ANATOMICAL VARIATION LEVELS OF DIOXIN CONGENERS IN HUMAN SEBUM

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Introduction

Humans are exposed to toxic dioxin congeners daily, mainly through diet. Their half-lives in the human body are generally long and they accumulate in fat tissue. Dioxin congeners are mainly excreted from feces and sebum. However, information about excretion characteristics of each congener is limited. Clarification of excretion characteristics is important, especially for prevention of adverse effects of dioxins to which humans are exposed accidentally. We previously analyzed levels of dioxin congeners in sebum from the face, mainly from the forehead and nose, and estimated the amount of daily excretion of dioxin from sebum[1)], based on the reports that daily excretion of sebum from the whole body is 1 g [2, 3)], and with an assumption that dioxin congener compositions in sebum from all parts of the whole body skin are the same. In the present study, we collected sebum separately, from 11 skin parts of the whole body of an adult male volunteer, and determined amounts of dioxin congeners. HRGC-HRMS was employed to determine the dioxin congener amounts.

Material and method

Chemicals

Authentic Co-PCBs, PCDDs and PCDFs, and $^{13}C_{12}$-Co-PCBs and $^{13}C_{12}$-PCDDs and $^{13}C_{12}$-PCDFs were purchased from Wellington Laboratories (Ontario, Canada). All solvents and Na_2SO_4 anhydrous were of dioxin-analysis grade (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Active-carbon-dispersed silica gel was of dioxin-analysis grade (Kanto Chemical Co., Inc., Tokyo, Japan). Distilled water was washed with hexane before use.

Sebum sample

The volunteer was a 31-year-old man. Sampling of sebum was made for 10 days. During the experimental period, the volunteer took the same meals every day. Before the start of the experiment, all the hair on his entire body, except for the eyebrows, was shaved, and then his body was thoroughly washed. He wore longunderwear connected with socks, a shirt connected with gloves, and a mask covering the whole of his head, made of cotton. The body skin area of the volunteer was divided into 11 parts, as shown in Fig.1; the top and back of the head, forehead, nose, ears and nuchal region, the area of eyebrows to jaw including cheeks, front of the torso, side of the torso, back of the torso, arms and legs. The skin surfaces of the 11 parts were wiped separately once a day for 10 days, with an appropriate sized towel (5 x 5, 10 x 10, 10x 18, 20 x 20 or 15 x 75 cm) made of cotton, wet with acetone. After 10 days, the clothing and mask were separated into 11 parts, towels and clothing were pooled for each skin part, and subjected to lipid extraction. Permission for analyzing dioxins was obtained from a volunteer.

Extraction of lipid fraction

Eleven pools of clothing (longunderwear, shirt and mask) and towels were spiked with internal standards, $^{13}C_{12}$-PCDDs, $^{13}C_{12}$-PCDFs and $^{13}C_{12}$-Co-PCBs, and then a Soxhlet extraction was performed with acetone for eight hrs and then with toluene for 16 hrs. The extracts were evaporated to dryness and dissolved in 5 ml n-hexane, then washed with water, dried over anhydrous sodium sulfate, evaporated to dryness, and the lipid residues obtained were weighed. The lipid was dissolved in 40 ml of n-hexane, and 10 ml of conc H_2SO_4 was added to remove most of the lipid. The n-hexane layer was washed with water, and evaporated to about 1 ml and loaded on an AS cartridge which was connected to a 0.5g active carbon-impregnated silica gel column (5 mm i.d.). The used AS cartridge included 10 % $AgNO_3$ (1 g)/silica gel (0.2 g)/44 % H_2SO_4 silica gel (3 g)/silica gel (0.2 g) (Supelco, Inc, Bellfonte, PA). The AS cartridge connected with the active carbon column was eluted with 50 ml of n-hexane at a flow rate of 3 ml/min. After disconnection of the AS cartridge, mono-*ortho*-Co-PCBs were eluted first with 20 ml of 25 % CH_2Cl_2 in hexane, and then PCDDs, PCDFs and non-*ortho*-Co-PCBs with 100 ml of toluene. The CH_2Cl_2-hexane eluate was evaporated to 2 ml in a rotary evaporator, and blown off to almost empty in a vessel with a stream of N_2, and 500 ul of toluene containing $^{13}C_{12}$-3,3',4,5'-TCB spiking substance was added to this vessel. The toluene eluate was evaporated to 2 ml in a rotary evaporator, blown off to almost empty in a vessel with a stream of N_2, and 10 ul of toluene containing $^{13}C_{12}$-1,2,3,4-TCDD, $^{13}C_{12}$-1,2,3,4,6,8,9-HpCDF and $^{13}C_{12}$-3,3',4,5'-TCB spiking substances was added to this vessel.

GC-MS analysis

PCDDs, PCDFs and Co-PCBs were analyzed by GC-MS, according to the method used by the EPA methods 1613, U.S. (Environmental Protection Agency, USA, Office of Water, 1994). The analytical conditions were as follows: gas chromatography was performed with an HP 6890 series unit (Hewlett-Packard, Palo Alto, CA) equipped with high resolution mass spectrometer (JMS-700, JEOL Ltd., Tokyo, Japan). The column used was an SP-Sil 8CB MS fused silica capillary column, 0.25 mm i.d.×30 m, with 0.25 um film thickness (Varian Association, Harbor City, CA) for mono-*ortho*, non-*ortho*-Co-PCB and 7-8Cl-PCDDs/Fs analysis, and an SP-2331 fused silica capillary column, 0.25 mm i.d.×30 m, with 0.2 um film thickness (Supelco, Inc, Bellfonte, PA) for 4-6Cl-PCDDs/Fs analysis. The SP-Sil 8CB MS fused silica capillary column temperature for mono-*ortho*- and non-*ortho*-Co-PCB analysis was maintained at 120 °C for 1 min, heated to 200 °C at a rate of 30 °C/min, to 270 °C at a rate of 5 °C/min, and maintained at 270 °C for 1 min. The SP-2331 fused silica capillary column temperature for 4-6Cl-PCDDs/Fs analysis was maintained at 140 °C for 1.5 min, heated to 180 °C at a rate of 20 °C/min, to 255 °C at a rate of 2 °C/min, to 270 °C at a rate of 2 °C/min, and maintained at 270 °C for 12 min. The SP-Sil 8CB MS fused silica capillary column temperature for 7-8Cl-PCDDs/Fs analysis was maintained at 120 °C for 1 min, heated to 200 °C at a rate of 30 °C/min, to 270 °C at a rate of 5 °C/min, and maintained at 270 °C for 12 min. The injection temperature was 270 °C, interface temperature was 270 °C, ion source temperature was maintained at 270 °C and the carrier gas (helium) flow rate was 1 ml/min. The ionizing current, ionizing energy and accelerating voltage were 700 uA, 42 eV and 10 kV, respectively. The resolution of the mass spectrometer was maintained at about 10,000 throughout the experiment, and the analysis was carried out according to selected ion monitoring (SIM) using 16, 12, and 40 selected ions for mono-*ortho*-Co-PCBs, non-*ortho*-Co-PCBs and PCDDs/Fs, respectively. M+ and M+2 were used for TCDD, TCDF, TCD and PeCDD, and M+2 and M+4 for other congeners. Deviations of ion intensity ratio values of samples were less than 15% of the theoretical values. A standard curve for quantification of each chemical was obtained using authentic congeners. Recoveries of all congeners were more than 60 %. Vehicle controls were lower than the detection limits for all congeners except for 2,3',4,4',5-PeCB examined in this study.

Results and Discussion

Daily excretion of sebum from the whole body was 0.783 g per day in an average over 10 days. The highest site of sebum excretion was the top and back of the head, followed by the forehead, ears and nuchal region. About 60 % of sebum was excreted from the whole head. Total-TEQ excreted from sebum of the whole body was 27 pg per day with the top and back of the head exhibiting the highest excretion rate. These were the same body parts that also excreted the most sebum. Total-TEQ excreted from the whole head was about 63 % of the whole body, coinciding well with the relative amount of sebum of the whole body. The total-TEQ of each of the 11 parts was between 1.8 and 3.9 pg TEQ /day, a more than two-fold difference between minimum and maximum. On the other hand, total-TEQ/g lipid was highest at the nose, being 45 pg TEQ/g lipid, followed by the ears and nuchal region sebum and the top and the back of the head, being 43 and 38 pg TEQ/g lipid, respectively. The total-TEQ/g lipid of 11 parts were between 27 and 45 pg, a difference of 1.7-fold the maximum, with an average of the whole body being 34 pg. Especially, congener composition of the forehead, the area from the eyebrows to jaw including cheeks or the whole head resembled that of the whole body.

We demonstrated that total-TEQ per g sebum of the whole body could be estimated with sebum sampled from the forehead, area from the eyebrows to jaw including cheeks or the whole head. This study clarified that about 60 % of dioxin congeners in terms of total-TEQ of that excreted from entire body is excreted from whole head.

References

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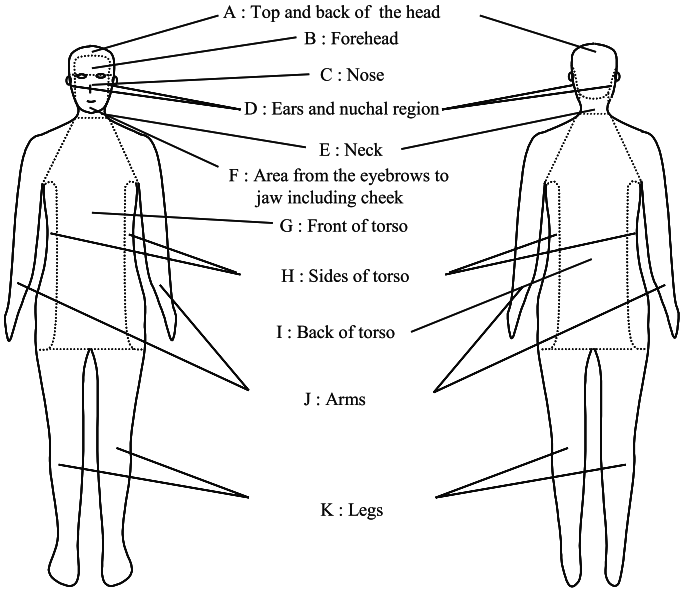

Fig. 1. The separate areas used to sample sebum from the entire human body

Table 1. Amount of sampled lipid by the body part area to sample of the sebum of the human whole body

Body part area	lipid (g/day)
A Top and back of the head	0.102
B Forehead	0.0995
C Nose	0.0412
D Ears and nuchal region	0.0869
E Neck	0.0703
F Area from the eyebrows to jaw including cheek	0.0633
G Front of torso	0.0713
H Sides of torso	0.0611
I Back of torso	0.0752
J Arms	0.0536
K Legs	0.0585
Head (A-F)	0.463
Body (G-I)	0.208
Limbs (J, K)	0.112
Whole Body	0.783

Table 2. Daily sebum excretion of PCDDs, PCDFs and Co-PCBs by the body part area

pg TEQ/day	A	B	C	D	E	F	G	H	I	J	K	Head (A-F)	Body (G-I)	Limbs (J, K)	Whole Body
PCDDs	1.4	1.3	0.77	1.3	0.82	0.86	0.79	0.83	0.75	0.95	0.93	6.4	2.4	1.9	11
PCDFs	0.92	0.85	0.51	0.89	0.50	0.50	0.49	0.49	0.51	0.55	0.54	4.2	1.5	1.1	6.8
Non *ortho*-Co-PCBs	0.98	0.98	0.36	0.95	0.36	0.56	0.54	0.36	0.54	0.27	0.27	4.2	1.4	0.54	6.2
Mono *ortho*-Co-PCBs	0.59	0.60	0.21	0.59	0.21	0.28	0.28	0.20	0.28	0.11	0.10	2.5	0.77	0.21	3.5
Total	3.9	3.7	1.8	3.7	1.9	2.2	2.1	1.9	2.1	1.9	1.8	17	6.1	3.7	27

A : Top and back of the head B : Forehead C : Nose D : Ears and nuchal region E : Neck F : Area from the eyebrows to jaw including cheek
G : Front of torso H : Sides of torso I : Back of torso J : Arms K : Legs

Table 3. Levels of PCDDs, PCDFs and Co-PCBs in sebum by the body part area

pg TEQ/g lipid	A	B	C	D	E	F	G	H	I	J	K	Head (A-F)	Body (G-I)	Limbs (J, K)	Whole Body
PCDDs	13	13	19	15	12	14	11	14	10	18	16	14	12	17	14
PCDFs	9.1	8.5	12	10	7.1	8.0	6.9	8.0	6.8	10	9.3	9.2	7.2	9.8	8.8
Non *ortho*-Co-PCBs	9.6	9.8	8.7	11	5.2	8.9	7.6	6.0	7.2	5.1	4.6	8.9	6.9	4.9	7.6
Mono *ortho*-Co-PCBs	5.8	6.0	5.0	6.8	3.0	4.5	4.0	3.3	3.7	2.0	1.8	5.2	3.7	1.9	4.2
Total	38	37	45	43	27	35	30	31	28	35	32	37	29	33	34

A : Top and back of the head B : Forehead C : Nose D : Ears and nuchal region E : Neck F : Area from the eyebrows to jaw including cheek
G : Front of torso H : Sides of torso I : Back of torso J : Arms K : Legs