JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Photodegradation of Phosmet in Wool Wax Models and on Sheep Wool: Determination of Wool Wax Bound Phosmet by Means of Isotope Ratio Mass Spectrometry

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The photochemical reactions of phosmet, an organophosphorus insecticide used for plant protection and for control of ectoparasites on productive livestock, were studied in the presence of wool wax. Induced by UV light, phosmet features numerous degradation pathways as well as photoaddition reactions with lipid structure moieties. In model irradiation experiments of phosmet in mixtures of solvents (cyclohexane, cyclohexene, 2-propanol) and fatty acid methyl esters (methyl stearate, methyl oleate, 12-hydroxymethyl stearate), both adjusted to the hydroxyl and iodine values of wool wax, half-lives were determined to be approximately 7 and 16 h, respectively. Irradiation of phosmet on crude sheep wool resulted in a degradation rate of 65% after 24 h. In tracer studies with stable isotope labeled phosmet ([^{15}N]phosmet) in commercial lanolin and on raw sheep wool, employing a sunlight simulator and natural sunlight, wool wax bound phosmet was formed. After extraction and measurement by elemental analyzer/isotope ratio mass spectrometry, δ^{15}N values of the phosmet-free wool wax fractions were notably increased as compared to the value of natural lanolin. Calculated from the δ^{15}N values, an average of 13.9/15.6% (sunlight simulator/natural sunlight) was bound to wool wax lipids after irradiation of thin films of commercial lanolin. In experiments with sheep wool, 13.2 and 15.4%, respectively, were detected as wax-bound.

KEYWORDS: Phosmet; photodegradation; wool wax; organophosphorus insecticide; EA/IRMS

INTRODUCTION

The use of pesticides on plants and animals plays an important role in the production of high-quality and high-yield agricultural products such as crops and productive livestock. With the moment of application on animal skin or hair, pesticides undergo degradation reactions induced by, among others, weathering effects such as sunlight and humidity.

In lipid environments, as found on plant surfaces as well as animal skin or sheep's wool (skin lipids and wool wax), pesticides undergo reactions different from those observed in aqueous environments. Under UV irradiation conditions, photolysis and photoaddition products with lipid components are likely to be found.

In the present study, the dithio-organophosphorus insecticide phosmet [phosphorodithioic acid, *S*-[(1,3-dihydro-1,3-dioxo-2*H*-isoindol-2-yl)methyl] *O*,*O*-dimethyl ester (**Figure 1**)], containing a phthalimide moiety, was used for photoreaction experiments in the presence of wool wax.

Only a few data of photolysis experiments on this substance are available to date. Tanabe et al. irradiated phosmet in the presence of diethyl ether and identified *N*-methylphthalimide and *N*-methoxymethylphthalimide as main products (*1*). Other groups described photolysis reactions of phosmet on silica gel plates and on apples (*2*, *3*).

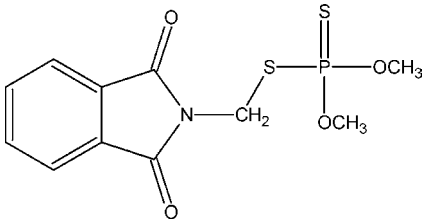

Figure 1. Molecular structure of phosmet.

Irradiation experiments with a number of other organophosphorus insecticides in the presence of wool were performed by Rammell (*4*), but degradation products were not identified.

Recently published photolysis studies in model solvents (methanol, 2-propanol, cyclohexane, and cyclohexene) and in fatty acid methyl esters showed potential reaction possibilities between phosmet and lipid constituents (*5*). A number of photoaddition products of phosmet and cyclohexene, a model for unsaturated lipid components, could be identified (*6*). Also, photoaddition products with methyl oleate, a more advanced model for wool wax, were identified by means of liquid chromatography−mass spectrometry (LC-MS) (*6*).

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10.1021/jf047834b CCC: $30.25
Published on Web 05/04/2005

Due to the complex composition of wool wax with a plethora of different ester structures, distinct photoaddition products are not likely to be identified. Because of this, a stable isotope labeled phosmet was synthesized (*7*) for photolysis experiments in wool wax and on raw sheep's wool. With ^{15}N-enriched samples, the fate of the labeled structure element can easily be monitored throughout all performed steps. δ^{15}N values of the resulting wool wax samples were measured by means of elemental analysis/isotope ratio mass spectrometry (EA/IRMS) for indicating bound phosmet in the case of increased values.

MATERIALS AND METHODS

Materials. All solvents used were of analytical grade or distilled prior to use. Water was purified by a Milli-Q 185 plus water purification system (Millipore Corp., Bedford, MA).

N-Hydroxymethylphthalimide and phthalimide (Fluka, Deisenhofen, Germany) were obtained as analytical grade standards. Phosmet-oxon and *N*-methoxymethylphthalimide were synthesized as described before (*5*). Phosmet was extracted from Imidan (Sigfried-Agro, Zofingen, Switzerland) and checked for purity as recently published (*5*). [^{15}N]-Phosmet was synthesized as described in ref *7*. Lanolin (anhydrous) was purchased from Sigma-Aldrich (Steinheim, Germany).

Raw sheep's wool was obtained from the back and lateral fleece of merino sheep (*Ovis aries*; Merino Land), which were not treated with pesticides. Coarse dirt was removed, and the wool was accurately mixed and stored at −18 °C. The wool contained on average 22.0% of wool wax [extracted with cyclohexane/ethyl acetate (1:1) by means of ASE].

General Techniques. High-performance liquid chromatography (HPLC) analyses of phosmet and photolysis products were carried out as described in ref *5*.

Isotope ratio mass spectrometry (IRMS) analyses were performed on a ThermoFinnigan DELTAplus XP system, coupled to a Euro EA elemental analyzer (Euro Vektor Instruments and Software, Hekatech, Wegberg, Germany) (oxidation furnace, 1020 °C; reduction furnace, 650 °C; carrier gas, 40 kPa; packed column temperature, 89 °C). Samples of 1.5−4 mg were weighed into tin capsules (5 × 9 mm) and sealed tightly to exclude air, which could interfere with the nitrogen isotope analysis. As standard material, apple leaves (Hekatech, SRM 1515 apple leaves, 2.25% N) were utilized. For data acquisition and processing, Thermo Electron ISODAT NT software, version 2.0, was used. δ^{15}N values are expressed in relation to atmospheric air as international standard. δ values (‰ difference from international standard) are defined as $\delta^{15}\text{N}\ (‰) = [(R_{smpl}/R_{std}) - 1) \times 1000$, in which R is the ^{15}N/^{14}N ratio of the sample and standard material, respectively.

Gel permeation chromatography (GPC) was performed on a Gilson system (Middleton, WI), consisting of a pump, an autosampler, a fraction collector, and a glass column (350 × 35 mm) filled with Bio-Beads S-X3 (Bio-Rad, Richmond, CA). As eluent, cyclohexane/ethyl acetate (50:50, v/v) at a flow rate of 2 mL/min was used.

Accelerated solvent extraction (ASE) was carried out with a Dionex ASE 200 extraction system (Sunnyvale, CA) with cyclohexane/ethyl acetate (50:50). Wool samples (1.25 g) were extracted twice in 22 mL extraction cells at 50 °C for 10 min at 10 MPa.

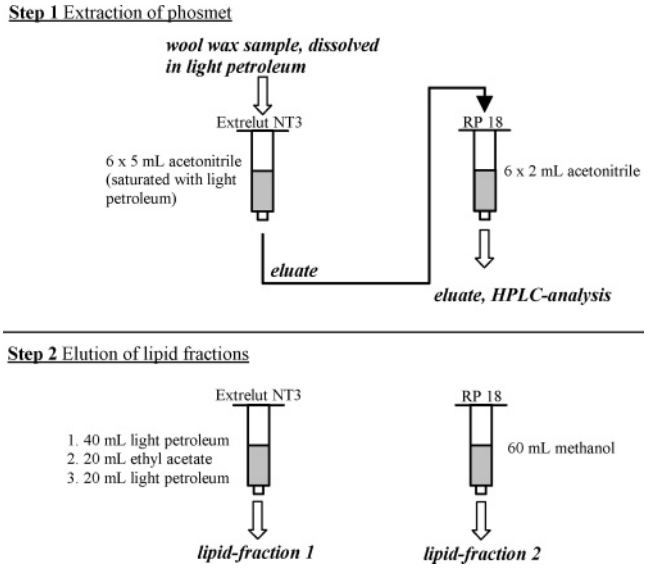
Irradiation Experiments. Irradiation in films and of raw sheep's wool was performed by employing a suntest CPS+ (Heraeus-Industrietechnik, Kleinostheim, Germany; xenon lamp; UV filters λ > 290 nm; air cooling; irradiance = 250 W/m^2; standard black temperature = 35 °C; dosis (24 h) 21600 kJ/qm).

Field irradiation experiments in the sun were performed in Stuttgart, Germany (9° 11′ E, 48° 47′ N, 391 m height above sea level) in the summer of 2004. The temperature was 25−27 °C, and the sky was generally cloudless.

For irradiation experiments in solutions, a metal halogen lamp (SOL 500, Dr. K. Hönle GmbH, Martinsried, Germany; 120 000 lx, 900 W/m^2) with a UV filter WG 295 (λ > 280 nm) (Schott Glaswerke, Mainz, Germany) was employed as light source.

Irradiation experiments in a mixture of model solvents were performed as follows. Phosmet (3.1 mmol/L, 1.00 g/L) was dissolved in a mixture of cyclohexane, cyclohexene, and 2-propanol [11.6:3.55:34.85, w/w/w, corresponding to the hydroxyl value (64.6) and iodine number (29.9) of wool wax] and degassed with nitrogen to prevent solvent oxidation. Subsequently, samples of 25 mL were irradiated for 7 h with stirring in water-cooled quartz cuvettes (60 × 35 mm) with Teflon caps. Samples of 0.5 mL were taken, diluted to 10 mL with methanol, and subjected to HPLC analysis.

Scheme 1. Sample Preparation of Wool Wax Samples, According to the Method of Diserens (*8*), Slightly Modified



For irradiation assays in a mixture of fatty acid methyl esters, Petri dishes (diameter = 9 cm, without cover) were used. Ten milligrams of phosmet and 1 g of the mixture of methyl stearate, 12-hydroxymethylstearate, and methyl oleate (17.9:47.2:35.0, w/w/w, also corresponding to the hydroxyl value and the iodine number of wool wax, as described above) were dissolved together in 50 mL of methanol. Five milliliters of the obtained solution was placed on the Petri dishes. After solvent evaporation at room temperature, 100 mg of fatty acid methyl ester containing 1 mg of phosmet resulted for each irradiation assay. The samples were irradiated for up to 24 h. For sample preparation, Petri dishes were rinsed with 25 mL of methanol, and the solution was used for HPLC analysis.

Irradiation experiments in films of lanolin were also performed in Petri dishes. Phosmet (20 mg, 1.2 at. % ^{15}N and 2 at. % ^{15}N, respectively) and lanolin (5 g) were dissolved in 50 mL of light petroleum ether. The two several enrichment levels were employed to control the comparableness of the results. Five milliliters of the obtained solution was placed on Petri dishes and evaporated, resulting in a film of 500 mg of lanolin containing 2 mg of phosmet in each irradiation assay. The samples were irradiated for 24 h in the suntest apparatus and in a field experiment in natural sunlight, respectively. For sample preparation, Petri dishes were rinsed with 50 mL of light petroleum ether and evaporated to dryness. Prior to HPLC analysis, the samples were fractionated using an SPE Extrelut NT3 and an SPE RP18 cartridge, largely following the procedure described by Diserens (*8*) (**Scheme 1**), however, slightly modified. Elution from the SPE Extrelut NT3 column was carried out six times with 5 mL of acetonitrile each (saturated with light petroleum ether) instead of four times, and elution from the SPE RP18 cartidge was performed four times with 2 mL of acetonitrile each instead of two times. The resulting sample was dissolved in methanol for HPLC analysis.

The lipid fractions, which remained on the respective SPE cartridges (Extrelut NT3 and RP 18), were eluted following **Scheme 1**.

Additionally, irradiated lanolin samples were also separated by means of GPC. Fractions of 10 mL (fractions 1−4 and fractions 12−19) and of 6 mL (fractions 4−11) were taken. Residual phosmet eluted in fractions 15−17. The high molecular weight fractions 4−12 were combined, solvent was evaporated, and the resulting residues were subjected to EA/IRMS.

For irradiation experiments on raw sheep's wool, samples of 2.5 g each were spread in a circle of 15 cm diameter. These samples were

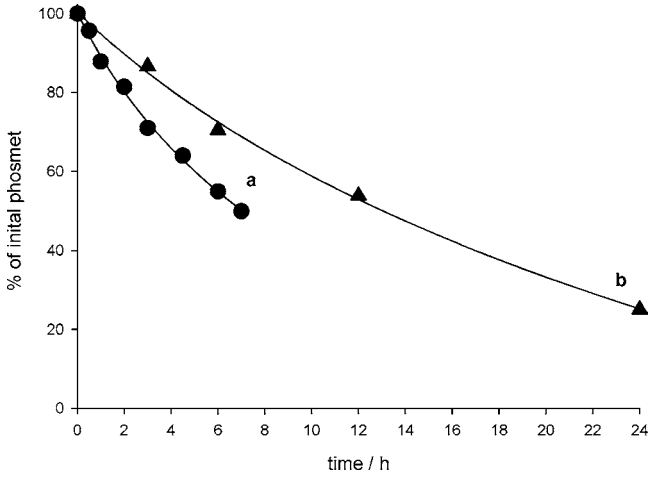


Figure 2. Photodegradation of phosmet in a mixture of cyclohexane, cyclohexene, and 2-propanol [11.6:3.55:34.85, w/w/w, 1 g/L phosmet, a (●)] and in a mixture of methyl stearate, 12-hydroxymethylstearate, and methyl oleate [17.9:47.2:35.0, w/w/w, 10 mg/g phosmet, b (▲)] both corresponding to the iodine (29.9) and hydroxyl value (64.6) of the used lanolin.

spiked with phosmet by spraying a solution of 2 g/L phosmet in methanol to accomplish initial contents of 4 and 8 mg/g of wool wax, respectively. Samples were dried at room temperature between spraying steps and before irradiation experiments. In each case, three samples of spiked wool were grouped into one Petri dish (quartz glass, 15 cm diameter, with cover). Samples were irradiated for up to 24 h in the suntest apparatus and in a field experiment in the sun, respectively. The Petri dishes were placed on small glass rings on wadded aluminum foil to allow for irradiation from all sides. Wool samples, spiked with labeled phosmet (1.2 at. % ^{15}N and 2 at. % ^{15}N, respectively) were prepared in the same way.

After extraction of the wool wax, the sheep's wool was pulverized in a mortar in the presence of liquid nitrogen, to allow also for EA/IRMS measurements.

RESULTS

Irradiation of Phosmet in Mixtures of Model Solvents Adjusted to the Hydroxyl and Iodine Values of Wool Wax. Recent studies showed considerable degradation rates in model solvents such as methanol, 2-propanol, cyclohexane, and cyclohexene (*5*). In these experiments, methanol and 2-propanol were used as models for primary and secondary alcohol groups as they occur in free fatty alcohols, hydroxy fatty acids, and sterols. Cyclohexane and cyclohexene were used as models for saturated and unsaturated constituents of wool wax and are also part of the backbone of sterols. As an intermediate step before the rather complex matrix of wool fat, irradiation experiments were performed in more advanced model systems representing the same hydroxyl and iodine values as wool wax. Therefore, phosmet was dissolved in a mixture of cyclohexane, cyclohexene, and 2-propanol and irradiated for up to 7 h. This resulted in rapid degradation of phosmet (**Figure 2**). The half-life was determined to be ~7 h.

Degradation products were formed in minute amounts, as can be seen in **Table 1**, whereas *N*-isopropoxymethylphthalimide and *N*-methylphthalimide are metabolites found only in the presence of pure 2-propanol or cyclohexene, respectively.

As a further step in the successive approximation to the matrix of wool lipids, irradiation experiments with phosmet, dissolved in a mixture of fatty acid methyl esters, adjusted to the hydroxyl and iodine values of wool fat, were performed. After 24 h of irradiation, only 25% of the initial phosmet content remained detectable (**Figure 2**). The half-life was reached at ~16 h.

Table 1. Photodegradation of Phosmet and Formation of Photoproducts in a Solution of Cyclohexane, Cyclohexene, and 2-Propanol (11.6:3.55:34.85, w/w/w) and in a Film of Methyl Stearate, 12-Hydroxymethylstearate, and Methyl Oleate (17.9:47.2:35.0, w/w/w), Both Corresponding to the Iodine (29.9) and Hydroxyl Values (64.6) of the Used Lanolin

	solution (mol %) (7 h)	film (mol %) (12 h)
N-hydroxymethylphthalimide	0.9	1.6
N-methoxymethylphthalimide	4.6	3.8
phthalimide	0.7	2.4
N-isopropoxymethylphthalimide	1.3	
N-methylphthalimide	1.0	
sum of photoproducts	8.5	7.8
turnover of phosmet	52.1	46.2

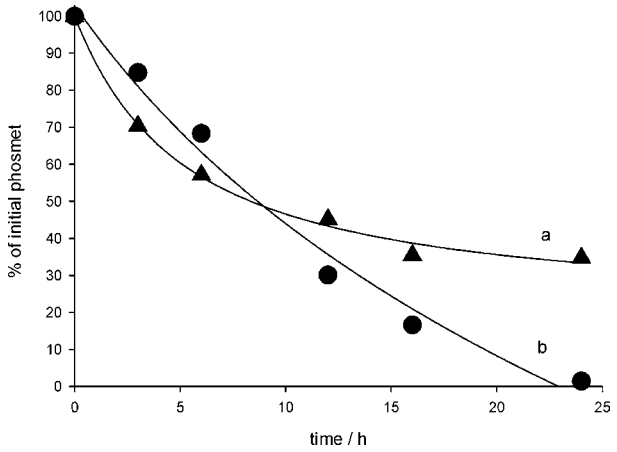


Figure 3. Photodegradation of phosmet (4 mg/g of wool wax) on raw sheep wool [a (▲)] in comparison to the photodegradation of phosmet (4 mg/g) in thin films of lanolin [b (●)] (data from ref *6*).

Common photodegradation products were found in small amounts. In **Table 1** are presented values after 12 h of irradiation, when photodegradation was comparable to the experiments in solutions (~50%).

Irradiation Experiments on Sheep's Wool. To study the photodegradation of phosmet on sheep's wool, aliquots of raw sheep's wool were spiked with phosmet to an initial level of 4.0 mg/g of wool wax. After irradiation in a sunlight simulator and extraction of the wool wax, the samples were cleaned up before HPLC analysis (*8*). Phosmet was rapidly degraded on sheep's wool as well (**Figure 3**). For comparison, the degradation curve in anhydrous lanolin (Sigma-Aldrich) [(*6*), initial phosmet content = 4 mg/g of lanolin], which was obtained in precedent experiments, is given as well in **Figure 3**.

After 24 h of irradiation on raw sheep's wool, a degradation of 65% was determined; surprisingly, the non-irradiated control sample also showed a phosmet degradation of 37 ± 8% ($n = 3$). A comparable high degradation rate in dark experiments was never observed in assays with phosmet in model solvents, fatty acid methyl esters, or even commercial lanolin. Non-irradiated control experiments were additionally performed with wool samples, which were heated to 80 °C for 15 min, to inactivate the wool microflora. However, also in these experiments, only 65% of the former phosmet was found unchanged after 24 h. Hydrolysis products such as *N*-methoxymeth-

Table 2. IRMS Results of [^{15}N]Phosmet-Spiked and UV-Irradiated Lanolin Samples before and after Lipid Fractionation[a]

	sample	1.2 at. %, 4 mg/g	2 at. %, 4 mg/g	1.2 at. %, 8 mg/g	2 at. %, 8 mg/g
		(A) Sunlight Simulator			
lanolin (before extraction)[b]	A	440 ± 21	679 ± 62	704 ± 35	1553 ± 7
	B	408 ± 19	772 ± 10	785 ± 19	1576 ± 20
lipid fraction 1	A	158 ± 7	250 ± 4	282 ± 9	528 ± 16
	B	151 ± 20	236 ± 9	274 ± 5	546 ± 36
lipid fraction 2	A	178 ± 4	305 ± 14	369 ± 23	746 ± 38
	B	149 ± 9	325 ± 8	372 ± 10	716 ± 53
		(B) Natural Sunlight			
lanolin (before extraction)[b]	A	476 ± 18	689 ± 50	839 ± 65	1593 ± 44
	B	465 ± 25	748 ± 12	852 ± 37	1563 ± 66
lipid fraction 1	A	118 ± 5	166 ± 5	202 ± 8	388 ± 28
	B	111 ± 7	175 ± 6	216 ± 7	406 ± 12
lipid fraction 2	A	233 ± 6	280 ± 23	407 ± 16	787 ± 65
	B	195 ± 23	295 ± 18	363 ± 33	839 ± 29

[a] δ^{15}N values are given in ‰ versus atmospheric air ± SD. Values are from three to five EA/IRMS measurements. [b] δ^{15}N of natural lanolin is 14.3‰.

ylphthalimide and phthalimide were detected in amounts of altogether 8% in the dark assays and cannot explain the loss of 35%.

After 24 h of irradiation, the photoproducts *N*-methoxymethylphthalimide and phthalimide were found in mole percentages of 0.8 and 4.6, respectively, whereas phosmet-oxon, which was the main degradation product during experiments in films of lanolin, could be observed in only traces of ~0.2 mol %.

Irradiation Experiments of [^{15}N]Phosmet in Thin Films of Lanolin. To investigate whether and to what extent photoaddition products of phosmet and lipid components of wool wax are formed, irradiation experiments with stable isotope labeled phosmet in wool wax were conducted. Commercial lanolin was spiked with 4 and 8 mg/g phosmet, respectively, and was subsequently irradiated for 24 h in a sunlight simulator. Additionally, the same experiments were performed under natural sunlight for 3 days in a row for a total irradiation time of 24 h. All samples were fractionated, employing a slightly modified extraction method introduced by Diserens (*8*) (**Scheme 1**).

Lipid fraction 1 mainly consisted of cholesteryl ersters and more unpolar components and was yielded in averages of 54 and 64% after irradiation under sunlight simulator and natural sunlight conditions, respectively. *Lipid fraction 2*, basically consisting of free cholesterol and more polar compounds, was yielded in 6 and 10%, respectively, of the original lanolin sample.

The resulting fractions, as well as the spiked lanolin samples, were subjected to EA/IRMS. The natural δ^{15}N of the lanolin used here was 14.3‰ at a nitrogen content of 0.08%. In **Table 2**, the obtained δ^{15}N values of lipid fractions 1 and 2 and the spiked wool wax after irradiation are shown. It is obvious that the δ^{15}N values of the phosmet-free lipid fractions 1 and 2 increased notably as compared to the value of natural lanolin. The increase of δ^{15}N correlated well with higher spiking level and higher enrichment of ^{15}N (**Table 2**).

Non-irradiated control samples with labeled phosmet and extracted in the same manner showed no increase of δ^{15}N values in lipid fractions 1 and 2. Also, when the irradiated but nonspiked wool wax samples were measured, no enrichment of ^{15}N of the lipid fractions during the extraction method could be observed.

In summary, the following observations were made (**Figure 4**). Using the sunlight simulator and natural sunlight, respectively, 10−18 and 23−33%—depending on the ^{15}N spiking and enrichment level—of the initial phosmet remained unchanged after 24 h of irradiation.

Phosmet-oxon, the main degradation product of experiments in thin films of lanolin, was formed in amounts of 21−27 and 21−22%, respectively. Other degradation products (*N*-methoxymethylphthalimide, *N*-hydroxymethylphthalimide, and phthalimide) were detectable in amounts of altogether 7−9% under both sunlight simulator and natural sunlight conditions. Calculated from the δ^{15}N values, lipid fractions 1 and 2 contained 11−14 and 1.5−3.4%, respectively, of lipid-bound phosmet after irradiation under sunlight simulator conditions. Under natural sunlight conditions, however, the bound phosmet was slightly shifted into lipid fraction 2 (3.5−4.5%) at the expense of lipid fraction 1.

For comparison, a different extraction method employing GPC, which separates by molecular size independent of the polarity, was used. The samples were irradiated at two spiking and two enrichment levels in the sunlight simulator for 24 h. The high molecular weight fractions, amounting to 55% of the former wool wax sample, which eluted before the fractions of low molecular weight, were combined and subjected to EA/IRMS. Calculated from the δ^{15}N values, total percentage of phosmet conjugated to lipid components varied from 16 to 21% of the initial phosmet (**Figure 5**).

Irradiation Experiments of [^{15}N]Phosmet on Sheep's Wool. To study whether the results of the lanolin film experiments can be transferred to the more complex matrix of raw sheep's wool, analogous experiments with spiked raw sheep's wool were carried out. Wool samples of 2.5 g each were spiked with phosmet at two spiking levels (4 and 8 mg/g of wool wax) by means of spraying application. The samples were irradiated for 24 h in the sunlight simulator and in a field experiment with natural sunlight. Subsequently, the wool wax was extracted from the sheep's wool by employing ASE and repeated column extraction as described above.

Lipid fraction 1 yielded 74 and 80% of the original lanolin sample after irradiation under sunlight simulator and natural sunlight conditions, respectively. Lipid fraction 2 afforded 6 and 8%, respectively.

The resulting lipid fractions 1 and 2, the spiked wool wax samples, and the extracted wool fiber were analyzed by means of EA/IRMS. The natural wool fiber showed an average nitrogen content of 12.3% and an average δ^{15}N of 6.6‰. Compared to the non-irradiated control samples, the δ^{15}N values of the fibers of irradiated wool samples did not change. This indicates that phosmet is not bound to the wool fiber. The nitrogen content of the extracted natural wool wax was 0.21% with a δ^{15}N value of 12.9‰.

However, the δ^{15}N values of phosmet-free lipid fractions 1 and 2 increased notably in comparison to the δ^{15}N value of extracted nonspiked wool wax (**Table 3**). As also observed in the experiments with lanolin, the increase of δ^{15}N correlated well with higher spiking and enrichment level of ^{15}N (**Table 3**).

The δ^{15}N values of the samples from irradiation experiments with sheep's wool were slightly below the δ^{15}N values of lanolin samples, although the spiking levels were comparable. A possible explanation for this observation is the higher nitrogen content of the extracted wax from sheep's wool as compared to commercial lanolin.

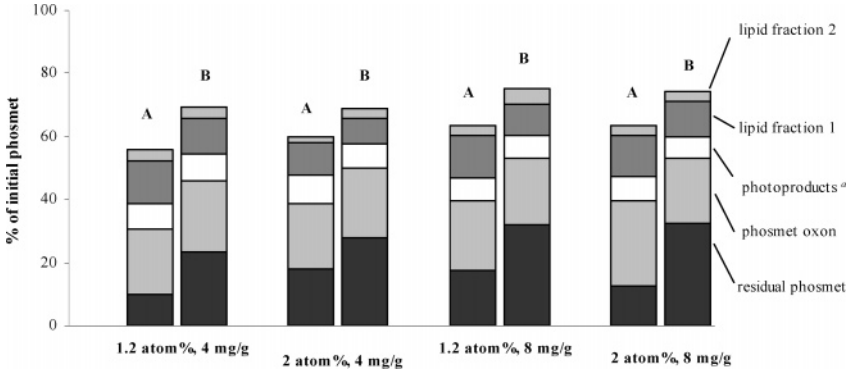


Figure 4. Distribution of phosmet and photoproducts in lanolin (spiking levels: 4 and 8 mg/g, each with 1.2 at. % ^{15}N or 2 at. % ^{15}N) after UV irradiation in a sunlight simulator (A) in comparison to natural sunlight (B). [a] *N*-Methoxymethylphthalimide, *N*-hydroxymethylphthalimide, phthalimide.

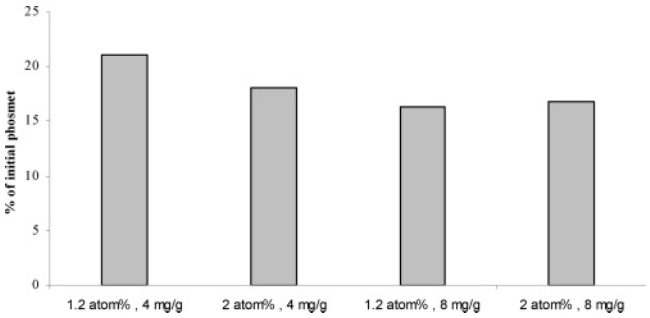


Figure 5. Amounts of wax-bound phosmet in the fractions of high molecular weights after separation of the irradiation assays by means of GPC.

Table 3. IRMS Results of [^{15}N]Phosmet-Spiked and UV-Irradiated Wool Wax Samples before and after Lipid Fractionation[a]

	sample	1.2 at. %, 4 mg/g	2 at. %, 4 mg/g	1.2 at. %, 8 mg/g	2 at. %, 8 mg/g
		(A) Sunlight Simulator			
wool wax (before extraction)[b]	A	132 ± 7	280 ± 25	271 ± 4	677 ± 17
	B	184 ± 21	283 ± 16	274 ± 12	701 ± 81
	C	147 ± 18	296 ± 19	423 ± 23	
lipid fraction 1	A	41 ± 6	60 ± 1	57 ± 2	174 ± 5
	B	33 ± 1	54 ± 2	59 ± 2	203 ± 8
	C	32 ± 1	79 ± 9	91 ± 3	
lipid fraction 2	A	198 ± 51	266 ± 43	370 ± 27	603 ± 40
	B	170 ± 8	374 ± 98	418 ± 20	786 ± 64
	C	192 ± 40	365 ± 32	376 ± 66	
		(B) Natural Sunlight			
wool wax (before extraction)[b]	A	208 ± 1	385 ± 10	381 ± 4	637 ± 7
	B	184 ± 2	266 ± 4	380 ± 6	730 ± 14
lipid fraction 1	A	55 ± 2	100 ± 1	82 ± 1	173 ± 1
	B	51 ± 1	84 ± 1	80 ± 1	129 ± 3
lipid fraction 2	A	199 ± 11	355 ± 25	332 ± 6	354 ± 3
	B	260 ± 8	329 ± 77	349 ± 44	514 ± 97

[a] δ^{15}N values are given in ‰ versus atmospheric air ± SD. Values are from three to five EA/IRMS measurements. [b] δ^{15}N of natural wool wax is 12.9‰.

Non-irradiated control samples with labeled phosmet and extracted in the same way as irradiated samples showed no increase of δ^{15}N values in lipid fraction 1 and only a very slight increase in lipid fraction 2. Also, on measuring irradiated but nonspiked wool wax samples, extracted from sheep's wool, no enrichment of ^{15}N of the lipid fractions during the extraction method could be found.

The following product distribution was obtained (**Figure 6**): Due to the higher variation of the irradiation experiments in the more complex environment of sheep's wool, no distinction can be made in terms of the amount of converted phosmet using a sunlight simulator compared to the natural sunlight.

After 24 h of irradiation, depending on the spiking and enrichment levels, 35−49 and 48−59% of the initial phosmet content were detectable after exposure in the sunlight simulator and natural sunlight, respectively. Phosmet-oxon, a main metabolite in the irradiation experiments of thin films of lanolin, was not detectable in the sheep's wool assays. The concentrations of other degradation products (*N*-methoxymethylphthalimide and phthalimide) were summed and averaged to 7−11 and 7−13%, respectively.

Calculated from the δ^{15}N values, lipid fraction 1 contained 7−13 and 10−16% of lipid-bound phosmet after sunlight simulator or natural sunlight irradiation, respectively. In lipid fraction 2 1.9−3.2 and 1.9−3.4% of lipid-bound phosmet were found.

DISCUSSION

As recently published, the organophosphorus insecticide phosmet is easily photodegraded in model solvents and fatty acid methyl ester films (*5*, *6*). In the presence of solvent mixtures (2-propanol/cyclohexane/cyclohexene) adjusted to the hydroxyl and iodine values of wool wax, the photodegradation corresponded quite well to those obtained in the pure solvents (*6*). The photoproducts *N*-methoxymethylphthalimide, *N*-hydroxymethylphthalimide, and phthalimide, commonly observed in all of the pure model solvents used, were also formed in relatively small amounts. However, it is important to note that the photoproducts formerly identified only in particular solvents, such as *N*-isopropoxymethylphthalimide in 2-propanol and both *N*-methylphthalimide and solvent photoaddition products in cyclohexene, were detected as well in the mixture of solvents. In a more realistic model for wool wax, irradiation experiments in mixtures of fatty acid methyl esters resulted in a degradation (∼75%) corresponding to the degradation rate found in pure methyl oleate (*6*) and indicating the influence of unsaturated lipids on the photochemistry. Consequently, the same groups of methyl oleate photoaddition products could be detected by HPLC in the mixture of fatty acid methyl esters, too. These results prove the suitability of the model systems used to first study the general photochemical possibilities of phosmet in lipid environments.

Irradiation of phosmet directly applied to natural sheep's wool resulted in a degradation of ∼65% after 24 h, in terms of turnover and kinetics, quite different from the results obtained in the presence of commercial lanolin (**Figure 3**). These differences can be attributed only to a hindered UV radiation accessibility to phosmet/wool wax inside the wool fibers.

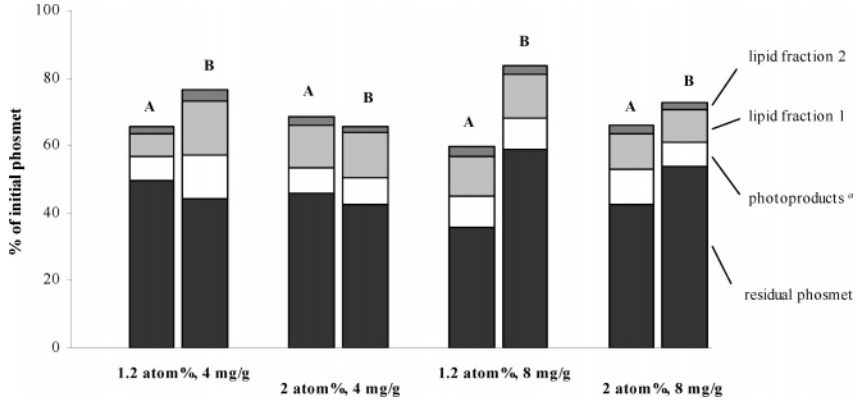


Figure 6. Distribution of phosmet and photoproducts in wool wax from sheep wool (spiking levels: 4 and 8 mg/g, each with 1.2 at. % ^{15}N or 2 at. % ^{15}N) after UV irradiation in a sunlight simulator (A) in comparison to natural sunlight (B). [a] *N*-Methoxymethylphthalimide, phthalimide.

Other and more surprising differences between wool/wool wax and lanolin were observed in dark control experiments. Although in the presence of lanolin (as well as in the presence of fatty acid methyl esters), phosmet was stable in the absence of light, on raw wool a mean degradation of 37% took place after 24 h. Also, heating of wool samples at 80 °C for 15 min to inactivate enzymatic or microbial activities before spiking did not really influence the dark reactivity of phosmet. Possibly, heat-resistant enzymes and microbes have not been totally inactivated.

The typical degradation products *N*-methoxymethylphthalimide and phthalimide, found at 8% altogether, cannot explain the high loss of phosmet. Consequently, there are additionally meaningful non-photochemical degradation pathways of phosmet on raw wool presently not understood. It is as well an open question if and to what extend the "dark reactions" also occur during UV irradiations, because there is presently no marker to distinguish between them.

After irradiation of phosmet in commercial lanolin, the main product was phosmet-oxon [up to 27% (*6*)], never found in experiments with solvents and fatty acid methyl esters and, therefore, photooxygenating properties of "wool wax" (lanolin) can be suggested. However, the assays on raw sheep's wool afforded only traces (0.2 mol %) of phosmet-oxon under the same irradiation conditions.

Due to the complex composition of wool wax, the analytical determination or isolation of distinct photoaddition products between phosmet and lipid components cannot be successful. Therefore, we used a different strategy employing a stable isotope (^{15}N) labeled compound as tracer and isotope ratio mass spectrometry as tracing analytical tool. After irradiation both in lanolin and on sheep's wool, a sample cleanup was carried out following a procedure introduced by Diserens (*8*) for residue analysis, using SPE and resulting in two lipid fractions free of phosmet and small degradation products. Both in the presence of lanolin and on wool, the δ^{15}N values of lipid fractions 1 and 2 remarkably increased by the action of UV light, which is a clear evidence for the formation of wax-bound phosmet. Generally, lipid fraction 1 (mainly sterol esters) contained ~10-fold bound phosmet as compared to lipid fraction 2 (mainly free sterols), indicating preferred binding to unsaturated fatty acids. In a comparison of the light sources, under natural sunlight the formation of bound residues was slightly enhanced (~15.5% of initial phosmet) as compared to sunlight simulator conditions (~13.5%). The different spiking levels of 4 and 8 mg/g of wool wax had no influence on the percentage of formation of wax-bound components. Despite the high formation of phosmet-oxon in the presence of lanolin, the same amounts of bound phosmet were found in lipid fractions 1 and 2. The extracted sheep's wool showed no increased δ^{15}N values, indicating that phosmet did not bind to the wool fiber matrix, and, therefore, leaving the dark reactivity of phosmet on wool unexplained.

As an alternate cleanup method, GPC (*9*) was used after irradiations of phosmet in lanolin, and the first fractions eluting before residual [^{15}N]phosmet were subjected to EA/IRMS. This extraction method did not reveal any observable differences in terms of the formation of lipid adducts in dependence of the spiking levels. An average of 18.0% of the initial phosmet was found in the form of photoaddition products, which was in the same range as the values obtained by the SPE method. This proves that both the extraction method introduced by Diserens and GPC are applicable for the analysis of wool wax bound pesticides or other xenobiotics, but in terms of simplicity and time consumption, we feel that SPE is more practicable.

In conclusion, the methods utilized here show that the organophosphorus insecticide phosmet is readily photodegraded on sheep's wool and potentially forms addition products with wool wax constituents when exposed to UV radiation. Yet, our studies do not provide any information about the molecular structures of the lipid-bound residues.

The comparableness of the results employing the sunlight simulator compared to natural sunlight clearly shows that results close to reality are obtained with the sunlight simulator.

From a couple of differences in the outcomes obtained on raw sheep's wool in comparison to commercial refined lanolin, especially concerning the formation of the oxon, it must be concluded that lanolin is not in all respects a suitable model to study the fate of pesticides on sheep's wool.

ABBREVIATIONS USED

EA/IRMS, elemental analyzer/isotope ratio mass spectrometry; GPC, gel permeation chromatography; ASE, accelerated solvent extraction.

ACKNOWLEDGMENT

We thank the Research Station for Husbandry and Organic Farming, University of Hohenheim, for the supply of merino sheep wool and W. Armbruster (Institute of Food Chemistry, University of Hohenheim) for support during EA/IRMS analysis.

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Received for review December 22, 2004. Revised manuscript received April 5, 2005. Accepted April 5, 2005.

JF047834B