Enantiomeric signatures of chiral polychlorinated biphenyl atropisomers in livers of harbour porpoises (*Phocoena phocoena*) from the southern North Sea

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Received 23rd January 2003, Accepted 28th March 2003
First published as an Advance Article on the web 23rd April 2003

The enantiomeric composition of polychlorinated biphenyl (PCB) atropisomers, including PCB 95, PCB 149 and PCB 132, was measured in 11 livers of harbour porpoises (*Phocoena phocoena*) from the southern North Sea. Non-racemic enantiomeric ratios (ERs) were found in some samples. The value of ERs in three of the four juvenile porpoises was equal or almost equal to one, while the ERs in all adults differed from racemic and ranged from 1.31 to 2.54 for PCB 95; from 1.19 to 1.81 for PCB 149 and from 0.45 to 0.94 for PCB 132. There were no relationships between the total concentration of PCBs and ERs. To understand the phenomena, the relationships between the ER value of individual chiral congener with age, concentration of total PCBs and PCB congener pattern were discussed. A model of intake and elimination kinetics was set up and it was tested using the ratio between concentration of PCB 153 and PCB 101 in the liver samples. There was a clear trend between the enantiomeric ratios and the ratio between PCB 153 and PCB 101. Considering that PCB 153 is one of the most persistent PCB congeners in marine mammals and PCB 101 is a relatively easy metabolised congener, this trend means that the enantiomeric ratio most likely reflects the proportion of the metabolised congener. The exposure period in contaminated conditions has a strong impact on ERs, and it is suggested that ERs in wildlife, combined with information on their anthropometric data, health status, diet and habitat conditions, might be good indicators of pollution in coastal ecosystems.

Introduction

It is well known that many organohalogenated compounds including those present in technical products such as DDT (*e.g. o,p′*-DDT), hexachlorocyclohexane (α-HCH), toxaphene, and chlordane are chiral.[1] Even if enantiomers of organohalogenated pollutants have identical physical/chemical properties in an achiral environment, their response to biological processes may differ. It follows that one enantiomer can be faster depleted than its mirror image and, therefore, it was suggested that enantioselective analysis might be suited for characterising their environmental fate.[2] Due to recent developments in gas chromatographic enantioseparation based on modified cyclodextrin stationary phases, the determination of enantiomeric composition of chiral pollutants in environmental samples at trace level is now possible.[3] Enantiospecific data for wildlife has shown that enantiomeric ratios (ERs) vary by compounds and bioaccumulate through the food chain.[4–11]

One important group of chiral environmental contaminants is polychlorinated biphenyls (PCBs).[12] 78 of 209 PCB congeners display axial chirality in their non-planar conformations, while 19 congeners with three or four *ortho* chlorine atoms exist as pairs of stable enantiomers at ambient temperatures as a result of restricted rotation abound the central C–C biphenyl bond.[13] Four of these congeners (PCB 95, 132, 149 and 174) are relatively abundant in commercial mixtures,[14] each accounting for over 2%. Recently, attention has been focussed on atropisomeric PCBs because these compounds are introduced into the environment as racemates and their uptake and metabolism by organisms may be enantioselective. The enantiomeric ratio of chiral PCBs in animals may give additional information on possible degradation pathways.[15] PCBs are found in almost every environmental sample including water, sediments, fish and marine mammals, but little information is know about the presence, distribution and fate of PCB atropisomers.

The harbour porpoise (*Phocoena phocoena*) is a marine mammal inhabiting coastal waters of Europe, including the North Sea. A sharp decline in the number of porpoises has been noticed[16] during the 1950s and early 1960s, and it has been suggested that this decline results from contamination with organochlorine compounds.[17,18] The harbour porpoise has a limited capacity to metabolise PCBs compared to pinnipeds or terrestrial mammals.[19] Furthermore, the porpoise has a relatively high daily food intake that corresponds to approximately 3.5% of their body weight. As fish is one major route for the entry of environmental PCBs in the food chain, there are reasons to believe that porpoises may be used as indicators of the extent of PCB contamination in marine ecosystems. Quantification of the enantiomeric occurrence and composition of chiral PCBs may reveal a number of important implications on the bioprocess of PCBs by marine mammals and thus can be a useful tool in understanding the behaviour of PCBs in food webs.

This study investigates the enantiomeric occurrence of four chiral PCB congeners (PCB 84, PCB 95, PCB 132 and PCB 149) found in harbour porpoises stranded on the Belgian and French North Sea coast and compares it to the congener pattern of achiral PCBs.

Materials and methods

Samples

Eleven out of twenty-one harbour porpoise livers, which had been prepared and analysed using achiral techniques,[20] were

Table 1 Anthropometric details and enantiomeric ratios of chiral PCBs for 11 liver samples collected from stranded harbour porpoises

Sample no.[a]	Stranding year	Age[b]	Sex	Lipid (%)	Length/ cm	Weight/ kg	ERs (GC-ECD)			ERs (GC-MSD)[c]		
							PCB 95	PCB 149	PCB 132	PCB 95	PCB 149	PCB 132
5	1997	a	m	4.06	151	39	1.31	1.30	0.57	1.31	1.20	ND
6	1998	a	m	9.17	144	33	2.40	1.63	0.45	2.54	1.70	0.44
7	1999	a	f	6.15	134	30	1.38	1.28	0.59	1.46	1.25	0.57
10	1999	j	m	7.12	103	16	0.99	1.00	0.97	1.01	1.01	0.98
12	1999	j	m	21.74	80	7	1.12	1.19	0.94	1.11	1.19	0.91
13	2000	j	m	10.75	99	16	1.11	0.96	0.99	1.05	0.98	0.99
15	2000	a	m	7.48	142	41	2.54	1.81	0.47	2.44	1.73	ND
17	2000	a	m	6.74	144	43	2.30	1.57	0.44	2.23	1.64	ND
18	2000	a	m	3.41	149	37	1.94	1.40	0.54	1.93	1.48	0.52
19	2000	j	m	11.96	103	27	2.22	1.55	0.54	ND	ND	ND
21	2000	a	m	3.24	150	39	2.31	1.31	0.59	2.37	1.34	ND

[a]The sample numbering is similar as in ref. 20. [b]a: adult; j: juvenile. [c]ND: not detected.

selected for the analysis of atropisomeric PCB. Samples chosen for enantioselective analysis contained relatively high concentrations of total PCBs (ranging from 36 to 404 μg g^{-1} lipid weight). Details of weight, size, sex and age of the porpoises are shown in Table 1. All animals were found dead along the Belgian and French North Sea coast between 1997–2000, but no information on the health condition at the time of stranding was available.

Chemicals and standards

All solvents were of pesticide residue analysis from Merck (Wesel, Germany). Florisil (60-100 mesh) was purchased from Supelco (Bornem, Belgium). Individual PCB congener standards, including 2,2′,3,3′,6-pentachlorobiphenyl (PCB 84), 2,2′,3,5′,6-pentachlorobiphenyl (PCB 95), 2,2′3,3′,4,6′-hexachlorobiphenyl (PCB 132), and 2,2′,3,4′,5′,6-hexachlorobiphenyl (PCB 149) were purchased from Dr. Ehrenstorfer (Augsburg, Germany) at a concentration of 10 μg ml^{-1} in isooctane. Standard solutions for quantification were prepared by diluting the individual stock solution with isooctane.

Determination of achiral PCBs

Tissue sampling and analytical methods used for achiral PCBs have been described elsewhere[20] and briefly presented here. Basically, the method included the extraction of homogenised samples with *n*-hexane–dichloromethane–acetone (3:1:1, *v*/*v*). The total lipid weight was determined gravimetrically after solvent evaporation. The extract was cleaned up by a dual SPE cartridge with 2 g alumina and 5 g acid silica, and the organochlorine pollutants, including PCBs, were determined by gas chromatography with mass spectrometry (GC-MS) in selected ion monitoring mode. Before the determination of enantiomeric ratios of chiral PCBs, all extracts were stored at −20 °C in glass vials with Teflon-lined caps. Enantiomeric ratios are not expected to change during storage since only interactions with other chiral molecules (*e.g.* enzymes, biomolecules) may change ERs.

Cleanup and fractionation of chiral PCBs

Before HPLC fractionation, the extracts were further cleaned up by Florisil column (8 g, activated at 130 °C for 12 h before use). One single fraction containing PCBs and some pesticides was eluted with 70 ml hexane and concentrated to 50 μl under a gentle stream of nitrogen. The fractionation of PCB congeners according to their substituted position was achieved by the method reported by Ramos *et al.*[14] with some modifications. The PCBs were separated by a Cosmosil 5-PYE column, 250 × 4.6 mm id, particle size 5 μm (Nacalai Tesque, Japan). The HPLC system consisted of a pump, UV/VIS detector (GILSON, Wisconsin, USA) and a Rheodyne injection valve equipped with a 20 μl loop. The detector was operated at 254 nm. Hexane was used as a mobile phase at a flow rate of 0.5 ml min^{-1} and the separated fractions were collected by a HeliRac LKB2212 fraction collector (LKB-Produkter AB, Sweden). Individual PCB congener standard solutions were injected into the HPLC system in order to determine their retention times. If most of the *ortho*-substituted PCBs were collected as one fraction, co-elution of the target compounds with other congeners during fractionation could not be excluded and this may have lead to errors in the determination of enantiomeric ratios. Therefore, heart cut technology was used in the process and 80 μl eluant was collected for each chiral congener according their retention times on the HPLC chromatogram.

Chiral analysis

A Hewlett-Packard 5890 series II GC equipped with a ^{63}Ni electron capture detector and a PTV injector (Optic, AI Cambridge, Cambridge, UK) was used for the analysis of chiral PCB congeners. The enantiomeric separation was carried out by a 30 m × 0.25 mm id Chirasil-Dex (β-cyclodextrin chemically bonded to dimethylpolysiloxane) column with 0.25 μm film thickness (Chrompack, Raritan, NJ). Helium was used as carrier gas and argon–methane (95:5) as make-up gas. To reduce the column bleed, a 3 m × 0.25 mm id DB-XLB column was connected to the main column with a Press-Tight connector (Agilent, Palo Alto, USA). The injector and detector temperatures were 200 °C and 250 °C, respectively. A 1 μl extract was injected into the column in splitless mode and splitless time was 1 min. The column temperature was programmed from 80 °C (hold for 2 min) to 140 °C at 10 °C min^{-1}, then to 190 °C at 1 °C min^{-1} and held for 70 min.

Although there were no indications of any signal distortions for the enantiomeric isomers of the four PCBs in chromatograms when ECD was used, the enantiomeric ratios were confirmed by GC-MS. All samples were re-analysed on an Agilent 6890 GC coupled with a 5973 MS detector. The GC parameters were similar with those for GC-ECD analysis. The interface and source temperatures were 280 °C and 230 °C, respectively. The quadrupole mass spectrometer was operated in electron impact ionisation at 70 eV. Selected ion monitoring (SIM) mode was used and three different chromatographic windows were defined for each PCB homologue. Two characteristic ions of each PCB homologue (m/z = 324 and 326 for the pentachloro-subtituted congeners PCBs 84 and 95, m/z = 360 and 362 for the hexachloro-substituted congeners PCB 132 and 149) were monitored. Identification of PCBs was based on retention time and the ion intensity ratio of sample peaks within 10% of the mean values obtained for the corresponding standards. However, even in SIM mode, the MSD has a poorer sensitivity for PCB congeners than ECD.

The enantiomeric ratio (ER) was defined according to Vetter[2] as the ratio of peak area of the first to the second eluting atropisomer peak (E1/E2) regardless of whether its optical rotation is known or not. For the validation of the chiral analytical procedure, enatiomeric ratios of the four PCB congeners were determined on ECD by five successive injections of standard solution at a concentration of 100 pg μl^{-1} with the following averages $\pm$ standard deviations: 0.992 $\pm$ 0.007, 0.979 $\pm$ 0.011, 0.992 $\pm$ 0.009, and 0.994 $\pm$ 0.010 for PCB 95, 84, 149 and 132, respectively. On MSD, the ERs were 0.997 $\pm$ 0.005, 1.005 $\pm$ 0.010, 0.999 $\pm$ 0.002 and 1.012 $\pm$ 0.015, respectively. This good reproducibility (RSD $<$ 1.5%) allows the determination of enantiomeric ratio changes of even a few percent with sufficient significance. A complete enantioseparation of PCB 84 atropisomer on Chirasil-Dex column could not be achieved (R = 0.7). On the basis of blank values and instrument sensitivity, the nominal absolute detection limits were 1 pg for the PCB atropisomers on ECD and 5 pg on MSD respectively.

Results

The results of ERs of chiral PCBs (PCB 95, 132 and 149) in porpoises investigated in this study are given in Table 1. The concentration of PCB 84 was very low in all samples. Due to the low signal-to-noise ratio and poor resolution, reliable quantification for atropisomers of PCB 84 in the samples was not possible. Good agreement was obtained between ER values on different detectors for all samples, except for some samples in which the concentrations of chiral congeners were below the limit of determination on MSD. The average percent difference in ERs was 0.9% (n = 11). Using heart cut technology in the fractionation process on the HPLC column, interferences for the enantiomeric determination are greatly reduced and the measurement of enantiomeric ratios is more accurate. However, the heart cut technology implies that only a fraction of the corresponding peak on the HPLC chromatogram for each compound is collected, and thus the method sensitivity is reduced. Typical ECD chromatograms for the enantiomers of PCB 95, 132 and 149 are shown in Fig. 1.

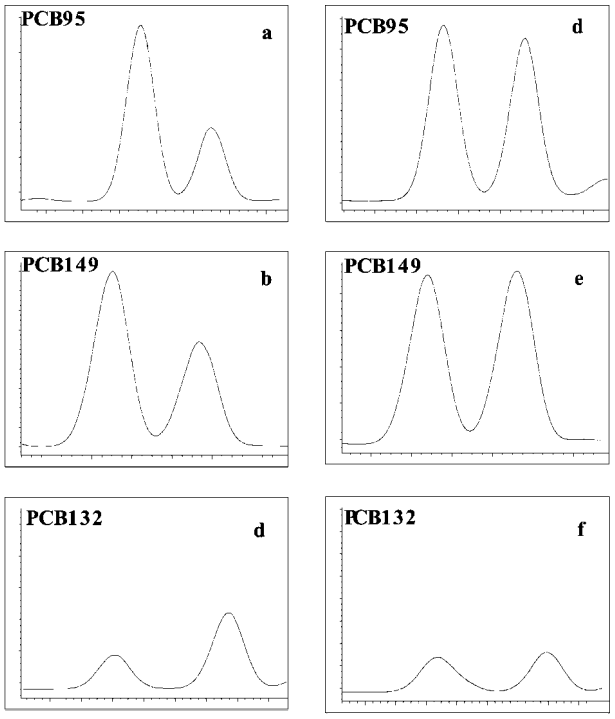

Fig. 1 Typical ECD chromatograms showing the separation of enantiomers of chiral PCB 95, 132 and 149 for adult porpoises (a, b, c) and for juvenile porpoises (d, e, f).

All samples of adult porpoises (sample nos. 5, 6, 7, 15, 17, 18 and 21) revealed non-racemic ERs with different enrichments for PCB 95, 132 and 149 (Table 1). Enrichments of the first eluted atropisomer of PCB 95 and PCB 149 were found, while the second eluted atropisomer of PCB 132 was enriched in all adult porpoises (Fig. 1a, b and c). The value of ERs in liver of adult porpoises ranged from 1.31 to 2.54 (average 2.03) for chiral PCB 95, from 1.28 to 1.81 (average 1.47) for PCB 149, and from 0.44 to 0.59 (average 0.52) for PCB 132. Since the elution order of enantiomers of PCB 132 on Chirasil-Dex was known[21] ((−) $<$ (+)), the (+)-enantiomer of PCB 132 is enriched in the liver of adult porpoises.

Contrary to the adult porpoises, racemic or nearly racemic ERs were found in three out of four juvenile animals (sample nos. 10, 12, 13). The ER values ranged from 0.99 to 1.12 for PCB 95; 0.96 to 1.19 for PCB 149 and 0.94 to 0.99 for PCB 132, respectively (Fig. 1d, e, f). The juvenile animal (sample no. 19) with a different behaviour had a weight of 27 kg that is closer to the weight of the adults (Table 1), rather than to the other three juvenile animals with body weights ranging from 7 to 16 kg. The enantiomeric ratios for all studied chiral PCBs in sample no. 19 were similar to those of adult animals (Table 1).

Discussion

Since the successful application of enantioselective separation of α-HCH from biota extracts,[22] more investigations have been done to study the environmental behaviour of chiral pollutants. Buser and Muller[4] have indicated that enantioselective degradation of α-HCH in sewage sludge was mainly biological and to a lesser degree chemical. Enantiomeric ratios of α-HCH in blubber of seals and cetacean species had only slight variation for animals from different regions.[23,24] Most of the studies on the enantioselective fate of α-HCH illustrated the utility of chiral analysis in understanding their processes in the environment. Unfortunately, the research field of chiral PCBs and their environmental behaviour was not as successful as for α-HCH. One of the problems in chiral PCBs investigation comes from interfering chromatographic peaks, especially from other PCB congeners, as well as from hydrophobic compounds commonly found in environmental samples. Even after fractionation by HPLC or multidimensional gas chromatography (MDGC), co-elution cannot be excluded and the interfering peaks may bring a large change in ER values.[3,25] Furthermore, complex clean up and analysis processes make a comprehensive survey of this field hardly possible by now. On the another hand, the enantioseparation may be carried out with different columns and the elution order has been determined for only a very few enantiomers. Considering that most of enantioselective values were given as ERs, reversed elution order on some chiral stationary phases confuses the comparison between different investigation results.

There are several investigations on the environmental behaviour and enantioenrichment of chiral PCBs. Racemic mixtures of chiral PCBs were observed in some sediment samples, suggesting little enantioselective biotransformation.[26,27] The most marked change in enantiomeric ratios of chiral PCBs was observed in biota. ERs ranging from 1.0 to 1.2 were found[28] for PCB 149, indicating a weak enantioenrichment in blue mussels (*Mytilus edulis L.*). In another investigation,[10] the enantiometric composition of PCB atropisomers measured in river and riparian biota from the Hudson and Housatonic River (fish, bivalves, crayfish, water snakes, barn swallows) was non-racemic for all measured PCBs. The enantioselective preference was reversed between fish and bivalves for PCB 95 (enantiomeric fraction EF $<$ 0.5 for fish, EF $>$ 0.5 for bivalves) and PCB 149 (EF $>$ 0.5 for fish; EF $<$ 0.5

for bivalves). No relations between the total PCBs concentration and EFs were apparent for any congener.[10] For marine fish, it was reported[5] that in Atlantic Ocean shark liver samples (*C. coelolepis*), ERs of PCB 95 and PCB 149 were racemic, while (+)-PCB 132 was enriched with ER (+/−) of 1.1–2.5.

Because they are at the top of the food chain, cetaceans are an important group of animals for marine pollution monitoring. In cetaceans from the Mediterranean Sea,[15] ERs ranged from 0.85 to 1.0, from 0.5 to 1.0, and from 0.7 to 1.05 for PCB 95, PCB 132 and PCB 149, respectively. Enantioenriched PCB 149 was found in the blubber of Icelandic harbour seals (*Phoca vitulina*) and grey seals (*Halichoerus grypus*),[29] while almost racemic composition (ER = 0.9 for PCB 149) was found in harbour seals from the North Sea and grey seals from the Baltic.[30]

All previously reported data show that a clear interpretation of ERs of atropisomeric PCBs is difficult at the moment. In most studies, only a weak enantioselective breakdown was found, but some samples (especially from marine mammals) presented exceptions. In this study, non-racemic mixtures of PCB 95, 132 and 149 were found in all adult porpoises, similarly with those already reported.[29,30] The differences in the enantiomeric ratios of individual chiral PCBs could not be explained by the theory about relationship between the congener molecular structure and the metabolism ability.[31]

North Sea harbour porpoises

All samples of adult porpoises studied revealed non-racemic ERs, while racemic or nearly racemic ERs were found in juvenile porpoises (3 out of 4 samples). While it was reported that the level of persistent organic pollutants in higher organisms increases with age,[32] in most investigations ERs tend to be independent of age and sex.[10] A change in ERs of chiral PCBs with the age is observed in this study, though the number of samples analysed and the age range are limited. This difference between our data and other studies may be due to the different chiral compounds or organisms studied, while differences in accumulation and metabolic capacities may be other possible reasons.

The ERs of individual chiral PCBs are different from each other in individual samples and are significantly correlated. In adult porpoises, ERs for PCB 95 and PCB 149 (ERs > 1) are different from the one for PCB 132 (ERs < 1). Whether this difference results from their different elution order or from reversed enantioselective enrichment in porpoises is unclear.

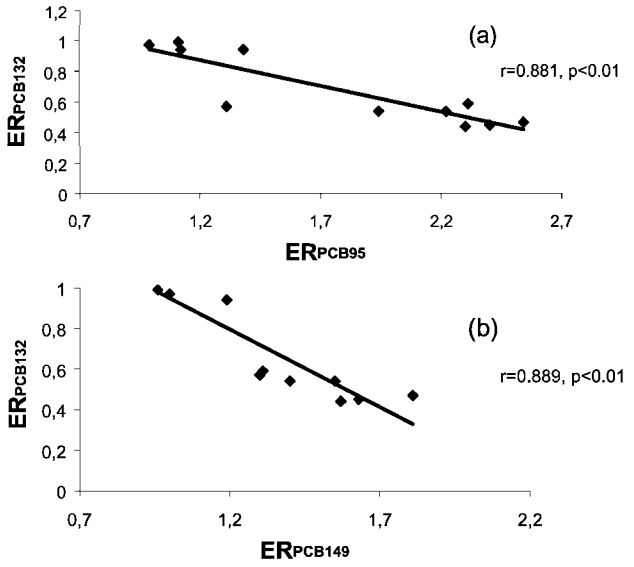

Fig. 2 Relationships between ER_{PCB132} and ER_{PCB95} (a), and ER_{PCB149} (b) in porpoise liver samples.

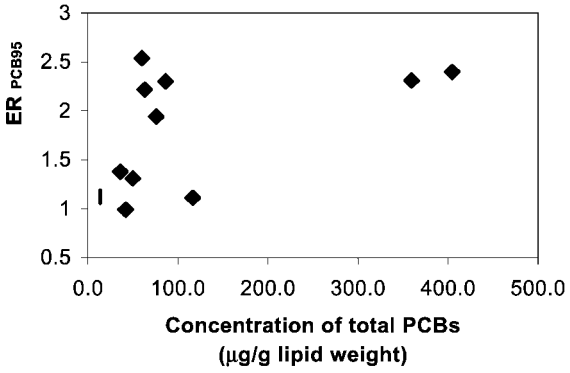

Fig. 3 Dependence of the concentration of total PCBs with ERs.

However, a good negative correlation (Fig. 2a and b) could be found between ER_{PCB132} and ER_{PCB95} ($r = 0.881$, $p < 0.01$), and between ER_{PCB132} and ER_{PCB149} ($r = 0.889$, $p < 0.01$). A pronounced enrichment of (+)-PCB 132 was observed in adult porpoises in this study, which is in agreement with other studies.[15] The same enrichment was also found in human milk samples,[33] meaning that (−)-PCB 132 tends to be more easy metabolised in bioprocesses than the (+)-PCB 132 atropisomer.

No significant correlation could be found between ER of chiral PCB 95 and the total concentration of 70 PCB congeners determined in the studied liver sample (Fig. 3). This result agrees with other investigations of chiral PCBs and α-HCH in environmental samples. For instance, the ERs of α-HCH in cetaceans ranged from 1 to 4, while the concentrations (sum of both enantiomers) in individual animals varied by 3 or 4 orders of magnitude. For aquatic and riparian biota, it was found that there were no trends in EFs with total PCB concentrations.[10] Moreover, they could not find a way to predict enantioselectivity in organisms nor to determine the extent to which specific species metabolise chiral PCBs enantioselectivity as compared to the extent to which they pass non-racemic residues with the same EF up the food chain.[10]

Metabolism and chirality

Being consistent fish consumers, porpoises may provide a more suitable model for a fish-based diet than do other mammals. Theoretically, in an environmental system, the predator accumulates pollutants from its prey and it can be concluded that the concentration of pollutants in the animal's body is a result of competing pseudo-first-order-kinetics processes, such as intake and elimination. If the pollutant is easily degraded or eliminated in the predator, the intake and elimination rates will become equal in a short period and the accumulation rate will be equal to zero.

Although this situation is often accepted by some researchers, it rarely happens in real environmental systems. For persistent organic pollutants (POPs), such as toxaphene, HCHs, and PCBs, the elimination and/or degradation rate is very slow. When the predator has a high daily consumption of contaminated food, the intake rate is much higher than the elimination/degradation rate. For example, porpoises consume approximately 3.5% of their body weight of fish per day.[34] If fish is highly contaminated by PCBs, it will be impossible for the animal to eliminate all ingested PCBs in the same period. In this situation, the PCB concentration will depend on time and it can be explained by the age dependency of total PCBs. Therefore, the concentration of individual PCB congeners and atropisomers in porpoises is best described by a kinetic model in which the congener pattern and ERs not only depend on concentration of PCBs in fish, but also on the exposure time.

If biological systems preferentially degrade one enantiomer, ERs will be shifted from the initial value and ERs will greatly

depended on exposure periods, but not on the total concentration of the pollutant alone. Thus, if initial ERs and other parameters are known, it is possible to estimate the residence times of these pollutants in the animal.

In reality, the contamination process is more complex than previously described and it is possible that the non-racemic amounts of PCBs observed in organisms were caused, at least in part, by previous bioprocesses whether in the sediment, water, or elsewhere in the food chain. In any case, the value of ER will mostly depend on exposure time or, in other words, it will depend on the length, but not on the level of contamination. Although a quantitative calculation is impossible in complex environmental systems, it supports a way to compare the ERs in different animal species. Upon this opinion the measurement of enantiomeric composition of chiral POPs in environmental samples is becoming more important in ecotoxicological investigations.

It should be noted that the above descriptions are based on few assumptions that cannot be checked directly. To test this hypothesis, it was necessary to find another parameter which depends also on exposure time. It is well known that the PCB congener patterns in animals are caused by the pollution source, distribution process, biotransformation and exposure period. Van Scheppingen *et al.*[35] have studied the congener pattern of PCBs in harbour porpoises stranded on the Dutch coast between 1990 and 1993. They have suggested that the amount of the most prominent and persistent congener, PCB 153, can be related to the feeding pattern of the male porpoises.[35] By the calculations of intake and metabolism and/or excretion, it was concluded that 65–90% of the PCB

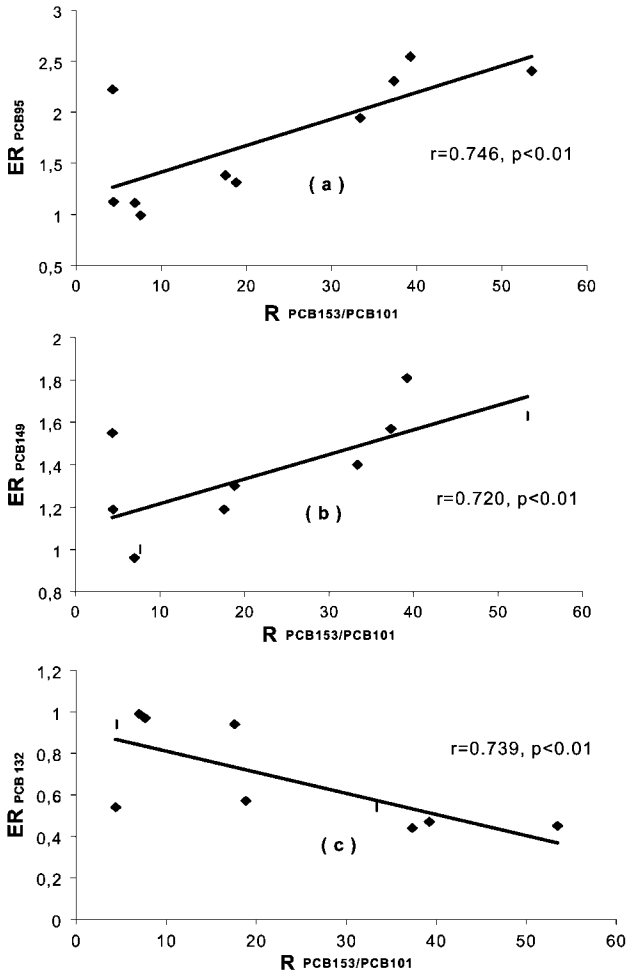

Fig. 4 Relationships between ER_{PCB95} and $R_{PCB153/PCB101}$ (a); ER_{PCB149} and $R_{PCB153/PCB101}$ (b); and ER_{PCB132} and $R_{PCB153/PCB101}$ (c).

contamination can be accounted for in the animal body, and PCB 153 is almost completely retained in the animal body.[35] Since PCB 153 constitutes about 20% of the total PCB concentration and can easily be quantified (no interference or co-elution in the analytical procedure), it can be used to evaluate trends in the PCB contamination of harbour porpoises. In contrast to PCB 153, the congener PCB 101 is relatively easily metabolised by porpoises, in contrast with fish such as cod.[36] Accordingly, the ratios of concentration of persistent congeners *versus* metabolizable congeners in porpoises will have a similar trend as ERs. The relationships between PCB 153/PCB 101 ($R_{PCB153/PCB101}$) and ER of PCB 95 (a) ($r = 0.746, p < 0.01$), PCB 149 (b) ($r = 0.720, p < 0.01$), and ER of PCB 132 (c) ($r = 0.739, p < 0.01$) are presented in Fig. 4. Data from sample No. 21 was not included, because $R_{PCB153/PCB101} = 146$, which is far outside the range of the other 10 samples (4.4 to 53). While a high ratio $R_{PCB153/PCB101}$ indicates that a high proportion of intake PCBs have been metabolized by porpoise, a low ratio should be associated with a low metabolic conversion of PCB congeners. Therefore, the non-racemic presence of these congeners is strong evidence for the contamination degree, but does not differentiate between the various elimination pathways. For enantiomers with similar volatility and partitioning properties, ERs are subject to biological removal mechanism processes (such as metabolism or selective passage through membranes), while physical/chemical properties do not affect them. Therefore, ER may be a complementary biomarker to the congener pattern for biological metabolism.

Enantioselective analysis may reveal important information in the environmental system. Our data have showed the utility of enantioselective analysis in understanding processes acting on chiral organic compounds in the environment. In conjunction with other tools, such as stable isotope analysis, *in vivo* laboratory experiments, enantioselective analyses may be a useful tool in assessing biological processes, such as health status and food quality associated with a wild population.

Acknowledgements

The authors wish to thank Dr. Krishna Das and Prof. Dr. Jean-Marie Bouquegneau (Department of Oceanography, University of Liège) for kindly supplying the harbour porpoise liver samples and to Dr. Thierry Jauniaux (University of Liège) for the necropsy of the animals.

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