Residues of Persistent Chlorinated Hydrocarbons in Human Tissues as Studied by Neutron Activation Analysis and Gas Chromatography

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Several persistent chlorinated hydrocarbons are found to be present in human adipose tissue and organs due to the widespread distribution of these compounds in the environment (KRAUL and KARLOG 1976, and references cited therein). Among the most dominant of these compounds are PCBs, DDT and DDE. The usual analytical technique for determination of organochlorinated compounds in such samples involves adequate clean-up and gas chromatography (GC) with electron capture (EC) detection. With this method it is inherent that non-volatile compounds do not pass the GC-column, thus the possibility exists that some organochlorine compounds escape detection.

By neutron activation analysis (NAA), it is possible to determine the total amount of chlorinated hydrocarbons present measured as chlorine (LUNDE and STEINNES 1975). In combination with NAA, GC-analysis results will give an estimate of the fraction of the total amount of chlorinated hydrocarbons present in the sample.

In the present paper we describe the determination of hexachlorobenzene (HCB), PCB and p,p'-DDE in liver and adipose tissue of 10 Norwegian subjects as determined by GC and NAA. The results are compared with similar studies from other countries.

EXPERIMENTAL

Samples. Samples of human autopsy material were provided by the Laboratory of Pathological Anatomy at the Oslo City Hospital (Ullevål sykehus). No special selection was made, with regard to age, sex or occupation. The samples were stored at -25 °C until analyzed.

Chemicals. All solvents used in this work were distilled in an all-glass distillation apparatus and their purity was carefully checked. Standards of the chlorinated hydrocarbons were purchased from commercial sources, and their purity checked by gas chromatography. All glassware used in the analysis were annealed by 550 °C.

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Clean-up. About 10 g of subcutaneous fat or liver was grinded and extracted twice by 25 ml cyclohexane/isopropanol (1:1) by vigorous shaking for 2 hours at room temperature. Water was added to the combined extracts and the cyclohexane phase transferred to a smaller tube. The oil yield was determined by removing the solvent in a water bath.

About 1 ml cyclohexane and 2 ml conc. sulfuric acid were added to 1 ml oil. The mixture was shaken for several minutes and centrifugated for 10 min. at 1500 rpm. The cyclohexane phase was transferred to a small column containing aluminium oxide activated at 800 ^{o}C and deactivated with 5 % water. Non-polar chlorinated hydrocarbons were eluted by 10 ml pentane. This solution was concentrated to about 3 ml in an aluminium heating block under a stream of highly purified nitrogen. About 2.5 ml was used for the NAA while the remaining part was used for the GC-analysis.

Gas chromatographic analysis. The GC-analysis was performed on a Perkin Elmer model 3920 GC equipped with a ^{63}Ni-electron capture detector. A stainless steel column of 2 m length and 2 mm inner diameter was packed with 3 % SE-30 on Supelcoport 100-120 mesh. Nitrogen was used as carrier gas and argon with 5 % methane as a make-up gas for the EC-detector. Injector- and detector temperatures were 275 ^{o}C and 300 ^{o}C, repectively, and the column temperature was programmed from 150 ^{o}C to 230 ^{o}C at 8 deg/min.

Identification and quantification of the peaks in the chromatograms were performed by comparing retention times and response factors with those of known standards. The GC-profile of PCB in the different samples was rather uniform and resembled most closely the commercial product Aroclor 1254, which therefore was used as a standard.

To confirm identification, samples were also chromatographed on a column filled with 1.5 % SP-2250/1.95 % SP-2401 on Supelcoport 100-120 mesh under the same chromatographic conditions.

Neutron activation analysis. The principles for determination of trace elements in non-polar extracts have been reported previously (LUNDE 1971). The samples were sealed in polyethylene vials and transferred to the reactor by means of a "rabbit" system, together with standards for chlorine, bromine and iodine. The neutron activation was carried out at a flux of about 1.5×10^{13} n/cm^2 sec. with an irradiation time of 5 minutes. The induced radioactivity was registered immediately after irradiation by means of a 33 cm^3 Ge-Li detector and a multichannel gamma spectrometer using ^{38}Cl (with 37.2 min. halflife) to quantify the chlorine. Simultanously, bromine and iodine were determined by ^{80}Br (halflife 17 min.) and ^{128}I (halflife 25 min.).

RESULTS

Gas chromatography. Among the chlorinated hydrocarbons, residues of HCB, PCB and p,p'-DDE were present in all the samples. The results of the analysis of the fat and liver samples are given in Table I. The values in Table I are calculated on basis of extractable fat. For each compound, an average value is also given. Occasionally, other peaks appeared in the chromatogram, but no attempt was made to identify these.

TABLE I

Residues of PCB, DDE and HCB in fat and liver samples as determined by gas chromatography (in ppm)[a]

Sample Number	Age	Fat			Liver		
		PCB	HCB	DDE	PCB	HCB	DDE
1	81	1.39	0.14	0.40	1.11	0.15	0.35
2	65	0.69	0.05	0.32	1.68	0.14	0.79
3	72	1.70	0.09	1.08	1.86	0.11	1.21
4	68	4.44	0.19	1.68	2.06	0.12	0.90
5	29	1.61	0.07	0.63	1.93	0.11	0.84
6	48	2.40	0.19	0.59	2.29	0.16	0.60
7	73	1.25	0.10	0.78	1.42	0.12	1.09
8	67	2.06	0.11	0.60	2.28	0.11	0.70
9	86	0.37	0.03	0.40	2.03	0.23	0.88
10	78	1.87	0.16	1.12	2.01	0.20	0.60
Average		1.78	0.11	0.76	1.87	0.15	0.80

a) µg/g extractable fat

Neutron activation analysis. The results of the NAA are presented in Table II. The results are calculated on the basis of extractable fat and are corrected for blank values. For the sake of completeness, the table also contains the values of persistent organic brominated and iodated hydrocarbons.

TABLE II

Determination of the total organic bonded halogens in fat and liver, as determined by neutron activation analysis (ppm)[a)]

Sample Number	Age (Years)	Fat			Liver		
		Total Cl	Total Br	Total I	Total Cl	Total Br	Total I
1	81	1.19	0.005	0.001	1.06	0.047	0.001
2	65	0.62	0.012	0.001	2.17	0.005	0.001
3	72	1.73	0.005	0.001	1.60	0.014	0.001
4	68	4.99	0.017	0.001	-	0.043	0.001
5	29	1.64	0.010	0.001	1.68	0.005	0.001
6	48	1.72	0.005	0.001	-	0.005	0.001
7	73	1.05	0.005	0.001	1.80	0.005	0.001
8	67	2.58	0.005	0.001	2.57	0.005	0.001
9	86	0.47	0.029	0.001	2.03	0.013	0.001
10	78	1.69	0.005	0.001	2.67	0.005	0.001
Average		1.77	-	-	2.08	-	-

a) µg/g extractable fat

DISCUSSION

Only one investigation of residues of chlorinated hydrocarbons (DDE, PCBs) in Norwegian subjects has previously been carried out (BJERK 1972). Recently, similar studies have been performed in Denmark (KRAUL and KARLOG 1976) and German Federal Republic (ACKER and SCHULTE 1974). A comparison of these results is given in Table III. The results may be difficult to compare due to the lack of a standarized procedure for the analysis of these compounds, and to the limited number of samples studied in the present work. However, a few points may be made from Table III. The two Norwegian studies both show that, on an average, PCB and DDE fat levels in Norwegians are lower than in Danes and Germans. The most apparent reason for this, is that the Norwegian studies lack the extreme maximum values of the other studies. In liver samples, the Norwegian mean value is also lower than the Danish, however, the difference being smaller compared to the fat values.

Only a limited number of determinations of HCB in human fat have been reported. As revealed by Table III, the level of HCB seems to be significantly lower in Norwegians than in German subjects.

TABLE III

Residues in human fat and liver in various countries (ppm)[a)]

Country Year	Sample	HCB	DDE	PCB	Reference
Denmark 1972-73	Fat	-	4.9 (0.31-18)	5.1 (1.0-49)	KRAUL & KARLOG 1976
	Liver	-	6.0[b)]	3.2 (0.94-27)	
GFR - Köln	Fat	2.9 (0.08-5.5)	2.9 (1.0-13)	6.9 (2.1-12)	ACKER & SCHULTE 1974
GFR - München	Fat	6.4 (1.2-21)	5.7 (1.1-19)	10 (2.1-52)	ACKER & SCHULTE 1974
Norway 1969-70	Fat	-	3.3 (0.19-12)	1.61 (0-6.87)	BJERK 1972
Norway 1975	Fat	0.11 (0.03-0.19)	0.76 (0.32-1.68)	1.78 (0.37-4.44)	This work
	Liver	0.15 (0.11-0.23)	0.80 (0.35-1.21)	1.87 (1.11-2.29)	

a) µg/g extractable fat

b) The actual value is Σ(DDT + DDE)

The NAA results show that persistent organochlorine compounds are present in human fat and liver averaging 1.77 ppm and 2.08 ppm, respectively, measured as chlorine on oil basis. The levels of persistent organobromine compounds are much lower, with all measured values less than 0.03 ppm. Persistent organoiodine compounds are not detected in this work. If present, the values are less than 1 ppb in the extractable fat.

It is also of interest to compare the total levels of organic bonded chlorine as determined by GC and NAA. In Table IV, the values of HCB, PCB and DDE given in Table I and II are converted to the equivalent amount of chlorine,

so that this number may be compared directly with the NAA results. In the last column the per cent of the NAA value that can be accounted for by the GC analysis is given. As shown in Table IV, the individual values range from 68 to 107 %, with an average of 80 %, for the fat and from 58 to 110 %, with an average value of 79 %, for the liver samples. These values are only estimates, and are given with rather large uncertainties. The major part of the chlorinated hydrocarbons are PCBs, and the choice of standard by quantification will influence the value of the chlorine equivalent. Nevertheless, the results indicate that most of the persistent chlorinated hydrocarbons present may be accounted for by PCB, DDE and HCB. Other compounds occasionally reported like DDT, DDD, dieldrin, the BHC-isomers, etc., apparently contribute only a small part of the total burden of chlorinated hydrocarbons.

TABLE IV

Comparison of results from neutron activation analysis and gas chromatography (ppm)[a]

Sample Number	Sample	PCB	HCB	DDE	Total Cl		Percent of NAA accounted for by GC
		as Cl	as Cl	as Cl	by GC	by NAA	
1	Fat	0.78	0.10	0.18	1.06	1.19	89
	Liver	0.62	0.11	0.16	0.89	1.06	84
2	Fat	0.39	0.04	0.14	0.57	0.62	92
	Liver	0.94	0.10	0.35	1.39	2.17	64
3	Fat	0.96	0.07	0.48	1.51	1.73	87
	Liver	1.04	0.08	0.54	1.77	1.6	110
4	Fat	2.50	0.14	0.75	3.39	4.99	68
	Liver	1.15	0.09	0.40	1.64	-	-
5	Fat	0.91	0.05	0.28	1.24	1.64	76
	Liver	1.08	0.08	0.38	1.54	1.68	92
6	Fat	1.35	0.14	0.26	1.75	1.72	102
	Liver	1.28	0.12	0.27	1.67	-	-
7	Fat	0.70	0.07	0.35	1.12	1.05	107
	Liver	0.80	0.04	0.49	1.38	1.80	77
8	Fat	1.16	0.08	0.27	1.51	2.58	59
	Liver	1.28	0.08	0.31	1.67	2.57	65
9	Fat	0.21	0.02	0.18	0.41	0.47	87
	Liver	1.14	0.17	0.39	1.70	2.03	84
10	Fat	1.05	0.12	0.50	1.67	1.69	99
	Liver	1.13	0.15	0.26	1.54	2.67	58

a) µg/g extractable fat

It is noteworthy that, in variance with these results, less than 20 % of the organochlorinated compounds determined by NAA in fish and other marine organisms from relatively uncontaminated areas are accounted for by GC (LUNDE and OFSTAD 1976).

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