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Comparison of Two Cleanup Procedures (Mills, Onley, Gaither *vs* Automated Gel Permeation) for Residues of Organochlorine Pesticides and Polychlorinated Biphenyls in Human Adipose Tissue

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The primary aim of this study was to determine if a less time consuming and more efficient cleanup procedure could be found for the residue analysis of human adipose samples. In the course of conducting analyses of several thousand adipose samples during the years for the National Human Monitoring Program, it became apparent that there existed several disadvantages in the acceptable MILLS, ONLEY, GAITHER (1963) (MOG) procedure. These disadvantages were: (1) poor recovery of some organochlorine pesticides that do not partition adequately, (2) necessity to perform the partitioning and Florisil column chromatography steps, (3) the excessive amount of solvent used in the above mentioned steps.

Automated gel permeation chromatography (AGPC) was selected for comparison with the MOG procedure because it could be continuously utilized over a 24 h period and its cleanup efficiency has been demonstrated by other investigators (JOHNSON et al. 1976, STALLING et al. 1972, TINDLE & STALLING 1972, GRIFFIT & CRAUN 1974).

AGPC has been applied to the cleanup of human adipose tissue samples prior to pesticide residue analysis by electron capture gas-liquid chromatography. Conservation of time and materials was realized over the traditional MOG procedure by the replacement of acetonitrile partitioning and Florisil column chromatography steps with AGPC.

The recoveries of 19 organochlorine pesticides of interest and PCBs, estimated as Aroclor 1260, were at least as good as, if not better than the recoveries achieved with the MOG procedure. A comparison of 21 human adipose tissue samples prepared for analysis using both procedures is presented to demonstrate the generally high correlation. In addition, compounds that partition poorly with acetonitrile/hexane such as aldrin and hexachlorobenzene were usually recovered in better yield with gel permeation cleanup.

EXPERIMENTAL

Apparatus. A gas chromatograph equipped with a ^{3}H electron capture detector operated in the d.c. mode was used with 1.8 m x 4 mm i.d. glass columns packed with 1.5% OV-17/1.95% OV-210 or 4.0% SE-30/6% OV-210 coated on 80/100 mesh Gas Chrom Q. Inlet, column, and detector temperatures were 220, 200, and 210° C, respectively; nitrogen carrier gas flow was 60 mL/min for the OV-17/OV-210 column and 90 mL/min for the SE-30/OV-210 column.

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An AutoPrep Model 1001 gel permeation chromatograph equipped with a 60 x 2.5 cm i.d. column packed with 50 g of Bio-Beads SX-3 (200-400 mesh) (Analytical Biochemistry Laboratories, Inc., Columbia, MO) was utilized.

Reagents and Materials. Solvents - hexane, diethyl ether, acetonitrile, pesticide grade (Fisher Scientific); dichloromethane, cyclohexane, distilled in glass, pesticide grade solvents (Burdick & Jackson Lab., Inc.).

Florisil - 60-100 mesh, PR grade, to be held and stored at 130 °C prior to use.

Analytical Pesticide and PCB Standards - supplied by U.S. EPA, HERL, ETD, RTP.

Sodium Sulfate - anhydrous granular.

Sodium Sulfate - 2% aqueous solution.

Chromatographic Columns - 25 mm o.d. x 300 mm long, with Teflon stopcocks, with coarse fritted glass plates and 300 mL reservoirs.

Whatman #41 Filter Paper - prerinsed with hexane.

Rotary Flash Evaporator - Buchler Instruments, Fort Lee, NJ.

N-Evap Evaporator - Organomation Associates, Inc., Northborough, MA.

AGPC Solvent - 15% dichloromethane and 85% cyclohexane.

Procedure. Sample analysis was limited to the determination of selected organochlorine pesticides and PCBs (Aroclor 1260).

Five g of each tissue sample was extracted with hexane and diluted to 100 mL according to the MOG procedure. Extracts were subdivided as follows: 20 mL (1 g) were removed for the gravimetric determination of lipid content after solvent evaporation at 37°C; 40 mL (2 g) were removed for AGPC cleanup; acetonitrile partitioning and Florisil cleanup was conducted on the remaining 40 mL (2 g) according to the MOG procedure.

The 40 mL for AGPC cleanup was rotary evaporated to less than 5 mL and quantitatively transferred to 15 mL graduated tubes. The extracts were then evaporated under a stream of nitrogen to less than 2 mL and adjusted to a final volume of 10 mL with AGPC solvent.

Five mL of each extract was injected into the AGPC AutoPrep 1001. The AGPC was adjusted to deliver 5 mL/min and was programmed to dump 24 min (0-120 mL), collect 56 min (121-400 mL) and wash 0 min. The eluates were rotary evaporated to less than 5 mL, transferred to 15 mL graudated tubes and evaporated under a stream of nitrogen to less than 0.5 mL to remove interfering dichloromethane. They were then diluted to appropriate volumes with hexane for gas chromatography.

RESULTS AND DISCUSSION

Recoveries of 19 organochlorine pesticides and 3 Aroclors after AGPC are shown (Table 1) together with the elution volume collected. The eluates were injected into the gas chromatograph after evaporation using a Rotary Flash Evaporator. These recoveries were obtained by preparing standard solutions of the pesticides and PCBs as individual solutions. Five mL of the standard solution, in AGPC solvent, were injected in the AGPC AutoPrep

TABLE 1. Recovery of Chlorinated Pesticides and Polychlorinated Biphenyls Standards After AGPC

Compound	% Recovery	Elution Volume Collected (mL)
α-BHC	94	121-200
β-BHC	94	251-400
Aldrin	99	121-200
Oxychlordane (Oxy)	94	121-200
t-Nonachlor (TNC)	96	121-200
Dieldrin (Diel)	96	151-200
o,p'-DDT	95	121-200
p,p'-DDT (ppT)	98	151-200
Hexachlorobenzene (HCB)	109	121-200
Lindane	96	121-250
Heptachlor	101	121-200
Heptachlor Epoxide (HE)	97	121-200
p,p'-DDE (ppE)	99	121-200
p,p'-DDD	99	151-200
o,p'-DDE	87	121-200
o,p'-DDD	97	151-200
Mirex	93	121-150
δ-BHC	106	201-350
Endrin	105	121-200
Aroclor 1242	105	121-250
Aroclor 1254	90	121-250
Aroclor 1260	93	121-250

1001. The pump was started and 50 mL fractions were collected up to 400 mL. Each fraction was then analyzed by gas chromatography to determine the amount of pesticide or PCB present. An additional 5 mL of standard solution were injected and a second fractionation was performed, collecting the 100 to 130 mL volumes in 10 mL increments. The results of these two tests indicated that the first 120 mL of elution volume could be discarded with no significant losses of the pesticides or PCBs.

To evaluate the cleanup efficiency of AGPC and the MOG procedure, 21 human adipose samples were prepared for comparison (Table 2). These 21 samples were randomly selected from samples submitted to this laboratory by the National Human Monitoring Program and represented general population pesticide exposures. In addition to these human samples, one internal quality control sample (QC-60*) was compared by both methods. As can be seen, the correlation between the two procedures is very good.

Using the Sign Test to determine if the AGPC procedure has a significantly ($\alpha=0.025$) higher recovery than the MOG procedure, 3 compounds showed a significant difference. Aldrin was 7% higher, hexachlorobenzene was 28% higher and p,p'-DDT was 13% higher. The higher recoveries of aldrin and hexachlorobenzene can be

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*Rendered chicken fat fortified with known quantities of pesticides.

TABLE 2. Comparison of Adipose Tissue Samples Analyzed by Both AGPC and MOG Preparative Methods

Sample No.	Method of Preparation	Compound, ppm									
		ppT	ppE	β-BHC	Aldrin	Diel	HE	Oxy	TNC	HCB	PCBs
206	AGPC	0.18	2.6	0.09	0.15	0.06	0.03	0.04	0.05	0.04	< 1
	MOG	0.21	2.6	0.08	0.14	0.04	0.03	0.05	0.04	0.03	< 1
207	AGPC	0.22	3.4	0.07	0.13	0.03	0.02	0.04	0.04	0.04	< 1
	MOG	0.21	3.5	0.07	0.13	0.04	0.02	0.04	0.04	0.03	< 1
208	AGPC	0.22	4.4	0.10	0.13	0.03	0.01	0.07	0.08	0.05	1-3
	MOG	0.22	4.7	0.11	0.13	0.02	0.01	0.07	0.08	0.04	< 1
209	AGPC	0.24	4.2	0.10	0.17	0.03	0.02	0.07	0.15	0.04	1-3
	MOG	0.27	4.4	0.08	0.16	0.04	0.03	0.08	0.14	0.03	1-3
211	AGPC	0.31	6.4	0.13	0.15	0.11	0.05	0.08	0.11	0.05	1-3
	MOG	0.34	5.8	0.11	0.15	0.10	0.06	0.08	0.12	0.04	1-3
212	AGPC	0.79	5.5	0.12	0.16	0.11	0.05	0.06	0.07	0.04	< 1
	MOG	0.71	5.2	0.11	0.15	0.10	0.04	0.05	0.06	0.03	< 1
218	AGPC	0.11	1.0	0.08	0.15	0.03	0.05	0.06	0.06	0.03	< 1
	MOG	0.09	1.0	0.09	0.17	0.04	0.04	0.06	0.06	0.02	< 1
219	AGPC	1.1	8.6	0.09	0.17	0.17	0.06	0.21	0.73	0.03	1-3
	MOG	1.2	8.6	0.10	0.14	0.14	0.04	0.20	0.71	0.03	1-3
220	AGPC	0.13	1.8	0.11	0.17	0.03	0.04	0.04	0.06	0.04	< 1
	MOG	0.11	1.9	0.11	0.16	0.03	0.02	0.03	0.06	0.04	< 1
222	AGPC	0.37	1.8	0.06	0.20	0.12	0.04	0.05	0.09	0.03	< 1
	MOG	0.24	1.5	0.05	0.17	0.12	0.05	0.04	0.08	0.02	< 1
223	AGPC	0.18	1.2	0.09	0.19	0.14	0.05	0.06	0.09	0.02	< 1
	MOG	0.14	1.0	0.09	0.15	0.14	0.05	0.05	0.08	0.01	< 1

TABLE 2 (continued)

Sample No.	Method of Preparation	Compound, ppm									
		ppT	ppE	β-BHC	Aldrin	Diel	HE	Oxy	TNC	HCB	PCBs
224	AGPC	0.55	1.7	0.07	0.15	0.25	0.05	0.07	0.09	0.02	< 1
	MOG	0.38	1.7	0.08	0.14	0.26	0.05	0.06	0.09	0.02	< 1
228	AGPC	0.14	0.59	0.03	0.16	0.06	0.03	0.06	0.12	0.03	< 1
	MOG	0.09	0.47	0.03	0.15	0.07	0.03	0.04	0.11	0.02	< 1
229	AGPC	0.33	2.0	0.13	0.17	0.03	0.05	0.09	0.19	0.03	1-3
	MOG	0.21	1.9	0.12	0.15	0.04	0.03	0.09	0.19	0.02	< 1
230	AGPC	0.22	1.1	0.04	0.16	0.04	0.05	0.05	0.06	0.03	< 1
	MOG	0.15	0.93	0.05	0.15	0.05	0.03	0.04	0.05	0.02	< 1
238	AGPC	0.55	5.2	0.23	0.15	0.09	0.06	0.05	0.07	0.02	< 1
	MOG	0.53	5.0	0.22	0.15	0.08	0.04	0.05	0.06	0.01	< 1
239	AGPC	0.17	0.91	0.17	0.14	0.03	0.02	0.02	0.02	0.02	< 1
	MOG	0.14	0.86	0.19	0.13	0.03	0.02	0.03	0.03	0.02	< 1
242	AGPC	0.22	3.0	0.07	0.13	0.04	0.04	0.07	0.14	0.03	< 1
	MOG	0.21	3.2	0.06	0.13	0.04	0.03	0.06	0.13	0.03	< 1
244	AGPC	0.10	0.90	0.26	0.17	0.02	0.04	0.05	0.05	0.03	1-3
	MOG	0.09	0.89	0.23	0.14	0.02	0.02	0.05	0.05	0.02	< 1
247	AGPC	0.17	2.4	0.26	0.15	0.09	0.03	0.03	0.05	0.03	< 1
	MOG	0.14	2.4	0.22	0.14	0.09	0.02	0.04	0.06	0.02	< 1
248	AGPC	0.50	4.9	0.20	0.23	0.13	0.04	0.06	0.29	0.04	< 1
	MOG	0.30	4.6	0.16	0.13	0.13	0.04	0.06	0.28	0.02	< 1
QC-60	AGPC	0.30	1.9	0.29	0.22	-	0.14	0.09	0.14	-	-
	MOG	0.28	1.9	0.28	0.19	-	0.14	0.09	0.13	-	-
	Theory	0.30	2.0	0.30	0.25	-	0.15	0.10	0.15	-	-

explained by their low partitioning efficiency in the MOG procedure. The higher p,p'-DDT recovery could be caused by increased PCB recoveries on the AGPC which interfere with p,p'-DDT quantitation. The partitioning of the PCBs should be studied further. The other compounds were not significantly higher but, using combined recoveries the AGPC procedure was 2.5% higher than the MOG procedure.

The PCBs were semi-quantitatively estimated as Aroclor 1260 using the isolated large PCB peak that elutes 8-12 cm beyond p,p'-DDT on the 1.5% OV-17/1.95% OV-210 (6.0-6.9 relative retention time to aldrin) and on 4% SE-30/6% OV-210 (4.6-5.0 relative retention time to aldrin). The PCBs are then reported as <1 ppm, 1-3 ppm or >3 ppm.

The results obtained from this study indicate that the AGPC procedure as applied to the cleanup of human adipose tissue samples can be used on a routine monitoring basis and is comparable to, if not better than, the accepted traditional methodology. In addition, the AGPC procedure uses about one half the solvents required by the MOG procedure and the time factor for cleanup of human adipose samples was reduced by approximately one hour per sample. Furthermore, the recoveries of aldrin and hexachlorobenzene were much better using the AGPC method of cleanup. The AGPC also has the advantage of processing 23 samples, unattended, and automatically shutting off when finished. These samples would be ready for further analytical techniques when the technician arrives for work the following day.

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