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Determination by Combustion of the Total Organochlorine Content of Tissues, Soil, Water, Waste Streams, and Oil Sludges

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Organochlorine compounds are produced by man and nature in large quantities (Pollak 1984, Harper 1985). Organochlorines are persistent chemicals and therefore tend to accumulate in the biosphere. When metabolized they are mostly bioactivated to give rise to free radicals, which lead to the propagation of further free radicals and/or lipid peroxidation (Reynolds and Moslen 1982, Link et al. 1984). Hence organochlorines have the potential of causing damage to the lipid bilayer of biological membranes (Slater 1984). It has been shown for some organochlorine pesticides that there is an age-related increase in their concentration in human adipose tissue (Mori et al. 1983). The distribution and concentration of organochlorine pesticides have been intensively investigated, but there is much less information on the total organochlorine overburden either in the environment or in man. The reason for this paucity of information seems to be that while there were many methods available for the determination of individual organochlorines, there was no simple method which permitted the determination of the total organochlorine content of biological and environmental samples. In this communication a method is described which is suitable for measuring g quantities of total lipid-soluble organochlorine.chloride in tissues, tissue fluids, water, soil, liquid industrial wastes and oil sludges. The method is simple and consists of three steps. The first step is a small volume extraction procedure, which extracts organic compounds containing chlorine, but excludes inorganic chlorides. The other two steps involve the degradation of organically bound chlorine to inorganic chlorides and a colorimetric assay of these chlorides.

MATERIAL AND METHODS

(a) Solvents: Hexane, Unilab, Ajax Chemicals; 2-propanol, Analar, B.D.H.; Extraction mixture (Dole 1955), 2-propanol 40 parts: hexane 10 parts: 0.5 M H_2SO_4 1 part. (V/V/V).
(b) Aluminium oxide for chromatographic analysis, Brockman, activity II, B.D.H. and silicic acid, 100 mesh powder, A.R. Mallinckrodt were prepared as described by Ahmad & Marolt (1986).

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(c) Sodium tetraborate, Analar, B.D.H.; ferric nitrate, Univar, Ajax Chemicals; mercuric thiocyanate, Sigma; anhydrous, granular sodium sulphate, Univar, Ajax Chemicals.
(d) Organochlorine standards: Aldrin (99.8%), Shell; Benzotrichloride, Lab. Reag.; Carbon tetrachloride, Analar, B.D.H.; Chloroacetamide, Purum, Fluka; Chloroform, redistilled; Chloraniline, Purissimum, Fluka; α-Chlordane, Anal. Ref. Std., Velsicol Chem. Corp.; 2,4-D (99.8%) technical, Farmco; p,p-DDE and p,p-DDT, Poly-science Corp. Kit No. 51 AX; Dichlorobenzene, A.R. Merck; 1,2-Dichloroethane, Lab. Reag., B.D.H.; Dichloromethane, A.R. Merck; Endrin, Polyscience Corp.; Heptachlor epoxide, Anal. Ref.Std., Velsicol Chem. Corp.; γ-Hexachlorocyclohexane, Purum, Fluka; 2,4,5-T Sigma; 1,1,1-Trichloroethane, Univar, Ajax chem.

Human adipose tissue was obtained from the Department of Clinical Pathology, Westmead Hospital and stored at - 20°C until assayed. Soil was collected with a standard tube soil sampler. Water, liquid industrial waste and oil sludges were obtained from the State Pollution Control Commission of New South Wales. Soil, water, industrial waste samples and oil sludges were stored at 4°C until assayed.

Tissue and soil samples were sonicated prior to solvent extraction. About 600 mg (between 500 and 700 mg) of sample were weighed out, 0.8 mL of water as added, and the sample was sonicated in a r.b., stoppered polypropylene tube (Kayline BSP8015(N)CE) in an icebath for 20 sec by using a Branson sonicator with a microtip at step 4 and maximal cavitation. After sonication, 3 mL of Dole mixture was added; the tubes were stoppered and the samples were shaken for 10 min on a IKA-Vibrax VXP shaker at 1600 cycles per min. For the determination of water samples, 100 mL were centrifuged to clear from particles, the supernatant was passed through a C18 Sep-Pak Cartridge (Millipore-Waters) which had previously been charged with methanol and washed with water. The C18 Sep-Pak cartridge was then eluted with Dole mixture, and the eluate was combined with the pellet obtained from centrifugation. The sample was shaken for 10 min at 1600 cycles per min like the tissue samples. Liquid industrial waste, and oil sludges (between 0.5 to 1.0 mL) were added to 3 mL of Dole mixture and shaken as described above. To these single-phase extraction mixtures, 2.0 mL of water and 1.5 mL of hexane were added and the tubes shaken as before for 10 min. The tubes were then centrifuged for 5 min to break the emulsion. The upper layers were removed quantitatively and placed into another stoppered polypropylene tube over 1.5 g of anhydrous sodium sulphate. The tubes were shaken for 10 min and the extract was removed with a syringe (the sodium sulphate was washed with a small volume of hexane). The extracts and the hexane washes were placed onto aluminium oxide - silicic acid columns. Each column consisted of 0.2 g of silicic acid layered over 0.3 g of aluminium oxide in a Pasteur pipet, which had been plugged with hexane-washed glass wool. The eluates from the columns were adjusted to a convenient volume.

A volume of the extract containing between 2 and 25 μg organochlorine.chloride (maximum volume 1 mL) was placed into an Inconel crucible (CBA460) of a Gallenkamp Bomb Calorimeter; if necessary the volume was made up to 1 mL with hexane. Mercerized cotton was used as a fuse, tied to the Pt-ignition wire. After filling the bomb with 20 bar of oxygen, the sample was ignited, the bomb was left for 10 min to cool, then the gas was released and the condensate of the bomb collected with 0.7 mL of 0.025 M sodium tetraborate and 0.7 mL of water. The two washes were combined and adjusted in a graduated centrifuge tube to 2.1 mL.

The method of Florence & Farrar (1971) was modified for a total volume of 2.5 mL. To the 2.1 mL of the combusted sample, 0.2 mL of ferric nitrate followed by 0.2 mL of mercuric thiocyanate reagents were added with vigorous mixing. Full colour development occurred after 5 min and the colour was found to be stable for at least 24 h. Sodium chloride standards were determined for every batch of combusted samples, and read at 460 nm.

RESULTS AND DISCUSSION

In our hands the modified Florence & Farrar (1971) method for the determination of inorganic chloride gave consistently satisfactory results, although the standard curve between 2 and 25 ug deviated from linearity (Fig. 1). In Fig. 1 the mean values and standard deviations of 33 standard curves generated by using NaCl are presented.

The recovery efficiency was established by combusting 18 individual organochlorines and also a mixture of 17 organochlorines in a Gallenkamp Bomb Calorimeter as described in the methods section. Each organochlorine compound was combusted at three concentrations (5, 10 and 20 μg organochlorine. chloride); at least four replicate samples were combusted and determined for each concentration. The percent recoveries for the organochlorines so tested are presented in Table 1. The results in Table 1 show that the combustion method gave consistently high recoveries for all 18 organochlorines that were tested.

For every ten samples, a blank of 1 mL of hexane was also combusted. The blank values were subtracted from the sample value. It was found that if the mercuric thiocyanate reagent was kept no longer than 2 mo, the blanks showed little variation and remained low, between 0.065 and 0.085 absorbance units.

During the extraction of organochlorines from tissues, soil, waste fluids, etc., the organic phase is separated from the aqueous phase as well as washed and subsequently put through a column which further ensures the elimination of inorganic ions from the organic phase. Nevertheless the possibility that inorganic chloride was present in the extraction mixture had to be examined. For this purpose replicate samples of human adipose tissue (600 mg) were sonicated either with water or with 2M NaCl.

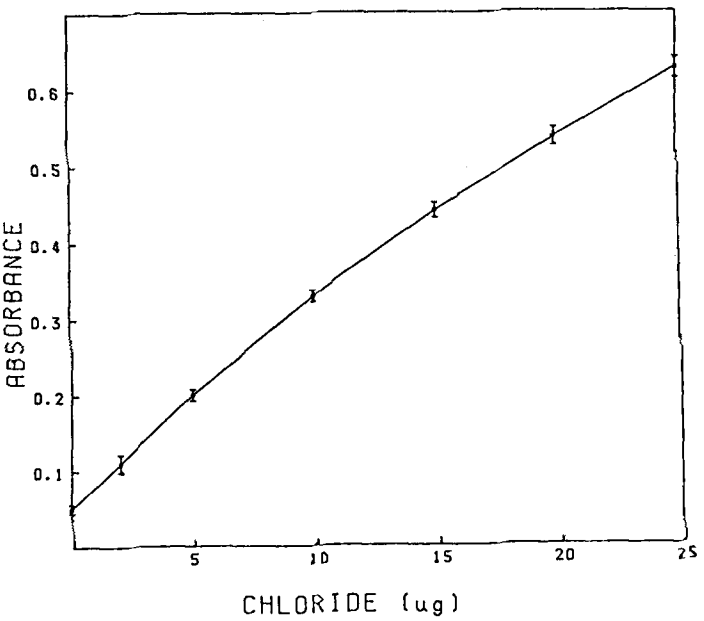

Figure 1. Standard curve for chloride. Sodium chloride was used to make up the standard solution. The values on the x-axis represent sodium chloride.chloride. The points plotted represent mean values of 33 experiments and the error bars show the standard deviation of the data.

The effectiveness of the extraction procedure to exclude any inorganic chloride present in water samples was tested by determining the organochlorine.chloride content of Sydney tap water (100 mL) in the absence and presence of added NaCl. (To 100 mL of tap water, 11.7 g of NaCl were added to ensure a NaCl concentration of at least 2M). The samples were extracted as described in the methods section. In Table 2, results are presented showing the amounts of total organochlorine.chloride found after the extraction and combustion of both the control samples and the samples in 2M NaCl. From these results, it emerges that the presence of inorganic chloride in the original sample does not interfere with the determination of total organochlorine.chloride.

In Table 3 the individual values of replicate determinations of total organochlorine.chloride from samples of adipose tissue, soil, industrial waste samples and water are presented.

In the present investigation the organochlorine values found in human adipose tissue were significantly higher than the sum of individual organochlorines determined in human adipose tissue in other investigations (Wassermann et al. 1976, Williams et al. 1984, Mes et al. 1985, Ansari et al. 1986). This suggests that this procedure included organochlorine compounds which were not detected by the chromatographic analyses of specific organochlorine compounds.

Table 1. Percent Recovery of Organochlorine.chloride after Combustion in a Bomb Calorimeter.

	Amounts of organochlorine.chloride combusted		
	5 μg	10 μg	20 μg
1. Aldrin	107 ±1.9(4)	101 ±1.1(5)	96 ±6.9(4)
2. Benzotrichloride	99 ±3.7(4)	95 ±0.9(4)	91 ±7.4(4)
3. Carbon tetrachloride	103 ±2.1(4)	88 ±1.5(6)	95 ±0.9(6)
4. Chloroacetamide	97 ±5.3(4)	97 ±3.4(4)	87 ±1.0(4)
5. Chloroform	92 ±3.3(5)	94 ±5.3(6)	98 ±3.3(5)
6. 2-Chloraniline	91 ±5.0(4)	93 ±2.0(4)	93 ±5.0(5)
7. Chlordane	99 ±4.5(5)	97 ±2.0(7)	99 ±3.9(6)
8. 2,4-D	92 ±7.2(4)	99 ±5.8(4)	110 ±5.5(4)
9. DDE	96 ±2.7(4)	87 ±3.4(4)	94 ±3.0(4)
10. DDT	110 ±4.8(4)	106 ±3.3(4)	108 ±4.1(4)
11. Dichlorobenzene	115 ±2.1(6)	98 ±4.0(8)	99 ±4.9(6)
12. Dichloroethane	94 ±2.2(4)	85 ±2.7(4)	93 ±3.2(4)
13. Dichloromethane	85 ±6.7(4)	78 ±3.2(6)	79 ±3.1(6)
14. Endrin	102 ±3.0(4)	92 ±3.0(4)	95 ±2.8(4)
15. Heptachlor epoxide	85 ±1.5(4)	93 ±5.0(4)	94 ±2.2(4)
16. -HCH	102 ±5.3(4)	96 ±6.5(4)	97 ±3.2(4)
17. 2,4,5-T	90 ±1.4(4)	92 ±1.6(5)	93 ±1.2(4)
18. 1,1,1-Trichloroethane	84 ±1.7(4)	91 ±2.9(4)	91 ±3.8(4)
Mixture of 1-9 & 11-18	98 ±2.0(4)	100 ±1.5(4)	96 ±3.5(4)

Combustion and chloride determination was performed as described in the Material and Methods section. The data represent the mean values of the percent recoveries ± S.E.M. The number of experiments are given in parentheses.

The method which has been described and tested above is therefore capable of extracting and measuring the total organochlorine content (expressed as total organochlorine.chloride) of adipose tissues, soil, water, industrial waste samples and oil sludges. The sensitivity of the method allows the total organochlorine content of 0.5 g of tissue, 1 mL of waste sample or 100 mL of water to be determined with acceptable accuracy. The method described in this investigation differs from other organochlorine assays in that it provides a measurement of total organochlorine.chloride instead of measuring the concentration of individual organochlorines. The individual organochlorines may also be determined by G.L.C. from the same extract if deemed necessary. However, since most organochlorines tend to promote lipid peroxidation which may lead to the damage of biological membranes, it is considered that a knowledge of the total organochlorine.chloride content of tissues may prove useful in determining the effects of the organochlorine overburden in the body.

Table 2. Effect of added NaCl on the determination of total organochlorine.chloride.

Sample		Additions	Total Organochlorine. Chloride µg/g	µg/L
Adipose tissue	2-3	-	12.2 ± 1.93 (6)	-
	2-3	+ 0.8ml 2M NaCl	11.6 (12.3, 10.8)	-
	2-14	-	15.9 (14.8, 17.0)	-
	2-14	+ 0.8ml 2M NaCl	15.1 (14.7, 15.4)	-
	3-18	-	19.0 ± 1.77 (5)	-
	3-18	+ 0.8ml 2M NaCl	19.8 (21.0, 18.5)	-
100 ml Sydney Tap Water		-	-	79.4 ±4.3(4)
100 ml Sydney Tap Water		+ 11.7 g NaCl	-	76.6 ±5.6(3)

The data present mean values ± S.D. where relevant. Numbers in parentheses represent either the number of samples analysed or the values of the individual samples.

The reproducibility of the extraction method, as well as that of the combustion step was evaluated by determining a number of different types of samples in duplicate.

Table 3. The Reproducibility of Duplicates of Total Organochlorine.Chloride Determinations from a Variety of Samples.

Sample		Total Organochlorine.Chloride of Duplicates µg/g		µg/mL		µg/L	
Adipose tissue	1-9	26.0,	27.7	-	-	-	-
	1-10	42.9,	44.1	-	-	-	-
	1-11	16.9,	17.5	-	-	-	-
	2-14	24.1,	25.9	-	-	-	-
Soil	R-A	22.0,	16.1	-	-	-	-
	R-19	11.9,	12.9	-	-	-	-
	R-22	12.9,	12.9	-	-	-	-
	R-51	14.4,	13.3	-	-	-	-
Liquid	15-5	-	-	68,	96	-	-
Industrial	15-6	-	-	10,	14	-	-
Waste	15-7	-	-	100,	140	-	-
	15-12	-	-	952,	1112	-	-
	15-13	-	-	100,000	97,000	-	-
	15-14	-	-	9,520	9,360	-	-
Laboratory Tap Water		-	-	-	-	84	74
	OC22	-	-	-	-	23	28
Water	OC23	-	-	-	-	23	23
	OC26	-	-	-	-	26	30

The method described in this investigation has the following advantages:

The extraction procedure is simple, reproducible, involves small volumes and is speedily performed.

The total organochlorine content in tissues and solids and fluids of environmental interest may involve a large number of different organochlorines, the analysis of which would be time-consuming. (e.g., in one investigation (Wasserman et al. 1976) 28 different organochlorine compounds were analysed and every one of these compounds showed a positive correlation with malignancy).

If the total organochlorine content is regarded as significantly high, portions of the extract may be analysed for specific organochlorine compounds by GLC- Mass spectrometry.

Conversely, samples with low organochlorine content, require no further analysis, therefore minimizing the use of the more time-consuming costly GLC- Mass spectrometry or similar instrumentation for the determination of a number of different organochlorine compounds.

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