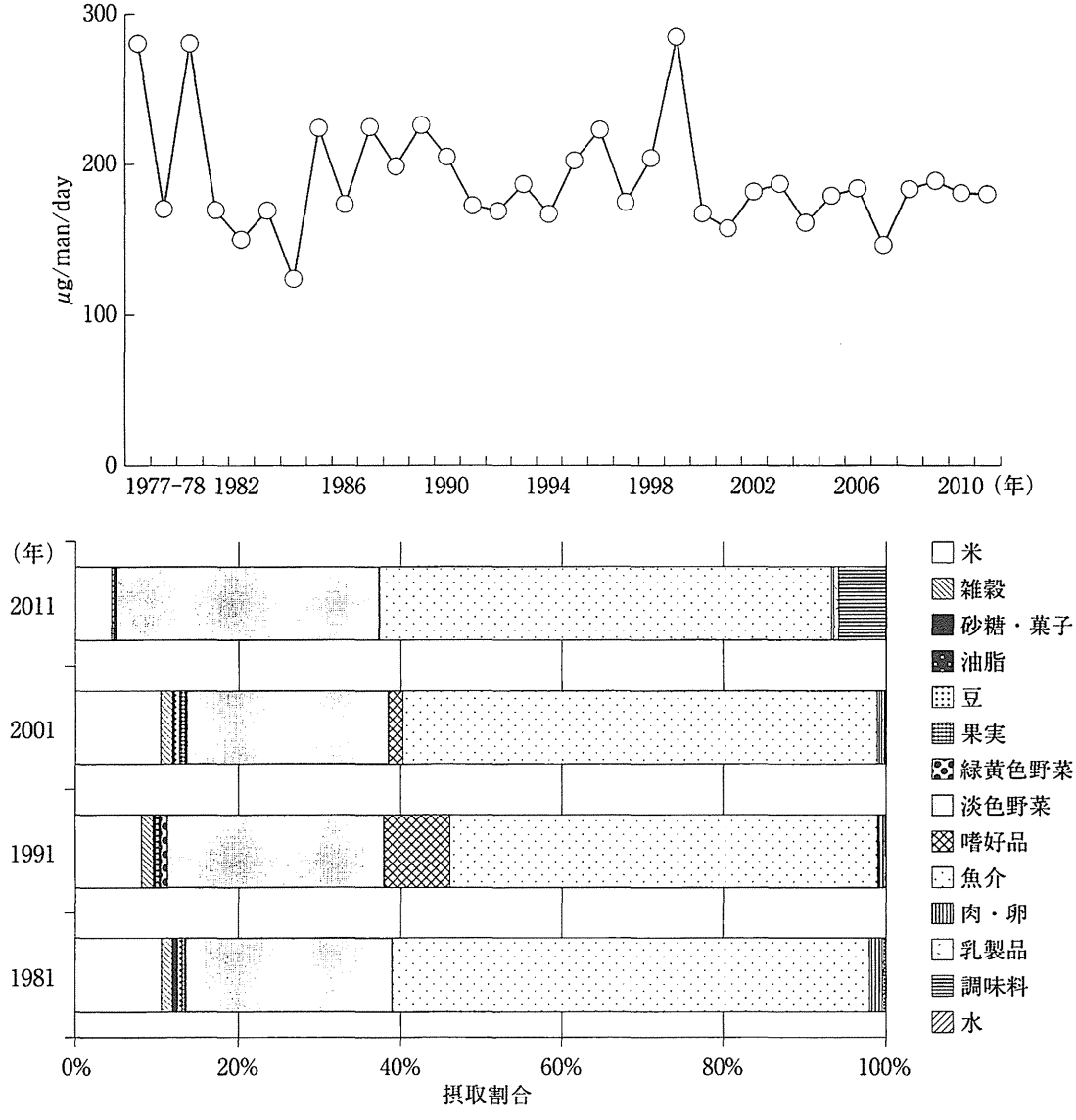

図４　総ヒ素の摂取量推移と食品群別摂取割合

た施策等も行われているが，顕著な効果は表れていないのが現状である。

Ⅳ　その他の手法による推定

次に，ダイオキシン類の摂取量をモンテカルロ法により推定した結果を紹介する。ダイオキシンの摂取量推定は1998年から継続しており，2011年のマーケットバスケット方式によるダイオキシン類の摂取量推定値は0.68 pgTEQ/kg bw/dayであり，日本におけるTDI（4 pgTEQ/kg bw/day）の約17%であった。ダイオキシン類の摂取量調査を開始した平成10年代には，2 pg TEQ/kg/日程度の摂取量であり，3分の1程度まで減少している。これは1999年に施行されたダイオキシン特別措置法による排出規制の効果と考えられる。

ダイオキシン類はPCBと同じく90%以上が魚介類から摂取されていたことを根拠として，魚介類中のダイオキシン濃度データと魚介類の摂取量分布データを用いて，モンテカルロ法による摂

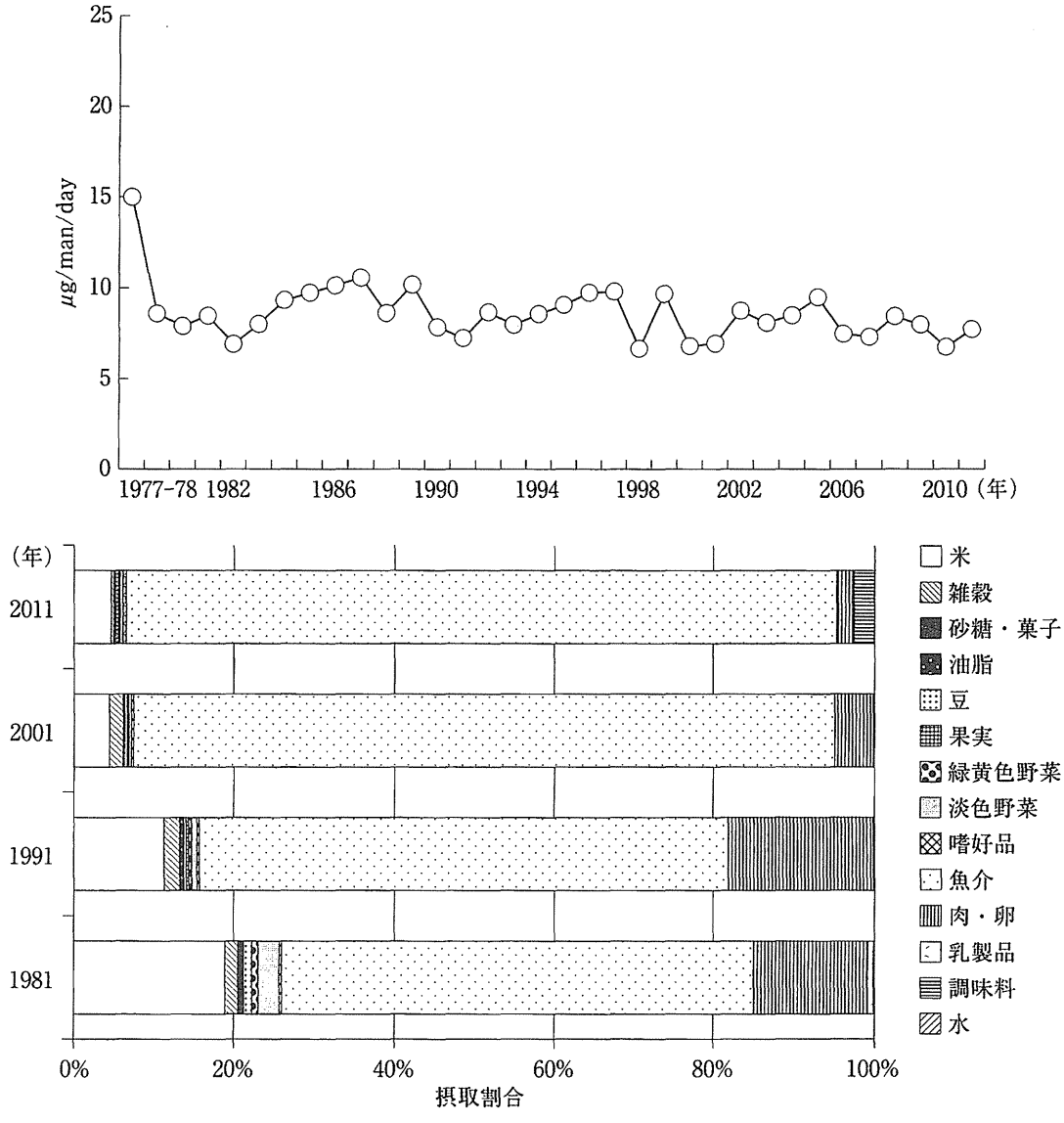

図５　総水銀の摂取量推移と食品群別摂取割合

取量推定を行った。平成15～19年度国民健康・栄養調査結果データから，魚介類を13区分（アジ・イワシ，サケ・マス，タイ・カレイ，マグロ・カジキ，その他の生魚，イカ・タコ，エビ・カニ，貝類，魚介乾物，魚介缶詰，魚介佃煮，魚介練り製品，魚肉ハム・ソーセージ）して集計した結果を，魚介類摂取量の分布とした。ヒストグラムの例を図6(A)に示す。左側に調査当日にその分類に該当する魚を食さなかった人の高いカラムがあり，適切な連続分布を当てはめることができないため，このままのデータをシミュレーションに使用した。魚介類中のダイオキシン類濃度データは，平成10～22年度の調査結果（約650試料）を用いた。魚介類を上記の13区分に分類し，ダイオキシン類濃度分布に対数正規分布を当てはめて，濃度分布として用いた。濃度分布を図6(B)に示す。

魚摂取量と濃度それぞれの分布に従う乱数を発生させ，ダイオキシン類摂取量とした。計算は魚類13区分ごとに行い，それらの総和を魚介類か

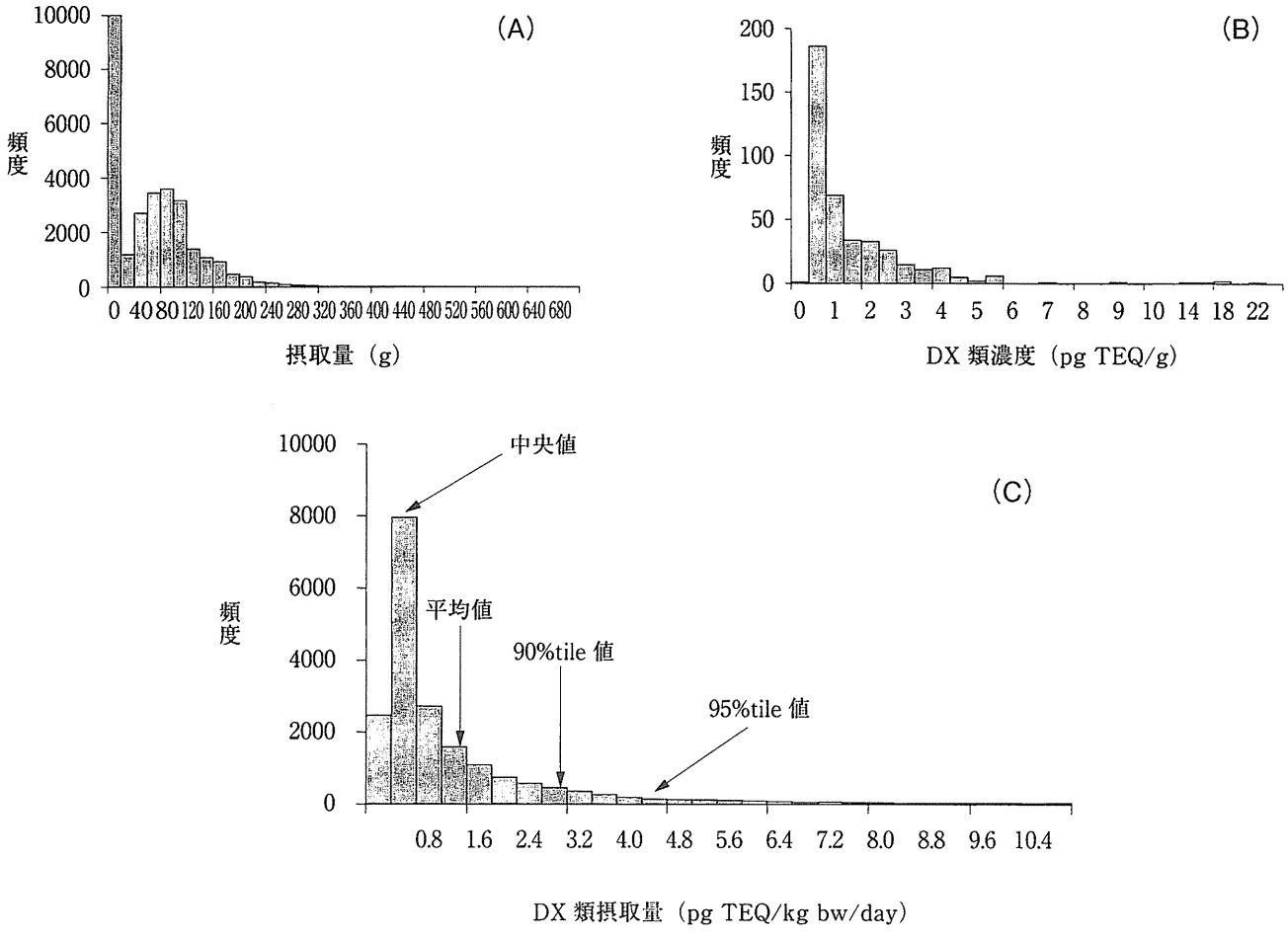

図６　モンテカルロ法によるダイオキシン類摂取量推定

A：魚類摂取量分布　　B: ダイオキシン類濃度分布　　C: ダイオキシン類摂取量分布

らのダイオキシン類摂取量とした。シミュレーションの試行回数は 20,000 回とした。得られた摂取量分布のヒストグラムを図 6(C)に示す。ダイオキシン類一日摂取量の平均値は 1.3 pg TEQ/kg/日であった。モンテカルロ法による摂取量推定では，平均値だけでなく，分布の形，中央値，90%タイル値等が得られる。これらの値を日本における TDI（4 pgTEQ/kg bw/day）と比較すると，95%タイル値がやや TDI を上回っている結果となった。中央値は 0.36 pg TEQ/kg/日，90%タイル値は 2.9 pg TEQ/kg/日，95%タイル値は 4.9 pg TEQ/kg/日であった。

モンテカルロ法による摂取量平均値の推定値 1.3 pgTEQ/kg/day は，2011 年のマーケットバスケット法による摂取量推定値 0.68 pgTEQ/kg/day より高い結果となった。魚介類中ダイオキシン濃度は，経年的に減少傾向にあり，過去の魚介類のダイオキシン類汚染データを使用しているため，現在よりもダイオキシン類濃度の高いデータが含まれていることから，シミュレーション結果が高くなったことが理由と考えられる。

V　摂取量推定値の評価

化学物質摂取量推定値が得られたら，国際的な枠組みのなかで毒性等を考慮して決められた耐容一日摂取量（TDI）等の数値と比較し，評価する。

TDIは，人が生涯にわたって摂取しても健康に対する有害な影響が現れないと判断される量であり，摂取量推定値がTDIに近ければ健康危害の発生の確率は高くなる。本稿で示した化学物質の2011年の摂取量推定値とTDIの比は，総HCHが0.003％，総DDTが0.15％，PCBが0.19％である。一方，鉛は6.8％，カドミウムは47％となっており，かなり高い比率で推移している。また，ダイオキシン類摂取量推定値のTDI比に対する比率は17％であった。

Ⅵ　今後の課題

化学物質の摂取量推定の今後の課題は，分析法に必要な性能基準の確立である。信頼性ある化学物質推定摂取量推定値を得るためには，適切な試料を作成することが第一であるが，その試料を使用する分析法の性能も非常に重要である。

正しい濃度が得られなければ，正しい摂取量推定値が得られないのは当然であり，分析法の真度が重要であることは，ほかの目的の分析と同じである。分析法によって測定値に偏りが生じては，推定が不正確になるので，70～120％程度の真度は必要となるだろう。では，精度はどの程度が必要か？多数の試料を測定することによる平均化の効果を考慮して必要な精度を決める基準は存在していない。

摂取量推定のための分析においては，特に分析法の検出下限が重要となる。Global Environment Monitoring System（GEMS）では，トータルダイエット試料分析の結果ND（検出せず）となった場合の扱いは，ND＝0とND＝LOD/2の2種類の計算を行うことが指示されている。この計算方式を採用するには，NDとなった試料が全体の60％以下であり，全試料の分析を通じて，検出下限（LOD）が一定とみなせることが前提となっている。したがって，LODが高く，ほとんどの試料がNDとなった場合には，信頼できる摂取量は推定できない。摂取量推定にトータルダイエット試料を用いる場合，その試料は多数の食品を混合して作成されており，目的とする化学物質の濃度は個別の食品よりもかなり低くなっていると考えられるので，十分低い検出限界を持つ分析法を採用する必要がある。モンテカルロ法を行う場合も，低濃度まで分析できる方法でなければ，濃度分布が推定できず，計算が不能となる。どこまでの検出限界を目指すべきかは，対象とする化学物質のTDIも考慮する必要がある。TDIに近い摂取量となる試料がNDになるような分析法は，十分な性能と言えないのは自明であるが，どの程度の検出限界が適正かの答えはまだない。

分析結果を使用する目的に予知，分析法に必要とされる性能が決まる。したがって，摂取量推定に使用する分析法の性能の妥当性を確認する基準は，推定された摂取量の信頼性はどのようにあるべきか，あるいはどのように信頼性を表示すべきかにより決まる。これが，摂取量推定において確立していくべき課題の1つである。

摂取量を推定する化学物質をどのように選択していくかは，もう一つの重要な課題である。DDTのように，既に摂取量がTDIよりはるかに低くなった化学物質の摂取量推定を，いつまでも続けることの意義には疑問がある。規制等の措置がとられた化学物質の摂取量は，施策効果が大きいほど急速に低下していく。特に，意図的に使用される化学物質の製造や使用が規制された場合などは，濃度が劇的に減少し検出が難しくなる。一方，新しく摂取量推定する化学物質の選択基準も，明確に決まっていない。

摂取量推定には時間・労力が必要であり，有限のリソースで最も効率よく実施するためには，どのような汚染物質をどのようなタイミングで摂取量推定の対象とするかの基準を決めていくことが

重要である。

最後に緊急の必要性から実施した摂取量調査の例を紹介する。平成23年3月の東京電力福島第一原子力発電所事故により，食品から放射性物質が検出される事態となった。国立医薬品食品衛生研究所食品部では，マーケットバスケット法により作成した試料を分析し，食品を介した内部被ばく線量の推定を行った。推定の目的は，当時の食品中の放射性物質規制が，健康リスクを十分に低くとどめる効果があるかの評価である。試料作製材料は，日本全体の平均的な地域として東京都，放射性物質汚染が懸念される地域として宮城県と福島県で購入した。生鮮食品は，可能なかぎり地元県産を購入し，入手できない場合には近県産，国産を優先した。

測定はゲルマニウム半導体検出器ガンマ線スペクトロメータ（Canberra社製，GC4019-7915-30-2002C）を使用し，試料約2kgを2Lマリネリ容器に入れ，24時間測定した。検出限界はCs-134およびCs-137が0.05 Bq/kg程度，K-40が0.5 Bq/kg程度であった。預託実効線量は以下の式により推定した。

1日摂取量(Bq/day)
＝濃度(Bq/g)×食品摂取量(g/day)

預託実効線量(Sv/year)
＝1日摂取量(Bq/day)×365 (day/year)×線量換算係数(Sv/Bq)

推定結果を図7に示す。白は放射性カリウム，灰色は放射性セシウムによる預託実効線量である。放射性セシウムの預託実効線量は東京都が0.0021 (0.0024) mSv/year，宮城県が0.017 (0.018) mSv/year，福島県が0.019 (0.019) mSv/yearであった。事故を起こした原子力発電所に近い福島県および宮城県での放射性セシウム預託実効線量が高く，遠い東京都で低い傾向が認められた。放射性セシウムの預託実効線量は，放射性カリウムに比較して小さく，放射性セシウム預託実効線量の放射性カリウムに対する比は，東京都では1.2%，宮城県では8.4%，福島県では10%であった。また，放射性セシウムの預託実効線量は，厚生労働省より示された許容線量1 mSv/yearを大きく下回っており，暫定規制値の設定および各地での食品の出荷制限等の施策が，食品中の放射性物質濃度を低レベルに保つための一定の効果を上げたことが示唆された。この事業は試料作成地域も10か所以上に拡大して，平成23年3月，および24年9月にも継続されている。

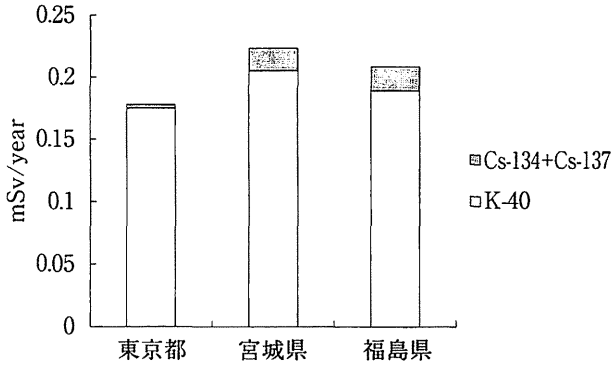

図7　放射性セシウムおよび放射性カリウムの預託実効線量

DIETARY EXPOSURE TO HEXABROMOCYCLODODECANES IN JAPAN

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Introduction

The economic benefit of adding various flame retardants to polyurethane products, such as home insulation, curtains, and plastics in PCs and other electrical appliances, has been proved. However, the disposal of these products has led to environmental pollution, and they pose a potential risk to human health. Hexabromocyclododecanes (HBCDs) are brominated flame retardants, and their high residuality in the environment and marine wildlife are problematic. Therefore, HBCDs, PBDEs, and PBBs are specified substances under the European Union Restriction of Hazardous Substances Directive and the Japanese Act on the Evaluation of Chemical Substances and Regulation of Their Manufacture, etc., (2004), and must be monitored. The consumption of HBCDs in Japan in 2003 was about 2400 t/year, which is close to that of decabromodiphenyl ether (2000 t/year), the only PBDE currently used in Japan. Human exposure to HBCDs through dietary intake has been studied by European, American, Chinese, and Japanese researchers. We have previously estimated the Japanese dietary intake of HBCDs based on the HBCD content of fish collected from the coast near Japan (1). In this paper, we present an analysis of the Japanese dietary intake of HBCDs by region and year as determined by an alternative method called total diet study (TDS). In the TDS, 13 food groups were used raw, or were prepared simply by boiling or grilling, depending on the food. The food was collected from 4 regions in 2007, 2008 and 2010 for HBCD isomeric analysis. Recently, the importance of HBCD enantiomeric analysis has been demonstrated (2,3). Therefore, the enantiomer fractions (EF) of fish which had a high HBCD content were determined. In this paper, we report the geographical changes in the average daily intake (EDI) of ΣHBCDs of the Japanese population and the enantiomeric profile of selected fish samples.

Materials and Methods

Food samples: The total diet (TD) food samples were prepared independently from 2007 to 2010 at four public laboratories located in Sendai City, Miyagi Prefecture, population ~1,000,000; Saitama City, Saitama Prefecture, population ~12,000,000; Osaka City, Osaka Prefecture, population ~8,800,000; and Fukuoka City, Fukuoka Prefecture, population ~1,450,000. The food samples were taken from 13 groups (I-XIII). However, the total number in one set of the TD food samples was 16, because additional samples were added from the X', XI' and XII' groups, which were prepared with alternative foods. More than 100 food items from 99 categories of foods commonly consumed in Japan were purchased for the preparation of food samples. The number of food items in each food group (from I to XIII) are shown in Table 1, and were determined based on actual Japanese nutrition surveillance data. In our study, group XIIII was not analyzed. Eight fish samples were collected from nearby markets for the determination of EF values.

Chemicals: Pesticide residue analysis grade dichloromethane (DCM), *n*-hexane (Hx), acetone and anhydrous sodium sulfate, (Kanto Chemical Co. Ltd., Tokyo), and reagent grade sodium chloride were used. The anhydrous sodium sulfate and sodium chloride were baked at 600 °C before use to reduce contamination. Native and $^{13}C_{12}$-labeled standards of α-, β- and γ-HBCD were purchased from Wellington Laboratories Inc. (Guelph, ON). LC/MS analysis grade methanol and distilled water were used (Kanto Chemical Co. Ltd., Tokyo), and the 44% sulfuric acid-impregnated silica gel was dioxin analysis grade (Wako Pure Chemical Industries Ltd., Tokyo).

Analysis of HBCDs: A clean-up spike of distilled water (5 mL), methanol (20 mL), and $^{13}C_{12}$-labeled HBCDs (1 ng) was added to each sample (5 g), and the mixture was homogenized. The mixture was filtered and the filtrate was collected in a 300 mL separating funnel. The residues on the filter were re-homogenized and filtered using methanol/10% DCM/Hx (20 mL, 1:1, v/v) and 10% DCM/Hx (20 mL). 5% NaCl (120 mL) was added to the collected filtrates and shaken. The organic layer was poured through anhydrous sodium sulfate into a 200 mL flask. The water layer was re-extracted twice more with 10% DCM/Hx. The collected extracts were concentrated and adjusted to 10 mL with acetone/cyclohexane (3:7, v/v). A portion of the extracts was volumetrically loaded onto a gel permeation column and fractionated at a flow rate of 5 mL/min with a mobile phase of acetone/cyclohexane. The HBCD fraction was re-purified through a mini column packed with 44% sulfuric acid impregnated silica gel prior to analysis by LC/MS/MS (Table 2). The detection limits of α-and γ-HBCD were 0.02 pg/g ww that for β-HBCD was 0.01 ng/g ww.

Table 1 Average composition of diet (2007).

Group	Foods in group	Wt, g/day
I	Rice and rice products,	343.9
II	Grains, seeds and potatoes	169.1
III	Sugar and confectionary	32.2
IV	Oils	10.3
V	Legume and legume products	59.2
VI	Fruits	125.7
VII	Carrots and green leafy vegetables	102.1
VIII	White leafy vegetables, mushrooms and seaweeds	208
IX	Beverages	601.5
X	Fish and fish products	84.1
XI	Meat and eggs	114.4
XII	Milk and milk products	125
XIII	Seasonings and other products	104.6
XIIII	Water	-
	Total	2080.1

Results and Discussion

Satisfactory recovery of HBCDs was achieved from food group X (fish and marine products) of the 2007 Kyushu TDS sample. HBCDs were detected in all the foods in group X of the TDS samples and the α- and γ- HBCD isomers were the most common. The α-isomer was the most abundant, although where the level of ΣHBCDs was very high, as in the food group X for Kyushu (24.7 ng/g), the γ-isomer was more abundant. We have previously observed this pattern in fish samples (1), and it may arise from a point source such as HBCD-contaminated sludge discharged from industrial facilities.

The mean of the ΣHBCD levels in each of the two food groups X and X' in 2007 was 1.16 ng/g (0.67, 1.64) for Kansai, 1.47 ng/g (0.62, 2.31) for Kanto and 1.71 ng/g (1.19, 2.23) for Kyushu. In 2010, the mean of the ΣHBCDs levels for Kansai, Kanto and Kyushu in each of the two food groups X and X' were 1.58 ng/g (1.27, 1.89) and 15.47 ng/g (6.22, 24.72), respectively. There was a slight increase from 2007 to 2010. The mean of the ΣHBCDs in the food groups X and X' for Tohoku prepared in 2008 was 0.97 ng/g (1.76, 0.17). The intake of HBCDs via food can be calculated by multiplying the level of ΣHBCDs by the average daily consumption per person of each food group. The daily estimated intake of HBCD in 2007 was 1.8 ng/kg bw/day for Kansai, 2.4 ng/kg bw/day for Kanto and 3.1 ng/kg bw/day for Kyushu, when assuming ND (not detected) = 0. These levels are very similar to those we estimated from the analysis of fish samples (Table 3) (1). The average EDI for ΣHBCDs in the three regions was 2.3 ng/kg in 2007 and 9.4 ng/kg in 2010, when assuming ND (not detected) = 0. The EDI of ΣHBCDs in three Japanese regions has on average increased 4.1-fold (Table 4). To clarify which marine item in food group X for Kyushu had the highest ΣHBCD level, the levels of HBCDs in all the food items were determined. Mackerel caught in the sea near Ishikawa Prefecture had the highest level (61.9 ng/g), followed by salmon (9.56 ng/g), and young yellowtail (2.54 ng/g). The Ishikawa Prefecture is located on the coast to the east of Korea and China, however, the pollution source of the mackerel was unclear.

Recently, the value of analyzing enantiomeric HBCD isomers has been demonstrated (4); therefore the EF values((+)E/((+)E+(-)E) of the HBCD enantiomers were also determined for several fish samples. A chiral column was used, and the EF values were obtained from the areas of the LC/MS/MS chromatograms (Table 5). However, there was no evidence of enantiomeric enrichment, in contrast to the pattern observed in human blood (3). This suggests that fish do not selectively metabolize one HBCD enantiomer.

The lowest observed adverse effect level (LOAEL) for HBCDs is 11.2 mg/kg bw/day in rat (4). The LOAEL was divided by a safety factor of 100, thus the tolerable daily intake should be 112 μg/kg bw/day. Therefore, the Japanese dietary intake of HBCDs is unlikely to present a serious threat to human

health. However, it is important to continue environmental monitoring of HBCDs, particularly in seafood, because they are persistent pollutants and may accumulate in the food chain.

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Acknowledgements
This work was supported by a Scientific Research Grant from the Ministry of Health, Welfare, and Labor of Japan. Govermental laboratories of Osaka, Saitama, and Miyagi prefectures are gratefully acknowledged for providing Total Diet samples.

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Table 2 LC/MS/MS analysis conditions.

Column	1.GL Sciences, Intertsil ODS-4(150×2.1 mm i.d., 5μm) 2.Marcherey-Nagel, Nucleodex b-PM(200×4.0 mm i.d., 5μm)
Column Temp. & Injection dose,	40℃; 5 μL
Mobile phase & flow rate	1. 10 mM Ammonium acetate:Methanol:Acetonitri=20:50:30(2min)～(linear gradient, 5min)～0:70:30, 0.2 μL/min 2. Methanol/ water (1:1)with 2 mM ammonium acetate:Methanol/ acetonitrile (3:7)=50:50(7min)～(linear gradient, 8min)～20:50:30, 0.5 μL/min
Aquisition mode, ESI negative MRM ; Monitor ions, 653>79、641>79, 639>79,	

Table 3 Estimated dietary intakes for an average Japanese person calculated from the HBCD concentration of fish.

Samples from which daily intake was estimated	Median of samples	EDI (ng/kg bw/day)
Five fish samples (Wild Anguilliformes, two species)	2.09	3.7
Five fish samples (farmed Salmoniformes)	1.29	2.3
30 fish samples (wild Performes, 10 species)	0.75	1.3

Table 4 Estimated intake (ng/kg bw/day) of ΣHBCDs for an average Japanese person, 2007-2010.

Region	2007		2008		2010		2007-2010	
	ND=0	ND=1/2× LOD	ND=0	ND=1/2× LOD	ND=0	ND=1/2× LOD	ND=0	ND=1/2× LOD
Kyushu	3.1	4.2			29.4	30.5	3.1-29.4	4.2-30.5
Kanto	2.4	3.4			3.6	4.7	2.2-3.6	3.4-4.7
Kansai	1.8	3.0			2.4	3.5	1.8-2.4	3.0-3.5
Tohoku			2.2	2.9			2.2	2.9

Table 5 HBCD levels and EF values for several fish samples.

na; not analyze

Fish name	Status	Sampling location	Fat (%)	α-HBCD ng/g	β-HBCD ng/g	γ-HBCD ng/g	Total HBCD ng/g	EF values
Saury 1	natural	Kanto, Japan	17.4	0.58	0.01	0.03	0.62	0.54
Saury 2	salted	Hokkaido, Japan	15.8	0.70	0.01	0.03	0.74	0.47
Salmon 1	salted	Russia, Imported	5.9	1.93	0.01	0.48	2.43	0.52
Salmon 2	salted	North America	5.9	0.13	0.00	0.00	0.13	0.50
Salmon 3	natural	Norway	9.6	0.21	0.00	0.03	0.23	0.66
Mackerel 1	salted	Norway	28.1	0.17	0.00	0.00	0.17	0.50
Mackerel 2	natural	Ishikawa, Japan	na	15.2	0.20	46.5	61.9	0.50
Young yellowtail	natural	Fukuoka, Japan	na	1.19	0.02	1.33	2.54	0.54

DETERMINATION OF BROMINATED FLAME RETARDANTS IN FOOD FROM JAPANESE MARKETS

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Introduction

Brominated flame retardants (BFRs), such as polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBBs), have been widely used in plastics and textile coatings throughout the world. The major commercial products made with the most commonly used PBDEs are penta-BDE, octa-BDE and deca-BDE (DeBDE). In Japan, although the use of low-brominated PBDEs has decreased, DeBDE is currently in use. PBDEs are additives to polymers such as polystyrene and are not chemically bound to the polymer. They are therefore easily released into the environment from waste products. It is predicted that, in Japan, the amount of waste Br from the plastics used in electrical appliances will increase until at least 2020 due to the increasing size of TV sets there[1]. This prediction suggests an urgent need to monitor these brominated compounds and to manage them in waste. For PBBs, the commercial products are mixtures containing hexa-BB, octa-BB, nona-BB, and deca-BB. Products made with PBBs have not been produced in Japan, but PBBs have been detected in environmental samples[2]. It is suspected that the contaminants in these samples have come from imported products or impurities in other BFRs. Decabromodiphenyl ethane (DBDPE) and bis(2,4,6-tribromophenoxy)ethane (BTBPE) are relatively new brominated flame retardants that came to market in the 1990s as alternatives to DeBDE. There is very little information about their toxicity or contamination levels.

In relation to BFRs, it is problematic that *de novo* synthetic compounds such as polybrominated dibenzo-*p*-dioxins, dibenzofurans (PBDD/DFs), and coplanar polychlorinated/brominated biphenyls (Co-PXBs) have been found in market fish[3)4)] and human samples[5)6)]. Co-PXBs may also be formed from BFRs and have toxicity levels similar to those of Co-PCBs due to their structural similarities.

It is important to investigate the levels of these brominated organic compounds in foods and to estimate their effects on humans. In our previous study, we developed a simultaneous-analysis method for brominated compounds, including new BFRs such as DBDPE and BTBPE[7]. In the present study, we analyzed BFRs in fish samples and market basket samples in Japan.

Materials and methods

Chemicals

DBDPE, BTBPE, and PBDE analytical standards were purchased from Wellington Laboratories. The PBB analytical standards were purchased from Wellington Laboratories and AccuStandard. Dichloromethane, *n*-hexane, and acetone used for extraction and cleanup were of dioxin analysis grade (Kanto Chemical). Silica gel (Wako Pure Chemical Industries) was heated for 3 h at 130°C. A sulfoxide cartridge column (6 g, 20 g glassware) was purchased from Sigma-Aldrich.

Analytical Methods and Instrumentation

The concentrations of DBDPE, PBDEs, Co-PXBs, and PBBs were determined using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS). The analytical conditions of HRGC/HRMS are shown in Table 1. HRGC/HRMS analysis was performed on a Micromass Autospec Ultima (Waters) connected to an HP6890 GC (Agilent).

Samples

The fish samples for the analysis of brominated compounds were purchased from fish markets in 3 different regions (Chubu, Chugoku-Shikoku, and Kyushu) of Japan. The edible parts of fish samples were blended using a food processor. The food mixtures were kept below –20°C until analysis.

For a market basket study, 120–200 kinds of foods were purchased from markets in 2 different regions (Kanto and Kyushu) of Japan. They were divided into 13 food groups and were weighed and cooked based on the daily consumption data calculated from the Japanese Nutrition Survey carried out by the Ministry of Health, Labor, and Welfare of Japan. The foods were blended with a food processor. The mixtures were kept below –20°C until analysis.

Sample Preparation

The analytical method for the brominated compounds was as follows. Each 50-g sample was freeze-dried using a model AD 2.0ES-BC (VirTis) freeze dryer. Dried samples were extracted with 10% (v/v) dichloromethane/*n*-hexane by an accelerated solvent extractor ASE300 (Dionex). The extraction temperature was 100°C; the time was 10 min. Extracts were treated with sulfuric acid three times and applied to a silica gel column. The column was prewashed with 100 ml *n*-hexane, and brominated compounds were eluted with 150 ml of 10% (v/v) dichloromethane/*n*-hexane. The eluate was evaporated and dissolved in 1 ml of *n*-hexane and treated with a sulfoxide cartridge column to remove the matrix. A sulfoxide column was prewashed with 20 mL of acetone and 20 mL of *n*-hexane. After the sample solution was loaded, the column was washed with 12 mL of *n*-hexane, and the fraction of target compounds was eluted with 25 mL of 50% (v/v) acetone/*n*-hexane. The eluted fraction was concentrated to a final volume of approximately 25 µl, and the samples were analyzed by HRGC/HRMS. The analytical method of BFRs is shown in Fig. 1.

Table 1 Analytical conditions of HRGC/HRMS

Compounds	Column	Injection temp.	Injection type /volume	Oven temp.	HRMS conditions
DBDPE	DB-5 15 m, 0.25 mm i.d., 0.1 µm film	260°C	Splitless 1 µl	100°C–(20°C/min)–200°C–(10°C/min)–320°C (7 min)	Electron energy: 38eV Filament current: 750µA Ion source temp: 270°C Resolution 10,000
PBDEs	DB-5 15 m, 0.25 mm i.d., 0.1 µm film	260°C	Splitless 1 µl	100°C–(20°C/min)–200°C–(10°C/min)–320°C (7 min)	
PBBs Co-PXBs	DB-5 15 m, 0.25 mm i.d., 0.1 µm film	280°C	Splitless 1 µl	130°C(1 min)–(20°C/min)–170°C(10 min)–(4°C/min)–210°C–(10°C/min)–300°C (3 min)	

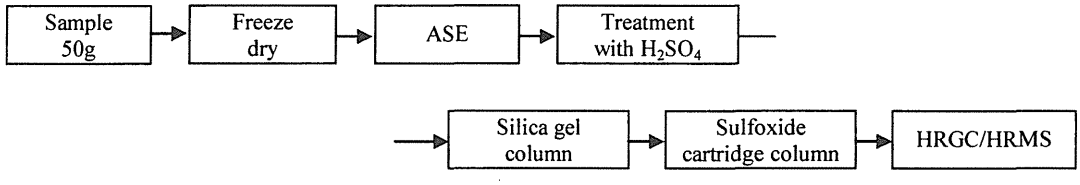

Fig. 1 Analytical method of BFRs (DBDPE, PBDEs, PBBs, and Co-PXBs)

Results and discussion

Concentrations of brominated compounds of fish samples

We have previously analyzed the PBDEs (23 congeners), PBBs (23 congeners), and Co-PXBs (7 congeners) in 12 kinds of fish[8]. In the present study, the other BFR compound, DBDPE was analyzed in the same fish samples by our previously developed method[7]. The results of the analysis are shown in Table 2. DBDPE was detected from 4 samples of fish; sea bream (1), conger eel (2), and flatfish (1). The concentrations of detected DBDPE were 5.86–8.68 pg/g wet weight (ww), 0.0541–0.533 ng/g lipid weight (lw). The detection limit of this method was 2 pg/g ww. The concentration of DBDPE was lower than that of PBDEs. Analysis of DBDPE in

fish samples has also been carried out in other countries, with values of 35–68 ng/g lw from Chinese fish and ND–3.3 ng/g lw from Canadian fish have been reported[9)10)]. The values obtained in our study are lower than those reported previously.

Table 2 Concentrations of DBDPE, PBDEs, PBBs, and Co-PXBs in fish samples

No.	Fish	Production regions of Japan	DBDPE		PBDEs[8)]	PBBs[8)]	Co-PXBs[8)]
			(pg/g ww)	(ng/g lw)	(ng/g ww)	(pg/g ww)	(ng/g ww)
1	Sea bream-1	Chubu	ND		0.100	0.230	ND
2	Sea bream-2	Chubu	8.08	0.292	0.247	0.813	ND
3	Sea bream-3	Kyushu	ND		0.016	0.105	ND
4	Sea bream-4	Chugoku-Shikoku	ND		0.018	ND	ND
5	Conger eel-1	Chubu	6.38	0.0541	0.818	2.24	ND
6	Conger eel-2	Chugoku-Shikoku	6.62	0.0670	0.406	1.83	ND
7	Flatfish	Chugoku-Shikoku	5.86	0.533	0.044	ND	ND
8	Shrimp	Kyushu	ND		0.033	ND	ND
9	Horse mackerel	Kyushu	ND		0.334	1.43	ND
10	Sand borer	Chugoku-Shikoku	ND		0.095	0.299	ND
11	Mackerel	Kyushu	ND		0.617	1.98	ND
12	Sardine	Kyushu	ND		0.167	0.827	ND

Daily intake of brominated compounds

Market basket samples were collected from 2 regions (A region: Kanto, B region: Kyushu) in Japan. They were analyzed for the estimation of the daily intake of BFRs. Daily intakes of DBDPE, PBDEs, PBBs, and Co-PXBs in each food group are shown in Table 3.

For DBDPE, the total daily intake was estimated as 1.27 ng/day for the A region and 0.190 ng/day for the B region, assuming that ND=0. In the case of 50 kg of body weight (bw), the daily intake was calculated as 0.0254 ng/kg bw/day and 0.0038 ng/kg bw/day, respectively (assuming ND=0). In the case of assuming that ND=1/2 limits of detection (LOD), the daily intake was calculated as 0.0690 and 0.0484 ng/kg bw/day.

For PBDEs, the daily intakes for the A and B regions were estimated to be 82.8 and 96.3 ng/day, respectively. In the case of 50 kg bw, the daily intake was calculated as 1.66 ng/kg bw/day for the A region and 1.93 ng/kg bw/day for the B region (assuming ND=0). In the case of assuming that ND=1/2 LOD, the daily intakes were calculated as 1.71 and 1.98 ng/kg bw/day, respectively. In a recent report, the lowest observed adverse effect level (LOAEL) value suggested as reasonable for compounds or mixtures belonging to the PBDE group were 1 mg/kg bw/day[11)]. Since the calculated value in this study was much less than this LOAEL value, the daily intake level of PBDEs was not considered a serious problem.

For PBBs, the daily intakes of A and B regions were estimated to be 0.393 and 0.119 ng/day, respectively. In the case of 50 kg body weight, the daily intake was calculated as 0.00786 ng/kg bw/day for the A region and 0.00238 ng/kg bw/day for the B region (assuming ND=0). In the case of assuming that ND=1/2 LOD, the daily intakes were calculated as 0.0662 and 0.0598 ng/kg bw/day, respectively. It was suggested that the total daily intake of PBBs should be less than 0.15 μg/kg bw/day by WHO Environmental Health Criteria. Compared with this value, the levels of the PBBs obtained in this study were not considered a serious problem.

Table 3 Daily intakes of DBDPE, PBDEs, and PBBs in each food group

No.	Food group	DBDPE (ng/day)		PBDEs (ng/day)		PBBs (ng/day)	
		A region	B region	A region	B region	A region	B region
1	Rice and rice products	0	0	1.72	9.30	0	0
2	Cereals seeds and potatoes	0	0	3.45	6.40	0	0
3	Sugars and confectioneries	0	0	0.304	1.05	0	0
4	Fats and oils	0.407	0	1.32	11.3	0	0
5	Pulses	0	0	2.33	1.45	0	0
6	Fruits	0	0	0.335	0.591	0	0
7	Green vegetables	0	0	1.62	0.681	0	0
8	Other vegetables and sea weeds	0	0	7.60	2.61	0	0
9	Beverages	0	0	7.99	5.44	0	0
10	Fish and shellfish	0.102	0	40.7	45.4	0.386	0.119
11	Meat and eggs	0.759	0.19	8.08	8.51	0.007	0
12	Milk and dairy products	0	0	1.30	2.65	0	0
13	Other foods (seasoning)	0	0	6.00	0.929	0	0
	Total	1.27	0.19	82.8	96.3	0.393	0.119

Daily intake calculated assuming that ND=0.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labor, and Welfare, Japan.

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