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Note

Quantitation of total *versus* selected polychlorinated biphenyl congeners in marine biota samples

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Environmental analyses of polychlorinated biphenyls (PCBs) involve two alternative approaches: (a) the use of extensive extraction and cleanup procedures, such as adsorption column chromatography or gel permeation chromatography in order to determine PCBs selectively from interfering compounds by high-resolution gas chromatography with electron-capture detection (HRGC–ECD)[1] and (b) the use of specific mass spectrometric (MS) detection methods, such as electron impact (EI)[2–5] or chemical ionization (CI)[5–9] to minimize the matrix effects. Concerning MS ionization modes, it has been alleged that negative ion chemical ionization (NICI) has between a 6 and 176 times higher sensitivity than EI for the different PCB congeners[5,8]. Nevertheless, this ionization mode has the disadvantage that chlorinated compounds should have at least 3–4 chlorine atoms to be easily identified[7]. As regards to the selectivity, NICI has proved to be slightly influenced by matrix effects in extracts containing compounds with high electron affinity[5,7,9].

On the other hand, the quantitation of PCBs has been accomplished either by employing a standard PCB mixture such as Aroclor[10,11] or by selected individual congeners[12,13], providing different sets of data that require some guide for comparison.

In this paper we describe a combined approach for determining PCBs in marine biota samples using simple extraction and cleanup procedures and HRGC–ECD for quantitation. In addition, NICI–MS is used as a confirmatory detection technique to facilitate the qualitative analysis of PCBs. Results on 38 samples of mussel (*Mytilus galloprovincialis*) and red mullet (*Mullus barbatus*) were obtained for total PCBs (as Aroclor 1254) and individual congeners and compared in terms of PCB profiles and individual isomeric distributions.

EXPERIMENTAL

Materials

All solvents (*n*-hexane, dichloromethane and diethyl ether) were glass distilled before use. Alumina and silica gel were supplied by Merck (Darmstadt, F.R.G.). Analytical-reagent grade individual PCB components (Promochem, Wesel, F.R.G.) were used with the following IUPAC numbers: 28 = 2,4,4′-trichlorobiphenyl;

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52 = 2,2',5,5'-tetrachlorobiphenyl; 101 = 2,2',4,5,5'-pentachlorobiphenyl; 118 = 2,3',4,4', 5-pentachlorobiphenyl; 138 = 2,2',3,4,4',5'-hexachlorobiphenyl; 153 = 2,2',4,4',5,5'-hexachlorobiphenyl; and 180 = 2,2',3,4,4',5,5'-heptachlorobiphenyl.

Sample work-up

Sample pre-treatment was carried out using slightly modified procedures derived from that recommended by the Association of Official Analytical Chemists[14].

Method 1. Light petroleum was replaced by *n*-hexane for extraction, as suggested by Sawyer[15], and the solvent extraction period was longer than 3–4 h in order to ensure recoveries of up to 90%[16]. A homogenized mixture of fish tissue (1–2 g) was mixed with 2–5 g of anhydrous sodium sulphate and extracted for 12 h with *n*-hexane in a Soxhlet apparatus. The *n*-hexane extract (10 ml) was cleaned up by vigorous shaking with 1 ml of sulphuric acid for about 3 min. As a result a good phase separation was obtained and the upper organic layer was removed with a pipette. Further cleanup involved the use of a short alumina column (1 g) previously deactivated with 5% of water. Subsequently, the solution was evaporated just to dryness and the residue was dissolved in 0.5 ml of isooctane.

Method 2. This method is based on that previously reported[10] for analysing hydrocarbons and organochlorine compounds: 1–2 g of whole fish was saponified with 15 ml of 6 *M* sodium hydroxide solution for 18 h at 30°C followed by triple extraction with 15, 10 and 10 ml of diethyl ether. The combined extracts were evaporated in a rotary evaporator nearly to dryness and 1 ml of *n*-hexane was added. The clean-up procedure involved the use of a silica–alumina column (8 g + 8 g) (35 cm × 9 mm I.D.), previously deactivated with 5% of water. In the second fraction, using *n*-hexane–dichloromethane (90:10), the PCBs were eluted[17].

The recoveries with methods 1 and 2 were 98% and 97%, respectively, referred to Aroclor 1254.

Chromatographic analysis

Samples of 1 μl were injected in the splitless mode (split opened after 35 s) in a Fractovap 4130 capillary gas chromatograph (Carlo Erba, Milan, Italy) equipped with a ^{63}Ni electron-capture detector at 310°C and a 30 m × 0.25 mm I.D. capillary column coated with 0.25 μm of chemically immobilized SPB-5 (Supelco, Bellefonte, PA, U.S.A.). The column was programmed from 80 to 290°C at 6°C/min keeping the final temperature during 15 min. The carrier gas was hydrogen at a linear flow-rate of 50 cm/s. High-purity nitrogen was used as the make-up gas with a flow-rate of 30 ml/min. The injector temperature was kept at 280°C. A Model 5888A quadrupole gas chromatograph–mass spectrometer connected on-line with a Model 36741B data station (Hewlett-Packard, Palo Alto, CA, U.S.A.) was employed. Methane was used as the reagent gas at 1.5 Torr. The transfer line, ion source and analyser temperatures were kept at 280, 150 and 130°C, respectively.

RESULTS AND DISCUSSION

Characterization of PCBs in biota extracts by HRGC–ECD and HRGC–NICI-MS

A general feature of the HRGC–ECD analysis of environmental PCBs is the complexity of the GC traces, related to the complexity of all commercial PCB for-

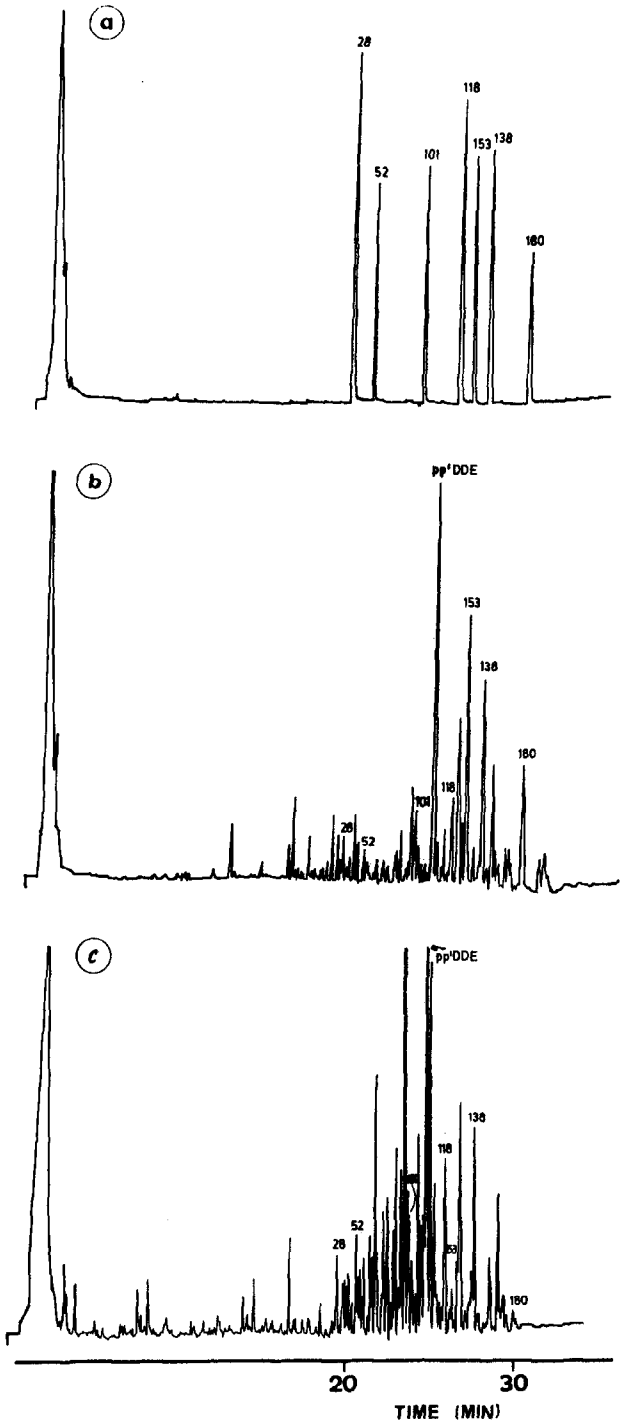

Fig. 1. HRGC–ECD traces of (a) external standard solution of the seven PCB congeners, concentration range from 0.42 ng/μl (28) to 0.15 ng/μl (180); (b) extract of *Gambussia affinis* (method 1); and (c) extract of *Mytilus galloprovincialis* (method 2).

mulations[18,19]. However, the GC profiles of extracts from diverse environmental matrices are usually different from those of commercial PCB mixtures, owing to the various environmental fates of the individual components. As an example, the HRGC–ECD traces of organic extracts from two aquatic organisms collected in the Ebro Delta (Spain) are shown in Fig. 1. These are *Gambussia affinis* (Fig. 1b), a valuable exterminator of mosquito larvae, and the mussel *Mytilus galloprovincialis* (Fig. 1c), processed according to methods 1 and 2, respectively.

In addition to the major component that was identified as *p,p'*-DDE, it is difficult in such profiles to identify conclusively all PCB components by simply matching the peaks with a commercial mixture. It should also be borne in mind that some PCB isomers coelute[20], thus making the identification even more uncertain. These problems will cause further difficulties in the accurate quantification of the samples. Therefore, the use of an alternative or complementary analytical technique is advisable.

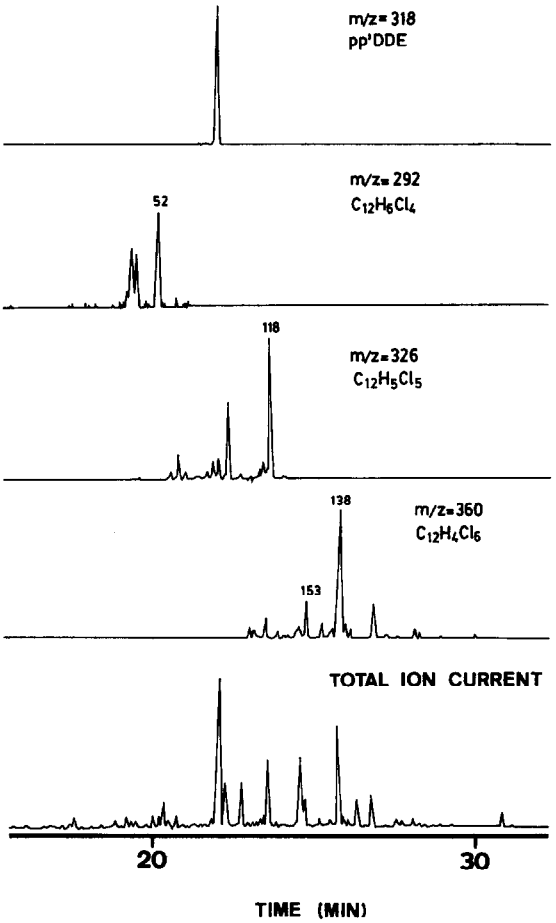

Fig. 2. HRGC–NICI-MS trace of the same mussel extract as in Fig. 1c. The ions monitored correspond to *p,p'*-DDE (m/z = 318) and the PCB isomeric groups $C_{12}H_6Cl_4$ (m/z = 292), $C_{12}H_5Cl_5$ (m/z = 326) and $C_{12}H_4Cl_6$ (m/z = 360).

Negative ion chemical ionization MS has shown high selectivity and sensitivity for molecules having low-lying vacant orbitals or virtual vacant orbitals such as chlorinated insecticides[7,21,22], polychlorinated biphenyls[1,7] and organophosphorus insecticides[23]. Ion-forming reactions in NICI-MS involve mechanisms of resonance capture of a thermalized electron, dissociative capture of a low-energy electron and ion–molecule reactions between ions and neutral species[24].

The analyses of the present biota extracts for PCBs and DDTs showed that the predominant mechanism of ion formation was electron capture. An example of this residue characterization is illustrated in Fig. 2 for a certain number of mass scans of the total ion current chromatogram, corresponding to the mussel extract shown in Fig. 1c. The ions monitored correspond to *p,p'*-DDE (m/z = 318) and the PCB isomeric groups $C_{12}H_6Cl_4$ (m/z = 292), $C_{12}H_5Cl_5$ (m/z = 326) and $C_{12}H_4Cl_6$ (m/z = 360).

With this technique it was easier to assign the different PCB congeners in the extracts, mainly owing to the high sensitivity and to the fact that the total ion current chromatogram is very little influenced by the sample matrix. It was also particularly useful for the confirmation of the 153/138 isomeric ratio (see Fig. 1c), which has been suggested as an indicator of the extent of metabolization in the PCB mixture[25] because the 153 congener does not contain adjacent unsustituted carbon atoms susceptible of metabolic breakdown[26]. Further, the different patterns of the PCB congeners, obtained by comparing the relative amounts of compounds 101, 153, 138 and 180, comes close to a 1:1 mixture of Aroclor 1254 and 1260. The occurrence of congener 28 can be taken as a leftover of an Aroclor 1242 PCB pollution[25]. Hence, the analyses of individual PCB congeners is advantageous because the profiles obtained for biota samples allow a prediction of the metabolism and fate of such pollutants in the environment.

Quantitative analysis of PCBs

The use of individual congeners for quantifying PCBs in environmental samples instead of commercial PCB mixtures has recently received increasing acceptance. Several EEC countries such as The Netherlands, U.K. and F.R.G. have defined maximum residue levels of PCBs in edible products based on specific congeners. The choice of these congeners (IUPAC numbers 28, 52, 101, 118, 138, 153 and 180) is

TABLE I

MEAN CONCENTRATION (n = 5) AND COEFFICIENT OF VARIATION (C.V.) OF INDIVIDUAL PCB CONGENERS IN FISH OIL USING AN EXTERNAL STANDARD MIXTURE

PCB congener	*x (ng/g)*	*C.V. (%)*
28	22	1
52	54	6
101	90	8
118	103	14
138	141	21
153	128	17
180	49	7

TABLE II

POLYCHLORINATED BIPHENYLS IN SAMPLES OF *MULLUS BARBATUS* AND *MYTILUS GALLOPROVINCIALIS* COLLECTED IN DIFFERENT LOCATIONS OF THE MEDITERRANEAN SEA

Concentrations expressed in ng/g fresh weight.

Sample	*PCB congener*							ΣPCB_{cong}	ΣPCB_{1254}
	28	*52*	*101*	*118*	*153*	*138*	*180*		
Mullus barbatus	0.1	0.2	0.3	0.7	1.7	2.0	0.7	5.7	19.0
	0.3	0.7	1.2	5.3	8.0	19.4	8.9	43.8	142.0
	–	–	0.5	2.6	4.6	10.6	5.4	23.7	84.1
	0.3	0.5	0.7	2.9	5.2	8.5	3.0	21.1	60.7
	8.7	17.3	37.6	60.6	96.6	145.9	44.3	411.0	1321.0
	0.2	0.3	0.3	1.3	1.0	3.5	0.4	7.0	22
	0.1	0.1	0.3	0.5	2.0	1.5	1.1	5.6	21.1
	0.1	0.2	0.5	4.1	6.6	13.0	4.5	29.0	101.4
	0.1	0.2	0.1	1.0	0.8	3.6	0.4	6.2	23.7
	0.3	0.4	1.6	5.5	8.0	11.0	5.2	32.0	130.0
	0.1	0.2	0.5	2.3	1.7	5.0	0.9	10.7	49.0
	0.1	0.2	0.2	1.8	1.2	5.5	0.7	9.7	34.3
	0.5	2.0	1.4	4.4	7.0	10.6	3.6	29.5	89.0
	2.2	9.4	14.8	14.4	15.2	26.7	8.2	90.9	272.0
	1.5	2.2	4.8	16.0	26.0	46.3	24.0	120.8	336.0
	0.8	1.7	2.1	4.4	8.4	10.5	5.0	32.9	95.0
	–	–	0.1	1.2	1.4	3.7	1.1	7.5	23.0
	0.4	0.3	0.5	1.7	5.3	4.8	3.1	16.1	48.7
Mytilus gallo-provincialis	1.1	1.4	1.9	2.4	2.4	3.8	1.0	14.0	37.1
	1.1	1.9	4.2	4.0	8.1	8.7	1.1	29.1	95.3
	0.4	0.3	0.6	0.5	1.0	1.2	0.1	4.1	30.0
	–	–	0.1	0.1	0.2	0.2	0.3	0.9	2.2
	0.1	0.2	0.5	0.6	1.2	1.6	0.1	4.3	18.5
	–	–	0.2	0.3	0.5	0.7	0.1	1.8	7.2
	0.2	0.3	1.0	1.5	2.5	3.0	0.8	9.3	29.3
	–	–	0.1	0.2	0.3	0.7	0.1	1.4	6.5
	0.1	0.2	0.7	1.1	1.6	2.3	0.3	6.3	24.4
	3.0	3.1	6.9	7.2	10.4	11.5	3.5	45.6	173.0
	0.1	–	0.4	1.0	2.6	3.1	2.1	9.2	40.1
	6.9	11.7	18.2	13.4	14.2	22.0	3.2	89.6	289.0
	3.8	16.0	15.0	19.1	15.7	33.1	4.6	97.2	287.3
	0.2	0.6	0.6	0.9	1.2	2.4	0.1	6.1	18.3
	0.3	0.2	0.7	1.0	1.3	1.9	0.2	5.6	22.6
	–	0.1	1.1	1.8	4.2	4.2	0.9	12.3	35.2
	0.3	2.6	0.6	1.2	0.9	1.9	0.1	7.6	25.0
	0.1	–	0.2	0.2	0.5	0.6	0.1	1.8	5.5
	–	0.1	0.3	0.7	0.8	1.6	0.1	3.6	8.5
	0.2	0.3	0.7	1.0	1.4	2.0	0.1	5.7	17.1

based on their separation by HRGC, their presence in technical mixtures and environmental samples and the value of the derived tolerance[2,7].

The Community Bureau of Reference is organizing intercalibration exercises within EEC countries for testing the accuracy of the methodologies and improving the reliability of the results[1,2]. In a recent study we obtained the results summarized

in Table I. The sample was a fish oil that was analysed according to method 1 described above and using an external standard solution of the indicated congeners. The coefficients of variation are of the same order of magnitude as those reported using 1,2,3,4-tetrachloronaphthalene (TCN) and dichlorobenzyl tetradecyl ether (DCBE-C14) as internal standards[12]. Nevertheless, when external quantitation is employed, several injections of the external standard solution are required in order to keep the instrument under linear calibration conditions and to select the injection standard closer to the sample to be analysed in order to minimize errors. The external standard solution of the seven PCB congeners was prepared from solutions ranging from 0.42 ng/μl (28) to 0.15 ng/μl (180), with the aim of obtaining a similar response as the number of chlorine atoms increased from three to seven.

The introduction of this quantitation procedure in long-term monitoring programmes in which the classical Aroclor standard mixture has been used raises the problem of the intercomparison of results within these series of data. Therefore, we proceeded to the concurrent analysis of a set of samples from two indicator organisms used in the Mediterranean Pollution Monitoring programme. namely the bivalve *Mytilus galloprovincialis* (mussel) and the fish *Mullus barbatus* (red mullet). These samples were analysed according to method 2 described above and the results are summarized in Table II.

PCBs are reported as the individual congeners Nos. 28, 52, 101, 118, 153, 138 and 180, and as ΣPCB_{cong}, which correspond to the sum of concentrations of the seven congeners. In addition, ΣPCB_{1254}, which represents the total PCBs expressed as Aroclor 1254, is also given. This provides a large range of data, from 2.2 to 1321 ng/g fresh weight and from 0.9 to 411 ng/g fresh weight of ΣPCB_{1254} and ΣPCB_{cong}, respectively, permitting the assessment of both series of values. The correlation was strikingly good (Fig. 3), with a correlation coefficient of 0.9984 and a mean ratio among values of 3.17, thus providing a satisfactory means of comparing sets of data generated with the two quantitation procedures.

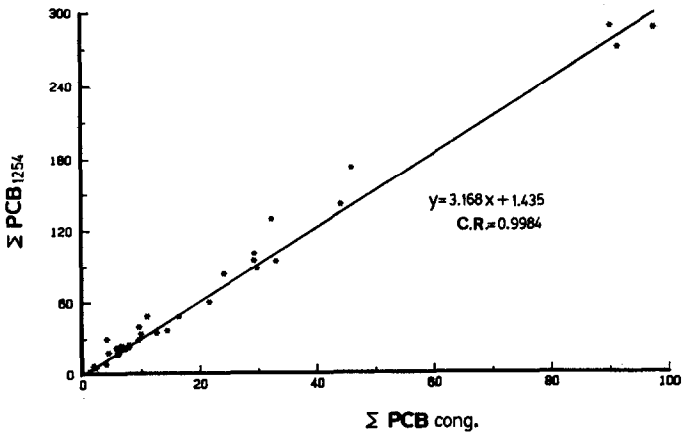

Fig. 3. Relationship between ΣPCB_{cong} and ΣPCB_{1254}, expressed in ng/g fresh weight, for different samples of the bivalve *Mytilus galloprovincialis* (mussel) and the fish *Mullus barbatus* (red mullet).

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