fetal development and after birth may have adverse effects on children. This exposure can be measured by PCB determination in cord serum, of which PCB levels are significantly lower than those of adults reflecting the lower lipid concentration of cord serum [9]. PCB concentrations were thus often calculated on a fat-mass basis.

Considering the lipophilic properties of PCBs, the general analytical procedure for the determination of PCBs in complex matrices includes the following steps: homogenisation and drying, extraction from the matrix (often together with fat and other lipophilic matrix components), clean-up and concentration, gas chromatographic separation and detection. Analytical procedures including a solvent extraction step are hardly routinely applicable in large studies, because they are very time-consuming and need large solvent volumes. The most frequently used detection method for PCBs is the electron-capture detection (ECD), because it is the most sensitive detector in routine use and is selective towards halogenated compounds. Despite the higher selectivity, mass spectrometric detectors are in some cases less useful, because of their lower sensitivity and higher costs. As in case of ECD identification of a compound is only performed by its retention time, dual-column chromatography on two capillary columns of different polarity can be applied for confirmation of the results. Although PCB mixtures may consist of up to 209 congeners, the determination of six selected standard congeners (IUPAC Numbers 28, 52, 101, 138, 153 and 180) is widely performed in most laboratories.

In this paper a procedure is described for the simultaneous determination of background levels of the PCB congeners 28, 52, 101, 138, 153 and 180 in human cord serum. The method consists of a column liquid chromatographic sample clean-up without a prior solvent extraction step. Determination was achieved either by dual-column capillary gas chromatography with ECD or by GC–MS.

The described procedure was applied in the context of a prospective field study on possible long-term developmental effects of prenatal and neonatal environmental exposure to PCBs [10]. The method allows the simultaneous determination of chlorinated pesticides such as Aldrine, DDT, DDE, BHC and HCB and is also applicable to other biological matrices such as bone marrow or tissues.

## 2. Experimental

### 2.1. Chemicals

*n*-Hexane, dichloromethane, toluene and acetone (for residue analysis) were obtained from Promochem (Wesel, Germany), sodium sulphate (for residue analysis) from Merck (Darmstadt, Germany).

Adsorbent: Florisil 0.150–0.250 mm, Merck.

Standard solutions: PCB and pesticide standards were purchased as solutions (certified if available) by Promochem (Wesel, Germany).

Test kit for determination of lipids: Merckotest "Total Lipids", Merck.

### 2.2. Sampling and sample storage

Blood and plasma/serum samples were taken in polyethylene monovettes. Immediately after sampling, blood samples were transferred into cleaned glass vials, serum and plasma samples were centrifuged in the monovettes and then transferred into glass vials immediately. Bone marrow and tissue samples were taken directly into glass vials. All samples were stored at −18°C until sample preparation.

All instruments and vials used during sample clean-up were made of glass, sample contact to plastic materials was reduced to a minimum in order to avoid adsorptive sample losses. Instruments and vials were cleaned with hexane and acetone and stored at 100°C until usage.

### 2.3. Chromatographic equipment and analytical conditions

GC–MS: A GC–MS System consisting of a HP 5890 gas chromatograph (Hewlett-Packard) with a 50 m×0.2 mm Ultra 2 column, film thickness 0.33 $\mu$m (Hewlett-Packard) coupled via a direct interface to a HP 5989A quadrupole mass spectrometer (Hewlett Packard) in the selected ion monitoring mode (GC–MS/SIM) was used. The oven programme was started at 60°C held for 1 min, programmed at 40°C $min^{-1}$ to 130°C, held for 2 min, then programmed at 15°C $min^{-1}$ to 270°C, held isothermal for 15 min. On column injection was performed at 60°C, after 0.1 min the injector was heated with a rate of 300°C $min^{-1}$ to a final temperature of 300°C. Injection

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# Determination of polychlorinated biphenyls and chlorinated pesticides in human body fluids and tissues

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**Abstract**

A fast and reliable method for the determination of polychlorinated biphenyls (PCBs) and chlorinated pesticides in human body fluids and tissues is presented. Sample clean-up and selective enrichment of analytes are carried out by liquid column chromatography without a prior solvent extraction step. Analytes are determined either by high-resolution capillary gas chromatography with mass spectrometric detection, or by dual-column GC followed by electron-capture detection. A procedure is described for the simultaneous determination of environmental levels of the PCB congeners 28, 52, 101, 138, 153 and 180 in human cord serum. The method allows the simultaneous determination of chlorinated pesticides such as aldrin, DDT, DDE, BHC and HCB and is also applicable to other biological matrices, such as bone marrow or tissues.

*Keywords:* Sample preparation; Pesticides; Polychlorinated biphenyls; Organochlorine compounds

## 1. Introduction

The intensive use of polychlorinated biphenyls (PCBs) as heat transfer and hydraulic fluids, additives in plastics and dyes etc., has led to an ubiquitous distribution in the environment. For they are lipophilic and poorly metabolised or biodegraded, they have a long half life and tend to accumulate in the food chains. Highest concentrations were thus usually found in human beings and higher animals being at the top of the food chains. Although in the meantime many industrial countries, including Germany, have banned the use of these chemicals, they are currently present in a variety of human biological matrices like adipose tissue, blood and blood compartments, cord blood and maternal milk [1–4]. The results available indicate that from the beginning of the 1980's the PCB levels tend to decline only very slowly, so that the end of the PCB exposure can hardly be expected in the near future [5]. Due to difficulties in the determination of ultratrace concentrations of these compounds in only small quantities of such complex matrices, a time trend can hardly be determined exactly.

Health effects of PCBs and chlorinated pesticides on humans are mainly known from occupational exposure or poisoning with the so called chloracne being the most common symptom. It has also been demonstrated that these organochlorines are transferred from mother to fetus and new-born babies through placenta and milk [1,4,6]. According to studies by Taylor et al. [7] and Leoni et al. [8], infants from mothers with higher, not environmentally related PCB concentrations were found to have significantly decreased birth masses and gestational ages and mothers with higher PCB levels have higher rates of miscarriages. It is suggested that environmental exposure to these pollutants through

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Table 3
Sample amounts used for Florisil clean-up

| Matrix | Sample amount | Activated Florisil (g) | Deactivated Florisil (g) |
|---|---|---|---|
| Whole blood | 2–5 ml | 6 | 9 |
| Serum | 2–5 ml | 6 | 9 |
| Plasma | 2–5 ml | 6 | 9 |
| Bone marrow | approx. 1 g | 4–6 | 15 |
| Tissues | 0.1–1 g | 4–6 | 15 |

tion procedure prior to the Florisil clean up. Sample amounts used are given in Table 3.

The clean-up procedure started by grounding the sample with Florisil (activated at 700°C for 12 h) after adding aldrin in hexane solution as an internal standard until a droughty powder was formed. This was transferred to the top of a prepacked column (I.D. 2.2 cm) containing the amount of Florisil deactivated with 3% water corresponding to the sample matrix (Table 3). The column was eluted stepwise with 60 ml hexane and 80 ml of hexane–dichloromethane (80:20, v/v). Both fractions were combined and the solvent was reduced in a rotavapor to approximately 5 ml and afterwards in a smooth nitrogen stream to a volume of 0.5 ml. This extract was used for gas chromatographic analysis.

## 3. Results and discussion

The method including the Florisil clean-up has been applied to a great variety of sample matrices for the determination of organochlorine pesticides and PCB congeners in our laboratories. The limits of detection with GC–MS/SIM respectively GC–ECD determination for this analytes are presented in Table 4 and Table 5, calculated using the baseline noise beneath the analyte signal. For the limit of detection a signal-to-noise ratio of 3, for the limit of determination a signal-to-noise ratio of 9 is chosen.

The method has been applied to 392 cord serum samples during a field study on possible long term neurodevelopmental effects of prenatal and neonatal background exposure to PCBs. A GC–ECD chromatogram of a cord serum sample is shown in Fig. 1. Since the analyte concentrations in the cord serum are very low due to their low fat content, dual-column GC–ECD was chosen for these samples. Detection limits obtained with ECD are approximately ten times lower than those obtained by MS SIM detection. The lack of selectivity shown by ECD could partially be compensated by the dual-column arrangement. The distribution of the PCB concentrations in cord blood samples is shown in Table 6.

The PCB content of 19 samples was below the limit of determination, these samples were not included in the calculations. Although the volume-based cord serum concentrations of the PCBs 138, 153 and 180 are rather low compared to those of adults' serum, they equal those of the adults' serum at the level of the fat based values [9]. This means, that the low PCB concentration in cord serum is caused by the low fat content of this matrix. The PCB concentrations measured during this study are in the range of PCB values measured in cord plasma during a Dutch study [12] as well as in studies from other countries [13–15]. A comparison of the PCB values from the Dutch study and from our study is given in Table 6, with our mean values being slightly higher.

In an additional study, the PCB and chlorinated pesticides concentrations in the bone marrow of 66 environmentally exposed persons were analyzed during a study to determine the correlation between the organochlorine compound concentrations in bone marrow and the appearance of diseases. The 66 samples analysed are the bone marrow samples of both, the patients and the control group. The results are shown in Table 7.

The results obtained are in agreement with literature values of the PCB content of bone marrow samples found by Dmochewitz and Ballschmiter in five bone marrow samples given in Table 7 as well [16]. As a remarkable result, the presence of PCB 52 in relatively high concentrations in 4 samples should be mentioned. PCB 52 normally does not occur in

volume was 1 μl using a retention gap of 1 m deactivated fused-silica (Hewlett Packard). Carrier gas was helium 5.0 at a constant flow-rate of 2.2 ml $min^{-1}$.

The MS interface was held on a temperature of 280°C, the MS source temperature was 200°C and ionisation energy was 70 eV. For quantification the masses shown in Table 1 were used.

GC–ECD: For the analysis of cord blood samples a dual-column gas chromatograph Mega 2 (Fisons Instruments) equipped with two programmed-temperature vaporisators (PTV injectors) with autosampler and two $^{63}Ni$ electron-capture detectors with nitrogen as make-up gas with a flow-rate of 60 ml $min^{-1}$ was used. Two columns were operated in parallel, a DB5-MS (30 m×0.32 mm, 0.25 μm) and a DB-1701 (30 m×0.32 mm, 0.25 μm; both J & W Scientific) the GC temperature program looked as follows: Initial 90°C held for 5 min then programmed at a rate of 15°C $min^{-1}$ to 270°C and held for 15 min.

Carrier gas was nitrogen at a column head pressure of 110 kPa. Make-up gas for the ECDs was nitrogen at a pressure of 150 kPa. 1 μl was injected splitless at injector temperature of 300°C.

### 2.4. Calibration and quantification

Calibration was performed using standard solutions of the analytes in hexane. Quantification was carried out using aldrin as an internal standard. Areas from the appropriate peaks, after correction relative to the aldrin response, were compared with peak areas from PCB congener standard solutions fortified with aldrin and analysed under the same conditions.

### 2.5. Recoveries

The recoveries of the analytes from serum after Florisil clean-up (Table 2) were used for calculation of the analyte content. Determination of recoveries was performed using bovine serum with a low native analyte content spiked to a level of 2 μg $l^{-1}$.

The serum was spiked by adding 100 μl of the analytes dissolved in hexane to 100 ml serum, after having been mixed on a rock-and-roller for 4 h the hexane was removed by blowing a gentle nitrogen stream over the serum surface. After final mixing this serum was used for the determination of recoveries.

### 2.6. Sample clean-up

A column liquid chromatographic clean-up technique based on a Florisil column [11] was modified and adapted to the different sample matrices. Human blood, serum and milk samples are prepared without sample pre-treatment, the determination of the fat content in serum samples was carried out with a commercial test kit for serum lipids. Samples like tissues or bone marrow must undergo a homogenisa-

Table 1
Masses used for quantification

| Analyte | Mass used for quantification ($m/z$) |
|---|---|
| Monochlorobiphenyl | 154 |
| Dichlorobiphenyl | 222 |
| Trichlorobiphenyl | 256; 258 |
| Tetrachlorobiphenyl | 292; 290 |
| Pentachlorobiphenyl | 326; 328 |
| Hexachlorobiphenyl | 360; 358 |
| Heptachlorobiphenyl | 394; 396 |
| Octachlorobiphenyl | 430 |
| Nonachlorobiphenyl | 460 |
| Decachlorobiphenyl | 498 |
| Aldrin | 263 |
| DDE | 246 |
| HCB | 284 |
| BHCs | 219 |

Table 2
Recoveries from serum after Florisil clean-up

| Analyte | Recoveries (%) | R.S.D. (%), $n=5$ |
|---|---|---|
| PCB-28 | 78 | 5 |
| PCB-52 | 77 | 2 |
| PCB-101 | 98 | 6 |
| PCB-138 | 110 | 3 |
| PCB-153 | 106 | 4 |
| PCB-180 | 112 | 8 |
| Aldrin | 91 | 5 |
| α-BHC | 87 | 6 |
| β-BHC | 77 | 8 |
| γ-BHC | 88 | 5 |
| HCB | 94 | 8 |
| $o,p$-DDE | 84 | 6 |
| $p,p$-DDE | 82 | 6 |
| PCB-69 | 81 | 5 |
| PCB-77 | 86 | 3 |
| PCB-118 | 86 | 5 |
| PCB-126 | 83 | 3 |

Table 4
Detection and determination limits of GC–MS

| | Analyte | Detection limit (pg abs) | Determination limit (pg abs) |
|---|---|---|---|
| Aldrin | (Internal standard) | 1.7 | 5 |
| α-BHC | α-Hexachlorocyclohexane | 4.0 | 12 |
| β -BHC | β-Hexachlorocyclohexane | 3.7 | 11 |
| γ -BHC | γ -Hexachlorocyclohexane | 4.0 | 12 |
| HCB | Hexachlorobenzene | 1.3 | 4 |
| o,p-DDT | | 2.3 | 7 |
| p,p-DDT | | 2.3 | 7 |
| o,p-DDE | | 2.7 | 8 |
| p,p-DDE | | 2.3 | 7 |
| PCB 8 | 2,4-Dichlorobiphenyl | 1.3 | 4 |
| PCB 28 | 2,4,4′-Trichlorobiphenyl | 1.0 | 3 |
| PCB 31 | 2,2′,5-Trichlorobiphenyl | 1.7 | 5 |
| PCB 40 | 2,2′,3,3′-Tetrachlorobiphenyl | 1.3 | 4 |
| PCB 47 | 2,2′,4,4′-Tetrachlorobiphenyl | 1.0 | 3 |
| PCB 52 | 2,2′,5,5′-Tetrachlorobiphenyl | 1.0 | 3 |
| PCB 69 | 2,3′,4,6-Tetrachlorobiphenyl | 2.0 | 6 |
| PCB 77 | 3,3′,4,4′-Tetrachlorobiphenyl | 2.0 | 6 |
| PCB 80 | 3,3′,5,5′-Tetrachlorobiphenyl | 2.0 | 6 |
| PCB101 | 2,2′,4,5,5′-Pentachlorobiphenyl | 2.0 | 6 |
| PCB118 | 2,3′,4,4′,5-Pentachlorobiphenyl | 2.7 | 8 |
| PCB126 | 3,3′,4,4′,5-Pentachlorobiphenyl | 2.0 | 6 |
| PCB138 | 2,2′,3,4,4′,5′-Hexachlorobiphenyl | 2.7 | 8 |
| PCB153 | 2,2′,4,4′,5,5′-Hexachlorobiphenyl | 2.7 | 8 |
| PCB169 | 3,3′,4,4′,5,5′-Hexachlorobiphenyl | 3.1 | 9 |
| PCB180 | 2,2′,3,4,4′,5,5′-Heptachlorobiphenyl | 10.0 | 30 |
| PCB185 | 2,2′,3,4,5,5′,6-Heptachlorobiphenyl | 10.0 | 30 |
| PCB209 | Decachlorobiphenyl | 23.3 | 70 |

Table 5
Detection and determination limits of dual-column GC–ECD

| Analyte | Detection limit (fg abs) | Determination limit (pg abs) |
|---|---|---|
| α-BHC | 400 | 1.2 |
| β-BHC | 400 | 1.2 |
| γ-BHC | 600 | 1.8 |
| HCB | 800 | 2.4 |
| p,p-DDE | 650 | 2.1 |
| Aldrin | 700 | 2.1 |
| PCB 28 | 350 | 1.1 |
| PCB 47 | 400 | 1.2 |
| PCB 52 | 500 | 1.5 |
| PCB 77 | 700 | 2.1 |
| PCB 101 | 400 | 1.2 |
| PCB 118 | 500 | 1.5 |
| PCB 138 | 300 | 0.9 |
| PCB 153 | 400 | 1.2 |
| PCB 180 | 300 | 0.9 |

human samples except for an actual exposure to this substance. An actual exposure of the four subjects with high PCB 52 levels could not be proven. As sample contamination, could be excluded and the same finding was revealed in former studies, we assume that bone marrow could be a compartment in which PCB can be stored without being accessible to metabolic degradation.

## 4. Conclusions

The presented method allows the sensitive and selective determination of chlorinated pesticides and PCBs in a great variety of human body fluids and tissues as shown on the examples of cord serum and bone marrow. High selectivity, required for the analysis of biological samples, has been achieved either by mass spectrometric detection or by the use

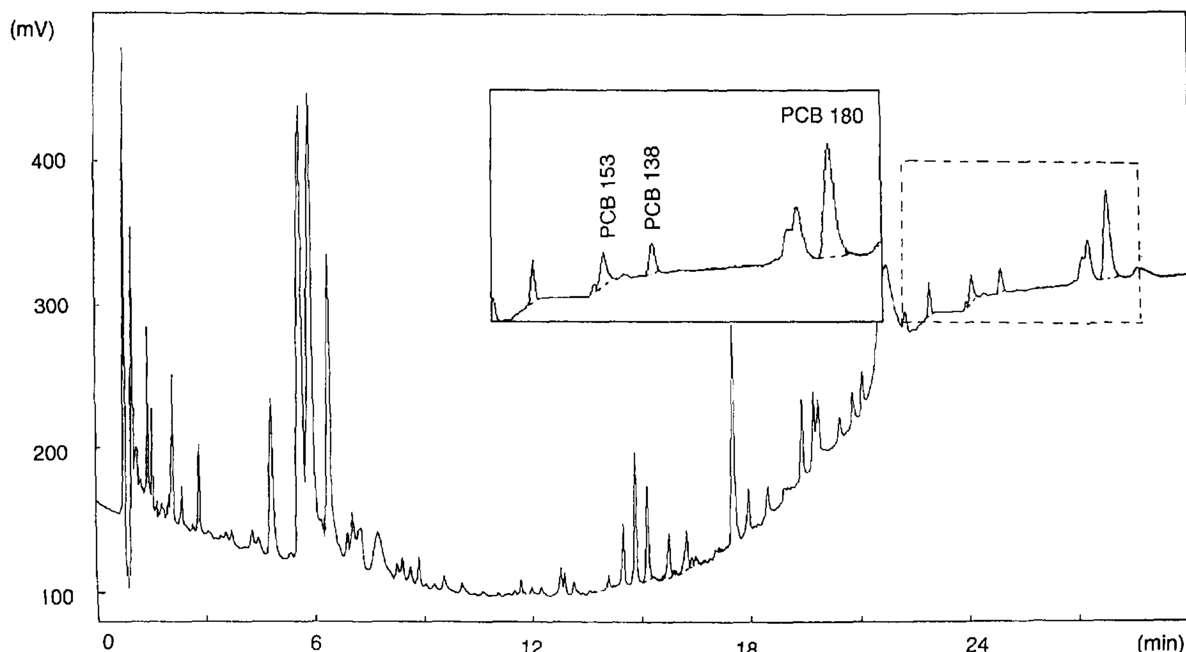


Fig. 1. GC–ECD chromatogram of a cord blood sample.

of a dual column set-up. The required sensitivity has been obtained by selected ion monitoring or by the use of ECD. As necessary for studies with environmental concern, the presented column clean-up allows a high sample throughput. Another way of a fast and reliable clean-up of biological samples is the selective extraction with supercritical fluids (SFE). A method involving SFE is in preparation [17].

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Table 6
PCB concentrations (μg/g) in cord serum samples

| Analyte | This study | | | | | Literature values [12] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Valid $n$ | Arithmetic Mean | S.D. | Minimum | Maximum | $n$ | Arithmetic Mean | Minimum | Maximum |
| PCB 138 | 373 | 0.17 | 0.07 | 0.05 | 0.73 (μg/l Serum) | 382 | 0.13 | 0.01 | 0.59 (ng/g Plasma) |
| PCB 153 | 373 | 0.21 | 0.08 | 0.06 | 0.78 (μg/l Serum) | 382 | 0.18 | 0.02 | 0.85 (ng/g Plasma) |
| PCB 180 | 373 | 0.18 | 0.07 | 0.05 | 0.64 (μg/l Serum) | 382 | 0.1 | 0.01 | 0.76 (ng/g Plasma) |
| PCB 138 | 373 | 0.06 | 0.03 | 0.01 | 0.26 (μg/g Lipids) | | | | |
| PCB 153 | 373 | 0.08 | 0.35 | 0.01 | 0.29 (μg/g Lipids) | | | | |
| PCB 180 | 373 | 0.07 | 0.03 | 0.01 | 0.21 (μg/g Lipids) | | | | |
| Total lipids | 376 | 2.98 | 1.18 | 0.66 | 7.69 (g/l Serum) | | | | |

Table 7
PCB concentrations ($\mu g/g$) in bone marrow samples

| Analyte | This study | | | | | Literature values [16] | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Valid $n$ | Arithmetic Mean | S.D. | Minimum | Maximum | Arithmetic Mean | $n$ | Minimum | Maximum |
| ß-BHC | 66 | 1.50 | 0.39 | 0.95 | 2.74 | 1.3 | 5 | 1.0 | 2.5 |
| $\gamma$-BHC | 66 | 1.03 | 0.27 | 0.61 | 1.83 | 0.1 | 5 | n.d. | 0.2 |
| HCB | 66 | 5.62 | 1.57 | 3.36 | 10.56 | 2.6 | 5 | 1.3 | 3.9 |
| PCB 52 | 4 | 1.05 | 0.27 | 1.25 | 1.87 | | | | |
| PCB101 | 66 | 0.10 | 0.027 | 0.07 | 0.18 | | | | |
| PCB138 | 66 | 2.99 | 0.83 | 1.88 | 4.91 | | | | |
| PCB153 | 66 | 4.74 | 1.27 | 3.05 | 8.47 | | | | |
| PCB180 | 66 | 3.42 | 0.96 | 2.17 | 5.67 | | | | |
| Sum PCB | | | | | | 6.4 | 5 | 1.1 | 11.8 |

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