

# Rapid Multiresidue Procedure for the Determination of Pesticides in Anhydrous Lanolin and Lanolin-Containing Pharmaceutical Preparations Utilizing Gel Permeation Chromatography Cleanup with Gas Chromatographic and Mass Spectrometric Techniques<sup>†</sup>

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The proposed multiresidue method has been used to quantify residues of 17 different pesticides found in anhydrous lanolin and lanolin-containing pharmaceutical preparations. Lanolin waxes are separated from the residues using automated gel permeation chromatography (GPC). Resulting eluates are injected directly into gas chromatographs using a variety of wide-bore capillary columns. A flame photometric detector in the phosphorus mode (FPD/P) is used to quantify organophosphorus pesticides. Organochlorine residues are determined through the use of electrolytic conductivity detection (ELCD). Several residues were identified or confirmed using GC with mass spectrometry. Results of analysis of samples of anhydrous lanolin collected in 1991 were similar in types and levels of residues to those analyzed in 1989. Diazinon residues were most prominent, with levels frequently in excess of 20 mg/kg. Additionally, some lanolin-containing pharmaceutical preparations collected in 1988 and 1992 were found to have patterns of residues similar to those encountered in anhydrous lanolin. However, the highest level of residues encountered in these preparations was only 6.82 mg/kg (diazinon). Recoveries from anhydrous lanolin samples fortified with 12 organochlorine and organophosphorus pesticides ranged from 97 to 110%.

## INTRODUCTION

Anhydrous lanolin is the highly purified product of the wax or grease secreted by the sebaceous glands of sheep. The crude wax (woolgrease) is removed from wool with an aqueous process, neutralized by saponification, and bleached and deodorized to produce anhydrous lanolin. This highly refined product is particularly compatible with the oils of the human skin and, consequently, has found an important use as a moisturizer in cosmetics and a convenient vehicle in pharmaceutical preparations (Goode, 1963; Clark, 1990). Unfortunately, samples of anhydrous lanolin have been found to contain a large number of pesticide residues at significant levels. Diserens (1989) analyzed a number of lanolin samples for both organochlorine and organophosphorus residues by modifying a solid-phase extraction (SPE) procedure developed by Di Muccio et al. (1987). He found the highest level of residues to be that of diazinon—in excess of 30 mg/kg in some samples. These pesticide residues appear to be carried over to lanolin-containing cosmetics and skin care items (Mueller and Schroeder, 1982). Cetinkaya (1988) analyzed a number of cosmetics and skin care products having natural ingredients of beeswax, plant oils, or lanolin. He concluded that the source of the majority of residues in cosmetics lies in the use of a lanolin base. A method consisting of a solid-liquid column chromatography extraction followed by a silica column chromatography cleanup was developed. By using capillary gas chromatography with element specific detection, samples were examined for approximately 40 different organochlorine and organophosphorus pesticides. Levels as high as 5.0 mg/kg (diazinon in baby cream) were reported. Miya-

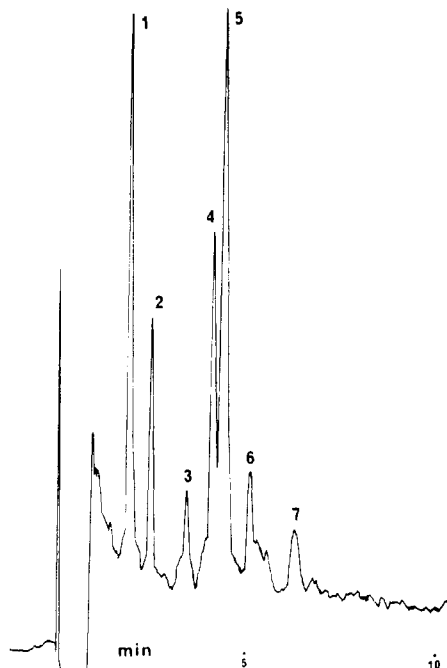
hara et al. (1992) were able to determine pesticides in lanolin utilizing a variety of detection techniques after isolation by Florisil column chromatography. In another study, Meemken et al. (1982) identified and determined residue levels of organochlorine insecticides and polychlorinated biphenyls in lanolin and lanolin-containing skin cream samples. It was demonstrated that the use of these creams by lactating women may result in the contamination of their milk with these residues.

The principal source of pesticides in woolgrease and processed lanolin lies in the practice of dermally dipping sheep in insecticidal solutions. Sheep are susceptible to a number of pests and parasites. Dermal dipping has proven to be an effective means of protecting both the quality of the wool and the health of the animal. Recently, the Australian government has approved an interim tolerance of 40 mg/kg total organophosphorus pesticides in lanolin (*Food Chemical News*, 1988).

This present study encompasses the analysis of both anhydrous lanolin and pharmaceutical preparations containing lanolin. Samples of anhydrous lanolin collected in 1989 and 1991, and lanolin-containing pharmaceutical preparations collected in 1988 and 1992 were analyzed using a gel permeation chromatography (GPC) method developed by Ault et al. (1979) and modified by Hopper (1982). This GPC system has been in continuous use in our laboratory for a number of years in the analysis of pesticide and industrial chemical residues found in a variety of table-ready food items (Pennington, 1983; Gunderson, 1988). Concentrated GPC eluates were analyzed without further cleanup. Wide-bore capillary gas chromatography with columns of diverse polarity with phosphorus-mode flame photometric detection (FPD/P) were used for the determination of organophosphorus pesticides and with electrolytic conductivity detection (ELCD) for the determination of organocholine residues.

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**Figure 1.** Wide-bore capillary gas chromatogram (DB-1, 30 m  $\times$  0.53 mm) of the GPC extract from an anhydrous lanolin sample analyzed in 1991 using an electrolytic conductivity detector (for conditions, see Experimental Procedures): (1) lindane, (2) dichlofenthion, (3) chlorpyrifos, (4)  $\alpha$ -chlorfenvinphos, (5)  $\beta$ -chlorfenvinphos, (6) bromophos ethyl, (7) dieldrin.

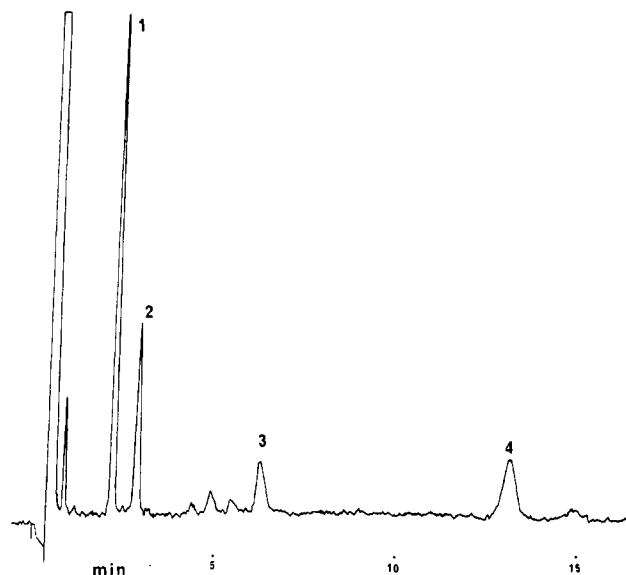
**Table I.** Retention Times of Pesticides Relative to Chlorpyrifos on Three GLC Wide-Bore Capillary Columns (for Conditions, See Experimental Procedures)

pesticide	$t_R$ , min		
	DB-1	DB-17	DB-225
chlorpyrifos	1.00	1.00	1.00
diazinon	0.52	0.40	0.40
dichlofenthion	0.67	0.56	0.64
$\alpha$ -chlorfenvinphos	1.17	1.40	1.58
$\beta$ -chlorfenvinphos	1.26	1.51	1.93
bromophos ethyl	1.51	1.45	1.42
proprymphos	0.48	0.42	0.70
lindane	0.48	0.47	0.69
$\alpha$ -BHC	0.40	0.35	0.48
$\beta$ -BHC	0.43	0.56	1.62
ethion	2.56	3.36	3.93
pirimiphos ethyl	1.14	1.14	1.03
dieldrin	1.91	1.84	1.87
carbophenothion	2.94	3.70	3.93
sulfoxide	5.40	10.0	17.0
sulfone	5.10	9.20	16.5

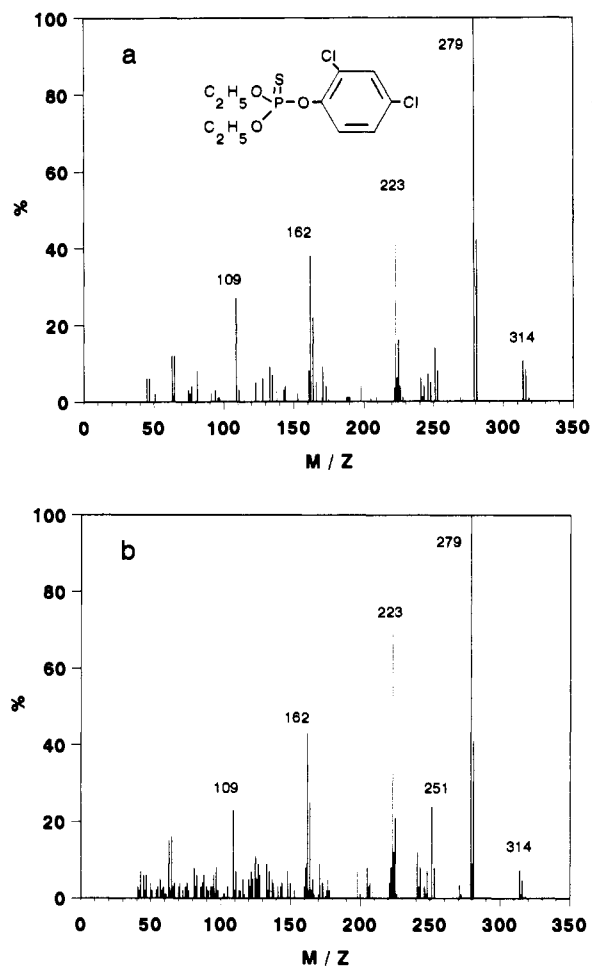
## EXPERIMENTAL PROCEDURES

**Preparation of Anhydrous Lanolin and Pharmaceutical Products.** Anhydrous lanolin samples, including ointments and creams, were melted on a steam bath, weighed directly (6 g) and dissolved in 50 mL methylene chloride-hexane (1:1, v/v) to form a solution of 0.12 g/mL anhydrous lanolin. All solutions were filtered with a 5.0- $\mu$ m filter (Prep-Disc, Bio-Rad Laboratories, Richmond, CA) prior to loading a 5-mL aliquot on an automated gel permeation chromatograph (GPC).

**Gel Permeation Chromatography.** An Auto-Prep 1002A (Analytical Biochemistry Laboratories, Columbia, MO) was equipped with a 30-cm  $\times$  2.5-cm (i.d.) columns (Kontes, Vineland, NJ) slurry packed with 33 g of Bio-Beads SX-3 resin (200-400 mesh) (Bio-Rad Laboratories, Richmond, CA) and compressed to a bed length of approximately 20 cm. The eluting solvent, methylene chloride-hexane (1:1, v/v), was pumped at a flow rate of 5.0 mL/min with an operating pressure of 8-11 psig. The GPC system was programmed for a cycle of a 12-min (60-mL)



**Figure 2.** Wide-bore capillary gas chromatogram (DB-17, 30 m  $\times$  0.53 mm) of the GPC extract of diaper rash ointment using a flame photometric detector (for conditions, see Experimental Procedures): (1) diazinon, (2) dichlofenthion, (3) bromophos ethyl, (4) ethion.



**Figure 3.** Electron ionization mass spectra of (a) standard dichlofenthion and (b) dichlofenthion identified in anhydrous lanolin sample. For mass spectrometric conditions, see Experimental Procedures.

dump, 16-min (80-mL) collect, and 2-min wash. The collected fractions were concentrated on a steam bath with Kuderna-Danish evaporators fitted with 5-mL calibrated receiver tubes (Kontes). After the volume reached approximately 2 mL during the concentration step, 50 mL hexane was added through the

**Table II. Levels (mg/kg) of Organophosphorus and Organochlorine Pesticides Determined in Anhydrous Lanolin Samples Analyzed in 1989**

pesticide	sample									
	1	2	3	4	5	6	7	8	9	10
diazinon	13	5.1	14	8.8	18	19	21	2.2	5.7	12
dichlofenthion	nd <sup>a</sup>	nd	nd	nd	nd	1.5	20	nd	2.0	nd
$\alpha$ -chlorfenvinphos	2.9	0.76	1.7	0.71	1.0	1.4	1.1	0.46	1.0	1.3
$\beta$ -chlorfenvinphos	5.9	2.0	1.2	2.2	3.4	2.1	2.1	0.60	2.3	3.3
bromophos ethyl	nd	nd	nd	nd	nd	14	0.37	13	nd	nd
propetamphos	0.58	0.35	0.59	nd	nd	0.40	0.54	nd	nd	0.55
lindane	nd	nd	0.60	nd	nd	2.4	0.93	1.0	nd	nd
$\alpha$ -BHC	nd	nd	2.3	nd	nd	8.2	nd	nd	nd	nd
$\beta$ -BHC	nd	nd	2.9	nd	nd	2.6	nd	nd	nd	nd
ethion	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
pirimiphos ethyl	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
toxaphene	nd	nd	nd	nd	nd	nd	5.8	2.8	nd	nd
chlorpyrifos	nd	nd	nd	nd	nd	nd	nd	0.84	nd	nd
dieldrin	0.19	nd	0.12	2.0	1.8	0.50	nd	0.28	2.1	0.37
carbophenothion	nd	nd	0.07	nd	0.64	nd	nd	nd	nd	nd
sulfoxide	0.91	nd	1.0	3.5	3.3	1.9	0.51	nd	2.9	3.5
sulfone	3.2	nd	1.5	2.7	5.0	2.4	0.78	nd	3.0	4.8

<sup>a</sup> nd, not detected.**Table III. Levels (mg/kg) of Organophosphorus and Organochlorine Pesticides Determined in Anhydrous Lanolin Samples Analyzed in 1991**

pesticide	sample									
	1	2	3	4	5	6	7	8	9	10
diazinon	28	54	15	13	14	7.2	8.9	7.6	27	8.3
dichlofenthion	nd <sup>a</sup>	nd	nd	2.3	3.7	0.63	1.3	nd	nd	0.44
$\alpha$ -chlorfenvinphos	2.1	0.55	0.36	2.7	0.99	0.86	0.64	0.36	0.45	0.35
$\beta$ -chlorfenvinphos	6.1	1.9	1.1	10	1.4	0.99	2.6	0.99	1.4	0.81
bromophos ethyl	nd	nd	nd	nd	nd	0.23	0.80	nd	nd	0.28
propetamphos	nd	1.9	0.90	1.7	nd	nd	nd	nd	nd	nd
lindane	nd	0.11	nd	nd	nd	0.20	nd	nd	nd	nd
$\alpha$ -BHC	nd	0.060	nd	nd	nd	nd	nd	nd	nd	nd
$\beta$ -BHC	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
ethion	nd	nd	nd	nd	nd	19	nd	nd	nd	nd
pirimiphos ethyl	nd	nd	nd	nd	nd	2.3	nd	nd	nd	nd
toxaphene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
chlorpyrifos	nd	nd	nd	nd	nd	0.22	nd	nd	nd	nd
dieldrin	nd	nd	nd	nd	nd	nd	0.33	nd	nd	nd
carbophenothion	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
sulfoxide	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
sulfone	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

<sup>a</sup> nd, not detected.

condenser, and the solvent was reconcentrated to a final volume of 3.0 mL (0.2 g/mL anhydrous lanolin). Samples eluates, thus prepared, were examined directly by GC and GC-MS.

**GC and GC-MS Analysis.** A Varian 3600 gas chromatograph (Sugar Land, TX) equipped with an FPD/P and a DB-17 fused silica wide-bore capillary column (30 m  $\times$  0.53 mm i.d., 1.0- $\mu$ m film) (J&W Scientific, Folsom, CA) was used in the initial analyses of the GPC eluates for the presence of organophosphorus pesticide residues. Organochlorine residues were initially determined with an ELCD and DB-1 fused silica wide-bore capillary column (30 m  $\times$  0.53 i.d., 1.5- $\mu$ m film). Alternately, either column was replaced with a DB-225 fused silica wide-bore column (15 m  $\times$  0.53 mm i.d., 1.0- $\mu$ m film) to aid in the interpretation of particularly complex chromatograms. All residues were confirmed with a second GLC column or with mass spectrometry. Usual operating conditions were as follows: injector temperature, 220 °C; detector temperature, 230 °C; column oven temperature 200 °C; He flow rate, 30 mL/min. Mass spectrometric analyses were accomplished with either a Varian 3700 gas chromatograph coupled through an open split interface to a VG 7070E-HF mass spectrometer or a Varian 3400 gas chromatograph with an open split interface to a Varian Saturn II mass spectrometer. Mass spectra were obtained by electron ionization (EI) with source temperatures of 230 °C and electron energy of 70 eV. GC columns used were the same as above.

## RESULTS AND DISCUSSION

The use of three GC columns of diverse polarity coupled with element-specific detection and mass spectrometry

provided positive identification and quantitation of all organophosphorus and organochlorine pesticides encountered in anhydrous lanolin and lanolin-containing pharmaceuticals. This system is part of a scheme used routinely to screen for approximately 400 pesticides and industrial chemicals in the Total Diet Study of FDA (Heikes, 1990). Additionally, Florisil column chromatography (FDA, 1991) was used to separate pesticide residues into different fractions to aid in the interpretation of complex chromatograms (e.g., toxaphene). However, since several of the pesticide residues found in anhydrous lanolin are not easily recovered from a Florisil column, certain determinations must be performed prior to Florisil fractionation.

Gas chromatographic retention data, relative to chlorpyrifos, for pesticides determined in this study are listed in Table I. Typical chromatograms generated from the analyses of anhydrous lanolin and lanolin-containing pharmaceutical preparations are represented as Figures 1 and 2, respectively. Mass spectrometry proved to be a valuable technique to identify or confirm several pesticide residues. This was especially true in the initial determinations of anhydrous lanolin. In all cases, close agreement of both retention data and mass spectra were required for positive identification. Figure 3 (parts a and b) represents

**Table IV. Levels (mg/kg) of Organophosphorus and Organochlorine Pesticides Determined in Lanolin-Containing Pharmaceutical Preparations Analyzed in 1988**

sample	pesticide						
	diazinon	dichlofenthion	bromophos ethyl	ethion	$\alpha$ -chlorfenvinphos	$\beta$ -chlorfenvinphos	carbophenothion
A & D ointment	1.1	0.15	0.83	nd <sup>a</sup>	tr <sup>b</sup>	0.090	nd
analgesic balm	0.19	1.4	0.73	nd	tr	0.11	nd
nitroglycerin cream	3.7	nd	nd	nd	0.55	1.1	nd
icathammol ointment	0.29	nd	0.20	nd	nd	nd	nd
physostigmine ointment	0.070	0.77	0.39	nd	nd	nd	nd
atropine sulfate ointment	0.16	1.1	0.58	nd	tr	0.080	nd
dibucaine ointment	6.8	0.92	5.1	nd	0.29	0.77	0.65
breast cream	0.22	nd	nd	nd	nd	nd	nd

<sup>a</sup> nd, not detected. <sup>b</sup> tr, trace, <0.05.

**Table V. Levels (mg/kg) of Organophosphorus and Organochlorine Pesticides Determined in Lanolin-Containing Pharmaceutical Preparations Analyzed in 1992**

sample	pesticides						
	diazinon	dichlofenthion	bromophos ethyl	ethion	$\alpha$ -chlorfenvinphos	$\beta$ -chlorfenvinphos	carbophenothion
A & D ointment	0.20	nd <sup>a</sup>	nd	nd	nd	nd	nd
analgesic balm	tr <sup>b</sup>	0.79	1.1	nd	nd	nd	nd
hemorrhoidal ointment	0.32	0.14	nd	nd	nd	nd	nd
diaper rash ointment	0.89	0.38	0.28	0.34	nd	nd	nd
antibiotic ointment	4.0	nd	nd	nd	0.14	0.32	nd
cold sore ointment	0.79	0.56	tr	0.47	tr	tr	nd
ophthalmic ointment	0.12	0.21	0.30	nd	tr	tr	nd
ophthalmic ointment	nd	nd	nd	nd	nd	nd	nd
breast cream	nd	nd	nd	nd	nd	nd	nd

<sup>a</sup> nd, not detected. <sup>b</sup> tr, trace, <0.05.

the mass spectra generated in the confirmation of the presence of dichlofenthion in one of the early anhydrous lanolin samples.

Since this GPC cleanup is used routinely in our laboratory, a great deal of data has been generated for the recovery of organophosphorus and organochlorine pesticides from fat of both plant and animal origin. Typical fortification levels are between 0.01 and 0.10 mg/kg (Johnson, 1989). However, for the purpose of this study, two anhydrous lanolin samples were fortified with 12 organophosphorus and organochlorine pesticides at levels ranging from 2.0 to 10 mg/kg. Recoveries of these residues using this procedure ranged from 97 to 110%.

Certain trends in types and levels of pesticide residues found in anhydrous lanolin in 1989 and 1991 could be projected from the data presented in Tables II and III, respectively. However, such comparisons would not be entirely valid. Samples analyzed in 1989 were part of a random collection of lanolin samples that was designed to determine the potential of danger to the health of the American public. Whereas, the latter series of samples represented a more calculated survey of anhydrous lanolin gathered from each of the several refiners in the U.S.

Lanolin may be present in pharmaceutical preparations as an oil, an alcohol, an alkyl ester, or anhydrous lanolin. Approximate contributions of lanolin to various formulations are as follows: baby cream, 10%; lip balm, 20%; and moisturizer cream, 30%. Levels of pesticide residues found in lanolin-containing pharmaceutical preparations examined in 1988 and 1992 are listed in Tables IV and V, respectively. FDA toxicologists have concluded that current levels of pesticide residues in lanolin-containing pharmaceutical preparations and cosmetics pose no imminent danger to the health of consumers (FDA, 1988).

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way constitute approval, endorsement, or recommendation by the Food and Drug Administration.

**Registry No.**  $\alpha$ -BHC, 319-84-6;  $\beta$ -BHC, 319-85-7; diazinon, 333-41-5; dichlofenthion, 97-17-6;  $\alpha$ -chlorfenvinphos, 470-90-6;  $\beta$ -chlorfenvinphos, 135373-33-0; bromophos ethyl, 4824-78-6; propetamphos, 31218-83-4; lindane, 58-89-9; ethion, 563-12-2; pirimiphos ethyl, 23505-41-1; toxaphene, 8001-35-2; chlorpyrifos, 2921-88-2; dieldrin, 9014-01-1; carbophenothion, 786-19-6; carbophenothion sulfoxide, 17297-40-4; carbophenothion sulfone, 16662-85-4; chlorine, 7782-50-5; phosphorus, 7723-14-0.