The Removal of Pesticide Residues from Wool Wax by Solvent Extraction

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ABSTRACT: A range of suitable solvents for the removal of dieldrin and diazinon residues from wool wax by solvent-solvent extraction was evaluated. Extraction of a 10% solution of wool wax in hexane with *N,N*-dimethylformamide was shown to be the most effective. These solvents were then used to measure the partition coefficients of 36 organochlorine, organophosphorus, and pyrethroid pesticides that have the potential to be found in wool wax. Repeated batchwise extraction of a raw wool wax, which had been spiked to produce typical pesticide residue levels, yielded a high-quality wax in which all pesticide residues had been reduced to below detectable levels. The treated wool wax was lighter in color with a lower acid number and a lower free alcohol content and had excellent water absorption characteristics. All detergents associated with the recovery of wool wax from an aqueous scour were also removed. *JAOCS 74,* 1241–1245 (1997).

KEY WORDS: Diazinon, dieldrin, *N,N*-dimethylformamide, lanolin, pesticide, solvent extraction, wool wax.

Wool wax is the lipid material secreted by the sebaceous glands of sheep. It is a complex mixture of a large number of high-molecular-weight esters, together with smaller amounts of free fatty and steroidal alcohols, free fatty acids, and a component of the wax that has been degraded by oxidation. In its refined form, this highly emollient mixture is particularly compatible with the oils of the human skin and consequently has found an important use in cosmetics, baby-care products, and various pharmaceutical preparations. In the 1980s, reports on the presence of pesticide residues in these products (1–6) raised serious concerns, and as a result, various public health authorities (7–9) have sought to introduce regulations to set minimal levels for pesticides in wool wax products that come in contact with human skin.

Most pesticide residues arise from the treatments used by farmers to control sheep parasites, such as scab-mite, lice, keds, and blowflies. In the major wool-growing countries of the Western world, only organophosphorus and pyrethroid pesticides are licensed for use on sheep (10). However, other pesticides of the organochlorine type are also found in some waxes. These organochlorine pesticides are very persistent in the environment, and low-level contamination may be a result of contact with, and/or by ingestion of the contaminated pasture and associated soils. Some illegal use may occur, but this has been substantially eradicated in major wool-producing countries by the use of test and trace-back schemes (11,12). This is not true in Eastern Europe, Commonwealth of Independent States, and some third-world countries where the use of some organochlorine compounds, such as lindane, is still widespread. The type of pesticide and the amount of residue present vary with the parasite to be controlled and the time of application relative to harvesting the wool. For most treatments applied immediately after shearing, the pesticides used substantially biodegrade on the sheep in the paddock and leave only small residual levels in the wax at shearing (10). Late-season treatments, however, may lead to more substantial levels, which, after scouring, contaminate the recovered wool wax.

Traditionally, wool wax has been refined to produce lanolin by a sequence of simple steps. The raw wool wax is first neutralized by extraction with aqueous or alcoholic alkali, bleached with peroxide or acid chlorite, and deodorized by vacuum steam stripping. These processes reduce the amount of pesticide residues in the refined wax but do not remove them entirely. As a result, "pesticide-free" grades of lanolin may only be produced by the selective purchase of raw wool wax with negligible pesticide residues or by the use of expensive refining processes, such as molecular distillation.

While many workers (13–18) have shown that most of the unwanted components of wool wax are more polar than the commercially valuable esters and can be removed by solvent extraction, no satisfactory solvent extraction procedures for the removal of pesticide residues from wool wax have been reported. Diserens (4) observed that pesticides in wool wax could be isolated for analytical purposes by solvent extraction of dilute solutions of wool wax in hexane with acetonitrile, but the procedure was prone to the formation of emulsions. He proposed a procedure in which the wool wax solution was absorbed onto a diatomaceous earth support.

This present study examines the suitability of various solvent combinations for the extraction of pesticide residues and the nature of the semirefined wool wax produced.

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MATERIALS AND METHODS

Pesticides. The pesticides used were obtained from ChemService, 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 wax. Raw wool wax, which contained less than 1 μg/g of total pesticide and was obtained by the commercial aqueous scouring of Australian merino wool with the nonionic detergent, Lissapol TN450 (ICI, Melbourne, Australia), was used throughout this study.

Solvents. All solvents used were of analytical grade.

Gas chromatography. Trace pesticide analysis of wool wax was performed as described elsewhere (19). Pesticide partition coefficients were determined with the aid of a Varian 3400 gas chromatograph (Varian Associates, Walnut Creek, CA), equipped with a split/splitless injector, an electron capture detector, and a short DB5 column (4 m × 0.3 mm i.d., 1 μm film). The system was used under the following operating conditions: injector temperature, 200°C; detector temperature, 300ºC; column oven, ramped from 120 to 280ºC at 25ºC/min and held for 2 min; and He carrier gas at 30 cm/min.

Volatile wool wax components and detergent trimethylsilyl (TMS) ethers were analyzed on a Varian 3400 gas chromatograph, equipped with an SPI temperature-programmable injector, a flame-ionization detector, and a short HT5 column (6 m × 0.22 mm i.d., 1 μm film) under the following operating conditions: injector temperature, ramped from 80 to 380ºC at 100ºC/min and held for 15 min; detector temperature, 400ºC; column oven, ramped from 100 to 380ºC at 10ºC/min and held for 5 min; and He carrier gas at 30 cm/min.

TABLE 1
Partitioning of Dieldrin and Diazinon in Wool Wax Between Various Solvents

Extraction solvent	Wool wax solvent[a]	Partition coefficient	
		Dieldrin	Diazinon
Methanol	Hexane	0.70	0.47
DMF	Hexane	0.17	0.24
Acetonitrile	Hexane	0.59	0.39
Dimethylsulfoxide	Hexane	0.33	0.57
Sulfolane	Hexane	0.35	0.58
N-Methyl-2-pyrrolidone	Hexane	0.22	0.26
Acetone/water (90:10)	Hexane	6.01	4.69
Acetone/water (80:20)	Hexane	7.85	6.15
Ethanol/water (95:5)	Hexane	0.98	0.67
Ethanol/water (90:10)	Hexane	1.05	0.79
2-Propanol/water (90:10)	Hexane	1.66	1.82
Dioxane/water (90:10)	Hexane	6.14	5.35
THF/water (90:10)	Hexane	>100	>100
DMF/water (95:5)	Hexane	0.26	0.42
DMF/methanol (80:20)	Hexane	0.38	0.35
DMF/ethanol (80:20)	Hexane	0.38	0.39
DMF/2-propanol (80:20)	Hexane	0.40	0.41
DMF/acetone (80:20)	Hexane	0.43	0.37
DMF	Pentane	0.18	0.25
DMF	Heptane	0.17	0.23
DMF	2,4,6-Trimethylpentane	0.15	0.22
DMF	Petroleum spirit 40–60	0.17	0.25
DMF	Cyclohexane	0.35	0.39

[a] Wool wax concentration 10% (wt/vol). Abbreviations: DMF, *N,N*-dimethylformamide; THF, tetrahydrofuran.

Measurement of partition coefficients. Wool wax, dissolved in the appropriate hydrocarbon solvent (10% wt/vol), was filtered under pressure through a bed of diatomaceous earth filter aid to remove suspended cellular debris (20). This solution (5 mL), together with the appropriate extraction solvent or solvent mixture (5 mL), was placed in a 12-mL screw-capped vial and spiked with the appropriate pesticide (100 μL of a 1000 μg/L solution in hexane). The contents of the vial were equilibrated for 30 min at 20ºC and shaken vigorously, and the phases were separated by centrifuging at 1500 × *g* for 3 min. Aliquots of the upper and lower phases (initially 100 μL) were diluted with toluene (1 mL) that contained 4 μg/L benzhydryl chloride as internal standard. These solutions were analyzed by gas chromatography. The size of the aliquots taken from the upper and lower phases was then adjusted to give similar-sized peaks to the internal standard, and the analysis was repeated. The partition coefficient "*P*" for each solvent combination was calculated from the formula:

$$P = C_{\text{light phase}}/C_{\text{heavy phase}} \qquad [1]$$

where $C_{\text{light phase}}$ and $C_{\text{heavy phase}}$ are the concentrations of the pesticide in the light and heavy phases. Each partition coefficient is the mean of at least six measurements.

Partition coefficients of Lissapol TN450. *N,N*-Dimethylformamide (DMF) (10 mL) and Lissapol TN450 (0.5 g) were added to a filtered solution of wool wax in hexane (25 mL, 10% wt/vol) in a heavy-wall 50-mL screw-capped vial. The contents of the vial were equilibrated at 20ºC and shaken vigorously, and the phases were separated by centrifuging at 1500 × *g* for 3 min. Aliquots of the upper (10 mL) and lower phases (100 μL) were evaporated to dryness under a stream of N_2 at 100ºC. The residue was taken up in hexane (10 mL), and the wool wax esters were separated from the detergent by elution from a Bond Elut Si (500 mg) cartridge with hexane/ethyl acetate (2:1, 5 mL). The detergent was recovered by elution with methanol (5 mL), and the oligomers were analyzed as their TMS ethers by gas chromatography.

Partition coefficients of wool wax acids, alcohols, and sterols. The free wool wax acids, alcohols, and sterols present in a solution of wool wax (5 mL, 10% wt/vol) in hexane were fortified by the addition of either a sample of wool wax acids (0.1 g) or lanolin alcohols (0.1 g), obtained from the hydrolysis of wool wax, and the mixture was partitioned with DMF (5 mL) as described above. An aliquot (200 μL) of each phase was evaporated to dryness under N_2 at 80ºC, dissolved in toluene (1 mL), and analyzed as the TMS derivative by gas chromatography.

Batchwise extraction of wool wax. Wool wax (100 g) was dissolved in hexane (1 L), filtered, and spiked to give levels of 5.2 μg/g of dieldrin and 60.3 μg/g of diazinon, based on the wax. This solution was extracted with DMF saturated with hexane (1 L) in a 5-L separating funnel for a total of five extractions.

RESULTS AND DISCUSSION

The initial evaluation of the suitability of different solvents to extract pesticides from wool wax was made by measuring the distribution of dieldrin and diazinon between a hydrocarbon solution of wool wax and a number of different solvents that were immiscible with the wool wax solution (Table 1). Only a limited number of solvents were immiscible with the hydrocarbon solution of wool wax, but a number of other solvents could be rendered so by the addition of small amounts of water. However, these were much less effective than the other pure solvents. The most effective extraction solvent was DMF. Only minor, almost insignificant, differences in extraction efficiencies were produced by varying the hydrocarbon wool wax solvent. Accordingly, the solvent system hexane/DMF was selected for further evaluation. As shown in Figure 1, the partition coefficients of dieldrin and diazinon were found to increase with increasing wool wax concentration. This is hardly surprising as the mixture of high-molecular-weight wool wax esters, which make up the bulk of the wool wax, are significantly more polar than hexane. Thus, an increase in wool wax concentration would be expected to decrease the polarity difference between the two phases, resulting in the observed increased solubility of DMF in the hexane-rich phase. This change in the composition of the phases also would be expected to affect the value of the pesticide partition coefficients; therefore, to easily compare results, all extractions were arbitrarily carried out at a 10% wt/vol wool wax concentration in the wool wax-rich phase.

The results for other pesticides are given in Table 2. The pesticides chosen cover all the organophosphorus and pyrethroid compounds that are registered for sheep treatments in the major wool-growing countries of the Western world (21–38), together with some other compounds that have the potential to be found in wool wax. The organochlorine compounds are generally only observed in waxes from certain Eastern European countries and are generally restricted to the isomers of benzene hexachloride.

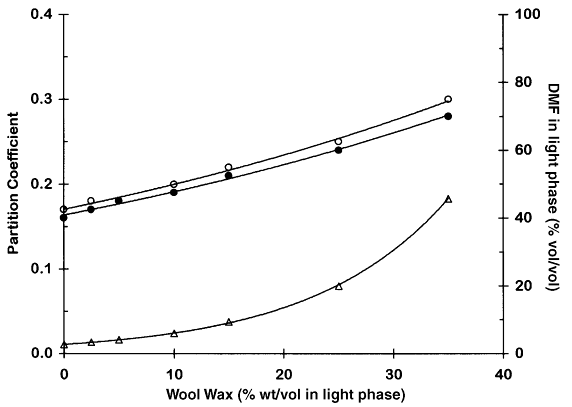

FIG. 1. Effect of wool wax concentration on the partition coefficients of dieldrin and diazinon and the solubility of *N,N*-dimethylformamide (DMF) in the hexane-rich phase. (○) Dieldrin; (●) diazinon; (△) DMF.

TABLE 2
Pesticide Partition Coefficients Between Solutions of Wool Wax (10% wt/vol) in Hexane and DMF

Pesticide	Partition coefficient	Pesticide	Partition coefficient
Aldrin	0.82	Bromophos methyl	0.33
α-BHC	0.18	Bromophos ethyl	0.37
β-BHC	0.14	Carbophenothion	0.091
δ-BHC	0.13	Chlorfenvinphos	0.056
Endrin	0.29	Chlorpyrifos methyl	0.065
Endosulfan-α	0.38	Chlorpyrifos ethyl	0.13
Endosulfan-β	0.18	Coumaphos	0.044
Heptachlor	0.39	Diazinon	0.24
Hexachlorobenzene	0.70	Dichlofenthion	0.22
DDD	0.064	Dichlorvos	0.054
DDE	0.22	Dioxathion	0.21
DDT	0.12	Ethion	0.055
Dieldrin	0.17	Fenchlorphos	0.11
Lindane	0.08	Malathion	0.051
Methoxychlor	0.11	Phosalone	0.065
		Pirimifos ethyl	0.31
Cyhalothrin[a]	0.042	Propetamphos	0.066
Cypermethrin	0.034		
Deltamethrin[a]	0.032		
Fenvalerate	0.024		

[a]Isolated from commercial formulations. Abbreviations: BHC, hexachlorocyclohexane; DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; and DDT, dichlorodiphenyltrichloroethane. See Table 1 for other abbreviation.

These partition coefficients indicate that, for most of the pesticides examined, one extraction with an equal volume of DMF would remove more than 70% of the pesticide residues. The two compounds aldrin and hexachlorobenzene, which have significantly poorer partition coefficients and would only be reduced by about 55%, are rarely found in raw wool wax.

To assess the quality of the wool wax after solvent extraction with DMF, a spiked sample was sequentially extracted five times. The resultant wax was of a high quality with undetectable levels of pesticide residues (Table 3). It was lighter in color with a slightly reduced melting point and a lower acid number. The level of free lanolin alcohols was also reduced, and although these alcohols are known to be responsible for the emollient properties of lanolin (39), the wax still displayed excellent water absorption capacity (Table 4). As shown by the partition coefficients in Figures 2 and 3, this extraction process preferentially removes the smaller and more polar members of the free acids and alcohols present in the wax, leaving substantial amounts of the higher members in the wax. This allowed the raffinate wax to retain its emollient properties, but meant that the wax still required a neutralization step to convert it to pharmaceutical grade.

During the refining of wool wax, it is essential to remove the detergent residues that arise from recovery of wax from aqueous wool scours. The presence of these residues is unde-

TABLE 3
Removal of Dieldrin and Diazinon from Wool Wax by Sequential Batch Extraction[a]

Extract number	Amount of wax extracted (%)	Residual pesticide levels in wool wax (μg/g) Dieldrin	Residual pesticide levels in wool wax (μg/g) Diazinon
0	—	5.2	60.3
1	12.0	0.87	13.21
2	4.1	0.14	2.72
3	1.3	0.022	0.57
4	0.4	n.d.	0.11
5	0.15	n.d.	n.d.

[a] n.d. = not detected. Total yield of raffinate wax = 82%.

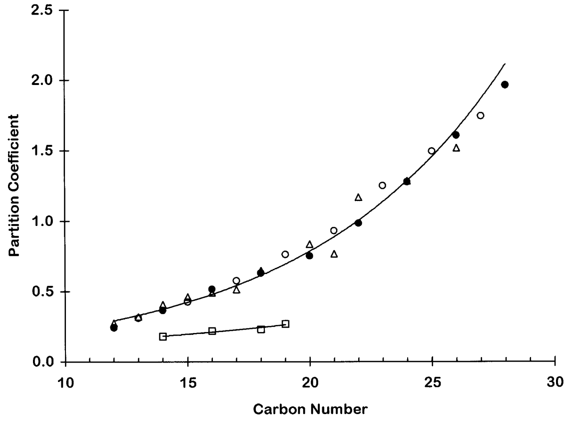

FIG. 2. Partition coefficient of wool wax acids as a function of chain-length. (○) *Iso*-acids; (●) *anteiso*-acids; (△) *n*-acids; (□) α-hydroxy acids.

sirable because they adversely affect the emollient properties of the lanolin and have been implicated in incidences of hypersensitivity to lanolin (18). Their removal is usually achieved by extraction of the molten wax, or a hydrocarbon solution of the wax, with an aqueous alcohol. As shown by the partition coefficients in Table 5, a single extraction with an equal volume of DMF will reduce the level of Lissapol TN450 residues by more than 98%. Similar results, which are not reported here, have been observed with other nonionic detergents that are commonly used to scour raw wool. This contrasts with only a 50% removal of detergent residues by the extraction of molten wool wax with aqueous isopropanol (17).

Clark (18) has reported that the prime allergen responsible for lanolin sensitivity is present in the natural free alcohols of wool wax and that the incidence of lanolin allergy is significantly reduced when the level of these free alcohols was reduced to below 3% by repeated, or continuous, extraction of the wool wax with aqueous isopropanol. While the exact alcohols responsible have not been identified, the use of a polar extraction solvent would suggest that the allergen is one of the more polar alcohols present and therefore would also be removed by the present extraction solvents.

The extracted wax is a highly complex mixture of the more polar components of the wool wax. It appeared to be similar to the "oxidized" wool wax fraction isolated by extraction of molten wool wax with refluxing methanol (15). It was heat-sensitive and darkened rapidly to a dark brown viscous "grease" after removal of the DMF solvent. From the amount of extract recovered during the sequential batchwise extraction, this material has an average partition coefficient of ≈0.47. About 15% of this material has a molecular weight range of 1000 to 2000 daltons, but the bulk of the material, 50%, was an intractable mixture of polar material with a similar molecular weight distribution to the wool wax esters. The balance of the material present contained the lower members of free wool wax acids, alcohols, and sterols. Free cholesterol was the single largest compound present and accounted for about 13% of the extract, i.e., about 60% of the amount present in the original wax.

TABLE 4
Characteristics of Raffinate Wool Wax

	Raw wool wax	Raffinate wool wax
Acid number (mg KOH/g)	6.5	2.17
Free lanolin alcohols (%)	11.5	4.9
Free cholesterol (%)	3.8	1.85
Detergent (% Lissapol TN450)	1.6	n.d.
Water absorption capacity (%)	85	275
Melting point (°C)	40.5	38
Color[a]		
yellow	30	9
red	4.7	1.3
green	1	0

[a] Lovibond 1/4-inch cell. n.d. = not detected. Lissapol TN450 (ICI, Melbourne, Australia).

The evaluation of these solvents in a continuous solvent-solvent extractor is discussed in the companion paper (40).

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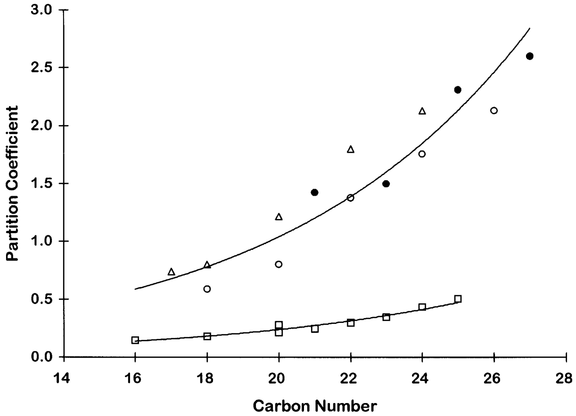

FIG. 3. Partition coefficient of wool wax alcohols as a function of chain-length. (○) *Iso*-mono alcohols; (●) *anteiso*-mono alcohols; (△) *n*-mono alcohols; (□) *iso* and *n*-1,2 diols.

TABLE 5
Partition Coefficients for Nonylphenol Ethylene-Oxide Oligomers Between Wool Wax Solutions in Hexane and *N,N*-Dimethylformamide

Ethylene-oxide number	Partition coefficient	Ethylene-oxide number	Partition coefficient
3	0.040	10	0.007
4	0.029	11	0.0055
5	0.028	12	0.0040
6	0.019	13	0.0030
7	0.014	14	0.0025
8	0.011	15	0.0020
9	0.009	16	0.0015

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