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Journal of Chromatography B, 791 (2003) 117–125
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Determination of polychlorinated biphenyls in serum using gas chromatography–mass spectrometry with negative chemical ionization for exposure estimation

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Received 22 July 2002; received in revised form 29 January 2003; accepted 11 March 2003

Abstract

A sensitive and selective method for the determination of 24 polychlorinated biphenyls (PCBs) by gas chromatography–mass spectrometry with negative chemical ionization (GC–MS-NCI) was applied for the recent needs of occupational exposure in waste incineration. The three most abundant ions were used in determining compounds with at least five chlorine atoms in the PCB molecule. Selecting ions Cl^{35} and Cl^{37} for di-, tri-, and tetrachlorinated PCBs resulted in reliable quantification of these compounds. The detection limits for the 24 individual compounds varied from 0.01 to 0.08 μg/l. The recovery of the method was 113±16%. Stability tests showed no degradation of the compounds studied during 6 weeks. The sum of 24 PCB compounds measured from the sera of workers in a disposal plant was 1.9–10.9 μg/l, and 0.3–3.0 μg/l for controls, respectively. The mean proportion of the low chlorinated PCB compounds (with four or less chlorine atoms) was 20% for workers in the disposal plant and 14% for the controls.

Keywords: Biological monitoring; Polychlorinated biphenyls

1. Introduction

Polychlorinated biphenyls (PCBs) are mainly regarded as ubiquitous environmental pollutants and due to their chemical stability and lipophilicity, they accumulate readily in the body and at high levels (60–3300 μg/l) they may cause harmful health effects [1,2]. They also still exist as occupational contaminants in elements of house renovation and in waste incineration [3–5].

Determination of PCB compounds has engaged many research groups since the 1980s and modern techniques are mainly based on gas chromatography with electron-capture detection (GC–ECD) [5–8], or more reliable methods, such as GC–mass spectrometry (MS) in the electron impact (EI) mode [9–13], that have been used in recent studies to identify individual compounds. Mass spectrometry with negative chemical ionization (NCI) has been found to be superior in sensitivity when analyzing most toxic compounds (PCBs 77, 126, 169) in sewage sludges [14].

For occupational and environmental exposure estimation, serum has been considered a suitable matrix, being homogenous and not readily coagulating during freezing [15]. The extraction of lipophilic PCBs from serum or plasma is mostly done with

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1570-0232/03/$ – see front matter
doi:10.1016/S1570-0232(03)00216-2

solvents or solvent mixtures like hexane–dichloromethane [14], hexane–diethyl ether [16], hexane [6] or acetonitrile [11]. The solvent extract is often washed with acid or base as an initial step to remove large quantities of organic coextractives to ensure that subsequent commonly used column chromatography procedures are not overloaded by organic material. Adsorbent columns reported for sample purification include silica gel [11,12,15], Florisil [7,12,14,17], carbon [9], basic alumina [12], potassium silicate [11], and acid-impregnated silica gel columns [11]. Also liquid–lipophilic gel partitioning as the lipid extractive step without acid treatment has been reported for blood samples [10,18]. The extraction solvents in the literature are chosen to match the clean-up step and give rather similar yields.

In Finland, a biological monitoring method using ECD has been used since the early 1980s to assess workers' exposure to PCBs in capacitor and transformer manufacture and service as well as in waste disposal work [19,20]. The main PCB compounds found in waste incineration originated earlier from capacitor and transformer oils. Therefore, nine low chlorinated PCB compounds (PCBs 8, 18, 28, 33, 44, 47, 66, 74, 101) have traditionally been measured from workers' serum to evaluate their exposure to PCBs. Nowadays, however, construction waste and contaminated soil containing mainly highly chlorinated PCBs (PCBs 101, 118, 138, 153, 180) [3,21–23], seem to be the main sources of PCBs in waste incineration.

The sensitivity of ECD for halogenated compounds has been the reason for its use in the trace analysis of PCBs. The disadvantage of the ECD is, however, its ready response to all negative ions which occasionally leads to false exposure estimations. To have a more specific method for the analysis of PCBs we applied a method based on negative chemical ionization and also tested it in waste incineration work and in controls not occupationally exposed PCBs.

2. Experimental

2.1. Materials

The following reagents were used: diethyl ether (Riedel-de Haen, Seelze, Germany), methanol (Rathburn, Walkerburn, UK) and hexane (Baker, Deventer, The Netherlands) were of HPLC grade. Sulfuric acid (suprapur) and sodium sulfate (analytical grade) were purchased from Merck (Darmstadt, Germany).

The PCB compounds were: standard solution (C-CCSEC) purchased from Accustandard (New Haven, CT, USA), and solid PCB compounds 30, 33, 47, 74, 116, 169 (IUPAC nomenclature) purchased from Dr. Ehrensdorfer (Augsburg, Germany). Serum was obtained from the Finnish Red Cross.

Commercial silica adsorbent columns (Bond Elut catalog No. 1210-2037, 500 mg) were obtained from Varian (Middelburg, The Netherlands).

2.2. Procedure

Preparation of PCB standard solutions

Stock solutions of the solid PCBs were made in hexane at a concentration level of about 250 mg/l. Internal standard (I.S.) stock solutions (PCBs 30 and 116) were combined and diluted 1:100 (v/v) in hexane. The hexane I.S. solution was diluted 1:25 (v/v) in acetone, and further 1:40 (v/v) in methanol. The final concentration of the I.S. mixture to be added to the samples was 2 μg/l for both PCBs 30 and 116. The stock solutions of PCBs 33, 47, 74, 169 were diluted 1:10 (v/v) and C-CCSEC standard solution 1.5/100 (v/v) in acetone. The analyte solutions were combined, giving a concentration of about 1.5 μg/ml. The working standard mixtures were made in hexane, the concentrations ranging from 0.5 to 9 μg/l for individual isomers. The concentration of internal standards was 4 μg/l.

Sample treatment

Fasted serum samples were collected at the end of the week or the exposure period. The samples were stored in a refrigerator (+6 °C) until analyzed.

The samples were prepared as previously described [5]. In short, a 2-ml volume of serum sample was pipetted into a screw-capped test tube, and 2 ml of internal standard in methanol and 6 ml of diethyl ether–hexane (1:1, v/v) were added. The sample was then mixed for 30 min. After separation of the phases, 4 ml of the organic layer was concentrated to 2 ml and to remove fat and polar materials mixed with 2.5 ml sulfuric acid. The organic layer (2 ml) was dried on sodium sulfate, and 1 ml of sample was added to a silica column which had been conditioned

with 20 ml of hexane before use. The PCBs were eluted with 2 ml of hexane. The sample was concentrated to 250 μl and transferred to autosampler bottles for gas chromatography. The following 24 PCB compounds were determined: PCBs 8, 18, 28, 33, 44, 47, 52, 66, 74, 77 (low chlorinated PCBs containing four or less chlorine atoms in the molecule); PCBs 101, 105, 118, 126, 128, 138, 153, 169, 170, 180, 187, 195, 206 and 209 (high chlorinated PCBs with more than four chlorine atoms).

Recovery

The recovery was investigated by applying known amounts of the PCBs into the serum. The levels of individual PCBs were 0.9 and 3.6 μg/l.

Stability tests

For stability testing, the serum was spiked with two levels of PCBs (0.9 and 3.6 μg/l) in triplicate. The samples were stored at +20 °C and at −20 °C for 4 days, 2 and 6 weeks before analysis.

2.3. Chromatographic equipment and analytical conditions

A Hewlett-Packard (HP) 5890 II gas chromatograph equipped with an ECD system (^{63}Ni) operating at 350 °C and a gas chromatograph, HP 6890 with mass-selective detector (HP 5973) with NCI using methane as reagent gas, was applied. The temperatures of the transferline, ion source and quadrupole were 250, 150 and 106 °C, respectively. The emission current was 49 mA. The MS system was operated in the selective-ion monitoring (SIM) mode. The following ion pairs were monitored: di-, tri- and tetrachlorinated PCBs, *m*/*z* 35/37; PCB 77, *m*/*z* 289.9/291.9/293.9; pentachlorinated PCBs, *m*/*z* 323.9/325.9/327.9; hexachlorinated PCBs, *m*/*z* 357.8/359.8/361.8; heptachlorinated PCBs, *m*/*z* 393.8/395.8/397.8; octachlorinated PCB, *m*/*z* 427.8/429.8/431.8; nonachlorinated PCB, *m*/*z* 461.7/463.7/465.7 and decachlorinated PCB, *m*/*z* 495.7/497.7/499.7. A fused-silica capillary column (PAS-1701, HP No. 19091S-033) 30 m×0.25 mm I.D., coated with cyanopropylphenyl–dimethyl (14:86) polysiloxane (0.25 μm film thickness) was used. An equivalent column was used with ECD. The column temperature programme, for GC–MS, was as follows: 80 °C, heating to 180 °C at 30 °C/min, heating to 188 °C at 4 °C/min, 9 min at 188 °C, heating to 230 °C at 5 °C/min, 25 min at 230 °C, and for GC–ECD: 80 °C, heating to 180 °C at 30 °C/min, heating to 190 °C at 4 °C/min, 9 min at 190 °C, heating to 230 °C at 5 °C/min, 50 min at 230 °C. Helium was used as a carrier gas at flow-rates of 1.9 ml/min (ECD) and 1.4 ml/min (MS). An autoinjector was used to introduce 3 μl samples to the gas chromatograph. The injection into the GC–MS system was operated in the pulsed splitless mode with a pulse pressure of 25 p.s.i. and a pulse time of 1.5 min (1 p.s.i.=6894.76 Pa). The inlet temperatures in both apparatuses were 250 °C.

The quantitation limits for the individual compounds were calculated by dividing the concentration of the spiked PCB amount in serum by the signal-to-noise ratio and multiplying the quotient by 3, which was used as a safety factor. The external quality of the analysis have been assured with the Quality Assurance assay in Occupational and Environmental Medicine run by University of Erlanden, Nurenberg, Germany. The PCB compounds in the program are PCBs 28, 52, 101, 138, 153, and 180.

2.4. Application

Fasted serum samples (10 ml) were obtained from 26 men (aged 22–63 years) working in a hazardous waste disposal plant, and from 21 controls (aged 30–57 years). The blood samples were collected in vacuum glass tubes. The serum was immediately separated by low-speed centrifugation and stored in precleaned centrifuge tubes made of polypropylene in a freezer (−20 °C) until further sample treatment. All 24 PCB compounds were determined and their concentration compared to the sum of nine PCB compounds traditionally used for exposure assessment in Finland.

3. Results and discussion

3.1. Determination

The recovery of the method including hexane–diethyl ether extraction, sulfuric acid treatment and silica column chromatography was calculated for the 24 PCB compounds analyzed. The overall recovery for single PCB compounds was 113±16% both at

level 0.9 μg/l (n=18) and 3.6 μg/l (n=15). For the nine PCB compounds used for exposure assessment, the overall recovery at both concentration levels was 102±6% for GC–MS which is comparable to that achieved earlier (84.6±11.4%) when using ECD [5] or by other purification systems.

The imprecision of the method used was estimated from control samples analyzed in every series of analysis. At a concentration level of 0.9 μg/l (n=18), the variation coefficient was 3–18% for individual compounds and 22% for the sum of PCBs. At a concentration level of 3.6 μg/l (n=15), the variation coefficient was 2–16% for individual compounds and 4.6% for the sum of PCBs.

Stability tests at +20 °C, +10 °C and −20 °C showed no degradation of the compounds studied during 6 weeks. The mean recovery after 6 weeks (n=3) for individual PCBs was 92% at 0.9 μg/l and 93% at 3.6 μg/l.

With MS application the 24 PCB compounds are fully separated with the semipolar cyanopropyl–dimethylpolysiloxane phase. The toxic coplanary PCBs (PCBs 77, 129, 169) have been reported to gain sensitivity with negative chemical ionization using the molecular ion as a target ion when compared to EI [11]. We found that in the NCI mode the molecular ion was too weak or non-existent for di-, tri-, and tetrachlorinated PCBs. Therefore, chlorine isotopes ^{35}Cl and ^{37}Cl were used to achieve reliable quantification for PCBs 8, 18, 28, 33, 44, 47, 52, 66, and 74.

In our study, the ECD and MS determinations for the sum of nine PCB compounds used earlier for exposure assessment (PCBs 8, 18, 28, 33, 44, 47, 66, 74, 101) gave a satisfactory correlation (r=0.97, n=10). PCB concentrations determined with ECD and MS are plotted in Fig. 1a. The distribution of residuals (Fig. 1b) on both sides of the zero axis strengthens the conclusion of comparability of the two detection methods used.

The detection and quantitation limits for the PCBs are presented in Table 1. MS-NCI, being more selective than ECD, gave a lower background (Fig. 2) and thus giving a better signal-to-noise ratio. The calculated concentrations for the sum of PCBs can be higher when using MS than the values given by ECD due to higher limit of quantitation of ECD. All the compounds were not quantifiable with ECD, i.e.,

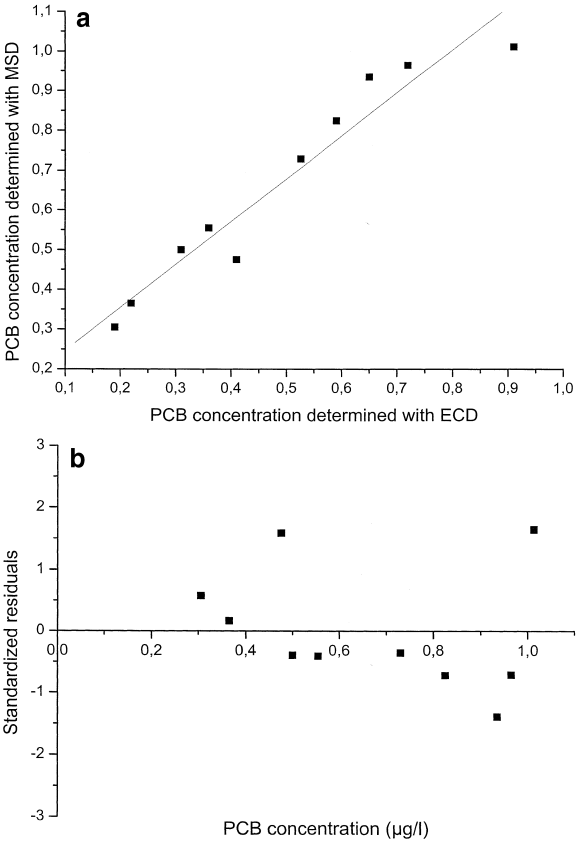

Fig. 1. Plot (a) of PCB concentrations determined with ECD and MS and the distribution of standardized residuals (b), i.e., calculated residual divided by overall residual standard deviation [34].

PCBs 66 and 187. The samples tested positive by GC–ECD may require further confirmation, e.g., dual columns have been used [7]. In the GC–MS the fragmentation pattern and the ratio of the ions chosen add a further dimension to the identity of the compounds, in addition to retention time. In our method, a deviation limit of the isotope ratio (IR) calculated as ±(0.1*IR+10)% from the relative intensities determined in the standard solution was considered acceptable. GC–MS-EI has been reported to be less sensitive than GC–ECD [8] [17], but this can be compensated by using GC–MS-NCI [14] where the molecular ion is the base peak for penta- to decachlorinated PCBs, and practically no fragmentation of these compounds can be observed. The fragmentation of di- and tetrachlorinated PCBs to

Table 1
The limits of detection and quantitation (μg/l)

Compound	LOD (GC–MS)	LOQ (GC–MS)	LOD (GC–ECD)	LOQ (GC–ECD)
PCB 8	0.02	0.12	0.12	2.1
PCB 18	0.08	0.17	0.09	0.3
PCB 28	0.07	0.08	0.10	0.3
PCB 33	0.08	0.24	0.05	1.0
PCB 44	0.01	0.11	0.07	0.5
PCB 47	0.01	0.08	0.10	0.3
PCB 52	0.01	0.12	0.06	0.4
PCB 66	0.03	0.08	0.09	*
PCB 74	0.02	0.09	0.10	0.1
PCB 77	0.01	0.04	0.11	1.5
PCB 101	0.01	0.02	0.07	0.3
PCB 105	0.01	0.03	0.05	0.6
PCB 118	0.02	0.03	0.04	0.6
PCB 126	0.01	0.02	0.04	2.8
PCB 128	0.01	0.01	0.06	1.6
PCB 138	0.02	0.05	0.03	1.1
PCB 153	0.01	0.06	0.03	0.4
PCB 169	0.01	0.01	0.34	4.0
PCB 170	0.02	0.05	0.08	1.4
PCB 180	0.02	0.03	0.07	1.4
PCB 187	0.01	0.01	0.03	*
PCB 195	0.01	0.01	0.09	2.7
PCB 206	0.01	0.01	0.12	1.4
PCB 209	0.01	0.01	0.11	1.8
Mean	0.02	0.06	0.09	0.7
SD	0.02	0.06	0.06	1.0

* No separation from serum background.

yield ^{35}Cl and ^{37}Cl ions is enhanced in MS-NCI still offering more selectivity than gained by determination with GC–ECD. Also, the ion intensities become more pronounced with an increased degree of chlorination of the molecules. In GC–ECD, the detection of the higher chlorinated PCBs is affected by the peak widening due to long retention times and increasing column bleed. The detection and quantitation limits for the 24 individual compounds in our study varied from 0.01 to 0.08 and 0.03–0.34 μg/l for MS-NCI, respectively (Table 1). The quantitation limits for individual PCB compounds were comparable to those reported for PCBs 77, 126, and 169, 50–100 fg per injection [14]. The quantitation limits for the sum of 24 PCB compounds were 0.1 μg/l for MS and 0.7 μg/l for ECD. For the sum of nine low chlorinated PCBs that have traditionally been measured from serum to evaluate workers' exposure to PCB in Finland, the quantitation limit was 0.5 μg/l when using ECD.

3.2. Application

The NCI technique was applied to routine analysis with serum samples from the waste incineration workers, because according to Council Directive 96/59/EC [24], waste containing PCBs should be disposed as hazardous waste. The PCB concentrations found in the serum of waste disposal workers, and of the controls not occupationally exposed to PCBs, are presented in Fig. 3a and b. The mean sum of the 24 PCB compounds was 3.4 μg/l (1.9–10.9 μg/l) and the median was 2.9 μg/l for the disposal plant workers and 1.6 μg/l (0.3–3.0 μg/l) and 1.5 μg/l for the controls, respectively. The mean proportion of the low chlorinated PCB compounds (PCBs

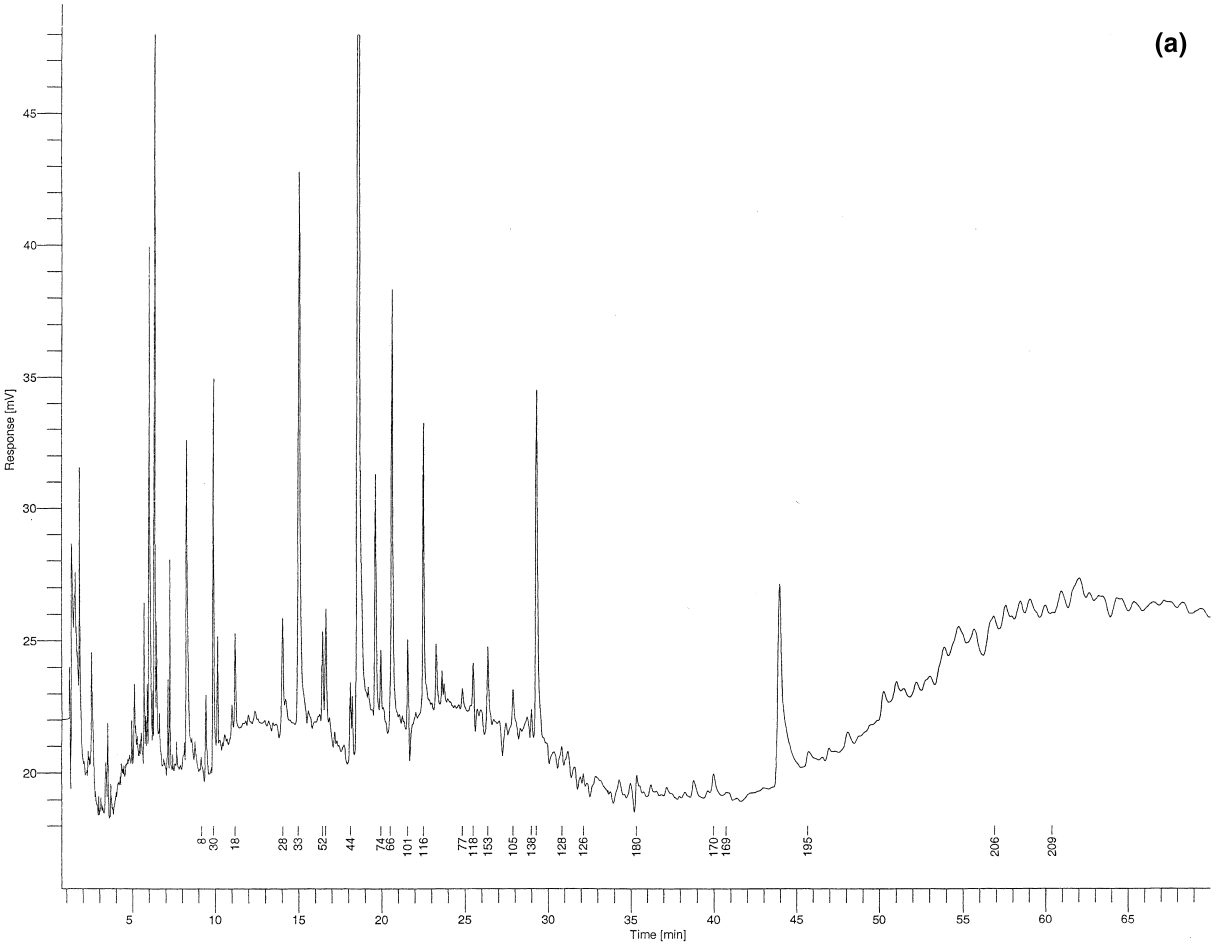

Fig. 2. (a) GC–ECD chromatogram of a serum sample containing 2 μg/l of PCB 8, 18, 28, 33, 44, 47, 66, 74 and 101. (b) GC–MSD/NCI total ion chromatogram of a serum sample containing about 2 μg/l of PCB 8, 18, 28, 33, 44, 47, 66, 74 and 101.

8, 18, 28, 33, 44, 47, 52, 66, 74, 77) in these samples was 20% (median 13%) for disposal plant workers and 14% (4%) for the controls, respectively.

The concentration levels of the nine PCB compounds the serum (PCBs 8, 18, 28,33, 44, 47, 66, 74, 101) which have traditionally been used as markers of the exposure to PCBs were for occupationally unexposed controls of the same magnitude as those determined in Finland in the early 1980s [5,25]. For the sum of these PCB compounds, there were only two workers in our study whose PCB concentrations (3.2 and 7.8 μg/l) were above the Finnish upper reference limit of the occupationally nonexposed population, which is estimated to be 3 μg/l [26]. In Finland, the reference limit is given to the sum of PCBs and it is not adjusted to the age of the persons investigated.

Concentrations of PCBs 28, 52, 101, 138, 153 and 180 have been reported in several studies. In the sera of disposal plant workers of our study, the concentration of PCB 28 varied from not detected to 2.3 μg/l, PCB 52 was not detected, PCB 101 ranged from not detected to 1.4 μg/l, PCB 138 from 0.1 to 1.3 μg/l, PCB 153 from 0.2 to 2.0 μg/l, PCB 180 from 0.4 to 1.6 μg/l. In the controls, the concentration of PCB 28 varied from not detected to 0.3 μg/l, PCB 52 from not detected to 0.2 μg/l, PCB 138 from 0.04 to 0.6 μg/l, PCB 153 from 0.1 to 1.1 μg/l, and PCB 180 from 0.05 to 0.7 μg/l. PCB 101 was not detected in any of the samples from the

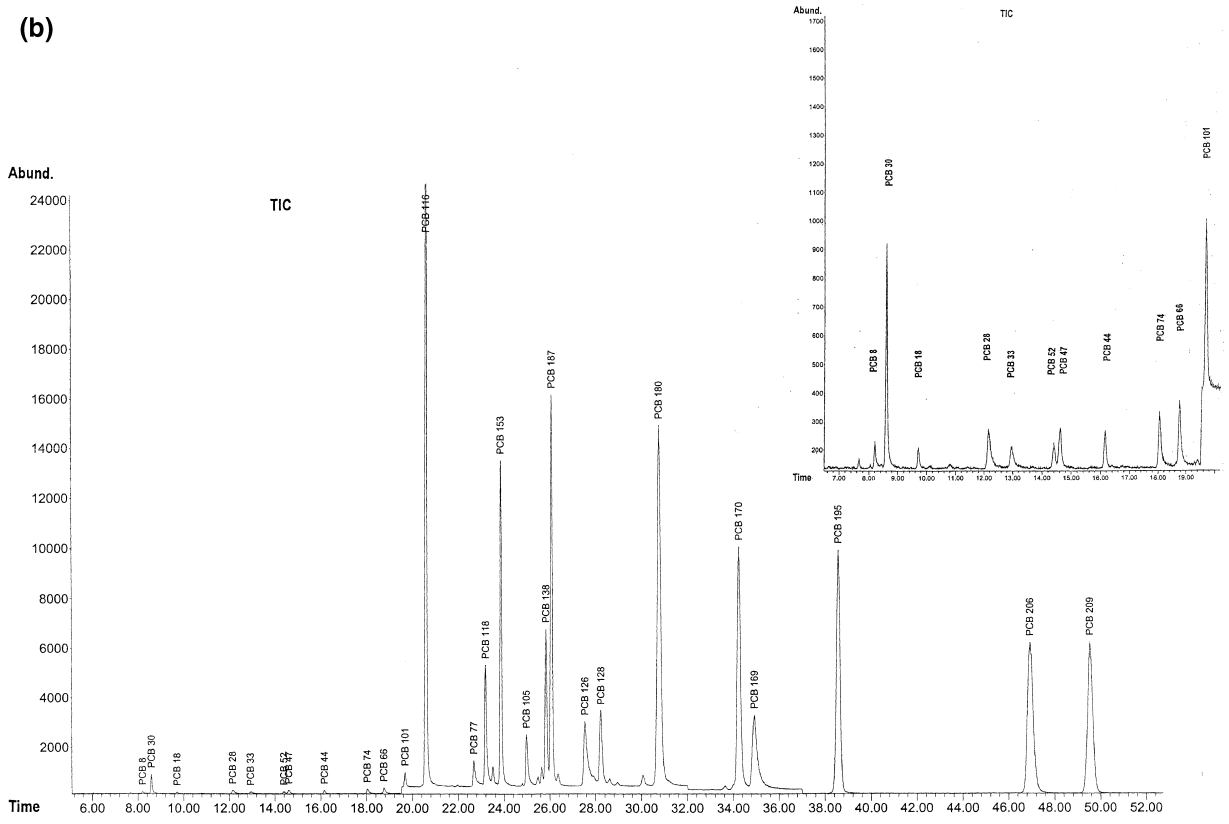

Fig. 2. (*continued*)

controls. In our study, the concentrations of these compounds in the sera of both the controls and the workers were lower than those for residents in Belgium [27], Germany [28,29], or in Spain [30,31], with the exception of PCBs 28 and 101 in the sera of disposal plant workers. In an other Spanish study [32], the levels of PCB 28 (0.004–0.39 μg/l) and PCB 101 (0.011–0.21 μg/l) in the sera from the workers in a new hazardous waste incinerator were lower than those in the sera of the workers in our study. Furthermore, the maximum concentrations of PCBs 138, 153 and 180 in sera of the workers in our study were nearly ten times lower than those in the workers of a German municipal waste incinerator (0.5–11.6, 0.70–15.1, 0.40–10.1 μg/l, respectively) [28].

Kappos et al. [33] have proposed reference values for PCBs 138, 153 and 180 in human blood and plasma. The concentrations of individual compounds are given for six age groups. According to these age groups, the concentrations of the above-mentioned PCB compounds in the sera of the workers in our study were well below the reference values proposed by Kappos et al. However, about 42% of the exposed workers had serum PCB concentrations for the sum of 24 compounds that exceeded the Finnish upper reference limit, i.e., 3 μg/l, for occupationally non-exposed people.

4. Conclusion

SIM in the MS determination of PCB compounds offers inherent selectivity and better sensitivity than previously used gas chromatographic methods based on ECD. In waste incineration, it is beneficial to determine PCB compounds with wide range of chlorination to be able to assess workers exposure to

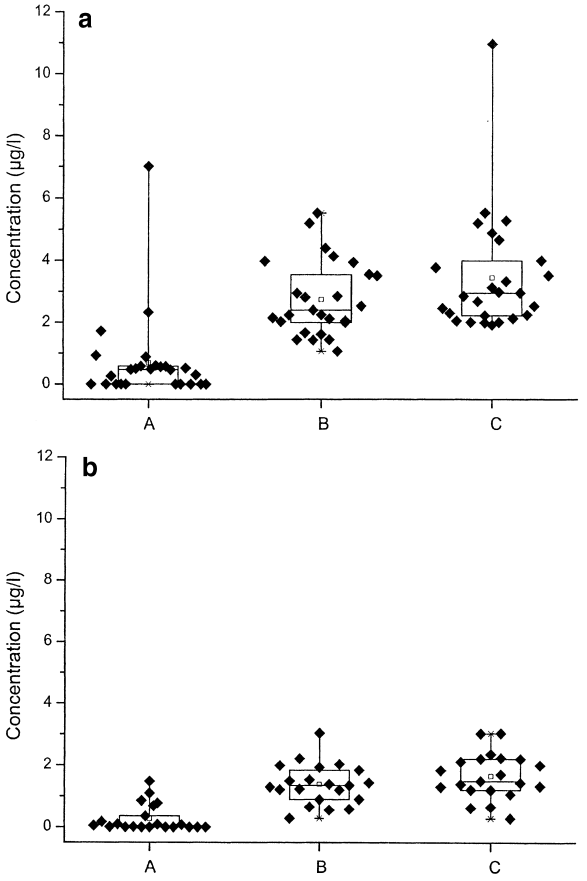

Fig. 3. (a) The PCB concentrations, μg/l, in the sera of waste disposal workers (n=26). (A) PCB compounds with four or less chlorine atoms. (B) PCB compounds with more than four chlorine atoms. (C) The sum of 24 PCB compounds determined. (b) The PCB concentrations, μg/l, in serum of controls not occupationally exposed to PCBs (n=21). (A) PCB compounds with less than four chlorine atoms. (B) PCB compounds with more than four chlorine atoms. (C) The sum of 24 PCB compounds determined. The bars represent minimum–maximum, the boxes the 25th, 75th percentiles and median.

PCBs containing waste originating from different sources.

Acknowledgements

We thank Ms. Tuula Karttunen for skillful technical assistance and Ms. Eevi Nieminen and Ms. Kirsti Nilsson for carrying out the sampling.

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