表 5 トータルダイエット試料からの有機フッ素化合物(PFOA/PFOS)一日摂取量

食品群	全国平均					
	ND=0			ND=LOD/2		
	PFOA	PFOS	PFOA+PFOS	PFOA	PFOS	PFOA+PFOS
1群(米、米加工品)	0	0	0	120.5	120.5	241.0
2群(米以外の穀類、種実類、いも類	0	0	0	65.5	65.5	131.0
3群(砂糖類、菓子類)	0	0	0	13.6	13.6	27.2
4群(油脂類)	0	0	0	5.4	5.4	10.8
5群(豆・豆加工品)	0	0	0	20.1	20.1	40.2
6群(果実、果汁)	0	0	0	30.8	30.8	61.6
7群(緑黄色野菜)	0	0	0	22.2	22.2	44.5
8群(他の野菜類、キノコ類、海草類)	0	0	0	47.8	47.8	95.5
9群(酒類、嗜好飲料)	0	0	0	144.6	144.6	289.3
10群(魚介類)	0	47.8	47.8	19.9	47.8	67.7
11群(肉類・卵類)	0	0	0	25.6	25.6	51.2
12群(乳・乳製品)	0	0	0	35.1	35.1	70.2
13群(調味料)	0	0	0	23.4	23.4	46.9
14群(飲料水)	2.9	1.3	4.2	2.9	1.3	4.2
総摂取量, ng/day	2.9	49.1	52.0	577.4	603.6	1181.0
総摂取量, ng/kg bw/day	0.06	0.98	1.04	11.5	12.1	23.6

単位:ng

表 6 個別食品中の有機フッ素化合物(PFOA/PFOS)濃度

試料		対象化合物	検出頻度	検出濃度(ng/g)		
			(陽性/全試料)	中央値	最大値	最小値
肝臓を原料に含む食品	蟹みそ	PFOA	2/4	0.2	0.8	0
		PFOS	4/4	1.7	2.2	0.8
	イカ塩辛	PFOA	0/4	0	0	0
		PFOS	0/4	0	0	0
	牛肝臓	PFOA	1/4	0	0.2	0
		PFOS	2/4	0.1	0.9	0
	鶏肝臓	PFOA	1/4	0	0.3	0
		PFOS	2/4	0.15	0.3	0
	健康食品(鮫肝油)	PFOA	0/5	0	0	0
		PFOS	0/5	0	0	0
魚介	マグロ	PFOA	0/5	0	0	0
		PFOS	2/5	0	2.1	0
	ブリ	PFOA	0/5	0	0	0
		PFOS	3/5	0.6	1.7	0
	サケ	PFOA	0/5	0	0	0
		PFOS	0/5	0	0	0
	イカ	PFOA	0/5	0	0	0
		PFOS	0/5	0	0	0
	エビ	PFOA	0/5	0	0	0
		PFOS	0/5	0	0	0
	カニ	PFOA	0/5	0	0	0
		PFOS	2/5	0	0.6	0
ファーストフード	ハンバーガー	PFOA	0/5	0	0	0
		PFOS	0/5	0	0	0
	ポテト	PFOA	0/3	0	0	0
		PFOS	0/3	0	0	0
	ピザ(グラタン)	PFOA	0/3	0	0	0
		PFOS	0/3	0	0	0
	クラムチャウダー	PFOA	0/1	0	0	0
		PFOS	0/1	0	0	0
	チキンナゲット	PFOA	0/1	0	0	0
		PFOS	0/1	0	0	0
菓子	ポップコーン	PFOA	0/3	0	0	0
		PFOS	0/3	0	0	0

表 7　市販魚中のベンゾトリアゾール類濃度(ng/g)

	(DBHP)BT	(DAHP)BT	(DBHP)CBT	(BMHP)CBT
マサバ	<0.08	0.4	0.5	<0.1
マダイ	<0.08	0.08	0.1	0.2
サケ	<0.1	0.1	<0.2	0.3
ブリ	<0.1	0.4	0.6	0.5
クロマグロ	<0.1	0.9	0.4	<0.2

表 8　魚介類個別試料における臭素系ダイオキシン類, PBDEs, PBBs 及び Co-PXBs の分析結果

魚種（産地）	脂肪含量(%)	臭素化ダイオキシン類* (pgTEQ/g ww)	ΣPBDEs (ng/g ww)	ΣPBBs (pg/g ww)	ΣCo-PXBs (pg/g ww)
タイ-1（名古屋）	0.48	ND	0.100	0.230	ND
タイ-2（名古屋）	2.77	ND	0.247	0.813	ND
タイ-3（鹿児島）	0.19	ND	0.016	0.105	ND
タイ-4（瀬戸内）	0.42	ND	0.018	ND	ND
タイ-5（大分）	0.44	ND	0.116	1.47	ND
タイ-6（青森）	0.79	ND	0.136	0.368	ND
アナゴ-1（名古屋）	11.8	0.009	0.818	2.24	ND
アナゴ-2（瀬戸内）	9.88	0.015	0.406	1.834	ND
アナゴ-3（福島）	12.2	0.0018	0.263	2.57	ND
カレイ-1（瀬戸内）	1.10	ND	0.044	ND	ND
カレイ-2（福島）	4.53	ND	0.180	ND	ND
エビ（鹿児島）	0.12	ND	0.033	ND	ND
アジ（鹿児島）	4.88	ND	0.334	1.432	ND
キス（瀬戸内）	0.60	ND	0.095	0.299	ND
サバ（鹿児島）	12.2	ND	0.617	1.975	ND
イワシ（鹿児島）	1.73	ND	0.167	0.827	ND

*暫定的に塩素化ダイオキシンの毒性等価係数(TEF, 1998)を用いて算出した。

表 9　3 地域における臭素系ダイオキシン類，PBDEs,PBBs 及び Co-PXBs の 1 日摂取量総括表

(1) 関東地区

異性体		1 群	2 群	3 群	4 群	5 群	6 群	7 群	8 群	9 群	10 群	11 群	12 群	13 群	合計	1 日摂取量*
	一日食事量(g)	332.8	175.4	32.1	11.0	59.6	125.4	100.3	209.1	540.8	84.8	111.3	137.7	94.5	2015	
臭素系ダイオキシン類	ND=0	0	0	0	0.073	0	0	0	0	0	0	0	0	0	0.073	**0.00145** pgTEQ/kg/日
pgTEQ/日	ND=1/2LOD	13.3	7.9	1.1	0.4	2.1	4.4	3.3	7.3	18.8	2.8	3.4	4.8	3.3	73.0	**1.46** pgTEQ/kg/日
Total PBDEs	ND=0	5.35	3.11	0.399	19.0	1.50	5.76	0.872	20.7	31.5	43.4	19.4	3.24	6.30	161	**3.21** ng/kg/日
ng/日	ND=1/2LOD	5.75	3.39	0.431	19.0	1.54	5.88	0.997	20.8	32.0	43.5	19.5	3.33	6.39	162	**3.25** ng/kg/日
Total PBBs	ND=0	0	0	0	0.013	0	0	0	0	0	0.327	0.038	0	0	0.378	**0.00755** ng/kg/日
ng/日	ND=1/2LOD	0.479	0.286	0.040	0.023	0.075	0.157	0.119	0.263	0.676	0.404	0.155	0.172	0.118	2.97	**0.0593** ng/kg/日
Total Co-PXBs	ND=0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0** ng/kg/日
ng/日	ND=1/2LOD	0.057	0.034	0.005	0.002	0.009	0.019	0.014	0.032	0.081	0.012	0.015	0.021	0.014	0.315	**0.00629** ng/kg/日

(2) 関西地区

異性体		1 群	2 群	3 群	4 群	5 群	6 群	7 群	8 群	9 群	10 群	11 群	12 群	13 群	合計	1 日摂取量*
	一日食事量(g)	341.4	174.2	35.1	10.6	57.5	120.8	92.8	184.1	616.3	82.2	121.4	142.9	92.9	2072	
臭素系ダイオキシン類	ND=0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0** pgTEQ/kg/日
pgTEQ/日	ND=1/2LOD	20.2	10.3	2.5	0.4	3.5	4.2	2.9	6.0	21.4	2.7	3.7	5.0	3.2	86.1	**1.72** pgTEQ/kg/日
Total PBDEs	ND=0	11.9	0.796	2.14	11.8	4.27	4.14	2.31	1.87	3.01	64.5	8.91	11.9	9.35	137	**2.74** ng/kg/日
ng/日	ND=1/2LOD	12.7	1.24	2.20	11.8	4.33	4.32	2.35	2.10	3.99	64.5	8.95	12.0	9.44	140	**2.80** ng/kg/日
Total PBBs	ND=0	0.000	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.000	0.141	0.020	0.000	0.000	0.169	**0.00337** ng/kg/日
ng/日	ND=1/2LOD	0.726	0.370	0.091	0.018	0.126	0.151	0.103	0.215	0.770	0.220	0.148	0.179	0.116	3.23	**0.0647** ng/kg/日
Total Co-PXBs	ND=0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0** ng/kg/日
ng/日	ND=1/2LOD	0.087	0.044	0.011	0.002	0.015	0.018	0.012	0.026	0.092	0.012	0.016	0.021	0.014	0.371	**0.00742** ng/kg/日

(3) 九州地区

異性体		1 群	2 群	3 群	4 群	5 群	6 群	7 群	8 群	9 群	10 群	11 群	12 群	13 群	合計	1 日摂取量*
	一日食事量(g)	357.1	162.9	33.1	10.4	59.9	106.2	90.5	196.0	581.6	81.5	114.7	144.5	86.2	2025	
臭素系ダイオキシン類	ND=0	0	0	0	0.087	0	0	0	0	0	0.105	0	0	0	0.192	**0.00384** pgTEQ/kg/日
pgTEQ/日	ND=1/2LOD	14.7	7.0	1.2	0.4	2.2	4.1	3.7	8.2	20.2	3.3	4.7	5.0	3.0	77.8	**1.56** pgTEQ/kg/日
Total PBDEs	ND=0	8.87	3.98	2.27	13.6	3.47	1.99	0.203	9.06	1.96	92.9	8.89	1.91	7.93	157	**3.14** ng/kg/日
ng/日	ND=1/2LOD	9.48	4.15	2.29	13.6	3.52	2.10	0.375	9.38	2.89	92.9	8.97	2.08	7.99	160	**3.19** ng/kg/日
Total PBBs	ND=0	0	0	0	0.006	0	0	0	0	0	0.309	0.010	0	0	0.324	**0.00648** ng/kg/日
ng/日	ND=1/2LOD	0.590	0.251	0.044	0.016	0.079	0.148	0.134	0.295	0.727	0.400	0.173	0.181	0.108	3.084	**0.0617** ng/kg/日
Total Co-PXBs	ND=0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0** ng/kg/日
ng/日	ND=1/2LOD	0.064	0.030	0.005	0.002	0.009	0.018	0.016	0.035	0.087	0.014	0.020	0.022	0.013	0.335	**0.00670** ng/kg/日

*　体重 50kg とした場合

表 10 国内 3 地域の魚介類における HBCDs の分析結果

購入場所	No.	魚名	天然・養殖の別	脂肪含量 (%)	α-HBCD ng/g ww	β-HBCD ng/g ww	γ-HBCD ng/g ww	ΣHBCD ng/g ww	ΣHBCD ng/g lw	ΣPBDE* ng/g ww	ΣPBDE* ng/g lw
九州	1	マアジ	天然	5.67	0.17	ND	ND	0.17	3.0	0.28	4.9
	2	アナゴ	天然	7.52	0.09	ND	ND	0.09	1.1	0.11	1.5
	3	アラカブ	天然	0.37	ND	ND	ND	0.00	0.00	0.05	12
	4	ヤリイカ	天然	0.38	ND	ND	ND	0.00	0.00	0.17	44
	5	コバイワシ	天然	0.74	0.05	ND	ND	0.05	6.7	0.13	18
	6	エビ足赤	天然	0.19	ND	ND	ND	0.00	0.00	0.01	2.8
	7	カマス	天然	9.88	0.73	ND	0.27	1.01	10	0.70	7.1
	8	キビナゴ	天然	1.82	0.11	ND	0.02	0.13	7.2	0.09	5.0
	9	クツゾコ	天然	1.42	0.04	ND	ND	0.04	2.6	0.08	5.3
	10	マサバ	天然	20.4	0.17	ND	0.18	0.35	1.7	0.30	1.5
	11	タイ	天然	1.01	ND	ND	ND	0.00	0.00	0.05	5.0
	12	タチウオ	天然	0.33	0.13	ND	ND	0.13	40	0.11	33
	13	ツケアミ	加工食品	1.02	ND	ND	ND	0.00	0.00	0.05	4.9
	14	ヒラメ	天然	0.30	ND	ND	ND	0.00	0.00	0.04	13
	15	モチ魚	天然	3.93	ND	ND	ND	0.00	0.00	0.06	1.7
	中央値			1.02	0.04	0.00	0.00	0.04	1.1	0.09	5.0
	最大値			20.4	0.73	0.00	0.27	1.0	40	0.70	44
	最小値			0.19	0.00	0.00	0.00	0.00	0.00	0.01	1.5
中国・四国	1	地アジ	天然	2.28	0.25	ND	ND	0.25	11	0.12	5.1
	2	アナゴ	天然	12.7	5.8	ND	3.2	9.0	71	0.31	2.5
	3	エビ	天然	0.49	ND	ND	ND	0.00	0.00	0.01	1.9
	4	カキ	養殖	2.26	0.14	ND	0.02	0.16	7.0	0.05	2.2
	5	カレイ	天然	0.35	ND	ND	ND	0.00	0.00	0.03	9.3
	6	ゲタ	天然	0.35	0.04	0.01	0.01	0.06	18	0.02	4.8
	7	サゴシ	天然	1.91	0.16	ND	0.33	0.49	26	0.41	21
	8	サヨリ	天然	0.92	0.22	ND	0.05	0.27	29	0.11	12
	9	マダイ	天然	1.10	ND	ND	ND	0.00	0.00	0.05	4.5
	10	マダイ	養殖	7.11	0.22	ND	ND	0.22	3.1	0.30	4.3
	11	イイダコ	天然	0.26	ND	ND	ND	0.00	0.00	0.02	6.2
	12	ハモ	天然	3.40	0.05	ND	ND	0.05	1.5	0.31	9.2
	13	マグロ	天然	0.51	ND	ND	ND	0.00	0.00	0.04	8.6
	14	ママカリ	天然	4.53	18	2.4	57	77	1700	0.53	12
	15	メバル	天然	0.50	0.05	ND	0.03	0.08	16	0.12	24
	中央値			1.10	0.05	0.00	0.00	0.08	7.0	0.11	6.2
	最大値			12.7	18	2.4	57	77	1700	0.53	24
	最小値			0.26	0.00	0.00	0.00	0.00	0.00	0.01	1.9
中部	1	アジ	天然	4.72	3.8	ND	1.1	4.9	100	0.66	14
	2	スルメイカ	天然	1.19	0.07	ND	0.08	0.15	12	0.06	5.5
	3	カマス	天然	4.50	3.8	ND	1.4	5.3	120	0.53	12
	4	キス	天然	0.46	0.23	ND	0.09	0.32	70	0.03	5.8
	5	サゴシ	天然	1.30	0.74	ND	1.8	2.5	190	0.41	32
	6	サバ	天然	13.7	14	ND	7.1	21	150	1.8	13
	7	サワラ	天然	11.3	5.8	0.24	18	24	210	2.9	26
	8	スズキ	天然	0.72	8.0	0.35	15	23	3300	0.33	46
	9	セイゴ	天然	0.98	1.9	ND	0.98	2.9	300	0.18	18
	10	タイ	養殖	8.12	0.31	0.02	ND	0.33	4	0.68	8.4
	11	タイ	養殖	9.36	0.71	ND	ND	0.71	8	0.42	4.5
	12	タイ	養殖	4.10	0.26	0.01	0.08	0.35	9	0.19	4.6
	13	タコ	天然	0.35	0.49	0.09	0.62	1.2	350	0.02	4.8
	14	ブリ	養殖	17.3	0.33	ND	ND	0.33	2	2.8	16
	15	ボラ	天然	1.69	0.28	ND	0.13	0.41	24	0.25	15
	中央値			4.10	0.71	0.09	0.62	1.2	100	0.41	13
	最大値			17.3	14	0.35	18	24	3300	2.9	46
	最小値			0.35	0.07	0.00	0.00	0.15	1.9	0.02	4.5

*：平成16年度報告書より
統計量(中央値、最小値、合計) はND＝0として計算

表 11 魚介類個別試料における HBCDs 及び TBBPA の分析結果

購入場所	魚介名	脂肪含量 (%)	α-HBCD (ng/g)	β-HBCD (ng/g)	γ-HBCD (ng/g)	Total HBCDs (ng/g)	TBBPA (ng/g)
東北	スズキ1	2.4	3.25	0.08	4.37	7.69	0.04
	スズキ2	3.4	2.31	0.02	1.86	4.19	0.04
	スズキ3	2.5	2.04	0.02	1.62	3.68	0.04
	スズキ4	1.4	1.40	0.02	1.17	2.59	0.08
中部	タイ①	0.48	0.21	ND	0.03	0.24	ND
	タイ②	2.8	5.28	0.04	2.21	7.54	0.31
	アナゴ	12	17.7	0.40	18.8	36.9	0.09
中国・四国	カレイ	1.1	ND	ND	ND	ND	0.05
	アナゴ	9.9	1.36	0.04	0.70	2.09	0.12
	タイ	0.6	0.05	ND	0.03	0.08	0.10
	キス	0.42	0.23	ND	0.05	0.28	0.03
九州	アジ	4.9	0.10	ND	0.02	0.12	0.05
	サバ	12	2.86	ND	0.95	3.80	ND
	イワシ	1.7	0.08	ND	0.02	0.10	ND
	エビ	0.12	ND	ND	ND	ND	0.04
	タイ	0.19	ND	ND	ND	ND	0.03

ND:α-HBCD, ＜0.02ng/g; β-HBCD,＜0.01ng/g; γ-HBCD, ＜0.02ng/g

表 12　3 地域における HBCDs 及び TBBPA の 1 日摂取量

(1) 関東地区

異性体		1 群	2 群	3 群	4 群	5 群	6 群	7 群	8 群	9 群	10 群	11 群	12 群	13 群	合計	体重 50kg と仮定した場合
	一日食事量(g)	332.8	175.4	32.1	11.0	59.6	125.4	100.3	209.1	540.8	84.8	111.3	137.7	94.5	2015	
HBCDs	ND=0	0	0	0	0	0	0	0	0	0	118.3	0	0	0	118.3	2.4 ng/kg/日
pgTEQ/day	ND=1/2LOD	9.6	5.7	0.9	0.3	1.5	3.1	2.4	5.3	13.5	118.5	2.5	3.4	2.4	169.0	3.4 ng/kg/日
TBBPA	ND=0	0	2.88	0	0	0.98	0	1.77	6.44	14.4	2.06	2.36	0.57	0	31.5	0.6 ng/kg/日
ng/day	ND=1/2LOD	1.91	2.88	0.18	0.06	0.98	0.63	1.77	6.44	14.4	2.06	2.36	0.92	0.47	35.1	0.7 ng/kg/日

(2) 関西地区

異性体		1 群	2 群	3 群	4 群	5 群	6 群	7 群	8 群	9 群	10 群	11 群	12 群	13 群	合計	体重 50kg と仮定した場合
	一日食事量(g)	341.4	174.2	35.1	10.6	57.5	120.8	92.8	184.1	616.3	82.2	121.4	142.9	92.9	2072	
HBCDs	ND=0	0	0	0	0	0	0	0	0	0	90.1	0	0	0	90.1	1.8 ng/kg/日
pgTEQ/day	ND=1/2LOD	14.5	7.4	1.8	0.3	2.5	3.0	2.1	4.3	15.4	90.3	2.7	3.6	2.3	150.2	3.0 ng/kg/日
TBBPA	ND=0	19.5	2.3	1.3	0	4.2	1.8	8.1	17.5	59.1	7.3	6.8	6.8	5.2	139.9	2.8 ng/kg/日
ng/day	ND=1/2LOD	19.5	2.3	1.3	0.1	4.2	1.8	8.1	17.5	59.1	6.2	8.3	8.3	5.3	142.1	2.8 ng/kg/日

(3) 九州地区

異性体		1 群	2 群	3 群	4 群	5 群	6 群	7 群	8 群	9 群	10 群	11 群	12 群	13 群	合計	体重 50kg と仮定した場合
	一日食事量(g)	357.1	162.9	33.1	10.4	59.9	106.2	90.5	196.0	581.6	81.5	114.7	144.5	86.2	2025	
HBCDs	ND=0	0	0	0	0	0	0	0	0	0	155.1	2.0	0	0	157.1	3.1 ng/kg/日
pgTEQ/day	ND=1/2LOD	10.6	5.0	0.9	0.3	1.6	3.0	2.7	5.9	14.5	155.3	4.6	3.6	2.2	210.1	4.2 ng/kg/日
TBBPA	ND=0	0	3.7	0	0.1	1.0	0	0	0	0	0.5	3.3	0	0	8.5	0.2 ng/kg/日
ng/day	ND=1/2LOD	2.1	3.7	0.2	0.1	1.0	0.6	0.5	1.2	2.9	0.7	4.7	0.7	0.4	18.8	0.4 ng/kg/日

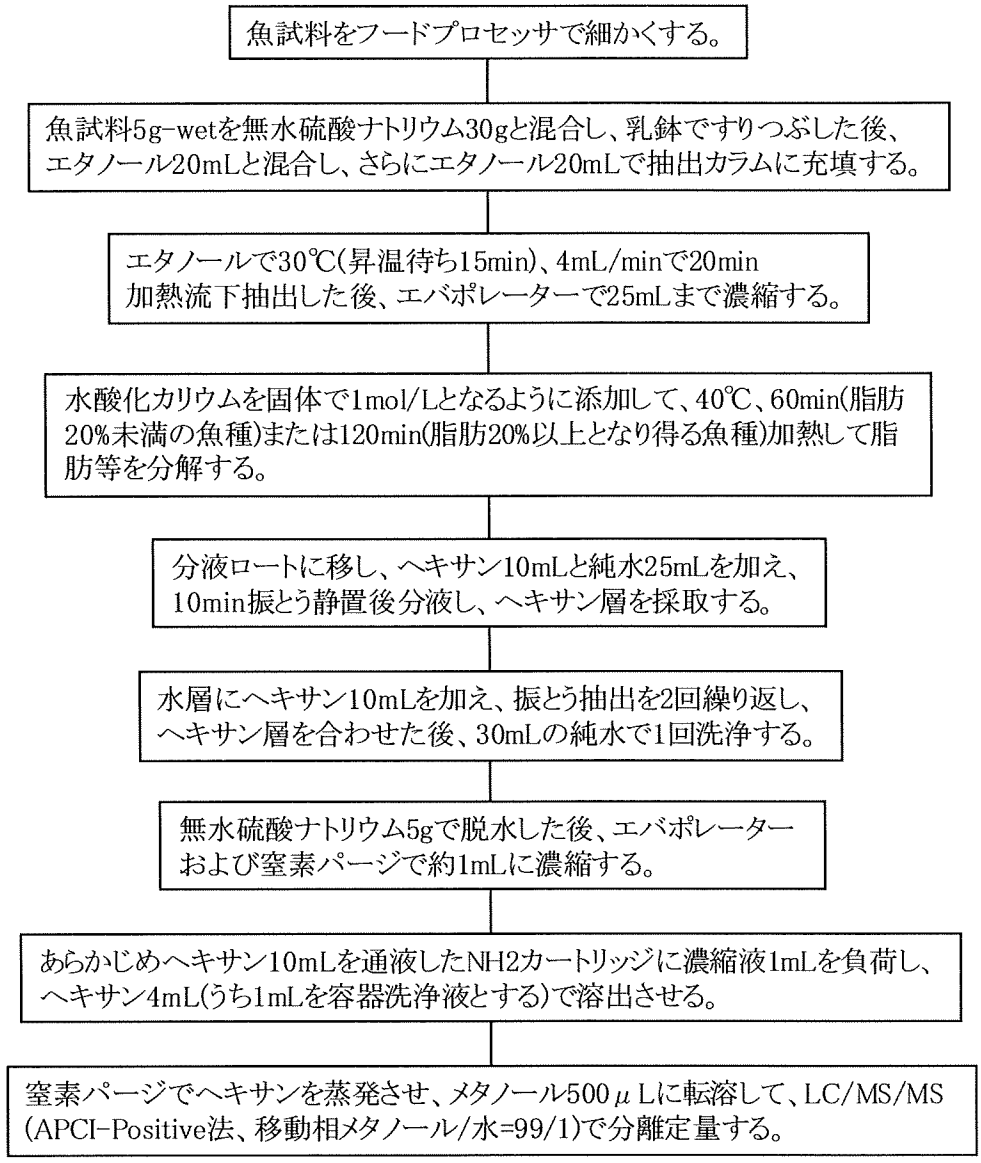

図1　魚中ベンゾトリアゾール類の分析手順

研究成果の刊行に関する一覧表

及び

研究成果の刊行物・別刷

研究成果の刊行に関する一覧表

雑誌

	発表者氏名	論文タイトル	発表誌名	巻号	ページ	出版年
1	Hori T, Yasutake D, Tobiishi K, Ashizuka Y, Kajiwara J, Nakagawa R, Iida T, Tsutsumi T, Sasaki K	Comparison of accelerated solvent extraction and alkaline digestion-hexane shaking extraction for determination of dioxins in animal-origin food sample	Organohalogen Compounds	69	1118-1121	2007
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3	Tsutsumi T, Amakura Y, Tanno K, Yanagi T, Kono Y, Sasaki K, Maitani T	Dioxins and other organohalogen compounds in fish oil supplements on the Japanese market	Organohalogen Compounds	69	2371-2374	2007
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その他(通知)

	通知名	通知番号	発出年
1	食品中のダイオキシン類の測定方法暫定ガイドライン	食安監発第0228003	2008

COMPARISON OF ACCELERATED SOLVENT EXTRACTION AND ALKALINE DIGESTION-HEXANE SHAKING EXTRACTION FOR DETERMINATION OF DIOXINS IN ANIMAL-ORIGIN FOOD SAMPLE

Hori T[1], Yasutake D[1], Tobiishi K[1], Ashizuka Y[1], Kajiwara J[1], Nakagawa R[1], Iida T[2], Tsutsumi T[3], Sasaki K[3]
[1]Fukuoka Institute of Health and Environmental Sciences, 39 Mukaizano, Dazaifu-shi, Fukuoka 818-0135, Japan; [2]Kitakyushu Life Science Center, 1-4 Nakabarushinmachi, Kitakyushu-shi, Fukuoka 804-0003, Japan; [3]National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

Abstract
We studied the progressive analytical method for dioxins in animal-origin food samples such as fish, meat and dairy products. This study aimed to establish a highly sensitive and rapid analytical method using HRGC/HRMS equipped with a solvent cut large volume (SCLV) injection system and accelerated solvent extraction (ASE). When ASE was applied to extract fat from dried milk powder, high fat amounts were obtained in the case where the temperature was set to 150 °C and acetone/n-hexane (1:1, v/v) was used as the extraction solvent. A high extraction efficiency in these conditions was also found in quantitative results for 29 kinds of dioxin congeners on the identical sample. Using these conditions, a freeze-dried *tuna* homogenate was extracted by ASE and we performed a standard alkaline digestion followed by a n-hexane shaking extraction on the identical sample. The concentrations of each dioxin congener were very similar in both extraction methods. Our analysis of 20 g of various animal-origin food items according to the present method, including the ASE and SCLV injection technique, showed recovery rates for labeled congeners within the range recommended by the Japanese analytical guideline of dioxins in food (40%-120%).

Introduction
We previously developed a highly sensitive method for determining dioxin content in food using a solvent cut large volume (SCLV) injection system coupled to a cyanopropyl phase capillary column[1]. The SCLV injection system coupled to a 40m-length Rtx-2330 column showed sufficient separation of 2,3,7,8-chlorine-substituted isomers and had at least five-times higher sensitivity than the conventional injection technique[2]. In the conventional method, a large volume of sample (generally 100g) must be treated collectively in order to attain the desirable limit of detection (LODs) at low ppt levels, namely, 0.01pg/g for 2,3,7,8-tetraCDD/F. The SCLV injection technique method allows the reduction of a sample volume from 100g to 20g when such usual LODs are demanded and is expected to improve the efficiency of laboratory performance, especially when it is coupled to an automated extraction method such as accelerated solvent extraction (ASE). In order to examine the applicability of ASE for the determination of dioxins in food samples, it is important to verify the extraction efficiency of this method against that of the conventional technique.
We reported the applicability of an ASE for the determination of dioxins in plant food samples and compared the method's performance with that of the standard conventional shaking extraction (separatory funnel extraction) regarding recovery rates and quantitative determination[3]. The results showed that ASE could extract dioxins at high efficiency using a low-volume solvent and could provide a high level of performance for various plant matrices, especially regarding those, such as seaweed powder, from which dioxins are difficult to extract using conventional shaking extraction.
In the present study, the applicability of the combined SCLV injection and ASE methodology is evaluated for use regarding animal-origin fatty food samples. It is considered that homogeneous tissue, such as dried milk powder, is suitable for the method's quantitative validation.

Materials and Methods
Dried milk powder on the market was used for the examination of extraction conditions. For the comparison of quantitative determinations, about 300 g of the edible parts of *tuna* were purchased at a market in Japan. They were homogenized using a food processor, freeze dried and homogenized again. For the examination of the recovery rate, extracts were prepared from homogenates of animal-origin food samples (cow's milk, cheese, yogurt, and so on). The recovery rates for 17 kinds of ^{13}C-labeled 2,3,7,8-substituted PCDD/Fs and 12 kinds of ^{13}C-labeled dioxin-like PCBs were evaluated.

The analytical procedures used in this study are summarized in Table 1. In Method 1, the conventional standard method, the sample was treated with 100 ml of 1 N potassium hydroxide/ethanol for two hours with stirring at room temperature. The alkaline hydrolyzate was extracted twice with 100 ml of n-hexane using a separatory funnel for one hour each time, and then the concentrated extract was treated with 15 ml of concentrated sulfuric acid. By contrast, in Method 2, automated extraction was performed using an ASE-300 (Dionex, CA) under conditions of 1500 psi. Four individual experiments and four simultaneous blank tests were performed for each extraction method.

Dioxins were analyzed using a model 6890 gas chromatograph (Agilent Technologies, CA) coupled to a model Autospec-Ultima mass spectrometer (Micromass, UK). We employed an Rtx-2330 (0.18mm x 40m) capillary column (Restek, PA) on an SCLV injection system (SGE, Australia) in order to determine tetra- and pentaCDD/Fs, and hexaCDFs. The details of the operating conditions for the SCLV injection system are described in another paper[2]. The LOD for each congener was determined according to the provisional guidelines for analysis of dioxins in foods issued by the Ministry of Health and Welfare of Japan in 1999 ("*Guideline*"): An absolute quantity corresponding to S/N = 3 was evaluated on HRGC/HRMS chromatograms using verification standards.

Table 1 Analytical procedures for determination of dioxins in food.

		Method 1	Method 2
Extraction		Alkaline digestion (KOH/ethanol) followed by shaking extraction* Sample size: 20g Time: 60 min x 2 (120 min) Solvent: n-hexane 200 ml (100ml x 2)	Accelerated solvent extraction (ASE) Sample size: 20 g Time: 25 min Solvent: acetone/n-hexane (1:1, v/v) 120 ml
Cleanup		Sulfuric acid treatment ↓ Multi-layer silica gel column ↓ Active carbon-dispersed silica gel column	
HRGC/ HRMS analysis	PCDD/DFs and non-*ortho* PCBs	SCLV injection Injection volume: 4 μL / 20μL Pre-column:BPX-5 (0.25mm x 5m) Analytical columns: a) Rtx-2330 (0.18mm x 40m) b) BPX-5 (0.15mm x 30m)	
	Mono-*ortho* PCBs	Splitless injection Injection volume: 1μL/20 μL Analytical column: HT8-PCB (0.25mm x 60m)	

* Method recommended for fat, fish and shellfish, meats, eggs, milk and dairy products in "*Guideline*".

Results and Discussion

Twenty grams of milk powder were extracted by ASE. After the extracts were evaporated and dried, fat contents were measured gravimetrically. Three individual experiments were performed for each extraction condition shown in Table 2. As a result, the largest fat content was obtained under the condition of 150 °C, acetone/n-hexane (1:1, v/v). This highest value agreed with that obtained from the standard fat extraction method by shaking its reconstituted aqueous solution with diethyl ether/petroleum ether (1:1, v/v). Data regarding the quantification of dioxin congeners in milk powder (pg/g whole weight basis) are shown in Table 3. Trace data showing the concentrations of the congeners under detection limits were re-evaluated and are shown in parentheses to compare concentrations between the methods. Generally, high concentrations and a large number of detected congeners were found under the condition of 150 °C, acetone/n-hexane (1:1, v/v), compared to other conditions. By contrast, there were no obvious differences among the computed data showing 29 kinds of labeled compound recoveries in each extraction condition (data not shown), all of which were adapted to the range recommended in the "*Guideline*" (40%-120%). The above results suggested that differences in quantification values between extraction conditions were due to differences in the extraction efficiency of dioxin molecules from the tissue. Hence, validation tests comparing ASE to the conventional method were carried out using the condition of "150 °C, acetone/n-hexane," which demonstrated the high extraction efficiency of the compounds and the fat content's similarity to the standard fat extraction method.

Table 2 Fat contents (%) of milk powder under various extraction conditions

	Temperature (℃)	Solvent	Trial	Milk powder weighed (g)	Fat obtained (g)	Fat contents (%)
ASE	100	n-hexane	1st	20.01	0.20	1.0
			2nd	20.34	0.31	1.5
			3rd	20.00	0.30	1.5
		acetone/n-hexane (1:1)	1st	20.21	0.69	3.4
			2nd	20.08	0.93	4.7
			3rd	20.01	1.12	5.6
	150	n-hexane	1st	20.16	3.02	15.0
			2nd	20.33	3.22	15.8
			3rd	20.27	3.13	15.5
		acetone/n-hexane (1:1)	1st	20.24	5.30	26.2
			2nd	20.27	5.24	25.8
			3rd	20.26	5.30	26.1
Shaking extraction		diethyl ether/petroleum ether (1:1)	1st	5.06	1.24	24.4
			2nd	4.96	1.19	23.9
			3rd	4.96	1.16	23.3

Table 3 Concentrations of dioxins (pg/g whole weight basis) in dried milk powder; comparison of temparature and solvent used.

Temperature(℃)		100						150					
Solvent		n-hexane			acetone/n-hexane			n-hexane			acetone/n-hexane		
Congener	LOD (pg/g)	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
2,3,7,8-TeCDD	0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1,2,3,7,8-PeCDD	0.01	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
1,2,3,4,7,8-HxCDD	0.02	nd	nd	nd	nd	nd	nd	nd	nd	0.021	nd	0.020	(0.011)
1,2,3,6,7,8-HxCDD	0.02	nd	nd	nd	nd	nd	nd	0.026	0.034	0.035	0.046	0.035	0.026
1,2,3,7,8,9-HxCDD	0.02	nd	nd	nd	nd	nd	nd	nd	nd	0.013	(0.018)	(0.018)	0.020
1,2,3,4,6,7,8-HpCDD	0.02	nd	nd	nd	(0.064)	(0.061)	0.13	0.25	0.19	0.21	0.34	0.38	0.31
OCDD	0.05	0.19	0.25	0.22	0.51	0.62	0.99	2.1	2.2	2.2	3.3	3.6	2.9
2,3,7,8-TeCDF	0.01	nd	nd	nd	0.019	0.016	0.017	0.055	0.055	0.049	0.082	0.080	0.067
1,2,3,7,8-PeCDF	0.01	nd	nd	nd	nd	nd	nd	0.037	0.021	0.030	nd	nd	nd
2,3,4,7,8-PeCDF	0.01	nd	nd	nd	nd	nd	nd	0.043	0.035	0.036	nd	nd	0.024
1,2,3,4,7,8-HxCDF	0.02	nd	nd	nd	nd	(0.0091)	(0.019)	0.031	0.026	0.029	0.051	0.064	0.036
1,2,3,6,7,8-HxCDF	0.02	nd	nd	nd	nd	nd	(0.012)	0.021	nd	(0.012)	0.029	0.030	0.027
1,2,3,7,8,9-HxCDF	0.02	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.014
2,3,4,6,7,8-HxCDF	0.02	nd	nd	nd	nd	nd	(0.0080)	0.022	(0.013)	(0.016)	0.028	0.032	0.020
1,2,3,4,6,7,8-HpCDF	0.02	nd	nd	nd	0.024	0.020	(0.019)	0.056	0.054	0.085	0.10	0.10	0.11
1,2,3,4,7,8,9-HpCDF	0.02	nd	nd	nd	nd	nd	(0.010)	nd	nd	nd	nd	nd	nd
OCDF	0.05	nd	nd	nd	(0.024)	(0.014)	0.056	0.14	0.15	0.16	0.25	0.19	0.20
3,3',4,4'-TeCB(#77)	0.1	(0.075)	(0.096)	(0.087)	(0.099)	(0.064)	0.10	0.13	0.11	0.15	0.14	0.15	0.22
3,4,4',5-TeCB(#81)	0.1	nd	nd	nd	(0.015)	nd	nd	(0.012)	nd	(0.011)	(0.013)	nd	(0.019)
3,3',4,4',5-PeCB(#126)	0.1	nd	nd	nd	(0.031)	(0.022)	nd	(0.082)	(0.099)	0.11	0.13	0.11	0.11
3,3',4,4',5,5'-HxCB(#169)	0.1	nd	nd	nd	nd	(0.0083)	nd	(0.057)	(0.048)	(0.052)	(0.061)	(0.074)	(0.063)
2,3,3',4,4'-PeCB(#105)	1	(0.23)	(0.31)	(0.32)	(0.53)	(0.55)	(0.88)	1.7	1.6	1.8	2.1	2.1	2.2
2,3,4,4',5-PeCB(#114)	1	(0.028)	(0.029)	(0.031)	(0.046)	(0.044)	(0.075)	(0.18)	(0.18)	(0.18)	(0.22)	(0.26)	(0.23)
2,3',4,4',5-PeCB(#118)	1	(0.81)	1.1	1.2	1.8	2.0	3.1	6.9	6.5	7.1	8.9	9.9	9.3
2',3,4,4',5-PeCB(#123)	1	(0.019)	(0.032)	(0.025)	(0.042)	(0.039)	(0.066)	(0.12)	(0.087)	(0.092)	(0.13)	(0.13)	(0.17)
2,3,3',4,4',5-HxCB(#156)	1	(0.12)	(0.11)	(0.16)	(0.33)	(0.38)	(0.56)	1.4	1.4	1.4	2.0	2.2	1.9
2,3,3',4,4',5'-HxCB(#157)	1	(0.033)	(0.045)	(0.036)	(0.095)	(0.11)	(0.16)	(0.39)	(0.36)	(0.40)	(0.50)	(0.51)	(0.47)
2,3',4,4',5,5'-HxCB(#167)	1	(0.054)	(0.070)	(0.064)	(0.12)	(0.14)	(0.21)	(0.50)	(0.48)	(0.47)	(0.72)	(0.75)	(0.73)
2,3,3',4,4',5,5'-HpCB(#189)	1	(0.022)	(0.030)	(0.027)	(0.050)	(0.056)	(0.11)	(0.22)	(0.20)	(0.20)	(0.26)	(0.37)	(0.28)

Table 4 shows the dioxin concentrations and RSD values obtained from the two extraction methods using freeze-dried *tuna* homogenates. RSD values in ASE ranged from 4% to 19%, similar to the results in alkaline digestion (1% to 27%). The concentrations of 29 kinds of dioxin congeners were close for both extraction methods other than OCDD; the ratios of estimated concentrations from ASE compared to those from the alkaline digestion-hexane shaking extraction method ranged from 0.96 to 1.4, except 2.0 for OCDD. It is considered that this result was due to ASE's high extraction efficiency compared with the shaking extraction; a tendency like this was observed in our previous examination using dried seaweed powder, in which the extraction efficiency of ASE was found to be superior to that of conventional separatory funnel extraction [3].

A recovery test in the present method, including the ASE and SCLV injection technique, was performed using 18 food items, mainly dairy products. The results showed that recovery rates for 29 kinds of labeled congeners ranged from 41% to 108 %, within the range recommended by the Japanese analytical guideline for dioxins in food (40%-120%). Our results suggest that the present method is available for rapid and sensitive determination

of dioxins in animal-origin fatty food samples of low sample size and requiring only a small volume of extraction solvent compared to the conventional extraction method. The ASE condition suited for dioxins in the animal-origin sample presented here is identical to that proposed for plant food samples[3]. Therefore, independent extraction conditions could be available for both animal- and plant-origin food samples. Moreover, fat content values obtained from the present extraction method of dioxins could be directly applied to the calculation of fat weight-based concentrations. The applicability of the combined SCLV injection and ASE methodology has been continuously verified for use regarding food mixture samples, e.g., total diet study samples.

Table 4 Concentrations of dioxins (pg/g whole weight basis) in dried *tuna* homogenates; conparison between ASE and alkaline digestion followed by hexane shaking.

Congener	ASE (n=4)				Alkaline digestion-hexane shaking (n=4)				a / b
	Range		Mean[a]	RSD(%)	Range		Mean[b]	RSD(%)	
2,3,7,8-TeCDD	0.61 -	0.67	0.64	4	0.60 -	0.72	0.67	7	0.96
1,2,3,7,8-PeCDD	0.75 -	0.83	0.80	5	0.76 -	0.80	0.77	3	1.0
1,2,3,4,7,8-HxCDD	0.023 -	0.035	0.028	19	0.020 -	0.030	0.024	16	1.2
1,2,3,6,7,8-HxCDD	0.20 -	0.22	0.21	5	0.20 -	0.22	0.21	4	0.99
1,2,3,7,8,9-HxCDD	0.026 -	0.037	0.032	17	0.022 -	0.028	0.025	12	1.2
1,2,3,4,6,7,8-HpCDD	0.058 -	0.067	0.065	7	0.055 -	0.058	0.057	2	1.1
OCDD	0.15 -	0.17	0.16	6	0.070 -	0.094	0.081	13	2.0
2,3,7,8-TeCDF	4.4 -	5.5	5.0	9	4.8 -	5.3	5.1	5	0.98
1,2,3,7,8-PeCDF	0.92 -	1.1	0.99	6	0.94 -	1.0	0.96	3	1.0
2,3,4,7,8-PeCDF	2.7 -	3.1	2.9	5	2.7 -	2.8	2.7	2	1.1
1,2,3,4,7,8-HxCDF	0.15 -	0.21	0.19	14	0.15 -	0.25	0.18	26	1.1
1,2,3,6,7,8-HxCDF	0.14 -	0.21	0.18	19	0.17 -	0.21	0.18	11	1.0
1,2,3,7,8,9-HxCDF	0.11 -	0.15	0.13	14	0.12 -	0.14	0.13	7	1.0
2,3,4,6,7,8-HxCDF	nd	nd	-	-	nd	nd	-	-	-
1,2,3,4,6,7,8-HpCDF	0.067 -	0.079	0.072	7	0.050 -	0.063	0.055	11	1.3
1,2,3,4,7,8,9-HpCDF	nd	nd	-	-	nd	nd	-	-	-
OCDF	nd	nd	-	-	nd	nd	-	-	-
3,3',4,4'-TeCB(#77)	220 -	270	240	9	260 -	280	270	3	0.91
3,4,4',5-TeCB(#81)	17 -	21	19	9	20 -	21	20	1	0.94
3,3',4,4',5-PenCB(#126)	210 -	250	230	6	230 -	240	230	1	0.99
33'44'55'-HxCB(#169)	26 -	29	27	5	28 -	28	28	1	0.97
233'44'-PeCB(#105)	60000 -	72000	64000	9	50000 -	68000	61000	14	1.1
2344'5-PeCB(#114)	2700 -	3400	3100	10	2700 -	2900	2800	4	1.1
23'44'5-PeCB(#118)	100000 -	120000	110000	6	110000 -	130000	120000	8	0.96
2'344'5-PeCB(#123)	2600 -	4100	3600	19	1900 -	3700	2700	27	1.4
233'44'5-HxCB(#156)	31000 -	37000	36000	9	32000 -	42000	39000	12	0.92
233'44'5'-HxCB(#157)	8500 -	10000	9400	8	8000 -	11000	9700	13	0.97
23'44'55'-HxCB(#167)	20000 -	23000	22000	7	20000 -	25000	23000	11	0.96
233'44'55'-HpCB(#189)	4900 -	5900	5600	8	4400 -	5300	5000	9	1.1

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BROMINATED FLAME RETARDANTS (HBCD,TBBPA AND ΣPBDES) IN MARKET BASKET FOOD SAMPLES OF NORTHERN KYUSHU DISTRICT IN JAPAN

Murata S[1], Nakagawa R[1], Ashizuka Y[1],Hori T[1], Yasutake D[1], Tobiishi K[1], and Sasaki K[2]

[1]Fukuoka Institute of Health & Environmental Sciences; 39 Mukaizano, Dazaifu, Fukoka, 818-0135,Japan
[2]National Institute of Health Sciences; 1-18-1 Kamiyoga, Setagaya-ku, Tokyo, 158-8501, Japan

Abstract
We developed an analytical method for HBCD in food samples using gel permeation chromatography and a mini-column coupled with LC/MS/MS. In this report, to estimate the trend of human exposure to brominated flame retardants (BFRs) via food as well as to describe our validation results using this method, we analyzed two sets of market basket food samples prepared in Fukuoka prefecture in the fiscal years of 2002 and 2005. The estimated dietary intakes of HBCD, TBBPA and ΣPBDEs by an adult were 2.2, 1.1, and 2.3 ng/kg b.w./day, respectively, in F2002, and were 1.4, 0.1, and 1.4 ng/kg b.w./day, respectively, in F2005 when calculated for ND=0. The BFR intake levels by Japanese populate were considered as not so concerned.

Introduction
Polybrominated diphenyl ethers (PBDEs), tetrabrominated bisphenol A (TBBPA), and hexacycrododecane (HBCD) have been revealed as ubiquitous contaminants in human and wild live tissues as well as in other environmental samples. As another noteworthy point, they are closely related to the occurrence of polybrominated dioxins (PBDD/DFs)[1]. For example, the detection of PBDD/Fs in a PBDE formula or in ashes caused from the burning of plastics with PBDE as a flame retardant is well-known as a significant evidence[2]. When we estimate human exposure to these pollutants, the route via food is the most important. In our previous study, we found PBDEs in almost all fish samples collected from the Japanese near coasts and also found a tendency for PBDD/Fs to be detected in fish accompanying with highy brominated diphhenyl ethers such as nona- and deca-brominated diphenylethers. From such a viewpoint, BFR monitoring in food is important in preventing health hazardous effects not only from BFRs themselves but also from PBDD/DFs. According to a domestic trade paper, TBBPA is the mostly used BFR in Japan, with as much as 35,000 tons as a demand in F2004, followed by HBCD(2600 tons) and decabrominated diphenyl ether (2000 tons). In Japan, PBDEs without decabrominated diphenyl ether are self-controlled by the trade, and the value of the domestic demand for DBDE is only officially announced. Basically, there is only limited data on food pollution by BFRs. The best of the convenient ways to estimate the trend of human exposure to BFRs via food is to analyze market basket food samples. However, market basket food samples usually contain too many matrices that make an analysis difficult. Therefore, we have to modify the basic analytical method depending on the characteristic conditions of food—for example, whether it is a fatty food or not, or whether it is chlorophyll-rich or not. The appropriate modification is the key point in determining pollutant concentration with accuracy. In this study, we designed a pre-treatment method for the HBCD analysis for market basket samples, paying attention to the fact that HBCD is a large molecule of MW 641 and has a non-polar structure. For the method validation and the preliminary estimation of BFR intakes, the market basket samples prepared in our laboratory were used

Materials and Methods
Market basket food samples: Thirteen mixed food samples were prepared following the method of the Market Basket Study, alternatively termed the Total Diet Study. More than one hundred food items were chosen from 99 categories of foods that the Fukuoka populace commonly consumes, and the respective amounts of food items composing each food group (from 1 to 13) were determined by referring to the data of the latest national and prefecture survey (2002 and 2005).

Analysis of HBCD: Five grams of an homogenized sample with an addition of $^{13}C_{12}$ -α,β, and γ-HBCD were extracted twice with 20mL of dichloromethane (DCM) using a Polytron®. The extracts were dried over sodium sulfate dehydrate and concentrated. (I) Each residue of groups 1 (rice and its products), 2 (other grains, seeds, potatoes), 3 (sugar and a confectionary) and 4 (oils) was dissolved in 20mL of methanol/water (15:5, v/v) and re-extracted with n-hexane. One-half of each hexane layer was purified by liquid-liquid partition with DMSO. The purified extracts were concentrated and dissolved into 0.2mL of acetone and loaded onto a column of gel permeation chromatography (GPC). (II) Each half residue of groups 6 (fruits), 7 (colored vegetables) and 8 (other vegetables, mushroom, sea weed) was purified by multi-layered column chromatography of 22% H_2SO_4-silica (4g) and 44% H_2SO_4-silica (3g). HBCD was recovered with 10% DCM/n-hexane as an eluate. Each eluate was concentrated and dissolved into 0.2mL of acetone and subjected to GPC. (III) Each residue of the others--groups 6 (beans), 9 (drink, beverage), 10 (fish), 11 (meat and eggs), 12 (milk), and 13 (seasoning)-- was dissolved in 10% DCM/n-hexane and was treated twice with 5mL of sulfuric acid. After centrifuging at 2000 rpm, the upper hexane layer was collected and evaporated. The residue was dissolved in 0.2mL of acetone, and a half of it was subjected to GPC.
HBCD was fractionated in 12 to 14 min after large molecules such as crude fatty acids eluted in 10 to 12 min. The fraction was re-purified with a cartridge mini-column (Varian BOND ELUT-PSA, 500mg) prior to analysis by LC/MS/MS (Table 1). Detection limits of α-and γ-HBCD were 0.02 pg/g wb. That for β-HBCD was 0.01 ng/g wb.

Analysis of PBDEs: Each food group sample (fifty to one hundred grams) except for group 4 (oils) was freeze dried. After being spiked with $^{13}C_{12}$-labelled 2,3,7,8-substituted PBDD/DFs (128-500pg), $^{13}C_{12}$-1-Br-2,3,7,8-TeCDD (50pg) and $^{13}C_{12}$-labelled PBDEs (500-2500pg), it was extracted by accelerated solvent extractor(100 ℃ ,1500psi, 10% DCM/n-hexane) and with sulfuric acid and two kinds of column chromatography with silica-gel activated overnight at 130 ℃ and florisil deactivated with 1% of water. Group 4(oil) sample was directly diluted with n-hexane and then cleaned up as same as the other group. The PBDE fraction was cleaned up by liquid-liquid partition with DMSO. Prior to measurement by HRGC/HRMS (Table 2), $^{13}C_{12}$- 2,2',3,4,4',5',6 -HpBDE was added. Detection limits of tetra-to hepta-isomers were 0.1 pg/g wb.

Analysis of TBBPA: A homogenized sample (5 g) was spiked with ^{13}C-labeled TBBPA (0.5 ng) as a clean-up standard and then extracted with methanol. The methanol extract (ca.50 mL) was defatted by liquid-liquid partition with 20 mL of hexane. Then, to the methanol layer, 120 mL of 5% sodium chloride solution was added and re-extracted with DCM. The extract was concentrated to dryness and then ethylated with diethyl sulfate under an alkaline condition. After that, TBBPA ethylate was extracted with n-hexane and was cleaned up with florisil mini-column chromatography. The purified eluate was concentrated, re-dissolved in 20 μL of nonane with 2.5 ng of chrysene-d_{12} as a syringe spike, and subjected to measurement by HRGC/HRMS (Table 2). The detection limit of TBBPA was 0.01ng/g wb.

Results and Discussion
HBCD are highly lipophilic chemicals and large molecules similar to PBDEs. There are three stereo isomers: α,β,and γ. So far, detecting α,β,and γ separately is difficult with GC/MS. Therefore, replacing GC/MS by LC/MS/MS is likely to be used in the analysis of HBCD. However, there is a basic problem in LC/MS/MS analysis: the suppression of ionization caused by co-eluting the matrix. Good recovery and good reproducibility are required, when developing an analytical method. Therefore, sufficient clean-up to reduce the adverse problem as much as possible is necessary. In this study, we employed GPC and an additional clean-up by mini-column chromatography. With GPC, HBCD and TBBPA eluted separately from a large molecule such as crude fatty acid of fish. In the experiment, using the group 10 sample (fish as the main food) prepared in 2007, we obtained satisfactory recoveries: a mean of 73.4% ranging from 62.2% to 81.9% for α–isomer, a mean of 83.4% ranging from 66.5% to 92.6% for β-isomer, and a mean of 73.4% ranging from 54.8% to 90% for γ –isomer, as well as satisfactory reproducibilities of 12.2%, 11.7% and 15.4% for α,β,and γ–isomers, respectively. And for the other various food group samples, the recoveries of HBCD were

42%~106% for α–isomer, 59%~130% for β-isomer and 53%~124% for γ-isomer, except for 182%~225% for group 9.

HBCD was detected in each group 10 of F 2002 and F 2005 and in group 11 of F 2002. TBBPA was detected in each group 10 of F2002 and F2005. It was also detected in the groups 3, 4, 5 and 11 of F 2002 and in the groups 3 and 11 of F 2005. On the other hand, due to the lower detection limit of each PBDE congener, PBDEs were sensitively detected in the almost samples of F 2002 and F 2005. In group 10 among the 13 food groups of each year set, PBDEs were most abundantly detected. The partition coefficient of n-octanol to water (Log K_{ow}) for TBBPA, HBCD and PBDEs was 4.5~5.3, 7.74 and 6.27 (as DeBDE), respectively. The very frequent detection of HBCD and PBDEs like PCBs in group 10 (fish) seems to be acceptable; in contrast, TBBPA was detected only in each group 10 of F2002 and F2005 but at a very low level, which would be due to the instability of the phenol moiety of TBBTA. TBBPA is reported to be rapidly metabolized biologically to sulfate or gluculonide conjugates in the environment[3]. On the basis of the above data, the daily intakes of HBCD, TBBPA and ΣPBDE were calculated by multiplying each pollutant's concentration by the daily food consumption amount by one person (Table 3). Assuming the average adult body weight as 50 kg, the daily intakes of HBCD, TBBPA and ΣPBDEs were 2.2, 1.1, and 2.3 ng/kg b.w./day, respectively, in F2002, and 1.4, 0.1, and 1.4 ng/kg b.w./day, respectively, in F2005, when calculated for ND=0. The daily intakes of HBCD, TBBPA and ΣPBDEs were 3.1, 1.3, and 2.3 ng/kg b.w./day, respectively, in F2002, and 2.4 ng, 0.3 ng, and 1.4 ng/kg b.w./day, respectively, in F2005 when calculated for ND=1/2xLOD. Only TBBPA varied greatly between F2002 and F2005. The dietary intakes of HBCD in F2002 and F2005 were at the same level as those of ΣPBDEs. In other reports, Swedish people's intake of BFRs was <3ng ng/kg b.w. day for HBCD[4] , and the UK people's intake of these brominated retardants in 2003 and 2004 was <5.9 ng/kg b.w./day for HBCD, <1.6 ng/kg b.w./day for TBBPA, and <5.9 ng/kg b.w./day for ΣPBDEs[5], respectively. The food standards agency of the UK that carried out the above market basket study concluded that the estimated adult dietary intake of HBCD, TBBPA and ΣPBDEs does not raise toxicological concerns. Although there is a report which simulates the increase of Br emission from TV casing waste in the near future, basically not only the available references about the intake data of BFRs, but also the fate of BFRs are too limited; the increase or decrease of emission and deposition into the environment are not completely clear. Therefore, we recommend continuing to collect more data in order to clarify the trend of food pollution by those BFRs and avoid probable human hazardous exposure.

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Table 1 The LC/MS/MS conditions for HBCD analysis

LC/MS/MS: Waters Quatro Micro API
Column:Inertsil ODS-3 (GL Sciences) 2.1mmi.d. x150m, 5μ
Injector volume.:5 uL
Column temp.:40℃
Flow rate: 0.2mL/min
Moving phase: 10mM ammonium acetate/methanol/acetonitrile (10:55:35)
Monitor Ions: native-HBCD, 641 >79 (Q1) , 639 >79(Q2)
$^{13}C_{12}$-HBCD; 653>79(Q1), 651>79(Q2)
Ionization;ES negative; Ion source temp.,130℃; Capillary energy ; 2.0kV

Table 2 The GC/MS conditions for PBDEs and TBBPA analysis

GC/HRMS: HP6890 (Hewlett Packard) /Autospec Ultima (MicroMass)
Electron energy,38eV; filament current, 750μA; ion source Temp.,270℃; resolution,10000
PBDE analysis: Column: HP-5MS(Agilent) 0.25mmi.d. x15m, film thickness 0.1μm
Injector temp.:260℃
Column temp.:120℃(2min)-20℃/min-200℃-10℃/min-300℃(7.5min)
TBBPA analysis: Column:DB-5 (J&W) 0.25mmi.d. x 30m, film thickness 0.25μm
Injector temp.:280℃
Column temp.:120℃(1min)-20℃/min–300℃(8min)

Table 3 The estimated dietary intakes of HBCD, TBBPA and ΣPBDEs

Food group		Food consumption (g/day) in F2002	Daily intake in F2002						Food consumption (g/day) in F2005	Daily intake in F2005					
			α-HBCD	β-HBCD	γ-HBCD	ΣHBCD	TBBPA	ΣPBDEs		α-HBCD	β-HBCD	γ-HBCD	ΣHBCD	TBBPA	ΣPBDEs
I	(rice)	459	ND	ND	ND	ND	ND	0.11	446.5	ND	ND	ND	ND	ND	0.19
II	(other grains, potatoes,seeds)	226.1	ND	ND	ND	ND	ND	0.37	209.9	ND	ND	ND	ND	ND	0.50
III	(sugar, confectionary)	36.6	ND	ND	ND	ND	0.33	0.17	33.7	ND	ND	ND	ND	0.33	0.67
IV	(oils)	15.2	ND	ND	ND	ND	0.15	1.85	11.5	ND	ND	ND	ND	ND	0.65
V	(legume and its products)	80.4	ND	ND	ND	ND	0.73	0.30	56.4	ND	ND	ND	ND	ND	0.13
VI	(fruits)	130.6	ND	ND	ND	ND	ND	0.00	136.0	ND	ND	ND	ND	ND	2.60
VII	(colored vegetable)	108.3	ND	ND	ND	ND	ND	0.05	96.6	ND	ND	ND	ND	ND	0.10
VIII	(other vegetables)	234.6	ND	ND	ND	ND	ND	0.03	219.0	ND	ND	ND	ND	ND	0.06
IX	(bebariges)	172.2	ND	ND	ND	ND	ND	0.02	504.2	ND	ND	ND	ND	ND	0.00
X	(fish)	100.8	57*	ND	19.7*	76.7*	50.7*	102*	90.7	49.3*	ND	19.7*	49.3*	2.72*	57.8*
XI	(meat and eggs)	157.9	34.5*	ND	ND	34.5*	4.42*	9.17*	133.6	ND	ND	ND	ND	0.67*	4.19*
XII	(milk and its products)	122.5	ND	ND	ND	ND	ND	1.04*	146.8	ND	ND	ND	ND	ND	0.74*
XIII	(seasonings)	38.1	ND	ND	ND	ND	ND	0.06	80.9	ND	ND	ND	ND	ND	0.65
XIV	(water)		-	-	-	-	—	—		-	-	-	-	—	—
Daily intake ng/day at ND=0			91.5	0	19.7	111	56.3	115		49.3	0	19.7	68.4	3.7	68.3
Daily intake ng/day at ND=1/2 xLOD			108.5	9.4	37.5	155	63.9	116		70.1	10.8	39.9	121	13.8	69.3
Daily intake ng/kg,b.w./day at ND=0						2.2	1.1	2.3					1.4	0.1	1.4
Daily intake ng/kg,b.w./day at ND=1/2xLOD						3.1	1.3	2.3					2.4	0.3	1.4

LOD: 0.01ng/g for β-HBCD and TBBPA; 0.02ng/g for α-HBCD and γ-HBCD; 0. 0001ng/g for each PBDE congener (from tetra- to hepta-brominated isomer)

* means an average of two food group samples prepared separately.

DIOXINS AND OTHER ORGANOHALOGEN COMPOUNDS IN FISH OIL SUPPLEMENTS ON THE JAPANESE MARKET

Tsutsumi T[1], Amakura Y[1], Tanno K[2], Yanagi T[2], Kono Y[2], Sasaki K[1], Maitani T[1]
[1]National Institute of Health Sciences, Kamiyoga 1-18-1, Setagaya-ku, Tokyo 158-8501, Japan; [2]Japan Food Research Laboratories, 52-1, Motoyoyogi-cho, Shibuya-ku, Tokyo 151-0062, Japan

Abstract
Dioxin (PCDD/Fs and DL-PCBs) concentrations of 30 fish oil supplements on the Japanese market were determined to estimate the dioxin intakes resulting from their consumption. The dioxin intakes from most products were under 10% of the tolerable daily intake (TDI) of dioxins (4 pg-TEQ/kg bw/day) set in Japan. However, only a product, no. 1, had extremely high dioxin concentrations and dioxin intake of the product greatly exceeded the TDI. Four products with relatively high dioxin concentrations, including product no. 1, were further analyzed in terms of PBDD/Fs, PXDD/Fs, PCBs and PBDEs. PCBs and PBDEs were found in all samples, especially product no. 1 had much higher concentrations of PCBs and PBDEs than the other products did. By contrast, PBDD/Fs and PXDD/Fs were detected much less often in all samples.

Introduction
Fish oil supplements are a source of long-chain *n*-3 polyunsaturated fatty acids, such as eicosapentaenoic and docosahexaenoic acid, which are thought to have health benefits. Recently, the popularity of these supplements has increased in Japan. They are produced from various types of fish, especially from fatty tissues, and can be a major source of persistent organic pollutants such as dioxins (PCDD/Fs and DL-PCBs) and PCBs. Recently, there has been increasing concern over brominated compounds such as PBDEs, which are used as flame retardants, as well as brominated and mixed chlorinated-brominated dioxins (PBDD/Fs and PXDD/Fs, respectively). Although it is important to determine the levels of these compounds in fish oil supplements, only a few previous studies have considered them[1–4]. Here, we examined the dioxin levels in fish oil supplements on sale in Japan and estimated the dioxin intakes resulting from their consumption. Products with relatively high dioxin concentrations were further analyzed in terms of PBDD/Fs, PXDD/Fs, PCBs and PBDEs.

Materials and Methods
Fish oil supplements: In total, 30 products (29 capsule formulations and 1 bottled formulation) were purchased between 2002 and 2005 from retail outlets, or by post, in Tokyo, Japan. The analysis of encapsulated products included the capsules. All samples were stored at 4°C until they were analyzed.

PCDD/F and DL-PCB analyses: Dioxins were extracted, prepared and analyzed as described previously[5]. The TEQ concentrations were calculated using WHO-TEFs (1998).

PBDD/F and PXDD/F analyses: Samples (5–20 g) spiked with $^{13}C_{12}$-labelled internal standards were stirred with aqueous potassium hydroxide (KOH) and then kept for 16 h at room temperature (RT). The alkaline hydrolysates were extracted with *n*-hexane. The extracts were treated with concentrated sulphuric acid, and then purified on a silica gel column followed by a Florisil column (deactivated with 1% water). After washing with *n*-hexane, the elute obtained with 60% dichloromethane/*n*-hexane was loaded onto an activated carbon column. This was washed by *n*-hexane followed by 25% dichloromethane/*n*-hexane, and the fraction containing PBDD/Fs and PXDD/Fs was eluted with toluene. The fraction was spiked with $^{13}C_{12}$-labelled recovery standards, and subjected to HRGC/HRMS. The determinations of 12 2,3,7,8-substituted PBDD/Fs (2,3,7,8-TeBDD, 1,2,3,7,8-PeBDD, 1,2,3,4,7,8/1,2,3,6,7,8-HxBDD, 1,2,3,7,8,9-HxBDD, OBDD, 2,3,7,8-TeBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, 1,2,3,4,7,8-HxBDF, 1,2,3,4,6,7,8-HpBDF and OBDF) and seven PXDD/Fs (2-Br-3,7,8-TrCDD, 1-Br-2,3,7,8-TeCDD, 2-Br-3,6,7,8,9-PeCDD, 1-Br-2,3,6,7,8,9-HxCDD, 1-Br-2,3,4,6,7,8,9-HpCDD, 3-Br-2,7,8-TrCDF and 1-Br-2,3,7,8-TeCDF) were performed using a DB-5HT and BP1 column. The WHO-TEFs for the chlorinated isomers were provisionally used to evaluate the toxicities of the corresponding PBDD/F and PXDD/F isomers.