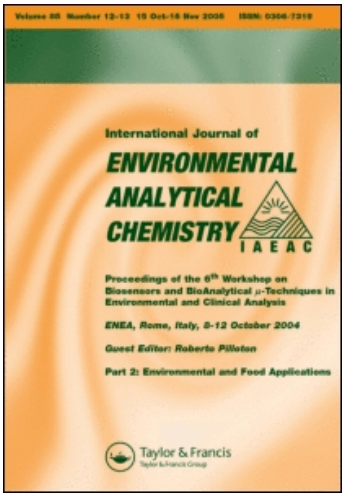

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Evaluation of the Sweep Co-Distillation Cleanup Technique for the Determination of Enviromental Contaminants in Human Adipose Tissue

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Evaluation of the Sweep Co-Distillation Cleanup Technique for the Determination of Environmental Contaminants in Human Adipose Tissue

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Beef and pork fat, corn, peanut, rapeseed and paraffin oils, as well as vegetable shortening were used to investigate their suitability as fortification media for environmental chemicals in the evaluation of the sweep co-distillation technique. The animal fats produced considerable gas chromatographic background interference, while the oils were partly carried over during the sweep co-distillation process, except for rapeseed and peanut oil.

Residue free rapeseed oil was fortified with 26 environmental chemicals in several groups at 20 and 200 ng/g of oil. Recoveries for most compounds were $>80\%$ with a coefficient of variation of ≤ 10. At the 20 ppb fortification level, recoveries for Aroclor 1260, Mirex and pentachlorobenzene were only 70–80%. A similar low recovery was observed for p,p′-DDT, Mirex, hexachloro-1,3-butadiene, while at the 200 ppb level, 1,2,4-trichlorobenzene was only 33% recovered.

The sweep co-distillation technique was further evaluated by using rendered human fat and the same fat diluted with residue free peanut oil. Residue levels in diluted and non-diluted fat were in good agreement, except for hexachlorobenzene. These residue levels were further compared with those obtained by two other cleanup procedures: Florisil–silicic acid column chromatography and low temperature precipitation. In general the sweep co-distillation technique compared favourably with these other cleanup procedures. There was evidence, however, that p,p′-DDT broke down into p,p′-TDE and varying operating procedures did not completely remedy this situation.

KEY WORDS: Sweep co-distillation, cleanup, recovery, human fat.

INTRODUCTION

Recently renewed interest has been shown in the sweep co-distillation technique of Storherr and Watts[1] as a cleanup procedure for the determination of pesticides in fats and oils.[2,3,4] Luke *et al.*[5] summarized recent advances in sweep co-distillation and described the use of a commercial apparatus, which would allow simultaneous cleanup of ten samples.

This paper reports on the evaluation of the sweep co-distillation technique, using this newly developed piece of equipment, called the Unitrex (Scientific Glass Engineering Pty, Ltd., Australia), with respect to analysis of environmental contaminants in human adipose tissue.

EXPERIMENTAL

Solvents and chemicals

All solvents were glass distilled and 300 ml aliquots were tested for interfering residues by gas liquid chromatography (GLC) after concentration to 1 ml.

Florisil, silicic acid, anhydrous Na_2SO_4 and glass wool were decontaminated as previously described.[6]

Octachlorostyrene and photomirex were gifts from Drs. H. Newsome and I. Chu (Health Protection Branch, Canada) respectively. All other standards were gifts from the Environmental Protection Agency (U.S.A.). Standards were 98–100% pure, except for photomirex (96%).

Animal fats and vegetable oils were locally obtained and reagent grade paraffin oil purchased from BDH Chemicals Canada. The human adipose tissue sample was obtained from the 1978 collection of samples.[7]

Fortification and controls

Standard solutions of each environmental chemical were made up at a concentration of 0.5 mg/ml hexane. Two fortification solutions were prepared by first diluting 1 ml of standard solution to 25 ml with hexane (solution A), followed by a further dilution of 1 ml of solution

A to 10 ml with hexane (solution B). Where possible, several residues were simultaneously determined by preparing fortification solutions containing more than one chemical. Five gram aliquots of rapeseed oil (free of interfering residues) were fortified with 50 μl of solution A or B to give residue levels of 200 and 20 ng/g of oil, respectively.

Rapeseed and peanut oil were used as controls to determine GLC background interference from the sweep co-distillation technique.

Sweep co-distillation

1) Animal fats, vegetable oils and fortified rapeseed oil. Rendered beef and pork fat, vegetable shortening (liquified by warming in a warm water bath), corn, peanut, rapeseed and paraffin oils were individually sweep co-distilled to determine their carry over and/or background interference in order to choose an appropriate fat or oil for the fortification experiment. The sweep co-distillation cleanup procedure using the Unitrex, may be briefly outlined as follows:

a) A fractionation tube was placed in each station of the Unitrex, supplied with a prepunctured septum head, connected to a nitrogen carrier gas supply and heated to 235°C.

b) Residue traps, containing Florisil deactivated with 1.5% water,[6] were connected to the fractionation tubes and a nitrogen flow rate established of 230 ml/min, as measured at the Florisil trap outlet.

c) Aliquots of 1.1 ml of oil or rendered fat were injected with a special 1.2 ml syringe (Scientific Glass Engineering Pty. Ltd., part no. 010512), which releases the oil through a tiny lateral opening near the rounded needle tip. Aliquots were checked for consistency of weight by gravimetric determinations in triplicate.

d) Sweep co-distillation was carried out for 30 min, after which the traps, containing residues and/or any oil carried over during the process, were removed. Residues were eluted from the Florisil with 15 ml of 60% dichloromethane (CH_2Cl_2) in hexane. This solvent mixture will remove all residues and only traces of lipids. The solvents were carefully evaporated to dryness on an all-glass rotary evaporator (<30°C) and the residues redissolved in hexane for injection onto the gas chromatographic column.

Triplicate samples of fortified rapeseed oil were sweep co-distilled as above. Observed breakdown of p,p'-DDT in the fortified samples was further investigated by varying the distillation time and temperature and paying special attention to the silanizing of fractionation tubes.

2) Human adipose tissue. Human fat was placed on a bed of glass wool in a funnel resting on a beaker and rendered in an oven at 130°C for 1 hr.

Duplicate samples were sweep-co-distilled as above. To anticipate small adipose tissue samples as often encountered in biopsies, 1 g of rendered human fat was diluted with 4 g of peanut oil, which had previously been cleaned-up by low temperature precipitation.[8]

Triplicate samples (1 g each) of this mixture (containing 0.2 g of rendered human fat/sample) were sweep co-distilled as earlier described.

Separation of residues

Since this study was limited to the evaluation of the sweep co-distillation technique *per se*, no effort was made to determine the efficiency of the Florisil trap in partly separating polychlorinated biphenyls (PCBs) from organochlorine (OC) pesticides or other environmental chemicals. In the case of human fat residues, this separation of PCBs and OC pesticides was achieved on a combined Florisil–silicic acid column as reported earlier.[6] Three fractions were collected from this latter column by elution with 40 ml each of 2, 20 and 60% CH_2Cl_2 in hexane.

To compare residue levels in human fat obtained by the sweep co-distillation with the conventional column chromatography cleanup technique, 0.99 g of rendered human fat was dissolved in 3 ml hexane and a 1 ml aliquot chromatographed on the Florisil–silicic acid column. All fractions were carefully evaporated to dryness as above and residues redissolved in an appropriate volume of hexane for identification and quantification by GLC.

Identification and quantification

Fractions were chromatographed as 0.5 μl aliquots on a Varian 1400 gas chromatograph with a splitless injector (Scientific Glass

Engineering), a DB-5 (J and W Scientific Inc., Rancho Cordova, Ca., U.S.A.) fused silica capillary column (0.3 mm × 30 m) and Scandium Tritide electron capture detector. Injector, column and detector temperatures were 268, 224 and 283°C respectively. Helium was used as carrier gas at a linear velocity of 30 cm/sec and nitrogen as make-up gas at a flow rate of 30 ml/min.

Quantification of spiked samples was carried out by diluting the fortification volume (50 μl) to the same volume as the final redissolved sample residues in hexane for direct comparison of peak heights in sample and standard. For Aroclors, this was accomplished by summation of peak heights.

Residues in human adipose tissue were quantitated by measuring the peak heights of corresponding peaks in sample and standards with the exception of PCBs, where designated peak heights were summed up.

RESULTS AND DISCUSSION

The rendered animal fats were unsuitable for fortification experiments as such, due to high GLC background response, even after Florisil cleanup. Corn, paraffin oils, as well as vegetable shortening, were equally unsuitable since they largely distilled over into the Florisil trap under the experimental conditions used. Rapeseed and peanut oil did not distill over to any extent during sweep co-distillation and their eluates from the Florisil trap were sufficiently free of contaminants, and therefore were considered suitable for preliminary fortification work (Figure 1a) and/or sample dilution.

When 1.1 ml aliquots of rape, peanut and human oils were dispensed from the injection syringe and weighed, the coefficient of variation was <0.2 for triplicate determinations. For practical purposes, a 1.1 ml aliquot of these oils is equivalent to 0.99 g.

Table I shows recoveries of a number of environmental contaminants from fortified rapeseed oil. Recovery of most compounds was $>80\%$ at the 20 ppb fortification level. The coefficient of variation was ≤ 10. Unexpected contamination of the Aroclor 1242 and 1254 and the polycyclic aromatic hydrocarbon eluates (possibly due to contaminated glassware) prevented meaningful quantification of these compounds at that level. Eluates of other compounds gave acceptable GLC background as shown in Figure 1b. At the 200 ppb

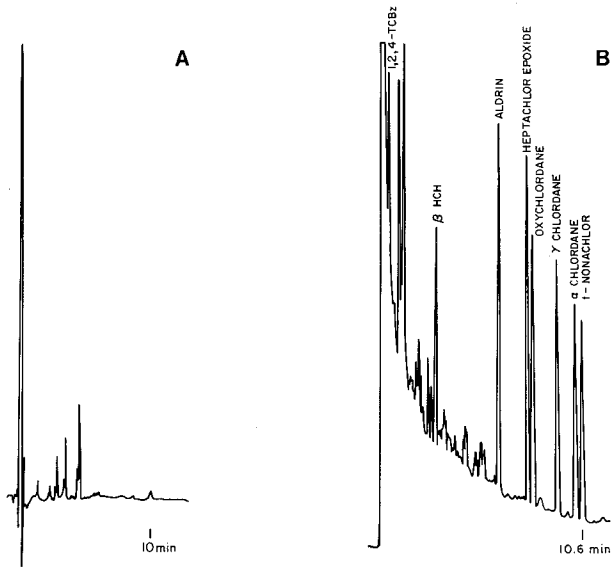

FIGURE 1 Tracings of GLC elution patterns from eluates of the Florisil trap after sweep co-distillation. (a) Rapeseed oil control. (b) Rapeseed oil fortified at the 20 ppb level.

fortification level, the recovery of most compounds was also $>80\%$ with a coefficient of variation ≤ 10. However, recovery of HCB and HCBD was considerably lower at 200 than at 20 ppb level of fortification.

Recovery of Mirex in both fortified experiments was lower than expected for such a non-polar and thermally stable compound.

The poor recovery of the volatile 1,2,4-TCBz may have been caused by losses during the solvent evaporation step rather than during the sweep co-distillation process. Such volatile compounds may have to be investigated further using different operating conditions.

The p,p′-DDT values reported in Table I were obtained at a sweep co-distillation temperature of 231°C rather than the 235°C used for all other compounds. It was observed that in rapeseed oil samples fortified with both p,p′-DDT and p,p′-TDE the recoveries at 235°C (actual temperature at that Unitrex setting measured 237°C by thermocouple) were relatively low and erratic for p,p′-DDT and

TABLE I

Recoveries of some environmental chemicals from fortified rapeseed oil using the Unitrex sweep co-distillation apparatus

Compound	% Recovery + S.D.[a]	
	Fortification level in ng/g	
	20	200
Aldrin	93 ± 2	95 ± 4
Anthracene		103 ± 1
Aroclor 1242		94 ± 10
Aroclor 1254		104 ± 6
Aroclor 1260	77 ± 8	86 ± 6
α Chlordane	94 ± 3	93 ± 1
γ Chlordane	94 ± 1	98 ± 3
p,p′-DDT[b]	82 ± 3	70 ± 1
Dieldrin	92 ± 5	90 ± 2
Hexachlorobenzene (HCB)	98 ± 4	81 ± 2
Hexachloro-1,3-butadiene (HCBD)	80 ± 2	69 ± 7
α Hexachlorocyclohexane (HCH)	95 ± 4	95 ± 1
β Hexachlorocyclohexane	83 ± 3	100 ± 5
γ Hexachlorocyclohexane	99 ± 5	91 ± 4
δ Hexachlorocyclohexane	96 ± 4	97 ± 2
Heptachlor[c]	92 ± 8	86 ± 3
Heptachlor epoxide	94 ± 2	99 ± 3
Mirex	72 ± 6	74 ± 12
trans-Nonachlor	92 ± 4	96 ± 3
Octachlorostyrene	92 ± 2	97 ± 3
Oxychlordane	101 ± 9	98 ± 11
Pentachlorobenzene (PCBz)	79 ± 4	99 ± 4
Photomirex	82 ± 5	87 ± 13
Pyrene		104 ± 8
p,p′-TDE	96 ± 6	94 ± 6
1,2,4-Trichlorobenzene (TCBz)		33 ± 7

[a]S.D. = Standard deviation of triplicate determinations.
[b]Operating temperature 231°C.
[c]Duplicate determination only.

too high for p,p′-TDE, suggesting possible breakdown of p,p′-DDT into p,p′-TDE. When p,p-DDT was measured by itself, it was always accompanied by p,p′TDE.

Several parameters were varied in an attempt to improve p,p′-DDT recovery. Resilanizing the fractionation tubes did not improve

p,p′-DDT recoveries. Lowering the operating temperatures to 200°C gave lower recoveries (<50%) than at 235°C, while no p,p′-TDE was observed, indicating insufficient sweep co-distillation of p,p′-DDT rather than breakdown. However, operation at 231°C gave consistent, but relatively low recoveries of p,p′-DDT.

Table II shows a comparison of residue levels found in human fat by using three different cleanup procedures. In general the sweep co-distillation procedure compared favourably with the other two cleanup procedures. Somewhat lower residue levels were found in the most recent analysis of the rendered fat using Florisil–silicic acid as cleanup procedure as compared to those found earlier using a low temperature precipitation technique for sample cleanup. However, six years of storage may possibly have affected residue levels.

TABLE II

Recovery of chlorinated hydrocarbon residues from human fat by sweep co-distillation as compared to other methods

	μg residue/g rendered human fat			μg residue/g human fat
	SCD[a]		FS[b]	LTP[c]
	Sample weight (grams)			
Compound	0.2	1.0	0.33	2.04
Aroclor 1260	0.857	0.875	0.885	1.354
p,p′-DDE	0.952	1.023	0.884	0.779
o,p′-DDT	0.036	0.015	N.D.[d]	N.D.
p,p′-DDT	0.138	0.158	0.248	0.352
Dieldrin	0.102	0.085	0.072	0.073
HCB	0.187	0.051	0.174	0.070
α HCH	0.001	0.002	N.D.	0.004
β HCH	0.110	0.088	0.083	0.095
Heptachlor epoxide	0.035	0.051	0.019	0.042
t-Nonachlor	0.052	0.043	0.023	0.058
Oxychlordane	0.056	0.041	0.041	0.065
p,p′-TDE	0.123	0.103	N.D.	N.D.
Total DDT[e]	1.249	1.299	1.132	1.131

[a]Sweep co-distillation.
[b]Florisil–silicic acid column chromatography.
[c]Low temperature precipitation; sample analysed in 1978 and calculated on a fat basis.
[d]N.D. = not detected.
[e]Expressed as p,p′-DDE + o,p′-DDT + p,p′-DDT + p,p′-TDE.

Dilution of the rendered human fat with peanut oil in order to facilitate small sample uptake by the injection syringe, proved to be acceptable for most residues in terms of their levels as compared to the undiluted samples. The HCB residue values for all cleanup procedures are difficult to evaluate. The observed low CV (<7) for duplicate and triplicate determinations of HCB residues would not be expected, if contaminants of identical GLC retention time or mechanical losses were reponsible for their over- and under-estimation respectively.

The possibility of p,p'-DDT breakdown during the sweep co-distillation process was again illustrated by the presence of considerable amounts of p,p'-TDE. The latter was not found using the other cleanup methods.

The coefficient of variation was <10 for all determinations in Table II, except for o,p'-DDT, heptachlor epoxide and dieldrin in the first column and t-nonachlor in the third column.

Figure 2 shows some relatively polar chlorinated hydrocarbon residues in human adipose tissue after sweep co-distillation cleanup of the fat sample and subsequent separation from other residues by Florisil–silicic acid column chromatography.

In general, the results indicate that the sweep co-distillation cleanup technique would be useful for the determination of many environmental chemicals in human fat and possibly other human tissues and fluids after prior extraction and collection of the lipids. Relatively small samples could be analysed by dilution with residue free oil. However, residue levels expressed on a rendered fat basis may not necessarily be the same as on a solvent extracted fat basis and therefore the fat preparation procedure should be specified when using the sweep co-distillation technique.

Although the sweep co-distillation technique was not tested with fortified samples of less than 1 g oil, the results of the diluted and non-diluted human fat samples seem to indicate that this technique is applicable to small quantities of oil without loss of sensitivity. This would be of particular interest in the analysis of human tissues and fluids of much lower fat content than adipose tissue.

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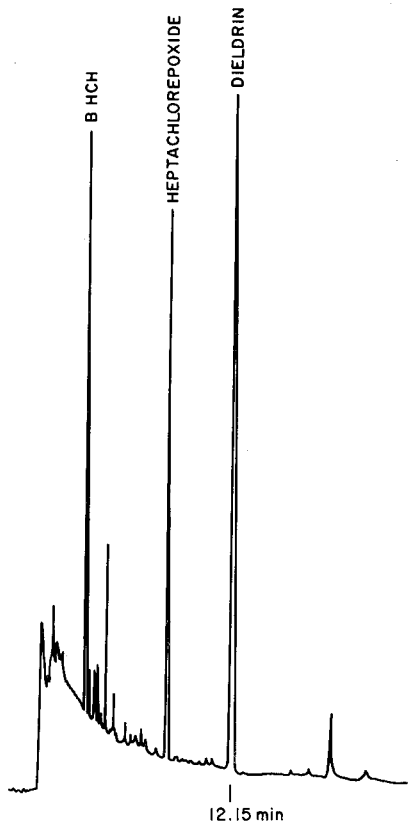

FIGURE 2 Tracing of a GLC elution pattern of some residues in human fat after sweep co-distillation and column chromatography.

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