表 10　3 地域における臭素系ダイオキシン類及び臭素化ジフェニルエーテルの一日摂取量総括表

(1) 北海道地区

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
群別一日食事量 (g)	340.1	190.4	30.4	11.1	43.9	135.4	85.4	211.2	412.8	120.2	105.6	193.8	90.3	1970.6
PBDD/DFs, MoBrPCDD/DFs Total TEQ(ND=0) pgTEQ/日	0	0	0	0.028	0	0	0	0	0	0	0	0	0	0.028
PBDD/DFs, MoBrPCDD/DFs Total TEQ(ND=1/2LOD) pgTEQ/日	11.9	14.2	1.5	0.4	1.6	4.7	2.7	7.1	14.4	5.3	4.8	6.8	3.2	78.6
Total PBDEs (ND=0) ng/日	3.70	3.38	2.77	13.17	0.47	0.05	0.06	0.06	0.15	50.44	13.45	4.06	4.23	95.99
Total PBDEs (ND=1/2) ng/日	4.11	3.97	2.80	13.17	0.53	0.32	0.21	0.47	0.98	50.56	13.50	4.23	4.27	99.10

(2) 東北地区

	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
群別一日食事量 (g)	382.0	164.8	30.9	10.6	58.9	128.2	95.7	213.7	389.5	92.8	104.7	162.9	78.0	1912.7
PBDD/DFs, MoBrPCDD/DFs Total TEQ(ND=0) pgTEQ/日	0	0	0	0.033	0	0	0	0	0	0	0	0	0	0.033
PBDD/DFs, MoBrPCDD/DFs Total TEQ(ND=1/2LOD) pgTEQ/日	13.4	5.9	1.1	0.4	3.4	4.5	3.1	6.9	13.6	2.9	3.1	5.7	2.7	66.7
Total PBDEs (ND=0) ng/日	8.44	2.59	2.09	15.76	1.64	0.38	0.70	1.42	1.30	30.41	5.83	4.91	4.73	80.19
Total PBDEs (ND=1/2) ng/日	8.85	2.77	2.10	15.76	1.76	0.58	0.84	1.72	1.91	30.46	5.87	5.08	4.78	82.46

(3) 中部地区

	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
群別一日食事量 (g)	346.4	171.1	35.6	10.3	59.7	130.2	92.3	196.2	499.2	89.0	110.3	177.5	81.0	1999.3
PBDD/DFs, MoBrPCDD/DFs Total TEQ(ND=0) pgTEQ/日	0	0	0	0.045	0	0	0	0	0	0	0	0	0	0.045
PBDD/DFs, MoBrPCDD/DFs Total TEQ(ND=1/2LOD) pgTEQ/日	18.4	9.1	1.7	0.4	2.2	4.6	2.8	6.6	17.5	3.9	4.4	6.2	2.8	80.6
Total PBDEs (ND=0) ng/日	1.16	2.27	3.84	31.80	3.03	0.23	0.26	0.40	0.88	26.73	7.61	2.96	16.51	97.69
Total PBDEs (ND=1/2) ng/日	1.97	2.64	3.86	31.81	3.08	0.44	0.38	0.68	1.68	26.84	7.68	3.14	16.54	100.75

* 10 群、11 群、12 群は、A と B の平均値を示す。

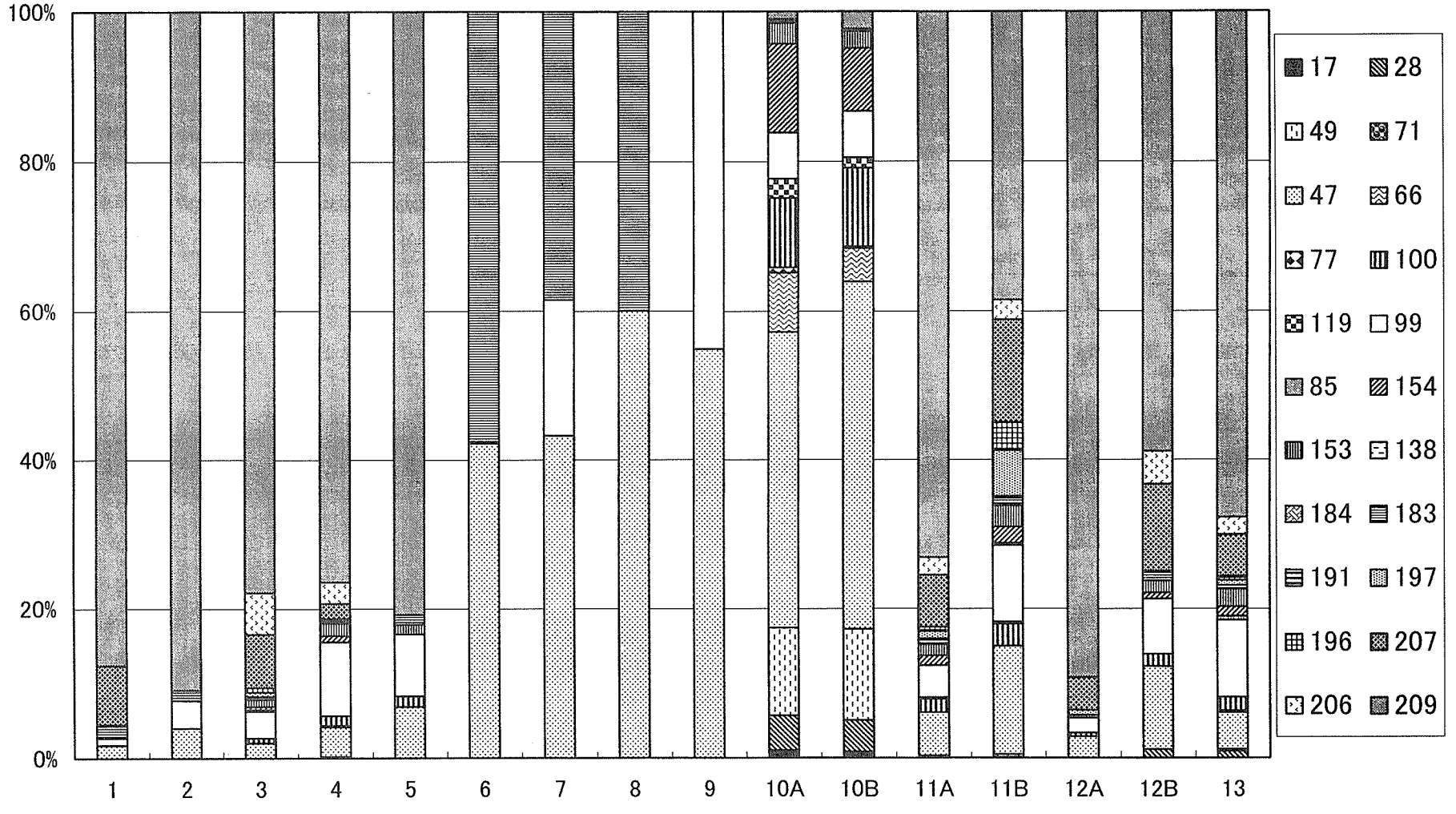

図 5　北海道地区マーケットバスケット試料における PBDE 異性体比

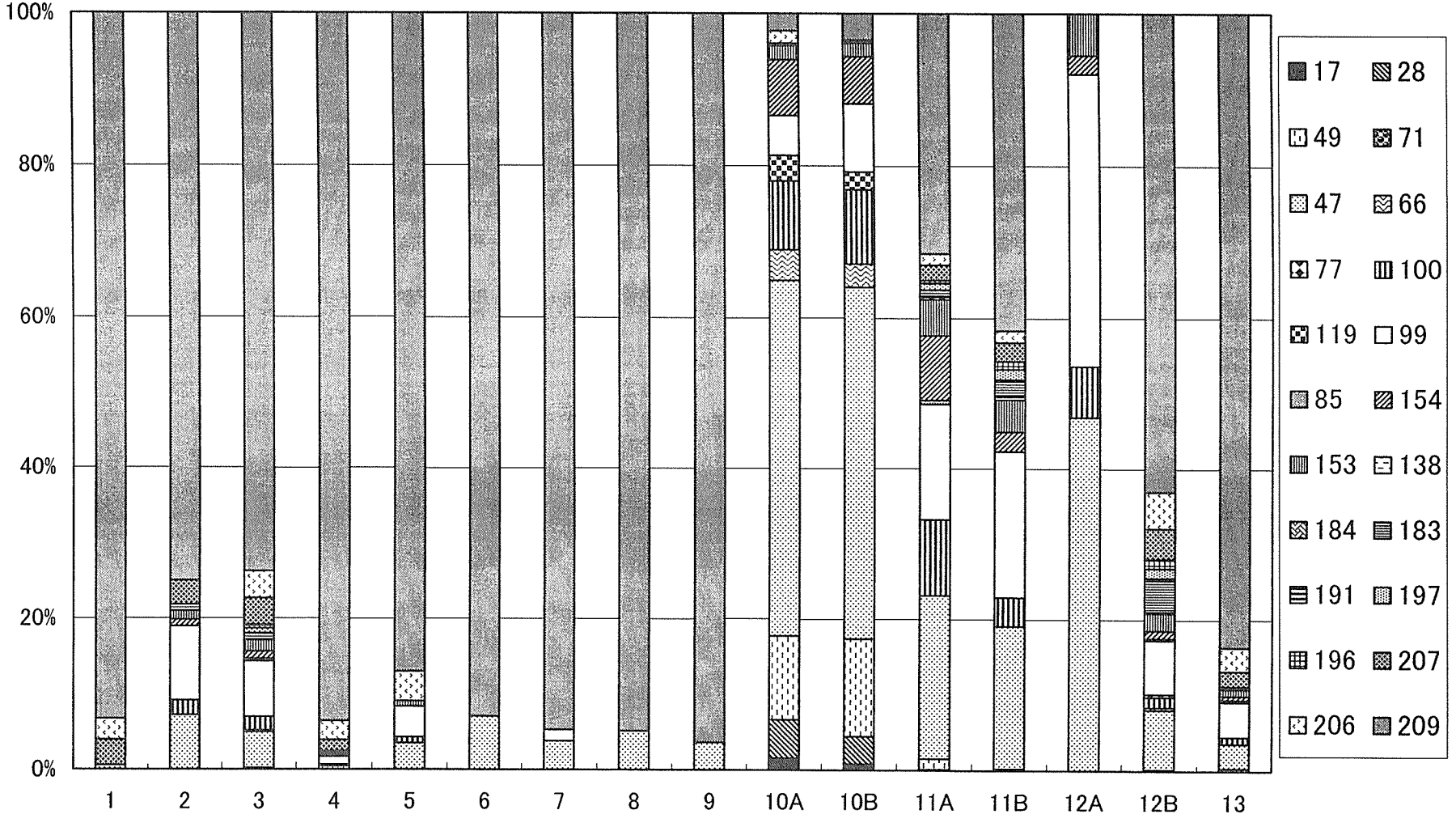

図 6 東北地区マーケットバスケット試料における PBDE 異性体比

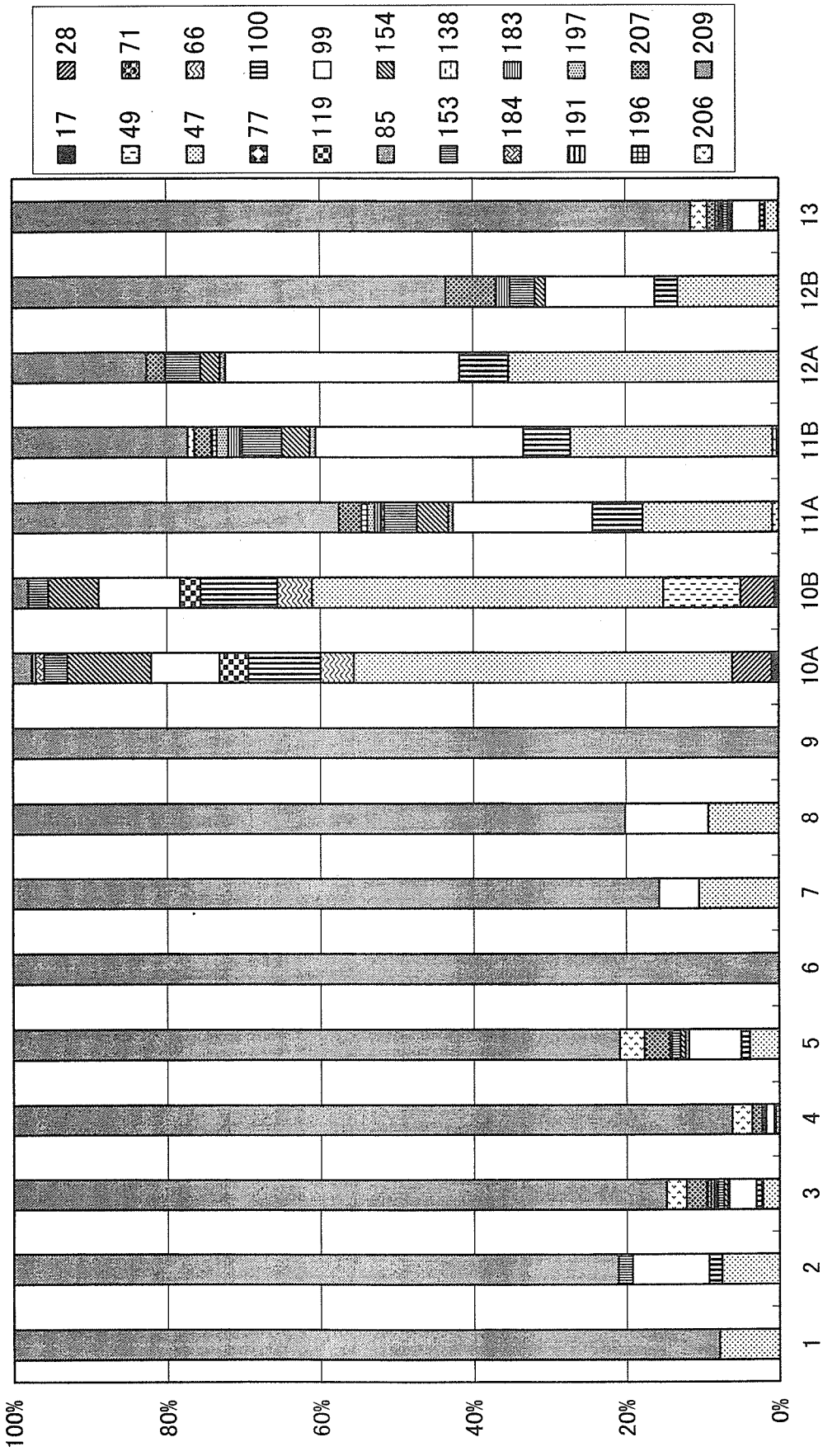

図7 中部地区マーケットバスケット試料における PBDE 異性体比

2 ヘキサブロモシクロドデカン(HBCDs)の分析

2-1 分析法のバリデーション

今回用いたHBCDsの分析方法について、第 10 群(魚介類)を用いた再現性試験(*n*=6)を実施した。結果は、表 11 に示したように、定量値はα-HBCD が 0.54 ng/g (RSD 7.7%)、γ-HBCD が 0.21 ng/g(RSD 15.0%)と良好であった。β-HBCD については検出されなかった。本分析法による回収率は $^{13}C_{12}$-α-HBCD については 62.2%～81.9% (平均 73.4% RSD 12.2%)、$^{13}C_{12}$-β-HBCD については 66.5%～92.6% (平均 83.4% RSD 11.7%)、$^{13}C_{12}$-γ-HBCD について 54.8%～90.0% (平均 73.4% RSD 15.4%)が得られた。以上の結果は、今回の添加量が、各 0.2 ng/g であったことから、残留農薬分析などの精度管理で用いられる、濃度範囲が 1ng/g 以下、試行回数 5 のとき、回収率は 50～120%、併行再現性(RSD)は30%以下とするCodex委員会の目標値に、満足する結果が得られたこととなる。

2-2 HBCDsの摂取量調査-マーケットバスケット方式による調査

北九州地区におけるマーケットバスケット試料の第 1 群から第 13 群までの分析を行った。今回の標準物質HBCDsは図8のように良好な異性体分離を示し、そのHBCDsの検出下限値はα,γは 0.02ng/g、βは 0.01ng/g であった。また、各食品群における添加回収率は表 12 に示したように、$^{13}C_{12}$ラベル体の添加量 0.2ng/g に対して第 9 群を除くと 40-130%の許容可能な回収率が得られた。第 9 群で回収率が極端に高い原因については、マトリックスの影響か否か現段階では不明である。今後、他の地区のマーケットバスケット試料でも共通に見られる現象か否かについて、明らかにしていきたい。マーケットバスケット試料の分析では、第 10 群の魚介類及び第 11 群の肉類からα、γ-HBCD が検出され(図 9)、そのΣHBCDs 濃度は第 10 群では 0.76～0.89ng/g wb(前者が 2002 年度、後者が2005年度試料;いずれも試料A及びBの平均値)、第 11 群では 0.22ng/g wb (2002年度;試料A及びBの平均値)であった(表 13)。なお、β-HBCD はいずれの検体からも検出されなかった。

表11 今回のHBCD分析法による第10群(魚介類)食品試料のHBCDs濃度と添加$^{13}C_{12}$-HBCD異性体の回収率*

No.	HBCD ng/g,wb				$^{13}C_{12}$-HBCD回収率(%)		
	α	β	γ	ΣHBCDs	α	β	γ
1	0.52	-	0.18	0.70	81.7	82.3	90.0
2	0.51	-	0.22	0.73	62.8	66.5	54.8
3	0.61	-	0.18	0.79	62.2	79.4	73.5
4	0.51	-	0.22	0.72	77.5	89.1	73.5
5	0.54	-	0.27	0.80	74.5	90.7	71.7
6	0.58	-	0.21	0.79	81.9	92.6	76.8
平均値	0.54	-	0.21	0.76	73.4	83.4	73.4
標準偏差	0.04	-	0.03	0.04	8.9	9.7	11.3
RSD(%)	7.7	-	15.0	5.5	12.2	11.7	15.4

*回収率は絶対検量線にて求めた

以上の分析結果を用いて、総HBCDsのそれぞれの年度ごとの各群からの一日摂取量を算出した(表 14)。検出された群、すなわち、第 10 群から、68.4～76.7ng/人/日(前者が 2005 年度、後者が 2002 年度試料)、第 11 群から、34.5ng/人/日(2002 年度)の摂取量であった。また、第 1 群から第 13 群までのトータルの一日摂取量を求める際、ND を 0 とした場合と、ND を検出下限値の 2 分の 1 とした場合の両方で計算を行った。それぞれの年度の各群の摂取量から換算すると、ND=0 とした場合、2002 年度では 111ng/人/日、2005 年度では 68.4ng/人/日 であった。なお、ND=1/2×LOD とした場合は、2002 年度では 155ng/人/日、2005 年度では 121ng/人/日であった 。

今回のマーケットバスケット試料から推定される北九州地区での平均的な総HBCDs摂取量は、参考資料(対象試料は表 13 及び表 14 と同一のマーケットバスケット試料)に示した 2002 年度及び 2005 年度のポリ臭素化ジフェニルエーテル(PBDEs:4～7 臭素化体)摂取量 115ng/人/日及び 68.3ng/人/日とほぼ近い値になることがわかった。ただし、その PBDEs 値には我が国での使用が多いデカブロモジフェニルエーテル(DeBDE)が含まれていないため、今後はDeBDEを含めて評価することが必要であろう。

2006 年、英国における摂取量調査[3]で、総 HBCDs 摂取量については 5.9ng/kg 体重/日未満というデータが示され、この値は毒性学的に見ても問題ないとの見解が示されている。一方、今回調査した日本人対象者の平均体重を 50kgと仮定するとその推定される総HBCDs摂取量は、ND=0のとき、平均 1.80(1.37～2.22)ng/kg 体重/日、 ND=1/2xLOD のとき、平均 2.77(2.42～3.11) ng/kg 体重 /日となり、英国での推定摂取量(＜5.9ng/kg 体重 /日)より、さらに低いため、健康上懸念される量ではないと考えられる。近年 PBDEsの

代替品として使用されるようになったが、PBDEsと同様に脂溶性が高く、残留性を有しているため、環境への排出量もPBDEsとともに今後増加することも予想される。したがって、昨年度報告したTBBPAに加えて、これからも注意深くその汚染推移を観察する必要があると思われる。

D 結論

1 臭素系ダイオキシン類(PBDD/DFs, MoBrPCDD/DFs)及び臭素化ジフェニルエーテル類(PBDEs)のマーケットバスケット方式による三地域(北海道、東北、中部地区)の摂取量調査では第4群の油脂類から1,2,3,4,6,7,8-HpBDFが検出されたが、その他の群では臭素系ダイオキシン類は検出されなかった。臭素化ジフェニルエーテルはすべての群から検出された。異性体別では四臭素化体#47、及び十臭素化体#209の寄与が大きかった。ND=0とした場合の臭素系ダイオキシン類の一日摂取量は平均で0.00071pgTEQ/kg体重/日、臭素化ジフェニルエーテル類の一日摂取量は平均で1.83ng/kg体重/日であった。

2 検討したHBCDsの分析法は再現性及び回収率も良好であった。また、予備調査での北九州地区における、HBCDsの一日摂取量はND=0とした場合2002年と2005年の平均では1.80 ng/kg体重/日であった。これは英国での推定摂取量(< 5.9ng/kg 体重 /日)より、さらに低いため、健康上懸念される量ではないと考えられた。

E 研究発表

1 論文発表

・ Nakagawa R, Ashizuka Y, Hori T, Yasutake D, Tobiishi K, Sasaki K. Determination of brominated flame retardants and brominated dioxins in fish collected from three regions of Japan. *Organohalogen Compounds*, 68(2006) 2166-2169.

・ Ashizuka Y, Nakagawa R, Hori T, Yasutake D, Tobiishi K, Sasaki K. Determination of brominated flame retardants and brominated dioxins in fish collected from three regions of Japan. *Molecular Nutrition & Food Reseach* (投稿中)

2 学会・協議会発表

・芦塚由紀, 中川礼子, 堀 就英, 村田さつき, 安武大輔, 佐々木久美子 食品における臭素化ダイオキシン及びその関連化合物の汚染実態調査. 第43回全国衛生化学技術協議会、鳥取県、11月1-2日、2006年.

・芦塚由紀, 中川礼子, 堀 就英, 安武大輔, 佐々木久美子 食品における臭素化ダイオキシン及び臭素系難燃剤の汚染実態調査. 第9回環境ホルモン学会、東京都、11月11-12日、2006年.

参考文献

1) 厚生労働科学研究「ダイオキシン類

による食品汚染実態の把握に関する研究」平成17年度 研究報告書
2) Darnerud, P. O., Eriksen, G. S., Jóhannesson, T., Larsen, P. B., Viluksela, M., Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. *Environ. Health Perspect.*, 109 supplement1 (2001) 49-68.
3) http://www.food.gov.uk/science/surveillance/fsisbranch2006/fsis1006

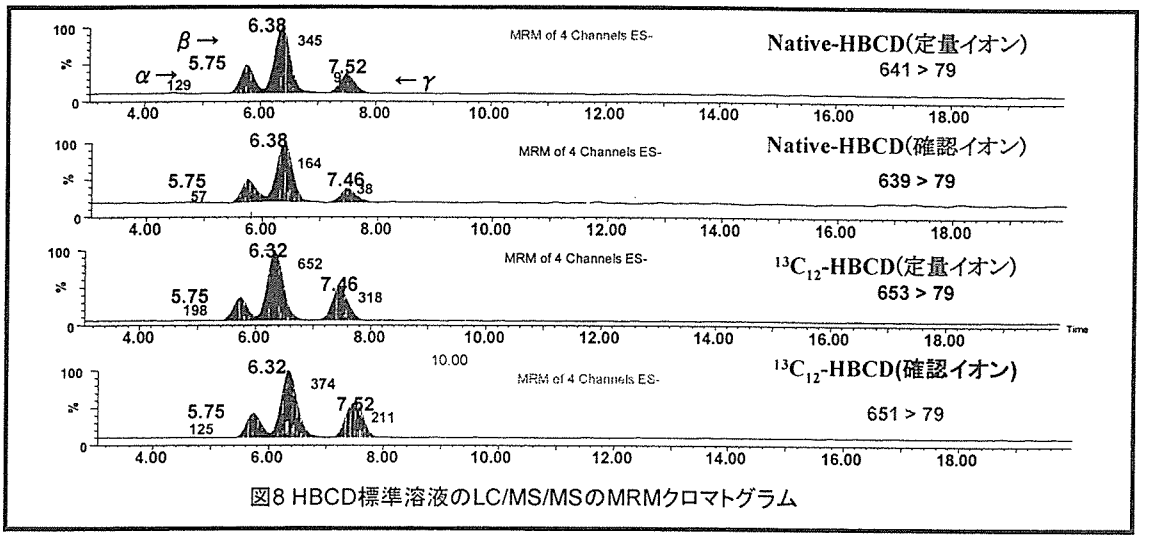

図8 HBCD標準溶液のLC/MS/MSのMRMクロマトグラム

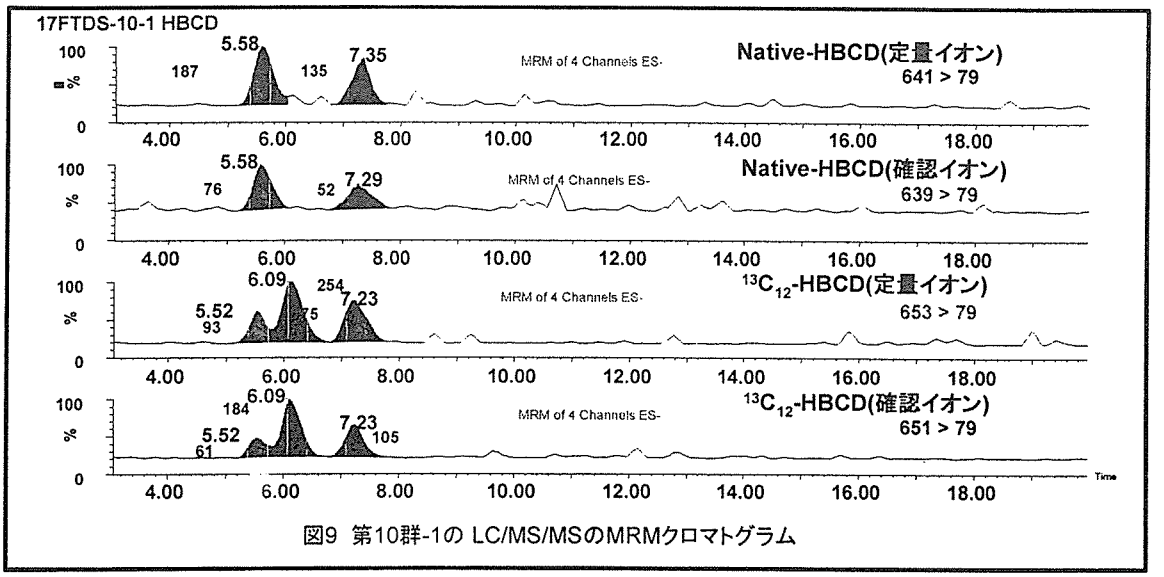

図9 第10群-1の LC/MS/MSのMRMクロマトグラム

表12　各$^{13}C_{12}$-HBCD異性体の添加回収試験の結果

マーケットバスケット試料		$^{13}C_{12}$-HBCD異性体の回収率(%)		
		α	β	γ
第1群	(米類)	64.3	75.1	58.8
第2群	(米以外の穀類)	53.8	64.6	68.9
第3群	(砂糖・菓子類)	42.1	61.2	53.5
第4群	(油脂類)	43.4	58.9	52.7
第5群	(豆類)	42.7	77.5	73.4
第6群	(果実類)	50.6	87.2	61.8
第7群	(緑黄色野菜)	77.7	122.1	123.6
第8群	(その他の野菜)	81.6	122.9	103.2
第9群	(調味嗜好飲料)	182.4	225.7	186.4
第10群	(魚介類)A	69.5	86.3	69.3
第10群	(魚介類)B	73.4	83.4	73.4
第11群	(肉・卵類)A	68.8	88.0	81.7
第11群	(肉・卵類)B	55.8	66.7	67.6
第12群	(乳類)A	85.5	99.6	97.1
第12群	(乳類)B	106.4	130.4	105.4
第13群	(調味料)	108.0	70.7	103.7

表13 マーケットバスケット試料(北九州地区)におけるHBCDs濃度

マーケットバスケット試料		2002年度 HBCD (ng/g,wb)				2005年度 HBCD (ng/g,wb)			
		α	β	γ	ΣHBCDs	α	β	γ	ΣHBCDs
第1群	(米類)	ND	ND	ND	ND	ND	ND	ND	ND
第2群	(米以外の穀類)	ND	ND	ND	ND	ND	ND	ND	ND
第3群	(砂糖・菓子類)	ND	ND	ND	ND	ND	ND	ND	ND
第4群	(油脂類)	ND	ND	ND	ND	ND	ND	ND	ND
第5群	(豆類)	ND	ND	ND	ND	ND	ND	ND	ND
第6群	(果実類)	ND	ND	ND	ND	ND	ND	ND	ND
第7群	(緑黄色野菜)	ND	ND	ND	ND	ND	ND	ND	ND
第8群	(その他の野菜)	ND	ND	ND	ND	ND	ND	ND	ND
第9群	(調味嗜好飲料)	ND	ND	ND	ND	ND	ND	ND	ND
第10群	(魚介類)A	0.59	ND	0.2	0.79	0.56	ND	0.47	1.03
第10群	(魚介類)B	0.54	ND	0.19	0.73	0.54	ND	0.21	0.75
第11群	(肉・卵類)A	0.43	ND	ND	0.43	ND	ND	ND	ND
第11群	(肉・卵類)B	ND	ND	ND	ND	ND	ND	ND	ND
第12群	(乳類)A	ND	ND	ND	ND	ND	ND	ND	ND
第12群	(乳類)B	ND	ND	ND	ND	ND	ND	ND	ND
第13群	(調味料)	ND	ND	ND	ND	ND	ND	ND	ND

表14　マーケットバスケット試料(北九州地区)におけるHBCDsの一日平均摂取量

マーケットバスケット試料	2002年度							2005年度						
	食品摂取量(g/日)	HBCD (ng/人/日)						食品摂取量(g/日)	HBCD (ng/人/日)					
		ND=0			ND=1/2×LOD				ND=0			ND=1/2×LOD		
		α	β	γ	α	β	γ		α	β	γ	α	β	γ
第1群　(米類)	459.0	ND	ND	ND	4.59	2.30	4.59	446.5	ND	ND	ND	4.47	2.23	4.47
第2群　(米以外の穀類)	226.1	ND	ND	ND	2.26	1.13	2.26	209.9	ND	ND	ND	2.10	1.05	2.10
第3群　(砂糖・菓子類)	36.6	ND	ND	ND	0.37	0.18	0.37	33.7	ND	ND	ND	0.34	0.17	0.34
第4群　(油脂類)	15.2	ND	ND	ND	0.15	0.08	0.15	11.5	ND	ND	ND	0.12	0.06	0.12
第5群　(豆類)	80.4	ND	ND	ND	0.80	0.40	0.80	56.4	ND	ND	ND	0.56	0.28	0.56
第6群　(果実類)	130.6	ND	ND	ND	1.31	0.65	1.31	136.0	ND	ND	ND	1.36	0.68	1.36
第7群　(緑黄色野菜)	108.3	ND	ND	ND	1.08	0.54	1.08	96.6	ND	ND	ND	0.97	0.48	0.97
第8群　(その他の野菜)	234.6	ND	ND	ND	2.35	1.17	2.35	219.0	ND	ND	ND	2.19	1.10	2.19
第9群　(調味嗜好飲料)	172.2	ND	ND	ND	1.72	0.86	1.72	504.2	ND	ND	ND	5.04	2.52	5.04
第10群　(魚介類)*	100.8	57	ND	19.7	57.0	0.50	19.7	90.7	49.3	ND	19.1	49.3	0.45	19.1
第11群　(肉・卵類)*	157.9	34.5	ND	ND	35.3	0.79	1.58	133.6	ND	ND	ND	1.34	0.67	1.34
第12群　(乳類)*	122.5	ND	ND	ND	1.23	0.61	1.23	146.8	ND	ND	ND	1.47	0.73	1.47
第13群　(調味料)	38.1	ND	ND	ND	0.38	0.19	0.38	80.9	ND	ND	ND	0.81	0.40	0.81
各HBCD摂取量 ng/日		91.5	0	19.7	108.5	9.4	37.5		49.3	0	19.1	70.1	10.8	39.9
ΣHBCDs推定摂取量 ng/日				111			155				68.4			121
ΣHBCDs推定摂取量 ng/kg体重/日				2.22			3.11				1.37			2.42

一日平均摂取量を算出する場合、第10,11,12群については各々平均摂取量を採用した。
α、γ-HBCDのLOD値は0.02ng/g、β-HBCDのLOD値は0.01ng/gとした。

参考資料　マーケットバスケット試料（北九州地区）のTBBPA及びΣPBDEs摂取量

マーケットバスケット試料	TBBPA （ng/人/日）				ΣPBDEs （ng/人/日）			
	2002年度		2005年度		2002年度		2005年度	
	ND=0	ND=1/2x LOD	ND=0	ND=1/2x LOD	ND=0	ND=1/2x LOD	ND=0	ND=1/2x LOD
第1群（米類）	0.00	2.05	0.00	2.23	0.11	0.40	0.19	0.43
第2群（米以外の穀類）	0.00	0.96	0.00	1.05	0.37	0.49	0.50	0.58
第3群（砂糖・菓子類）	0.33	0.33	0.33	0.33	0.17	0.19	0.67	0.67
第4群（油脂類）	0.15	0.15	0.00	0.06	1.85	1.86	0.65	0.66
第5群（豆類）	0.73	0.73	0.00	0.28	0.30	0.33	0.13	0.16
第6群（果実類）	0.00	0.57	0.00	0.68	0.00	0.09	2.60	2.65
第7群（緑黄色野菜）	0.00	0.43	0.00	0.48	0.05	0.11	0.10	0.14
第8群（その他の野菜）	0.00	0.92	0.00	1.10	0.03	0.18	0.06	0.20
第9群（調味嗜好飲料）	0.00	0.86	0.00	2.52	0.02	0.13	0.00	0.33
第10群（魚介類）*	50.7	51.4	2.72	2.95	102	102	57.8	57.8
第11群（肉・卵類）*	4.42	4.68	0.67	1.00	9.17	9.21	4.19	4.21
第12群（乳類）*	0.00	0.61	0.00	0.73	1.04	1.10	0.74	0.79
第13群（調味料）	0.00	0.19	0.00	0.41	0.06	0.08	0.65	0.68
推定摂取量 ng/日	56.3	63.9	3.7	13.8	115	116	68.3	69.3
推定摂取量 ng/kg/日	1.13	1.28	0.07	0.28	2.30	2.32	1.37	1.39

10,11,12群についてはn=2の平均摂取量を採用した。
TBBPAのLOD値は、0.01ng/g、各PBDE同族体のLOD値は0.0001ng/g。

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	Nakagawa R., Ashizuka Y., Hori T., Yasutake D., Tobiishi K., Sasaki K.	Determination of brominated flame retardants and brominated dioxins in fish collected from three regions of Japan.	Organohalogen Compounds	68	2166-2169	2006
2	Tsutsumi T., Amakura Y., Matsumoto T., Ito Y., Kurihara H., Sasaki K., Maitani T.	Removal of dioxins from retail fish by high-speed solvent extraction	Organohalogen Compounds	68	2473-2476	2006
3	Tsutsumi T., Amakura Y., Okuyama A., Tanioka Y., Sakata K., Sasaki K., Maitani T	Application of an ELISA for PCB 118 to the screening of dioxin-like PCBs in retail fish	Chemosphere	65	467-473	2006
4	Tsutsumi T., Amakura Y., Sasaki K., Maitani T	Dioxin concentrations in the edible parts of Japanese common squid and saury	J. Food Hyg. Soc. Japan	48	8-12	2007

DETERMINATION OF BROMINATED FLAME RETARDANTS AND BROMINATED DIOXINS IN FISH COLLECTED FROM THREE REGIONS OF JAPAN

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Introduction

In Japan, tetra brominated bisphenol A (TBBPA) totaling nearly 32,000 tons was consumed in 2001, which is ten times more than deca brominated diphenyl ether (DecaBDE), the only polybrominated diphenyl ether (PBDE) used in Japan (2,200 tons). Due to the worldwide usage of these brominated flame retardants (BFRs), their detection has been reported not only in environmental samples such as effluents from BFR manufacturing plants and textile plants, but also in human breast milk[1]. Therefore, the social relevance of the environmental pollution and human toxicities of BFRs is of great concern. In addition, PBDEs and TBBPA are suspected as polybrominated dioxin (PBDD/DFs) originating chemicals, because such dioxins were found in formulations of the BFRs. PBDEs and PBDD/DFs are lipophilic and likely to be bio-accumulated in organisms through the food web. Consequently the pollution in environmental samples, in particular that in food, should be cleared as quickly as possible. Additionally, it is important to investigate whether there are differences in the pollutant levels among sampling areas, to judge the pollution status of BFRs and PBDD/DFs in Japan. Here, we report the pollution levels of PBDEs, TBBPA, and PBDD/DFs in marine products purchased at food market stores in three regions of Japan from 2004 to 2005. The regions were Kyushu, a less industrialized area; Seto Inland Sea, an industrialized area; and Nagoya, a commercialized and industrialized area. Additionally, we estimate the daily intakes of PBDEs, TBBPA, and PBDD/DFs by multiplying each analyte concentration with fish weight consumed by an average Japanese adult.

Materials and Methods

Marine products: fifteen marine products were collected from each of the three regions of Nagoya, Seto Inland Sea, and Kyushu.

Analysis of PBDEs and PBDD/DFs: Forty-five marine products in total were analyzed for PBDEs and PBDD/DFs, following the procedure described in our previous report[2]. The analytical procedure consisted of freeze-drying, accelerated solvent extraction (100℃,1500 psi, n-hexane) and purification with sulfuric acid treatment, and three kinds of column chromatography of silica gel, florisil, and active carbon. Measurement was conducted by an isotope dilution method using a high resolution gas chromatograph/ high resolution mass spectrometer (HRGC/HRMS) (Table 1). Detection limits of PBDD/DFs were 0.01 pg/g, wb for tetra and penta brominated DD/DFs, 0.05 pg/g, wb for hexa brominated DD/DFs, and 0.1 pg/g, wb for hepta brominated DF. Detection limits of PBDEs were 0.1 pg/g, wb for from tetra- to octa-BDE, and 0.2 pg/g, wb for nona-BDE and 1.0 pg/g, wb of DeBDE (#209).

Analysis of TBBPA: A homogenized sample (5 g) was spiked with $^{13}C_{12}$-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 10 mL of n-hexane. Then, to the methanol layer, 120 mL of 5% sodium chloride solution was added and re-extracted twice with 25 mL of dichloromethane. The extract was concentrated to dryness, and then 1mL of 1N potassium hydroxide / ethanol and 0.2 mL of diethyl sulfate were added, and the mixture was kept at 30 ℃ for 30 min. To the mixture, 4 mL of 1N potassium hydroxide / ethanol was added and kept at 70℃ for 1 hour. Then 3 mL of water was added, and it was re-extracted with n-hexane. The n-hexane extract was cleaned with florisil mini-column chromatography using an elution solvent of 8 mL of 2% diethyl ether/n-hexane The final 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 1). The detection limit of TBBPA was 0.01ng/g, wb.

Table 1 The HRGC/HRMS conditions for PBDD/DF, PBDE AND TBBPA analysis

HRGC: HP6890 (Hewlett Packard)
PBDD/DF analysis : Column:DB-5 (J&W) 0.25mmi.d. x 30m, film thickness 0.1μm **Injector temp.:260℃** **Column Temp:130℃(1min)-20℃/min-240℃-5℃/min-320℃(7.5min)**
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)**
HRMS: Autospec Ultima (MicroMass) **Electron energy,38eV; filament current, 750μA; ion source Temp.,270℃; resolution,10000**

Results and Discussion

The levels of PBDEs, PBDD/DFs, and TBBPA in 45 marine products from the three regions are summarized in Table 2. PBDEs were detected in all of the samples. The means and ranges of ΣPBDEs were 0.75 ng/g, wb and 0.01 to 0.70 ng/g, wb for Nagoya (N), 0.16 ng/g, wb and 0.01 to 0.53 ng/g, wb for Seto Inland Sea (S), and 0.15 ng/g, wb and 0.01 to 0.70 ng/g, wb for Kyushu (K). TBBPA was detected in a part of the samples. The detection rates were 53.3% for S and N and 86.7% for K. The means were 0.01 to 0.02 ng/g, wb for all regions. The mean of TBBPA was about one-tenth or less, compared with those of ΣPBDEs. PBDD/DFs were only detected in eight fish of the S region. They are 1,2,3,4,6,7,8-heptabrominated dibenzofuran (25.6 pg/g wb of Pike eel shown in Fig. 2, 0.42 pg/g wb of flatfish, 0.276 pg/g wb of natural sea bream A, 0.217 pg/g wb of conger eel, 0.114 pg/g wb of sole, 0.175 pg/g wb of young seerfish, 0.104 pg/g wb of sea bream B), 2,3,7,8-tetrabrominated dibenzo-p-dioxin (0.016pg/g wb of natural sea bream A shown in Fig. 3), 2,3,7,8-tetrabrominated dibenzofuran and 3-bromo-2,7,8-trichlorinated dibenzofuran (0.029 pg/g wb and 0.020 pg/g wb shown in Fig. 4 and 5) of conger eel. The mean and range of PBDD/DFs in the S region was 0.19 pg TEQ/g wb and ND to 0.256 pg TEQ/g wb. These findings would support that the S region has been polluted by PBDD/DF emissions from industry plants. Assuming that an adult person every day consumes 82.2 g of fish contaminated with the means of the three regions, that is, 0.35 ng/g of ΣPBDEs, 0.02 ng/g of TBBPA, and 0.006pgTEQ/g of ΣPBDD/DFs, the estimated daily intake of ΣPBDEs, TBBPA, and ΣPBDD/DFs from fish would result in 28.8 ng of ΣPBDEs, 1.64 ng of TBBPA, and 0.49 pg TEQ of ΣPBDD/DFs. In particular, compared with the Japanese daily intake of polychlorinated dioxins from fish (44.11 pg TEQ) investigated in 2004, the above intake of ΣPBDD/DFs is very low.

Table 2 The mean levels and ranges of PBDEs,TBBPA and PBDD/DFs in marine products from three regions of Japan

	Nagoya	Seto Inland Sea	Kyushu	Mean
ΣPBDEs (ng/g,wb)	0.75(0.01-0.70)	0.16(0.01-0.53)	0.15(0.01-0.70)	0.35
TBBPA(ng/g, wb) ND=0	0.01(ND-0.04)	0.02(ND-0.10)	0.02(ND-0.11)	0.02
ΣPBDD/DFs(pgTEQ/g, wb) ND=0	0.000	0.019(ND-0.256)	0.000	0.006

Fig. 1a and 1b show the correlations between ΣPBDEs and fat content (Fig. 1a) or fish size (Fig. 1b). Since PBDE is lipophilic, good correlation between ΣPBDEs and fat content (R^2=0.431) is reasonable. However, better correlation (R^2=0.618) was obtained between ΣPBDEs and fish size. In Table 2, one reason why ΣPBDEs in fish from the N region were higher than those in fish from other regions might have been the difference in fish size. On the other hand, TBBPA didn't correlate with fish size or fat content. Table 3

shows PBDE congener levels of cultivated or natural sea breams and conger eel. The cultivated sea breams contained relatively higher levels of ΣPBDEs than those of the natural sea breams. As a source of PBDE in cultivated fish, feed used for cultivation is suspected. For the cultivated sea breams, congeners #47 and #100 were big contributors. For the natural sea breams, the contribution of congener #47 had decreased, but that of congener #209 had increased. Particularly, for the pike eel with a high level of ΣPBDEs, contribution of #209 was also large (25%; the same as that of #47). Additionally, congeners # 206 and #207 contributed nearly 10% each. In the pike eel, 1,2,3,4,6,7,8-HpBDF was detected abundantly compared with the levels detected in the other six fish. Therefore, we speculated that congeners #209, #206, and #207 are closely related to 1,2,3,4,6,7,8-HpBDF. This is also supported by the finding of 1,2,3,4,6,7,8-HpBDF in the sea water into which effluents from DBDE(#209) manufacturing plant[3] were possibly dumped.

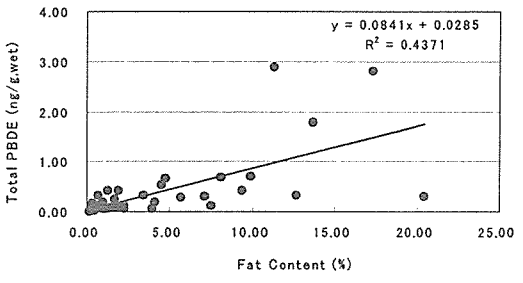

Fig.1aCorrelation between Total PBDE and Fat Content

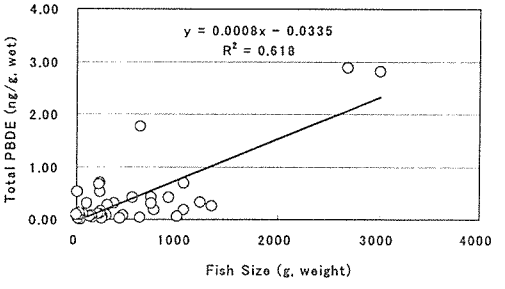

Fig.1b Correlation between Total PBDE and Fish Size

Table 3 PBDE congener levels(pg/g) in cultured and natural sea breams and a pike eel in which 1,2,3,4,6,7,8-HpBDF was detected .

	Cultivated Sea	Cultivated Sea	Natural Sea	Natural Sea bream(K)	Natural Pike eel
2,,4,4'-TriBDE(#28)	28.9	25.9	2.2	2.0	4.8
2,2',4,5'-TeBDE(#49)	52.6	4.5	0.5	1.3	20.2
2,2',4,4'-TeBDE(#47)	365.1	236.0	18.0	18.1	80.3
2,3',4,4'-TeBDE(#66)	19.2	19.8	1.7	1.8	4.5
2,2',4,4',6-PeBDE(#100)	93.0	60.4	2.2	5.0	16.6
2,3',4,4',6-PeBDE(#119)	0.0	9.6	0.3	0.8	6.2
2,2',4,4',5-PeBDE(#99)	17.8	12.2	1.9	0.8	11.2
2,2',4,4',5,6'-HxBDE(#154)	41.4	39.7	4.2	5.5	24.1
2,2',4,4',5,5'-HxBDE(#153)	4.7	1.5	1.5	0.4	8.1
2,2',3,3',4,4',5,6,6'-NoBDE(#207)	2.6	0.6	0.8	0.9	26.2
2,2',3,3',4,4',5,5',6'-NoBDE(#206)	2.2	0.0	0.7	0.7	24.5
DeBDE(#209)	51.4	5.7	13.7	10.8	79.6
Total PBDEs*	681.1	419.8	50.1	50.1	306.3

* Total PBDEs includes minor PBDE congeners not shown in the Table.

Acknowledgements
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References

1. Fängström B, Strid A, Grandjean P, Weihe P, Bergman Å. *Environmental Health:A Global Access Science Source* 2005;4:12.
2. Ashizuka Y, Nakagawa R, Tobiishi K, Hori T, Iida T. *J. Agric. Food Chem.* 2005;53;3807-3813.

3. http://www.env.go.jp/air/report/h16-12/index.html *Investigation Report on the emission of poly-brominated dioxins (in Japanese)*2005.

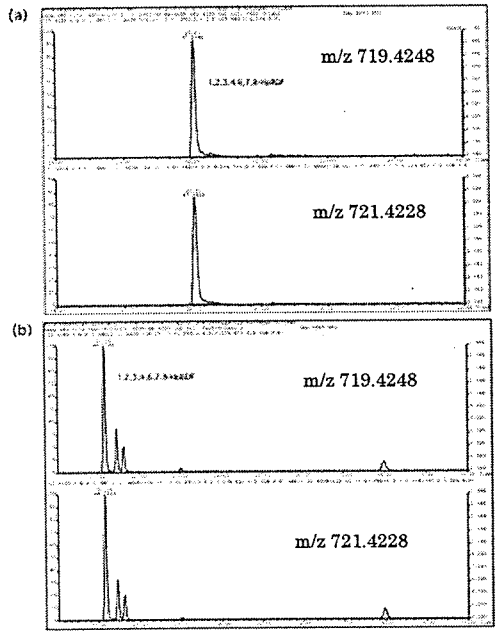

Fig. 2 MS chromatograms of1,2,3,4,6,7,8-HpBDFin natural Pike eel
(a) Column: DB-5 (b) Column: MP65HT

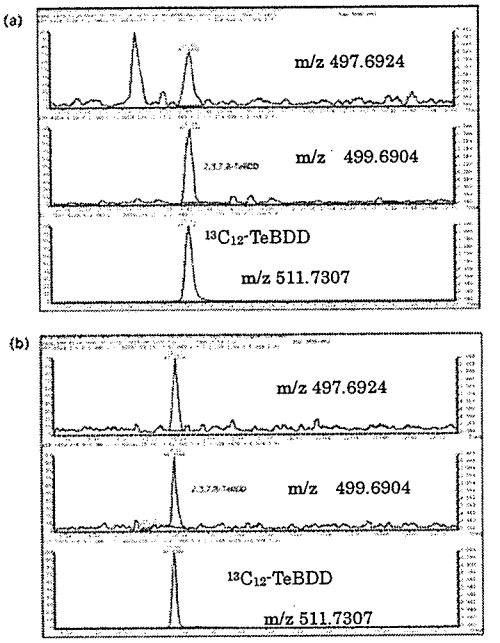

Fig. 3 MS chromatograms of1,2,3, 7,8-TeBDD in natural Sea bream
(a) Column: DB-5 (b) Column: MP65HT

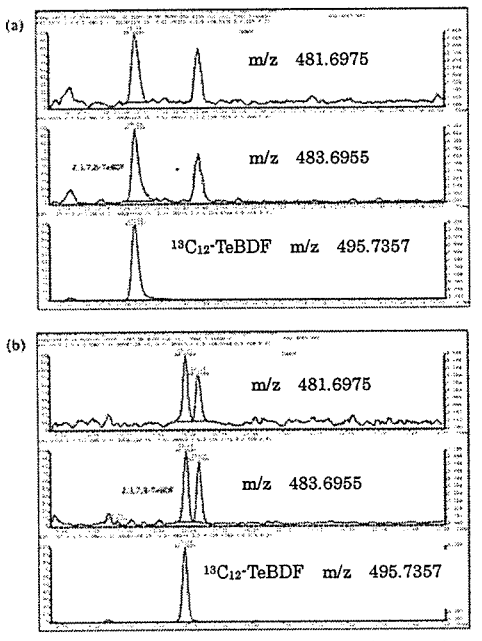

Fig. 4 MS chromatograms of1,2,3, 7,8-TeBDF in natural Conger eel
(a) Column: DB-5 (b) Column: MP65HT

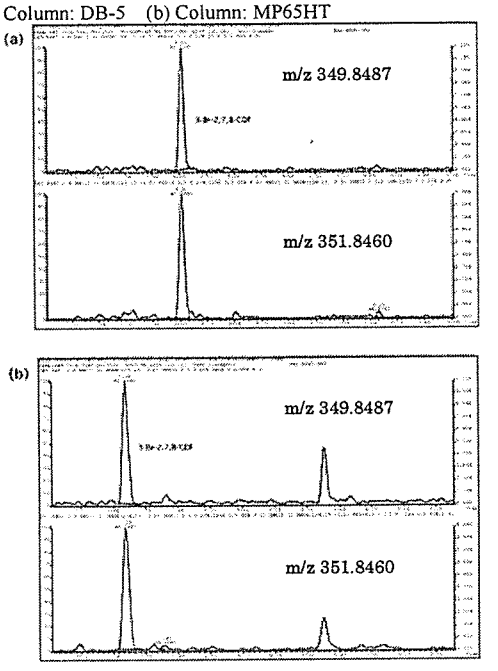

Fig.5 MS chromatograms of1 3-Br-2,7,8-CDF in natural Conger eel
(a) Column : DB-5 (b) Column: MP65HT

REMOVAL OF DIOXINS FROM RETAIL FISH BY HIGH-SPEED SOLVENT EXTRACTION

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Introduction

Studies of the Japanese diet have identified fish and shellfish as the main sources of PCDD/Fs and dioxin-like PCBs (dioxins).[1,2] Assessing the risk posed by dioxins in retail fish requires the development of rapid quantitative methods. HRGC/HRMS is the standard technique for dioxin analysis; however, the lengthy extraction process makes it time consuming. The most widely used method for extracting fish dioxins is Soxhlet extraction, although alkaline digestion followed by solvent extraction is also often used in Japan. Both conventional methods take over 16 h. Some new techniques, such as pressurised liquid extraction (or accelerated solvent extraction) have been applied to dioxins, but rarely to those in fish samples. We recently developed a high-speed method based on extraction in heated liquid solvents under near-atmospheric pressure. This technique has been used for dioxin extraction from contaminated soil and fly ash, yielding similar concentrations to Soxhlet extraction but much more rapidly.[3] Here, we report the first data from the application of this method to the extraction of dioxins from retail fish.

Materials and Methods

Samples: Retail fish samples were purchased during the years 2004 and 2005 from supermarkets in Tokyo, Japan. The muscular parts of the samples were homogenised using a food cutter and stored at –20°C until required for analysis.

High-speed solvent extraction: A model SE-100 (Dia Instruments Co., Ltd., Japan) high-speed solvent extractor was employed. Homogenised fish samples (20 g) and sodium anhydrous sulphate (80 g) were ground into powder using a mortar and pestle. The samples were then packed into 160-ml stainless-steel extraction cells. The dead volume was filled with extraction solvents and the top of the cell was sealed with a cap. $^{13}C_{12}$-labelled internal standards were used to spike samples before extraction, and also to spike extracts in order to determine the following optimal extraction conditions: 30°C and 80°C when using acetone/*n*-hexane (1:1) and toluene, respectively, as extraction solvents. The flow rate was set at 6 ml/min. A schematic diagram of the extractor is shown in Figure 1.

Alkaline digestion followed by hexane extraction: The extracts were prepared as described previously.[4] Homogenised fish samples (20 g) spiked with $^{13}C_{12}$-labelled internal standards were incubated in aqueous KOH for 16 h at room temperature. The alkaline hydrolysates were added to methanol and extracted three times by mechanically shaking with *n*-hexane.

Cleanup and HRGC/HRMS analysis: The cleanup and analysis of dioxins generally followed the methods reported previously.[4] Briefly, the extracts were treated with concentrated sulphuric acid and then purified on a silver nitrate/silica gel column. The elute obtained with *n*-hexane was loaded onto an alumina column. After washing with *n*-hexane, the first fraction (containing mono-*ortho* PCBs) was eluted with 2% dichloromethane/*n*-hexane, while the second fraction (containing non-*ortho* PCBs and PCDD/Fs) was eluted with 60% dichloromethane/*n*-hexane. The second fraction was then loaded onto an activated carbon column and eluted with toluene. Both fractions were spiked with $^{13}C_{12}$-labelled recovery standards. The quantification of dioxins was conducted using an HP6890-plus gas chromatograph coupled to a JEOL JMS-700 mass spectrometer. The determination of 2,3,7,8-chlorine-substituted PCDD/Fs was performed in DB-5MS and DB-17 columns. The determination of dioxin-like PCBs was performed in an HT-8 column. The limits of quantification were 0.01–0.2 pg/g for PCDD/Fs and non-*ortho* PCBs, and 0.5–3.0 pg/g for mono-*ortho* PCBs. The TEQ concentrations were calculated using the WHO-TEFs.

Results and Discussion

We initially determined the extraction conditions for the fish dioxins using the high-speed extractor with various extraction times and solvents. Two types of fish, sea bass and yellowtail, were treated with acetone/*n*-hexane for up to 4 h, followed by toluene for 1 h (Figure 2). The cumulative concentrations of 2,3,7,8-chlorine-substituted PCDD/Fs and dioxin-like PCBs reached a plateau after 1 h of extraction with acetone/*n*-hexane in both samples. Although the sea bass samples contained relatively high amounts of dioxin-like PCBs, the 1-h extraction period was sufficient to extract them fully. This was therefore selected as the recommended extraction condition for the practical analysis of fish dioxins.

The suitability of the high-speed solvent extraction method for analysing fish dioxins was compared with that of the conventional alkaline digestion extraction. Table 1 shows the concentrations and relative standard deviations (RSDs) for the two methods when applied to yellowtail samples. The concentration ratios of the two methods were 0.9–1.1, indicating that the concentrations of each isomer were similar for both extractions. The RSDs of the quantified isomers using the novel method were acceptable (0.0–17.4%), and were similar to those obtained using the conventional method (0.0–24.2%). The recoveries of the internal quantification standards using the new method were 72.8–109%, and were similar to those obtained using the conventional method (67.5–105%). The selected ion-mode chromatograms obtained from both extractions were visually inspected, but showed no differences in the homologous groups of dioxins present (data not shown). These results suggest that the methods tested achieved similar extraction efficiencies for dioxins to the conventional extraction method.

Finally, we used the high-speed extraction method to determine the TEQ concentrations of samples of 12 popular retail fish from Japan compared with those obtained by the conventional extraction. As shown in Figure 3, the TEQ concentrations produced by both extractions showed excellent correlations for both PCDD/Fs (r = 0.99) and dioxin-like PCBs (r = 0.99), with the slopes and y-intercepts of the linear regression equations being close to 1 and 0, respectively. This confirmed that the TEQ concentrations obtained using the present method were comparable to those obtained with the conventional extraction method.

Overall, our results indicate that high-speed solvent extraction is a useful method for extracting dioxins from retail fish. The main advantage of this method is the short extraction time (~1 h) compared with the alkaline digestion extraction method (~20 h). This method allows the rapid determination of dioxins and will therefore be a valuable tool for monitoring dioxin levels in retail fish.

Acknowledgements

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References

1. Tsutsumi T, Yanagi T, Nakamura M, Kono Y, Uchibe H, Iida T, Hori T, Nakagawa R, Tobiishi K, Matsuda R, Sasaki K, Toyoda M. *Chemosphere* 2001;45:1129.
2. Sasamoto T, Ushio F, Kikutani N, Saitoh Y, Yamaki Y, Hashimoto T, Horii S, Nakagawa JH, Ibe A. *Chemosphere* (in press).
3. Kato M, Urano K, Shimizu Y, Oguchi M, Ito Y, Kurihara H. *12th Symposium on Environmental Chemistry*, Programs and Abstracts, Japan Society for Environmental Chemistry 2003:616.
4. Tsutsumi T, Amakura Y, Sasaki K, Toyoda M, Maitani T. *Analytical and Bioanalytical Chemistry* 2003;375:792.