Multiresidue Analysis of Pesticides in Wool Wax and Lanolin Using Gel Permeation and Gas Chromatography

F. William Jones

CSIRO, Division of Wool Technology, P.O. Box 21, Belmont 3216, Australia

The analysis of 48 different pesticide residues in raw wool wax and lanolin by the use of gel permeation and gas chromatography is described. The gel permeation conditions have been optimized for the recovery of the pyrethroids, which are currently widely registered for use on sheep to control ectoparasites. Coeluted wool wax peroxides, which were believed to be responsible for the initial failure of the narrow bore capillary gas chromatography columns used, were either removed or destroyed by passing the gel permeation eluant through alumina or by treatment with aqueous sodium metabisulfite. Wool wax was spiked with pesticides at three levels. Average recoveries at all levels ranged from 70% to 108%.

Keywords: *Analysis; gas chromatography; gel permeation chromatography; lanolin; pesticides; wool wax*

INTRODUCTION

Trace levels of pesticides occur in most raw wool waxes. These residues are mainly the result of the use of a variety of pesticides necessary to control sheep ectoparasites. The type of pesticide and the amount of residue found vary with the parasite to be controlled and the time of treatment relative to the harvesting of the wool. While the level of these residues may be reduced during the refining of raw wool wax, their presence in lanolin, lanolin-based baby care products, cosmetics, and pharmaceuticals has increasingly become a matter of public concern. As a result, various public health authorities (*Food Chemical News*, 1988; USP, 1989) have sought to introduce regulations to set maximum levels for pesticides in the lanolin used in these products. To enforce the regulations and to monitor the presence of pesticide residues in raw wool waxes, an adequate analytical procedure is required. Wool wax is a much more complex matrix than animal fats or vegetable oils, with a molecular mass distribution spanning 100−2000 Da. As a result, analytical methods developed for the determination of pesticides in meat or vegetable oils are not entirely satisfactory. The AOAC backwash method (AOAC, 1980) of partitioning the pesticide residues between light petroleum and acetonitrile may be used, but it is labor intensive and, when used to analyze wool wax, it is prone to the formation of emulsions that are difficult to eliminate (Diserens, 1989). Diserens proposed an alternative method in which the partitioning was carried out on a solid-phase diatomaceous earth column followed by a secondary cleanup on a C_{18} solid phase column. This procedure is simpler and uses less solvent than the AOAC backwash method, but the use of the disposable Extrelut and C_{18} columns is expensive for the routine analysis of a large number of samples. Sweep codistillation has been used by a number of laboratories and was considered as a possible cleanup procedure for inclusion as a test procedure in the USP lanolin monograph (USP, 1989). This procedure, however, has serious shortcomings, giving variable low recoveries of the more volatile pesticides, and is completely unsuitable for thermally labile residues. By far the most suitable method is that based on gel permeation chromatography (GPC) adopted by the USP (1993) as its official test method and further described by Heikes and Craun (1992). While this USP test method specifies 34 agricultural pesticide residues, it does not include all of the chemicals registered for use as sheep treatments in the various wool-growing countries. Pyrethroids have found wide spread use in the control of sheep ectoparasites. Presumably because of their relatively low mammalian toxicity, members of this class of compounds have not been specified in the USP regulations and, accordingly, the USP test procedure has not been optimized for the analysis of these compounds.

The present study examines the effects of modifications to the USP GPC protocol on the analysis of 48 pesticide residues that have the potential to be found in raw and refined wool waxes.

EXPERIMENTAL PROCEDURES

Pesticides. The pesticides used were obtained from Chem-Service, Inc. (West Chester, PA) or, where indicated in Table 1, were isolated from commercial formulations by preparative liquid chromatography on a Prep LC/System 500 (Waters Associates, Milford, MA). Samples were eluted on a silica gel cartridge column with mixtures of ethyl acetate and hexane.

Wool Waxes. Pesticide-free raw wool wax was obtained by the solvent extraction of fleeces obtained from Border Leicester × Corriedale sheep with a known history in a pesticide-free environment. Pesticide free grade lanolin was supplied by Westbrook, U.K. A variety of commercial raw wool waxes from different sources were supplied by various European and New Zealand wool wax suppliers and Australian woolscours. Raw wool samples from the 1991 clip were supplied as blended core samples by the then Australian Wool Corp.

Preparation of Wool Wax. Raw wool (20 g) was extracted with a mixture of hexane−diethyl ether (9:1, 150 mL) in a Soxhlet extractor. The hexane−ether extract was centrifuged at 1500*g* for 10 min to remove dirt and proteinecaceous material, and the solvents were removed from the wax by rotary evaporation on a water bath at 70 °C under a reduced pressure of 30 kPa.

Wool wax (2.5 g) was dissolved in dichloromethane (25 mL) and spiked with ethion (15 μg/g) as an internal standard. [Ethion is not used as a sheep treatment in Australia and accordingly is not found in wool waxes of Australian origin. However, ethion has been detected as a real residue in lanolin by the U.S. FDA (J. C. Craun, personal communication, 1996)

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Table 1. Recovery of Pesticides from Spiked "Pesticide-Free" Raw Wool Wax and Lanolin

pesticide	spike level[a] (μg/g)	recovery (%)	spike level[a] (μg/g)	recovery (%)	spike level[b] (μg/g)	recovery (%)
α-BHC	2.5	89	0.25	83	0.05	71
β-BHC	2.5	93	0.25	90	0.05	81
δ-BHC	2.5	95	0.25	90	0.05	85
aldrin	2.5	98	0.25	94	0.05	82
bromophos ethyl[c]	6.5	99	0.65	84	0.07	91
bromophos methyl[c]	11.0	101	1.1	105	0.11	98
carbophenothion	13.3	100	1.3	99	0.13	92
chlorfenvinphos[c] [*E*] + [*Z*]	15.0	102	1.5	104	0.15	104
chlorpyriphos ethyl	9.9	98	1.0	96	0.10	97
chlorpyriphos methyl	10.4	100	1.0	108	0.10	100
coumaphos	17.1	101	1.7	108	0.20	102
cyhalothrin[c]	15.0	98	1.5	91	0.15	86
cypermethrin	21.4	102	2.1	105	0.21	98
deltamethrin[c]	22.2	101	2.2	103	0.22	98
diazinon	30.2	105	3.0	99	0.30	105
dichlofenthion	14.2	98	1.42	95	0.14	102
dieldrin	2.0	96	0.20	93	0.02	98
endosulfan α	2.5	99	0.25	98	0.05	91
endosulfan β	1.0	99	0.10	97	0.05	98
endrin	2.5	100	0.25	104	0.03	89
ethion	10.6	95	1.06	101	0.11	97
fenchlorphos	8.9	101	0.89	99	0.09	98
fenvalerate	27.0	97	2.7	95	0.27	88
heptachlor	1.0	101	0.10	108	0.05	100
heptachlor epoxide	1.0	102	0.10	85	0.05	85
hexachlorobenzene	1.0	99	0.10	91	0.06	93
lindane	2.5	90	0.25	87	0.05	79
malathion	10.0	101	1.0	99	0.10	99
methoxychlor	4.6	105	0.46	107	0.05	93
o,p′-DDD	2.5	103	0.5	102	0.05	95
o,p′-DDE	2.5	105	0.5	103	0.05	96
o,p′-DDT	5.0	89	1.0	90	0.6	94
p,p′-DDD	5.0	95	1.0	82	0.5	89
p,p′-DDE	2.5	100	0.5	99	0.05	82
p,p′-DDT	2.5	100	0.5	105	0.05	97
permethrin	10.3	106	1.03	108	0.1	99
phosalone	10.2	107	1.02	106	0.1	104
pirimiphos ethyl ethyl	9.8	82	0.98	75	0.10	73
propetamphos[c]	26.6	100	2.7	89	0.27	99
tetrachlorvinphos	10.4	104	1.04	100	0.10	99
tecnazene	1.0	95	0.25	71	0.05	71

[a] Pesticide level in raw wool wax. [b] Pesticide level in lanolin. [c] Pesticides isolated from commercial formulations.

and therefore may not be suitable as an internal standard for all samples.] The wool wax solution was filtered through a fluted filter paper (Whatman grade 2, no. 1202-150) and the dichloromethane partially removed under N_2 to give a 25−30% w/v solution of wool wax.

GPC. Wool wax dissolved in dichloromethane was loaded via a 2 mL sample loop (Rheodyne injection valve model 7125) onto a 450 mm × 25 mm i.d. column (Pharmacia catalog no. SR25/45) slurry-packed with Bio-Beads SX-3 resin (35 g, 200−400 mesh) which were swollen in hexane−dichloromethane (1:1) and compressed to a bed length of 200 mm. The eluting solvent, dichloromethane−hexane (1:1), was pumped at a rate of 5.0 mL/min by an Altex Model 100 chromatography pump (Beckmann, Australia). The first 50 mL of eluant was discarded, and the next 80 mL was passed through a disposable neutral alumina extraction cartridge (ALN 500 mg, 2.8 mL column volume, Varian AI-121020-49) and collected. A keeper in the form of liquid paraffin (5 drops, 5% v/v in hexane) was added and the collected eluant reduced to about 1 mL by rotary evaporation on a water bath at 70 °C under a reduced pressure of 30 kPa. Hexane (4 mL) was added to the residue, which was then analyzed by gas chromatography (GC).

Gas Chromatography. A Varian 3400 gas chromatograph (Varian, Harbor City, CA) fitted with a 1093 septum-equipped programmable injector and either a DB-5 capillary column (15 m × 0.25 mm i.d., 0.25 μm film, J&W Scientific no. 123-5032) or a DB-1701 capillary column (15 m × 0.25 mm i.d., 0.15 μm film, J&W Scientific no. 122-0731) was used. The capillary column outlet was split and coupled to the electron capture and thermionic specific detectors via a 1:1 outlet splitter (VSOS, Scientific Glass Engineering, Australia, no. 123630) using 2 × 30 cm lengths of deactivated 0.22 mm i.d. fused silica tubing. The usual operating conditions were as follows: injector temperature, 65 °C programmed to 280 °C at 100 °C/min and held for 20 min; column oven temperature, 75 °C, programmed to 295 °C at 15 °C/min and held for 4 min; detector temperature, 350 °C; He flow rate 43 cm/s; and injection volume, 0.5 μL.

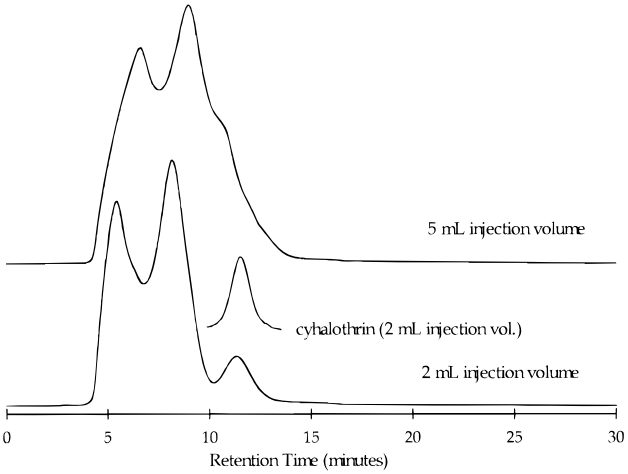

Figure 1. Effect of injection volume on the elution of wool wax and cyhalothrin from the GPC column. Detection was by UV at 240 nm.

Pesticide Recovery Study. Pesticide-free raw wool wax and a Pesticide-Free grade of commercial lanolin were spiked with the pesticides at three different levels. The pesticides that have been registered for use as sheep treatments were added at levels ranging from 30 to 1 μg/g, while those pesticides that may not be legally used as sheep treatments in Australia were added at lower levels ranging from 3 to 0.05 μg/g. The spiked wool wax/lanolin samples were analyzed in triplicate as described above.

RESULTS AND DISCUSSION

Although the GPC cleanup procedure adopted by the USP (1993) and described by Heikes and Craun (1992) has been shown to be suitable for the analytical separation of a wide range of organophoshorus and organochlorine pesticides from wool wax, we have found that it gave low, variable recoveries of pyrethroid pesticides. These pesticides have higher molecular weights than the organophosphorus class of pesticides and, accordingly, complete recovery of these residues could only be obtained by using an earlier collection time than specified in the USP procedure. However, under these conditions unacceptably high levels of wool wax esters were collected in the analyte solution. This was largely due to the large injection volume (5 mL) specified by the USP procedure. Injecting a smaller volume (≤2 mL) of a more concentrated wool wax solution produced less band spreading, and the pyrethroid residues were eluted clear of the wool wax esters (Figure 1). With this modification the amount of the original wool wax found in the analyte solution was typically reduced to between 5% and 10% for raw wool wax and to between 1% and 5% for refined lanolin. By comparison of GC/MSD and GC/FID chromatograms of the analyte solution obtained from a raw wool wax many of these wool wax components were identified (Figure 2). Free wool wax sterols, principally cholesterol, were the major components present. These sterols which have a similar molecular

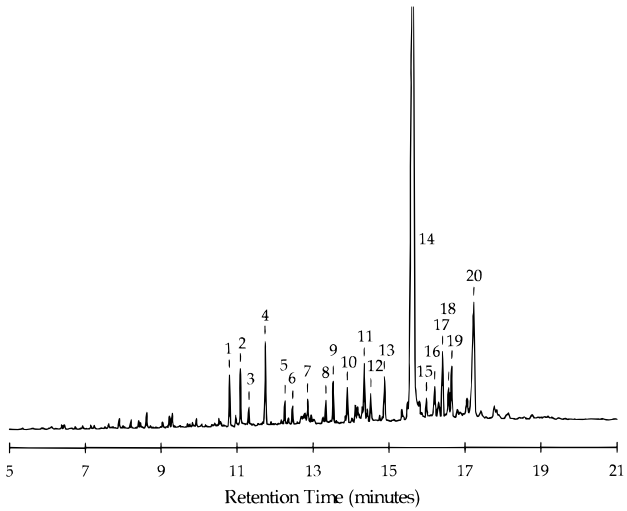

Figure 2. GC trace of residual wool wax components in GPC analyte solution of bulk raw wool wax sample A1. Flame ionization detection (for conditions, see Experimental Procedures): long-chain mono alcohols, (1) *anteiso*-C_{19}, (2) *iso*-C_{20}, (3) *n*-C_{20}, (4) *anteiso*-C_{21}, (5) *iso*-C_{22}, (6) *n*-C_{22}, (7) *anteiso*-C_{23}, (8) *iso*-C_{24}, (9) *n*-C_{24}, (10) *anteiso*-C_{25}, (11) I-C_{26}, (12) *n*-C_{26}, (13) *anteiso*-C_{27}, (15) *anteiso*-C_{29}; sterols, (14) cholesterol, (16) cholestanone, (17) dihydrolanosterol, (18) cholestenone, (19) lanosterol, (20) 7-oxocholesterol.

size to the pyrethroid pesticides, were eluted after the pesticide residues on both the GC capillary columns used. Except for a small decrease in the detection limit of deltamethrin, they did not significantly interfere with the analysis. Although the free wool wax alcohols and acids are generally smaller in size than the wool wax sterols, only a fraction of these compounds originally present in the wool wax were found in the analyte solution. This was probably due to the association of these polar compounds in the GPC solvent to form dimers or possibly micelles, causing the bulk of these compounds to be eluted with the wool wax esters. The small amount of these polar compounds present in the analyte solution coeluted with the pesticides on the GC columns used and contributed to the level of background interference observed. As a result, they affected the detection limits that could be achieved. The better resolution obtained with narrow bore capillary columns compared to the use of the wide bore GC capillary columns described by previous workers (Diserens, 1989; Heikes and Craun, 1992) was found to enhance both the detectability and the identification of the pesticide residues.

In the initial stages of this study the GC columns used were rapidly degraded, particularly when old, highly oxidized samples of wool wax were analyzed. Peroxides produced by the autooxidation of the wool wax sterol esters exposed to air (Scotney and Truter, 1968) were thought to be responsible as peroxides could be detected in the analyte solution by the oxidation of sodium ferrothiocyanate (Gordan and Ford, 1972). These peroxides were destroyed (Hamstead, 1964) by shaking the analyte solution with a couple of drops of aqueous Na_2SO_5 or by the use of a short, disposable, alumina column fitted to the outlet of the GPC column. The use of these alumina columns was preferred as they also reduced the level of background interference in the GC analysis and were routinely used in this study. All of the pesticides examined were readily eluted from the alumina column by the GPC eluant under the conditions described.

Table 2. Practical Detection Limits[a] for Pesticide Residues in Raw Wool Waxes and Lanolins

	detection limit (μg/g)			
	raw wool wax[b]		lanolin[c]	
pesticide	ECD	TSD	ECD	TSD
α BHC	0.02		0.01	
β-BHC	0.1		0.04	
δ-BHC	0.1		0.04	
aldrin	0.1		0.01	
bromophos ethyl	0.1	0.32	0.03	0.03
bromophos methyl	0.2	0.21	0.05	0.07
carbophenothion	0.3	0.05	0.07	0.02
chlorfenvinphos [*E*]	1.5	1.2	0.05	0.08
chlorfenvinphos [*Z*]	4.2	0.1	0.05	0.06
chlorpyrifos ethyl	0.2	0.01	0.05	0.004
chlorpyrifos methyl	0.1	0.02	0.05	0.05
coumaphos	0.2	0.4	0.1	0.03
cyhalothrin	0.4		0.08	
cypermethrin	0.5		0.07	
deltamethrin	0.2		0.15	
diazinon	4.3	0.1	1.3	0.07
dichlofenthion	0.5	1.2	0.1	0.06
dieldrin	0.5		0.1	
endosulfan I	0.02		0.03	
endosulfan II	0.6		0.1	
endrin	0.5		0.1	
ethion	0.6	0.2	0.04	0.03
fenchlorphos	0.1	0.03	0.02	0.02
fenvalerate	0.1		0.1	
heptachlor	0.04		0.01	
heptachlor epoxide	0.4		0.005	
hexachlorobenzene	0.01		0.01	
lindane	0.04		0.01	
malathion	3.4	0.01	1.7	0.01
methoxychlor	0.5		0.01	
o,p′-DDD	0.1		0.001	
o,p′-DDE	0.03		0.04	
o,p′-DDT	1.0		0.5	
p,p′-DDD	1.0		0.01	
p,p′-DDE	0.4		0.2	
p,p′-DDT	0.3		0.01	
permethrin	1.1		0.37	
phosalone	0.7	0.5	0.03	0.05
pirimiphos ethyl		0.5		0.05
propetamphos	0.8	0.02	0.14	0.12
tetrachlorvinphos	0.4	0.5	0.21	0.03
tecnazene	0.1		0.04	

[a] Defined as 3 times the background noise in the retention time window. [b] Average detection limit for 2 samples of pesticide free raw wool wax. [c] Average detection limit for 7 samples of commercial "pesticide-free" grade lanolins.

Pesticide Recovery. Excellent recovery of all the pesticides from both spiked raw wool wax and lanolin was achieved, with the recoveries ranging from 70% to 100%, most being >80% (Table 1). In particular, quantitative, highly reproducible recovery of all the pyrethroids was achieved. In all cases the coefficient of variation was within acceptable limits (Horwitz, 1982), ranging from 3% for the higher residue concentrations to up to 25% at the lowest concentrations. The lower and more variable recoveries were generally associated with the more volatile pesticides. This variability could be reduced by the use of a "keeper" and by ensuring that the GPC eluant fraction containing the pesticide residues was never evaporated to dryness.

Detection Limits. Samples of raw wool wax that were known to be pesticide free and wool waxes refined to produce commercial pesticide-free grades of lanolins were used to estimate the degree of background interference and hence practical detection limits for the pesticides used in this study (Table 2). The pesticide-free grades of lanolins exhibited much lower background interference and, accordingly, higher detection limits

Table 3. Retention Times of Pesticides Relative to Diazinon on Two GC Capillary Columns (for Conditions, see Experimental Procedures)

pesticide	t_R DB-5	t_R DB-1701
tecnazene	0.806	0.842
α-BHC	0.894	0.957
hexachlorobenzene	0.909	0.879
β-BHC	0.945	1.139
lindane	0.953	1.021
propetamphos	0.980	1.043
δ-BHC	0.998	1.172
diazinon	1.000	1.000
dichlofenthion	1.058	1.065
chlorpyriphos methyl	1.073	1.092
heptachlor	1.081	1.049
fenchlorphos	1.096	1.103
aldrin	1.137	1.087
malathion	1.144	1.184
chlorpyriphos ethyl	1.158	1.181
bromophos methyl	1.184	1.181
pirimiphos ethyl	1.200	1.178
heptachlor epoxide	1.205	1.193
chlorfenvinphos [*E*]	1.207	1.219
chlorfenvinphos [*Z*]	1.225	1.230
bromophos ethyl	1.253	1.243
o,p′-DDE	1.256	1.215
endosulphan (I)	1.262	1.229
tetrachlorvinphos	1.270	1.290
dieldrin	1.301	1.281
p,p′-DDE	1.307	1.261
o,p′-DDT	1.319	1.306
endrin	1.337	1.310
endosulfan (II)	1.352	1.379
o,p′-DDD	1.371	1.325
p,p′-DDD	1.376	1.370
ethion	1.384	1.381
carbophenothion	1.410	1.396
p,p′-DDT	1.430	1.393
methoxychlor	1.549	1.479
phosalone	1.564	1.586
cyhalothrin 1	1.586	1.563
cyhalothrin 2	1.602	1.580
cis-permethrin	1.662	1.581
trans-permethrin	1.673	1.597
coumaphos	1.675	1.703
cypermethrin 1	1.737	1.692
cypermethrin 2	1.745	1.704
cypermethrin 3 (alpha)	1.752	1.708
cypermethrin 4	1.774	1.716
fenvalerate 1	1.811	1.770
fenvalerate 2	1.827	1.790
deltamethrin	1.892	1.850

than raw wool waxes as many of the smaller wool wax components that contribute to this interference had been removed during the refinement processes.

While the detection limits for individual pesticides shown in Table 2 give an indication of the efficacy of the GPC−alumina cleanup procedure, they can only be used as an approximate guide when applied to other GC systems as the amount and type of wool wax matrix coeluted will vary with the conditions used.

Pesticide Identification. The identities of the pesticides found in the wool wax samples during this study were confirmed and the amounts quantified by the use of two GC columns of differing polarity together with the use of electron capture and/or thermionic specific detectors. For most of the residues this procedure was more than adequate; however, there is some doubt about the identification of a residue found in the raw wool wax samples which had retention times similar to that of fenchlorphos on both GC columns used. This residue when quantified by both TSD and ECD using fenchlorphos as a standard gave significantly different results for each detector. This residue was generally associated with the presence of significant quantities of diazinon, and it is thought it may be a degradation product of diazinon. Formal identification of this residue will be the subject of a future publication.

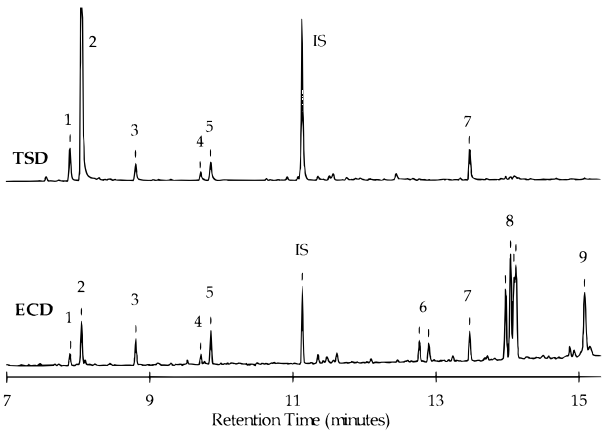

Figure 3. GC trace of pesticide residues in bulk raw wool wax sample A1 using dual thermionic and electron capture detection (for conditions, see Experimental Procedures): (1) propetamphos, (2) diazinon, (3) unknown (believed to be a degradation product of diazinon), (4) (*E*)-chlorfenvinphos, (5) (*Z*)-chlorfenvinphos, (IS) internal standard (ethion), (6) cyhalothrin isomers, (7) coumaphos, (8) cypermethrin isomers, (9) deltamethrin.

Retention times relative to diazinon for the two columns used are listed in Table 3. In general the nonpolar DB-5 column was used for the routine work. Typical chromatograms generated by samples of raw wool wax are presented in Figure 3. The more polar DB-1701 column was used for retention time confirmation, but due to a lower maximum operating temperature and the tendency for the pyrethroids to isomerize on this column, it was not used for routine analysis. In most cases the pyrethroids present in wool wax samples could be readily identified by both their retention time and isomer distribution on the single DB-5 column.

Pesticide Analysis in Raw Wool Waxes. This procedure has been used in these laboratories to routinely analyze the wool wax extracted from a large number of raw wool samples as well as raw wool wax samples obtained from a variety of commercial sources. In general only one or two different pesticide residues have been found in the wool wax obtained from the raw wool samples but a much larger range of pesticide residues was observed in wool waxes obtained from commercial sources. This is a result of both the blending of different lots of raw wool before scouring and the subsequent blending of wool wax from a variety of sources to make up commercial shipments. The principal residues found could all be linked to the pesticides registered to treat sheep for ectoparasites, and the only quantifiable levels of the banned organochlorine pesticides were found in the wool waxes that were not of 100% Australian origin. This may be attributed largely to the successful Australian Wool Corp./International Wool Secretariat "Test and Trace Back Scheme" (AWC, 1991), which was first introduced in 1988.

Conclusion. By modifying the USP gel permeation cleanup procedure as described, multiresidue GC analysis of all pesticides likely to be encountered in wool wax or lanolin may be readily carried out.

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LITERATURE CITED

AOAC. *Official Methods of Analysis*, 13th ed.; Horwitz, W., Ed.; AOAC: Arlington, VA, 1980; p 471, sec. 29.014.

AWC. Testing for Illegal Pesticide Residues; Australian Wool Corp.: Melbourne, Australia, Sept 1991.

Diserens, H. Simplified extraction and cleanup for multiresidue determination of pesticides in lanolin. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 991−993.

Food Chemical News. Australian's Set Pesticide Tolerance in Lanolin; *Food and Chemical News*: Washington, DC, Sept 19, 1988; p 23.

Gordan, A. J.; Ford, R. A. In *The Chemist's Companion*; Wiley: New York, 1972; p 437.

Hamstead, A. C. Destroying peroxides of isopropyl ether. *Ind. Eng. Chem.* **1964**, *56*, 37−42.

Heikes, D. L.; Craun, J. C. Rapid multiresidue procedure for the determination of pesticides in anhydrous lanolin and lanolin-containing pharmaceutical preparations utilizing gelpermeation chromatography cleanup with gas chromatographic and mass spectrometric techniques. *J. Agric. Food Chem.* **1992**, *40*, 1586−1590.

Horwitz, W. Evaluation of analytical methods used for regulation of foods and drugs. *Anal. Chem.* **1982,** *54*, 67A−76A.

Scotney, J.; Truter, E. V. Auto-oxidation of lanostenyl acetate: the major compounds. *J. Chem. Soc.* **1968**, *15*, 1911−1913.

USP. Process revision: lanolin and anhydrous lanolin monographs. In *Pharmacopeial Forum*; The United States Pharmacopeial Convention: Washington, DC, May−June 1989; pp 5127−5131.

USP, Official lanolin and modified lanolin monographs. In *USP XXII-NF XVII*, 9th suppl.; The United States Pharmacopeial Convention: Washington, DC, Sept 15, 1993; pp 3473−3475.

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