Bull Environ Contam Toxicol (2007) 78:299–303
DOI 10.1007/s00128-007-9159-x

Enhanced Skin Absorption and Fly Toxicity of Permethrin in Emulsion Formulation

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Received: 23 March 2007 / Accepted: 29 April 2007 / Published online: 10 July 2007

In the course of developing new formulations for fly control, we examined the skin disposition of insecticide permethrin, using excised pig skin and evaporation/penetration cells. This model has been shown to be predictive of human skin absorption (Reifenrath et al. 1984; Reifenrath and Hawkins 1986). Since permethrin formulations are intentionally applied to the skin of livestock, we also studied the disposition on cattle skin using similar methods. In many cases, dermal absorption studies of pesticides are conducted in simple solvents, such as acetone or ethanol (Shah et al. 1987), and may not be representative of skin absorption from other types of vehicles. Dust formulations of pesticides are frequently used for fly control in livestock. Emulsifiable concentrates (EC) are diluted and water and the resultant emulsion is applied as space sprays to inanimate surfaces as well as to livestock. Therefore, we determined the evaporation and skin penetration characteristics of permethrin in ethanol solution, clay dust (cattle skin only), and emulsion vehicles. Since the evaporation and skin penetration properties of the insect repellent *N*,*N*-diethyl-m-toluamide or DEET have been extensively studied, we included this compound in our study for reference purposes.

Finally, as a parallel to the skin absorption measurements, we examined the knock-down ability of permethrin formulations against house flies. Formulations which enhanced mammalian dermal absorption may also have enhanced insect absorption and toxicity.

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Materials and Methods

[^{14}C-carbonyl]*N*,*N*-diethyl-m-toluamide was originally synthesized by New England Nuclear, Boston, MA and repurified by Moravek Biochemicals, Brea, CA. [^{14}C-benzyl ring UL] *cis*, *trans* permethrin (*cis*/*trans* ratio 46/49) was obtained from Sigma, St. Louis, MO.

Technical grade permethrin (97%, *cis*/*trans* ratio 43/57), and permethrin in emulsifiable concentrate (Permethrin 10% EC 2784) were obtained from McLaughlin Gormley King Co. (MGK), Minneapolis, MN. Permethrin analytical standard (*cis*/*trans* 46/52) was obtained from Chem Services, West Chester, PA. *N*,*N*-diethyl-m-toluamide (97%) was obtained from Sigma-Aldrich, Milwaukee, WI. All other chemicals/solvents were of reagent grade.

Radiochemical purities were determined by HPLC/radiometric flow detector by the manufacturer (95% for permethrin, 99.3% for DEET) and similarly re-determined in-house at the conclusion of this study (95% for permethrin in EC, 100.3% for DEET in ethanol solution). HPLC conditions for permethrin analysis were as follows: the solvent methanol:water (85:15) was pumped under isocratic conditions through a C18 column (Discovery C18, 15 cm × 4.6 mm, 5 μ) using a Spectra Physics SP8800 pump. Column effluent was monitored at a wavelength of 254 nm. The UV monitor output was processed with a PC using Turbochrome software (Perkin Elmer). A Perkin Elmer NCI 900 was used to interface the monitor with the PC. A Spectra-Physics SP8780XR auto sampler with an Alltech 500 µL sample loop was used to inject 25 µL samples onto the column. Under these conditions, the two isomers of permethrin had a retention time of 7.4 (*trans* isomers) and 8.8 (*cis* isomers) min. These conditions, along with a radiometric flow detector (B-RAM, Model 2,

INUS, Tampa, FL), were used to determine radiochemical purity. For DEET, the solvent (60% methanol) was pumped under isocratic conditions through a C-18 column (Supelco Sil LC18S, 5 μm, 4.6 × 250 mm). Under these conditions, DEET had a retention time of 9.5 min.

Methods for the determination of evaporation and penetration with pig skin have been published (Reifenrath et al. 2002). These procedures were used for cattle skin, with the following notes. Cattle skin was obtained at a local slaughter house within an hour of slaughter, with care taken to avoid exposure to salt or other chemicals. Whole skin was transported to the laboratory on wet ice. After cleaning with cold water only, an electric clipper was used to reduce hair length to 0.25 in. Strips of skin were dermatomed (Brown electrodermatome, Zimmer USA, Warsaw, IN) to an overall thickness of 0.5–0.7 mm, similar to pig skin. At completion of the 24-h diffusion period, pig skin epidermis was heat separated. This was not possible with cattle skin; instead, skin samples were clipped of remaining hair, and the hair was collected on two tape strips (Magic transparent tape, 3 M, Minneapolis). The skin surface was tape stripped 22 times to remove the stratum corneum. Hair and stratum corneum tape strips (groups of 2, 5, 5, 5 and 5) were placed in separate liquid scintillation counting (LSC) vials. After dissolution of the tape by addition of 2–4 mL of tetrahydrofuran (Sigma-Aldrich, St. Louis, MO) to the LSC vials and standing for 48 h, LSC fluid (Optifluor O, Perkin Elmer) was added to the vials and radioactivity assayed by liquid scintillation (Perkin Elmer TR 2300). Radiolabel remaining in the viable epidermis and dermis of cattle skin was determined by combustion (Perkin Elmer Model 307 sample oxidizer, Norwalk CT) in the case of black angus cattle skin or solubilization (Soluene 350, Perkin Elmer) for white faced cattle skin. Each test was replicates 3–4 times per animal, with skin samples from two different animals used for permethrin emulsion (P/EC) and two different pigs for DEET (see Tables 1, 2). Data were analyzed by a two-group t test ($p = 0.05$) for the effect of different animals' skin within replicates for P/EC and DEET (Tables 1, 2). Two-way analysis of variance and Student–Newman–Keuls multiple range test were used to determine the effect of test substance and skin type on percutaneous absorption (Table 3). All analyses were carried out at the $p = 0.05$ level of significance using BMDP Statistical Software (Los Angeles, CA).

House fly toxicity tests were conducted in Plexiglas cages, designed by Bradley A Mullens, Dept. of Entomology, University of California Riverside. The floor measured 8 in. by 8 in., with walls 2 in. in height. To prevent chemical vapors from accumulating in the cage, the center of the removable ceiling was fitted with an air outlet tube (1/4 in. inside diameter with a screen to prevent insect entry) that was connected to a vacuum pump via an air flow gauge set at 2 L per minute. Vacuum pump exhaust was directed external to the laboratory. The test cage floor contained four felt pads (approximately 4 in. on a side). Two non-adjacent pads received test formulation and the remaining two pads received control carrier. The walls of the cage each had two screened 1 in. diameter holes (centers located 2 in. from the corners) to allow fresh air entry. To give a 1% (w/w) permethrin emulsion, Evercide (10% permethrin) was diluted one part Evercide and nine parts water. The control vehicle (–P/Evercide) was also diluted (1 part –P/Evercide plus 9 parts water). Permethrin emulsion was applied to the felt treatment grids at a permethrin dose of 0.055 mg/cm^2 (0.55 g/m^2). An equal volume of –P/Evercide was applied to the control grids. In a separate experiment, permethrin formulated as a 1% dust (Minugel 200, Floridan, Quincy, FL) was applied to treatment grids at a dose of 0.033 mg/cm^2 (0.33 g/m^2) in 300 mg of dust per grid. An equal amount untreated dust was applied to control grids.

Fifty flies (*Musca domestica*, L., 2–4 day old adults of mixed sex, raised from pupae supplied by Carolina Biological Supply, Burlington, NC) were gently pipetted into the test cage and the cage positioned over a fluorescent light (glow box) which helped to orient the flies to the floor of the cage. Flies were observed for knockdown or incapacitation up to 2.5 h after fly introduction. Where possible, the time (minutes) to reach 50% fly incapacitation following fly introduction was recorded. Following removal of the flies, cage lids were removed to expose treatments to the laboratory atmosphere (indirect lighting). Treatments were allowed to age for 1 week and were retested with fresh flies. Percent repellency was calculated as the number of flies on the control grids divided by the total number of flies on all the grids and multiplication of the quotient by 100.

Results and Discussion

The skin disposition of radiolabeled test compounds for pig and cattle skin was given in Tables 1 and 2, respectively. Total skin penetration (receptor fluid plus dermis) for cattle and pig skin was given in Table 3. DEET/ethanol (0.5 mg/cm^2) was extensively and rapidly absorbed by excised cattle skin, and the value obtained in this study (69 ± 5%) was similar to the value (73 ± 8%) reported for live cattle at a dose of 10 mg/kg (about 1 mg/cm^2), also applied in ethanol (Taylor et al. 1994). Skin penetration of DEET and permethrin were both higher for cattle skin as compared to pig skin (Table 3). This finding is consistent with the general observation of higher skin permeability for densely haired animals. For all formulations and skins, evaporative loss of permethrin was low (1% or less), as might be ex-

Table 1 Disposition of ^{14}C-labeled compounds on excised pig skin[a]

Percent of applied radioactive dose[b]					
Isotope/formulation	Evaporation	Dermis	Receptor fluid (RF)	Dermis + RF	Total recovery
[14C-P]2%Permethrin EC (skin 1)	0.1 ± 0.1	24 ± 3	0.1 ± 0.0	25 ± 3	91 ± 15
[14C-P]2%Permethrin EC (skin 2)	0.2 ± 0.1	37 ± 10	0.1 ± 0.0	37 ± 10	93 ± 4
[14C-P]2%Permethrin EC grand average[c]	0.2 ± 0.1			31 ± 9	92 ± 10
[14C-P]Permethrin/ethanol	0.6 ± 0.1	2.5 ± 0.1	0.2 ± 0.1	2.7 ± 0.1	96 ± 1
[14C-DEET]/ethanol[d]	66 ± 5	8 ± 6	15 ± 5	23 ± 3	92 ± 2

[a] An emulsifiable concentrate formulation of 2% permethrin (P/EC, 125 μg/cm^2), a solution formulation of 0.32% permethrin in ethanol (P/ETOH, 40 μg/cm^2), and an 8% solution of *N*,*N*-diethyl-m-toluamide in ethanol (DEET, 500 μg/cm^2) were applied to excised pig skin. Percutaneous absorption (Dermis + RF) was calculated as the sum of label remaining in the dermis and receptor fluid

[b] Mean and standard deviation of three replicates. P is permethrin, EC is Evercide emulsifiable concentrate (MGK), Skin 1 is skin from pig 1 and Skin 2 is skin from a second pig. For the EC formulations, a 5 μL dose of EC formulation was diluted with 13.5 μL of distilled water to create an emulsion on the skin surface

[c] Means were not significantly different between experiments (different animals, *t* test, $p > 0.05$), therefore replicates were combined

[d] Data from Olson et al. (2003)

Table 2 Disposition of ^{14}C-labeled compounds on excised cattle skin[a]

Percent of applied radioactive dose[b]								
Exp no.	Formulation	Evaporation	Skin surface	Hair	Skin	Receptor fluid (RF)	RF + skin	Total recovery
10	P/MG	0.11 ± 0.01	93 ± 4	1.0 ± 0.3	0.2 ± 0.1	0.04 ± 0.02	0.2 ± 0.1	96 ± 4
2	P/ETOH	0.09 ± 0.02	–	36 ± 14	15 ± 8	0.19 ± 0.10	15 ± 8	82 ± 5
10	P/EC	0.09 ± 0.04	–	16 ± 3	63 ± 5	0.22 ± 0.06	63 ± 5	95 ± 2
12	P/EC	1 ± 1	–	17 ± 5	62 ± 7	0.6 ± 0.2	62 ± 7	97 ± 11
P/EC grand average[c]		0.6 ± 0.6					63 ± 5	96 ± 7
11	DEET	15 ± 2	–	4 ± 1	14 ± 8	57 ± 9	70 ± 4	93 ± 2
12	DEET	13 ± 5	–	8 ± 2	7 ± 3	60 ± 4	67 ± 5	92 ± 1
DEET grand average[c]		14 ± 1					69 ± 5	92 ± 1

[a] A dust formulation of 1% permethrin in Minugel FG (P/MG,40 μg/cm^2), a solution formulation of 0.32% permethrin in ethanol solution (P/ETOH, 40 μg/cm^2), an emulsifiable concentrate formulation of 2% permethrin (P/EC, 125 μg/cm^2) and an 8% solution of *N*,*N*-diethyl-m-toluamide in ethanol solution (DEET, 500 μg/cm^2) were applied to excised cattle skin. Percutaneous absorption was calculated as the sum of label in receptor fluid (RF) and label remaining in skin after hair was clipped and surface was tape stripped 22 times

[b] Mean ± SD of three replicates (four for permethrin in experiment 2 and four for DEET in experiment 11). Exp no., experiment; skin surface, residue recovered by vacuuming the skin surface 24 h post-application of dust formulation; hair, labeled residue in hair clipped from the skin 24 h post-application; total recovery, which includes label removed by 22 tape strips; grand average is mean of combined replicates

[c] Means were not significantly different between experiments (different animals, *t* test, $p > 0.05$), therefore replicates were combined

Table 3 Comparison of pig and cattle skin percutaneous absorption for permethrin in ethanol (P/ETOH), permethrin in emulsifiable concentrate (P/EC), and *N*,*N*-diethyl-m-toluamide (DEET/ETOH)

Percent of applied rad. dose*			
Compound/vehicle/dose	Percutaneous absorption, pig skin (N)	Percutaneous absorption, cattle skin (N)	Percutaneous absorption ratio, cattle/pig
P/ETOH/ 40 μg/cm^2	2.7 ± 0.1 (3)[a]	15 ± 8 (4)[b]	5.6
P/EC/ 125 μg/cm^2	31 ± 9 (6)[c]	63 ± 5 (6)[d]	2.0
DEET/ETOH/500 μg/cm^2	23 ± 3 (6)[b]	69 ± 5 (7)[d]	3.0

* Mean ± SD of N replicates. Means with the same alphabetic superscript were not significantly different ($p > 0.05$, ANOVA, Student–Newman–Keuls multiple range test)

pected from its low vapor pressure (1.5×10^{-8} mm Hg at 25°C for a 52/48 *cis/trans* mixture) (Laskowski 2002). Almost all the permethrin label that penetrated the skin accumulated in the dermis, with very little penetrating completely through the skin and into receptor fluid. Permethrin absorption was relatively low for ethanol or dust formulations, and skin absorption (2.7%, receptor fluid plus dermis) for the ethanol vehicle was very similar to the value (4.5% residue in tape striped skin plus 0.1% in receptor fluid) reported by Baynes et al. (2002) for excised pig skin at the same dose of 40 μg/cm^2. We observed high skin absorption and skin reservoir formation of permethrin (31 ± 9%) when applied to pig skin in emulsion formulation and twice that level when applied to cattle skin (63 ± 5%, Table 3).

Emulsifiable concentrate (EC) vehicles are popular because of economy (shipment of concentrate only for mixing with water at treatment site) and enhanced effect (knock-down) likely due to increased absorption across the protective barrier or cuticle of the insect. Permethrin in emulsion formulation caused incapacitation of all house flies in less than 10 min. This happened shortly after application ($t = 0$) or 1 week after application. Incapacitation was too rapid to allow a determination of fly preference for control or permethrin treated grids (i.e., repellency). In contrast, permethrin in a dry formulation (1% permethrin in Minugel 200) at $t = 0$ required 38 ± 7 min (five separate experiments) to incapacitate 50% of the house flies and 71 ± 20 min (4 experiments) at 1 week. Repellency was only 59 ± 18% at $t = 0$ and 54 ± 4% at 1 week (a score of 50% indicates no preference for control or treated grids). It is likely that the presence of surfactants in the Evercide formulation enhance the absorption of permethrin in the fly, causing rapid incapacitation. It is not likely due to the presence of volatile solvents, since rapid incapacitation also occurred at a week after application, when solvents would have evaporated. Evercide without permethrin (–P/Evercide) generally caused less than 50% incapacitation over the 2.5 h of observation. These results parallel the poor mammalian skin absorption characteristics of permethrin in a dry formulation (Minugel 200), and high absorption in emulsion formulation.

Traynor et al. (2007) showed that butoxyethanol penetrated excised human skin up to six fold more rapidly from aqueous solution (50%) than from the neat solvent. Likewise, ethoxyethanol penetration increased threefold in the presence of water (50%). There was an increase in the apparent permeability coefficient as the glycol ether was diluted with water, reaching a maximum at 90% water for butoxyethanol and at 50% water for ethoxyethanol. The authors suggest disruption of the stratum corneum lipid bilayer, hydration with water mixtures, and the physical properties of the glycol ether–water mixtures were involved in the enhancement. Baynes and Riviere (1998) found increased percutaneous absorption of carbaryl when sodium lauryl sulfate was added to aqueous formulations of carbaryl in 40% acetone and 80% acetone, with higher values obtained for formulations with more dilute acetone. They also implicated alteration of the lipid phase of the stratum corneum barrier layer by the combined effect of surfactant and hydration.

Our findings are in contrast to a Presidential Advisory Committee comment that "In laboratory animal studies, dermal absorption of permethrin is low" (http://www.gulflink.osd.mil). These differing results are likely due to vehicle effects. Many of the military-issued pesticides used during the Gulf War were mixed with water as an inert carrier (EC type formulations) (Winkenwerder 2003) and this type of formulation continues in widespread use. Skin absorption following pesticide spray of surfaces, such as buildings, tents or clothing, has been recognized as one aspect of human exposure (Winkenwerder 2003).

For cattle, the role that EC vehicles might play in food residues of permethrin is beyond the scope of this report. However, it is clear that alternate vehicles can reduce the levels of potentially absorbable dermal residues.

In summary, an EC vehicle resulted in higher skin penetration of permethrin by a factor of ten. The formation of a dermal reservoir has the potential to convert an acute exposure to a chronic one. We suspect that this effect could be general, as the surfactants and solvents in EC vehicles can reduce the barrier properties of the protective layer of the skin.

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