Bull. Environ. Contam. Toxicol. (1987) 39:848–856
© 1987 Springer-Verlag New York Inc.

Bulletin of Environmental Contamination and Toxicology

Organochlorine Recovery from Small Adipose Samples with the Universal Trace Residue Extractor (Unitrex)

Susan L. Head and Virlyn W. Burse

Division of Environmental Health Laboratory Sciences, Center for Environmental Health, Centers for Disease Control, Public Health Service, U.S. Department of Health and Human Services, Atlanta, Georgia 30333

Preparing adipose tissue for the determination of certain lipid soluble compounds, particularly pesticides and polychlorinated biphenyls is labor intensive. The methods used generally involve the coextraction of lipid and analyte from the fat, followed by cleanup procedures designed to isolate the analyte before analysis. The common cleanup procedures for the lipid extract involve one or more of the following: 1) liquid-liquid partitioning (Mills 1959); 2) treatment with acid (Murphy 1972) or base (Young and Burke 1972); and 3) adsorption chromatography with Florisil^{R} (Mills et al. 1963), silica gel (Holden and Marsden 1969), or alumina (Kueseth and Brevik 1979). More recently, gel permeation chromatography (MacLeod et al. 1982) has been used for this purpose.

Forced volatilization uses heat and gas flow to separate the more volatile chlorinated compounds (subsequently trapped by a scrubber) from the less volatile lipids. Over the years, this technique has been demonstrated in various forms (Ott and Gunther 1964; Storherr and Watts 1965; Dingle 1975; Heath and Black 1980). The most recent commercially available form is the Universal Trace Residue Extractor (Unitrex) (Luke et al., 1984). This apparatus seemed to require a minimum amount of analyst's time and, with its capability for handling 10 samples simultaneously, offered the possibility for high sample throughput.

This study evaluated the Unitrex by comparing it with four analytical procedures with regard to: 1) recovery of added analyte (in vitro); 2) recovery of analyte (in vivo) from human breast fat; and 3) efficiency of lipid removal.

MATERIALS AND METHODS

Silica gel, (Woelm) 70-150 mesh (Needham 1981). Sodium sulfate, Mallinckrodt (Needham 1981). Alumina (Gillespie and Walters 1984). Sulfuric acid (Smrek and Needham 1982). All solvents were of "distilled-in-glass" quality, from Burdick and Jackson.

Send reprint requests to Susan L. Head at the above address.

Analytical standards of chlorinated pesticides were obtained from the U.S. Environmental Protection Agency, Research Triangle Park, NC. Aroclors 1254 and 1260 were obtained from Monsanto Chemical Company. Polybrominated biphenyl (Firemaster FF-1) was obtained from Michigan Chemical Company. Adsorption chromatography columns, Chromaflex (Kontes) - 7 x 200 mm and 9 x 330 mm, with 50-mL reservoir were used. Tissue grinders (Smrek and Needham 1982). Keeper solution, 1% w/v Fisher parafin oil, viscosity 125/135 in hexane.

A 1.83 m X 2 mm (i.d.) glass column packed with 3% SE-30 on 80/100 mesh Gas Chrom Q was used to analyze for Hexachlorobenzene, p,p'-DDE, Aroclors 1254/1260, Firemaster FF-1 (major hexabromo peak), and Mirex, and a 1.83 m X 2 mm (i.d.) glass column packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 mesh Supelcoport was used to analyze for the remaining analytes.

The Unitrex was used without modification as purchased from Scientific Glass Engineering, Inc. (Austin, Texas). The operational setup and recommended optimum parameters were similar to those previously recorded (Luke et al. 1984), except the injection volume of lard or tissue adipose extract was 100 µL.

Contents of trap (ratio of sodium sulfate:deactivated silica gel) was about 1:4.5 and was prepared, just prior to being used. The steps are as follows: 1) plug the cone section with a small amount of silanized glass wool; 2) add 0.20 g anhydrous sodium sulfate; 3) add 0.9 g of silica gel (5%); 4) plug the outlet with silanized glass wool. Be sure the trap is completely packed so that the packing will not settle after it has been installed.

To determine the absolute recovery of methods compared with the Unitrex, we prepared in-vitro spikes, in commercially available lard. To 5.0 g of lard in a tared 125-mL Erlenmeyer flask was added 0.5 mL of a pesticide standard in isooctane, and the mixture was reweighed. The resulting spike (µg/gm), based on the total weight, was HCB (0.156), γ-BHC (0.128), ß-BHC (0.163), Oxychlordane (0.155), Heptachlor Epoxide (0.145), Trans-Nonachlor (0.173), p,p'-DDE (1.28), Dieldrin (0.179), Endrin (0.038), and p,p'-DDT (0.173).

Individual in-vitro spikes in lard were also prepared by adding 0.5 mL of the following compounds to 5.0 g of lard in separately tared 125-mL Erlenmeyer flasks and reweighing the mixture. The resulting spikes (µg/g), based on the total weight, were: Aroclors 1254/1260 (1:1) (2.55), Mirex (0.550), o,p'-DDT (1.15), p,p'-DDT (1.15), o,p'-DDE (1.15), Chlordane (1.15) and Toxaphene (1.37). We evaluated one additional in-vitro spike that contained 0.5 mL of a Firemaster FF-1 standard added to 5.0 g of rendered control rat adipose tissue in a separately tared 125-mL Erlenmeyer flask. The mixture was reweighed, resulting in an in-vitro spike of 35.0 (µg/g). Five grams of unspiked lard was added to an Erlenmeyer flask. All of these spikes were mixed by shaking in a 70°C water bath for 24 h. One-tenth to two-tenths milliliter

of each spike was pipetted into 2-dram vials and stored at 0°C.

One hundred milligrams of lard containing the in-vitro spiked pesticide mixture or single components was homogenized in a Kontes tissue grinder three times with successive 3-mL portions of petroleum ether. After each extraction, the petroleum ether extract was added to a 15-mL centrifuge tube. Anhydrous sodium sulfate was added to the combined extracts, which were vortexed and transferred to a tared 30-mL beaker. The combined extracts were evaporated just to dryness, desiccated overnight and weighed to determine percent lipids. The lipid residue was redissolved in a minimum volume of petroleum ether, transferred to a 15-mL centrifuge tube with a few drops of keeper solution, and evaporated with a gentle nitrogen stream, at room temperature, to about 100 µL. If lipids were not to be determined, a few drops of keeper solution are added to the dried extract, and then the extract was concentrated to about 100 µL with a gentle nitrogen stream at room temperature.

To determine the in-vivo "recovery" of methods compared with the Unitrex, we obtained specimen of breast fat from women undergoing elective breast surgery in a local metropolitan hospital.

Four hundred milligrams of breast-fat samples was homogenized in a Kontes tissue grinder three times with successive 3-mL portions of petroleum ether. After each extraction, the petroleum ether extract was transferred to a 15-mL centrifuge tube. The combined extracts were dried with anhydrous sodium sulfate, transferred to a calibrated 15-mL centrifuge tube, and diluted with petroleum ether to 12 mL. Three-milliliter aliquots (equivalent to 100 mg wet weight fat) were transferred to tared 30-mL beaker if lipids were to be determined; if they were not, the aliquot was added to a 15-mL centrifuge tube, with a few drops of keeper solution, and concentrated to about 100 µL with a gentle nitrogen stream at room temperature.

One hundred microliters of the fat extract was injected into the Unitrex followed by 2- X 50-µL rinses of the sample container and syringe. After 35 min of fractionation, the trap was removed, and the contents were transferred to the top of a 9- X 330-mm Chromaflex column that contained 4.5 g of 5% deactivated silica gel (prerinsed with 30 mL of hexane) so that the composition of the final adsorption chromatography column was (top to bottom) glass wool, sodium sulfate (10 mm), silica gel (total weight, 5.4 g), sodium sulfate (10 mm), and glass wool. The walls of the trap were then rinsed with hexane onto the top of the column. The column was further eluted with hexane until 35 mL of eluate was collected (Fraction - I), followed by elution with benzene until 20 mL was collected (Fraction - II). Keeper solution was added to each fraction, and the fractions were evaporated just to dryness in a 40°C water bath, with a gentle nitrogen stream, and reconstituted in 1.0 mL of hexane.

One hundred microliters of fat extract of the pesticide mixture

in-vitro spike was evaluated by using method A, which combined deactivated alumina (Gillespie and Walters, 1984) for lipid adsorption, extraction with 20% acetone/acetonitrile (Crist and Moseman, 1976), and elution from 5% deactivated silica gel columns. One hundred microliters of fat extract was transferred to 16- X 125-mm round bottom tubes that contained 2.5 g of 16% deactivated alumina. The samples were mixed for 15 min on a Kraft Big Vortexer. Three milliliters of 20% acetone/acetonitrile was added; the alumina/fat mixture was extracted for 15 min on a mechanical rotator and centrifuged at 1,800 rpm for 15 min, and the solvent was transferred to a 50-mL round-bottom tube. The extraction was repeated two more times, and the extracts were combined. Twenty-five milliliters of a 2% sodium sulfate solution was added, and the solution mixed. This mixture was extracted three times, with 5 mL of hexane per extraction, by rotation on a mechanical rotator for 15 min. The combined hexane extracts were dried with anhydrous sodium sulfate, evaporated to 1.0 mL in a 40°C water bath with a gentle nitrogen stream, and eluted through the silica gel adsorption chromatography column in a manner synonymous with the Unitrex procedure.

One hundred microliters of fat extracts of the pesticide mixture in-vitro spike was evaluated by using method B (Crist and Moseman, 1976), which involved a triple extraction with 3 mL of 20% acetone/acetonitrile per extraction on a mechanical rotator for 15 min. The combined extracts were mixed with 25 mL of 2% sodium sulfate, and the mixture was extracted three times with 5 mL of hexane per extraction by using the mechanical rotator. The combined hexane extracts were dried with anhydrous sodium sulfate and evaporated to 1.0 mL in a 40°C water bath, with a gentle nitrogen stream, and eluted through the silica gel adsorption chromatography column in a manner synonymous with the Unitrex procedure.

One hundred microliters of fat extracts from in vitro spikes of single components were evaluated by method C, which is similar to that of Smrek and Needham (1982). The fat extracts were eluted through chromatographic columns, 7 X 200 mm, containing a silanized glass wool plug, 1.0 g of silica gel Activity I, and 0.52 g of 44% sulfuric acid on silica gel. The packed column had been prerinsed with 10 mL of hexane. The fat extracts were eluted in 15 mL of hexane (Fraction I), and then a second 15 mL of benzene, Fraction II. Keeper solution was added to all eluates before they were concentrated just to dryness; the concentrate was reconstituted in 1.0 mL of hexane.

Aliquots of the breast-fat extract equivalent to 100 mg were evaporated to about 100 µL, with a gentle nitrogen stream at room temperature, and extracted three times with 3 mL of acetonitrile per extraction. This method (Method D) is similar to the method in the EPA Analytical Manual (Thompson, 1977). The extracts were combined with 25 mL of 2% sodium sulfate and extracted three times with 5 mL hexane per extraction. The hexane extract was dried with sodium sulfate and concentrated to

1.0 mL in a 40°C water bath with a gentle nitrogen stream. The concentrate was eluted through the silica gel adsorption chromatography column as in the Unitrex procedure.

Methods were also evaluated to determine the amount of lipid carryover by each cleanup procedure. Petroleum ether extracts of control lard (≥ 100 mg) were processed through each procedure. The amount of residue found in the adsorption chromatography eluates (combined if two fractions were collected) was determined after the eluates were evapoarated to complete dryness in a tared vessel, desiccated overnight, and reweighed.

RESULTS AND DISCUSSION

Recoveries of chlorinated hydrocarbons in the in-vitro spike pesticide mixture in lard by the Unitrex, and by Methods A and B are shown in Table 1. Data reported are mean percent ± standard deviation for five replicates. The spiking levels are geometric mean values taken from Environmental Protection Agency National Human Adipose Survey For Fiscal Year 1977, except for Endrin. Considering only measurable recoveries, the Unitrex ranged from 41.5% for γ-BHC to 118.9% for Dieldrin. Recoveries for Method A ranged from 37.6% for Endrin to 89.9% for HCB. Recoveries for method B ranged from 41.8% for Endrin to 103% for HCB. Endrin was not recovered, and Dieldrin had the highest mean recovery, with the Unitrex used as a cleanup technique. Methods A and B produced considerably better precision for all pesticides than the Unitrex. Neither of the methods evaluated produced quantitative recovery of Endrin at this concentration. Recoveries of those compounds singly in-vitro spiked in lard are shown in Table 2. Since only duplicates were analyzed, we will make no quantitative statement about the data. The procedure we used for this comparison, Method C, gave higher recoveries, and the precision was qualitatively as good or better than that of the Unitrex.

Table 1. Recovery (%) of in vitro fortified pesticide mixture in lard

Pesticide	Spiking Level (ppm)	Unitrex $\bar{x}$ ± SD	Method A $\bar{x}$ ± SD	Method B $\bar{x}$ ± SD
HCB	0.156	87.5 ± 20.0	89.9 ± 3.0	103.0 ± 3.3
γ-BHC	0.128	41.5 ± 10.1	82.0 ± 4.4	85.0 ± 2.0
ß-BHC	0.163	58.4 ± 17.8	81.0 ± 3.3	87.2 ± 1.0
Oxychlordane	0.155	72.5 ± 20.7	59.3 ± 5.5	87.5 ± 0.9
Heptachlor Epoxide	0.145	58.0 ± 19.6	83.3 ± 2.4	90.7 ± 2.0
Trans-Nonachlor	0.173	80.6 ± 27.1	81.5 ± 4.2	91.0 ± 2.7
p,p'-DDE	1.28	66.5 ± 17.4	87.5 ± 2.6	96.4 ± 4.5
Dieldrin	0.179	118.9 ± 23.2	88.3 ± 3.5	92.5 ± 1.5
Endrin	0.038	No Peak Detected	37.6 ± 1.6	41.8 ± 0.7
p,p'-DDT	0.173	54.3 ± 74.2	71.2 ± 6.3	89.8 ± 3.0

Method D was compared with the Unitrex for analysis of unknown breast fats; the results are shown in Table 3. Data reported in ppm based on wet weight of tissue. γ-BHC and Endrin were not detected by either method in any specimens at lower detection limits of 0.01 and 0.02 ppm respectively. Aroclors are quantitated using a summation technique of peak areas and a standard Aroclor mixture of 1:1 Aroclor 1254:Aroclor 1260. Three samples were analyzed in duplicate by each method. The compounds detected by both methods are essentially the same as those reported in the EPA monitoring program that have a frequency of occurrence greater than 10%, except for o,p'-DDT and p,p'-DDT, which were not detected in any of these samples by either method.

Use of the Unitrex results in more variability and lower concentrations for the compounds reported in these unknowns except for p,p'-DDT and Dieldrin, which are found at higher concentrations.

Table 2. Recovery (%) of separate analytes in vitro fortified in lard

	Spiking	Unitrex		Method C	
Compound	Level (ppm)	Orig.	Dupl.	Orig.	Dupl.
AR 1254/1260(1:1)	2.55	59.1	89.4	71.4	102.9
Mirex	0.55	73.7	35.3	104.6	107.3
o,p'-DDT	1.15	70.6	65.1	99.8	102.0
p,p'-DDT	1.15	67.0	54.2	100.6	99.4
o,p'-DDE	1.15	80.5	92.8	99.2	101.0
Chlordane	1.15	86.7	86.1	96.7	108.0
Toxaphene	1.37	49.4	43.6	107.0	107.6
Firemaster FF-1	35.0	51.5	52.3	70.9	76.9

Table 3. Concentration of a select number of chlorinated pesticides and aroclors found in human breast fat

	Sample	Unitrex		Method D	
Compound	I.D.	Orig.	Dupl.	Orig.	Dupl.
HCB	270	0.008	0.011	0.009	0.013
	271	0.018	0.033	0.022	0.019
	272	0.022	0.010	0.017	0.022
P,P'-DDE	270	0.530	0.454	1.04	1.03
	271	1.17	0.844	2.35	2.10
	272	0.618	0.307	0.712	0.739
AR(1254/1260)	270	0.528	0.348	0.950	0.945
	271	0.400	0.298	0.814	0.727
	272	0.356	0.239	0.468	0.450
B-BHC	270	0.114	0.111	0.190	0.181
	271	0.236	0.200	0.454	0.413
	272	0.054	0.026	0.061	0.069
P,P'-DDT	270	0.061	0.035	<0.02	<0.02
	271	0.047	0.016	<0.02	<0.02
	272	0.028	0.036	<0.02	<0.02

Table 3. Continued

Compound	Sample I.D.	Unitrex Orig.	Unitrex Dupl.	Method D Orig.	Method D Dupl.
Oxychlordane	270	0.005	0.008	0.029	0.034
	271	0.035	0.027	0.073	0.061
	272	0.015	0.006	0.020	0.014
Heptachlor Epoxide	270	<0.02	<0.02	0.035	0.032
	271	<0.02	<0.02	0.142	0.111
	272	<0.02	<0.02	0.125	0.121
Trans-Nonachlor	270	0.078	0.530	0.111	0.116
	271	0.065	0.009	0.140	0.113
	272	0.051	<0.01	0.081	0.073
Dieldrin	270	0.210	0.136	0.058	0.060
	271	0.253	0.088	0.030	0.025
	272	0.043	0.037	0.011	0.009

For the in-vitro studies, only Dieldrin gave a higher recovery by the Unitrex. The recovery of p,p'-DDT in the in-vitro studies produced the highest variation with the Unitrex, and the unknowns also produced high variability. We have found disparity between recovery for in-vitro spikes and in-vivo samples before. We believe that the inability to mimic the in-vivo binding that takes place between the analyte and the matrix accounts for part of the discrepancy.

The results of the experiment designed to determine the amount of lipid carryover by each technique are reported in Table 4. The average weight of lipid residue was the combined weight of adsorption chromatography eluates after evaporation to dryness and dessication overnight. The Unitrex is as efficient as any of these methods in removing lipids. These data also indicate that 100 mg of lipid is the upper limit for micro cleanup procedures, if the amount in the sample extract before analysis by gas chromatography is to be minimized.

Table 4. Lipid carryover by various analytical methods

Method	Average Lard Weight Extracted (mgs)	Average Weight (mgs) of Lipid Residue	Average % Carryover	N
Unitrex	109.0	0.12	0.12	4
A	103.1	6.40	6.2	2
B	135.8	10.02	7.6	3
C	114.6	1.40	1.2	2
D	177.5	12.13	7.0	3

In this evaluation, the Unitrex did not yield data comparable to the recovery or precision reported by Luke et al. (1984) for chlorinated pesticides added to beef fat. Several differences between our studies and theirs might account for the discrepancies: 1) Sample Size: We need methods capable of handling sample sizes of $\leq$ 100 mg, because of the limitations of

our sampling technique. Luke et al. (1984) used a sample size of 1.0 g. 2) Use of solvent for injection: Because of the difficulty of rendering small adipose samples, our plan to determine percent lipids, and the need to quantitatively transfer the sample to the Unitrex, we used solvent injection and washes. Heath and Black (1979) demonstrated that the use of solvent reduced the recovery of DDTs and Dieldrin but enhanced the recovery of HCB and Lindane. We found, the recoveries of Dieldrin and HCB acceptable, though variable, using solvent injection and wash 3) Use of silica gel instead of Florisil for a scrubber: Because of the need to determine polychlorinated biphenyls and chlorinated hydrocarbons, silica gel was chosen over Florisil^{R} (Bevenue and Ogata, 1970). In limited studies, using the mixed pesticide in-vitro spike we found no apparent improvement in recovery when Florisil (activated only at 130°C) was substituted for silica gel. Luke and Richards (1984) suggest, at least for organophosphorus compounds, that the ratio of sodium sulfate to Florisil and the activity of the Florisil are critical in obtaining acceptable recovery. In our studies the ratio of the sodium sulfate to the adsorbent, silica gel or Florisil was about 1:4.5 and 1:4.2, respectively. We did not investigate the effect of varying these ratios.

The variable that differs most according to the reported data relating to this technique is sample size. We did not investigate increasing the size of the sample, since our primary objective was to evaluate methods that would accommodate small ($\leq$ 100 mg) samples of adipose tissue.

Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or the U.S. Department of Health and Human Services.

REFERENCES

Bevenue A, Ogata JN (1970) A note on the use of florisil adsorbent for the separation of polychlorobiphenyls from chlorinated pesticides. J Chrom 50:142-144.

Crist HL, Moseman RF (1976) Improved recovery of hexachlorobenzene in adipose tissue with a modified micro-multiresidue procedure. J Chrom 117:143-147.

Daum SM, Kittle J, Rosenman K, Rom WN, Holstein EC (1978) A simple technique for fat biopsy of PBB-exposed individuals. Environ Health Perspect 23:183-185.

Dingle JHP, (1975) Automation of the storherr sweep co-distillation method and associated techniques. Environmental Quality Safety Supplement 3:1-7.

Gillespie AM, Walters SM (1984) Alumina blending technique for separation of pesticides from lipids. JAOAC 67:290-294.

Heath AB, Black RR (1980) Improvements to assisted distillation cleanup of pesticide residues in animal fats. JAOAC 63:529-531.

Holden AV, Marsden K (1969) Single-stage clean-up of animal tissue extracts for organochlorine residue analysis. J Chrom 44:481-492.

Kueseth NJ, Brevik EM (1979) Column chromatographic method for cleaning up extracts from biological material and simultaneous separation of PCBs and DDE. Bull Environ Contam Toxicol 21:213-218.

Luke BG, Richards JC (1984) Recent advances in cleanup of fats by sweep co-distillation. Part 2. Organophosphorus residues, JAOAC 67:902-904.

Luke BG, Richards JC, Dawes EF (1984) Recent advances in cleanup of fats by sweep co-distillation. Part 1. Organochlorine residues, JAOAC 67:295-298.

MacLeod KE, Hanisch RC, Lewis RG (1982) Evaluation of gel permeation chromatography for clean up of human adipose tissue samples for GC/MS analysis of pesticides and other chemicals. J Anal Tox 6:38-40.

Mills PA, (1959) Detection and semiquantitative estimation of chlorinated organic pesticide residues in foods by paper chromatography. JAOAC 42:734-740.

Mills PA, Onley JH, Gaither RA (1963) Rapid method for chlorinated pesticide residues in nonfatty foods. JAOAC 46:186-191.

Murphy PG (1972) Sulfuric acid for the cleanup of animal tissues for analysis of acid-stable chlorinated hydrocarbon residues. JAOAC 55:1360-1362.

Needham LL, Burse VW, Price HA (1981) Temperature-Programmed gas chromatographic determination of polychlorinated and polybrominated biphenyls in serum. JAOAC 64: 1131-1137.

Ott DE, Gunther FA (1964) Forced volatilization cleanup of butterfat for gas chromatographic evaluation of organochlorine insecticide residues. J Agr Food Chem 12:239-243.

Smrek AL, Needham LL (1982) Simplified cleanup procedures for adipose tissue containing polychlorinated biphenyls, DDT, and DDT metabolites. Bull Environ Contam Toxicol 28:718-722

Storherr RW, Watts RR (1965) A sweep co-distillation clean up method for organophosphate pesticides I. Recoveries from fortified crops. JAOAC 48:1154-1158.

Thompson JF (ed) (1977) Analysis of pesticide residues in human and environmental samples. U.S. Environmental Protection Agency, Research Triangle Park, North Carolina.

Young SJV, Burke JA (1972) Micro scale alkali treatment for use in pesticide residue confirmation and sample cleanup. Bull Environ Contam Toxicol 7:160-167.

Received June 23,1986; accepted May 22,1987