# BFRs in Arctic marine mammals during three decades. Not only a story of BDEs.

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## Introduction

Polybrominated diphenyl ethers (BDEs) have been widely used as flame retardants incorporated, for ex ample, into textiles and polymers. PBDEs were first found in the environment in 1981, but is was not until 15 years later that these compounds were reported in marine mammals in Europe (Haglund et al 1997, de Boer 1998, Lindström et al. 1999, Rigét et al. 2006), in Asia (Takasuga et al. 2006, Tanabe et al. 2008, Tanabe 2008) and the US (She et al. 2002, Shaw et al. 2008). Now BDE have attracted worldwide attention and levels of BDEs have been reported for a large variety of environmental compartments such as air, sediment and biota (De Wit 2002). This has resulted in the inclusion of BDE on the list of Stockholm convention persistent organic pollutants (POPs) .

Recently structurally similar methoxylated BDEs (MeO-BDEs), have been found in top predators such as harbour seals and harbour porpoises (Weijs et al., 2009). Although MeO-BDEs are structurally related to BDEs they are not known to be commercially produced. Me-O-BDEs are most likely of natural origin (Teuten et al. 2005).

Both BDEs and MeO-BDEs were analyzed in Pilot whale (*Globicephala melas*) from the Faroes Islands, Ringed seal (*Phoca hisida*) from Greenland, Minke whale (*Balaenoptera acutorstrata*) from Norway and Minke whale and Fin whale (*Balaenoptera physalus*) from Iceland covering a time period of three decades (1986-2006). The same sample were also screened for a large number of other BFRs by using NCI monitoring m/z 79 and 81 or high resolution GC/MS including TBECH and PBDD/DFs.

## Material and methods

### Chemical Analysis

For the analysis of brominated POPs Internal standards BDEs, 13C-labeled BDE #77 was purchased from CIL (Andover, MA, USA), and BDE #139 from Wellington Laboratories (Guelph, Ontario, Canada). A 13C-labeled PCB recovery standard was used, containing PCB #81, #114, and #178. The quantification standard for the Tri-through Hepta BDEs consisted of the following BDE congeners; BDE#28, #47, #66, #85, #99, #100, #138, #153, #154, and #183 from Wellington Laboratories (Guelph, Ontario, Canada). A separate standard for the analysis of Deca BDE #209, BDPE (1,2-bis(pentabromophenyl)ethane) and BTBPE (1,2-Bis(2,4,6-tribromophenoxy)ethane also from Wellington laboratories was prepared for the quantification standard of the 'higher' brominated BFRs. The quantification standard for the 'lower' brominated BFRs consisted of PBT (pentabromotoluene), HBBz (hexabromobenzene) and HBCD (hexabromocyclo dodecane). Screening experiments for TBECH tetrabromoethyl cyclohexane using isomeric mixtures of both the α- and β- isomers and the γ- and δ- isomers.

Open column chromatography was applied for approximately 1 gram tissue sample. Sample clean-up was performed using three open columns (multilayer silica, AlOx and active carbon). For a selected number of samples an additional clean up and fractionation on planarity an active carbon column, containing Carbopack C (Supelco) dispersed on Celite 545, which performed to obtain a fraction containing planar compounds including brominated dioxins (PBDD/Fs). Addition of the $^{13}$C-labeled recovery standards (including BDE #77, BDE #138 and BDE #209) was done prior to instrumental analysis. Throughout the sample preparation the samples were kept shielded from UV light to avoid photo degradation.

GC low resolution MS was performed using a HP 6890 GC coupled to a HP 5973 MS working in the negative chemical ionization (NCI) mode monitoring m/z 79 and m/z 81 for BDEs, DeDBethane, PBT and HBB. Analytes were separated on a 30 m DB-5MS (0.25 mm id, 25 µm) column. For BTBPE fragment ions 257.8 and 259.8 and for Deca BDE#209 fragment ions 484.7 and 486.7 were monitored simultaneously in the NCI mode. Quantification was done using by calculating relative response factors against a number of different BFRs mixtures (Wellington Laboratories, Guelph, Ontario) relative to the added $^{13}$C labelled standards. Method performance was controlled by extracting $^{13}$C-labeled internal standards allowing recovery values between 50-150 %. With every batch of samples extracted an extraction blank was also prepared and analyzed. The detection levels were in the range 0.1-1 ppb, depending on congener, sample amount and type of tissue.

The confirmation analysis for TBECH were performed on a Micromass high resolution GC/MS system coupled to a HP 6890 GC operating at 10 000 resolution monitoring the three most abundant isomers of the molecular ion cluster. Also confirmation of the co-eluting of BDE #154 and PBB #153 was done on the high resolution GC/MS system. For the analysis of the higher brominated BDEs a 15m column (0.25 mm id, 25 µm) was used instead of the routinely 30 m monitoring the two most abundant molecular ions of the bromine cluster for both BDE #153 (m/z 641.53, 643.53), PBB #153 (m/z 625.54, 627.53), the internal standard BDE #138 (m/z 653.57, 655.57) and the recovery standard PCB #178 (m/z 405.84, 407.84). Also the analysis of a subset of samples of PBDD/DFs was performed on the high resolution GC/MS system after a full 'dioxin' clean up (multilayer silica, AlOx, carbon column). The MTM laboratory participates on a regular basis in international intercalibration studies. In studies organized by AMAP, QUASIMEME and the Norwegian Institute of Public Health the MTM laboratory qualified with z-scores between -2 and 2.

**Results and discussion**

In all samples BDE were present at relatively high concentrations with the highest levels found in the end of the 1990s, beginning 2000. However the 3 pooled samples from each year did show large variation in the sum of BDEs (tetra through nona) and different trends for the individual BDE congeners.

In addition to the BDEs, HBB and PBB #153 were found in all samples in all time periods. BTBPE, BDE #209 and DecaBDethane were not found in levels above the LOD (0.5 ng-3.0 ng/g). Interesting when using NCI and analyzing m/z 79 and 81 two peaks at the correct retention time for TBECH were found, however when these results could not be confirmed using high resolution GC/MS operating in the EI mode at 10 000 resolution. This compound has been found in Beluga Whales from the Arctic (Tomy et al. 2008).

All samples were screened for unknown Br containing organic compounds. All samples contained several peaks that could not be identified as 'known' BFRs. This is illustrated below by a chromatogram of the screening of the Pilot Whale sample from 1986. Several 'candidate BFRs are currently under further investigation and by confirmatory analysis using high resolution GC/MS.

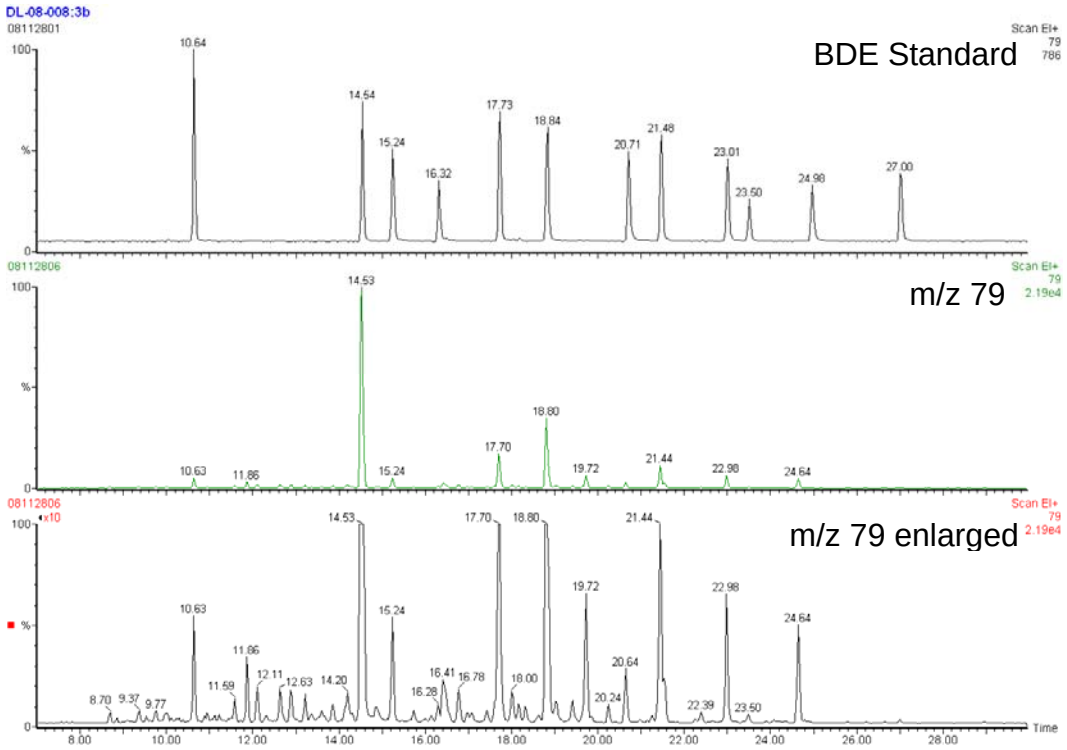


**Figure 1. Screening m/z 79 for organic bromo compounds in Pilot Whale, Faroe Island illustrating a large number of unidentified bromine containing compounds when comparing the sample to a standard BDE mix.**

## Conclusion

Monitoring brominated organic compounds in marine mammals during three decades showed decreasing trends or at least a levelling of the total levels for BDEs. For other BFRs this trend was not seen. In addition all samples seem to contain a large number of organic bromine compounds of which the identity has not yet been established. Relatively high level of Me-O-BDE were found in the Pilot Whale samples, a compound which probably is of 'natural' origin.

**References**

de Boer J, Wester P G, Klamer H J C, Lewis W E, Boon J P, Nature 1998, 29-30.
De Wit CA (2002). Chemosphere 46: 583–624.
Haglund PS, Zook DR, Buser HR & Hu J; Environ Sci Technol 1997, 31, 3281-3287.
Kajiwara N, Ueno D, Takahashi A, Baba N, Tanabe S. et al. 2004. Environ Sci Technol 38: 3804.
Lindström G, Wingfors H, Dam M and van Bavel B. Arch. of Environ. Contam. Toxicol. 36 (1999) 355-363
Rigét F, Vorkamp K, Dietz R. and Rastogi S.C. J. Environ. Monit., 2006, 8, 1000-1005.
Shaw SD, Brenner D, Berger ML, Fang F, Hong CS, Addink R, Hilker D. Chemosphere. 2008 73 (11):1773-80.
She J, Petreas M, Winkler J, Visita P, McKinney M, Kopec D, Chemosphere 46, 697-707. 2002.
Takasuga T, Matsukami H, Watanabe K, Takemori H, Takahashi S, Tanabe S. 2006. Organohalogen Compd 68: in press.
Tanabe S, Ramu K, Isobe T, Takahashi S. J Environ Monit. 2008 10(2):188-97.
Tanabe S. Mar Pollut Bull. 2008 57(6-12):267-74
Teuten, E.L., Xu, L., Reddy, C.M. (2005): Science, 307, 917-920.
Tomy GT, Pleskach K, Arsenault G, Potter D, McCrindle R, Marvin CH, et al. 2008. Environ Sci Technol 42(2): 543–549.
Weijs L, Losada S, Das K, Roosens L, Reijnders PJH, Santos JF, Neels H, Blust R, Covaci A (2009). Environment International 35: 893–899