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Analysis of TBBP-A and HBCD in human blood

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Preface

In the context of discussions around the draft European Union chemical law, 'REACH', the World Wide Fund For Nature, (WWF Toxics Programme), in cooperation with the Co-Operative Bank, decided to bring attention to the exposure of humans (and wildlife) to persistent, bioaccumulative and endocrine disruptive chemicals by organizing a "Chemicals Check-Up". At least 40 European members of parliament participated by donating blood in December 2003. This blood was analysed by several labs in Europe for a variety of chemicals (ca. 70) in order to illustrate the level of environmental contamination that can be expected in European citizens. The laboratory of the Netherlands Institute for Fisheries Research contributed to the WWF initiative by analysing samples for the brominated flame retardants (BFRs) hexabromocyclododecane (HBCD) and tetrabromobisphenyl-A (TBBP-A) in the blood samples. The results of this study are presented in this report.

Summary

The brominated flame retardants, hexabromocyclododecane (HBCD) and tetrabromobisphenyl-A (TBBP-A) were analysed in samples of human blood from 40 different individuals. In only one individual was HBCD detected. In about half of the individuals, TBBP-A was detected. When they were detected, the amounts of both chemicals were near the limits of quantification. That the chemicals were detected confirms that certain individuals were exposed to sources of these substances. The toxicological implications of these low levels are not well known. Very few studies of the toxicology of these compounds have been performed to date.

1. Introduction

Brominated flame retardants (BFRs) such as hexabromocyclododecane (HBCD) and particularly tetrabromobisphenyl-A (TBBP-A) are widely used in Europe and elsewhere in such products as polystyrene foams, upholstery textiles and electronic equipment [de Wit 2002]. While flame retardants are highly important for their fire-retardant properties, they also have disadvantages. They can escape during production processes or from the finished products and be emitted to the environment. The emissions become distributed among the different environmental compartments (*e.g*. air, sediments, biota, water).

Due to their physical-chemical properties, these compounds are rather poorly soluble in water but have a high affinity for organic matter, lipids and biological tissues. These kinds of chemicals have already been detected in marine and freshwater sediments, in wastewater treatment plants and landfill leachates but also in aquatic biota, birds, mammals, including human serum and mothers' milk [*e.g.* Wantanabe *et al*. 1983; Sellström *et al.* 1998; de Boer *et al*. 2002a; Thomsen *et al.* 2001, 2002, 2003; Jacobsson *et al.* 2002; Sellström *et al.* 2003; Zegers *et al*. 2003; Tomy *et al.* 2003]. Studies on the distribution, behaviour and toxicity of TBBP-A and in particular, HBCD are limited in number [de Wit 2002]. Blood is a common sample type for the assessment of human exposure because compounds are more often present in a non-metabolised form than in urine, for example. While TBBP-A has been detected in human plasma [Klasson Wehler *et al.* 1997; Thomsen *et al.* 2001] and serum [Thomsen *et al*. 2002; Jacobsson *et al*. 2002] to date, no published data on HBCD concentrations in blood exist to our knowledge.

The objective of this present study was to analyse concentrations of hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A) in human blood samples.

2. Chemical structures of analytes

Tetrabromobisphenol-A

TBBP-A is a phenolic BFR (Figure 1). Because it is ionic, it can be protonated or deprotonated depending on the pH. This can affect its ability to interact or react with other molecules such as organic material in sediments or biological macromolecules in living organisms. TBBP-A has a high octanol-water partition coefficient, (log K_{ow} 5.9) and is more soluble under acidic conditions (low pH) than when the medium is alkaline. At a pH of 7.4, such as in human blood, TBBP-A can be expected to be partially deprotonated and susceptible to interactions with proteins and other macromolecules in the blood.

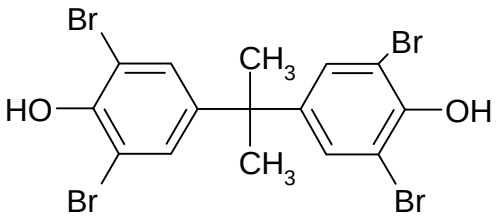

Figure 1. *Chemical structure of tetrabromobisphenol-A.*

Hexabromocyclododecane

Three diastereomers of HBCD, ?-, ?- and ?-HBCD (Figure 2) can be identified. Assays of isomer distribution in a number of different samples of commercial HBCD, [Peled *et al*. 1995] showed the technical mixture of HBCD to be predominantly composed of ?-HBCD (ca. 76%), while ?- and ?-HBCD made up about 13% and 11% respectively. Studies to date indicate that the patterns of bioaccumulation in organisms tend to be different than the composition of technical mixture would suggest, with a relatively large contribution of ?-HBCD to the total HBCD body residue being found to date [de Boer *et al.* 2002a].

Analysis by gas chromatography (GC) leads to changes in the isomeric ratio of HBCD at the high temperatures (>160°C) in the gas chromatograph, although total HBCD concentrations are still measurable by GC/MS [Covaci *et al.* 2003]. International laboratory studies have shown that only a few laboratories worldwide are able to carry out reliable GC/MS and LC/MS analyses of HBCD [de Boer *et al.* 2002b]. The different HBCD isomers can be separated and quantified using LC/MS. However, this method requires relatively high concentrations of analyte compared to the GC/MS method. Because of the analytical challenges, diasteromer-specific concentration data for environmental matrices or human tissues are currently very limited.

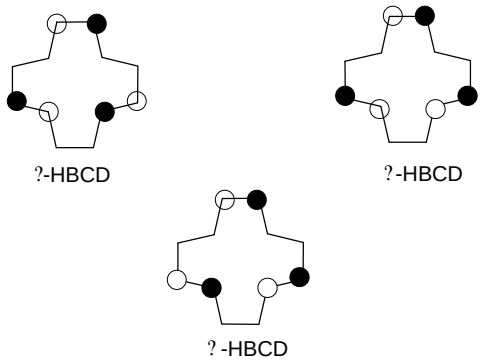

Figure 2. *Structures of hexabromo-cyclododecane diastereomers, ?-HBCD (1R,2R,5S, 6R,9R,10S), ?-HBCD (1S,2S,5R, 6S,9R,10S) and ?-HBCD (1R,2R, 5R,6S,9S,10R). Black circles indicate that the Br-atom is positioned into the plane of the paper, open circles indicate Br is positioned out of the plane of the paper [from Peled* et al. *1995]*.

3. Materials and Methods

Human blood of 46 individuals was drawn in Brussels on 2 and 3 December 2003, and collected in either glass (SST) or plastic (SST-II) BD Vacutainer vials. Blood was sent (frozen and on dry ice) to the Netherlands. Forty samples with the highest sample volumes were analysed for HBCD and TBBP-A. The method of extraction, clean up and chemical analysis is based on a standardized procedure for measuring flame retardants in biota [Hesselingen and Brandsma, 2003] of the Netherlands Institute for Fisheries Research.

Soxhlet extraction, GPC and GC-MS analysis

Blood samples (excluding the silica gel contents of the vacutainer vials) were weighed and dried by mixing with sodium sulphate and then extracted by Soxhlet extraction with hexane:aceton as solvent. Following Soxhlet, internal recovery standards were added to the blood extracts. For TBBP-A the internal standard was ^{13}C-TBBP-A, for HBCD, BDE116 was used. To ensure that TBBP-A was in the protonated form and retained in the organic phase before proceeding with the next steps, the Soxhlet extract was treated with a sulphuric acid solution (pH 2).

Extracts were then purified with the gel permeation chromatography (GPC) technique to remove large biomolecules such as lipids before injecting into analytical apparatus, as these would interfere with the analysis. The organic layer was further cleaned with silica gel to remove other matrix compounds. Finally, the purified extracts were transferred to vials for analysis with GC/MS.

TBBP-A and HBCD in blood extracts were measured by gas chromatography/mass spectrometry (GC/MS) in four series. Each series included two blanks, an internal reference material sample (eel), and a recovery standard. Due to limitations of the blood sample volume, no duplicate extraction of a blood sample was performed. Quantification was performed using external standards. Final concentrations were corrected for percent recovery determined by recovery standards. No LC-MS analysis of the extracts was performed because TBBP-A was analysed with GC-MS, and HBCD concentrations were too low to detect the separate HBCD diastereomers in an LC-MS chromatogram.

4. Results

TBBP-A was detected in about half of the blood samples in the ng/kg blood range (see Appendix). In the other blood samples, no TBBP-A was detected. The concentrations in the blood extracts were reported if the measured concentration was ?5 times higher than the blank signal. If the value was ?3 times higher than the blank signal it was reported with an indication of this. All extracts had concentrations below the lowest standard, but since the peaks in the chromatograms could be detected and integrated, these values were not reported as 'under detection limit' in this case. Normally these concentrations would be reported as <L.O.D. so it must be stressed that this lends uncertainty to the reported values. HBCD was not detected in the blood samples with the exception of one individual, where the concentration, 63 ng/kg, was well above the blank (Appendix). Because of the low concentration (below detection limits of LC/MS analysis), it was not possible to measure specific HBCD diastereomers in this sample. Analysis of the internal reference material (eel) and recovery standards in each of the four series indicated that the extraction and GC/MS analysis of the BFRs proceeded according to quality standards at the Netherlands Insitute for Fisheries Research.

5. Discussion

Tetrabromobisphenol-A. Earlier studies published in the literature have reported TBBP-A to be detectable in humans [Klasson Wehler *et al.* 1997; Thomsen *et al.* 2001]. Investigating an occupationally exposed group, Jakobsson *et al.* [2002] quantified TBBP-A in four serum samples from Swedish computer technicians and reported TBBP-A concentrations between <1 and 3.4 pmol/g lipid weight. The highest concentrations measured in whole blood in this current study are roughly a factor 10-20 higher than these values (after correction for the different units). In Norway, TBBP-A concentrations measured in a larger number of human blood plasma samples from different occupation groups were determined to be between 0.3 and 1.8 ng/g lipid [Thomsen *et al.* 2001].

The differences between previous studies of plasma and serum and this study, using whole blood, may have to do with some difference in the distribution of TBBP-A among the different blood compartments.

In a study of occupationally exposed workers in Sweden, Hagmar *et al.* [2000] estimated the halflife of TBBP-A in blood serum to be about 2 days. TBBP-A is known to be quickly metabolised in other mammals, such as the rat. One study showed that the half-life after oral administration in rat was <3 days [WHO/IPCS, 1995]. Rapid metabolism of TBBP-A has been reported in more recent studies in rats [Meerts *et al.* 1999] and quail [Halldin *et al.* 2001].

A known possible metabolite of TBBP-A is tetrabromobisphenyl-A (Me-TBBP-A) [Hakk & Letcher 2003]. This compound was also screened in the same GC/MS chromatograms generated for the other analytes, although none of this possible metabolite was detected in any samples above the level of blanks. Because this is more lipophilic than the parent compound, it probably would have a longer half-life in the body than the parent compound, and thus we suspect this is not a metabolic route in the individuals tested. Debromination is another possible metabolic pathway.

Hexabromocyclododecane. HBCD has not been reported in human blood to date, therefore it was interesting to observe it in one individual in the current study (Table 1). HBCD has been found in human mother's milk at concentrations between 250 and 2000 ng/kg lipids [Thomsen *et al.* 2003]. This is considerably higher than found in the current study, which can be at least party due to the higher lipid content in milk than whole blood. The higher lipid content means that the milk has a larger capacity to contain this lipophilic compound.

Exposure and Toxicology. What do these data mean from a toxicological standpoint? Levels of TBBP-A and HBCD were either undetectable or low in the blood of the individuals tested. The occupation of the blood donors does not belong to a high-risk category. The exposure for subjects in the current study was either low, or a high rate of elimination from the body must have been taking place. There are indications that TBBP-A can be eliminated quickly, as mentioned above.

The higher TBBP-A levels in some individuals could also reflect different exposure scenarios, linked to geographical distribution of the chemical's applications and usage. Information such as country of residence of the blood donors with higher TBBP-A concentrations in blood would give more insight into such possible correlations. Because the main uptake route for TBBP-A is probably inhalation [Sjodin *et al.* 2003], the type of diet in this case may not be correlated to TBBP-A levels in blood. BFR concentrations are also believed to be independent of age of the adult *e.g.* [Thomsen *et al*. 2002].

The toxicology of these chemicals is not yet sufficiently elucidated. Legler & Brouwer [2003] have reviewed the literature for evidence of endocrine disruption of TBBP-A. While it is sometimes suggested that TBBP-A may act as an endocrine disruptor (at least *in vitro*), a cytotoxicant and an immunotoxicant, there is currently somewhat more evidence that it disrupts thyroid system functioning [Birnbaum & Staskal 2004] as for example was demonstrated in two recent articles [Meerts *et al.* 2000; Kitamura *et al.* 2002].

The WWF's Chemicals Check-up Project is one of the few studies to date to present data on the levels of TBBP-A or HBCD in human blood.

6. Conclusion

TBBP-A was detected in 26 out of 40 human blood samples in the range of 2 - 330 ng/kg whole blood. These levels were higher compared to earlier reports from Sweden and Norway, but differences may be due to lower TBBP-A levels present in serum and plasma, sampled in those studies, compared to whole blood.
TBBP-A is most likely less persistent than other BFRs (such as PBDEs), or PCBs. The TBBP-A concentrations found may be the result of exposure in the days before the sampling of the blood.
HBCD was found in only one blood sample at a concentration of 63 ng/kg whole blood. However, this HBCD value is the first positive HBCD value in human blood ever reported.

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Appendix

Excel file with concentration data for TBBP-A and HBCD