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Can the ratio of selected congeners be used to assess the degradation status of polychlorinated biphenyls in biological matrices?

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1. Introduction

Commercial PCB mixtures are composed of a high number of congeners: it was reported[1] that only about 80 out of the 209 possible congeners are "absent" (present at a concentration lower than 0.05% w/w) from all of the most widely used mixtures; each commercial mixture contains about 50-80 congeners, out of which only about 25 are present at concentrations higher than 1% w/w.
The CB contamination of the environment can be considered as caused by a combination of the variuos commercial mixtures.
PCB contamination patterns of matrices from living organisms often show relevant differences with respect to industrial and environmental matrices: metabolic activity probably causes this phenomenon[2,3]. Some major components of the industrial mixtures are almost missing in the contamination profile of many samples from organisms at the top of the food-chain; their presence at unusually high levels generally indicates a possible laboratory contamination.[3,4]
In this paper, starting with milk, we will try to investigate whether the ratio of selected congeners, especially in biological matrices, can be used to assess the status of biodegradation of the PCB.

2. Materials and methods

Many of the data reported were recovered from previous studies; the experimental details are available from published reports[4-6]; here information will be briefly given only on experimental procedures used for the new data.
Mollusc and fish samples from various breedings located at different sites of the Venice lagoon were obtained from breeders; some samples were directly collected from polluted zones.
Analytical procedure: all the samples were spiked with a mixture of isotopically labeled standards, then homogenized in a laboratory mixer and lyophilized. Extraction and purification were performed by means of a Hewlett-Packard 7680T supercritical fluid extractor (SFE). The tests performed to assess the method efficiency and reliability will be described in a paper by La Rocca et al. still in preparation. The instrumental conditions were the following: $C0_2$ of 99.998% purity was used, with a density of 0.75 g/mL, at a pressure of 13300 kPa and a temperature 40°C, 2mL/min flow rate; the trap was packed with ODS and *n*-hexane was used as desorption solvent. Swift collection and analysis are described in a poster presented at this symposium.[7]
PCB determination was performed on a HP5989A GC-MS system equipped with on column injector and a HP Ultra 2 capillary gas cromatographic column (50-m-long 0.2 mm-i.d.). The 149/153 ratio was calculated as peak area ratio; peak integration was automatically performed and visually checked.

3. Results and discussion

Six congeners (99, 118, 153, 138, 180, 170) account for about 80% of the total PCB contamination of cow milk, which is mainly characterized by a dozen congeners.

On 36 samples of milk and dairy products analyzed[4], we found that the mean value of the sum of these 6 congeners accounted for 0.79 of total PCB with a standard deviation of 0.037. In a paper reviewing PCB congeners in human foodstuff[5], Jones reported slightly different figures for slightly different choices of congeners and number of congeners determined.
Such a constancy of composition suggests that the PCB contamination pattern of milk is under metabolic control, as is observed in milk from different areas and with strongly different contamination levels, which is to say in milk from animals with different exposures, both qualitative and quantitative. It also suggests that the congeners 99, 118, 153, 138, 180, 170 are, among the major components of the environmental mixtures presumably present in food, the more resistant to cow metabolism.
Many PCB congeners, such as 95, 101, 110, 149, 174 and others are comparatively very low in milk, and in human tissues[8], with respect to industrial and environmental mixtures; they are probably to some extent metabolized .
It was suggested by Zell and Ballschmiter[3]that metabolic alteration of the PCB profile performed by living organisms increased going from phytoplankton feeding fishes to predatory fishes, increasing even more in warm blooded species (birds and mammals); however, it is very difficult to get a simple quantitative indicator of the alteration of a complex pattern.
Pursuing this purpose, we choosed a pair of abundant congeners of industrial PCB mixtures, one degradable (149) and one resistant (153)[8], and checked whether their ratio in samples from different environmental and biological matrices could give information on the degradation status of the PCB in the matrix.

Table 1. Values of the ratio 149/153 measured in matrices from animals at different levels in the food chain, in some sediment and whole diet samples.

Mollusc(left) and(right) ** sediment from same area	*Mytilus gallo-provincialis*, A **0.34**	Sediment, A **0.4-0.5**	*Mytilus gallo-provincialis*, B **0.36**	Sediment, B **0.51**
Mollusc(left) and(right) ** sediment from same area	*Mytilus gallo-provicialis*,C **0.41**	Sediment, C **0.65**	*Tapes semidecussatus* **0.53**	Sediment **0.74**
fish **	*Liza ramada* **0.41**	*Sparus aurata* **0.16**		
From reference 10: seal (left) and its food (right)	*Phoca vitulina*, Wadden Sea **0.12***	Wadden sea Fish mainly plaice **0.24***	*Phoca vitulina*, Atlantic **0.10***	Atlantic fish mainly mackerel **0.38***
From reference 9: sole (sides), its food(center)	*Solea solea*, before treatment **0.15***	contaminated food **0.31***	*Solea solea*, after treatment **0.22***	
Swift(*Apus apus)* tissues	heart **0.053**	muscles **0.028**	liver **0.024**	
English birds, from ref.12	*Buteo buteo* **0.04***	*Falco peregrinus* **0.06***	*Tyto alba* **0.05**	*Milvus milvus* **0.62***
English birds, from ref.12	*Ardea cinerea* **0.13***	*Uria aalge* **0.19***	*Alca torda* **0.23***	*Sula bassana* **0.29***
Milk, cheese and human fluids.	mean from 36 samples±SD **0.037±0.012**	Human milk **0.013**	Human blood (from ref. 13) **0.015**	
Various: sediments (left columns) diets (right column) 149/153, range	background, low pollution **0.4-0.56**	industrial, high pollution **0.61-0.75**		Whole diets **0.31-0.47**

* *Value calculated from a bar-graph by the authors of the present article.*
** *Samples from Venice Lagoon.*

The reference value of the ratio of the two congeners in the polluting mixtures, in the moment they are released into the environment, was then estimated: the ratio 149/153 was measured[4] in 55 samples of electric capacitor oil. These samples may be considered to be representative of what, from electric apparatuses, is released into the environment. The mean value of the ratio was 0.7. A series of seven Aroclor standard mixtures was also analyzed. The mean ratio 149/153 was 0.76. Schulz et al.[1] have also reported the composition of eight commercial PCB mixtures with different chlorine content; the mean value was 0.7.
Based on these results, a reference value slightly higher than 0.7 was assumed for the 149/153 ratio of the mixtures entering the environment.
The 149/153 congener ratio was then carefully checked in several environmental and biological matrices being analyzed or previously analyzed, and listed, together with some literature data, in Table1.
It is visible at a glance that the values reported in Table 1 are generally in agreement with the observation of Zell and Ballschmiter[3]: molluscs and plankton eating fishes display a higher value than predator fishes, which in turn display higher values than warm blooded species; additionally, almost all of the values reported are lower than the reference value.
It is important to stress that some of the values listed were taken from the literature, and were obtained with different techniques; it is reasonable that results are in some cases biased. This may be particularly true for results obtained with ECD. It is however evident that each set of data from each laboratory is —with one exception— internally coherent on the general trend and that quantitative discrepancies between different laboratories may be due to different analytical techniques.
It may be worth noticing in Table 1 that, in the data from the Venice lagoon, the 149/153 ratio of molluscs seems to be influenced by the 149/153 ratio of the sediment of the same area: the higher the value of the ratio in the sediments, the higher the value in the molluscs. The thin lipped grey mullet (*Liza ramada*), omnivorous fish feeding on fine mud, has an higher ratio than the predator sea bream (*Sparus aurata*).
The same indication seems to emerge from the data reported by Boon[9-11] et al. in papers in which PCB contamination profiles of different marine animals are reported. It is interesting to highlight the results of the experiments in which both the animal subject of the study and its food were analyzed. In the first case, sole were fed with PCB contaminated food, and the value of the 149/153 ratio after treatment was lower than in the food but higher than before the experiment. In the second case, two groups of seals were fed respectively with fish (mainly plaice) from the contaminated Wadden sea, and with fish (mainly mackerel) from the Atlantic ocean, and the two groups displayed greatly different PCB contamination levels, but essentially the same ratio 149/153, even if their food had different 149/153 ratios. These data seem to indicate that mammals may keep PCB contamination profile under metabolic control, while other organisms display a limited, but observable, ability to degrade PCB.
The values reported by Boumphrey et al.[12] on British birds show that all but one of the animals considered had low value of the ratio 149/153; birds of prey (with one exception) had lower ratios than seabirds.
The values of the 149/153 ratio measured in various swift tissues are also interesting: in all cases they are very low (0.03-0.06) and close to those measured in cow's milk. The PCB contamination patterns of these insectivorous birds recall those of high predators[7].
The mean 149/153 ratio measured on 36 different milk and dairy products samples was 0.032, with a standard deviation of 0.012.
The lowest values of the ratio were measured in human milk and blood samples: 0.01[4,13].
In a previous paper[4], the 149/153 ratio of marine sediments was shown to be lower in low contaminated samples, which supposedly reflect a time averaged background pollution and in which a partial degradation may have occurred, and higher in highly contaminated sediments, reflecting more recent inputs from industrial activities (Table1).
Finally, mean diet samples from various Italian areas[4], show ratios generally between 0.31 and 0.47. The presence in such samples of food of animal origin together with other items directly reflecting environmental pollution is compatible with this intermediate value of the ratio (Table1).
An implication of the proposed meaning of the ratio 149/153 is its use as an indicator of laboratory contamination. In a previous paper[4], evidence was presented that the ratio 149/153 could be used

as an indicator of laboratory contamination of some kinds of samples, and some examples were given of cases of laboratory contamination, not effecting the procedure blank, detected with the aid of the 149/153 ratio.
Extensions of the present study are the analysis of a wider variety of matrices from different ecosystems and the evaluation, as possible indicators, of other pairs of congeners such as 101/118 and 174/180. A deeper search of the literature could also add information, altough there are some difficulties, such as the difference of analytical methods used and the fact that data on such congeners as 149 are not often reported.

4.References

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