Patterns and toxic potency of persistent PCDD and PCDF congeners in liver of mustelids from the Netherlands

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1. Introduction

For some obvious and some obscure reasons, terrestrial mammals belong to the least known species as far as environmental contamination is concerned. Decline of various species has prompted research into contaminant levels in various countries. In particular levels of chlorinated aromatic hydrocarbons have been suspected because of the sensitivity of mink towards these compounds. In the Netherlands, the population decline of several members of the mustelid family has led to a survey of polychlorinated biphenyl (PCB) levels, including the toxicologically relevant non-*ortho* PCB[1]. The present study, a continuation of that survey, investigates individual 2,3,7,8-substituted polychlorinated dibenzo-*p*-dioxin (PCDD) and dibenzofuran (PCDF) concentrations in livers of five members of the mustelid family, viz. stoat, weasel, polecat, pine marten and otter and assesses the PCDD/F contribution to the total TCDD equivalent concentration.

2. Experimental

Samples of stoat (*Mustela herminea*), weasel (*Mustela nivalis*), polecat (*Mustela putorius*), pine marten (*Martes martes*) and otter (*Lutra lutra)* were collected in the field as road victims or animals accidently trapped. All samples came from the province of Friesland in the northern part of the Netherlands. Between three and five animals were collected for each species. Animals were stored at -20°C. After dissection, livers were stored at -35°C.

The samples were initially Soxhlet extracted with dichloromethane/n-pentane (1/1 v/v) after homogenising with anhydrous sodium sulphate in a mortar. The extracts were taken up in n-hexane.

n-Hexane extracts (containing between 20 and 80 mg lipid) were spiked with a mixture of 17 ^{13}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.

3. Results and Discussion

Table 1 presents the results of individual congener analysis of livers from five mustelid species. The mean result for each species is given. Two samples of other tissue were also analysed, viz. a sample of adipose tissue from a pine marten and a sample of muscle from otter. These results are included in Table 1 for comparison. The results show that total PCDF levels amount to roughly the same level (10 - 15 ng·g^{-1} lipid) in various mustelid species, with the exception of polecat, where a significantly lower mean value of 3.5 ng·g^{-1} was found. As for the total PCDD levels, some more variation is found between species. Pine marten and weasel show the highest levels (10 ng·g^{-1}), otter and stoat have levels around 5 ng·g^{-1} and polecat again shows the lowest levels (2.5 ng ·g^{-1}). OCDD exhibits invariably the highest concentration among the PCDD congeners. Among the PCDF congeners, 2,3,4,7,8-penta-, 1,2,3,4,7,8-hexa-, 2,3,4,6,7,8-hexa-, 1,2,3,4,6,7,8-hepta- and octa-CDF are the major congeners.

A remarkable difference of about one order of magnitude is found between liver and adipose tissue levels of both PCDD and PCDF in Pine marten. Although the adipose tissue level is based on only one sample at the moment, this may indicate that body distribution is not in equilibrium or that specific binding sites for these compounds exist in Pine marten liver, as has been suggested for rodents[2], where similar differences have been reported between liver and adipose tissue. An increasing liver/adipose tissue ratio is found with increasing chlorination degree within the PCDD congeners, in agreement with findings for rat and monkey[3]. For PCDF, however, this ratio is more or less constant over the entire congener range.

Muscle and liver from otter display similar levels on a lipid base, pointing at equilibrium distribution within this species.

Table 1. Mean concentrations (in pg·g^{-1} lipid) of 2,3,7,8-substituted PCDD and PCDF in liver of mustelids from the Netherlands

PCDD Species	tcdd	1-penta	14hexa	16hexa	19hexa	146hepta	octa	Total
Stoat (n=5)	107	154	269	911	218	1240	1964	4863
Weasel (n=3)	137	271	323	855	381	2297	6173	10437
Polecat (n=4)	33	55	100	360	53	774	1075	2450
Pine marten (n=4)	33	84	135	613	97	2626	6457	10045
adipose tissue (n=1)	8	17	26	71	20	210	579	931
Otter (n=4)	38	116	102	793	76	724	2966	4815
muscle (n=1)	76	121	275	356	139	938	3102	5007

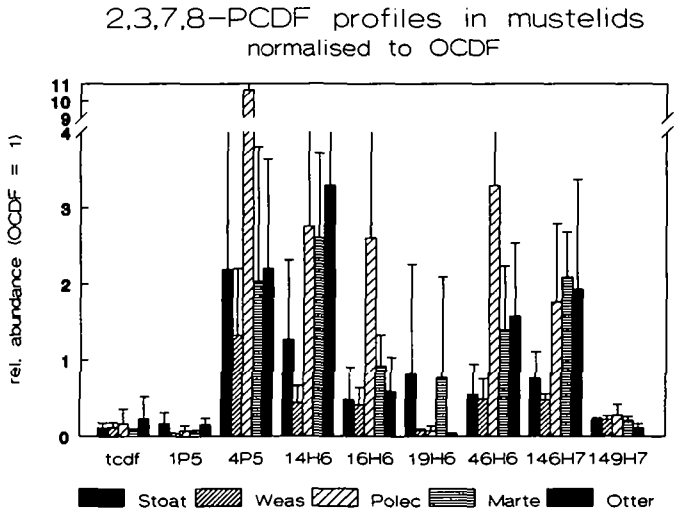
PCDF Species	tcdf	1penta	4penta	14hexa	16hexa	19hexa	46hexa	146hepta	149hepta	octa	Total
Stoat	154	173	2058	1495	564	561	1044	1220	530	2435	10252
Weasel	301	205	2335	1161	1061	387	1137	2226	1333	4888	15034
Polecat	23	11	1345	414	370	9	532	282	50	449	3485
Pine marten	68	47	1699	2592	879	435	1794	2203	213	1086	11016
adip. tissue	7	7	148	131	73	126	38	131	19	86	766
Otter	144	115	1597	2280	409	28	1215	1345	149	2849	10131
muscle	203	276	451	722	444	203	434	2199	803	4086	9821

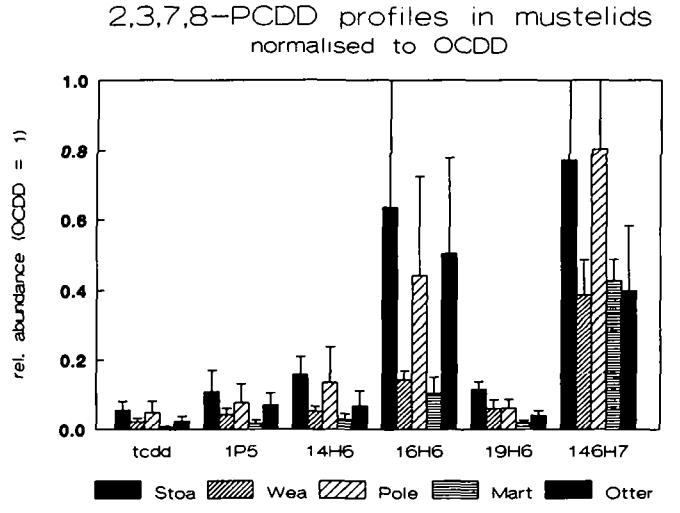
The congener patterns can be displayed more explicitly by normalizing the individual PCDD and PCDF abundance to that of OCDD and OCDF, respectively, which is shown in figure 1. This figure shows that interspecies differences exist in relative abundancies, in particular for 1,2,3,4,7,8-; 1,2,3,7,8,9- and 2,3,4,6,7,8-hexaCDF and 1,2,3,4,6,7,8-heptaCDF; and for 1,2,3,6,7,8-hexaCDD. For the PCDD, a consistent pattern is found when species are compared: stoat, polecat and otter appear to have much higher relative PCDD abundancies than weasel and pine marten. This may point at a relatively better developed metabolic system for the latter two animals. For PCDFs, in weasel a similar finding is shown, whereas pine martin data show a more intermediate position for this animal when compared to polecat or otter. Further evidence for individual congener metabolite formation is currently being investigated through analysis of various types of prey that mustelids feed on.

The contribution from the PCDDs and PCDFs to the total TCDD equivalent concentration calculated in liver amounts to about 50%, the other half being contributed by planar polychlorinated biphenyls[4]. The major part (90%) of the PCDD/F contribution stems from PCDFs, with a prime contribution from the 2,3,4,7,8-pentaCDF.

Figure 1.

PCDF and PCDD profiles in five mustelid species from the Netherlands (1P5 = 1,2,3,7,8-penta-isomer, etc.)





4.References

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