An integrated approach towards understanding the biotransformation of chlorinated biphenyls (CBs) and the degree of MFO induction in coastal fauna from an accurate measurement of CBs in sea water

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Introduction:

Polychlorinated biphenyls (PCBs) are universal pollutants identified in every possible ecosystem in the world. Analytical tehniques have been improved in recent years to analyse these chemicals on a congener-specific basis and several toxic congeners have been successfully quantitated in wildlife specimens at ultra trace levels (Duinker et al. 1988, Kannan et al. 1989 & De Voogt et al. 1990). However, measurement of these congeners in water has been achieved only recently (Schulz et al. 1989, 1990 & Iwata et al. 1993). Studies on the distribution of CBs in water, sediment and aquatic organisms are very rare because the concentration in water is usually very low (in sub-pg dm^{-3} range). An *in-situ* water pumping system has been developed in our Institute that enables sampling volumes >700 dm^{-3}. Multidimensional Gas Chromatography - ECD techniques that offer accurate and unambiguous measurement of coeluting compounds have been successfully utilized for the determination of CBs in environmental samples (Duinker et al. 1988). In view of these recent developments a study has been undertaken to discern knowledge on the uptake, accumulation and biotransformation of CBs in a coastal ecosystem integrating work on water.

Experimental:

Water:

Water samples were extracted using "in situ pumps", capable of running for over 12 hr with capacity 60 dm^{-3}/ h . The water was passed through a GF/F filter (14 cm Ø) and a XAD-2 resin column (5 bed volumes/min). Organics including CBs were eluted

from XAD-2 resin columns in a modified Soxhlet apparatus for 6 hours with 150 ml of acetonitrile containing 15% water. The extract was concentrated to about 50 ml in a flash vacuum evaporator and shaken three times with 10 ml portions of *n* -hexane.The extracts were combined and dried with anhydrous sodium sulphate, removed with a Pasteur pipette, concentrated to 250 µl in a rotary evaporator under a N_2 blanket and cleaned-up by high performance liquid chromatography (HPLC). The second HPLC fraction, containing the CBs quantitatively, was concentrated down to 20-50 µl with a gentle stream of N_2 at room temperature and analysed by GC-ECD.

Sediment:

Sediment samples were collected with box core samplers. The upper 3 cm layer of sediment was taken and stored in a glass petri dish at -20°C. 30 g were dried in an oven at 60°C until constant weight. Organic carbon content was determined by loss-on-ignition (LOI) method using a muffle furnace maintained at 400°C for 24 hr. The sediment thus obtained was mixed thoroughly with anhydrous sodium sulphate and extracted in *n* -hexane as discribed for water analysis. The extract was cleaned by alumina column chromatography and silica gel HPLC. The extract was cleaned with copper (activated with HCl) to remove elementary sulphur.

Organisms:

Diatoms were collected with 50 µm plankton nets in the upper 1-3 m. Zooplankton was collected with 200 µm plankton nets. Phytoplankton (50-250 µm) and zooplankton (~250 µm) were separated in the laboratory using filters. Planktonic species were identified by microscopy after fixing of a portion of the sample in 4% formalin.

The marine worms (*Nephtys* spec.) and mussels (*Artica islandica*) were taken from the box core samplers. Shrimps (*Crangon crangon*) and flounders (*Platichthys flesus*) were netted. The herrings (*Clupea harengus*) were obtained from commercial fishermen. The female harbour porpoise (*Phocoena phocoena*) was found strangulated in gill nets.

Approximately 3 g of biological sample and 20 g of sediment were taken for analysis. Phytoplankton and zooplankton were represented by a pool of planktonic communities. For mussel, worm and shrimp an aliquot was taken from an homogenate of 3 entire organisms. Muscle tissue of individual flounder and herring, pre-spawn ovary of herring and blubber of the porpoise were taken for CB analysis according to the method of Petrick et al. (1988). In brief, the samples were thoroughly mixed with anhydrous

sodium sulphate in a mortar and extracted in a soxhlet apparatus using 100 ml of *n* - hexane. An aliquot of this extract was used for the (n-hexane extractable) lipid weight determination. The volume of the extract was reduced initially using a rotary evaporator and finally to smaller volumes (100-200 µl) using a gentle stream of nitrogen for the CB determinations by GC-ECD after clean-up in alumina and silica gel chromatography and finally by HPLC. CBs in cleaned-up extracts were measured using single capillary column GC-ECD and multidimensional GC-ECD. Single column analysis was carried out using a Siemens SiChromat I - GC equipped with a SE-54 coated column and the latter with a Siemens SiChromat-2 MDGC-ECD with two ^{63}Ni ECDs. More details of this technique are available elsewhere (Duinker et al. 1988 & Schulz et al. 1989). MDGC-ECD was applied for some important non- and mono- *ortho* Cl CBs with toxic properties. Quantitation was performed using a mixture of individual chlorobiphenyl congener standard solutions of high purity (>99%)

Results and Discussion:

Concentrations of CBs in solution were very low ($\sum$CB values in the order of 9.0 pg dm^{-3}) being several orders of magnitude lower than other values reported for coastal waters in the literature. The most toxic non- *ortho* Cl CBs reported in other studies for sediment and organisms were absent in our study (< 1 pg/g lipid). Bioconcentration factors (on lipid weight basis) of CBs from water to sediment and lower aquatic organisms were between 10^5 and 10^8. A CB pattern recognition technique was carried out with principal component analysis. Water, diatom, sediment and mussels appeared in one group; also prey-predator organisms such as copepod-herring and nereid worm-flounder appeared in one group. Harbour porpoise and shrimp are placed separate from the other organisms. Metabolic efficiency of the organism plays a role in the noticed CB pattern. The relationship between bioaccumulation factor and the octanol-water partition coefficient for all congeners studied showed a negative correlation, contradicting the theoretical expectation.

A chemico-toxicological model was developed from the content of chlorinated biphenyls (CBs) in sea water, diatom, copepod, shrimp, annelid worm, flounder, herring and harbour porpoise and from a biochemical understanding of a structure-activity relationship. The CB congeners were grouped into four categories depending on the position of vicinal H-atoms in the biphenyl molecule: CBs with *meta-para* and *ortho-meta* vicinal H-atoms (easily metabolizable), with only *meta-para* vicinal H-atoms (metabolized by Cytochrome *P* -450 II B isozymes), with only *ortho-meta* vicinal H-atoms (metabolized by Cytochrome *P* -450 1A isozymes) and with no vicinal H-atoms (non-metabolizable). The extent of these biotransformations was understood from "metabolic slopes" derived

from the model. The degree of enzyme induction could also be derived similarly from our model. Induction of both Cytochrome *P* -450 II B and Cytochrome *P* -450 1A isozymes in several aquatic organisms could also be deduced. The ecotoxicological stress was greatest in harbour porpoise that was at the top of the food chain. The predictability of our model is confirmed from several recent field studies using bioassay techniques. The sensitivity of our model over regular biomonitoring methods is demonstrated.

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