Extraction Efficiency of Polychlorinated Biphenyl, Organochlorine Pesticides and Phthalate Esters from Human Adipose Tissue

Jos Mes and David S. Campbell

Department of National Health & Welfare
Health Protection Branch
Food Directorate, Tunney's Pasture
Ottawa, Canada

Introduction

The extraction of organochlorine (OC) pesticides from human fatty tissues has been carried out by many investigators in a variety of solvents such as hexane (OLOFFS et al. 1974), petroleum-ether (WASSERMANN et al. 1972) and acetonitrile (PESENDORFER et al. 1973). Polychlorinated biphenyls (PCBs), being also non-polar and fat soluble, have been extracted under the same conditions as the organochlorine pesticides (ZITKO and CHOI 1971). More polar components such as phthalate esters have also been found in human fatty tissues and extracted with mixtures of chloroform and methanol (JAEGER and RUBIN 1973) or benzene and acetone (MES et al. 1974).

Simultaneous extraction of PCBs, OC pesticides and phthalate esters would be desirable as time and cost saving factors in monitoring programs where large numbers of samples have to be analysed and the available quantity of human tissue necessarily limited.

This paper reports on the recovery of pesticides and other industrial contaminants from three distinct human adipose tissue samples as a function of lipid extraction by different homogenizing techniques and a variety of solvents.

Sampling

Human adipose tissue was collected during autopsies on accident victims. The fat samples were deposited in glass jars, previously acid washed and rinsed with residue free acetone and hexane. The caps were lined with aluminum foil. Samples were immediately frozen and kept frozen until analysed.

Bulletin of Environmental Contamination & Toxicology,
Vol. 16, No. 1 © 1976 by Springer-Verlag New York Inc.

Analytical Methods

Solvents were of the glass-distilled residue free type, except n-butanol and diethyl ether, which were checked for residues by gas chromatography (GC).

Aroclor 1260 was obtained from Monsanto Chemical Co. All pesticides were 99% pure as verified by GC.

Extraction. The fatty tissue in each jar was cut up into small pieces and thoroughly mixed. For total lipid and subsequent residue determination three 5 g portions of each sample were separately extracted with a mixture of 100 ml of methanol and methylene chloride (2:1 v/v) for 3 min in one of the following homogenizers: Silverson Mixer (Silverson Machines Ltd., Chesham, England), Waring Blendor and Virtis Model 23.

To evaluate the solvent effect on lipid and residue extraction, 5 g samples were extracted in the Silverson homogenizer for 3 min with 100 ml of the following solvents or solvent mixtures: hexane, hexane and acetone (2:1 v/v), methanol and methylene chloride (2:1 v/v) and (1:1 v/v), methylene chloride, benzene, benzene and acetone (1:19 v/v), water saturated butanol (n-butanol saturated with distilled water at 6°C) and petroleum ether (boiling range 30-60°C) and diethyl ether (1:1 v/v).

The extracts were quantitatively filtered through a 2 cm layer of anhydrous Na_2SO_4, prewashed with the extraction solvent and the filtrate concentrated on an all-glass rotatory-evaporator (<30°C) and made up to 50 ml. A 1 ml aliquot was evaporated in a preweighed aluminum dish to determine lipid content.

Cleanup and separation. The fat was removed by low temperature precipitation (McLEOD and WALES, 1972) and the fat-free extract thus obtained evaporated to dryness on an all-glass rotatory-evaporator (<30°C). The residue was redissolved in 1-2 ml of hexane and applied to a 2% Florisil column (deactivated with 2% water after heating for 8 hrs at 300°C) (McLEOD and RITCEY, 1973). The PCBs and OC pesticides were eluted in 250 ml of 30% methylene chloride in hexane (v/v), followed by the phthalate esters in 100 ml of 10% ethyl acetate in hexane (v/v). The phthalate ester fraction was evaporated to dryness and redissolved in 5 ml of hexane.

The PCBs and pesticides were partly separated on a silicic acid column according to ARMOUR and BURKE (1970), except that the silicic acid was prewashed with the eluate used for the pesticides, deactivated with 5% water and the column chromatography itself scaled down to 1/5 of the original quantity of adsorbent, flow-aid and solvents.

The PCB and pesticide fractions were made up to 1 ml in hexane before gas chromatography.

Identification and quantification. A 5 ml aliquot of the PCB or OC pesticide fraction was injected into a Varian Aerograph Series 1400 GC with electron capture detector (Tritium foil) under the following conditions:

Column: 180 x 0.6 cm, packed with 6% OV-210 + 4% SE-30 on chromosorb W (AW) 60/80 (0.6 gm OV-210 + 0.4 gm SE-30 on 10 gm solid support).

Temperatures: Injection: 200°C
Column : 210°C for PCBs; 203°C for OC pesticides
Detector : 225°C

To give a retention time of 13 min for p,p'-DDT an approximate flow rate of 50 ml N_2/min was used. Standard solutions were made up to contain 50 x 10^{-5} µg/µl of Aroclor 1260 or 0.5 - 5.0 x 10^{-5} µg/µl of pesticide, depending upon the individual response of the pesticide (e.g. 5 x 10^{-5} µg/µl of p,p'-DDT).

A 5 µl injection of standard solution was made before and after every two sample injections.

PCBs and OC pesticides in the same fraction were quantitated by integration of peak areas, while other pesticides were quantitated by measuring peak heights. Peaks 8, 10 and 11-15 in Aroclor 1260, according to the numbering system of REYNOLDS (1969) and JENSEN AND WIDMARK (1967), were used for quantification.

Phthalate esters were identified and quantitated as reported by MES et al (1974).

Results and Discussion

The data in Table 1 show considerable variation, which makes it difficult to evaluate the efficiency of the extraction technique. Nevertheless, the Silverson appears to be slightly superior over the other two techniques.

The effect of the different extraction techniques on the final residue level is shown in Tables 2 and 3. To limit the scope of the study only a few of the OC pesticides found in human adipose tissues are reported here. Higher lipid extractions are not necessarily followed by higher residue levels as shown for PCBs and some of the phthalate ester values. These results also indicate that the Silverson and Waring Blendor are equivalent with respect to residue extraction, although not necessarily for the same residues. A prerequisite for the calculation of residue levels on a fat basis in human adipose tissue is the complete extraction of the latter. For this reason and the fact that the Silverson gave reasonable overall residue recovery, homogenized the samples better and was easier to handle than the Waring Blendor (no gaskets which could cause contamination; no chance of leaking; no cleaning of Blendor jars in between samples; shaft

TABLE 1

Effect of extraction technique on lipid content of 3 different human adipose tissue samples extracted with a mixture of methanol and methylene chloride (2:1 v/v).

Homogenizer	% Lipid extracted from samples no.		
	1	2	3
Silverson	81	89	73
Blendor[a]	68	70	75
Virtis	61	80	51

[a]Waring Blendor

TABLE 2

Determination of PCB and OC residue levels in 3 different human adipose tissue samples as affected by different extraction techniques.

Homogenizer	µg/g wet weight											
	PCB			HCB			p,p'-DDE			p,p'-DDT		
	1	2	3	1	2	3	1	2	3	1	2	3
Silverson	1.14	0.40	0.51	0.13	0.04	0.09	2.31	1.13	1.68	0.58	0.32	0.33
Blendor	1.28	0.74	0.78	0.10	-[a]	0.12	2.01	1.45	1.64	0.44	0.06	0.38
Virtis	1.15	0.50	0.56	0.08	0.04	0.06	1.59	1.22	1.11	0.58	0.29	0.28

[a]none detected

easily rinsed and cleaned in between samples without dismantling) for routine analysis, it was chosen as the instrument for investigating the solvent effect on lipid and residue recovery from human adipose tissue.

TABLE 3

Determination of DBP and DEHP in 3 different human adipose tissue samples as affected by different extraction techniques.

Homogenizer	µg/g wet weight					
	DBP			DEHP		
	1	2	3	1	2	3
Silverson	0.57	0.75	0.49	1.17	0.69	1.29
Blendor	0.81	1.26	1.44	1.30	0.64	1.82
Virtis	0.77	1.02	0.59	0.41	0.51	0.85

The average lipid content ± S.D. in all three samples in Table 4 indicate the limited solvent effect on the total amount of lipid extracted from human adipose tissue.

TABLE 4

The effect of solvents on lipids extracted from 3 different human adipose tissue samples.

Solvent	% lipid extracted from samples no.		
	1	2	3
1. Hexane	70	84	73
2. Hexane:Acetone (2:1 v/v)	84	91	76
3. MeOH[a]:CH_2Cl_2 (2:1 v/v)	81	89	73
4. MeOH:CH_2Cl_2 (1:1 v/v)	83	93	79
5. Methylene chloride	86	90	73
6. Benzene	79	88	78
7. Benzene:Acetone (1:19 v/v)	78	92	75
8. H_2O sat'd butanol[b]	81	86	74
9. pet. ether:DEE (1:1 v/v)[c]	75	90	76
Average ± S.D.[d]	80±6	89±3	75±3

[a]Methanol; [b]saturated; [c]petroleum ether:diethyl ether
[d]standard deviation

The data in Tables 5,6 and 7 show the effect of the various solvents on the residue levels in human adipose tissue.

If the residue level obtained was proportional to the lipid extracted a lower relative standard deviation for the residue calculated on a total fat basis would be expected. That the amount of residue obtained is not necessarily related to the lipid extracted is indicated by the close relative standard deviations for both averages as shown in Tables 5 and 6.

The benzene and acetone (1:19 v/v) mixture ranked highest among all the solvents used with respect to the highest PCB, HCB, p,p'-DDE and DBP residue levels. The same mixture ranked last in the case of DEHP, which cannot be readily explained, except that the data showed more than the usual variation. Although the benzene-acetone achieved the highest ranking among the solvents for most residue extractions investigated here, it was not the best solvent for total lipid extraction.

TABLE 5

Solvent effect on extractability of p,p'-DDE and p,p'-DDT from 3 different human adipose tissue samples.

	µg/g wet weight					
Solvent	p,p'-DDE			p,p'-DDT		
No.	1	2	3	1	2	3
1	1.81	1.07	1.83	0.48	0.30	0.33
2	2.09	1.17	1.56	0.50	0.31	0.27
3	2.31	1.13	1.68	0.58	0.32	0.33
4	2.43	1.10	1.66	0.61	0.31	0.34
5	2.50	1.26	1.63	0.57	0.34	0.34
6	2.25	1.15	1.66	0.56	0.30	0.35
7	2.35	1.16	1.75	0.55	0.26	0.35
8	2.33	1.07	1.48	0.64	0.27	0.26
9	2.25	1.12	1.49	0.56	0.30	0.27
$\bar{x}_{ww}$ ±R.S.D.[a]	2.26 9%	1.14 5%	1.64 7%	0.56 8%	0.30 8%	0.31 11%
$\bar{x}_{L}$ ±R.S.D.[b]	2.84 6%	1.28 5%	2.18 8%	0.71 8%	0.34 8%	0.42 12%

[a]Average of µg/g wet weight ± relative standard deviation (100 S.D./$\bar{x}$); [b]Average of µg/g lipid (calculations based on the lipid content given in Table 4).

Nevertheless, the actual differences between residue levels obtained with the various solvents are relatively small and ranking of solvents may not be of any practical significance. On that basis, all solvents tested can be used for the extraction of PCB, OC pesticides, DBP and DEHP from human adipose tissue.

This information should be of interest when comparing residue data in human adipose tissue.

TABLE 6

Solvent effect on the extractability of DBP and DEHP from 3 different human adipose tissue samples

Solvent No.	µg/g wet weight DBP 1	DBP 2	DBP 3	DEHP 1	DEHP 2	DEHP 3
1	0.39	0.59	0.77	1.16	0.44[b]	0.62
2	0.53	0.54	0.56	1.20	2.28[b]	0.53
3	0.57	0.75	0.49	1.17	0.69	1.29
4	0.59	0.65	0.51	1.05	0.74	0.91
5	0.53	1.17	0.50	0.99	0.70	0.51
6	0.45	0.93	0.57	4.42[a]	1.15	0.85
7	0.63	0.82	0.57	0.90	0.66	0.38
8	0.46	0.72	0.62	1.37	0.29	0.35
9	0.50	0.94	0.53	1.34	0.42	0.46
$\bar{x}_{ww}$ ±R.S.D.	0.52 14%	0.79 25%	0.57 15%	1.15 14%	0.64 42%	0.65 47%
$\bar{x}_{L}$ ±R.S.D.	0.65 12%	0.89 25%	0.76 17%	1.45 17%	0.70 41%	0.87 47%

[a] and [b] not included in average.

TABLE 7

Solvent effect on extractability of PCB and HCB from 3 different human adipose tissue samples.

Solvent No.	µg/g wet weight PCB 1	PCB 2	PCB 3	HCB 1	HCB 2	HCB 3
1	0.86	0.40	0.51	0.08	0.04	0.09
2	0.83	0.42	0.51	0.10	0.04	0.11
3	1.14	0.40	0.51	0.13	0.04	0.09
4	1.21	0.40	0.59	0.14	0.05	0.10
5	1.20	0.41	0.51	0.14	0.05	0.09
6	1.29	0.41	0.54	0.14	0.05	0.09
7	1.40	0.46	0.54	0.15	0.05	0.10
8	1.27	0.38	0.55	0.13	0.05	0.08
9	1.20	0.36	0.65	0.12	0.05	0.09
$\bar{x}_{ww}$ ±R.S.D.	1.16 16%	0.41 7%	0.54 9%	0.13 16%	0.05 11%	0.09 7%
$\bar{x}_{L}$ ±R.S.D.	1.45 16%	0.45 6%	0.72 8%	0.16 14%	0.05 10%	0.13 6%

Acknowledgement

The authors thank the Field Operations Directorate of the Health Protection Branch for procuring the samples and Drs. J. Lawrence and C. Mendoza for reviewing this paper.

References

AMOUR, J.A., and BURKE, J.A.: J. Ass. Offic. Anal. Chem. 53, 761 (1970).

JAEGER, R.J., and RUBIN, R.J.: Environ. Health Perspect. Exp. Issue 3, 95 (1973).

JENSEN, S., and WIDMARK, G.: Organization for Economic Cooperation and Development (O.E.C.D.) Report 1966-1967.

McLEOD, M.A., and RITCEY, W.R.: Analytical Methods for Pesticide Residues in Foods. Manual of the Health Protection Branch of the Canada Department of National Health and Welfare, (1973).

McLEOD, M.A., and WALES, P.J.: J. Agri. Food Chem. 20, 624 (1972).

MES, J., COFFIN, D.E., CAMPBELL, D.S.: Bull. Environ. Contam. Toxicol. 12, 6 (721) (1974).

OLOFFS, P.C., HARDWICK, D.F., SZETO, S.Y., and MOERMAN, D.G.: Clin. Biochem. 7, (297) (1974).

PESENDORFER, H. von., EICHLER, I., and GLOFKE, E.: Wien. Klin. Wochenschr. 85, 14, (218) (1973).

REYNOLDS, L.M.: Bull. Environ. Contam. Toxicol. 4, (128) (1969).

WASSERMANN, M., NOGUEIRA, D.P., TOMAKIS, L., ATHIE, E., WASSERMANN, D., DJAVAHEIRAN, M., and GUTTEL, C.: Ind. Med. 41, 3 (1972).

ZITKO, V., and CHOI, P.M.K.: Technical Report No. 344 of the Fisheries Research Board of Canada, (1972).