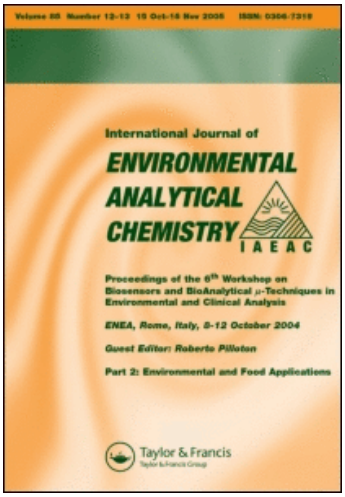

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The Determination of Polychlorinated Biphenyl Congeners and Other Chlorinated Hydrocarbon Residues in Human Blood, Serum and Plasma. A Comparative Study

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THE DETERMINATION OF POLYCHLORINATED BIPHENYL CONGENERS AND OTHER CHLORINATED HYDROCARBON RESIDUES IN HUMAN BLOOD, SERUM AND PLASMA. A COMPARATIVE STUDY

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A minimum acceptable volume of blood for the simultaneous determination of the lipid and chlorinated hydrocarbon content was established at 50 ml. At that volume the coefficients of variation for lipid and chlorinated hydrocarbon residue determinations were < 10 and 20% respectively. Comparing chlorinated hydrocarbon levels in whole blood with serum and plasma from the same blood pool, indicated that approximately 25% of the tri-to penta chlorobiphenyls were found in the serum and 40% in the plasma. In contrast, 55–83% of the hexa-to octachlorobiphenyls were found in the serum and plasma. From among the organochlorine pesticides, between 80–110% of the trans-nonachlor and DDTs appeared in the serum and plasma, but for others this range was considerably lower (40–71%). In general no appreciable differences were observed in the recoveries of some chlorinated hydrocarbons from fortified blood and serum.

KEY WORDS: Blood, serum, plasma, PCBs, distribution.

INTRODUCTION

The literature on the determination of polychlorinated biphenyls (PCBs) and organochlorine (OC) pesticides in human blood reveals that some investigators used whole blood[1,2], many others used serum, especially in monitoring studies[3,4], while a few preferred using plasma[5,6]. Comparison of the results of these studies has often been hampered by omission of information on haematocrit values and/or the distribution of the chlorinated hydrocarbon residues in the different blood components. In some instances however this distribution has been determined for specific organic chlorine pesticides in selected blood components. For example Radomski *et al.*[7] compared some OC pesticide levels in human blood and plasma as affected by dietary intake,

while Mick *et al.*[8] studied levels of dieldrin and aldrin in various blood fractions including erythrocytes and plasma. Although specific PCB congeners have been determined previously[6,9,10] in separate studies of human blood, serum and plasma, the authors of this paper are not aware of any data available on their distribution in blood and its components obtained from the same blood pool.

This paper reports on the results of a study to determine the minimum volume of blood necessary to efficiently carry out lipid and residue analyses and to evaluate the use of blood, serum and plasma in the determination of various chlorinated hydrocarbon residues, using serum and plasma from the same blood pool.

EXPERIMENTAL

Materials

All glassware was heat-treated and rinsed with acetone and hexane before use as reported earlier by Mes[11]. Glass-distilled solvents were tested for interfering residues by gas chromatography (GC) after a 300-fold concentration. Glasswool and adsorbents were washed in a soxhlet with dichloromethane as described previously[11]. All PCB congeners (numbered according to Ballschmiter and Zell[12]) were 90–99% pure, except for the 2,3,4,2'-tetrachlorobiphenyl (PCB No. 41) which was only 34% pure, and were purchased from Ultra Scientific Inc. (Hope, R.I., U.S.A.) or Wellington Laboratories/Guelph, Ont., Canada). Other chlorinated hydrocarbons were >95% pure and were gifts from the Environmental Protection Agency (Triangle Park, N.C., U.S.A.) or purchased from Ultra Scientific Inc. Three mixtures of chemical standards (A, containing PCBs, trans-nonachlor, chlorobenzenes, and p,p'-DDE; B, containing hexachlorocyclohexanes, oxychlordane and p,p'-DDT; C, containing heptachlor epoxide and dieldrin) were made up, containing 1–58, 0.5–5 and 2 pg per μl hexane respectively and with concentrations of each chemical depending on their GC detector response. In addition a fortification solution was prepared with concentrations of the various residues in hexane as given in Table 1. A ten- and hundred-fold dilution of this stock solution was used for fortifying blood and its components at lower concentrations.

Sampling

All blood, serum and plasma samples were collected and/or prepared by the Blood Centre of the Canadian Red Cross Society in Ottawa (Ont., Canada) and prepared as follows, using citrate as anticoagulant: ~500 ml of whole blood (sample I), kept at 4°C, was delivered in a plastic blood pack and immediately used for preliminary work as outlined below. Furthermore 3 units of approximately 500 ml of whole blood each, having the same blood group, were pooled (sample II) and a 200 ml aliquot removed to a transfer pack and labelled pooled whole blood (sample II B). A further two aliquots (sample II) of 500 ml each were similarly removed for the preparation of pooled serum and pooled plasma. The serum was prepared by addition of 14 ml of 0.5 M $CaCl_2 . 2H_2O$ to one of the 500 ml aliquots. The blood was allowed to

Table 1 Concentrations of various chemicals in the stock solution used for fortification experiments

Compound	*Concentration (pg μl^{-1})*
2,4,4'-Trichlorobiphenyl (PCB No. 28)	498
2,5,2',5'-Tetrachlorobiphenyl (PCB No. 52)	410
2,4,5,3',4'-Pentachlorobiphenyl (PCB No. 118)	592
2,3,4,2',4',5'-Hexachlorobiphenyl (PCB No. 138)	436
2,4,5,2',4',5'-Hexachlorobiphenyl (PCB No. 153)	664
2,3,4,5,2',4',5'-Heptachlorobiphenyl (PCB No. 180)	456
2,3,5,6,2',3',4',5'-Octachlorobiphenyl (PCB No. 210)	435
Hexachlorobenzene	598
t-Nonachlor	476
β Hexachlorocyclohexane	549
Dieldrin	503
p,p'-DDT	503

coagulate for 2 hrs at 37°C, continuing overnight at 4°C. The resultant supernatant serum was expressed into a transfer pack and marked 'pooled serum' (sample II S). The remaining 500 ml aliquot was centrifuged at 7000 g for 7 min at 4°C and the supernatant also transferred to a transfer pack and designated as 'pooled plasma' (sample II P). A platelet count was carried out on a small aliquot of plasma in order to confirm that a platelet poor plasma was obtained.

A final supply of pooled blood (sample III B) and pooled serum (sample III S), prepared as above, was received, kept at 4°C and used for the fortification experiment as described below.

Lipid determination

In order to determine the minimum volume of blood, which would result in the most precise gravimetric lipid determination and at the same time supply sufficient chlorinated hydrocarbons to provide the necessary GC sensitivity to quantitate these residues with a certain degree of confidence, triplicate analysis of 25, 50 and 100 ml blood samples were carried out. Samples were weighed in 250 ml centrifuge tubes and extracted with an equal volume of benzene using a Silverson homogenizer as described earlier[13]. After centrifugation of the extract for 15 min at 600 g, 3/4 of the supernatant was removed by volumetric pipette and filtered through anhydrous Na_2SO_4. The filtrate was evaporated to just dryness on a rotary—evaporator (<30°C) and redissolved in hexane. The concentrated filtrate was then transferred to a preweighed aluminum dish, evaporated to dryness in the fumehood and the benzene extractable lipids weighed. Serum triglycerides were measured by the Canadian Red Cross in sample II S, using the method of Gottfried and Rosenberg[14] and triolein as a reference.

Separation and cleanup

The lipids were redissolved in hexane and chromatographed on 6.5 g activated (300°C) Florisil®. Three fractions were collected in the following order: 50 ml hexane (fraction 1), 60 ml of 20% CH_2Cl_2 in hexane (fraction 2) and 60 ml of 60% CH_2Cl_2 in hexane (fraction 3). The first fraction contained all chlorinated benzenes, PCBs, t-nonachlor (t-NCl) as well as most p,p'-DDE. The second fraction contained all oxychlordane (OCl), pp'-DDT and most hexachlorocyclohexane isomers and some p,p'-DDE. Heptachlor epoxide and dieldrin were eluted in the last fraction. The fractions were concentrated to ~1 ml and quantitatively transferred to 15 ml stoppered centrifuge tubes. The volumes were appropriately adjusted for GC analysis either by concentration under a gentle stream of N_2 or by dilution with hexane.

Identification and quantitation

Residues in fractions 1 and 2 were identified and quantitated on either a Varian Series VA3400 or VA3500 gas chromatograph, containing a 30 m × 0.24 mm (i.d.) fused silica column, coated with 0.25 μm DB-5 (J & W Scientific Inc., Folson, CA, U.S.A.) and a ^{63}Ni electron capture detector (ECD). The injector temperature was programmed from 70°C, held for 0.5 min, to 240°C at 160° min^{-1} and kept at this final temperature for the duration of the chromatographic run. The initial column temperature of 70°C was held for 1 min and then increased to 130°C at 50° min^{-1} held for 1 min and further increased to 190°C at 4° min^{-1} and finally to 230°C at 2° min^{-1} and held at that temperature until the end of the chromatographic run. The detector temperature was 300°C. The linear velocity of the He carrier gas was 50 cm/sec^{-1} and the flowrate of the nitrogen make-up gas was 30 ml min^{-1}. Aliquots (1 μl) of standards A and B, for fractions 1 and 2 respectively, were injected before and after every three sample injections (1 μl).

Residues in fraction 3 were identified and quantitated using a Varian VA 3700 GC with a 0.6 × 183 cm glass column packed with 6% OV-210 + 4% SE-30 on chromosorb W(AW) 100/120 mesh. Injector, column and detector temperatures were 232, 218 and 303°C respectively. The N_2 carrier gas flowrate was 30 ml min^{-1}. Aliquots (5 μl) of standard C and samples were injected in the same sequence as above.

The detection limit by GC/ECD for all chemical residues reported in this paper was 10 pg g^{-1}, except for the 1,2,3-trichlorobenzene (TCBZ) and 2,4,2',5'-tetrachlorobiphenyl (PCB No. 49) residues, both of which had detection limits at 40 pg g^{-1}.

Confirmation

The tri- to decachlorobiphenyls were confirmed by mass spectrometry (MS) using a Hewlett-Packard 5971 A MSD instrument with the same capillary column as mentioned above. The on-column injector temperature was programmed from 70° to 280°C at 100°C min^{-1}. The column temperature was successively increased from 70°C (held for 2 min) to 210°C (held for 4 min), again to 250°C (held for 5 min) and finally to 270°C at rates of 20, 5 and 15°C min^{-1}, respectively. The final temperature

was kept for the duration of the chromatographic run. Multiple ion detection (MID) analysis of PCBs in pooled fractions of blood, serum and plasma were carried out using the following ions (m/z): 256 and 258 for tri-, 290 and 292 for tetra-, 324, and 326 for penta-, 360 and 362 for hexa-, 394 and 396 for hepta-, 428 and 430 for octa-, 462 and 464 for nona-, and 498 and 500 for decachlorobiphenyls. The dwell time ranged from 35–50 msec, depending on the ion. The following organochlorine pesticides were similarly monitored: trichlorobenzenes (m/z = 180, 182), HCB (m/z = 284, 286), HCH isomers (m/z = 181, 183), oxychlordane (m/z = 387, 389), t-nonachlor (m/z = 407, 409), p,p'-DDE (m/z = 246, 248) and p,p'-DDT (m/z = 235, 257).

Quality control

Fifty gram samples of whole blood and serum were fortified with the compounds listed in Table 1 at the 0.1, 1 and 10 ng g^{-1} level. The samples were thoroughly mixed and allowed to equilibrate for 45 min before analysis. The fortification experiment was carried out in quadruplicate, while all other samples were analyzed in triplicate. Solvent blanks were run through the entire analytical procedure.

RESULTS AND DISCUSSION

The effect of sample volume on the precision of the blood lipid determination is shown in Table 2. The results indicate that by increasing the sample volume from 25 to 50 or 100 ml, the coefficient of variation (CV) improved from 21 to <10, although the mean % lipid remained relatively constant. Therefore a blood sample volume of 50 ml was considered the minimum acceptable volume in this study with regard to the blood lipid determination. To establish whether this volume would be

Table 2 The effect of blood volume on the precision of the blood lipid determination

Aliquot[a] *No.*	*% Lipid*		
	Blood volume (ml)		
	25	*50*	*100*
1	0.17	0.22	0.21
2	0.24	0.22	0.18
3	0.25	0.22	0.21
Mean[b]	0.22 (21)[c]	0.22 (0)	0.20 (8)

[a] Aliquots of pooled blood sample 1.
[b] Arithmetic.
[c] Figure between ( ) is coefficient of variation.

Table 3 The effect of blood volume on the precision of residue determination

Residue	*ng g⁻¹ Whole blood*					
	25 *ml*		50 *ml*		100 *ml*	
	Mean[a]	*CV*	*Mean*	*CV*	*mean*	*CV*
HCB	0.08	38	0.04	14	0.09	4
β HCH	0.10	30	0.10	14	0.09	17
γ HCH	0.09	41	0.07	7	0.08	7
Oxychlordane	0.06	31	0.05	5	0.05	8
t-Nonachlor	0.08	12	0.07	8	0.05	15
Dieldrin	0.09	40	0.06	3	0.06	12
Heptachlor epoxide	0.03	9	0.06	13	0.02	17
p,p'-DDE	1.44	34	1.41	7	1.26	7
p,p'-DDT	0.10	22	0.08	3	0.08	6
PCBs (as Aroclor 1260)[b]	1.00	0	0.90	11	1.00	0

[a] Arithmetic.
[b] Quantitiated according to Mes *et al.* (1986)[15].
[c] Based on triplicate determinations.

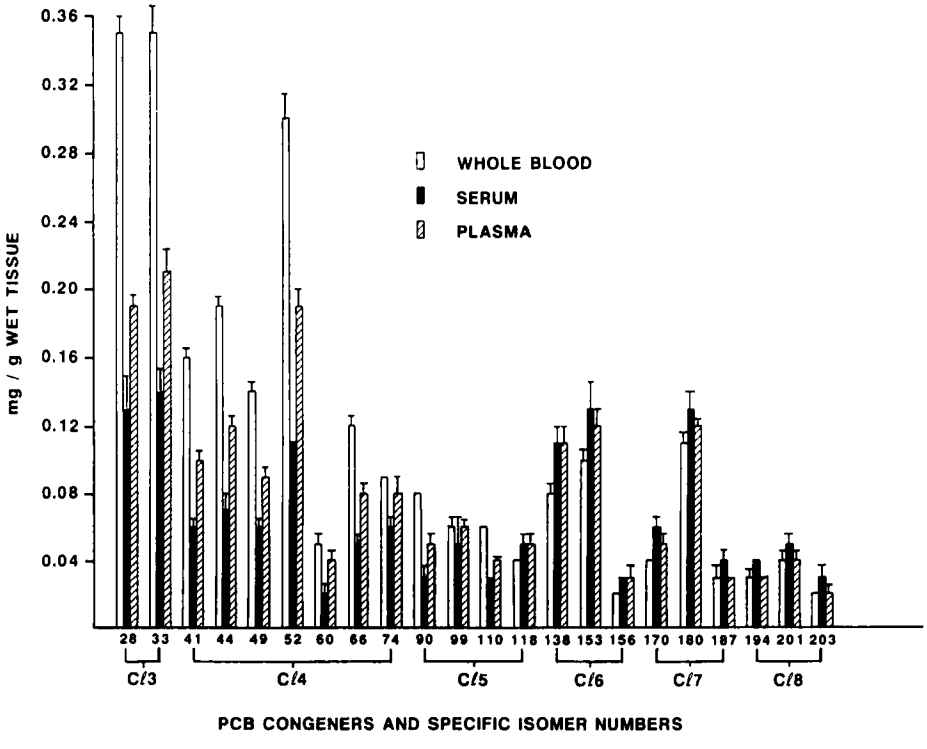

Figure 1 Levels of PCB congeners in whole blood, serum and plasma from the same pooled blood sample.

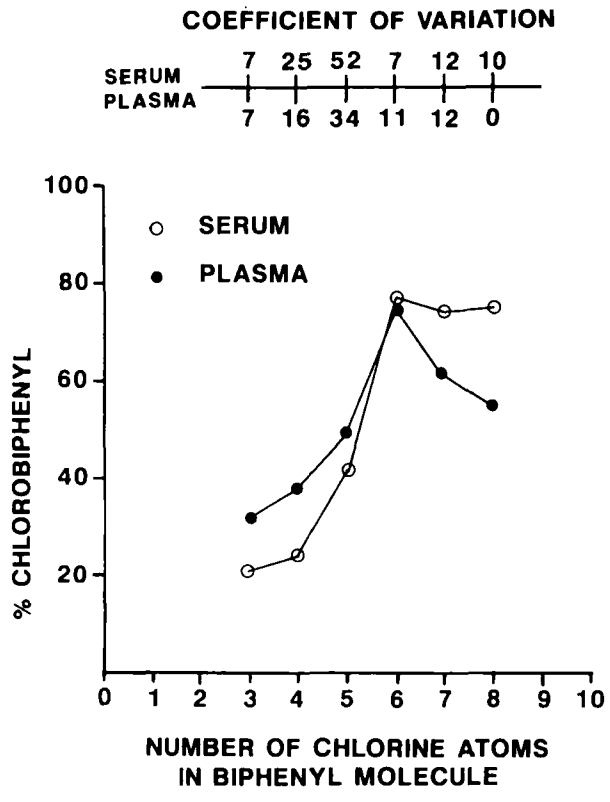

Figure 2 Relationship between the degree of chlorination of the biphenyl molecule and the percent distribution of PCB homologs in serum and plasma.

sufficient for the analysis of chemical residues, a selected number of residues were determined in 25, 50 and 100 ml volumes of the same blood pool as used for the lipid determination above. The results are summarized in Table 3.

The Student t-test showed no significant differences in residue levels between the three blood sample volumes used, except for heptachlor epoxide. For HCB a significant difference was only observed between residue levels in the 50 and 100 ml blood samples. It is difficult to explain these discrepancies, although the possibility of external contamination during one of the analytical steps may not be dismissed, especially considering the very low residue levels. Furthermore the data in Table 3 indicate that with few exceptions, the CV's are lower for residue levels in blood samples greater than 25 ml. Therefore the use of 50 ml blood volumes for residue analysis in further experimental work seemed to be justified.

Having established a minimum workable sample size, a comparison was made between residue levels in whole blood (sample II B), serum (sample II S) and plasma (sample II P). The results are graphically illustrated in Figures 1 and 3. All values are the result of triplicate determinations with the standard deviations indicated by

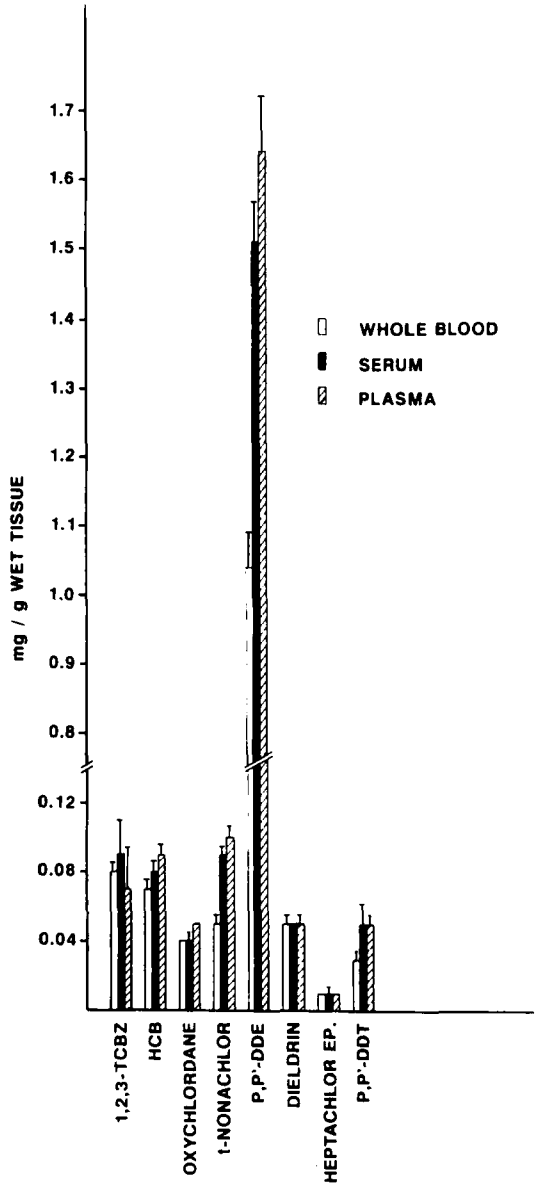

Figure 3 Levels of some organochlorine pesticides in whole blood, serum and plasma from the same pooled blood sample.

the vertical lines on each bar. The serum values, however, were not corrected for the slight dilution during serum preparation. The PCB congeners reported were confirmed by GC-MS, except for the 2,4,3',4'-tetrabiphenyl (PCB No. 66) congener for which no standard was available at the time. All tri- and tetrachlorobiphenyl

Table 4 Percent distribution of PCB congeners in serum and plasma

PCB No.[a]	*% PCB congener*		*PCB No.*	*% PCB congener*	
	Serum	*Plasma*		*Serum*	*Plasma*
28	20	30	110	28	37
33	22	33	118	69	69
41	21	34	138	76	76
44	20	35	153	72	66
49	22	33	156	83	83
52	20	35	170	83	69
60	22	44	180	65	60
66	23	37	187	73	55
74	37	49	194	73	55
90	21	34	201	69	55
99	46	55	203	83	55

[a] Numbering system according to Ballschmiter and Zell[12].

congeners and two of the pentachlorobiphenyls (PCB Nos. 90 and 110) were considerably higher in the whole blood than in the serum and plasma, while in turn these congener levels in plasma were higher than in serum. For the higher chlorinated biphenyls the reverse was true. Based on the average haematocrit value of 0.45, the percent distribution of each congener in serum and plasma was calculated and the data shown in Table 4. The results indicate that most of the lower chlorinated biphenyls in the blood are associated with the red blood cell fraction under the given conditions of serum and plasma preparation. This assumption is supported by earlier work of other investigators[16,17], where the lower chlorinated biphenyls were not associated with the serum lipids. Indirectly the work of Dewailly *et al.*[6] may point to the same phenomenon. These authors found considerably lower levels of pentachlorobiphenyls than hexa- or hepta chlorobiphenyls in plasma. Therefore the use of serum or plasma for the determination of these congeners is questionable. The relationship between the number of Cl-atoms in the biphenyl molecule and the

Table 5 Percent distribution of organochlorine pesticides in serum and plasma

Residue	*% Residue*	
	Serum	*Plasma*
1,2,3-Trichlorobenzene	62	48
Hexachlorobenzene	63	71
Oxychlordane	55	69
Trans-nonachlor	99	110
p,p'-DDE	80	87
Dieldrin	55	55
Heptachlor epoxide	55	55
p,p'-DDT	92	92

Table 6 Recoveries of selected chlorinated hydrocarbons from whole blood and serum at various levels of fortification

Chemical residue	*% Recovery*					
	Level of fortification (ng g^{-1})					
	0.1		1.0		20.0	
	III B[ab]	IIi S[c]	III B	III S	III B	III S
PCB No. 28	182	176	160	149	155	147
PCB No. 52	104	107	101	95	99	97
PCB No. 118	99	103	93	87	93	91
PCB No. 138	100	114	95	91	99	95
PCB No. 153	66	78	68	64	72	67
PCB No. 180	105	101	98	91	103	93
PCB No. 201	136	122	122	112	131	118
HCB	89	65	76	120	99	114
β HCH	85	79	91	100	103	99
t-Nonachlor	105	125	112	111	121	115
Dieldrin	112	66	94	86	115	74
p,p'-DDT	88	110	105	109	120	104

[a] Based on quadruplicate determinations and corrected for background levels.
[b] Blood.
[c] Serum.

percent distribution of the PCB congeners in serum and plasma is shown in Figure 2. The percent distribution in the serum and plasma increases with increased chlorine content of the biphenyl molecule up to the hexa- chlorobiphenyls, after which it remains constant in serum, but slightly decreases in plasma.

The residue levels in Figure 3 show that for many of the OC pesticides the residue levels in serum and plasma were equal to or somewhat larger than those in whole blood, except for trans-nonachlor, p,p'-DDE and p,p'-DDT, which levels were considerably higher. The percent distribution of the OC pesticides in serum and plasma is given in Table 5, the results indicate that only t-nonachlor, p,p'-DDE and p,p'-DDT could accurately be determined in serum or plasma as an alternative to whole blood. For other OC's a conversion factor based on the percent distribution, would be required. Radonski *et al.*[7] estimated that 25% of the p,p'-DDT was in the red blood cells. In our study this percentage appears to be somewhat lower.

The mean lipid content based on triplicate determinations for the whole blood (II B), serum (II S) and plasma (II P) was 0.17, 0.18 and 0.14% respectively, with a CV < 1 in all cases. The triglyceride content, measured as triolein, in the serum was 0.11%, indicating that the benzene extracted more than just the triglycerides. The reported chlorinated hydrocarbons in Figure 3 were confirmed by GC-MS, except for dieldrin and heptachlor epoxide, where interferences from co-eluted lipids made this difficult. Although β and γ HCH were measured by GC-ECD, their presence could not be confirmed in blood, serum or plasma of pooled blood sample II. This is remarkable especially for β HCH, where in the GC-ECD elution pattern a large

peak appeared with identical retention time to β HCH. In addition β HCH has been frequently reported not only in serum[18], but also in human breast milk[14] and adipose tissue[19].

To eliminate the possibility that the differences in residue levels between whole blood and serum and/or plasma were partly the result of the applied methodology, a fortification experiment was carried out for whole blood (sample III B) and serum (sample III S). The results are given in Table 6 for three levels of some selected chlorinated hydrocarbons. Actual residue levels of most of these selected chlorinated hydrocarbons found in the blood and serum samples used for fortification were close to the 0.1 ng g^{-1} level. At this fortification level reasonably good agreement between recoveries from blood and serum were obtained except for dieldrin. The poor recovery of this compound relative to the whole blood, may be reflected in the percent distribution in serum and plasma (Table 5). Acceptable recoveries from either blood or serum were obtained for all compounds at all levels of fortification, except for PCB No. 28, 153 and 201, while HCB, t-nonachlor and dieldrin were occasionally either under- or overestimated. Nevertheless overall recoveries of t-nonachlor, dieldrin and p,p'-DDT were similar to those reported by Burse *et al.*[20] at the 1–10 ng g^{-1} of human serum, while Bristol *et al.*[21] recorded overall recoveries of HCB and β HCH of 72 and 91% from whole blood for the same fortification levels, but poor recovery of t-nonachlor (56%).

The overestimation of PCB congener 28 and 201 may be partly due to the fact that these two congeners are difficult to determine unambiguously on a DB-5 capillary column, due to interferences from other PCB congeners and/or chlorinated hydrocarbons other than PCBs. The consistent underestimation of PCB No. 153 could possibly be attributed to an extraction effect similar to that reported by Roboz *et al.*[22], where the precipitation of proteins prior to extraction was necessary to obtain full recovery of 2,4,5,2',4',5'-hexabromobiphenyl from serum. However in our laboratory no previous recovery problems were encountered with PCB congeners, except when blood was used which had been stored for a considerable length of time. In that case recoveries of PCB congeners, including PCB No. 153, decreased to $<50\%$ (unpublished data), as compared to those obtained from 'fresh' blood. Although the storage time of the blood and serum for the above fortification experiment was <1 month, it may have been sufficient to affect the recoveries of some of the PCBs and chlorinated hydrocarbons tested. The results of this study indicate that under our experimental conditions, certain chlorinated hydrocarbons determined in serum and/or plasma may not necessarily reflect their levels in whole blood. Whether this applies to other experimental conditions where for example a different extraction solvent is used, remains to be investigated.

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