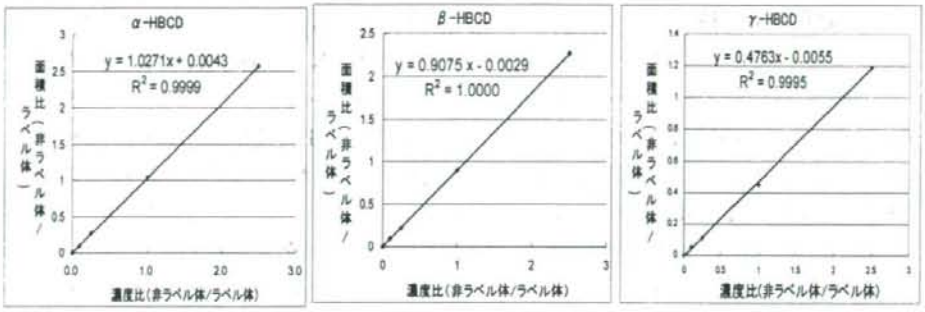

図 7 HBCDs 検量線

2-1-4 TBBPA の検量線と分析精度

100 mL 容ナス型フラスコに非ラベル化 TBBPA を各 0、0.2、0.5、1.0、1.5 ng 添加した 5 組に、それぞれラベル化 TBBPA 1.0 ng を添加し、それぞれ試料と同様の方法で前処理・測定して検量線を作成した。検量線は直線性を示した(R^2=0.9981、図 8)。また、実験方法に従い、第 10 群及び第 12 群のマーケットバスケット食事試料を用いて得られた $^{13}C_{12}$-TBBPA の回収率を内標準法で求めると、第 10 群と第 12 群で平均 100.5%と 90.2%で、RSD（併行精度）は、10 群が 7.4%、12 群が 4.8%と良好な結果であった(表 25)。

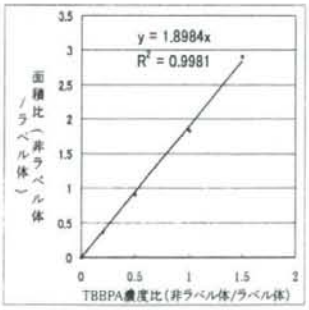

図 8 TBBPA 検量線

表 25 第 10 群（魚介類）及び第 12 群（乳類）食品試料の TBBPA 濃度と添加 $^{13}C_{12}$-TBBPA の回収率*

No.	第10群		第12群	
	TBBPA (ng/g,wb)	$^{13}C_{12}$-TBBPA 回収率(%)	TBBPA (ng/g,wb)	$^{13}C_{12}$-TBBPA 回収率(%)
1	0.035	96.2	0.032	92.1
2	0.052	90.9	0.028	88.7
3	0.045	99.9	0.024	91.4
4	0.045	106.0	0.036	83.6
5	0.032	109.5	0.048	95.2
平均値	0.042	100.5	0.034	90.2
標準偏差	0.008	7.5	0.009	4.4
RSD(%)	19.0	7.4	27.4	4.8

*回収率は内標準法にて求めた

2-2　試料の HBCDs 及び TBBPA 分析結果

2-2-1　魚介類個別試料

東北、中部、中国四国及び九州の 4 地域の個別試料について、HBCDs 及び TBBPA を測定した。その結果と脂肪含量を表 26 に示す。今回使用した試料は全て天然魚介であるが、脂肪含量は九州 エビの 0.1% から九州 サバの 12.2%まで広範囲であった。16 検体の内、HBCDs は 13 検体で検出され、特に中部地方のアナゴは 36.9 ng/g という非常に高い濃度が検出された。HBCDs 異性体の中ではα-HBCD が高濃度(最大値：中部アナゴの 17.7 ng/g)で検出される場合が多く、ついでγ-HBCD であり、β-HBCD は最大でアナゴに 0.4ng/g 検出されたが、他は低濃度であった。TBBPA は 13 検体で検出され、最高値は中部 タイ②の 0.31 ng/g であった。

化学物質の生物体内への蓄積性は化学物質の疎水性(脂質への溶けやすさ)とよく相関することが知られている。そこで、各地域別に脂肪含量と HBCDs 及び TBBPA 濃度との相関関係の有無を調べた。図 9、10 より、HBCDs は脂肪含量が多いほど濃度が高い傾向がみられたが、TBBPA に明瞭な相関関係は認められなかった。図 9 及び 10 は、ND を 1/2LOD として図示した。

表 26　個別食品での HBCD s 及び TBBPA 分析結果

	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

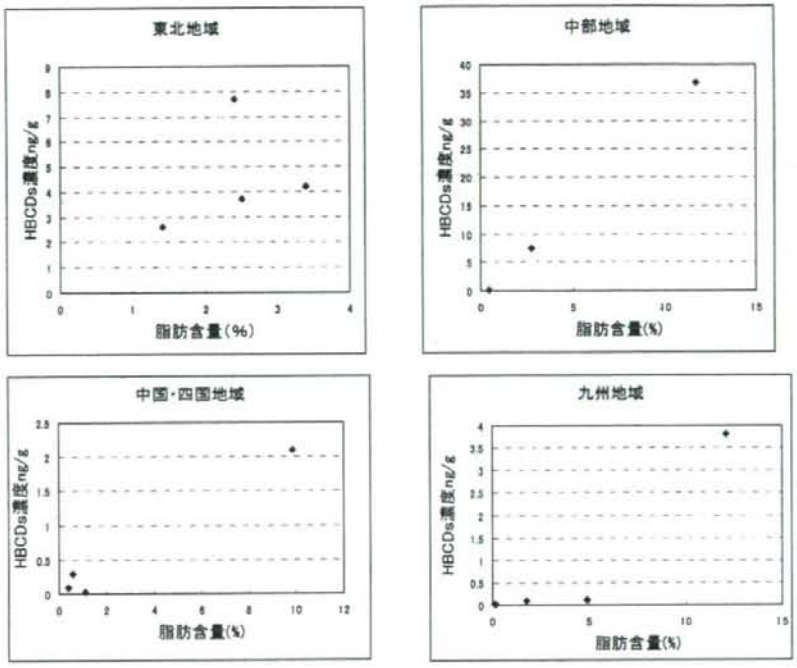

図 9 HBCDsと脂肪含量の相関(地域別)

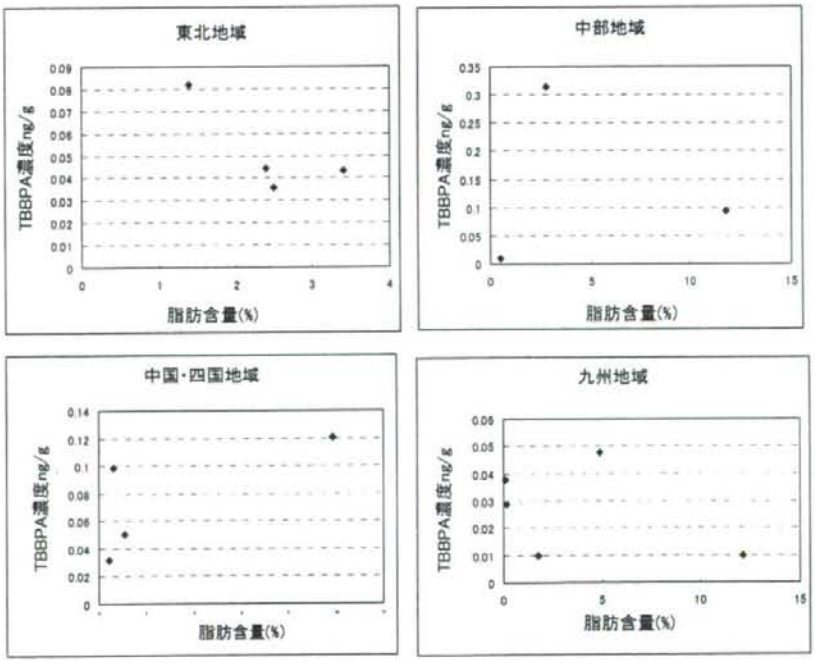

図 10 TBBPA 濃度と脂肪含量の相関(地域別)

2-2-2 マーケットバスケット試料

関東・関西の地域から集められたマーケットバスケット試料について HBCDs を測定した。2 地域の HBCDs 濃度及び一日推定摂取量の食品群別にまとめたものを表26-29 に示す。 マーケットバスケット試料の HBCDs 分析では第 10 群(魚介類)から関東で 0.62 及び 2.31 ng/g (平均 1.47 ng/g)、関西で 0.67 及び 1.64 ng/g (平均 1.16 ng/g)、検出されたが、その他の食品群からはほとんど検出されなかった。HBCDs 異性体の中では α-HBCD が一番高濃度(最大値:関東地区の 2.21 ng/g)で、次いで、γ-HBCD、β-HBCD の順であった。β-HBCD は最大でも 0.02 ng/g と、ほとんど検出されなかった。

表10の最終分析試料重量とHBCDs汚染濃度を乗じて算出した一日摂取量は、関東が、118.3 ng/人/日(ND=0)及び169.0 ng/人/日 (ND=1/2LOD)、関西が、90.1 ng/人/日(ND=0)及び150.2 ng/人/日

(ND=1/2LOD) となった。2地域で平均して得られた平均摂取量は104.2 ng/人/日(ND=0)及び159.6 ng/人/日 (ND=1/2LOD)となり、日本人の平均体重を50 kgと仮定したとき、上記の摂取量は2.1 ng/kg/日 (ND=0)、3.2 ng/kg/日 (ND=1/2LOD) と計算された。平成18年度の北九州地域での予備的摂取量調査[5]では、2002年度及び2005年度試料の平均で1.8 ng/kg/日

(ND=1/2LODの場合は2.8 ng/kg/日) であった。今回の調査結果はほぼ同程度の値と考えられる。動物試験の結果から日本では10.2 mg/kg/日が無毒性量(NOAEL)とされている[10]。HBCDsのヒトへの影響は、長期に亘って摂取するという仮定の下では、安全係数100(動物種差10×個体別差10)で除した量(耐容一日摂取量)[11]と比較することが妥当と考えられており、HBCDsの場合は102 μg/kg/日と比較することとなる。今回得られた平均摂取量2.1 ng/kg/日 (ND=0)、3.2 ng/kg/日

(ND=1/2LOD) は耐容一日摂取量の約50,000分の一から30,000分の一となり、ただちに健康に問題がある量ではないと考えられた。しかし、性特異的な脂質代謝に係る酵素合成系への影響[12]や、甲状腺ホメオスタシスの妨害[13]、チトクロムP-450の誘導[14]などin vivoでの作用が報告されていることから、今後も食品における汚染や摂取量の推移の観察は必要である。

一方、関東・関西の地域から集められたマーケットバスケット試料のTBBPA分析結果を表30-33に示す。関東地域の食品群別では、第1、3、4、6、12B、13群がTBBPA不検出であったが、その他の群では、0.01-0.03 ng/gと微量ながら検出された。したがって、その検出頻度は16試料中10試料と62.5%であった。一方、関西地域では4群のみ不検出で、その他の群で0.01-0.11 ng/g 検出された。その検出頻度は16試料中15試料と93.8%であり、平成18年度研究報告での福岡地域での検出頻度37.5%と比較して高かった。TBBPAの日本での使用量が3万トンであるということを考慮すると、代謝されやすい物質であるとしても、検出頻度の増加が懸念される。また、TBBPAの一日摂取量は、関東地域が31.5 ng/人/日(ND=0)及び35.1 ng/人/日 (ND=1/2LOD)、関西が、139.9 ng/人/日(ND=0)及び142.1 ng/人/日

表 26　マーケットバスケット試料(関東) における HBCDs 濃度

マーケットバスケット試料		試料調製時基礎となったの食品摂取量(g/日)	調製後の試料群重量(g/日)	2007年度関東 調製試料中HBCD濃度 (ng/g,wb)			
				α	β	γ	ΣHBCDs
第1群	(米類)	332.8	382.8	ND	ND	ND	ND
第2群	(米以外の穀類)	175.4	228.4	ND	ND	ND	ND
第3群	(砂糖・菓子類)	32.1	36.1	ND	ND	ND	ND
第4群	(油脂類)	11	11.0	ND	ND	ND	ND
第5群	(豆類)	59.6	59.6	ND	ND	ND	ND
第6群	(果実類)	125.4	125.4	ND	ND	ND	ND
第7群	(緑黄色野菜)	100.3	95.5	ND	ND	ND	ND
第8群	(その他の野菜)	209.1	210.2	ND	ND	ND	ND
第9群	(調味嗜好飲料)	540.8	540.8	ND	ND	ND	ND
第10群	(魚介類)A	84.8	80.7	0.57	0.01	0.04	0.62
第10群	(魚介類)B	84.8	80.8	2.21	ND	0.10	2.31
第11群	(肉・卵類)A	111.3	102.3	ND	ND	ND	ND
第11群	(肉・卵類)B	111.3	93.7	ND	ND	ND	ND
第12群	(乳類)A	137.7	137.7	ND	ND	ND	ND
第12群	(乳類)B	137.7	137.7	ND	ND	ND	ND
第13群	(調味料)	94.5	94.5	ND	ND	ND	ND

α、γ-HBCDのLOD値は0.02ng/g、β-HBCDのLOD値は0.01ng/g である。

表 27 マーケットバスケット試料(関西) における HBCDs 濃度

マーケットバスケット試料		試料調製時基礎となったの食品摂取量(g/日)	調製後の試料群重量(g/日)	2007年度関西 調製試料中HBCD濃度 (ng/g,wb)			
				α	β	γ	ΣHBCDs
第1群	(米類)	341.4	581.0	ND	ND	ND	ND
第2群	(米以外の穀類)	174.2	295.7	ND	ND	ND	ND
第3群	(砂糖・菓子類)	35.1	72.6	ND	ND	ND	ND
第4群	(油脂類)	10.6	10.6	ND	ND	ND	ND
第5群	(豆類)	57.5	101	ND	ND	ND	ND
第6群	(果実類)	120.8	120.8	ND	ND	ND	ND
第7群	(緑黄色野菜)	92.8	82.4	ND	ND	ND	ND
第8群	(その他の野菜)	184.1	171.8	ND	ND	ND	ND
第9群	(調味嗜好飲料)	616.3	616.3	ND	ND	ND	ND
第10群	(魚介類)A	82.2	78.6	0.43	0.02	0.22	0.67
第10群	(魚介類)B	82.2	78.3	1.19	ND	0.44	1.64
第11群	(肉・卵類)A	121.4	102.6	ND	ND	ND	ND
第11群	(肉・卵類)B	121.4	111.2	ND	ND	ND	ND
第12群	(乳類)A	142.9	142.9	ND	ND	ND	ND
第12群	(乳類)B	142.9	142.9	ND	ND	ND	ND
第13群	(調味料)	92.9	92.9	ND	ND	ND	ND

α、γ-HBCDのLOD値は0.02ng/g、β-HBCDのLOD値は0.01ng/g である。

表 28　マーケットバスケット試料(関東地区)における HBCD s の一日平均摂取量

マーケットバスケット試料	2007年度								
	試料調製時基礎となったの食品摂取量(g/日)	HBCD (ng/人/日)							
		ND=0				ND=1/2×LOD			
		α	β	γ	ΣHBCDs	α	β	γ	ΣHBCDs
第1群　(米類)	332.8	0	0	0	0.0	3.83	1.91	3.83	9.6
第2群　(米以外の穀類)	175.4	0	0	0	0.0	2.28	1.14	2.28	5.7
第3群　(砂糖・菓子類)	32.1	0	0	0	0.0	0.36	0.18	0.36	0.9
第4群　(油脂類)	11	0	0	0	0.0	0.11	0.06	0.11	0.3
第5群　(豆類)	59.6	0	0	0	0.0	0.60	0.30	0.60	1.5
第6群　(果実類)	125.4	0	0	0	0.0	1.25	0.63	1.25	3.1
第7群　(緑黄色野菜)	100.3	0	0	0	0.0	0.96	0.48	0.96	2.4
第8群　(その他の野菜)	209.1	0	0	0	0.0	2.10	1.05	2.10	5.3
第9群　(調味嗜好飲料)	540.8	0	0	0	0.0	5.41	2.70	5.41	13.5
第10群　(魚介類)*	84.8	112.3	0.4	5.7	118.3	112.3	0.61	5.65	118.5
第11群　(肉・卵類)*	111.3	0	0	0	0.0	0.98	0.49	0.98	2.5
第12群　(乳類)*	137.7	0	0	0	0.0	1.38	0.69	1.38	3.4
第13群　(調味料)	94.5	0	0	0	0.0	0.95	0.47	0.95	2.4
各HBCD摂取量 ng/日		112.3	0.4	5.7	118.3	132.5	10.7	25.9	169.0
ΣHBCDs推定摂取量 ng/kg体重/日					2.4				3.4

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。

表 29　マーケットバスケット試料(関西地区)における HBCD s の一日平均摂取量

マーケットバスケット試料	2007年度								
	試料調製時基礎となったの食品摂取量(g/日)	HBCD (ng/人/日)							
		ND=0				ND=1/2×LOD			
		α	β	γ	ΣHBCDs	α	β	γ	ΣHBCDs
第1群　(米類)	341.4	0	0	0	0.0	5.81	2.91	5.81	14.5
第2群　(米以外の穀類)	174.2	0	0	0	0.0	2.96	1.48	2.96	7.4
第3群　(砂糖・菓子類)	35.1	0	0	0	0.0	0.73	0.36	0.73	1.8
第4群　(油脂類)	10.6	0	0	0	0.0	0.11	0.05	0.11	0.3
第5群　(豆類)	57.5	0	0	0	0.0	1.01	0.51	1.01	2.5
第6群　(果実類)	120.8	0	0	0	0.0	1.21	0.60	1.21	3.0
第7群　(緑黄色野菜)	92.8	0	0	0	0.0	0.82	0.41	0.82	2.1
第8群　(その他の野菜)	184.1	0	0	0	0.0	1.72	0.86	1.72	4.3
第9群　(調味嗜好飲料)	616.3	0	0	0	0.0	6.16	3.08	6.16	15.4
第10群　(魚介類)*	82.2	63.5	0.8	25.9	90.1	63.5	1.0	25.9	90.3
第11群　(肉・卵類)*	121.4	0	0	0	0.0	1.07	0.53	1.07	2.7
第12群　(乳類)*	142.9	0	0	0	0.0	1.43	0.71	1.43	3.6
第13群　(調味料)	92.9	0	0	0	0.0	0.93	0.46	0.93	2.3
各HBCD摂取量 ng/日		63.5	0.8	25.9	90.1	87.4	13.0	49.8	150.2
ΣHBCDs推定摂取量 ng/kg体重/日					1.8				3.0

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。

(ND=1/2LOD) となった。2地域での格差は4.4倍あり、その平均摂取量は85.7 ng/人/日(ND=0)及び88.6 ng/人/日 (ND=1/2LOD) となり、日本人の平均体重を50 kgと仮定したとき、上記の摂取量は1.7 ng/kg/日 (ND=0)、1.8 ng/kg/日 (ND=1/2LOD) と計算された。平成18年度の北九州地域での予備的摂取量調査[5]では、2002年度及び2005年度試料の平均で0.6 ng/kg/日 (ND=1/2LODの場合は0.8 ng/kg/日) であり、今回の調査結果はその倍に相当した。地域や年度、マーケットバスケット試料調整時に選択した食品種の差異もあり、平均摂取量の把握にはある程度の期間観察する必要がある。毒性面については、1995年にIPCS/WHO[15]に

よって報告されたNOAEL値 700 mg/kg体重がある。ヒトへの外挿値として、安全係数100で除した数値 7 mg/kg体重と比べて、今回得られた平均摂取量は、かなり低いレベルであり、ヒトへの健康影響はないと考えられる。しかし、現在もマウスにおいて胎児性暴露による神経発達障害や肝臓・腎臓での組織障害などの報告[16)-18)]がみられることから、TBBPA摂取量の推移にはやはり注意すべきである。

表 30　マーケットバスケット試料(関東) における TBBPA 濃度

マーケットバスケット試料	試料調製時基礎となったの食品摂取量(g/日)	調製後の試料群重量(g/日)	2007年度関東調製試料中TBBPA濃度(ng/g,wb)
第1群　(米類)	332.8	382.8	ND
第2群　(米以外の穀類)	175.4	228.4	0.01
第3群　(砂糖・菓子類)	32.1	36.1	ND
第4群　(油脂類)	11	11.0	ND
第5群　(豆類)	59.6	59.6	0.02
第6群　(果実類)	125.4	125.4	ND
第7群　(緑黄色野菜)	100.3	95.5	0.02
第8群　(その他の野菜)	209.1	210.2	0.03
第9群　(調味嗜好飲料)	540.8	540.8	0.03
第10群 (魚介類)A	84.8	80.7	0.03
第10群 (魚介類)B	84.8	80.8	0.02
第11群 (肉・卵類)A	111.3	102.3	0.03
第11群 (肉・卵類)B	111.3	93.7	0.02
第12群 (乳類)A	137.7	137.7	0.01
第12群 (乳類)B	137.7	137.7	ND
第13群 (調味料)	94.5	94.5	ND

TBBPAのLODは0.01ng/gである。

表 31　マーケットバスケット試料(関西) における TBBPA 濃度

マーケットバスケット試料	試料調製時基礎となったの食品摂取量(g/日)	調製後の試料群重量(g/日)	2007年度関西調製試料中TBBPA濃度(ng/g,wb)
第1群　(米類)	341.4	581.0	0.03
第2群　(米以外の穀類)	174.2	295.7	0.01
第3群　(砂糖・菓子類)	35.1	72.6	0.02
第4群　(油脂類)	10.6	10.6	ND
第5群　(豆類)	57.5	101	0.04
第6群　(果実類)	120.8	120.8	0.02
第7群　(緑黄色野菜)	92.8	82.4	0.10
第8群　(その他の野菜)	184.1	171.8	0.10
第9群　(調味嗜好飲料)	616.3	616.3	0.10
第10群 (魚介類)A	82.2	78.6	0.08
第10群 (魚介類)B	82.2	78.3	0.11
第11群 (肉・卵類)A	121.4	102.6	0.08
第11群 (肉・卵類)B	121.4	111.2	0.05
第12群 (乳類)A	142.9	142.9	0.05
第12群 (乳類)B	142.9	142.9	0.05
第13群 (調味料)	92.9	92.9	0.06

TBBPAのLODは0.01ng/gである。

表 32　マーケットバスケット試料(関東地区)における TBBPA の一日平均摂取量

マーケットバスケット試料	2007年度		
	試料調製時基礎となったの食品摂取量(g/日)	TBBPA (ng/人/日)	
		ND=0	ND=1/2×LOD
第1群　(米類)	332.8	0.00	1.91
第2群　(米以外の穀類)	175.4	2.88	2.88
第3群　(砂糖・菓子類)	32.1	0.00	0.18
第4群　(油脂類)	11	0.00	0.06
第5群　(豆類)	59.6	0.98	0.98
第6群　(果実類)	125.4	0.00	0.63
第7群　(緑黄色野菜)	100.3	1.77	1.77
第8群　(その他の野菜)	209.1	6.44	6.44
第9群　(調味嗜好飲料)	540.8	14.40	14.40
第10群　(魚介類)*	84.8	2.06	2.06
第11群　(肉・卵類)*	111.3	2.36	2.36
第12群　(乳類)*	137.7	0.57	0.92
第13群　(調味料)	94.5	0.00	0.47
TBBPA摂取量　ng/日		31.5	35.1
TBBPA推定摂取量　ng/kg体重/日		0.6	0.7

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。

表 33　マーケットバスケット試料(関西地区)における TBBPA の一日平均摂取量

マーケットバスケット試料	2007年度		
	試料調製時基礎となったの食品摂取量(g/日)	TBBPA (ng/人/日)	
		ND=0	ND=1/2×LOD
第1群　(米類)	341.4	19.5	19.5
第2群　(米以外の穀類)	174.2	2.3	2.3
第3群　(砂糖・菓子類)	35.1	1.3	1.3
第4群　(油脂類)	10.6	0.0	0.1
第5群　(豆類)	57.5	4.2	4.2
第6群　(果実類)	120.8	1.8	1.8
第7群　(緑黄色野菜)	92.8	8.1	8.1
第8群　(その他の野菜)	184.1	17.5	17.5
第9群　(調味嗜好飲料)	616.3	59.1	59.1
第10群　(魚介類)*	82.2	7.3	6.2
第11群　(肉・卵類)*	121.4	6.8	8.3
第12群　(乳類)*	142.9	6.8	8.3
第13群　(調味料)	92.9	5.2	5.3
TBBPA摂取量　ng/日		139.9	142.1
ΣHBCDs推定摂取量　ng/kg体重/日		2.8	2.8

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。

D 結論

1 魚試料の汚染調査では、アナゴから4臭素化ダイオキシンが微量に検出されたが、その他の魚からはPBDD/DFsは検出されなかった。PBDEsではすべての魚から#28、#47、#99、#154、#206、#207、#209などの異性体が検出され、PBBsでは7件中5件の魚から4-6臭素化体の異性体が検出された。Co-PXBsは今回の魚試料からはいずれの異性体も検出されなかった。

マーケットバスケット方式による国内2地域の摂取量調査では、一日摂取量は臭素系ダイオキシン類が平均 0.000073 pgTEQ/kg/日、PBDEsが平均 3.23 ng/kg/日、PBBsが平均0.00547 ng/kg/日であった。Co-PXBsは2地域ともいずれの食品群別試料からも検出されなかった。

2 汎用性の高い食品中HBCDsの分析法を検討・開発した。その方法を用いて、東北、中部、中国・四国、九州の魚試料の汚染調査を実施した。16試料のうち、12試料からHBCDsを検出、最高値はアナゴ（中部）の36.9 ng/gであった。一方TBBPAは最高値でもタイ（中部）の0.31 ng/gであり、総じてHBCDsに比し、1-2桁低汚染であった。

マーケットバスケット方式での国内2地域の摂取量調査では、HBCDsは平均2.1 ng/kg/日（ND=0）、3.2 ng/kg/日（ND=1/2LOD）、TBBPAは平均1.7 ng/kg/日 ND=0)、1.8 ng/kg/日（ND=1/2LOD）であった。

E 研究発表

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Ⅲ. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

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PCB 118 and Aryl Hydrocarbon Receptor Immunoassays for Screening Dioxins in Retail Fish

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PCB 118 and Aryl Hydrocarbon Receptor Immunoassays for Screening Dioxins in Retail Fish

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The efficacy of a combination of two enzyme-linked immunosorbent assay (ELISA) kits was examined for screening the toxic equivalent (TEQ) concentrations of dioxins in retail fish. The coplanar PCB-EIA system, which is a competitive immunoassay specific for polychlorinated biphenyl (PCB) 118, was tested as a screening method for mono-*ortho* PCBs. The Ah immunoassay (Ah-I), which is an ELISA-based aryl hydrocarbon receptor binding assay, was analyzed for its screening ability for non-*ortho* PCBs, polychlorinated dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs). Dilution and recovery tests using purified fish extracts revealed no major interference of the matrix in the PCB-EIA and suggested that the matrix effect was minimized in the Ah-I. Finally, the results for the fish samples (n = 20) showed a strong correlation between this method and high-resolution gas chromatography coupled to high-resolution mass spectrometry for the determination of the TEQ concentrations of mono-*ortho* PCBs (r = 0.99) and non-*ortho* PCBs and PCDD/Fs (r = 0.97). These data indicate that our method is suitable for screening retail fish to determine the TEQ concentrations of dioxins.

KEYWORDS: Dioxins; immunoassay; bioassay; Ah receptor; fish; screening

INTRODUCTION

Fishery products have been identified as the main source of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-like polychlorinated biphenyls (PCBs)—collectively referred to as dioxins—in the Japanese diet (*1*, *2*). We previously carried out a nationwide survey of dioxin concentrations in various fishery products available on the Japanese market during the past few years (*3*–*5*) and found that fish often showed high toxic equivalent (TEQ) levels of dioxins. It is therefore important to develop screening methods for the determination of dioxin TEQs in retail fish in order to carry out risk assessments.

High-resolution gas chromatography coupled to high-resolution mass spectrometry (HRGC-HRMS) is generally viewed as the most reliable method for determining the TEQ concentrations of dioxins. This technique is sensitive and reproducible; however, it is also time-consuming and requires expensive instruments, which limits its capacity. A reporter-gene assay, such as the chemical-activated luciferase gene expression (CALUX) assay, is currently considered to be the best screening method for the TEQ concentrations of dioxins in food [as reviewed by Hoogenboom et al. (*6*)]. The CALUX assay detects dioxin-like compounds based on their activation of the aryl hydrocarbon receptor (AhR), which increases the expression of the luciferase reporter gene as reviewed by Behnisch et al. (*7*) and Overmeire et al. (*8*). The response for a sample containing dioxin-like compounds can be converted into 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) equivalents, which are known as CALUX-based TEQs, using a 2,3,7,8-TCDD standard curve. The CALUX assay has been applied to the detection of dioxins in fish and fishery products (*9*–*13*); however, its drawbacks include the need for cell culture, which requires skilled personnel and elaborate equipment, and the likely requirement of a license for the assay.

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An enzyme-linked immunosorbent assay (ELISA) based screening tool, in the form of a commercially available kit, might be a simpler alternative that does not require cell culture. The objective of the current study was therefore to evaluate two commercially available ELISA kits for dioxin TEQ concentration screening in retail fish. We recently developed the coplanar PCB-EIA system, which is a competitive ELISA kit, using a monoclonal antibody (mAb) that is highly specific for 2,3′,4,4′,5-pentachlorobiphenyl (PCB 118) (*14*). According to the HRGC-HRMS data produced by our Japanese national survey (*15*), PCB 118 accounts for about 50% of the total TEQ concentrations of the mono-*ortho* PCB isomers in fish. The PCB-EIA method is rapid, taking approximately 2 h, and performed well in the analysis of PCB 118 in fish samples after purification (*16*); we therefore consider it to be a good screening method for mono-*ortho* PCB TEQ concentrations in fish.

The Ah immunoassay (Ah-I) kit is a hybrid of an immunoassay and an in vitro AhR-binding assay (*17*). Dioxin-like compounds bind to the AhR and form complexes with the AhR nuclear translocator (ARNT) and dioxin-responsive element (DRE) DNA oligomer. The complexes are then detected by an immunoassay-based color reaction using an enzyme-conjugated Ab. The response for a sample containing dioxin-like compounds in the Ah-I can be converted into 2,3,7,8-TCDD equivalents using a 2,3,7,8-TCDD standard curve. The assay is rapid, taking approximately 6 h, and has a simple-to-use format without the need for live cell culture. We previously examined the ability of the Ah-I to screen dioxins in flue gas, soil, ash, and wastewater samples (*18, 19*). We also applied it to detect dioxins in fish samples (Tsutsumi et al., unpublished work) and obtained a positive reading for non-*ortho* PCBs and the PCDD/F fraction, although we were unsuccessful in detecting mono-*ortho* PCBs, probably due to strong antagonistic effects in the fraction. Therefore, the Ah-I was introduced as a method for screening the TEQ concentrations of non-*ortho* PCBs and PCDD/Fs in fish.

In the present study, we assessed the efficacy of combining the PCB-EIA (as a screening method for mono-*ortho* PCBs) and the Ah-I (as a screening method for non-*ortho* PCBs and PCDD/Fs).

MATERIALS AND METHODS

Reagents. The solvents used in this study (acetone, dichloromethane, *n*-hexane, methanol, and toluene) were obtained from Kanto Kagaku (Tokyo, Japan). Silica gel S-1, 22% sulfuric acid-impregnated silica gel, dimethyl sulfoxide (DMSO), and 2,3,7,8-TCDD were obtained from Wako Pure Chemicals Co. (Osaka, Japan). The 10% silver nitrate−silica gel and 44% sulfuric acid-impregnated silica gel were obtained from GL Sciences Inc. (Tokyo, Japan). Alumina B-Super I was obtained from ICN Pharmaceuticals Inc. (Costa Mesa, CA).

A multilayer silica gel column was prepared by filling it from bottom to top with 2.0 g of anhydrous sodium sulfate, 0.9 g of silica gel, 4.5 g of 44% sulfuric acid-impregnated silica gel, 6.0 g of 22% sulfuric acid-impregnated silica gel, 0.9 g of silica gel, 3.0 g of 10% silver nitrate−silica gel, and 1.5 g of anhydrous sodium sulfate. An alumina column was prepared by filling it from bottom to top with 2.0 g of anhydrous sodium sulfate, 15 g of alumina, and 0.5 g of anhydrous sodium sulfate. A sulfuric acid−silica gel column was prepared by filling it from bottom to top with 2.0 g of anhydrous sodium, 0.2 g of silica gel, 6.0 g of 44% sulfuric acid-impregnated silica gel, and 0.5 g of anhydrous sodium sulfate.

The PCB-EIA kit was purchased from EnBioTec Laboratories (Tokyo, Japan). The Ah-I kit was purchased from Kubota Corp. (Osaka, Japan).

Fish Samples. The fish samples (bonito, mackerel, mullet, salmon, sea bass, tuna, and yellowtail) were actual retail products purchased during 2003 and 2005 from supermarkets in Tokyo, Japan. The samples were skinned, and the muscular parts of the samples were homogenized using a GM200 food cutter obtained from Retsch Co., Ltd. (Haan, Germany) and stored at −20 °C until required for analysis.

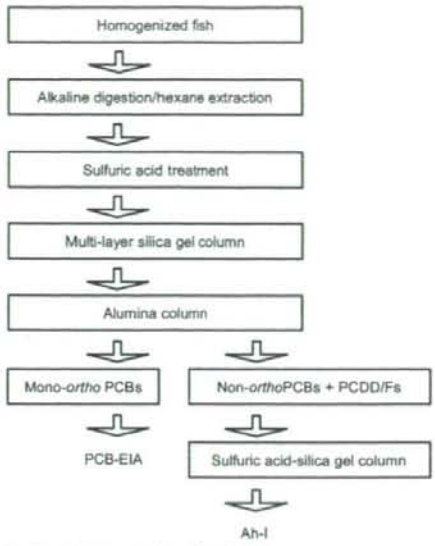

Figure 1. Sample preparation of retail fish.

Sample Preparation for the PCB-EIA and the Ah-I. The procedure for preparing the fish samples is shown schematically in **Figure 1**. Samples (20 g) of retail fish were homogenized and incubated in 100 mL of 2 M aqueous KOH for 16 h at room temperature. The alkaline hydrolysates were added to 150 mL of methanol and extracted three times by mechanical shaking for 10 min with 100 mL of *n*-hexane; the *n*-hexane layers were then washed twice with 150 mL of 2% (w/v) aqueous NaCl. The extracts were treated several times with concentrated sulfuric acid (H_2SO_4) and were passed through anhydrous sodium sulfate. The eluate was evaporated to near dryness at 40 °C using a rotary evaporator and then was loaded onto a multilayer silica gel column. The eluate obtained with 200 mL of *n*-hexane was evaporated to near dryness at 40 °C using a rotary evaporator and was loaded onto an alumina column. After washing with 150 mL of *n*-hexane, the first fraction (containing mono-*ortho* PCBs) was eluted with 150 mL of 2% (v/v) dichloromethane/*n*-hexane, and the second fraction (containing non-*ortho* PCBs and PCDD/Fs) was eluted with 200 mL of 60% (v/v) dichloromethane/*n*-hexane. The first fraction was evaporated to near dryness at 40 °C using a rotary evaporator and was dried under nitrogen at room temperature. The residue was redissolved in 100 μL of DMSO and then used in the PCB-EIA. The second fraction was evaporated to near dryness at 40 °C in a rotary evaporator and was further purified on a sulfuric acid−silica gel column. This reduced the background AhR-agonist activity in the Ah-I from the alumina column. The eluate obtained with 100 mL of *n*-hexane was evaporated to near dryness at 40 °C in a rotary evaporator and was evaporated to dryness under nitrogen at room temperature. The residue was then redissolved in 20 μL of DMSO and used in the Ah-I.

PCB-EIA. The PCB-EIA kit was used according to the manufacturer's instructions (EnBioTec Laboratories) (*20*). Samples (12.5 μL/well) or various concentrations of 3,3′,4′-trichloro-4-methoxybiphenyl, which is a surrogate standard for PCB 118, were mixed with competitor−horseradish peroxidase conjugate (1:3) and added to a microtiter plate (50 μL/well) coated with an mAb against PCB 118 and then incubated for 30 min at room temperature with gentle shaking. After washing with the solution provided, the enzyme–substrate solution containing 3,3′,5,5′-tetramethylbenzidine was added to each well (50 μL/well) and incubated for 20 min. The enzyme reaction was stopped with 0.5 M H_2SO_4 (50 μL/well), and the absorbance at 450 nm was measured. All of the experiments were conducted in duplicate. The standard curves were fitted using a four-parameter logistic model. The PCB-EIA had a detection limit for PCB 118 of 10 ng/mL (125 pg/well), corresponding to 50 pg/g in the test samples.

Ah-I. The Ah-I kit was used according to the manufacturer's instructions (Kubota Corp.) (*21*). Samples (up to 2 μL/well) or various

concentrations of 2,3,7,8-TCDD were mixed with a reagent containing DRE DNA oligomers, ARNT, and cytosol components containing AhR. The mixtures were added to microtiter wells (200 μL/well) coated with DRE-binding protein and then incubated for 2 h at 30 °C. The presence of dioxins promoted the formation of AhR−ARNT complexes, which then bound DRE DNA oligomers, and so bound to the wells. After washing with the solution provided, an anti-ARNT Ab solution was added to each well (200 μL/well) and incubated for 1 h at 30 °C. After another washing, a second Ab conjugated to alkaline phosphatase solution was added to each well (200 μL/well) and incubated for 1 h at 30 °C. After yet another washing, an enzyme–substrate solution was added to each well (200 μL/well) and incubated for 30 min at 30 °C, and then the absorbance at 405 nm was measured. All of the experiments were conducted in triplicate wells for standard solutions and in a single well for serially diluted fish extracts. The assay used a standard curve with various concentrations of 2,3,7,8-TCDD, for which the detection limit was 5.0 pg/mL (1.0 pg/well). The standard curves were fitted using a cubic polynomial model. The measurements for samples containing dioxin-like compounds were converted into Ah-I-based 2,3,7,8-TCDD equivalents (dioxin equivalents or DEQs) and were corrected by subtracting the blank concentration for the sample preparation procedure. The minimum concentration measurable in the samples was 1.0 pg-DEQ/g.

HRGC-HRMS Analysis. Extraction, cleanup, and analysis of the dioxins were performed as described previously (*22*). Briefly, 50 g of homogenized fish sample was spiked with a $^{13}C_{12}$-labeled internal quantification standard mixture (containing 17 2,3,7,8-chlorine substituted PCDD/Fs and 12 dioxin-like PCBs) and then digested with 2 M aqueous KOH. The alkaline hydrolysate was extracted three times with *n*-hexane. After treatment with concentrated sulfuric acid, the extract was purified on a silver nitrate−silica gel column followed by an alumina column. On the alumina column, mono-*ortho* PCBs were eluted with 2% (v/v) dichloromethane/*n*-hexane, and non-*ortho* PCBs and PCDD/Fs were eluted with 60% (v/v) dichloromethane/*n*-hexane. The latter fraction was further purified on an activated carbon column. Both fractions were spiked with $^{13}C_{12}$-labeled recovery standards (3,3′,4,5′-tetrachlorobiphenyl and 1,2,3,4-TCDD) and concentrated before HRGC-HRMS analysis.

The quantification of 17 2,3,7,8-chlorine-substituted PCDD/Fs, 4 non-*ortho* PCBs, and 8 mono-*ortho* PCBs was performed by HRGC-HRMS using an HP-6890 plus gas chromatograph coupled to a JEOL JMS-700 MStation mass spectrometer (Tokyo, Japan). The determination of 2,3,7,8-chlorine substituted PCDD/Fs was performed in DB-5MS and DB-17 fused silica capillary columns (J&W Scientific, Folsom, CA). The determination of non-*ortho* and mono-*ortho* PCBs was performed in an HT-8 fused silica capillary column (SGE, Austin, TX). The TEQ was calculated using the World Health Organization Toxic Equivalency Factor (WHO-TEF) scheme (*23*). The limits of quantification (LOQ) were around 0.01 pg/g for TCDDs/tetrachlorodibenzofurans (TCDFs) and pentachlorodibenzo-*p*-dioxins (PeCDDs)/pentachlorodibenzofurans (PeCDFs), 0.02 pg/g for hexachlorodibenzo-*p*-dioxins (HxCDDs)/hexachlorodibenzofurans (HxCDFs), and heptachlorodibenzo-*p*-dioxins (HpCDDs)/heptachlorodibenzofurans (HpCDFs), 0.05 pg/g for octachlorodibenzo-*p*-dioxin (OCDD)/octachlorodibenzofuran (OCDF), 0.1 pg/g for non-*ortho* PCBs, and 1.0 pg/g for mono-*ortho* PCBs. Calculations of the total TEQ in a sample were carried out by assuming that all isomer concentrations lower than the LOQs were equal to zero.

RESULTS

Recovery of Dioxins in the Sample Preparation Step for the PCB-EIA and Ah-I. The recovery of WHO-TEF dioxin isomers in the sample preparation step for the PCB-EIA and the Ah-I was determined by HRGC-HRMS analysis. Two varieties of fish contaminated in the natural environment were purified by the sample preparation procedure, their extracts were spiked with the $^{13}C_{12}$-labeled internal quantification standard mixture, and HRGC-HRMS analysis was carried out. As shown in **Table 1**, dioxin isomer recovery in the fish samples was good in the mono-*ortho* PCB fraction (80.1–103.5%) and the non-*ortho* PCB and PCDD/F fraction (76.6–103.3%). In addition, the standard deviations (SDs) of the dioxin isomer recovery percentages were relatively small (<19.7%). These data indicate that no significant loss of dioxins occurred during the sample preparation step for the PCB-EIA and Ah-I.

Table 1. Recoveries of Dioxins from Fish in the Sample Preparation for the PCB-EIA and Ah-I Determined by HRGC-HRMS Analysis[a]

dioxin isomer	recovery[b] (%) mullet	recovery[b] (%) sea bass
non-*ortho* PCBs and PCDD/Fs fraction		
PCDDs		
2,3,7,8-TCDD	76.9 ± 6.8	86.9 ± 15.8
1,2,3,7,8-PeCDD	92.7 ± 18.8	78.5 ± 4.7
1,2,3,4,7,8-HxCDD	–[c]	–
1,2,3,6,7,8-HxCDD	96.3 ± 10.2	80.8 ± 8.5
1,2,3,7,8,9-HxCDD	–	–
1,2,3,4,6,7,8-HpCDD	92.5 ± 14.2	80.7 ± 8.0
OCDD	95.8 ± 4.9	76.6 ± 6.7
PCDFs		
2,3,7,8-TCDF	94.0 ± 1.1	96.8 ± 5.3
1,2,3,7,8-PeCDF	94.3 ± 19.7	93.9 ± 7.8
2,3,4,7,8-PeCDF	96.5 ± 5.9	89.6 ± 3.9
1,2,3,4,7,8-HxCDF	96.9 ± 12.5	103.3 ± 11.4
1,2,3,6,7,8-HxCDF	99.2 ± 6.9	80.5 ± 16.6
1,2,3,7,8,9-HxCDF	–	–
2,3,4,6,7,8-HxCDF	89.2 ± 12.1	84.7 ± 8.3
1,2,3,4,6,7,8-HpCDF	–	–
1,2,3,4,7,8,9-HpCDF	–	–
OCDF	–	–
non-*ortho* PCBs		
3,3′,4,4′-TCB (77)	95.7 ± 7.8	92.0 ± 3.5
3,4,4′,5-TCB (81)	92.8 ± 2.5	88.9 ± 7.4
3,3′,4,4′,5-PeCB (126)	93.3 ± 6.7	94.3 ± 5.9
3,3′,4,4′,5,5′-HxCB (169)	99.1 ± 3.0	94.5 ± 9.2
mono-*ortho* PCBs fraction		
mono-*ortho* PCBs		
2,3,3′,4,4′-PeCB (105)	101.5 ± 5.3	89.4 ± 3.5
2,3,4,4′,5-PeCB (114)	97.0 ± 11.7	80.1 ± 4.7
2,3′,4,4′,5-PeCB (118)	94.7 ± 3.8	89.6 ± 3.8
2′,3,4,4′,5-PeCB (123)	103.5 ± 6.1	85.0 ± 3.0
2,3,3′,4,4′,5-HxCB (156)	93.9 ± 6.1	89.4 ± 6.9
2,3,3′,4,4′,5′-HxCB (157)	91.2 ± 6.2	86.3 ± 9.0
2,3′,4,4′,5,5′-HxCB (167)	96.5 ± 4.3	94.4 ± 4.8
2,3,3′,4,4′,5,5′-HpCB (189)	84.4 ± 11.2	96.6 ± 0.0

[a] Natively dioxin-contaminated samples (1.3 pg-TEQ/g in mullet and 2.9 pg-TEQ/g in sea bass) were extracted and cleaned up following the sample preparation for the PCB-EIA and Ah-I under Materials and Methods. The cleaned-up fractions were then spiked with the $^{13}C_{12}$-labeled internal quantification standard mixture. The mono-*ortho* PCBs fraction was analyzed by HRGC-HRMS. The non-*ortho* PCBs and PCDD/Fs fraction was further purified on an activated carbon column and then analyzed by the HRGC-HRMS. Three examinations were carried out on different days ($n = 3$). [b] Recoveries of dioxin isomers in the sample preparation step were calculated with respect to the concentrations obtained by the HRGC-HRMS analysis under Materials and Methods. [c] Concentration below the LOQs.

Table 2. LOQs of the PCB-EIA and Ah-I with the Sample Preparation

	blank value[a] (mean ± SD)	LOQ in kit	LOQ with sample preparation
PCB-EIA (pg/well)	–[b]	125	125
Ah-I (pg-DEQ/well)	1.5 ± 0.25	1.0	2.0

[a] Blank values were determined by replicate analyses of the procedural blank samples on four different days ($n = 4$). [b] Not detected.

Determination of the LOQs for the PCB-EIA and Ah-I Combined with the Sample Preparation Procedure. We performed procedural blank tests on four different days to determine the assay LOQs using the sample preparation procedure. As shown in **Table 2**, no procedural blanks were observed in the PCB-EIA, and the LOQ was equal to that defined by the kit. By contrast, a slight procedural blank was observed in the Ah-

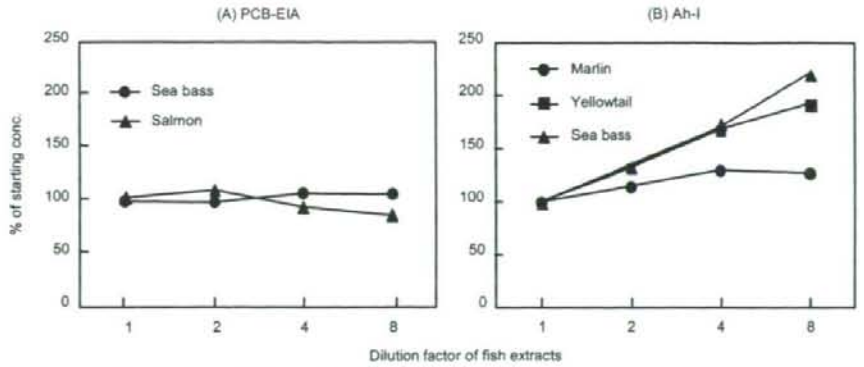

Figure 2. Effect of the dilution factor on the determination of dioxins in fish. Cleaned-up extracts from natively contaminated fish were serially diluted with DMSO and assayed by the (**A**) PCB-EIA and (**B**) Ah-I in duplicate.

Table 3. Recovery of Dioxins from Spiked Fish Extracts after Sample Preparation[a]

PCB-EIA			Ah-I		
composite extract	spiked PCB 118 (pg/well)	recovery (%) (mean ± SD)	composite extract	spiked TCDD (pg-DEQ/well)	recovery (%) (mean ± SD)
tuna and yellowtail	190	115.1 ± 6.9	salmon and yellowtail	10	104.8 ± 6.4
	500	117.7 ± 5.3			
	1250	102.9 ± 3.2			
	2500	96.5 ± 14.7			
mackerel and sea bass	500	103.6 ± 11.5	sea bass and yellowtail	10	89.7 ± 18.3

[a] The two kinds of cleaned-up composite extracts from fish samples were spiked with known quantities of PCB 118 or 2,3,7,8-TCDD and analyzed repeatedly by the PCB-EIA and Ah-I ($n = 3$).

I. Sample measurements in the Ah-I were corrected by subtracting the mean blank value, and the LOQ was defined as 2.0 pg-DEQ/well, which corresponded to 8 SDs of the blank value. Testing a 20 g fish sample revealed the LOQ to be 50 pg/g in the PCB-EIA and 1.0 pg-DEQ/g in the Ah-I.

Effect of Fish Matrix on the PCB-EIA and Ah-I. Purified extracts of fish samples contaminated in the natural environment were subjected to 2-fold serial dilutions with DMSO before being assayed. In the PCB-EIA, the measured concentrations were 83.5–107.9% of those expected from the starting concentrations (**Figure 2A**), suggesting that the matrix did not greatly interfere with the assay performed using this sample preparation technique. By contrast, the Ah-I-measured concentrations of some samples, especially sea bass and yellowtail, appeared to increase with dilution (**Figure 2B**). This indicates that the dilution process might eliminate the matrix effect in the Ah-I. For this reason, serial dilutions of fish extracts (dilution factors of 1, 2, 4, and 8) were measured in the Ah-I, and the maximum concentration was used to reduce the rate of false-negative results. When the highest dilution is used, the LOQ will be 8 times higher than when using the lowest dilution.

A recovery test using purified composite fish extracts was also carried out to further examine the effect of the matrix on the assays. In the PCB-EIA, purified extracts spiked with various concentrations of PCB 118 were assayed. In the Ah-I, purified extracts spiked with 2,3,7,8-TCDD were assayed with serial dilutions. Recovery over the tested range was 96.5– 117.7% (SD = 3.2–14.7%) for the PCB-EIA and 89.7–104.8% (SD = 6.4–18.3%) for the Ah-I (**Table 3**). These results were satisfactory, suggesting that the assays can detect and measure dioxins with good accuracy following sample preparation.

Reproducibility of the PCB-EIA and Ah-I. The reproducibility of the PCB-EIA and Ah-I using the sample preparation procedure described above was tested by analyzing replicate fish samples. The fish were extracted, cleaned, and assayed in three separate analyses on different days. The coefficients of variation for two varieties were 0.5–5.0% for the PCB-EIA and 21.1–23.5% for the Ah-I (**Table 4**), which indicated an acceptable level of precision for dioxin analysis.

Table 4. Reproducibility of the PCB-EIA and Ah-I Combined with the Sample Preparation Procedure[a]

PCB-EIA			Ah-I		
sample	pg/g (mean ± SD)	CV (%)	sample	pg-DEQ/g (mean ± SD)	CV (%)
mullet	3466 ± 17	0.5	mullet	3.8 ± 0.8	21.1
sea bass	832 ± 41	5.0	yellowtail	3.4 ± 0.8	23.5

[a] The fish contaminated in the natural environment (1.5 pg-TEQ/g in mullet, 1.3 pg-TEQ/g in sea bass, and 1.3 pg-TEQ/g in yellowtail) were extracted, cleaned up, and assayed by the PCB-EIA and Ah-I in three separate runs on different days ($n = 3$).

Comparison of the PCB-EIA and Ah-I with HRGC-HRMS Analysis. Dioxin concentrations were measured by the PCB-EIA and Ah-I in 20 retail fish samples and compared to the TEQ concentrations obtained by HRGC-HRMS analysis. Both the concentrations of mono-*ortho* PCBs obtained by the PCB-EIA (**Figure 3A**) and the concentrations of non-*ortho* PCBs and PCDD/Fs obtained by the Ah-I (**Figure 3B**) showed good correlations with the TEQ concentrations measured by HRGC-HRMS ($r > 0.98$ and $r = 0.97$, respectively). These results show that a combination of the PCB-EIA and Ah-I is a practical method for estimating the TEQ concentrations of dioxins in retail fish.

Although the PCB-EIA is specific to PCB 118, it has slight cross-reactivity with other PCB isomers (*16, 20*), many of which are found in fish samples. To interpret the positive PCB-EIA readings, we therefore compared the results with the PCB 118

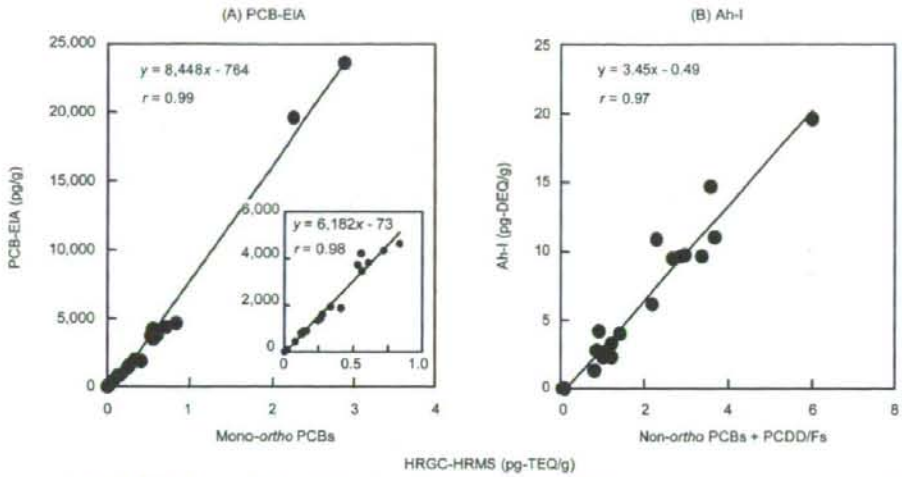

Figure 3. Comparison of the (**A**) PCB-EIA and (**B**) Ah-I with HRGC-HRMS measurements of fish samples. In total, 20 samples (bonito, two mackerels, mullet, four salmon, three sea bass, three tuna, and six yellowtail) were analyzed by the PCB-EIA and Ah-I and by HRGC-HRMS. Undetectable data in the PCB-EIA (one sample) and the Ah-I (two samples) were assigned a value of zero. For PCB-EIA concentrations <5000 pg/g, the regression equation is illustrated in the inset of **A** ($n = 18$).

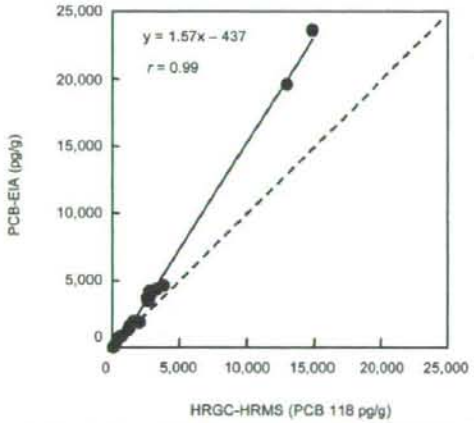

Figure 4. Comparison of PCB-EIA concentrations with HRGC-HRMS measurements of PCB 118 concentrations in fish samples ($n = 20$). The dashed line represents $x = y$.

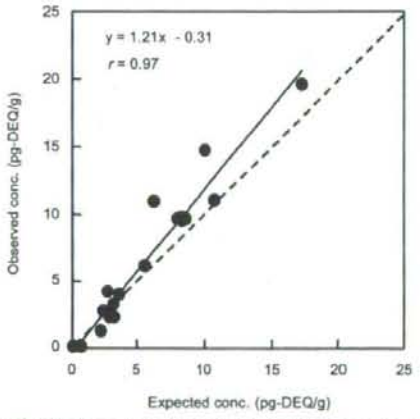

Figure 5. Comparison of observed and expected Ah-I concentrations ($n = 20$). The dashed line represents $x = y$.

concentrations measured by HRGC-HRMS analysis of the 20 fish samples (**Figure 4**). As shown in **Figure 4**, a good correlation was obtained between the two methods, and the linear regression slope was approximately 1, suggesting that a positive reading in the PCB-EIA was mainly attributable to PCB 118 in most samples.

The Ah-I has the potential to estimate all of the compounds acting as AhR agonists, whereas HRGC-HRMS analysis is restricted to the target dioxin isomers assigned by the WHO-TEF scheme. To assess whether the positive Ah-I readings were consistent with the target dioxins, we compared the Ah-I results with the expected concentrations based on the HRGC-HRMS results of the 20 fish samples. The expected concentrations were calculated by multiplying the concentrations of four non-*ortho* PCBs and 17 PCDD/Fs determined by HRGC-HRMS and their relative potency values in the Ah-I (*18*). As shown in **Figure 5**, a good correlation was observed between the obtained and expected values, with the slope of the linear regression equation approximating 1. This suggests that a positive Ah-I reading was largely attributable to the target compounds in the samples.

DISCUSSION

In the present study, we found a good correlation between our combined PCB-ELA and Ah-I results and the HRGC-HRMS analysis, suggesting that this method is suitable for measuring dioxin concentrations and screening for TEQ concentrations in retail fish. **Figure 6** shows the 95% prediction interval for the regression lines in the comparative study of 20 fish samples. To eliminate distortion of the results, two highly contaminated PCB-EIA samples and samples with undetectable levels in both assays were excluded. For example, 0.5 pg-TEQ/g mono-*ortho* PCBs in the HRGC-HRMS analysis corresponded to 2300–3800 pg/g in the PCB-EIA. Similarly, 3 pg-TEQ/g non-*ortho* PCBs and PCDD/Fs in the HRGC-HRMS analysis corresponded to 6.7–13 pg-DEQ/g in the Ah-I. Each assay easily detected the lowest concentration of the predicted interval, as these were higher than the LOQ of the assay. Future assessments of the assays will require more practical data points in a comparative study in order to determine variation in the assay results and allow the accurate monitoring of TEQ concentrations.

TEF values have been revised recently (*24*). The WHO advises that the new TEFs (TEF_{2005}) be used because they

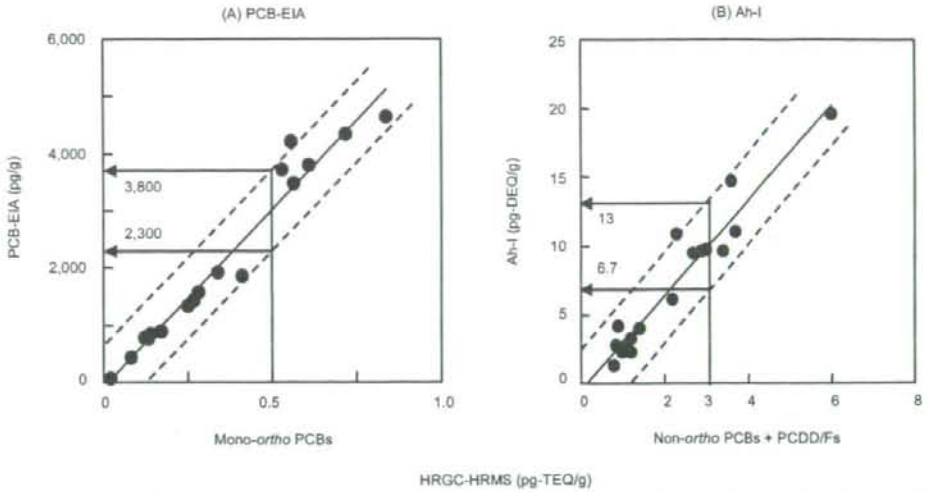

Figure 6. Combined use of the PCB-EIA and Ah-I as a screening method for TEQ concentrations of dioxins in fish samples. The dashed lines represent the 95% prediction interval for regression lines. Two highly contaminated PCB-EIA samples (>19000 pg/g) and one sample with undetectable levels were excluded from the regression calculation in **A** ($n = 17$). Two Ah-I samples with undetectable levels were excluded from the regression calculation in **B** ($n = 18$).

replace the previous TEFs (*23*) reported in 1998. As a result of recalculation using TEF_{2005} in the 20 samples employed in the comparison study, the contribution of mono-*ortho* PCBs to the total TEQ decreased significantly (data not shown). This is mainly because the TEF_{2005} of PCB 118 (0.00003) is slightly lower than the TEF_{1998} value (0.0001). However, the contribution of mono-*ortho* PCBs still accounted for 10−20% of total TEQ concentrations in three fish samples, such as mullet and sea bass. It would therefore be better to screen mono-*ortho* PCBs along with non-*ortho* PCBs and PCDD/Fs. Good correlations were also observed between the assay's results and TEQ concentrations calculated by TEF_{2005} in the 20 fish samples: the linear regression equations $y = 36165x - 640$ ($r = 0.99$) and $y = 3.56x - 0.50$ ($r = 0.97$) were obtained for PCB-EIA versus mono-*ortho* PCBs and Ah-I versus non-*ortho* PCBs and PCDD/Fs, respectively.

Recently, the European Commission set the maximum limits for combined PCDD/Fs and dioxin-like PCBs in consumer foods available on the European market at 8 pg-TEQ/g in fish muscle on a fresh weight basis (*25*). As our screening method measures dioxin TEQ concentrations in two separate fractions, it is unable to directly determine the sum of the PCDD/Fs and dioxin-like PCBs. However, our HRGC-HRMS data previously revealed that mono-*ortho* PCBs account for approximately 15% of the total amount of dioxins in fish samples, with non-*ortho* PCBs and PCDD/Fs making up the remainder (*15*). Therefore, fish samples containing a combined PCDD/F and dioxin-like PCB level of at least 8 pg-TEQ/g are likely to give positive PCB-EIA and Ah-I readings, although close attention must be paid to the variable ratios of mono-*ortho* PCBs and non-*ortho* PCBs and PCDD/Fs in fish samples.

The positive PCB-EIA results were mainly caused by the reactivity with PCB 118 in the samples (**Figure 4**). However, the slope of the liner regression equation was slightly larger than 1, suggesting that fish samples appeared to contain other PCB isomers recognized by the PCB-EIA, along with PCB 118. The PCB-EIA is known to have slight cross-reactivity with PCB 31, PCB 66, and PCB 70 (12.9–17.8% of PCB 118) (*16, 20*), which can be fractionated into mono-*ortho* PCB fractions. The presence of high concentrations of these isomers might have influenced the PCB-EIA results, although it is not certain that these compounds were present in the samples.

Many compounds, such as polycyclic aromatic hydrocarbons (PAHs), brominated dioxins, and non-2,3,7,8-substituted chlorinated dioxins, also possess relatively strong AhR-agonist activity in the Ah-I (*18*), in addition to the target dioxins assigned by the WHO-TEF scheme. As the behaviors of brominated and non-2,3,7,8-substituted chlorinated dioxins in the sample preparation step are similar to those of target dioxins, it is difficult to exclude them. However, surprisingly, our data suggested that the positive Ah-I readings were mainly attributed to target dioxin isomers in the samples (**Figure 5**). In general, brominated dioxins have not been detected in fish (*26–28*) or have been identified less often and at lower concentrations than chlorinated dioxins (*29*). Additionally, the major non-2,3,7,8-substituted chlorinated dioxins that are frequently found in retail fish samples—that is, 1,3,6,8-TCDDs and 1,3,7,9-TCDDs (*5, 30*)—are insensitive to the Ah-I (*18*).

The main advantage of our combined method is that it is less likely to produce false-negative results than cell-based assays, such as the CALUX assay. Some mono-*ortho* PCBs, such as PCB 118, show a relatively weak response in the CALUX assay (*31–33*), and so samples containing high levels of mono-*ortho* PCBs might be underestimated (*13*). However, as shown in the present study, the PCB-EIA strongly reacts to PCB 118, which is a good indicator of the TEQ concentrations of mono-*ortho* PCBs. Indeed, the two highly contaminated samples in our study were shown by HRGC-HRMS analysis to contain high concentrations of mono-*ortho* PCBs (2.3 and 2.9 pg-TEQ/g) and were not underestimated by the PCB-EIA (**Figure 3A**). Moreover, sample cytotoxicity causes false-negative CALUX results, but does not affect the PCB-EIA and Ah-I because they are cell-free tests.