Supercritical Fluid Extraction-HRGC-HRMS Determination of PCB in Lyophilized Samples of Antarctic Krill

Luigi Turrio-Baldassarri, Susana Bayarri, Alessandro di Domenico, Annarita Fulgenzi, Cinzia La Rocca, Nicola Iacovella

Istituto Superiore di Sanità, Laboratorio di Tossicologia Comparata ed Ecotossicologia, Viale Regina Elena 299, 00161 Roma, Italy

Introduction

In the course of a pilot study aimed at the preparation of a reference material based on antarctic krill for metal analysis (1), the possibility was also considered of performing on the same matrix organic micropollutant analysis, and in particular to determine polychlorobiphenyls (PCB).
The term krill indicates small crustaceans living in Antarctic waters in huge schools.
The availability of materials from low contamination areas it is of utmost importance for environmental research; for PCB determination of such samples it is necessary to use techniques with high sensitivity and implying very low contamination of the procedural blanks. Supercritical fluid extraction (SFE) was succesfully employed in PCB analysis (2-3) and proved to be scarcely affected by blank contamination (4).

Materials and Methods

The provenience of the sample and its preparation are described in detail in ref. 1; briefly, the crustaceans making up the sample were catched by three different scientific expeditions to Antarctica between 1993 and 1995. They were kept frozen and susequently lyophilized, homogenized and bottled by the Institute for Reference Materials and Measurements of the European Commission in Geel, Belgium. The samples came in 10 mL amber bottles with PTFE lined stoppers, each containing about 1 g of lyophilized sample. They were gently provided by dr. S. Caroli of our Institute.
The SFE extraction was performed by means of a Hewlett-Packard model 7680 T supercritical fluid extractor, with the following experimental conditions:
extraction fluid CO_2 99.9999 wt % purity (from Sapio); density: 0.75 g/ml; pressure 134 bar; flow rate 2 ml/min; chamber temperature 40 °C; extraction time 10 min; extract trap conditions: nozzle temperature 45 °C; trap temperature 20 °C; trap packing ODS; fraction output: 1 rinse step with 1.5 ml *n-hexane*, at a rate of 1 ml/min; nozzle temperature 45 °C; trap temperature 40 °C.
The whole content of a bottle was spiked with a solution containing 13 fully ^{13}C labelled PCB congeners (from Cambridge Isotope Laboratories-CIL, Andover, Massachussets, USA), then left to equilibrate and to allow solvent to evaporate. Finally, it was mixed with aluminum oxide (1/2, w/w), previously washed by means of an accelerated solvent extractor (ASE 200, Dionex) with dichloromethane at 100 °C, and used as such, and packed into a 7-ml extraction cell. On the top (extraction fluid

direction is upward), 4 grams of alumina were added for lipid retention. An extraction of an empty cell was performed before and after each sample.
GC-MS determination was performed on the extract with no further treatment but volume reduction to 50 µL with a solution of injection standard (^{13}C labeled chlordane). A high resolution magnetic instrument was used (VG Autospec) operating in single ion recording (SIR) at 5000 resolution, equipped with a Fisons (Carlo Erba) GC 8000 series, a cold on-column (Carlo Erba) injector and a HP Ultra 2 capillary gas chromatographic column (50 m - long, 0.32 mm - i.d., 0.17µm film thickness).
Analysis was performed on three different bottles together with a procedural blank.

Results and Discussion
The recovery yields of the isotopically labeled ranged from a minimum 67% to a maximum 107% for all 13labeled internal standards.
In Table 1 for each congener is listed the result obtained on triplicate analises after blank subtraction. Also listed is the ratio between the peak area of the congener in the sample and in the blank.

Table 1. PCB congener concentration (pg/g lyophilized sample) in krill. S-B = sample-blank; S/B = ratio sample to blank; CV = coefficient of variation on triplicate analysis.

CONGENER	S-B	S/B	CV	*CONGENER*	S-B	S/B	CV
T_3CB 18	217	8.4	16%	P_5CB 105	35	3.6	31.9%
T_3CB 28	502	32.1	11.0%	H_6CB 155	6	/	39.0%
T_4CB 52	317	10.8	2.2%	H_6CB 136	55	6.3	23.0%
T_4CB 49	157	16.1	14.3%	H_6CB 151	123	6.0	26.2%
T_4CB 47	1229	162.9	4.0%	H_6CB 135	68	5.2	33.4%
T_4CB 44	148	14.0	22.9%	H_6CB 149	193	4.6	13.7%
T_4CB 41+64	565	55.2	2.0%	H_6CB 146	51	5.9	8.1%
T_4CB 74	105	19.5	18.0%	H_6CB 153	332	6.5	19.7%
T_4CB 70	191	12.8	16.1%	H_6CB 141	63	4.1	21.0%
T_4CB 80	183	17.1	4.7%	H_6CB 137	14	/	32.0%
T_4CB 60+56	109	15.4	9.8%	H_6CB 138+163	258	3.6	19.2%
P_5CB 95	235	7.9	4.8%	H_6CB 128	28	4.0	25.0%
P_5CB 91	36	/	28.0%	H_7CB 187	58	3.5	14.0%
P_5CB 101	385	8.8	1.1%	H_7CB 183	27	2.9	23.0%
P_5CB 99	154	10.0	28.0%	H_7CB 174	29	2.4	54.3%
P_5CB 97	55	6.3	38.0%	H_7CB 177	27	3.3	25.2%
P_5CB 87	107	7.6	20.2%	H_7CB 171	10	2.2	36.4%
P_5CB 85	42	/	21.4%	H_7CB 180	60	2.4	14.4%
P_5CB 110	139	4.8	6.2%	H_7CB 170	20	2.2	25.3%
P_5CB 118	155	5.3	17.5%	*PCB, sum*	*6513*	*8*	*8%*

The limit of determination (LOD) is 6 pg/g of freeze-dried sample, with a signal to noise ratio equal to 5. The slash in the S/B column indicates that the blank is lower than the LOD.
It is clearly visible that the profile is richer of light congeners (3 or 4 chlorine atoms) and that the ratio sample-to-blank decreases steadily going from the tri-tetrachloro congeners to the heptacholoro congeners. For the heptachloro congeners the ratio is so low that the determination has limited significance. This is also reflected by the high coefficients of variation.
The blank run was performed filling the extraction cell of the SFE apparatus with alumina previously spiked with the labeled internal standard solution in the same conditions as for the samples. When referred to a 1 g size sample, the blank yields a total PCB load of about 800 pg. This amount is somewhat higher than what we experienced previously (4). It is however low enough to allow determination of most of the relevant congeners in a low-contaminated sample.
An impressive feature of these data is the relevance of T_4CB 47 (Figure 1), which is the prevailing congener and accounts for 19% of the total PCB content. The prevalence of this congener is quite unusual even in samples, like the present one, in which the lower chlorinated molecules predominate. This congener is reported (5) to be a minor component, about 1% or lower, even in the light industrial mixtures such as Clophen A30 or Aroclor 1016 and 1242.
We have no explanation for this. It is worth reminding that the determination was performed at 5000 resolution, and that the isotopic ratio and the cromatographic retentiont time were both correct.
The ratio sample-to-blank, the very little manipulation performed in the laboratory and the repetition of the same result on a second set of four samples and two blanks (analysis performed with low resolution MS) seem to exclude the possibility of a laboratory contamination.
All the same it is very difficult to presume a contamination occurred during sampling, storage or preparation because one should think of a source of a single congener.
We had a previous experience with this same congener on one milk sample, and also in that case the laboratory contamination could be ruled out: the blank was good and, at that time, we did not have in the laboratory the standard of the pure compound.
An interesting feature of Figure 1 is that the prevalence of the light congeners suggests that krill contamination occurs mainly thruough air.

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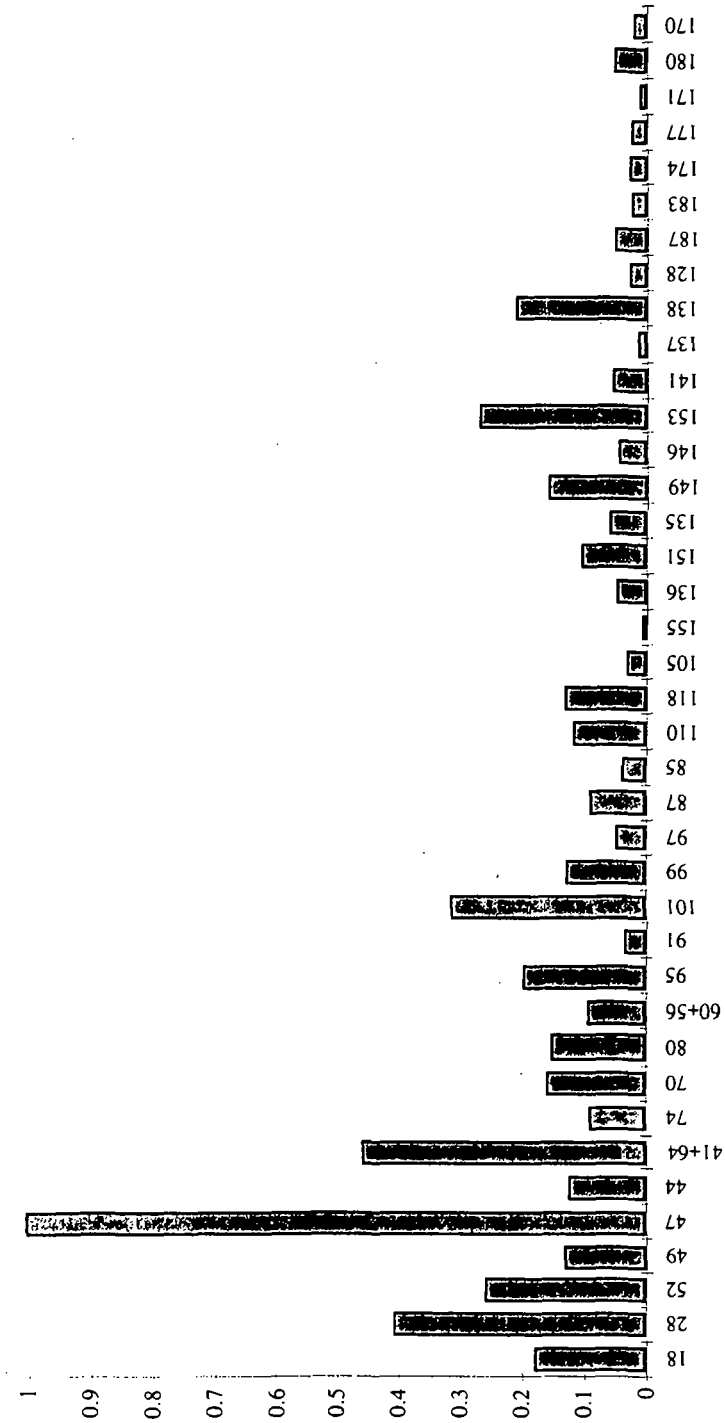

Figure 1. PCB congener profile of Antarctic krill. T_4CB 47 = 1.