

CHANGES IN THE STRUCTURE OF BROMINATED FLAME RETARDANT MOLECULES INFLUENCE THEIR BIOACCUMULATION IN THE FOOD-WEB

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Brominated flame retardants are widely applied to diminish the chance of fires. This is a great bonus for the safety of a wide variety of goods, such as electronic equipment, foams and upholstery in furniture, carpets and building insulation materials. The other side of the medal of this increased safety are increased levels of these compounds in the environment. The bioaccumulation of these compounds in the food-web is closely related to their resistance to environmental processes causing changes in their molecular structures. It was found that the molecular structures of some compounds can be changed more easily by the cytochrome P450 liver enzymes of fish and marine mammals than others, but large differences between species occurred.

Brominated flame-retardants (BFRs; Fig.1) enhance the fire resistance of many materials. Drawbacks of this great safety feature are environmental problems, since some representatives have become known as persistent organic pollutants (POPs). These 'POPs' accumulate in lipid-rich tissues of animals and have a high resistance to environmental degradation processes. A good understanding of the fate of BFRs in the environment is important to assess the long-term toxic effects on animals. An important step in the structure-effect cascade is whether the structure of the compounds can be changed by the action of enzymes in organisms. This process is called biotransformation or metabolism. Enzymes of the cytochrome P450 family play a key-role in this process. These enzymes are present in many tissues of animals, but for the biotransformation of man-made pollutants the liver is the key-organ.

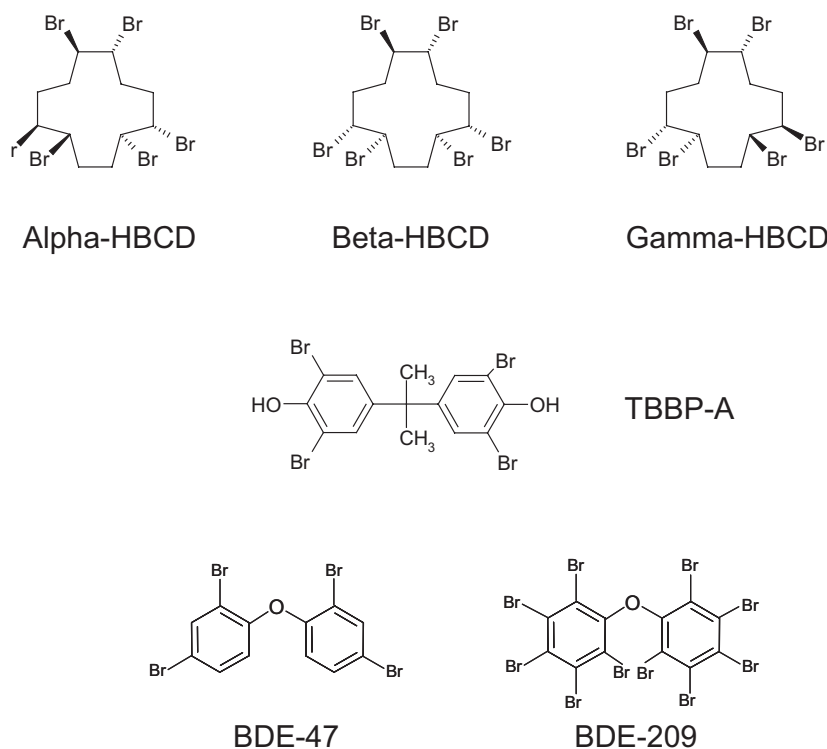


Fig. 1. Structures of brominated flame-retardants.

To study this in whole fish is very elaborate and time-consuming, and a study with marine mammals is impossible for ethical as well as logistical reasons. However, the process can also be studied with isolated fractions from livers which

were taken out of the animals. In the case of fish, the livers originated from animals that were caught in the Wadden Sea with the NIOZ research vessel Navicula. The livers were excised on board and they were subsequently frozen

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in liquid nitrogen. The samples of marine mammals were taken from animals that were sampled shortly after the animals died. The samples of pilot whale were a gift of Dr. Maria Dam of the Food- Veterinary and Environmental Agency of the Faroe Islands

The liver tissue is homogenised and centrifuged twice to get tiny subcellular organelles called the smooth endoplasmic reticulum, in which the biotransformation enzymes are embedded like small protein islands in an ocean of phospholipids. The final fractions are called microsomes.

For the experiments, small amounts of microsomes equivalent to 10mg of protein ml⁻¹ were put in Erlenmeyer flasks, to which the different brominated test compounds were also added in a small volume of an organic solvent that mixes well with water. The biochemical reaction occurs only when the reaction is fuelled by an energiser in the form of the electron donor NADPH. Three classes of bromi-

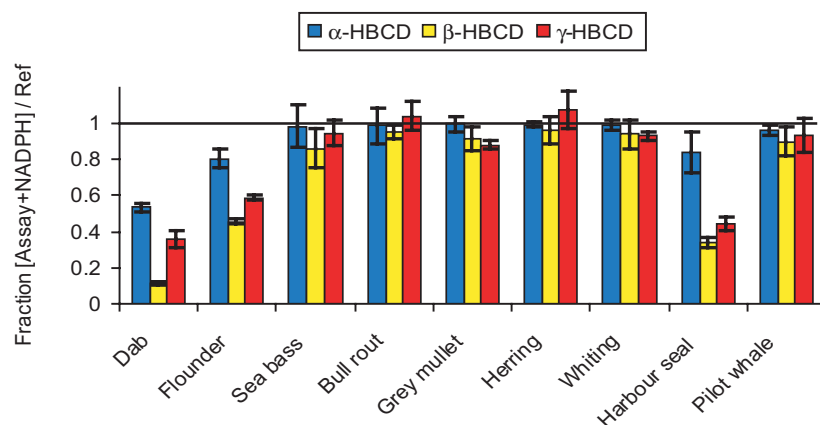


Fig. 2. Fraction recovered hexabromocyclododecane, corrected with the internal standard BDE153, and calculated against the reference assays after 90min biotransformation assays with hepatic microsomes of fish and marine mammals at 25°C and 35.5°C, respectively. The values in the presence of NADPH, were calculated as fraction against reference assays, to which no NADPH was added. A value of 1 means no changes in the presence of NADPH. Results are the mean and standard deviation (error bars) of four assays. The biotransformation studies were performed with the individual HBCD stereoisomers.

nated flame retardants were investigated: different types of hexabromocyclododecanes (HBCDs), tetrabromobisphenol-A (TBBP-A) and a mixture of polybrominated diphenyl ethers (PBDEs).

Of seven fish species belonging to six families, including bottom-dwelling and pelagic fish, only the microsomes of the flatfish species

dab and flounder (*L. limanda* and *P. flesus*, respectively) were capable of metabolizing all three types of HBCD molecules. The experiments showed for the first time that α-HBCD can also be metabolised by cytochrome P450. A 60% reduction by biotransformation of β and γ-HBCD occurred also in the assays with harbour seal microsomes, but no significant reduction was detected for α-HBCD (Fig. 2). In tissue residues from animals originating from different European seas, the α-HBCD isomer gets strongly enriched compared to especially γ-HBCD. The β-HBCD isomer is small in industrial mixtures and in the environment and thus plays a minor role. The formation of hydroxylated metabolites was tentatively confirmed by gas chromatography- mass spectrometry.



TBBP-A was significantly metabolized by microsomes of sea bass (*Dicentrarchus labrax*) and harbour seal (*Phoca vitulina*; Fig. 3). This compound is also more polar than HBCDs and PBDEs and it can be coupled to compounds naturally present in the animal body, which increases the polarity and speed of excretion further. In contrast to the HBCDs and PBDEs, TBBP-A is chemically bound to the matrix of the products in which it is applied, and therefore the escape to the environment is probably much lower.

The PBDEs were not reduced by any of the fish or marine mammal microsomes within the 90 minutes assay time frame. For comparison, similar assays have also been carried out with microsomes of rats of which the cytochrome P450 system was boosted prior to the in vitro experiments by exposing them to the model inducer compound phenobarbital. These microsomes were very active and showed metabolism of several PBDEs. However, metabolism of the fully brominated deca-BDE compound could not be established with rat microsomes either. Thus, this compound was at least stable in our assay and this route does not seem to contribute to the observed debromination of this compound in humans, soils and the atmosphere. The less brominated PBDEs were metabolised according to similar structural requirement rules as we found about twenty years ago for the structurally related polychlorinated

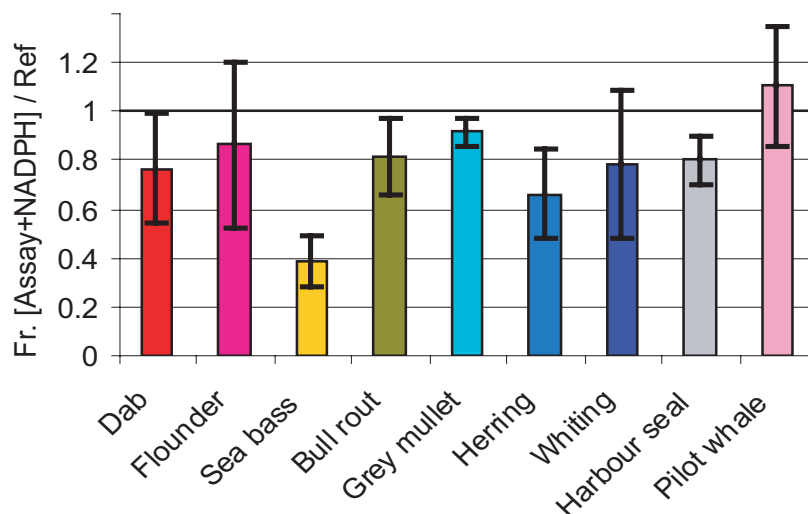


Fig. 3. Fraction tetrabromobisphenol-A, corrected with the internal standard ^{13}C -TBBP-A, recovered after 90min biotransformation assays with hepatic microsomes of fish and marine mammals at 25°C and 35.5°C, respectively. The values in the presence of NADPH, were calculated as fraction against reference assays, to which no NADPH was added. A value of 1 means no changes in the presence of NADPH. Results are the mean of four assays \pm standard deviation.

biphenyls (PCBs) in harbour seals. However, the rates for PBDEs are apparently too low in wildlife to register them in our in vitro test system. Nevertheless, this system allows a first screening of the biotransformation potential of many species towards a specific compound without using large numbers of animals in experiments. The FIRE project has shown that there is often a close relation between biotransformation and biochemical effect.

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