

Comprehensive Analysis for Organo-bromine Compounds in Environmental Samples with GC/MS (EI/NCI)

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Introduction. Brominated flame retardants (BFRs) are chemicals that are added to polymers that are used in plastics, rubber, textiles and other materials to prevent fires. Similarly to other organic pollutants, BFRs have persistence and bioaccumulation potential in organisms. Recently, BFRs have become of increasing concern in environmental samples, due to the increase of the concentration of BFRs in contrast to that of organo-chlorine compounds such as PCDDs/DFs and PCBs. PBDEs have been emerged the level of contamination in environment (water, air, sediment, sewage sludge, organism) and human (blood, milk, tissue) at great endeavor by a large number of researchers. However, other BFRs scarcely emerge the actual circumstances of contamination, although tribromophenol has acute toxicity against aquatic organisms, tetrabromobisphenol A (TBBPA) has persistence and endocrine disruption potential, and hexabromocyclododecane (HBCD), bis(tribromophenoxy)ethane (BTBPE), and Decabromodiphenylethane (DBDPE) have persistence and bioaccumulation potential similarly to PBDEs. Moreover, as one of the actual circumstances of contamination by BFRs, it has reported that some BFRs are transformed to organo-bromine compounds that have a higher bioaccumulation potential than their parent compounds in environment. For example, dimethylated derivative of TBBPA may have some use as a flame retardant, but may also be the result of methylation of TBBPA in sediment, or in organism. Similarly, tribromoanisole may be the result of methylation of tribromophenol. And PBDEs are transformed to hydroxylated and methoxylated PBDEs through metabolism in vivo. Considering these facts, in products such as plastics, in environment such as water, sediment, and organisms, it is supposed that various pieces of organo-bromine compounds are contained, which are 1) known BFRs such as PBDEs, tribromophenol, TBBPA, HBCD, BTBPE, and DBDPE, 2) unknown BFRs, 3) organo-bromine compounds transformed from BFRs, and 4) natural organo-bromine compounds. Therefore, investigation of various organo-bromine compounds is required to evaluate the actual circumstances of contamination by BFRs in products and environmental samples. In previous study, we developed the comprehensive analytical method for various organo-bromine compounds by gas chromatography/mass spectrometry (GC/MS) with electron impact (EI) or negative ion chemical ionization (NCI), established suitable clean-up method, and these GC/MS and clean-up techniques were applied for blubber of finless porpoise sample. In the present study, we analyzed plastic products and blubber of finless porpoise samples, identified known or unknown organo-bromine compounds, investigated the level of contamination in various pieces of organo-bromine compounds, and quantified organo-bromine compounds which ¹³C-labelled internal standard were available, such as PBDEs, HBCD, TBBPA and TBBPA dimethyl ether that is the metabolite of TBBPA, tribromophenol and tribromoanisole that is the metabolite of tribromophenol, BTBPE, DBDPE.

Materials and methods. Plastic sample (0.25 g) was extracted with tetrahydrofuran, and fractionated to non- and slightly-polar components, and polar components with silicagel. Polar fraction was ethylated, both of

fractions were mixed and passed through sulfuric acid-silicagel column, and measured by GC/MS. Blubber sample (1 g, finless porpoise) was extracted with dichloromethane, fractionated non- and slightly-polar components and polar components with silicagel. Polar components fraction was ethylated, each of fractions were passed through sulfuric acid-silicagel column, and measured by GC/MS. The procedure of comprehensive analytical method by GC/MS was followed. Firstly, GC/LRMS NCI scanning was

performed for assignment of peaks of organo-bromine compounds from mass chromatograms of $m/z = 79, 81$. Secondly, GC/LRMS EI scanning was done for identification of organo-bromine compounds by examination of the mass spectrum in assigned peaks. GC condition for EI scanning was the same as NCI. Thirdly, GC/HRMS EI scanning was done for determination of molecular formula of unknown compounds with this accurate mass measurement. Fourthly, GC/HRMS EI SIM analysis in using all available ^{13}C -labelled internal standards was done for the target compounds quantification.

We used Thermoelectron Finnigan MAT95XL for LRMS EI or NCI scanning, Micromass AutoSpec-Ultima for HRMS EI scanning. And we selected the GC column that its length is short (15 m), its film thickness is thin (0.1 μm), and its phase is slightly polar ((5%-phenyl)-methylsiloxane) due to avoid thermal digestion of organo-bromine compounds in GC column, controlled thermal digestion in injector by on-column injection, and controlled digestion by light by using amber glassware and UV cut off light.

Results and discussion. Figure 2 shows the result of plastic product. From mass chromatogram of bromine ion ($m/z 79, 81$) obtained by GC/LRMS NCI scanning, 7 peaks of organo-bromine compounds were assigned. From mass spectrum obtained by GC/LRMS EI scanning, its retention time was the same as in NCI, peak 1 was identified as tribromoanisole, peak 2 was tribromo-phenol, peak 3 was tetrabromodibenzo- p-dioxin (TeBDD), peak 4-6 were nonabromodiphenyl ethers (NBDEs), peak 7 was decabromodiphenyl ether (DeBDE). This result suggested that tribromophenol and DeBDE were intentionally added as BFR, and

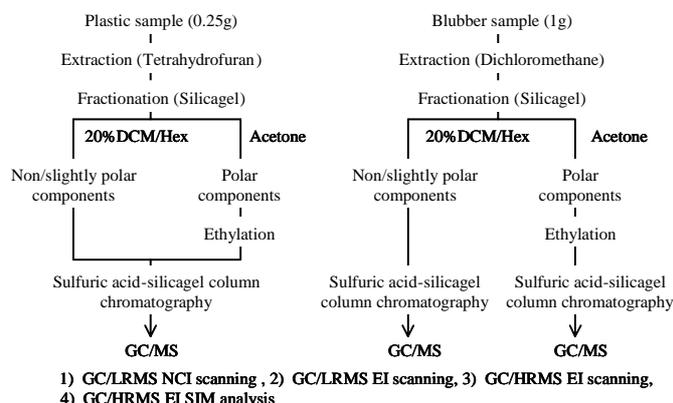


Figure 1. Procedure of comprehensive analysis for organo-bromine compounds in plastic and blubber sample

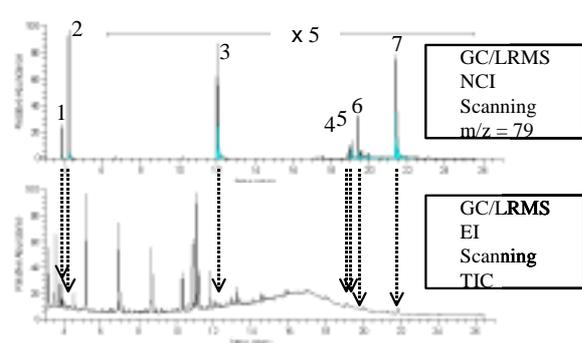


Figure 2. Chromatograms obtained from plastic product. Upper: Mass chromatogram of $m/z = 79$ (Br^+) by GC/LRMS NCI scanning. Lower: TIC chromatogram by GC/LRMS EI scanning. 1: Tribromoanisole, 2: Tribromophenol, 3: TeBDD, 4-6: NBDEs, 7: DeBDE

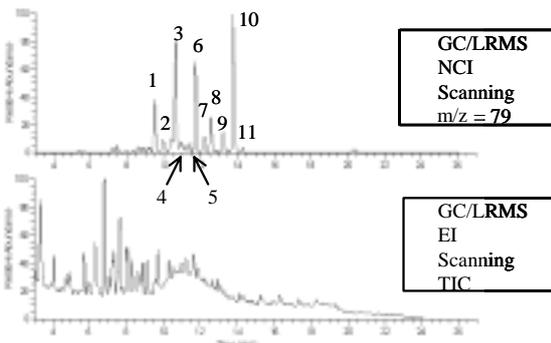


Figure 3. Chromatograms obtained from non and slightly polar components fraction in blubber of finless porpoise. Upper: Mass chromatogram of $m/z = 79$ (Br^+) by GC/LRMS NCI scanning. Lower: TIC chromatogram by GC/LRMS EI scanning. 1: TeBDE, 2: PeBDE, 3: Unknown 1, 4-5: PeBDE, 6: Unknown 2, 7-9: HxBDE, 10: Unknown 3, 11: HpBDE

tribromoanisole and TeBDD were unintentionally produced with methylation and combination of tribromophenol. Figure 3 shows the result of non- and slightly- polar components fraction in blubber sample. By GC/LRMS NCI and EI scanning, peak 1 was identified as tetrabromodiphenyl ether (TeBDE), peak 2, 4, and 5 were Pentabromodiphenyl ethers (PeBDEs), peak 7-9 were Hexabromodiphenyl ethers (HxBDEs), and peak 11 was Heptabromodiphenyl ether (HpBDE). Peak 3, 6, and 10 were unknown compounds, therefore, HRMS EI scanning was performed for determination of molecular formula of unknown compounds 1-3 with these accurate mass measurement. Figure 4 shows the theoretical accurate mass spectrum of methoxylated TeBDE (MeO-TeBDE), and the accurate mass spectrum obtained from unknown 1 by GC/HRMS EI scanning. The difference of accurate mass between theoretical and observed spectrum was ± 10 ppm. This result suggested that unknown 1 was MeO-TeBDE.

Figure 5 shows the theoretical accurate mass spectrum of tribromohexahydro-1*H*-xanthene derivatives (TrBHD), and the accurate mass spectrum obtained from unknown 2 by GC/HRMS EI scanning. TrBHD is one of the halogenated natural products that are widespread in nature. The difference of accurate mass between theoretical and observed spectrum was ± 10 ppm. This result suggested that unknown 2 was TrBHD.

Figure 6 shows the theoretical accurate mass spectrum of tetrabromohexahydro-1*H*-xanthene derivatives (TeBHD), and the accurate mass spectrum obtained from unknown 3 by GC/HRMS EI scanning. TeBHD is also one of the halogenated natural products that are widespread in nature. The difference of accurate mass between theoretical and observed spectrum was ± 10 ppm. This result suggested that unknown 3 was TeBHD.

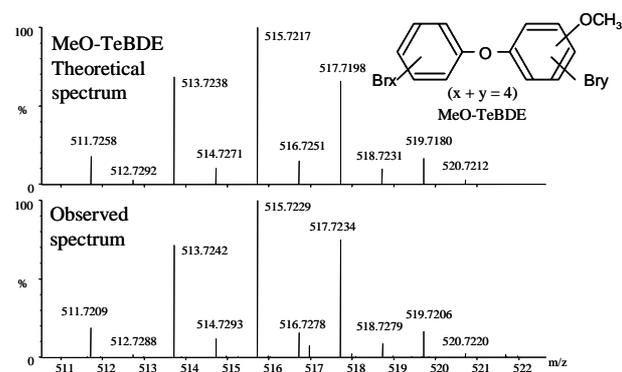


Figure 4. Theoretical spectrum of MeO-TeBDE and observed spectrum of unknown 1 by GC/HRMS EI scanning

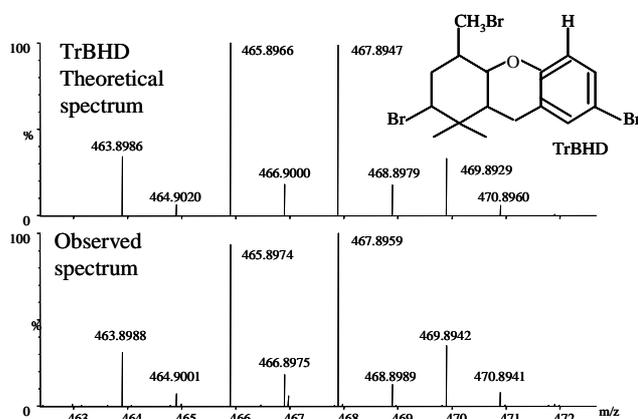


Figure 5. Theoretical spectrum of TrBHD and observed spectrum of unknown 1 by GC/HRMS EI scanning

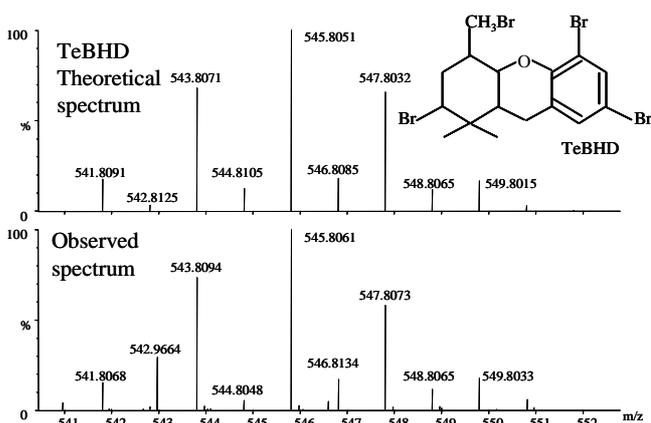


Figure 6. Theoretical spectrum of TeBHD and observed spectrum of unknown 1 by GC/HRMS EI scanning

We compared mass chromatogram of MeO-TeBDE with that of TeBDE, it emerged that MeO-TeBDE was detected the same intensity of TeBDE. This result suggested that MeO-TeBDE was contained the same level of concentration of TeBDE in blubber of finless porpoise.

Moreover, GC/HRMS EI SIM analysis was performed for the target compounds quantification in this sample by using all available ¹³C-labelled internal standards. BFRs such as PBDEs and HBCD were detected relatively high level, while the concentration of BDE-47 200 ng/g was the highest among PBDEs detected. BDE-209 was also detected although the concentrations were lower than those of other congeners. The concentrations and composition of PBDEs found in the blubber of finless porpoise were similar to those observed in the blubber of coastal cetaceans in Japanese waters. DBDPE is also detected in the blubber of finless porpoise. To our knowledge, this is a first report on the detection of DBDPE and the level is higher than DeBDE, indicating its increasing use as an alternative BFR of DeBDE. The usage of HBCD, DBDPE and BTBPE are gradually increased since 1990's in Japan. TBBPA dimethyl ether and tribromoanisole, which are metabolites of TBBPA and TrBPh, respectively, are also detected at higher concentrations than their parent compounds. Consequently, it is necessary to investigate the extent of pollution by these compounds and their accumulation in the ecosystem.

Table 1. Concentration of organo-bromine compounds in blubber of finless porpoise sample by GC/HRMS EI SIM analysis

Compounds	TrBDEs	TeBDEs	PeBDEs	HxBDEs	HpBDEs	OBDEs	NBDEs	DeBDEs
Concentration (ng/g)	31	280	180	370	76	39	0.22	0.27
Compounds	TBBPA	TBBPA dimethyl ether	TrBPh	Tribromo anisole	HBCD	BTBPE	DBDPE	
Concentration (ng/g)	0.089	0.13	0.46	1.1	86	0.10	7.3	

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