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Method for the Determination of PCB Congeners and Chlorinated Pesticides in Human Blood Serum

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Determination of organochlorine compounds (OCCs) in human blood has engaged the attention of many analytical chemists for many years (Burse et al 1990; Gill et al 1996; Greizerstein et al 1997; Wolff et al 1991). This is because blood is regarded as a good matrix for monitoring of human exposure to OCCs and other xenobiotics. Some laboratories have carried out measurements on human adipose tissue which, though effective, involves a more complicated sampling procedure than blood. Blood is, however, a very complex matrix susceptible to a large variation in the determinations of fat or OCCs between laboratories or even within a laboratory. These problems can often be connected with sampling strategy, sample type (whole blood, serum or plasma), storage, extraction method or adequate quality control. The analytical techniques used are often extensive, expensive and tedious but significant progress has opened numerous possibilities for measuring low levels of OCCs in sera or plasma.

In this work serum is considered a suitable matrix since it is quite homogeneous and does not readily coagulate during freezing. Serum or plasma is usually the chosen matrix in many laboratories and this makes comparisons of results possible. In this paper results are presented from the development of a routine method for the determination of polychlorinated biphenyls (PCB) and chlorinated pesticides in human sera. The validity of the method has been checked by recovery experiments, repeated analysis of a quality control sample and by participation in WHO's intercalibration study on blood plasma. The method has been applied to more than 500 serum samples collected in different areas of Sweden. The results of those studies will be published separately.

MATERIALS AND METHODS

Blood was collected in 15 mL glass tubes without any anticoagulant. The serum was obtained by centrifugation (3000 rpm for 10 min) within 1 hr of collection and was kept frozen at -20 °C until analysis. Most of the PCB congeners and chlorinated pesticides used as standards were obtained from Dr Ehrenstorfer (Germany) or Community Bureau of Reference (Belgium). Diethyl ether, methanol, potassium carbonate (all three analytical quality, Merck), n-hexane (trace analysis quality, Merck), sulphuric acid (analytical quality, Acros Organics)

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and destilled water could be used without any further purification. The silica gel (Kiselgel 60 0.063-0.200 mm, Merck) was activated over night at 360 °C and subsequently deactivated with 3 % (w/w) of destilled water.

Thawed serum (4 g) was mixed with methanol (4 mL) by vortexing in a 16 mL test tube with a Teflon-lined screw-cap. After addition of 200 µl of an internal standard solution, containing 2.2 ng of PCB 189 and 5 ng of *o,p'*-DDD, the mixture was extracted with 5 mL of n-hexane / diethyl ether (1:1 v/v). The test tube was left on a rotatory mixer (Falc intruments) for 15 min and then centrifuged at 2500 rpm for 10 min (Hettich Rotanta /T). The organic upper layer was collected in a weighed test tube and the extraction was repeated twice. The solvents from the combined organic extracts were removed using a gentle stream of nitrogen and the fat weight determined gravimetrically.

In order to remove the lipids and other polar materials the extracted fat was re-dissolved in 2 mL of n-hexane and treated with 8 mL of concentrated sulphuric acid. After separation (centrifugation at 3000 rpm for 15 min) and collection of the organic phase, the acid phase was extracted once more with another 2 mL portion of n-hexane. The organic phases were combined and the volume reduced to 0.5 mL by a gentle stream of nitrogen. The extract was transferred to an open silica gel column (8 mm id, 4.5 g of 3% water deactivated silica gel with a 0.5 cm layer of potassium carbonate added on the top). Elution of the column with about 30 mL of n-hexane gave a fraction (F1) containing the PCB congeners, HCB and *p,p'*-DDE. The exact volume of F1 was earlier determined by a test run with a standard solution containing DDT and its metabolites. DDT should not be eluted into F1. A second fraction (F2), containing mainly the chlorinated pesticides, was eluted with 40 mL of n-hexane / diethyl ether mixture (3:1 v/v). The obtained fractions (F1 and F2) were each reduced to 1 mL and treated with 2 mL of concentrated sulphuric acid. The organic phases were collected after centrifugation at 2500 rpm for 2 min. The acid phase was extracted in each case with another 1 mL of n-hexane and the organic phases were combined. The fractions were concentrated to 200 µL and were kept in Teflon-lined capped microvials until analysis.

Further fractionation of the silica gel fraction F1 was performed for some samples on HPLC in order to confirm the levels of the PCB-congeners. A part of the concentrated fraction (100 µL) was injected into a previously equilibrated HPLC system. The system consisted of Gilson 305/307 pumps, a Rheodyne 7725 valve injector equipped with a 100 µl loop, a Gilson 118 UV/Vis detector operating at 254 nm, a Gilson fraction collector (FC 205) and a hypercarb column packed with 7 µm porous graphite carbon (100 x 4.7 mm, Shandon Scientific Ltd, Cheshire, England). Hexane was used as the mobile phase at a flow rate of 1 mL/min. Two fractions were collected at 0-4 mL and 4-10 mL containing mainly the di-*ortho* and mono-ortho congeners, respectively. In order to clean the column after the elution of a series of samples, a motor-controlled valve actuator (Gilson Model 817) was used to reverse the direction of the column flow and the mobile phase

was changed to dichloromethane (2 mL/min). The planar PCBs are normally obtained in this manner (Atuma and Hansson 1994).

Gas chromatographic (GC) analysis was performed on a Hewlett-Packard Model 5890A instrument equipped with a HP 7376 auto sampler, a dual capillary column system, two electron capture detectors (ECD, ^{63}Ni) and a Hewlett-Packard 3365 data system. Two different columns were used, an Ultra-2 column, 50 m x 0.20 mm with a 0.33 µm film (Hewlett-Packard) and a DB-17 column, 60 m x 0.25 mm with a 0.25 µm film (J & W Scientific). Helium was used as a carrier gas at a flow rate of 0.6 and 1.3 mL/min, respectively, and nitrogen as the make-up gas (40-60 mL/min). The initial oven temperature was 105 °C, held for 1.3 min and programmed to 210 °C at 20 °C/min, held for 2 min, then to 260 °C at 2 °C/min, held for 5 min, and finally to 270 °C at 5 °C/min. The final temperature was held for 8 additional min. The injector and detector temperatures were set at 200 °C and 300 °C, respectively. Sample volumes of 2 µL were injected in the splitless mode with the valve closed for 90 sec. The identification of the different substances was based on their retention times relative to the internal standards. Quantification was performed using multi-level calibration graphs obtained by injection of standard solutions of at least four different concentrations. In the graphs peak height ratios [OCC response / internal standard response] were plotted against the concentrations of the OCC.

In order to test the performance of the method the recovery of 10 PCB congeners and 10 chlorinated pesticides (Table 1) in 4 g of serum was tested by a standard addition procedure. 5 unspiked and 8 spiked samples from the same batch of pooled serum (an in-house control sample) were analysed. The PCB congeners were added at two levels (0.5 and 3 ng) in duplicate samples and the chlorinated pesticides were added at one or two levels between 0.1 - 3 ng (duplicate samples at each level).

A reagent or extraction blank, consisting of destilled water in equal amount to the sample, was included in every batch of samples.

RESULTS AND DISCUSSION

The limits of detection (LOD) for the specific compounds were determined as 3 times the standard deviation of the blank (Long and Winefordner 1983). The LODs varied between 1 and 7 pg/g serum for the PCB congeners and between 2 and 7 pg/g serum for the chlorinated pesticides. The quantification limits were set at levels corresponding to the lowest standard concentration used, which was 10 pg/g serum for the PCB congeners, HCH-isomers and chlordanes, 20 pg/g for *p,p'*-DDD, *p,p'*-DDT and *o,p'*-DDT, 50 pg/g for HCB and 200 pg/g for *p,p'*-DDE.

The concentration reported, for each substance, was the average of the values obtained from the two GC columns when the difference between the values was less than 20 %. If the difference was more, the lower level was reported. PCB 167

Table 1. Recoveries of PCBs and chlorinated pesticides in serum spiked at two levels (duplicate samples at each level). The incurred levels can be found in Table 2.

Compound	Level 1		Level 2	
	Added amount (pg/g serum)	Recovery (%)	Added amount (pg/g serum)	Recovery (%)
PCB 28	130	102	784	91
PCB 52	115	96	691	88
PCB 101	131	116	784	98
PCB 118	131	103	785	95
PCB 153	-	-	805	112
PCB 105	124	77	748	90
PCB 138	125	92	752	87
PCB 167	126	104	882	93
PCB 156	114	92	686	97
PCB 180	-	-	768	94
HCB	-	-	125	118
α-HCH	50	84	-	-
β-HCH	-	-	483	88
γ-HCH	25	78	-	-
Oxychlordane	42	93	424	96
Transnonachlor	91	90	905	91
p,p'-DDE	-	-	625	91
p,p'-DDD	250	100	-	-
p,p'-DDT	212	104	-	-
o,p'-DDT	250	88	2120	100

and P-HCH were always quantified on Ultra-2 since there were problems with co-elution on DB-17. Furthermore, PCB 138 was always quantified on DB-17 because of co-elution on Ultra-2.

Separation of the mono-*ortho* and di-*ortho* PCB congeners was performed on HPLC to confirm the analytical results for the PCBs. The levels of PCB congeners obtained by analysis directly after silica gel separation were in good agreement with those obtained by analysis of the HPLC fractions. Since the HPLC separation seemed unnecessary in this case it was only applied to a small number of samples.

The reproducibility of the method was demonstrated by 21 replicate determinations using an in-house control serum sample (Table 2). The results were obtained from analyses performed by different analysts using different equipment during a period of ca 1.5 years. The coefficients of variations (CV) were less than 13 % for most of the compounds. For PCB 28 and PCB 105,

Table 2. Reproducibility of the method demonstrated by 21 replicate determinations of individual PCBs and chlorinated pesticides in one batch of pooled blood serum. The concentrations reported are based on serum fresh weight.

Compound	Range (pg/g)	Mean (pg/)	CV(%)
PCB 28	19-41	31	22
PCB 52*	-	<10	-
PCB 101*	-	<10	-
PCB 118	212-288	248	8
PCB 153	1130-1560	1310	9
PCB 105	22-44	33	20
PCB 138	536-752	638	10
PCB 167	42-62	50	12
PCB 156	85-123	110	7
PCB 180	854-1060	955	6
HCB	385-522	440	11
α-HCH*	-	<10	-
β-HCH	543-927	743	13
γ-HCH*	-	<10	-
Oxychlordane	63-90	76	9
Transnonachlor	127-153	137	6
p,p'-DDE	4000-6140	5260	10
p,p'-DDD*	-	<20	-
p,p'-DDT	120-218	177	12
o,p'-DDT*	-		-

* PCB 52, PCB 101, α-HCH, γ-HCH, *p,p'*-DDD and *o,p'*-DDT were either not detected or detected but not quantifiable.

however, the CVs were 22 and 20 % respectively, which can be attributed to the low concentrations of these compounds. The average fat content of the control sample, which was determined gravimetrically, was 0.60 % with a CV of 4 %. The analytical results for two PCB congeners and *p,p'*-DDE are presented in Figure 1. The reliability of the method is shown by non-significant increase or decrease of the levels during the period of 1.5 years.

The recoveries of the 10 PCB congeners and 10 chlorinated pesticides analysed, were investigated by a standard addition experiment. The results from spiking the in-house serum sample at two levels for both the PCBs and chlorinated pesticides are shown in Table 1. The incurred levels in the sample were quite high (Table 2) and therefore the quantification of the added amounts of the OCCs was in some cases difficult. The average recoveries of the different PCB congeners were 98 ± 12 % and 94 ± 8 % for 0.1 and 0.8 ppb levels, respectively. The recoveries for the chlorinated pesticides varied from 78 to 118 %.

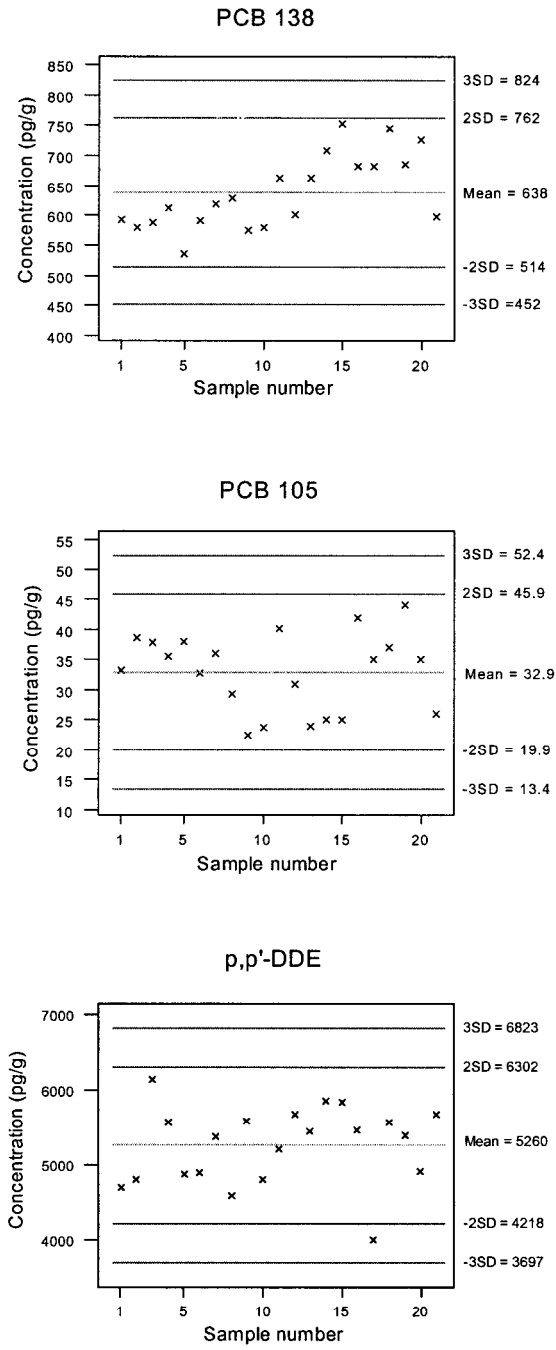

Figure 1. Results for two PCB congeners and *p,p'*-DDE in 21 analytical runs of an in-house serum control sample.

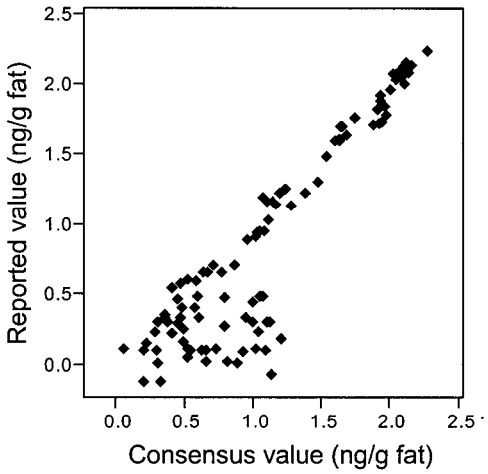

Figure 2. Reported values versus consensus values of PCBs in WHO's inter-calibration study on blood plasma. The values are log-transformed.

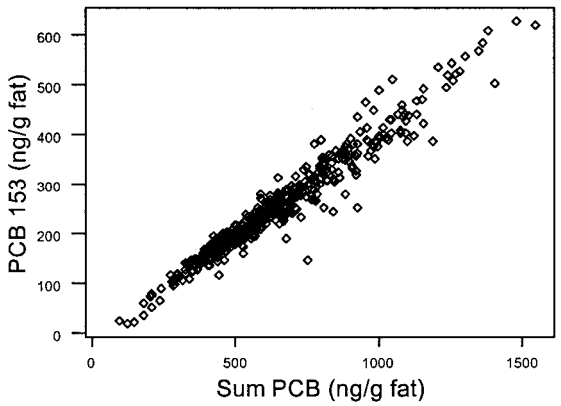

Figure 3. Correlation between the concentration of PCB 153 and the sum of PCB in 460 serum samples from Swedish men and women (r = 0.95).

Apart from the above quality control measures the laboratory participated in the fourth round of "WHO's interlaboratory quality assessment study on human milk and blood" which included analysis of PCBs in blood plasma (WHO 1997). In Figure 2 our results are compared with the consensus values for 8 samples of blood plasma. They were in good agreement with the consensus values. The largest deviations were found for PCB congeners detected at lower levels.

PCB 153, the major PCB congener in the serum samples, has earlier been shown to correlate to the total amount of PCB in some studies of e.g. human milk and fish (Atuma et al 1998a; Atuma et al 1998b). Analysis of 460 serum samples from Swedish men and women (40-75 years) gave a high correlation (r = 0.95) between PCB 153 and the sum of the analysed PCB congeners (Figure 3). Therefore, if the

interest in a monitoring study is limited to the total PCB, savings can be made by analysing only PCB 153, which then serves as an indicator or marker.

In conclusion, a simple method for the analysis of individual PCB congeners and chlorinated pesticides in human blood serum has been developed and validated. The method can be used for measuring low-level exposures which can be essential in epidemiological studies.

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