatant was washed twice with 100 ml and then 50 ml of ultra-pure water. The pooled

Table 1. PCDD/F/Co-PCB Levels in Daily Meals

	pg/day				pg TEQ/day
	Sample				
	1	2	3	Mean ± S.D.	Mean ± S.D.
Congener					
2,3,7,8-TCDD	7.5	1.0	5.3	4.6 ± 3.3	4.6 ± 3.3
1,2,3,7,8-PeCDD	22.0	16.0	33.0	23.3 ± 8.6	23.3 ± 8.6
1,2,3,4,7,8-HxCDD	17.0	26.0	22.0	21.5 ± 4.1	2.2 ± 0.5
1,2,3,6,7,8-HxCDD	47.0	31.0	56.0	44.9 ± 12.9	4.5 ± 1.3
1,2,3,7,8,9-HxCDD	21.0	19.0	17.0	19.1 ± 1.8	1.9 ± 0.2
1,2,3,4,6,7,8-HpCDD	279.0	351.0	272.0	300.6 ± 44.1	3.0 ± 0.4
OCDD	1664.0	1896.0	1907.0	1822.5 ± 137.0	0.2 ± 0.0
2,3,7,8-TCDF	6.0	10.4	9.2	8.5 ± 2.3	0.9 ± 0.2
1,2,3,7,8-PeCDF	16.0	14.0	14.0	14.5 ± 1.1	0.7 ± 0.1
2,3,4,7,8-PeCDF	36.0	29.0	34.0	33.1 ± 3.3	16.6 ± 1.6
1,2,3,4,7,8-HxCDF	25.0	36.0	34.0	31.8 ± 5.8	3.2 ± 0.6
1,2,3,6,7,8-HxCDF	32.0	35.0	40.0	35.6 ± 4.0	3.6 ± 0.4
2,3,4,6,7,8-HxCDF	60.0	45.0	53.0	52.6 ± 7.8	5.3 ± 0.8
1,2,3,7,8,9-HxCDF	13.0	21.0	19.0	17.6 ± 4.5	1.8 ± 0.5
1,2,3,4,6,7,8-HpCDF	84.0	115.0	96.0	98.3 ± 15.6	1.0 ± 0.2
1,2,3,4,7,8,9-HpCDF	14.0	17.0	26.0	19.1 ± 6.4	0.2 ± 0.1
OCDF	76.0	65.0	72.0	70.7 ± 5.5	0.0 ± 0.0
3,3′,4,4′-TCB	185.0	171.0	183.0	179.8 ± 7.7	0.0 ± 0.0
3,3′4,4′,5-PeCB	110.0	108.0	99.0	105.3 ± 5.8	10.5 ± 0.6
3,3′4,4′5,5′-HxCB	64.0	56.0	70.0	63.5 ± 6.7	0.6 ± 0.1
Congener-Group					
PCDDs	2057.5	2340.0	2312.3	2236.6 ± 155.7	40.0 ± 11.2
PCDFs	362.0	387.4	397.2	382.2 ± 18.2	33.1 ± 1.67
Co-PCBs	359.0	335.0	352.0	348.7 ± 12.3	11.2 ± 0.5
Total	2778.5	3062.4	3061.5	2967.5 ± 164	84.2 ± 12.5

water fraction obtained was washed with hexane and the hexane fraction was washed with ultra-pure water once. Hexane fractions were pooled and dried by passing through a column containing 10 g Na_2SO_4, concentrated to about 1 ml by a rotary evaporator, and then spontaneously dried by standing at room temperature. The lipid was then weighed.

Cleanup —— Cleanup was achieved by passing through a multi-layer column composed of Na_2SO_4, 10% (w/w) $AgNO_3$-silica gel, silica gel, 22% (w/w) H_2SO_4-silica gel, silica gel, 44% (w/w) H_2SO_4-silica gel, silica gel, 2% (w/w) KOH-silica gel and silica gel. After elution with 150 ml of *n*-hexane the specimen was evaporated to a small volume.[21] The concentrate was applied to an active carbon-impregnated silica gel column,[22] washed with 200 ml of 25% (v/v) dichloromethane/*n*-hexane, and then eluted with 200 ml of toluene. The elute obtained was evaporated at room temperature almost to dryness. Five μl of *n*-nonane containing $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD spiking substances was added to this vessel.[23] Samples dissolved in hexane were subjected to GC/MS analysis.

GC/MS Chromatography —— PCDDs, PCDFs and Co-PCBs were analyzed by GC-MS, according to the method by US. Environmental Protection Agency (EPA).[24] The analytical conditions were as follows: gas chromatography was performed with an HP 6890 series unit (Hewlett-Packard, Palo Alto, California, U.S.A.) equipped with a Finnigan MAT-95S (Finnigan MAT GmbH, Bremen, Germany). The column used was a DB5MS fused silica capillary column, 0.25 mm i.d. × 60 m, with 0.25 μm film thickness (J&W Scientific, Folsom, California,

Table 2. Daily Excretion of PCDD / F / Co-PCB from Feces*

	pg/day				pg TEQ/day
	A (N=7)	B (N=7)	C (N=6)		
	Mean ± S.D.	Median ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Congener					
2,3,7,8-TCDD	1.3 ± 0.7	0.9 ± 0.5	0.9 ± 0.2	1.0 ± 0.5	1.0 ± 0.5
1,2,3,7,8-PeCDD	6.9 ± 2.4	6.7 ± 6.2	5.1 ± 1.4	6.3 ± 3.9	6.3 ± 3.9
1,2,3,4,7,8-HxCDD	1.7 ± 0.5	1.1 ± 0.6	1.5 ± 0.4	1.4 ± 0.6	0.2 ± 0.1
1,2,3,6,7,8-HxCDD	15.7 ± 5.5	10.2 ± 2.6	13.6 ± 3.3	13.0 ± 4.5	1.3 ± 0.5
1,2,3,7,8,9-HxCDD	3.0 ± 1.1	1.9 ± 0.8	2.5 ± 0.8	2.5 ± 1.0	0.3 ± 0.1
1,2,3,4,6,7,8-HpCDD	32.2 ± 8.9	25.8 ± 12.9	28.5 ± 10.9	28.8 ± 10.8	0.3 ± 0.1
OCDD	143.1 ± 33.1	119.7 ± 45.3	136.4 ± 36.3	131.2 ± 38.0	0.0 ± 0.0
2,3,7,8-TCDF	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.1 ± 0.0
1,2,3,7,8-PeCDF	0.6 ± 0.3	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.0 ± 0.0
2,3,4,7,8-PeCDF	9.6 ± 3.5	6.9 ± 2.4	7.9 ± 1.8	8.1 ± 2.8	4.1 ± 1.4
1,2,3,4,7,8-HxCDF	5.8 ± 1.8	4.6 ± 0.8	4.3 ± 1.0	5.0 ± 1.4	0.5 ± 0.1
1,2,3,6,7,8-HxCDF	8.3 ± 3.1	6.2 ± 0.9	6.4 ± 1.4	6.9 ± 2.2	0.7 ± 0.2
2,3,4,6,7,8-HxCDF	8.3 ± 1.7	7.1 ± 0.8	7.3 ± 0.9	7.6 ± 1.3	0.8 ± 0.1
1,2,3,7,8,9-HxCDF	0.5 ± 0.2	0.4 ± 0.3	0.4 ± 0.3	0.4 ± 0.2	0.0 ± 0.0
1,2,3,4,6,7,8-HpCDF	33.5 ± 37.5	19.0 ± 1.8	18.7 ± 2.2	23.8 ± 22.3	0.2 ± 0.2
1,2,3,4,7,8,9-HpCDF	1.4 ± 0.4	0.9 ± 0.6	1.5 ± 0.6	1.2 ± 0.6	0.0 ± 0.0
OCDF	6.4 ± 2.5	5.5 ± 1.0	6.1 ± 0.9	6.0 ± 1.6	0.0 ± 0.0
3,3′,4,4′-TCB	16.8 ± 5.3	13.1 ± 3.6	12.9 ± 2.5	14.5 ± 4.2	0.0 ± 0.0
3,3′4,4′,5-PeCB	27.1 ± 5.6	21.2 ± 2.8	23.4 ± 4.3	24.0 ± 4.9	2.4 ± 0.5
3,3′4,4′5,5′-HxCB	20.0 ± 4.9	15.9 ± 2.4	17.0 ± 3.0	17.7 ± 3.9	0.2 ± 0.0
Conger-Group					
PCDDs	204.0 ± 47.1	166.3 ± 58.9	191.2 ± 50.2	184.3 ± 52.2	9.3 ± 4.4
PCDFs	75.1 ± 37.7	51.8 ± 4.1	51.0 ± 6.2	60.1 ± 24.3	6.4 ± 1.9
Co-PCBs	63.9 ± 12.4	50.2 ± 7.8	53.0 ± 9.5	56.2 ± 11.3	2.6 ± 0.5
Total	343.1 ± 83.3	268.3 ± 61.8	308.8 ± 54.3	300.6 ± 72.6	18.3 ± 5.9

*Three subjects, A, B and C took the same meals for nine days. Values are mean ± S.D. of collected feces during the experimental period from day three to nine.

U.S.A.). The column temperature was maintained at 100°C for 1 min, heated to 220°C at a rate of 17°C/min, heated to 310°C at a rate of 3°C/min, and maintained at 250°C for 15 min. The injection temperature was 260°C, ion source temperature was maintained at 250°C and the carrier gas (helium) flow pressure was 12 ml/min. The ionizing current, ionizing energy and accelerating voltage were 1 mA, 60 eV and 5 kV, respectively. The resolution of the mass spectrometer as maintained at about 10000 throughout the work, and the analysis was carried out according to a selected ion monitoring (SIM) using 50 selected ions. A standard curve for quantitation of each chemical was obtained using authentic congeners.

RESULTS

Levels of Dioxins in Meal

Table 1 shows the levels of dioxins in one-day diet. Variation among the meals over three days was small, except for 2,3,7,8-TCDD, and the average intake of congener-groups of dioxins, PCDDs, PCDFs, and Co-PCBs were 2236.6 ± 155.7, 382.2 ± 18.2 and 348.7 ± 12.3 pg/day, respectively, and 40.0 ± 11.2, 33.1 ± 1.6 and 11.2 ± 0.5 pg TEQ/day, respectively. The total TEQ was 84 pg TEQ/day, cor-

Table 3. Sebum Levels of PCDD/F/Co-PCB

	pg/g lipid				pg TEQ/g lipid			
	Subjects				Subjects			
	A	B	C	Mean ± S.D.	A	B	C	Mean ± S.D.
Congener								
2,3,7,8-TCDD	1.5	1.3	1.2	1.3 ± 0.1	1.5	1.3	1.2	1.3 ± 0.1
1,2,3,7,8-PeCDD	4.2	4.8	3.5	4.2 ± 0.7	4.2	4.8	3.5	4.2 ± 0.7
1,2,3,4,7,8-HxCDD	1.3	1.3	1.5	1.4 ± 0.1	0.1	0.1	0.2	0.1 ± 0.0
1,2,3,6,7,8-HxCDD	14.1	7.1	9.5	10.2 ± 3.5	1.4	0.7	1	1.0 ± 0.4
1,2,3,7,8,9-HxCDD	3.7	1.6	2.1	2.4 ± 1.1	0.4	0.2	0.2	0.2 ± 0.1
1,2,3,4,6,7,8-HpCDD	206.6	127.9	171.1	168.5 ± 39.4	2.1	1.3	1.7	1.7 ± 0.4
OCDD	4206.9	2567.9	3541.7	3438.8 ± 824	0.4	0.3	0.4	0.3 ± 0.1
2,3,7,8-TCDF	15.9	7.2	7.7	10.2 ± 4.9	1.6	0.7	0.8	1.0 ± 0.5
1,2,3,7,8-PeCDF	4.6	1.8	2.7	3.0 ± 1.4	0.2	0.1	0.1	0.2 ± 0.1
2,3,4,7,8-PeCDF	15.8	8.9	10.3	11.7 ± 3.7	7.9	4.5	5.2	5.8 ± 1.8
1,2,3,4,7,8-HxCDF	7.2	4.0	5.0	5.4 ± 1.6	0.7	0.4	0.5	0.5 ± 0.2
1,2,3,6,7,8-HxCDF	9.8	5.1	5.2	6.7 ± 2.7	1	0.5	0.5	0.7 ± 0.3
2,3,4,6,7,8-HxCDF	15.5	7.8	10.6	11.3 ± 3.9	1.6	0.8	1.1	1.1 ± 0.4
1,2,3,7,8,9-HxCDF	4.4	1.9	1.0	2.5 ± 1.8	0.4	0.2	0.1	0.2 ± 0.2
1,2,3,4,6,7,8-HpCDF	26.6	13.3	17.0	19.0 ± 6.9	0.3	0.1	0.2	0.2 ± 0.1
1,2,3,4,7,8,9-HpCDF	4.7	2.5	1.2	2.8 ± 1.8	0.1	0.0	0.0	0.0 ± 0.0
OCDF	16.4	10.7	16.2	14.4 ± 3.2	0.0	0.0	0.0	0.0 ± 0.0
3,3′,4,4′-TCB	593.7	296.7	332.1	407.5 ± 162.2	0.1	0	0	0.0 ± 0.0
3,3′4,4′,5-PeCB	61.3	39.7	45.1	48.7 ± 11.2	6.1	4	4.5	4.9 ± 1.1
3,3′4,4′5,5′-HxCB	19.2	13.0	16.5	16.3 ± 3.1	0.2	0.1	0.2	0.2 ± 0.0
Congener-Group								
PCDDs	4438	2712	3731	3627 ± 867.9	10	8	8.0	8.9 ± 1.1
PCDFs	120.9	63.3	76.9	87.0 ± 30.1	14	7.3	8.4	9.8 ± 3.4
Co-PCBs	674.2	349.5	393.8	472.5 ± 176.1	6.4	4.1	4.7	5.1 ± 1.2
Total	5233.5	3124.6	4201.3	4186.4 ± 1054.5	30	20	21	23.8 ± 5.6
Extracted lipid (g)	0.21	0.19	0.17	0.19 ± 0.02				

*Three subjects took the same meals for nine days. Daily lipid excretion is estimated to be 1 g. Values are means of wiped out sebum during experimental days three to nine.

responding to 1.2 pg TEQ/kg b.w./day.

In terms of pg per day, OCDD was the most abundant dioxin in meals, and the amounts (averages of the same chloride-number congeners) increased with an increase of the number of chlorides among PCDDs. A similar tendency was also observed with PCDFs, although it was opposite for Co-PCBs. In terms of TEQ, 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF and 3,3′,4,4′,5-pentachlorobiphenyl (3,3′,4,4′,5-PeCB) were the most abundant congeners, being 23.7, 16.6 and 10.5 pg TEQ/day, respectively. TEQ values of PCDDs, PCDFs and Co-PCBs were about 1/60, 1/10 and 1/30 of their weights, indicating that PCDD congeners of low toxic equivalency factors (TEFs) were abundant in diet.

Excretion from Feces

Table 2 shows the levels of dioxins in feces obtained from the three volunteers on experimental days three to nine. Daily and individual variations were relatively small and no significant differences were detected among them. Thus, data were evaluated from the average values of 20 samples, seven for A, seven for B and six for C.

Total daily excretion of dioxins from feces was 300.6 ± 72.6 pg, corresponding to 18.3 ± 5.9 pg TEQ.

Table 4. Blood Levels of PCDD/F/Co-PCB

	pg/g lipid						pg TEQ/g lipid					
	Subjects						Subjects					
	A	B	C	Mean	±	S.D.	A	B	C	Mean	±	S.D.
Congener												
2,3,7,8-TCDD	1.5	1.1	0.9	1.2	±	0.3	1.5	1.1	0.9	1.2	±	0.3
1,2,3,7,8-PeCDD	4.5	3.5	5.4	4.5	±	1.0	4.5	3.5	5.4	4.5	±	1.0
1,2,3,4,7,8-HxCDD	1.7	1.2	0.9	1.2	±	0.4	0.2	0.1	0.1	0.1	±	0.0
1,2,3,6,7,8-HxCDD	18.3	13.1	11.5	14.3	±	3.5	1.8	1.3	1.2	1.4	±	0.4
1,2,3,7,8,9-HxCDD	2.4	1.5	1.5	1.8	±	0.6	0.2	0.2	0.2	0.2	±	0.1
1,2,3,4,6,7,8-HpCDD	25.0	9.6	14.1	16.2	±	7.9	0.3	0.1	0.1	0.2	±	0.1
OCDD	240.2	82.9	83.7	135.6	±	90.6	0.0	0.0	0.0	0.0	±	0.0
2,3,7,8-TCDF	1.0	0.5	1.2	0.9	±	0.4	0.1	0.1	0.1	0.1	±	0.0
1,2,3,7,8-PeCDF	0.5	0.3	0.0	0.3	±	0.2	0.0	0.0	0.0	0.0	±	0.0
2,3,4,7,8-PeCDF	9.7	8.4	6.2	8.1	±	1.8	4.8	4.2	3.1	4.1	±	0.9
1,2,3,4,7,8-HxCDF	3.6	3.8	3.2	3.5	±	0.3	0.4	0.4	0.3	0.4	±	0.0
1,2,3,6,7,8-HxCDF	5.2	4.2	3.4	4.3	±	0.9	0.5	0.4	0.3	0.4	±	0.1
2,3,4,6,7,8-HxCDF	4.1	3.2	3.8	3.7	±	0.4	0.4	0.3	0.4	0.4	±	0.0
1,2,3,7,8,9-HxCDF	0.0	0.3	0.0	0.1	±	0.2	0.0	0.0	0.0	0.0	±	0.0
1,2,3,4,6,7,8-HpCDF	3.1	3.6	8.7	5.1	±	3.1	0.0	0.0	0.1	0.1	±	0.0
1,2,3,4,7,8,9-HpCDF	0.0	0.0	0.0		ND		0.0	0.0	0.0		ND	
OCDF	1.6	0.8	0.0	0.8	±	0.8	0.0	0.0	0.0	0.0	±	0.0
3,3′,4,4′-TCB	6.6	3.8	6.6	5.7	±	1.6	0.0	0.0	0.0	0.0	±	0.0
3,3′4,4′,5-PeCB	62.1	18.3	55.6	45.3	±	23.6	6.2	1.8	5.6	4.5	±	2.4
3,3′4,4′5,5′-HxCB	35.3	24.6	33.8	31.3	±	5.8	0.4	0.3	0.3	0.3	±	0.1
Congener-Group												
PCDDs	293.6	113.0	118.0	174.8	±	102.8	8.5	6.3	7.9	7.6	±	1.1
PCDFs	28.7	25.2	26.5	26.8	±	1.7	6.3	5.5	4.4	5.4	±	1.0
Co-PCBs	104.0	46.8	96.0	82.3	±	31.0	6.6	2.1	5.9	4.9	±	2.4
Total	426.2	185.0	240.5	283.9	±	126.3	21.4	13.9	18.1	17.8	±	3.8
Extracted lipid (%)	0.34	0.42	0.32	0.36	±	0.05						

Three subjects, A, B and C took the same meals for nine days. Blood were obtained from three subjects on the experimental day four or five.

OCDD was the most abundant in weight, followed by 1,2,3,4,6,7,8-HpCDD, 3,3′,4,4′,5-PeCB, and 1,2,3,4,6,7,8-HpCDF, accounting for 69% of the total dioxins in feces.

PCDDs contributed about 50% in TEQ, followed by PCDFs and Co-PCBs, and the congener that had the highest TEQ value was 1,2,3,7,8-PeCDD, followed by 2,3,4,7,8-PeCDF and 3,3′,4,4′,5-PeCB, the contribution of these three congeners being 70% in TEQ of the total in feces.

Excretion from Sebum

Table 3 shows the dioxin levels excreted from sebum for the three volunteers. It is estimated that the sebum lipid-excretion of a man is 1 g per day.[12] Thus, the value of pg/g lipid in Table 3 is equal to that excreted daily. Excretion of dioxins was 4186.4 ± 1054.5 pg/day, corresponding to 23.8 ± 5.6 pg TEQ/day. The most abundant congener was OCDD contributing more than 80% of the total in weight, and the most abundant conger in TEQ was 2,3,4,7,8-PeCDF, followed by 3,3′4,4′,5-PeCB and 1,2,3,7,8-PeCDD, accounting for 62% of the total TEQ. The most abundant congener-group was PCDFs in TEQ.

Blood Levels

Table 4 shows the blood levels of the three volunteers. The most abundant congener was OCDD

Table 5. Daily Excretion from Feces and Sebum in Ratio to the Intake from Meals

	%		%
	Feces	Sebum	Feces plus sebum
	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
Congener			
2,3,7,8-TCDD	22.1 ± 12	28.9 ± 2.9	50.8 ± 8.8
1,2,3,7,8-PeCDD	27.0 ± 17	17.9 ± 2.9	44.6 ± 6.8
1,2,3,4,7,8-HxCDD	6.7 ± 2.6	6.4 ± 0.6	13.1 ± 1.6
1,2,3,6,7,8-HxCDD	29.0 ± 10	22.8 ± 7.9	51.8 ± 13.9
1,2,3,7,8,9-HxCDD	13.0 ± 5.1	12.7 ± 5.7	25.7 ± 8.5
1,2,3,4,6,7,8-HpCDD	9.6 ± 3.6	56.1 ± 13	65.6 ± 14.2
OCDD	7.2 ± 2.1	189 ± 45	195.9 ± 45.9
2,3,7,8-TCDF	6.4 ± 2.4	120 ± 57	126.6 ± 58.3
1,2,3,7,8-PeCDF	4.0 ± 1.6	20.9 ± 9.8	24.9 ± 10.2
2,3,4,7,8-PeCDF	24.5 ± 8.4	35.3 ± 11.0	59.7 ± 15.0
1,2,3,4,7,8-HxCDF	15.8 ± 4.3	17.1 ± 5.1	32.8 ± 7.4
1,2,3,6,7,8-HxCDF	19.4 ± 6.3	18.9 ± 7.5	38.1 ± 11.0
2,3,4,6,7,8-HxCDF	14.4 ± 2.4	21.4 ± 7.4	35.8 ± 8.6
1,2,3,7,8,9-HxCDF	2.4 ± 1.4	14.0 ± 10	16.4 ± 10.3
1,2,3,4,6,7,8-HpCDF	24.2 ± 23	19.3 ± 7.0	43.2 ± 15.6
1,2,3,4,7,8,9-HpCDF	6.3 ± 2.9	14.5 ± 9.4	20.9 ± 10.1
OCDF	8.4 ± 2.3	20.4 ± 4.6	28.8 ± 5.1
3,3′,4,4′-TCB	8.1 ± 2.3	227 ± 90	234.7 ± 91.4
3,3′4,4′,5-PeCB	22.8 ± 4.6	46.3 ± 11	69.0 ± 13.4
3,3′4,4′5,5′-HxCB	27.9 ± 6.1	25.6 ± 4.9	53.5 ± 8.1
Congener-Group*			
PCDDs	25.1 ± 16	23.7 ± 7.4	49.1 ± 19.2
PCDFs	19.3 ± 5.7	29.7 ± 10	49.3 ± 12.3
Co-PCBs	23.0 ± 4.5	45.3 ± 9.2	68.3 ± 10.8
Total*	22.1 ± 8.0	28.5 ± 6.6	51.0 ± 11.2

*TEQ

in weight (pg/g lipid), and in TEQ, 3,3′,4,4′,5-PeCB, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF were the highest with almost equal levels, and accounted for 73% of the total TEQ in blood lipid.

Congener Patterns in Sebum, Feces, Diet and Blood

Balance of intake and excretion: Table 5 shows excretion ratios of each dioxin congener from feces and sebum relative to the dietary intake. Those of congener-groups, PCDDs, PCDFs and Co-PCBs and total were expressed in terms of TEQ. The daily excretion of each congener was in the range of 2.4–29% from feces and 6.4–227% from sebum as a percentage of the daily intake. Total excretion from feces and sebum was 51% of the intake in TEQ, and respective excretion values of each congener-group were in the range of 49.1–68.3% of their intake, with the highest excretion ratio being for Co-PCBs.

It is worthy to note that excretion of OCDD, 2,3,7,8-TCDF and 3,3′,4,4′-TCB from sebum exceeded the dietary intake. The sum of excretion from feces and sebum for these congeners reached 196%, 127% and 235% of the dietary intake, respectively. All these compounds were well absorbed from intestine, and excreted efficiently from sebum. 1,2,3,4,7,8-HxCDD and 1,2,3,7,8,9-HxCDF were also well absorbed from the intestine, but their excretions from sebum were relatively low, and the sum of excretion from feces and sebum was less than 20% of the intake.

Comparison of Congener-Patterns among

Table 6. Comparison of Congener Patterns among Meal, Blood, Sebum and Feces

	Mean (pg/g lipid)				Ratio			
	Meal	Blood	Feces	Sebun	Feces/Meal	Sebum/ Meal	Feces/blood	Sebun/Blood
Congener								
2,3,7,8-TCDD	4.6	1.2	1.0	1.3	0.22	0.29	0.86	1.13
1,2,3,7,8-PeCDD	23.3	4.5	6.3	4.2	0.27	0.18	1.40	0.93
1,2,3,4,7,8-HxCDD	21.5	1.2	1.4	1.4	0.07	0.06	1.16	1.11
1,2,3,6,7,8-HxCDD	44.9	14.3	13.0	10.2	0.29	0.23	0.91	0.71
1,2,3,7,8,9-HxCDD	19.1	1.8	2.5	2.4	0.13	0.13	1.38	1.35
1,2,3,4,6,7,8-HpCDD	300.6	16.2	28.8	168.5	0.10	0.56	1.78	10.39
OCDD	1822.5	135.6	131.2	3438.8	0.07	1.89	0.97	25.36
2,3,7,8-TCDF	8.5	0.9	0.5	10.2	0.06	1.20	0.62	11.61
1,2,3,7,8-PeCDF	14.5	0.3	0.6	3.0	0.04	0.21	2.17	11.34
2,3,4,7,8-PeCDF	33.1	8.1	8.1	11.7	0.24	0.35	1.00	1.44
1,2,3,4,7,8-HxCDF	31.8	3.5	5.0	5.4	0.16	0.17	1.41	1.54
1,2,3,6,7,8-HxCDF	35.6	4.3	6.9	6.7	0.19	0.19	1.61	1.56
2,3,4,6,7,8-HxCDF	52.6	3.7	7.6	11.3	0.14	0.21	2.05	3.05
1,2,3,7,8,9-HxCDF	17.6	0.1	0.4	2.5	0.02	0.14	3.85	22.63
1,2,3,4,6,7,8-HpCDF	98.3	5.1	23.8	19.0	0.24	0.19	4.63	3.70
1,2,3,4,7,8,9-HpCDF	19.1	ND	1.2	2.8	0.06	0.15	—	—
OCDF	70.7	0.8	6.0	14.4	0.08	0.20	7.46	18.03
3,3′,4,4′-TCB	179.8	5.7	14.5	407.5	0.08	2.27	2.56	71.86
3,3′4,4′,5-PeCB	105.3	45.3	24.0	48.7	0.23	0.46	0.53	1.07
3,3′4,4′5,5′-HxCB	63.5	31.3	17.7	16.3	0.28	0.26	0.57	0.52
Congener-Group*								
PCDDs	40.0	7.57	9.3	8.92	0.23	0.22	1.23	1.18
PCDFs	33.1	5.37	6.4	9.83	0.19	0.30	1.19	1.83
Co-PCBs	11.2	4.85	2.6	5.08	0.23	0.45	0.53	1.05
Total*	84.2	17.78	18.3	23.82	0.22	0.28	1.03	1.34

*TEQ

Meals, Blood, Feces and Sebum: Table 6 shows the ratio of each congener in sebum and feces to those in meals and blood. The ratios of the feces levels to the intake were between 0.02–0.29, and to the blood were between 0.46 and 7.46-fold. In contrast, the ratio of sebum levels to the meals and blood were between 0.06–2.27 and 0.52–71.86, respectively. Thus, it is considered that very specific types of congeners are selectively excreted from sebum irrespective of body burden.

DISCUSSION

It has been assumed that the major pathway of dioxin intake is through meals, although some may be from air by respiration. Under normal conditions, the contribution of respiratory pathway would be small, being less than 10%.[23] In this study, it was clarified that about 50% of total TEQ of dioxins ingested through meals is excreted from feces and sebum, at levels of 22% and 29%, respectively. Remaining 50% TEQ of daily intake would be accumulated if they are not metabolized. However, metabolisms of dioxins have been suggested. In experimental animals, the presence of various dioxin metabolites, such as hydroxylation derivatives at unsubstituted positions or, at a substituted position, and ring-opened derivatives, with their β-glucuronide/sulfate diconjugate, sulfate conjugate monoglucuronide ether and glucuronide methyl ester were demonstrated in urine.[25]

It was also found that 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF and 3,3′,4,4′,5-PeCB are most

abundant in TEQ, in meals, feces, sebum and blood.

Schrey *et al*.[17] and Rohde *et al*.[7] reported that fecal excretion of most dioxins examined exceeded the daily intakes from meals. This might be due to the current high body burden with decreased concentrations in current diet. However, in our study, fecal levels were less than meal levels and all congeners in feces were in the range of 0.02–0.29 those in meals (Table 6). In contrast, three of 20 congeners examined were excreted from sebum at more than 100 % of daily intake (Table 5). These were OCDD, 2,3,7,8-TCDF and 3,3′,4,4′-TCB, and there is a possibility that these congeners are formed *in vivo*. The other possibility is exposure from other pathways than meals. The excretion rations of other congeners were in a range of 6–56 % of the intake.

We also examined congener levels in blood as a marker of body burden. It was demonstrated that sebum congener-pattern did not correlate well with blood congener-pattern. However, it has been reported that there is a good correlation between blood levels and sebum levels among Yusho patients for each congener, although correlation coefficients differed from each other.[12] Thus, each congener might have a different metabolism in the human body.

We previously found that dioxins are secreted into bile (Kitamura *et al*., in preparation), and TEQs of three congener-groups were relatively well correlated with those in blood. Using the correlation coefficients for each group, we estimated average bile TEQ levels of these three volunteers (Table 7). The data leads to the deduction that 14.8–19.5% of each congener-group present in meals and bile is excreted from feces. In other words, meals ingested will be mixed with the bile and 80.5–85.2% TEQ of each congener-group might be absorbed from the intestine. Analysis of metabolites is needed for clarification of the metabolisms of dioxin congeners in the human body. Further, development of prevention of absorption from the intestine of dioxins secreted into bile would be useful to reduce the body burden resulting from accidental high exposure.

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Table 7. Fecal Excretion of Congener Groups Present in Meal and Bile

	Daily dose in the intestine		Excretion
	%TEQ		%TEQ
	Bile	Meal	Feces
PCDDs	16.0	84.0	19.5
PCDFs	17.4	82.6	15.9
Co-PCBs	35.7	64.3	14.8
Total	15.6	84.4	18.3

Bile levels were calculated using the correlation coefficients between blood and bile levels (Kitamura *et al*., in preparation).

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