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Congener Specific Analysis of Polychlorinated Biphenyls (PCBs) in Human Blood Serum from Croatia

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A gas-chromatographic method on capillary columns is described for measuring concentrations of total PCBs and of six PCB congeners, PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 and PCB-180, in human blood serum. Recovery of compounds was evaluated, and the repeatability and reproducibility of the results tested on samples analysed on the same day and over a period of two years. The method was verified in an international AQA study in three rounds of measurements. The method was applied for the analysis of 45 serum samples collected in Zagreb, Croatia. All samples contained PCB-138 and PCB-153; the incidence of the other congeners was between 80 and 98%.

Key words: polychlorinated biphenyls, PCBs, PCB congeners, human blood serum.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are a group of 209 congeners differing in the number and position of chlorine on the biphenyl. They have been in use for numerous purposes (capacitors, transformers, textile and paper industry) for more than half a century, always in mixtures containing up to 60% of chlorine. No congener was used for any other purpose but research. Thanks to the discovery of PCBs presence in environmental samples by S. Jensen[1] and his »The PCB Story«, PCBs have been found in almost all envi-

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ronmental and human samples; they are also the group of most investigated compounds. PCB mixtures used commercially contain up to 100 congeners and they have been found in environmental and human samples depending on the exposure, time elapsed from the exposure and on degradation conditions. The analysis of PCBs in human and environmental samples faces the problem of low concentration levels and of the large number of congeners in every mixture. The method of choice is gas chromatography (GC) with electron capture detection (ECD), particularly linked to mass spectrometry. Due to different toxicities of the congeners, high resolution gas chromatography on capillary columns is applied, enabling congener specific analysis.[2]

In the past, PCBs were determined on packed GC columns and total PCB levels were determined by the summation of several peaks in comparison to the sum of the corresponding peaks in the solution of commercial PCB mixtures.[3,4] Due to the differences in chlorine contents of commercial mixtures and differences in chromatographic conditions, results obtained in different laboratories are not always comparable because different PCB peaks are evaluated. Furthermore, it was not possible to identify individual congeners because every peak represented more than one compound. To overcome these problems, the perchlorination procedure was introduced into the analysis of PCBs in order to measure total PCBs, expressed as decachlorobiphenyl.[5] Toxicological research showed, however, that PCB congeners differ greatly in their toxic potential and the information on the distribution of individual congeners is important. Therefore, the perchlorination method has been discarded.

EXPERIMENTAL

Analysed Compounds and Analytical Procedure

The PCBs analysis in human blood started in our laboratory in 1985 on packed GC columns using Aroclor 1260, one of the commercial PCB mixtures, containing 60% of chlorine and Aroclor 1016 containing 41.5% of chlorine as standards for PCBs determination.[4]

Humans are exposed to various mixtures of PCBs. The PCB pattern in human body depends therefore on the source of exposure. Due to the different stability and rate of metabolic degradation of PCB congeners not all congeners remain present in the human body at measurable levels. Aroclor 1260 and Aroclor 1016 contain almost a hundred congeners each, but under chromatographic conditions applied for packed gas chromatographic columns congeners are not separated and appear in the chromatogram in nearly twenty well defined peaks. Therefore, each peak represents more than one congener. When quantitation was done on packed columns, more than six PCB congeners (but not individually identified) were quantified and therefore total PCB levels were higher than the sum of the invidually analysed congeners.

When monitoring of the general population is performed, individual results for each person are not of general interest. Therefore, establishing a national ratio between the results obtained on packed columns using Aroclors for quantification and the results obtained in comparison with selected PCB congeners is of interest for the future research and monitoring as well as comparison of old data with those obtained using high resolution GC and quantitation as compared to individual PCB congeners.

This paper presents the analysis of six selected congeners, most frequently found in human and environmental samples. The aim was to introduce congener specific analysis of PCBs in human serum/plasma using high resolution GC with capillary columns, and compare the obtained results with the levels of total PCBs. Samples were therefore also analysed in comparison with Aroclor 1260. This mixture was chosen because it best represents the compounds present in human blood. The compounds were determined either in plasma or serum because no difference was found between these two media.

The six analysed PCB congeners are listed in Table I. They were purchased from Promochem GmbH, Germany. Aroclor 1260 containing 60% chlorine was obtained from the US Environmental Protection Agency.

Blood samples were collected by venipuncture in heparinized or nonheparinized test-tubes from donors of the general population and from exposed workers. Serum/plasma were separated by centrifugation and stored at –20 °C until analysis.

Extraction was performed in the following way: 1.0 mL of serum/plasma was mixed with 1.0 mL formic acid and 1.0 mL n-hexane for l min. Compounds were extracted four times with 3.0 mL n-hexane. Extracts were combined and mixed with 1.0 mL 5% K_2CO_3 solution. Extracts were evaporated to about 4 mL and purified with 5 mL concentrated sulfuric acid. Extracts were evaporated to dryness and residues were dissolved in 1.0 mL n-hexane prior the GC analysis.

The method was previously used for the determination of organochlorine pesticides and total PCBs,[3] and is a modification of the method published by Dale and Miles.[6]

TABLE I

Chemical names and IUPAC numbers of six PCBs congeners

IUPAC number	Chemical name
PCB-28	2,4,4'-trichlorobiphenyl
PCB-52	2,2',5,5'-tetrachlorobiphenyl
PCB-101	2,2',4,5,5'-pentachlorobiphenyl
PCB-138	2,2',3,4,4',5-hexachlorobiphenyl
PCB-153	2,2',4,4',5,5'-hexachlorobiphenyl
PCB-180	2,2',3,4,4',5,5'-heptachlorobiphenyl

Gas Chromatographic Analysis

Instruments

– TRACOR 550 dual channel with two ^{63}Ni electron capture detectors and two packed columns.

Columns: glass columns 2 m long, 2 mm I.D.; 3% OV-1 on Chromosorb WHP 80/100 mesh; 5% OV-101 on Chromosorb DMCS AW 80/100 mesh.

Carrier gas: nitrogen. Temperature: inlet, 250 °C; columns, 210 °C; detectors, 260 °C.

– ATI UNICAM 610 dual channel capillary column gas chromatograph with two ^{63}Ni electron capture detectors and split/splitless injectors.

Columns: 30 m capillary column fused silica 0.25 mm I.D., SPB-1701 0.25 μm film thickness; 60 m capillary column fused silica 0.25 mm I.D., SPB-5 0.25 μm film thickness.

Carrier gas: nitrogen. Injection: splitless, 3–5 μL.

Temperature programme: 30 m column – initial temperature 110 °C, ramp 4 °C/min, upper temperature 240 °C for 55 min; 60 m column – initial temperature 100 °C, ramp 4 °C/min, upper temperature 240 °C for 55 min.

All samples were analysed on two columns. Only peaks identified on both columns were quantified as compared to the external standard *via* peak heights.

RESULTS AND DISCUSSION

Recovery of Compounds and Reproducibility of Results

Recovery of compounds was tested by adding 1.0 mL of the compound solution in n-hexane to a mixture of serum/plasma and formic acid, and the procedure was continued as described above. Compounds were added at two concentration levels and ten parallel samples at each concentration level were extracted on the same day. Results are presented in Table II. When compounds were added in the range 1.5–4.1 ng mL^{-1} (depending on the compound), recoveries were 68–87% and relative standard deviations of repeatability of the results were 14–38%. At higher concentration ranges (3.7–10.2 ng mL^{-1} depending on the compound), recoveries were not markedly higher (range 80–112%), but the repeatability of the results was better, with the exception of PCB-52 where overlapping peaks of unknown impurity influenced the results. The average recovery for both levels was 76–95% and the relative standard deviation for reproducibility was 86% for PCB-52 and 16–34% for the other five PCB congeners.

Recovery and reproducibility were also tested on a long-term basis by analysing 13 samples over a period of two years. Four solutions of compounds were used for recovery testing. Concentration ranges of compounds

TABLE II

Recovery of compounds in 10 paralel samples at each concentration level analysed on the same day

Compound	Level I		Level II		Mean recovery[a] %
	Conc. ng mL^{-1}	Recovery[a] %	Conc. ng mL^{-1}	Recovery[a] %	
PCB-28	1.9	86 (20)	4.8	95 (14)	90 (22)
PCB-52	1.5	79 (31)	3.7	112 (72)	95 (87)
PCB-101	3.8	68 (38)	9.5	84 (15)	76 (34)
PCB-138	3.5	87 (14)	8.8	92 (12)	89 (16)
PCB-153	3.2	83 (18)	8.0	92 (13)	87 (18)
PCB-180	4.1	78 (18)	10.2	80 (17)	79 (20)

[a]Relative standard deviation in parentheses.

TABLE III

Recovery of compounds in 13 samples analysed over a period of two years

Compound	Conc. range ng mL^{-1}	Mean recovery[a] %
PCB-28	1.98–3.05	66 (25)
PCB-52	0.76–2.77	83 (29)
PCB-101	1.82–2.77	60 (18)
PCB-138	2.02–3.18	79 (57)
PCB-153	1.22–3.20	86 (61)
PCB-180	1.56–4.10	65 (34)

[a]Relative standard deviation in parentheses.

added are given in Table III. Recoveries were in the range 60–86% depending on compounds and relative standard deviation for the reproducibility of the results was in the range 18–61%. The highest RSD were obtained for PCB-138 and PCB-153.

Analytical Quality Assurance

In order to be comparable with other laboratories in different countries, we have participated in quality assurance studies for the analysis of PCB congeners in human plasma.

The method was tested in three rounds of an international quality assurance study (1994–1995) co-ordinated by the Deutsche Gesellschaft für Arbeitsmedizin und Umweltmedizin e.V., Erlangen, Germany. Plasma samples were spiked with PCB congeners at four different concentrations: two concentrations at environmental level and two concentrations at occupational exposure levels. Acceptance criterion: the results were accepted when they were within ± 3 standard deviations of the mean value reported by the reference laboratories.[7]

Our results are summarized in Table IV and compared with the results reported from the other participating laboratories. The number of the other

TABLE IV

Results of the Interlaboratory Quality Assurance Study, expressed as percent of positive results[a]

Compound	Level I			Level II			Both Levels	
	Conc.	Rounds 1–3		Conc.	Rounds 1–3		All Rounds	
	ng mL^{-1}	IMI	Others	ng mL^{-1}	IMI	Others	IMI	Others
PCB-28	0.25–4.30	100	67	1.60–6.74	83	59	92	64
PCB-52	0.15–2.09	67	65	0.97–5.35	33	57	50	62
PCB-101	0.15–2.03	50	60	0.97–4.07	50	53	50	57
PCB-138	0.43–3.02	100	64	2.99–7.73	100	51	100	59
PCB-153	0.71–3.24	67	64	3.02–7.32	17	48	42	57
PCB-180	0.48–3.26	50	53	2.18–5.41	100	54	75	53

[a]IMI refers to our Institute.

laboratories varied from 10 to 26 depending on the round. In all rounds and on both levels we have correctly determined the PCB-138 concentration; PCB-28 was correctly determined in three rounds of the lower concentration level, and PCB-180 in three rounds of the higher concentration level. The number of positive results for PCB-52, PCB-101 and PCB-153 was about the same as reported for the other laboratories.

Concentrations of PCB Congeners in Human Serum Samples

The method outlined above was applied to the analysis of PCB congeners in the serum of 15 workers (14 male and 1 female, 31–58 years old) occupationally exposed to PCBs and in the serum of 30 individuals (25 male

and 5 female, 14–83 years old) from the general population (Table V). In the group of occupationally exposed workers, total PCBs were also measured and concentrations were determined as compared to Aroclor 1260 (Table V).

TABLE V

Median concentrations / μg L^{-1} and concentration ranges (in brackets) of PCB congeners and total PCBs in serum samples collected from exposed workers (in 1994) and from the general population (in 1995 and 1997)[a]

Compound	Exposed workers[8]	General population[8,9]	
	1994 (*N* = 15)	1995 (*N* = 14)	1997 (*N* = 16)
PCB-28	0.4 (0–3.8)	0.1 (0–0.3)	0.2 (0–0.5)
PCB-52	1.6 (0–4.6)	0.7 (0.3–1.5)	2.5 (0.5–9.1)
PCB-101	0.6 (0–0.7)	0.4 (0–3.4)	0.5 (0–2.4)
PCB-138	0.9 (0.3–4.6)	0.5 (0.2–1.2)	0.5 (0.2–4.6)
PCB-153	1.3 (0.3–5.2)	0.5 (0.3–1.6)	0.5 (0.1–2.4)
PCB-180	0.9 (0–2.8)	0.3 (0.2–2.7)	0.3 (0–0.9)
Sum PCB	6.6 (1.1–20.5)	2.4 (1.5–6.4)	4.4 (1.9–11.4)
Tot PCB	9 (3–29)	–	–

[a]Zero stands for concentrations below detection limits. Sum PCB is the sum of six congener concentrations. Tot PCB is the concentration of total PCBs. *N* is the number of serum samples.

In 13 sera, out of 15, the concentration of total PCBs was higher than the sum of the six congeners. The mean ratio of total PCBs/sum of congeners was 1.8, and the median ratio was 1.4. This indicates that more than six congeners were probably present in these sera. The correlation coefficients between total PCB concentrations and concentrations of PCB-180, PCB-153 and PCB-138 were high: 0.94, 0.92 and 0.84 resp. For the other three congeners these correlations were below 0.65.

All 45 samples contained PCB-138 and PCB-153. The incidence of the other four congeners was 80% (PCB-28 and PCB-101), 96% (PCB-180) and 98% (PCB-52). Concentrations of the six congeners were on average slightly higher in the sera of exposed workers than in the general population. The concentrations of PCB-153 correlated best with the sum of the six congeners (r = 0.79), while the corresponding coefficients for PCB-138 and PCB-180 were 0.72 and 0.66 resp., and for the other three compounds they were below 0.60.

If the concentration of a given congener showed a high correlation with the concentration of total PCBs, or with the sum of all congeners, that compound might be suggested as a marker in monitoring studies. No data are available on the levels of the individual PCBs in the population of Croatia, except for the small group described in this paper. However, a study on 800 Swedish male individuals showed a high correlation ($r = 0.98$) between total PCBs and PCB-153, and the authors suggested that in monitoring studies PCB-153 could be used as an indicator of total PCBs.[10] Further congener specific studies are required before a recommendation can be given for the population of Croatia.

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SAŽETAK

Specifična analiza pojedinih poliklorbifenila (PCB) u serumu ljudi iz Hrvatske

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Opisana je plinskokromatografska analiza visoke djelotvornosti za određivanje koncentracije ukupnih PCB i šest pojedinačnih PCB (PCB-28, PCB-52, PCB-101, PCB-138, PCB-153 i PCB-180) u serumu ljudi. Evaluirana su iskorištenja i utvrđena ponovljivost i usporedivost rezultata analize uzoraka tijekom jednog dana i unutar razdoblja od dvije godine. Metoda je potvrđena sudjelovanjem u tri kruga mjerenja međunarodnog programa provjere kvalitete analize PCB u serumu. Provjerenom metodom analizirano je 45 uzoraka seruma sakupljenih u Zagrebu. Svi uzorci sadržavali su PCB-138 i PCB-153, a ostali PCB nađeni su u 80–98% uzoraka.