

## **PCB metabolites, chlorinated dioxins and furans in tissues of mustelids from the Netherlands**

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Exposure to chlorinated aromatic compounds, in particular the polychlorinated biphenyls (PCB), may be an important factor in the decline of populations of species of the mustelid family. Relatively much is known about the effects of PCB on mink from experimental studies, and the decline of the otter in Europe is attributed partly to PCB due to the assumed similarity between otter and mink. Concentrations of PCB in otters found dead in the field in the Netherlands amount to levels far above the levels considered to have no adverse effects in mink<sup>1</sup>. Both otter and mink primarily feed on aquatic food, such as, e.g., fish and amphibians. Terrestrial members of the mustelid family, such as polecat, weasel, marten and stoat have a much larger variety in their diet, ranging from aquatic (amphibians) to terrestrial preys (rodents, birds). Several European populations of these species are decreasing. Apart from the apparent habitat destruction, exposure to PCB has been suggested as an additional factor<sup>2</sup>.

In a large Swedish project recently published<sup>3</sup>, results of an extensive experimental study on mink showed that non- and mono-ortho substituted PCB were responsible for the observed reproductive impairment, whereas polychlorinated naphthalenes (PCN) and dibenzofurans (PCDF) had no adverse effects. It was also shown that relatively high concentrations of methyl sulfonyl metabolites of PCB (MSF-PCB) were found in animals exposed to PCB<sup>4</sup>. The possible role of MSF-PCB in PCB toxicity in mink remains to be clarified. Despite being a product of metabolism, MSF-PCB themselves bioaccumulate because of their hydrophobic nature. MSF-PCBs have been shown to bind to tissue-specific proteins<sup>5</sup> and, as a result, selectively accumulate in e.g., lung and kidney. Tissue accumulation may lead eventually to toxic effects.

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Effects of chlorinated aromatic hydrocarbons presumably are mediated by the Ah receptor and are thought to occur as a result of the same mechanism of action. This concept is the basis for the TCDD toxic equivalency factor system. If reproductive impairment is a result of the same mechanism of action, then not only PCB, but also PCDD, PCDF and other relevant chlorinated aromatic compounds need to be taken into account when evaluating contaminant levels in wildlife.

The objectives of this study were the identification and quantitation of MSF-PCB and toxic PCDD and PCDF in different tissues of mustelids from the Netherlands. The levels and patterns of MSF-PCB will be related to the PCB patterns found in the animals, in order to establish the source of the MSF-PCB (foodchain accumulation or metabolism in the animal itself) and the implications of the structure of the parent chlorinated biphenyl congener (CB) for the occurrence of MSF-PCB.

## Methods

### *MSF-PCB*

Samples of liver and adipose tissue were Soxhlet extracted with dichloromethane:pentane (1:1) and taken up in n-hexane. The hexane extracts (containing between 30 and 100 mg lipid) were spiked with internal standard (4-methyl-3-methylsulfonyl-2',3',4',5,5'-pentachlorobiphenyl) and mixed (1:1 v:v) with concentrated sulphuric acid to bring about partitioning of the MSF-PCB into the acid layer. The acidic phase was diluted 1:5 with cold distilled water and the MSF-PCB were back extracted into n-hexane. The acid partitioning procedure was repeated with the original hexane layer and the resulting n-hexane was combined with the n-hexane from the first back extraction. The combined hexane extract was concentrated and cleaned up over alumina (2% H<sub>2</sub>O). Hexane-dichloromethane (9:1 v:v, 12 ml) and dichloromethane 10 (ml) were used as eluents. The MSF-PCB elute in fraction 12 - 16 ml. The concentrated clean extract was spiked with an internal standard and analysed by HRGC-ECD and HRGC-MS. Identification and quantitation was based on MS and authentic MSF-standards, which were a gift from Dr. Åke Bergman, Stockholm University.

### *PCDD/PCDF*

n-Hexane extracts (containing between 20 and 80 mg lipid) were spiked with a mixture of 17 <sup>13</sup>C-PCDD/F standards (all 2,3,7,8-substituted) and transferred to an active carbon (Carbosphere) column. The PCDD/F (fraction 3) were separated from ortho substituted PCB (fraction 1) and non-ortho PCB (fraction 2) by refluxing in dichloromethane, toluene and - after turning the Carbosphere column - again toluene, respectively. The fractions were cleaned up by adsorption chromatography on silver nitrate impregnated silica and basic alumina, eluting with a 9:1 n-hexane:carbon tetrachloride mixture. The resulting

fractions were spiked with an additional internal standard and analysed by HRGC-HRMS.

## Results and Discussion

More than 10 MSF metabolites of CBs containing 4 to 6 chlorine atoms and at least one isomer of MSF-DDE were detected by GC-MSD in adipose tissue and liver of mustelids, based on their  $m/z$  value. Some of these could be identified on the basis of the relative retention times of the authentic standards. Thus, we identified for example MSF-metabolites of CBs 87, 101 and 149 and DDE in liver tissue. All parent CB compounds mentioned have *m,p*-vicinal H atoms, confirming this structural requirement for transformation. As was shown in the previous paper<sup>6</sup>, CBs 101 and 149 indeed are metabolised to a high extent in mustelids. In adipose tissue MSF-PCB levels (on lipid basis) were generally lower than in liver samples. The congener pattern of MSF-PCB in liver distinctly differs from that in adipose tissue, whereas PCB patterns in these tissues are similar<sup>6</sup>. This suggests a selective retention of MSF-PCB in various tissues, such as has been reported in earlier findings<sup>7,8</sup>. Relatively high levels of 3-MSF-DDE were found both in liver and adipose tissue. Concentration levels of MSF-PCB in mustelid liver range from 10 to 1000  $\text{ng.g}^{-1}$  (lipid weight). The estimated total MSF-PCB concentrations amount to between 2 and 10 % of the total PCB concentrations. The ratio of 3-MSF-DDE to total PCB was higher than corresponding ratios reported in the literature. This may point to a relatively high exposure to DDT in terrestrial ecosystems compared to aquatic ecosystems.

The levels of toxic isomers of PCDD and PCDF in liver and adipose tissue of mustelids range from 5 to 4000  $\text{pg.g}^{-1}$  (lipid weight). The highest concentrations are found for 1,2,3,4,7,8,9-H7CDD, OCDD, 2,3,4,7,8-PCDF, 1,2,3,4,7,8- and 1,2,3,7,8,9-H6CDF and 1,2,3,4,6,7,8-H7CDF. In liver, concentrations on a lipid base are between 5 and 10 times as high as those in adipose tissue.

The contribution from the PCDD/Fs to the total TCDD equivalent concentration found in mustelid liver is around 50%, the other half being contributed by PCBs<sup>6</sup>. The major part (90%) of this 50% is from the PCDFs, with a prime contribution from 2,3,4,7,8-pentachlorodibenzofuran.

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