Determination of α, β, and γ Hexabromocyclododecane (HBCD) in two marine food webs from Norway.

Kine Bæk[1], Eugen G. Sørmo[2], Siri Føreid[1], Bjørn M. Jenssen[2], Elisabeth Lie[1], Janneche U. Skåre[1,3]
[1]Norwegian School of Veterinary Science, P.O.Box 8146 Dep, 0033 Oslo, Norway
[2]Norwegian University of Science and Technology, Department of Biology, 7491 Trondheim, Norway
[3]National Veterinary Institute, P.O. Box 8156 Dep, 0033 Oslo, Norway

Introduction. Due to their environmental stability, persistence and high production volume 1,2,5,6,9,10-hexabromocyclododecan (HBCD) and polybrominated diphenyl ethers (PBDEs) are among the most abundant brominated flame retardants (BFRs) detected in the environment, wildlife and human tissue (de Wit 2002). Over the last decades there has been an increasing interest in the determination of BFRs and especially the PBDEs. The use of pent- and octa-BDEs in all applications for the EU market is banned during this period. The demand for replacement BFRs, e.g. HBCD, has seen an increase. More recently HBCD has gained attention in the field of environmental monitoring (Morris et al. 2004). The commercial product HBCD consist of three (α, β, and γ) isomers. Although γ-HBCD is the most dominant enantiomer in technical mixtures and sediments, α- HBCD is the primary congener detected in biota samples (Knudsen et al. 2005, Morris et al. 2004). In the present study, we aim to examine the isomer pattern and biomagnification of HBCD in two food webs from Norway.

Material and Methods. All organisms sampled in the outer Oslofjord (Hvaler and Torbjørnskjær archipelago in south-eastern Norway) were collected during spring and/or summer 2003 and 2004 (Table 1). Plankton net, shovel fine-meshed beach seine, fishing rods and rifle was used to collect the samples. The polychaeta lugworm was held in a tank of seawater for 24 hrs to empty their intestine. Samples from Svalbard were collected during 2002-2003 as described in Sørmo et al. 2006 (Table 1). For invertebrates and fish samples entire animals were stored. Blubber samples were collected from seals. All samples were kept frozen at -20° prior to analysis.

For the polar bear, the seal and the Atlantic cod the fat, blubber and liver were analysed respectively. For the rest of the species the whole animals were homogenised and analysed. The samples from polar bear, seal and cod were analysed individually while the rest of the species were analyzed by pools. The number of samples from each specie varied from 2-16.

Table 1: The HBCD are determined in different species from Svalbard and outer Oslofjord.

Svalbard
▪ ice-amphipod (*Gammarus wilkitzkii*)
▪ polar cod (*Boreogadus saida*)
▪ ringed seal (*Phoca hispida*)
▪ polar bear (*Ursus maritimus*)
Outer Oslofjord
▪ glass shrimps (*Palaemon adspersus/P.elegans*)
▪ northern shrimp (*Pandalus borealis*)
▪ sandeel (*Ammodytes spp.*)
▪ saithe (*Pollachius virens*)
▪ whiting (*Merlangus merlangus*)
▪ Atlantic cod (*Gadus morhua*)
▪ harbour seals (*Phoca vitulina*)

Samples preparation and chemical analyses of HBCD were done in the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science. The laboratory is accredited by Norwegian Accreditation for testing BFRs in biological material of animal origin according to the requirements of the NS-EN ISO/IEC 17025 (TEST 137). The method for total HBCD determination includes liquid extraction, clean up with sulphuric acid and GC-MS analysis and is further described in Sørmo et al. 2006. For determination of α-, β- and γ-HBCD, the extracts were analysed using an API 3000 LC-MS-MS system (triple quadrupole) (Applied Biosystem, USA) connected to a C18 column (15 cm x 2.1 mm, 5 µm) (Supelco). As mobile phases Amoniumacetate in water (A) and Amoniumacetate in 99% acetonitrile and 1% water (B) were used with a flow of 0.2 ml/min and gradients of 70 % B to 100 % B in 10 min, hold 5 min at 100 %B. The detection was performed by MRM (multiple reaction monitoring) and the mass transition ion-pair was selected as m/z 640.7-m/z 80.8.

Results and Discussion. Our main results are that α-HBCD dominate the isomere pattern inn all species analyzed (Fig 1c and 1d) and that total HBCD increase through the food web (Fig 1a and 1b), thus showing high potential for biomagnification. This is in line with results found in other studies (Covaci et al. 2006, de Wit 2002).

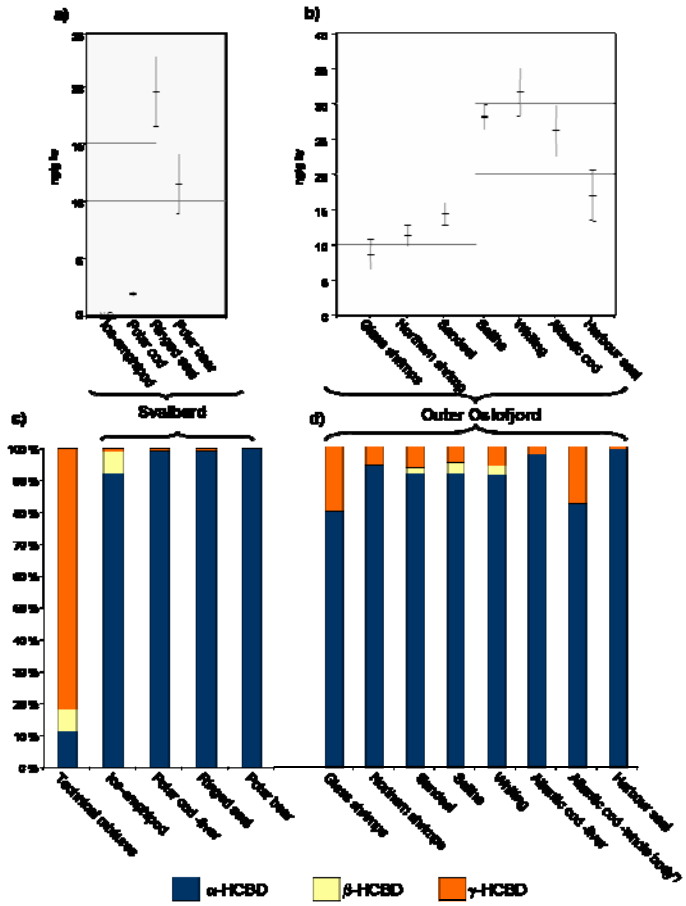

Figure 1: Mean (std. error) lipid weight concentrations (ng/g lw) of HBCD in different species from a) Svalbard (Sørmo et al. 2006) and b) Outer Oslofjord. The contribution of the different diastereomers of HBCD in a technical mixture and in the different species analyzed at c) Svalbard and d) outer Oslofjord. *) Whole body except liver.

All species sampled from outer Oslofjord and Svalbard (Fig. 1) are presented with expected increasing trophic levels (Sørmo et al. in prep, Sørmo et al 2006). Due to large diversity and complexity of the food web of North Sea our samples will not represent the complete picture of exposure to and bioaccumulation of HBCD in the harbour seal food chain, but covers some of the main pelagic and benthic links in their food web.

The α-, β-, and γ- isomers of HBCD were determined in samples of glass shrimps, northern shrimp, black goby, sandeel, sand goby, saithe, whiting, Atlantic cod and harbour seals from Outer Oslofjord (Fig 1d). α-HBCD was the dominating isomers in all species (more than 75%), whereas γ-HBCD dominates in the technical mixtures. We can observe a trend that the α-isomer is more dominating in the top-predator (seal) compared to a lower level (scrimps) in the food chain. It is also noteworthy that we see a much higher part of the γ- isomers in the whole body compared to liver of Atlantic Cod. From Svalbard the α-, β-, and γ- isomers of HBCD were determined in samples of ice-amphipod, polar cod, seal and polar bears (Fig 1c). α-HBCD was the dominating isomers in all species (more than 90%). The results are in line with other studies (Covaci et al. 2006, Knudsen et al. 2005, Morris et al. 2004).

Regarding total HBCD from Outher Oslofjord, we see that the levels increases from invertebrates to small fish and further to piscivorous fish (saithe and whitting), but we found no biomagnification in harbour seals (Fig 1b). The relative low levels of HBCD in the harbour seals compared to saithe, whiting and Atlantic cod can mainly bee explained by the fact that three of the four seals where young (< 2 years) and lower levels have to be expected. Additionally reasons will be discussed elsewhere (Sørmo et al. in prep). The results from Svalbard (Fig 1a) have been presented and discussed elsewhere (Sørmo et al. 2006). The main findings were that HBCD biomagnifies in the food chain up to ringed seal. However, we found no biomagnifications from ringed seal to polar bear. This result indicates that HBCD are biodegradable in the polar bears. The same has also been observed for PBDEs (Sørmo et al. 2006). Total HBCD for the ice-amphipod is not detected in the GC-MS analysis, but due to a lover detection limit in the LC-MS system, it was possible to determinate the isomers.

The spatial trends show that the highest levels are found at outer Oslofjord compared to Svalbard. We can see that the polar cod at Svalbard only contains 10% of the levels found in the Atlantic cod in the outer Oslofjord. However the difference between the sites is smaller compared to the PBDE levels (Bytingsvik et al. 2004).

In conclusion, we have found that α-HBCD is the dominating isomer in all the species studied and that HBCD biomagnify in different food chains. Due to low sample size, different biological matrices, and the reports of increased levels of HBCD the last decades (Covaci et al. 2006) there is a need for further research on HCBD in environmental samples.

Acknowledgements. This work was partly funded by the FIRE (Flame retardant Integrated Risk assessment of Endocrine disruption [http://www.rivm.nl/fire] project (Contract No: QLT4-CT-2002-00596) under the European Commission 5th Framework Programme for research, technological development and demonstration activities (Quality of Life and Management for Living Resources, Key Action 4: Environmental and Health). The contents herein do not represent the opinion of the European Community.

References

Bytingsvik J, Gaustad H, Salmer MP, Sørmo EG, Bæk K, Føreid S, Ruus A, Skaare JU, Jenssen BM. 2004. Organohalogen Comp 66:3869

Covaci A, Gerecke AC, Law RJ, Voorspoels S, Kohler M, Heeb NV, Leslie H, Allchin CR, de Boer J. 2006. Environ. Sci. Technol, in press.

de Wit CA. 2002. Chemosphere 46:583-624.

Knudsen LB, Gabrielsen GW, Verreult J, Barrett R, Polder A, Skaare JU. 2005. Report, The Norwegian Pollution Control Authority (SFT). ISBN: 82-7655-497-0.

Morris S, Allchin CR, Zegers BN, Haftka JJH, Boon JP, Belpaire C, Leonards PEG, van Leeuwen SPJ, de Boer J. 2004. Environ. Sci. Technol 38:5497.

Sørmo EG, Bæk K, Jenssen BM, Skaare JU. In prep.

Sørmo EG, Salmer MP, Jenssen MB, Hop H, Bæk K, Kovacs KM, Lydersen C, Falk-Petersen S, Gabrielsen GW, Lie E, Skaare JU. 2006. Environ Toxicol Chem 25:2502.