Considerations in the analysis of 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane and identification in beluga (*Delphinapterus leucas*) from the Canadian Arctic

Tomy GT[a], Pleskach K[a], Arsenault G[b], Chittim B[b], McCrindle R[b], Yeo B[b], Marvin CH[c], Sverko E[c], Tittlemier SA[d]
[a] Department of Fisheries and Oceans, Winnipeg, Manitoba, R3T 2N6, Canada
[b] Wellington Laboratories Inc., Research Division, Guelph, Ontario, N1G 3M5, Canada
[c] Environment Canada, Burlington, Ontario, L7R 4A6 Canada
[d] Food Research Division, Health Canada, Ottawa, Ontario, K1A 0L2, Canada

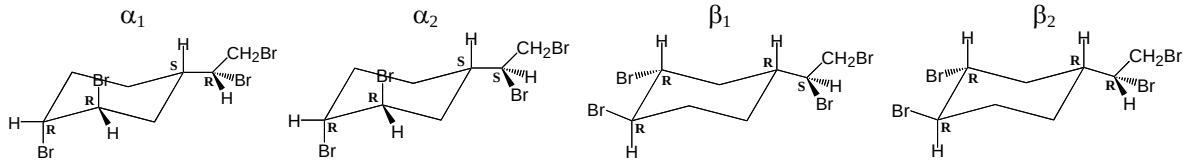
Abstract

A gas chromatography mass spectrometric (GC/MS) based method for the analysis of 1,2-dibromo-4-(1,2-dibromoethyl)cyclohexane (TBECH), has been developed for the two main diastereomers (α_1 and α_2) present in the technical mixture. Careful selection of GC-capillary column length is critical in resolving the two isomers: a column length of 30m (0.25 μm film thickness) results in incomplete separation of the two isomers while a 10m column results in baseline separation. While electron capture negative ion (ECNI) MS is more sensitive than electron ionization (EI), no characteristic ions in the ECNI mass spectrum of TBECH was evident, and because the α_2-diastereomer co-eluted with BDE-15 on the short column, more specificity was required. In EI, the dominant ions in the mass spectrum corresponded to a loss of HBr and Br from the molecular ion. The biggest peak in this ion cluster (*m/z* 266.9) was used for quantitation and the second biggest peak (*m/z* 264.9) was used for confirmation. Beluga (*Delphinapterus leucas)* extracts of animals from the Canadian arctic were quantified using low resolution (LR) MS and high resolution (HR) MS run at a resolving power of 10 000 (ions monitored: *m/z* 266.9908 and 264.9228). The LRMS technique gave numbers that were 1.5 to 2 times greater than that of HRMS measurements suggesting a small interference arising at nominal mass. Observed concentrations as measured by HRMS ranged from 6.5 to 8.7 ng/g (lipid weight).

Introduction

1,2-Dibromo-4-(1,2-dibromoethyl)cyclohexane, also known as tetrabromoethylcyclohexane (TBECH) is used primarily as an additive flame retardant in expandable and extruded polystyrene insulating boards; some secondary applications include use as adhesives in vinyl lamination, coatings in electric cables, and in high-impact plastic parts of appliances[1,2]. Information on production and usage is sparse but one report list production for 2002 between 4.5 to 226 MT[3].

Technical grade TBECH consists of equimolar amounts of two diastereomers, α_1 and α_2 (Figure 1). Interestingly, at temperatures above 150°C, a small amount of thermal conversion of both isomers occurs, resulting in the formation of two isomers, referred to as β_1 and β_2. Incorporation of hexabromocyclododecane (HBCD) as a flame retardant into polystyrene, which is used in similar applications to TBECH, is conducted at elevated temperatures resulting in thermal conversion of the γ to the α-isomer. Not surprisingly, both of these HBCD isomers are detected in the environment. Analogously then, while the technical TBECH formulation consist primarily of α_1 and α_2, like HBCD, we might expect to detect the thermally formed isomers, β_1 and β_2 in the environment.



α2-TBECH ≡ *rac*-(1*R*,2*R*)-1,2-dibromo-(4*S*)-4-((1*S*)-1,2-dibromoethyl)cyclohexane
α1-TBECH ≡ *rac*-(1*R*,2*R*)-1,2-dibromo-(4*S*)-4-((1*R*)-1,2-dibromoethyl)cyclohexane
β_1-TBECH ≡ *rac*-(1*R*,2*R*)-1,2-dibromo-(4*R*)-4-((1*S*)-1,2-dibromoethyl)cyclohexane

β_2-TBECH ≡ *rac*-(1*R*,2*R*)-1,2-dibromo-(4*R*)-4-((1*R*)-1,2-dibromoethyl)cyclohexane

Figure 1. Structures and nomenclature of the four possible TBECH diastereomers[4]

A recent report has suggested that TBECH binds and activates the human androgen receptor[1]. TBECH has also been shown to bioaccumulate in fish in laboratory based studies[5]; this is not too surprising considering that the log K_{ow} value of 4.96[2] (no information on which isomer was measured) is essentially at the accepted optimum bioaccumulation potential of log K_{ow} 5. Despite these two important studies, little is known about the environmental fate and behavior of TBECH. The goals of this study then are to develop an analytical method for the detection of the isomers of TBECH in environmental samples based on mass spectrometry and to determine the extent of contamination in select samples from the Canadian environment.

Experimental

Chemicals

Standard solutions of α_1 and α_2, TBECH were from Wellington Laboratories (Guelph, ON).

Environmental Samples

Extracts of Canadian Arctic beluga (*n=3*, 2004) were available from our other studies on bromine-based flame retardants. Blubber (0.1g) were spiked with a suite of recovery internal standards and extracted with hexane:dichloromethane:acetone (4.5:4.5:1) using a Polytron. Lipids were removed by automated gel permeation chromatography using a column packed with SX3-Biobeads. Further clean-up was done on a column containing Florisil. Extracts were reduced in volume using UHP nitrogen and spiked with an instrument performance internal standard to monitor changes in instrument performance.

Gas Chromatography/Mass Spectrometry

Low resolution analyses were performed on Agilent 5973 GC-mass selective detectors (Mississauga, ON, Canada) fitted with either a 30m or a 10m DB-5 capillary column (0.25 μm film thickness x 0.25 mm i.d; J&W Scientific, Folsom, CA, USA). On-column injections of 0.5 μL were made onto an injector set initially at 90ºC. The column program started at 90ºC with no hold time, ramped at 20ºC/min to 310ºC, and held for 5 min. The MS analysis was performed in either ECNI mode using methane as the buffer gas or EI mode. Source and quadrupole temperatures were both set to 150ºC. The same 10 m column was used in our HRMS analysis: *m/z* 266.9908 and 264.9228 ions corresponding to a loss of HBr and Br from the molecular ion were used in our analysis.

Results and Discussion

Elution behavior α_1 and α_2 isomers

Figure 2 shows the elution profiles of the α_1 (1st eluting peak) and α_2 (2nd eluting peak) isomers on a 30m and 10m column. Interestingly, the isomers are baseline resolved using the 10m column while incomplete resolution is observed with the 30m column. This suggests that longer residence times on the analytical column can lead to thermal breakdown of the isomers.

Selection of MS ionization mode

The use of ECNI is well established for the analysis of brominated flame retardants (BFRs). Brominated diphenyl ether (BDE) congeners, as one example, produce an intense *m/z* 79/81 signal allowing for routine trace (pg/g) analysis. Because this method offers little specificity, care is required for positive identification of the congeners.

While high resolution mass spectrometry (HRMS) offers a high degree of specificity, the technique is not available to most laboratories but was still employed in the EI mode to verify the measurements made under LRMS conditions.

Our initial attempt was to develop a method that was based on ECNI using the *m/z* 79/81 ions so that it could be incorporated easily into our existing protocols. However, because of our short analytical column, there was co-

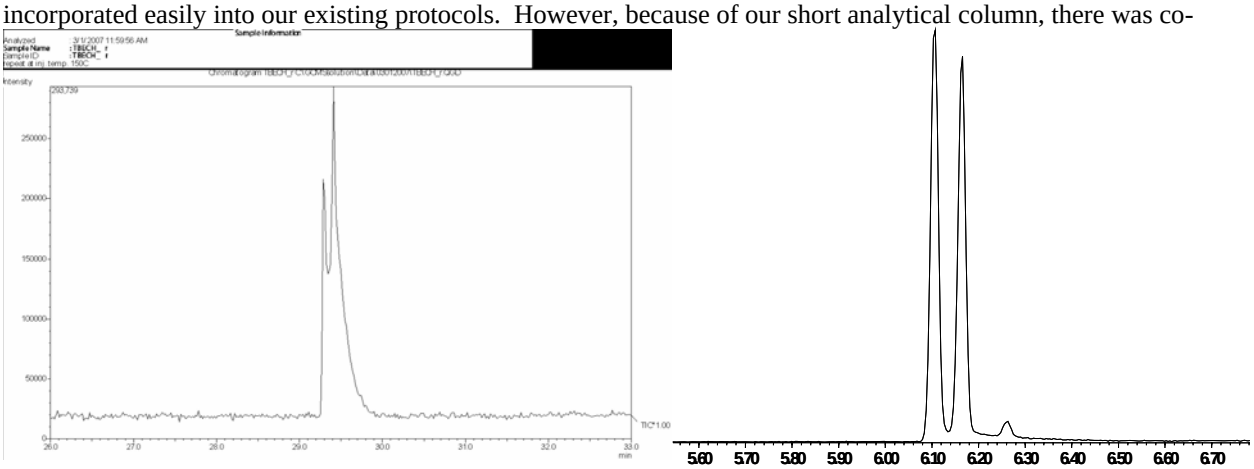


Figure 2. Elution of α_1 and α_2 isomers using a 30m (left panel) and a 10m (right panel) DB-5 columns.

elution of the α_2 isomer with BDE-15. By doing a full scan ECNI we had hoped to find an ion(s) more specific to TBECH which would allow us to mass resolve it from the BDE-15. This proved futile as the ECNI mass spectrum of TBECH showed only two clusters one at *m/z* 79 (Br^-) and the other at *m/z* 160 ($2Br^{-\bullet}$); neither being specific to TBECH.

Our next step was to try low resolution MS in the EI mode. A full scan EI mass spectrum showed a base peak corresponding to a loss of HBr and Br from the parent molecule; from this ion cluster, the biggest peak *m/z* 266.9 was selected as our quantitation ion and the second biggest peak *m/z* 264.9 was used for confirmation. These two ions were not evident for BDE-15. The theoretical ratio of the *m/z* 264.9/266.9 for TBECH is 1.96; under LRMS, the observed ratio was 1.98 ± 0.05 (arithmetic mean ± 1×standard error). Under HRMS EI, the ratio was determined to be 1.95 ± 0.04.

Quality control

Two important criteria were used for confirmation of α_1 and α_2 isomers in our beluga samples. First, the elution time of the isomers must be within ± 2 seconds in the sample and external standard. Second the ratio of the quantitation to confirmation ion must be within ± 20% of the observed value in our standard. Blanks contained undetectable concentrations of both isomers suggesting that minimal contamination is occurring in our extraction and workup procedures.

Application of the method

The isomers of TBECH are not discriminated against in our laboratories extraction and workup procedures of other BFRs and so extracts that were used previously for other BFRs were applicable for this study. The GC

chromatogram under LRMS showed a peak corresponding to the α_2 isomer in all three beluga samples with a range in concentration of 11.2 to 18.9 ng/g (lipid weight); the first eluting isomer, α_1, was not detected in any of our samples. These concentrations are in good agreement other BRFs in these animals: total BDE and HBCD concentrations, for example, in these animals ranged from 34-50 and 0.9 to 2.5 ng/g (lipid weight), respectively.

To verify these measurements, the same samples were analyzed under HRMS conditions. Table 1 shows the comparison of concentrations obtained using the two techniques. Not surprisingly, LRMS gives concentrations that are between 1.5 and 2 times greater than those determined using HRMS. The ratio of the quantiation to confirmation ions appear to be within acceptable values in samples analyzed by both LRMS and HRMS with the later method having values closer to the observed value and that are more consistent.

Table 1. Comparison of concentrations of α_2 and ion ratios of quantitation to confirmation using LRMS and HRMS.

Beluga sample #	Concentrations (ng/g, lipid weight)		Ratio of quantitation to confirmation ions	
	LRMS	HRMS	LRMS	HRMS
1	11.19	7.75	1.88	1.61
2	14.30	6.55	1.57	1.69
3	18.96	8.75	1.53	1.66

Conclusions

A method was developed for the analysis of the α_1 and α_2 isomers of TBECH using a short 10 m column and EI-HRMS that monitored characteristic ions corresponding to a loss of HBr and Br from the molecular ion. ECNI proved problematic in our analysis as there was co-elution of the second TBECH isomer (α_2) with that of BDE-15 and there was no diagnostic TBECH ion that could be used to discriminate it against BDE-15. LRMS EI analysis resulted in TBECH concentrations that were slightly greater than under HRMS conditions. The α_2 isomer was identifed in blubber of beluga sampled from the Canadian Arctic at concentrations smaller than that of ΣBDEs but similar to ΣHBCD. Interestingly, the α_1-isomer was not detected in any of our samples. This is surprising considered that the technical mixture contains close to equimolar amounts of the two isomers. Further work is ongoing to examine the extent of contamination by the TBECH isomers in the Canadian environment.

References

1. Larsson, A., Eriksson, L.A., Andersson, P.L., Ivarson, P., Olsson, P.-E. *J. Med. Chem.* 2006; 49: 7366-7372.
2. US-EPA 1,2-Dibromo-4-(1,2-dibromoethyl) cyclohexane (TBEC) Test Results: Decision Not to Test;50 FR 19460; May 8, 1985; Federal Register, May 8, 85.
3. EPA. TSCA Chemical Substance Inventory 2002, public portion; Environmental Protection Agency: Washington, DC, 2002.
4. Arsenault, G., Lough, A., McAlees, A., McCrindle, R., Pleskach, K., Riddell, N., Tomy, G., Yeo, B. *Organohalogen Compounds* 2007.
5. Rattfelt J, Norman A, Norrgren L, Haglund P, Andersson PL. *Organohalogen Compounds* 2006; 68: 2007-2010.