GAS CHROMATOGRAPHIC ENANTIOMER SEPARATION AND POTENTIAL TOXICITY OF ATROPISOMERIC POLYCHLORINATED BIPHENYLS IN MARINE BIOTA: HAS THE TOXIC EQUIVALENCE FACTOR [TEF] CONCEPT TO BE EXPANDED?

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1. Introduction

In the last few years, increasing attention has been paid to the relationship between the conformation of polychlorinated biphenyls [PCBs] and their impact on marine and terrestrial biota (refs.[1-7] and literature cited therein). In particular, emphasis has been placed upon the toxicity of so-called "coplanar" PCBs, i.e., congeners that are able to attain a coplanar conformation of the two aryl systems due to lack of *ortho*-chlorine substituents.

Basically, the toxic potency of PCB mixtures in environmental samples can be estimated with the help of a toxic equivalence model. Within the scope of this model, toxic equivalence factors (TEFs) were defined for the coplanar PCB congeners that induce aryl hydrocarbon hydroxylase (AHH) activity in a similar manner as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD). Each of these PCBs has been assigned a TEF value based on its toxicity relative to 2,3,7,8-TCDD, which got a TEF of 1 by definition.

A number of different toxic equivalence factor (TEF) schemes have been developed for coplanar ("dioxin-like") PCBs [3-7]. Recognizing the necessity for a more consistent approach towards setting internationally agreed TEFs, the WHO-European Centre for Environment and Health (WHO-ECEH) and the International Programme on Chemical Safety (IPCS), initiated a project to create a data base containing information relevant to the setting of TEFs, and, based on the available information, to assess the relative potencies and to derive consensus TEFs for coplanar PCBs. As a result, it was concluded that the available data could be used to set interim TEFs for selected PCBs. Furthermore, it was recommended that the interim data base for TEFs should be updated every two years. In particular, the following criteria for including a compound in a TEF-scheme should be met:

- It should show structural relationship to the PCDDs and PCDFs;
- it should bind to the Ah-receptor;
- it should elicit dioxin-specific biochemical and toxic responses;
- it should be persistent and accumulate in the food chain.

As a consequence, many PCB congeners were not included because of their weaker activity as Ah-receptor agonists and their lack of biological significance in the context of TEFs for dioxin-like chemicals. Furthermore, as stressed by Ahlborg et al.[7], it can not be excluded that certain congeners have not been taken into account due to lack of data.

In the present paper, attention will be drawn to a class of PCBs that is not included in the present TEF-scheme for the reasons outlined above, the so-called atropisomeric PCBs. Atropisomeric PCBs have been neglected thus far in marine chemistry with regard to their enantioselective degradation in marine biota and their toxicity. The reason for this gap is assumed to be the analytical difficulty to determine these PCB congeners by capillary gas chromatography [cGC] at low levels encountered in environmental samples. Only recently, König et al. [8,9] and Schurig et al. [10, 11] presented experimental approaches that overcome this obstacle.

As a consequence, we are able to present for the first time data of atropisomeric PCBs in samples of marine biota. The feasability of this approach will be demonstrated by the analysis of blue mussel (*Mytilus edulis* L.) samples collected at six different sites of the German Bight. Blue mussels often serve as indicator organism because of their capability of accumulating lipophilic pollutants and their relatively small enzymatic degradation potential, in order to assess the water quality of marine ecosystems [12-15]. Herein, emphasis will be placed upon a comparison between atropisomeric PCBs in mussel samples collected during the spring and the autumn period. Furthermore, based upon literature data, evidence will be presented that the PCB enantiomers may exert quite different toxic responses. Finally, the question will be raised as to whether or not the present TEF concept should be modified by including atropisomeric PCBs.

2. Experimental

The blue mussels (*Mytilus edulis L.*) were collected during spring and autumn 1993 at different locations in the German Bight (Fig. 1). The mussel samples were stored in a refrigerator at about 248 K prior to sample preparation. The mussels were defrosted and homogenised with anhydrous Na_2SO_4 (1:3) supplying a powder that was extracted in a Soxhlet apparatus for eight hours with 200 ml *n*-hexane of analytical-reagent grade. The extract was concentrated to 5 ml at 360 mbar and 313 K followed by column chromatography over a partially deactivated Al_2O_3-column (5 % water; for details see [16]). After elution with 80 ml *n*-hexane and subsequent concentration of the eluate to 1 ml, higher-molecular lipids were separated by means of conc. H_2SO_4 of analytical-reagent grade ("H_2SO_4 clean-up"). The remaining *n*-hexane solution was concentrated by a weak nitrogen stream to 50 µl for the following GC analysis. The coplanar and the atropisomeric PCBs were determined in the respective same solutions.

The cGC analysis for determining the coplanar PCBs was performed on a Mega 5300 gas chromatograph (Carlo Erba) equipped with a CP-Sil 5/C 18 CB fused-silica capillary column (Chrompack; length 100 m; 0.32 i.d.; film thickness 0.1 µm). Detection was carried out with a ^{63}Ni-electron capture detector (ECD; make-up gas argon/methane 95/5). Temperature program: 343 K, 5 min ⟶ 30 K/min ⟶ 363 K, 2 min ⟶ 40 K/min ⟶ 433 K, 2 min ⟶ 1 K/min ⟶ 533 K, 50 min; carrier gas helium (250 kPa); cold on-column injection. For peak identification a standard mixture containing 60 individual PCB congeners was used. The quantification was accomplished with ε-HCH as internal standard. The concentrations are expressed in the unit ng/g extractable organic matter (EOM).

The chiral cGC was performed on a Fisons 8000 series gas chromatograph using a 25 m fused-silica capillary column coated with 50 % heptakis(6-*O*-*tert*-butyl-dimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin and 50 % OV-1701 (w/w, i.d. 0.25 mm, film thickness 0.125 µm). Detection was carried out with a ^{63}Ni-electron capture detector (ECD; make-up gas nitrogen); column temperature program: initial 333 K, increased at 10 K/min to 423 K, 170 min isothermal; carrier gas hydrogen (60 kPa); on-column injection.

3. Results and Discussion

The mussels were collected at six different locations in the German Bight (Fig. 1). The first three sampling sites were chosen along the so-called "Cuxhaven Leitdamm" (short

LTD-Cux.), which is located in the Elbe river estuary, at a distance of 0 m, 2000 m and 3000 m from the beginning. The next site was positioned in the Weser estuary, and the last two sites were the so-called "Wilhelmshaven Leitdamm" (short: Wilhelmsh.) located within the Jade Gulf and a site in the inner Jade Bay underneath the Niedersachsen Bridge (short: Jade).

Basically, 78 congeners of the 209 PCBs possess axial chirality ("atropisomeric PCBs"), however, as pointed out by Kaiser [17], the existence of only nine of the major, and ten of the minor, constituents of commercial mixtures like Aroclors 1242, 1254 and 1260 in chiral conformations can be expected. These molecules derive chirality from the resistance to free rotation about the C-C-axis due to the interference of the chlorine substituents. The activation enthalpy required for the interconversion of enantiomers ($\Delta G^{*} \sim 105 - 240$ kJ mole^{-1}) has been estimated to be greater than the thermal energy usually available in the ecosystem[17]. In line with these pioneering results, Püttmann et al. [18] presented evidence that at least the following 19 of the 78 unsymmetrically-substituted PCBs will be stable to racemization at room temperature : PCB 45, PCB 84, PCB 88, PCB 91, PCB 95, PCB 131, PCB 132, PCB 135, PCB 136, PCB 139, PCB 144, PCB 149, PCB 171, PCB 174, PCB 175, PCB 176, PCB 183, PCB 196, PCB 197. For three of these atropisomeric PCBs, i.e., PCB 88, PCB 139, and PCB 197, the enantiomers were enriched or separated on triacetylcellulose by Mannschreck et al. [19] and by Püttmann et al. [18]. Furthermore, König et al. [8, 9] were able to separate the enantiomers of PCB 45, PCB 84, PCB 88, PCB 91, PCB 95, PCB 131, PCB 132, PCB 135, PCB 136, PCB 139, PCB 149, PCB 174, PCB 175, PCB 176, and PCB 183 by chiral cGC using modified cyclodextrin phases, while Schurig et al.[10, 11] reported the separation of the enantiomers of PCB 84, PCB 91, PCB 95, PCB 132, PCB 136, and PCB 149. Based upon these pioneering publications, we were encouraged to start an investigation about the distribution of atropisomeric PCBs in the marine environment.

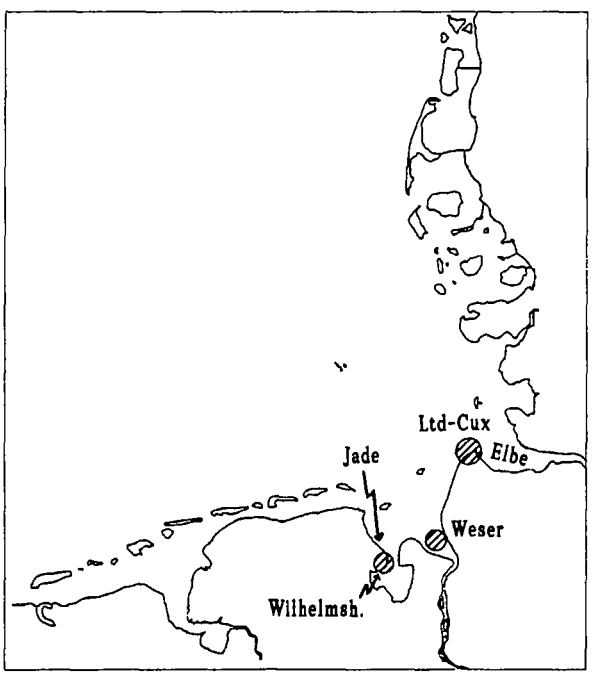

Figure 1: *Locations in the German Bight at which the blue mussels were collected during spring and autumn 1993.*

Concentrations of Atropisomeric PCBs in Blue Mussels

As summarized in Table 1, five of the above nineteen atropisomeric congeners, possibly present in commercial PCB formulations, were found in the blue mussel samples investigated herein: PCB 88, PCB 149, PCB 183, PCB 174, and PCB 171. In all cases, the concentrations encountered in mussels collected during the autumn period were significantly lower than in those of spring. It is tentatively assumed that these differences have to be largely attributed to the seasonal variation of the lipid matrix. Potential local variations of the contamination are not reflected in the data set of the atropisomeric PCBs.

PCB 149 was of particular interest, because sufficiently high concentrations were encountered both during spring and autumn, which allowed separation of the enantiomers by chiral cGC. A typical gas chromatogram of PCB 149 is shown in Figure 2 both for a standard and for a blue mussel sample of the "Jade" site. The resulting enantiomeric ratios, defined as quotient of the first eluting and the second eluting peaks in the gas chromatogram, are also given in Table 1. In spite of the reduced metabolising potential of mussels a tendency of a preferential degradation of the second eluting enantiomer can be inferred from the enantiomeric ratios. However, it should be noted that the enantiomeric ratio of 1.1 determined in six samples only reflects a weak degradation at the limit of the methodical error (i.e., a "tendency"), while the two enantiomeric ratios of 1.2 indicate a significant preferential enzymatic degradation of the second eluting enantiomer.

In conclusion, the present data set shows that only weak enzymatic degradation of atropisomeric PCBs can be stated for blue mussels. This result is fully in line with corresponding observations of Pfaffenberger et al. [20] for enzymatic degradation of α-HCH in blue mussels and of Glausch et al. [11] for PCB 95, PCB 132 and PCB 149 in human milk, doe liver and eel samples. Therefore, we intend to repeat this investigation for marine biota at higher trophic levels, which are expected to exhibit enzymatic systems more appropriate for the degradation of PCBs.

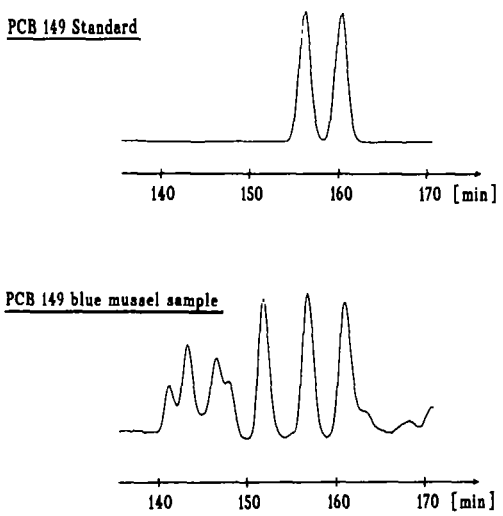

Figure 2: Enantiomeric separation of the atropisomeric PCB 149 on a 25 m fused silica capillary column coated with 50 % heptakis(6-*O*-*tert*-butyl-dimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin and 50 % OV-1701 (w/w, i.d. 0.25 mm, film thickness 0.125 μm); ^{63}Ni-electron capture detector (ECD; make-up gas nitrogen); column temperature program: initial 333 K, increased at 10 K/min to 423 K, 170 min isothermal; carrier gas hydrogen (60 kPa); on-column injection. Top: PCB 149 standard; bottom: PCB 149 in a blue mussel sample of the "Jade" site.

Table 1:
Concentration of PCBs which may exist as atropisomers (in brackets enantiomeric ratio) at six sites of the German Bight during the spring and autumn period, 1993 (in ng/g EOM).

IUPAC numbers	Weser	Jade	Wilhelmsh.	LTD-Cux 0 m	LTD-Cux. 2000 m	LTD-Cux 3000 m
			Spring			
PCB 88	2.21	0.42	0.71	0.97	0.0	0.97
PCB 149	248.1	172.1	170.2	236.6	287.6	243.5
	(1.1)	(1.0)	(1.2)	(1.2)	(1.1)	(1.0)
PCB 183	14.34	15.59	13.66	19.37	23.32	21.81
PCB 174	1.31	0.98	1.18	2.03	2.17	1.13
PCB 171	3.48	4.73	5.20	6.05	5.28	3.70
			Autumn			
PCB 88	0.004	0.0	0.12	0.71	0.25	0.04
PCB 149	114.9	140.3	87.8	152.9	108.0	85.3
	(1.0)	(1.0)	(1.1)	(1.1)	(1.1)	(1.1)
PCB 183	0.03	0.02	0.06	0.10	0.26	0.45
PCB 174	0.90	0.003	0.07	0.29	0.52	0.02
PCB 171	1.22	1.73	1.53	2.03	1.58	1.28

Toxic Potency of Atropisomeric PCBs

A risk assessment of the toxic potential of atropisomeric PCBs is difficult, because the present concept of TEFs is confined to dioxin-like PCBs as outlined above and thus not applicable to atropisomeric PCBs. However, some basic insights, which may give rise to an alternative concept that will include atropisomeric PCBs, can be inferred from the pioneering investigation published by Rodman et al. [21] . The autors investigated the atropisomers of PCB 88, PCB 139, and PCB 197 in the chick embryo hepatocyte culture to determine if chirality plays a role in the recognition events associated with the induction of cytochrome P450 and the accumulation of uroporphyrin (URO). Concentration-related induction of cytochrome P450 content, ethoxyresorufin-*O*-deethylase (EROD) and benzphetamine N-demethylase (BPDM) activities were measured. Rodman et al. determined a rank order of potency for total cytochrome P450 induction of PCB 139 > PCB 197 ≥ PCB 88. The (+)- and (-)-enantiomers of PCB 88 and of PCB 197 were of equal potencies as inducers of cytochrome P450, whereas potency of the (+)-enantiomer of PCB 139 was greater than that of the corresponding (-)-enantiomer. PCB 139 was a much more potent inducer of EROD activity than was either PCB 88 or PCB 197. Furthermore, EROD activity was induced to much greater extent by the (+)-enantiomers of all compounds, with the (-)-enantiomers of PCB 88 and PCB 197 being inactive. As coplanar PCBs are also able to induce EROD activity (ref.[22] and literature cited therein), it has to be investigated as to whether this parameter can be used to infer total toxic potencies including both coplanar and atropisomeric PCBs. Particularly encouraging are the significantly different responses of the EROD activity to the respective enantiomers of atropisomeric PCBs.

According to Rodman et al. [21] BPDM activity was induced in the order of PCB 197 ≥ PCB 139 > PCB 88. In this case, the (-)-enantiomers were more potent inducers of BPDM activities than were the (+)-enantiomers, except for PCB 139, in which the (+)-enantiomer was more potent than the (-)-enantiomer.

Furthermore, analysis of porphyrin accumulation in cultures treated with δ-aminolevulinic acid revealed that the (+)-enantiomer of PCB 139 caused the greatest percent URO accumu-

lation, which also correlated with the greatest increase in EROD activity. All other enantiomers caused up to 47 % URO accumulation, which did not correlate with an increase in EROD activity. In addition, chiral effects in the induction of drug-metabolizing enzymes by atropisomeric PCBs were reported by Püttmann et al. [23] . In conclusion, much evidence is available that supports the assumption that chirality of atropisomeric PCBs plays an important role in many recognition events associated with enzymatic degradation processes. However, systematic investigations are necessary, in order to develop risk assessment concepts that may compete with the TEF concept used for coplanar PCBs.

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