CONGENER-SPECIFIC BIOACCUMULATION OF PCBs IN DIFFERENT WATER BIRD SPECIES

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1. Introduction

Water birds are exposed to various toxic, halogenated compounds such as for example PCBs. Such exposures have resulted in a number of adverse effects on their reproductive potential, such as deformities and lethality of the embryos [10,11] and can cause population declines. Considerably, high levels of PCB congeners have previously been demonstrated in water birds.

However, most analyses so far have been restricted to top predator species. The contribution of other preceding trophic levels is still largely unknown. Of special interest are the non-ortho chloro-substituted coplanar PCB congeners because they show similar biochemical and toxic activity as the highly toxic 2,3,7,8-TCDD mediated by interaction with the Ah-receptor. Therefore, the concept of toxic equivalency factors (TEFs) and resulting TCDD toxic equivalents (TEqs) reasonably should also include the dioxin-like coplanar PCB congeners.

The aims of this study were the analysis of the species-specific PCB congener patterns including the mono- and non-ortho substituted congeners, and the organ-specific differences in content and/or congener pattern between liver and muscle tissue of four water bird species. Also the estimated transfer rate of PCB congeners from one trophic level to the next higher was investigated. The bioaccumulation behaviour of PCBs through the food chain was evaluated with special emphasis on coplanar congeners. On the basis of these data an assessment of the potential health hazards for different trophic levels should be possible.

2. Materials and methods

The whole procedure will be described and discussed elsewhere[1].

Sample collection:
All samples were collected in1993 in the Linth channel region. The Linth channel is the fast flowing drain of the relatively clean and oligotrophic Lake Walen. This area is virtually free of industrialisation.
Species of interest were tufted ducks *(Aythya fuligula)* , hooded grebes *(Podiceps cristatus)*, herons *(Ardea sinerea)* and cormorants *(Phalacrocorax carbo sinensis)*.

Fat extraction, clean up and separation of coplanar PCB congeners:
The lipid content of all the samples was determined gravimetrically. The fat extracts were saponified and then extracted with n-hexane.
After purification on silica gel impregnated with H_2SO_4, the sample was fractionated on activated carbon into three fractions containing di- to tetra-, mono-, and non-ortho PCB congeners. The whole fractionation procedure will be described and discussed elsewhere [2)]. A glass column was packed with silica gel and silica gel with activated carbon AX-21. The non-coplanar di- to tetra-ortho PCBs were eluted with n-hexane, and the mono-ortho PCB congeners with a mixture of n-hexane/1% toluene. Finally the flow direction of the column was reversed and the non-ortho congeners were eluted with a mixture n-hexane/10% toluene. For GC analysis the samples were redissolved in isooctane.

Instrumental analysis, Identification and Quantification:
The di- to tetra-ortho fractions were analysed using gas chromatography/electron capture detection (GC-ECD), whereas the mono- and non-ortho fractions were analysed with gas chromatography/high resolution mass spectrometry(GC-HRMS). Congeners were identified by co-chromatography with reference PCB congeners.

The quantification was based on peak areas and was carried out by comparing peak areas with those of external (GC-ECD) or ^{13}C-labelled internal (GC-HRMS) standards. The PCB congeners determined were 28 (2,4,4'-trichlorobiphenyl), 37 (3,4,4'-trichlorobiphenyl), 52 (2,2',5,5'-tetrachlorobiphenyl), 70 (2,3',4',5-tetrachlorobiphenyl), 77 (3,3',4,4'-tetrachlorobiphenyl), 81 (3,4,4',5-tetrachlorobiphenyl), 101 (2,2',4,5,5'-pentachlorobiphenyl), 105 (2,3,3',4,4'-pentachlorobiphenyl), 114 (2,3,4,4',5-pentachlorobiphenyl), 118 (2,3',4,4',5-pentachlorobiphenyl), 126 (3,3',4,4',5-pentachlorobiphenyl), 138 (2,2',3,4,4',5'-hexachlorobiphenyl), 153 (2,2',4,4',5,5'-hexachlorobiphenyl), 156 (2,3,3'4,4',5-hexachlorobiphenyl), 169 (3,3',4,4',5,5'-hexachlorobiphenyl), and 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl).
A total TEq value was calculated as the sum of the individual TEq values of 14 PCB congeners quantified in the biological samples. The individual TEq values represent the detected concentration multiplied by the corresponding mammalian TEF value [3)] of the PCB congener.

3. Results and discussion

Table 1 shows the levels of PCB congeners in the liver tissue of the investigated birds.

Seasonal variations:
The values of individual birds were not shown here. The rather high inter-individual differences may obscure possible seasonal differences.

For this reason, all birds of a species analysed were summarised to one group. When comparing the birds of the same species which were shot at three different seasons of the year, only herons show seasonal differences in the PCB body burden (data not published here). In spring time the PCB levels are slightly higher than in autumn and winter. This means that total PCB and individual congener concentrations vary greatly within one bird species group [7].
Higher values in PCB body burdens in spring could be explained by the fact that in addition to fish herons also feed on amphibians, belonging to a higher trophic level than fish.
Another important point for bioaccumulation is the age of an animal. The age of the analysed birds was not known. Therefore, the important PCB accumulation by age can not be interpreted in this study.

Species differences:
Hooded grebes, herons, and cormorants are fish eating birds, whereas the tufted ducks feed on zebra mussels exclusively. Table 1 shows that the fish eating bird species show higher PCB levels than the mussel feeding ducks. This can most likely be explained by the fact that fish belong to a higher trophic level than mussels. Furthermore, this tendency also can be demonstrated by the fact that herons and cormorants, both feeding on rather big fish (especially carnivorous trouts and omnivorous burbots), seem to have higher PCB body burdens than grebes, which only gulp down smaller fish (young fish feeding on plankton).

Organ differences:
The concentrations of single PCB congeners measured in liver tissues of the different bird species are shown in Table 1. Earlier measurements [1] of corresponding (same birds) muscle tissue PCB contents were in the same order of magnitude as the liver levels presented here. There do not seem to exist any significant differences between the concentrations or the pattern of PCB congeners between these two selected organs. Therefore, PCB congeners seem to be homogeneously distributed in the extractable lipids of the body.

PCB patterns and bioaccumulation:
The contribution of the di-ortho substituted congeners to the TEq value was always approximately 20%. The major contributors to the TEq were the mono-ortho congeners with 40 to 60 %, whereas the non-ortho congeners contributed between 20 and 40 %. The concentrations of the single PCB congeners for the herons from this study were in a range which is comparable to black-crowned night-heron embryos analysed from relatively uncontaminated areas [5]. In addition the pattern found in this study was similar to the one reported by other studies in birds [7].
The bioaccumulation behaviour of the coplanar PCBs is of special interest. The concentrations of the non-ortho substituted PCB congeners relative to the total PCB content did not significantly increase with higher trophic levels. However, in herons the contribution of the coplanar congeners relative to the total PCB content was higher than in tufted ducks.
This may indicate that while the congener pattern itself does not change, the coplanar PCBs may have a slightly higher tendency for bioaccumulation throughout increasing trophic levels.

Table 1: Average values of PCB congener concentrations (ng/g lipids) in the liver tissue of four different water bird species. In parentheses: range. Total PCB was calculated by multiplication of the sum of the congeners 153, 138 and 180 by 1.7 [4]. For the TCDD-TEq values the mammalian TEFs proposed by Safe [3] were used.

PCB congeners	TUFTED DUCKS (n = 8)		HOODED GREBES (n = 9)		HERONS (n = 9)		CORMORANTS (n = 6)	
52	16	(< 0.9 - 38)	23	(10 - 52)	44	(4 - 125)	32	(6 - 78)
101	42	(19 - 104)	117	(40 - 341)	468	(29 - 1'400)	283	(105 - 469)
153	364	(96 - 804)	3'326	(795 - 10'762)	7'245	(860 - 14'609)	6'295	(1'836 - 15'414)
138	412	(233 - 993)	2'393	(669 - 7'199)	6'827	(721 - 16'112)	5'350	(1'713 - 11'719)
180	163	(< 25 - 367)	986	(274 - 3'324)	4'381	(249 - 12'421)	3'055	(460 - 7'473)
sum di-ortho	997	(450 - 2'252)	6'855	(1'801 - 21'550)	18'964	(1'863 - 44'667)	15'013	(4'129 - 35'152)
28	8	(1 - 25)	185	(25 - 589)	137	(15 - 395)	302	(97 - 524)
70	4	(1 - 11)	36	(12 - 62)	87	(9 - 277)	74	(34 - 110)
118	143	(68 - 274)	2'499	(434 - 5'424)	4'677	(639 - 13'667)	2'636	(940 - 3'892)
114	2	(1 - 3)	45	(6 - 104)	150	(6 - 821)	62	(17 - 100)
105	49	(15 - 116)	683	(21 - 1'632)	1'987	(161 - 9'605)	876	(271 - 1'004)
156	22	(8 - 48)	483	(66 - 1'154)	1'562	(88 - 6'894)	461	(103 - 811)
sum mono-ortho	228	(110 - 452)	3'931	(653 - 8'583)	8'601	(920 - 31'659)	4'412	(1'461 - 6'650)
37	1.0	(0.1 - 3)	0.3	(0.05 - 0.6)	0.4	(0.1 - 1.3)	0.4	(0.3 - 0.6)
81	0.1	(0.01 - 0.1)	0.3	(0.06 - 0.7)	0.9	(0.1 - 3.5)	0.8	(0.4 - 1.8)
77	1.2	(0.3 - 2)	2	(0.6 - 5)	9	(0.7 - 41)	5	(2 - 7)
126	1.5	(0.5 - 3)	17	(5 - 53)	69	(4.1 - 327)	34	(11 - 59)
169	0.4	(0.2 - 0.6)	4	(1 - 15)	16	(0.8 - 80)	7	(1.3 - 15)
sum non-ortho	4.1	(2.3 - 9.1)	24	(8 - 74)	95	(6 - 453)	47	(15 - 81)
Lipid content (%)	*4.3 ± 0.7*		*3.8 ± 0.7*		*4.0 ± 1.8*		*4.2 ± 1.2*	
TOTAL PCB	1'598	(688 - 3'678)	11'399	(2'956 - 36'185)	31'370	(3'110 - 73'341)	24'988	(6'815 - 58'830)
Total TCDD-TEq	0.6	(0.3 - 1)	9	(2 - 18)	20	(2 - 78)	11	(3 - 17)

The observation that tufted ducks showed higher levels of congener 77 compared to other coplanar PCBs could be explained by lower metabolic capacities of some duck species for planar congeners [8].
On the other hand, the differences in PCB body burdens between bird species suggest that PCB pattern can be more influenced by dietary inputs than by the bioaccumulation abilities of a species. Comparison of the levels in the food sources [1] of these different bird species, leads to a biotransfer factor from zebra mussels to tufted ducks of approximately 2. In comparison, the biotransfer factors from fish to herons ranged from 4 to 15, depending on the individual congener. The biotransfer factors of the sum of the non-ortho substituted PCB congeners were higher (BTF = 9) than the mono-ortho congeners (BTF = 6) and the di-ortho congeners (BTF = 4). Within the coplanar PCBs, congener 169 seemed to have the highest biotransfering potency (BTFs for congener 77, 126 and 169 from trout to heron were 3, 11 and 15, respectively).
The PCB body burdens of the analysed trout cannot be compared to the values observed in hooded grebes, as only adult fish, which were surely too big to be consumed by the hooded grebes, were caught for analysis.

The PCB contents of these fish and those of the grebes, however, were in the same order of magnitude. In contrast to the grebes, cormorants stay in Switzerland only during winter time and breed during the summer months in Danish breeding colonies. The PCB levels in tissues of European cormorants are reported to range up to 160 µg/g lipid weight [9]. These findings stand in contrast to the low levels found in the present study. However, even if these migratory birds had accumulated higher PCB levels in their summer colonies, the residence time of 4 to 6 months in the relatively low contaminated Swiss aquatic ecosystem is sufficient to decrease possible high body burdens to the observed levels.

Toxicological evaluation and risk assessment:
The reproductive impairment is the most important and also almost the only visible parameter to indicate toxic effects of environmental xenobiotics in wildlife species as more subtle effects like organ toxicity are not directly detectable. Therefore, the reproductive success of a species can be used as an indicator or biological monitor for toxic PCB effects in the field.
To judge the hazard of PCB contamination for waterfowl, different parameters can be employed. For example, the total PCB content of bird species can be compared to species which already show impairment of their reproduction, e.g. reduced hatchability or chick deformities, and thus give an indication as to how close the bird species of interest is to become endangered by toxic effects of PCBs.
In livers of cormorants showing reproductive impairment, the total PCB content was around 100 µg/g lipids (24 - 301 µg/g lipids) [9]. In comparison, the total PCB concentrations in bird species determined in the study presented here (Table 1) were in the same range, especially these of the herons and cormorants. However, in Switzerland, these water bird species do not seem to be directly affected by PCB contamination. Indeed, hooded grebe and heron populations appear to be healthy and growing. This may also indicate that not all bird species are equally sensitive to the toxic effects of PCBs, as already suggested earlier by Tillit and coworkers [15].
Another example for the devastating effects of PCB toxicity is the fish otter (*Lutra lutra*), a mammalian top predator and fish consumer, which is now extinct in Switzerland. The role of PCB contamination in the food sources of the otters is currently assumed to be responsible for the impaired reproduction of these animals. In livers of fish otters total PCB values between 2 to 190 µg/g lipid were detected [14]. Comparing these PCB concentrations with those found in the birds of the study presented here, as well as assuming that birds are as sensitive to PCBs as mammals, one would have to expect that bird species, e.g. hooded grebes, herons and cormorants, would have to become extincted due to reproductive impairment.
This fact clearly shows that the comparison of total PCB content is the wrong way to do a risk assessment, especially as PCB sensitivity appears to be clearly species-specific [6]. It is also obvious, that birds and mammals may have totally different uptake rates and metabolism of the PCB congeners. Variations in the elimination half lives of single PCB congeners may lead to varying accumulation behaviour.

In order to include the species specific congener pattern for risk assessment, the TEF concept was used to weigh the relative toxicities of the individual PCB congeners. However, the TEF approach for the risk assessment of PCBs must be used with considerable caution. It must be emphasised that due to the fact that the TEF concept exists for avian eggs only, this cannot be extrapolated to the adult birds and thus the generally accepted TEF concept applied for mammals or mammalian cells [3] had to be employed.

To compare the data of adult water bird species to literature the corresponding TEq values were calculated (Table 1). Most congener-specific bird analyses have been done in eggs or embryos. In eggs of cormorants [12] and terns [13] TEq values up to 12 ng/g lipids were found. In these breeding colonies the reported reduced hatching rates and chick deformities seemed to be linked to PCB contamination. In this study the tissues of adult birds were analysed. The calculated TEq values (based on mammalian TEFs) for liver tissue of the four water bird species investigated here were in the same range (Table 1), almost demanding that reproductive impairment would have to be observed. This clearly indicates that mammalian TEFs are not suitable for risk assessment in adult birds and strongly suggests that further investigations to develop and evaluate bird specific TEF values are needed.

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4. References

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