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Transdermal Penetration of Atrazine, Alachlor, and Trifluralin: Effect of Formulation

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Commercial formulations of herbicides contain surfactants and other compounds to increase absorption by targeted plants. These chemicals, however, are also potential penetration enhancers for mammalian skin. The effect of formulation on dermal absorption of the herbicides atrazine, alachlor, and trifluralin and their commercial formulations Aatrex, Lasso, and Treflan was determined. *In vitro* absorption studies were performed by placing hairless mouse skin in a Bronough flow-through diffusion system. Donor solution was spiked with ^{14}C-labeled herbicide, and its penetration through the skin was monitored in 90-min fractions. Results demonstrate that dermal penetration of commercially formulated compound was significantly greater ($p < 0.05$) than that of the pure compound at the same concentration. The physical properties of a herbicide predicted penetration ($r^2 = 0.97–0.99$) for commercial formulations but were not as effective at predicting absorption for the pure compounds ($r^2 = 0.51–0.71$). The solvents associated with the hydrophobic herbicide Treflan altered dermal penetration of the more hydrophilic herbicides Lasso and Aatrex. Furthermore, although the most hydrophobic compound had the least penetration, it accumulated in the stratum corneum at the greatest rate. These studies can have important implications on future experiments performed to predict percutaneous penetration of herbicides.

Key Words: **skin absorption; dermal uptake; atrazine; alachlor; trifluralin; herbicide.**

Herbicides have been linked to a variety of diseases. There are three major routes by which these chemicals enter the body: inhalation, oral ingestion and dermal absorption. Skin absorption is the most common exposure route for herbicide mixers and applicators (Parat *et al*., 1997). Dermal penetration is often predictable based on physical properties of the compound, such as size and lipophilicity, and usually it is linearly related to exposure concentration and surface area (Potts and Guy, 1992).

Commercial herbicide formulations contain surfactants and other compounds to increase absorption by targeted plants. These chemicals, however, are also potential penetration enhancers for mammalian skin (Walters *et al*., 1997). Inert ingredients and solvents can also alter dermal absorption of herbicides, with effects being dependent on solvent specificity and concentration (Baynes *et al*., 1996; Baynes and Riviere, 1998). Furthermore, transdermal penetration of methyl parathion dissolved in acetone was found to be significantly less than when applied in a commercial formulation (Sartorelli *et al*., 1997). Most dermal penetration studies use pure herbicides diluted to the desired concentration in a solvent such as water, ethanol, or acetone.

Pesticides are rarely applied individually. Examination of the 1996 Guide for Herbicide Use in Nebraska (Nebraska Cooperative Extension, 1996) indicates that virtually all compounds are applied in combinations. Commercially, there are a variety of herbicides formulated to include multiple pesticides. Some examples include Bicep (metolachlor + atrazine, Novartis), Lariat and Bullet (alachlor + atrazine, Monsanto), Cannon (alachlor + trifluralan, Monsanto), and Shotgun (atrazine + 2,4-D, VAP). Farming practices frequently require the use of single herbicides that are applied together. For example Bladex (cyanizine) and Aatrex (atrazine) are used together in cornfields, as well as Bladex and Dual (metolachlor) and Lasso (alachlor) and Treflan (trifluralin). These products come from multiple manufacturers, and it is not evident that their interactions have been readily studied.

The work presented in this manuscript examines the effect of formulation on the percutaneous penetration of three herbicides (atrazine, alachlor, and trifluralin), selected for their different physical properties (Table 1). The following questions were addressed:

- Is *in vitro* dermal penetration of the commercially formulated compound significantly greater than that of the pure compound?
- Does penetration efficiency change as a function of concentration?
- Is there a difference in the amount of herbicide remaining in the stratum corneum for the different herbicides?
- Can physical properties of a herbicide predict penetration for both commercial and pure formulations?
- Do interactions between compounds influence absorption?

These studies can have important implications for experiments performed to predict percutaneous penetration of herbicides.

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TABLE 1
Properties of Herbicides Used For Dermal Penetration

Compound	Chemical type	Commercial formulation	MW	Water solubility	Log Kow
Atrazine	Triazine	Aatrex	215.68	28	2.7
Trifluralin	Dinitroaniline	Treflan	335.28	0.3	5.1
Alachlor	Chloroacetamide	Lasso	269.77	242	2.9

Note. MW, molecular weight. Log Kow, log octanol/water partition coefficient. Water solubility is given in mg/l.

MATERIALS AND METHODS

Chemicals. Buffer chemicals and liquid scintillation fluid were obtained from Sigma Chemicals (St. Louis, MO) and Fisher Scientific (Springfield, NJ). [^{14}C] Trifluralin (specific activity 13.6 μCi/mg), [^{14}C] alachlor (specific activity 0.5 μCi/mg) and [^{14}C] atrazine (specific activity 3.2 μCi/mg) and their nonradioactive and commercial counterparts were a gift from Dr. Pat Shea (School of Natural Resources, University of Nebraska). Commercial formulations tested were Aatrex 4L (Ciba-Geigy), Lasso-Microtek (Monsanto), and Treflan MTF (Dow Elanco). The exact ingredients of each formulation are proprietary and therefore not available to the authors; the following information is from the MSDS of each product. Aatrex 4L contains 40.8% atrazine (408 g/l or 1.9 M), ethylene glycol, and surfactants. Lasso- Microtek consists of 45.1% (451g/l or 1.7 M) alachlor, 20% C9 aromatics (including 1,2,4-trimethylbenzene, mixed trimethylbenzenes, xylene, and cumene), and 7% emulsifiers. Treflan MTF comprises 41.2% trifluralin (412g/l or 1.2 M) and 58.8% inert ingredients.

Pure herbicides were diluted in methanol (atrazine) or acetone (alachlor, trifluralin) to the same concentration as full-strength commercial formulations listed above. Both pure and commercial formulations were then further diluted 1:40 or 1:10 with water to 10.2 and 40.8 g/l for atrazine, 11.3 and 45.1g/l for alachlor, and 10.3 and 41.2g/l for trifluralin. These dilutions were chosen because they reflect concentration ranges commonly used by agricultural workers.

Diffusion experiments. All animal studies were performed in an AAALAC facility in accordance with the University of Nebraska IACUC guidelines. Dorsal skin from female hairless mice CRL:SK1 (8–16 weeks old) was removed from recently sacrificed animals. The skin was placed in a Bronaugh diffusion cell system (0.89 cm^2 surface area; PermeGear, Riegelsville, PA). It was then sandwiched between two pieces of the polymer Kel-F, with the epidermal side facing upward while being exposed to the donor solution (test compound) and the dermal side in contact with the receiver fluid. The receiver chamber was perfused with physiological buffer that then passed to a fraction collector via Teflon tubing. The perfusion buffer was Hanks balanced saline solution (0.95 mM $CaCl_2$, 5.37 mM KCl, 0.44 KH_2PO_4, 0.81 mM $MgSO_4$, 137 mM NaCl, 0.34 mM Na_2HPO_4, 5.6 mM glucose) supplemented with 4% bovine serum albumin (BSA). BSA enhances partitioning of lipophilic molecules such as herbicides into the aqueous receiver solution and can maintain skin viability and metabolic capacity for more than 24 h (Bronaugh, 1991). The skin was equilibrated for 90 min prior to introduction of the test compound. One hundred microliters of herbicide (Table 1) spiked with 25 nCi ^{14}C-labeled herbicide was placed on the skin and allowed to remain for the 24 h of the experiment. Receiver fluid was collected in 90-min fractions and transport determined by assaying for radioactivity by liquid scintillation counting (LSC; Packard, Tricarb Model 1600CA).

Penetration studies involving herbicide combinations followed the procedure described above. The donor solution contained two herbicides of interest, one of which, was ^{14}C labeled. Experiments were performed with herbicides at a final dilution of 1:40 of the full-strength commercial formulation. For example, the effect of Treflan on the dermal absorption of Aatrex was determined by adding 25 nCi of ^{14}C-labeled atrazine to a solution containing 1.02 mg Aatrex and 1.04 mg Treflan in 0.1 ml of water. Flux was quantitated by monitoring ^{14}C atrazine via LSC. This procedure was followed for each set of herbicides tested.

Tape stripping. Upon completion of the transport experiments, the skin was removed from the diffusion chamber and washed three times with a 10% soap solution. Each successive layer of the skin's principal barrier component, the stratum corneum, was then removed by placing a 1 $\times$ 2 in. piece of Bookman tape evenly across the skin and quickly peeling away. Ten tape strips were used to sequentially remove the stratum corneum (Van Der Valk and Maibach, 1990). The herbicide remaining in each layer of the stratum corneum was quantitated by counting individual tape strips by LSC. The percent radiation recovered in each tape strip was summed, and the total percentage recovered for the 10 strips is presented in Table 2. The remaining skin was dissolved in nitric acid, neutralized, and counted using LSC.

Data analysis. Individual experiments were performed from 3 to 14 times. Data were analyzed by calculating both flux (μg/cm^2/h) and cumulative penetration (μg/cm^2) as well as log permeability coefficients (Kp, given in cm/s). Lag time was defined as the x-intercept derived from the linear regression line that best described the linear portion of the cumulative penetration curve. The Kp were determined by dividing the steady-state flux by the donor concentration. The steady-state flux is the slope of the linear portion of the cumulative penetration curve. Statistics were performed on the Kp, although the data are presented in Table 2 as log Kp. Herbicide remaining in the stratum corneum was quantitated as % recovered in tape strips (counts in tape strips/counts applied $\times$ 100). The amount of herbicide transported was compared among different herbicides, and statistical differences were determined by ANOVA followed by a Bonferroni post-test on selected pairs using the GraphPad Prism program (GraphPad,, San Diego, CA). Posttests were performed only on relevant data (within a compound or between compounds at same concentrations).

RESULTS

Commercial versus Pure Herbicide

Table 2 provides a summary of the peak flux and cumulative penetration, time to peak penetration, lag times, log Kp, and amount recovered in tape strips for each of two concentrations of each of the six formulations tested.

Cumulative penetration was significantly greater for the commercial formulation over the pure compound for all test conditions, as demonstrated in Table 2 and Figure 1 (Aatrex/atrazine 1:10, $p < 0.001$, and 1:40, $p < 0.005$; Lasso/alachlor 1:10, $p < 0.01$, and 1:40, $p < 0.001$; and Treflan/trifluralin 1:10, $p < 0.01$). The 1:40 dilution of Treflan did not produce significantly different penetration through the skin than the trifluralin. Peak penetration demonstrated the same trends, with commercial formulation being greater than the pure counterpart for each of the 1:10 dilutions, but not the 1:40 dilutions (Table 2 and Fig. 1).

No significant differences in lag times as a function of either herbicide or formulation were observed ($p > 0.05$). The time to reach peak penetration was significantly slower for alachlor

TABLE 2
Percutaneous Penetration of Individual Herbicides

Herbicide	Dilution	*n*	Peak penetration	Time to peak (h)	Cumulative penetration	Lag time (h)	Log Kp (cm/s)	% Recovered
Atrazine	1:10	6	23.13 ± 3.30^{a}	3.30 ± 0.33	229.60 ± 88.25^{h}	0.42 ± 0.30	$-6.67 \pm 0.16^{\circ}$	4.47 ± 2.22
Atrazine	1:40	6	3.52 ± 0.99	2.70 ± 1.26	11.21 ± 2.51	0.29 ± 0.36	-7.33 ± 0.34	8.67 ± 3.77
Aatrex	1:10	7	$208.20 \pm 25.40^{a,b}$	4.00 ± 0.32	$1172.78 \pm 200.44^{h,i}$	1.49 ± 0.25	$-5.56 \pm 0.0^{\circ,p}$	1.81 ± 0.28
Aatrex	1:40	13	56.70 ± 7.30^{b}	5.36 ± 0.26	335.92 ± 30.03^{i}	1.54 ± 0.21	-6.20 ± 0.11^{p}	2.13 ± 0.17^{t}
Trifluralin	1:10	4	2.92 ± 0.84^{c}	4.13 ± 0.72	33.15 ± 6.42^{j}	1.63 ± 0.06	-7.11 ± 0.23	5.36 ± 2.17
Trifluralin	1:40	5	1.53 ± 0.05	4.50 ± 0.61	13.23 ± 1.23	0.34 ± 0.20	-7.47 ± 0.20	4.82 ± 1.74
Treflan	1:10	4	$39.01 \pm 2.09^{c,d}$	4.20 ± 0.56	$216.23 \pm 47.25^{j,k}$	0.13 ± 0.27	-6.55 ± 0.24^{q}	6.01 ± 1.64
Treflan	1:40	14	11.39 ± 3.08^{d}	$5.79 \pm .38$	50.73 ± 7.56^{k}	1.55 ± 0.16	-7.02 ± 0.08^{q}	$11.8 \pm 3.44^{t,u}$
Alachlor	1:10	4	24.00 ± 7.66^{e}	10.88 ± 0.94	102.73 ± 30.31^{l}	3.44 ± 0.30	-6.98 ± 0.14^{r}	4.14 ± 0.71
Alachlor	1:40	5	15.90 ± 1.39	12.75 ± 0.25	148.82 ± 18.42^{m}	3.40 ± 0.69	-6.74 ± 0.19	3.33 ± 0.25
Lasso	1:10	5	$128.5 \pm 28.53^{e,f}$	10.13 ± 1.78^{g}	$924.60 \pm 128.52^{l,n}$	2.41 ± 0.37	$-5.78 \pm 0.10^{r,s}$	1.97 ± 0.47
Lasso	1:40	10	61.48 ± 7.89^{f}	6.60 ± 0.84^{g}	$510.83 \pm 59.47^{m,n}$	2.02 ± 0.25	-6.07 ± 0.05^{s}	2.51 ± 0.60^{t}

Note. Peak penetration given in $\mu g/cm^2/h$; cumulative penetration given in $\mu g/cm^2$; % Recovered, % compound recovered in tape strips. Cells with the same letter are significantly different from each other at the following levels: $^{a}p < 0.001$, $^{b}p < 0.001$, $^{c}p < 0.01$, $^{d}p < 0.01$, $^{e}p < 0.001$, $^{f}p < 0.01$, $^{g}p < 0.05$, $^{h}p < 0.001$, $^{i}p < 0.001$, $^{j}p < 0.001$, $^{k}p < 0.001$, $^{l}p < 0.001$, $^{m}p < 0.01$, $^{n}p < 0.01$, $^{\circ}p < 0.001$, $^{p}p < 0.001$, $^{q}p < 0.01$, $^{r}p < 0.01$, $^{s}p < 0.05$, $^{t}p < 0.05$, $^{u}p < 0.01$.

than for either atrazine or trifluralin ($p < 0.01$), but no differences were found between commercial and pure formulations for a given herbicide. Lasso 1:10 was significantly slower than Lasso 1:40 ($p < 0.05$).

Permeability coefficients (Kp, cm/s) were calculated to determine if penetration efficacy is affected by dose. Each of the three commercial formulations demonstrated a penetration efficiency that increased with higher concentration, as demonstrated by the larger log Kp values (Table 2).

Tape Stripping

The total percent of herbicide recovered when the 10 tape strips are pooled is shown in Table 2. This portion of the experiment was performed either 3 or 4 times. The total amount of Treflan remaining in the stratum corneum, as determined by the total quantity remaining in the tape strips, for the 1:40 dilution was significantly greater than both Aatrex ($p < 0.05$) and Lasso ($p < 0.01$). No statistical differences were found between Aatrex and Lasso ($p > 0.05$). These differences were not seen for the pure compounds at 1:40 dilution.

Physical Properties

Potts and Guy (1992) have proposed the following model for predicting the skin permeability constant based on the physical parameters molecular weight (MW) and octanol-water partition coefficient (Kow):

$$\log \text{Kp (cm/s)} = -6.3 + 0.71 * \log \text{Kow} - 0.0061 * \text{MW},$$

where

Kp (cm/s)

= steady-state flux ($mg/cm^2/s$)/concentration (mg/cm^3).

The values predicted from the equation were plotted against the log Kp values measured from transport studies based on the 1:40 dilution data. Penetration of the commercial formulation was well predicted by this equation ($r^2 = 0.99$). Pure compound penetration, however, was not well modeled ($r^2 = 051$; Fig. 2a).

Alachlor is approximately nine times more water soluble than atrazine, but is only two times more soluble in octanol. The Potts and Guy model does not include a water solubility factor. We therefore examined absorption as a function of water solubility. Penetration of the commercial formulations was also well predicted by both a log of the water solubility ($r^2 = 0.97$), but the permeability of the pure counterpart was not well predicted by this factor ($r^2 = 0.71$; Fig. 2b).

Combinations

Interactions between the three commercial formulations are shown in Table 3. Aatrex decreased Treflan penetration by a nonsignificant factor of 1.2 and inhibited Lasso penetration ($p < 0.05$). Lasso did not influence Aatrex absorption, but did decrease Treflan dermal absorption by a factor of 2.8 ($p < 0.05$). Aatrex penetration was enhanced 2.1-fold by Treflan ($p < 0.05$). Lasso absorption was inhibited by both Treflan ($p < 0.05$) and Aatrex ($p < 0.05$).

Combination studies were repeated with trifluralin instead of Treflan to determine if the influence of Treflan on Aatrex and Lasso penetration was due to the active ingredient. Trifluralin inhibited Aatrex penetration by a factor of 7.8 ($p < 0.01$) and Lasso absorption by a factor of 5.7 ($p < 0.01$), indicating that the active ingredient is a key component in this interaction.

DISCUSSION

The effect of formulation on the percutaneous penetration of three model herbicides was quantitated. The herbicides were

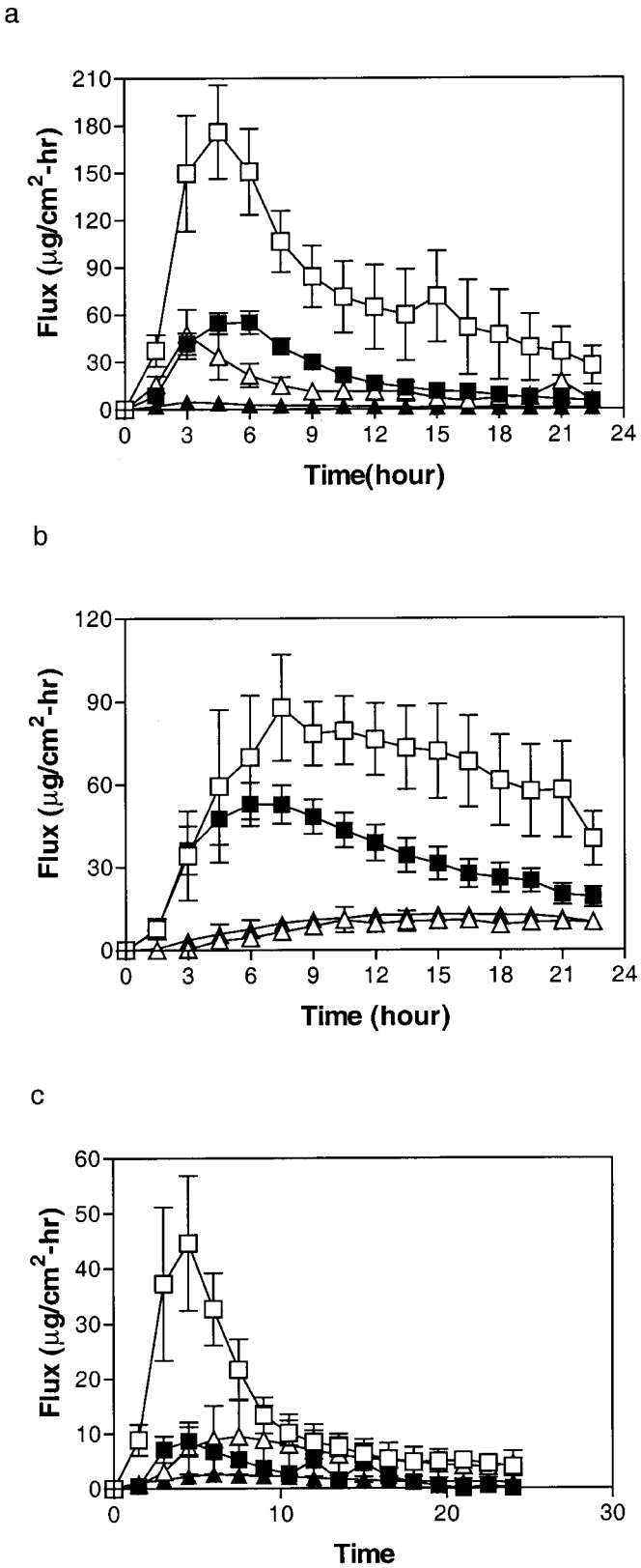

FIG. 1. *In vitro* dermal penetration of both commercial and pure herbicide diluted to a final concentration of 1:40 and 1:10 through hairless mouse skin. Filled squares, commercial 1:40; open squares, commercial 1:10; filled triangles, pure 1:40; open triangles, pure 1:10. (a) Aatrex/Atrazine. (b) Lasso/Alachlor. (c) Treflan/Trifluralin. Data are plotted as mean ± SEM.

selected because they are commonly used in agriculture, both individually and in combination. Additionally, they have different solubility properties, including a hydrophilic, moderately hydrophilic, and hydrophobic compound. For all herbicides tested, dermal penetration of the commercially formulated compound is significantly greater than that of the pure compound. Lipophilicity was correlated with the amount of herbicide remaining in the stratum corneum after 24 h. When two herbicides are placed in the skin, interactions between herbicides become more significant, with greater differences in solubility properties.

Transdermal penetration occurs as molecules partition into the lipid matrix surrounding the corneocytes. They remain in the lipid matrix and move through the tortuous lipid pathway surrounding the corneocytes in a transcellular fashion (Elias, 1983). Compounds that alter dermal lipids are known to act as penetration enhancers (Walters *et al*., 1997). This supports the hypothesis that surfactants and other nonactive chemicals used to improve uptake into plants also act as penetration enhancers in the skin.

The ideal method to perform occupational exposure studies is to place test chemicals on the skin of human volunteers and monitor blood and urine levels. This is not feasible because of the ethical issues associated with purposely exposing people to potentially toxic compounds. Therefore, *in vitro* models similar to the one used for the studies presented in this manuscript have been employed as a method to predict *in vivo* penetration. Overall, *in vitro* systems provide reasonable predictions of *in vivo* absorption for pesticides. Good correlation between *in vitro* and *in vivo* skin permeability was found for eight proprietary pesticides (Scott *et al*., 1992), for N,N-diethyl-m-toluamide (DEET; Moody and Nadeau, 1993), and for diazinon (Moody and Nadeau, 1994).

The effect of concentration on dermal absorption of herbicides is quite variable (Franklin, 1989). If transport efficacy decreases, the mass delivered still increases, just not by as much as would be predicted (Wester and Maibach, 1985). Several herbicides have demonstrated increasing flux efficiency with decreasing concentration, including Lasso (Bucks *et al*., 1989), atrazine (Shah *et al*., 1987b), parathion (Chang and Riviere, 1998), and carbofuran (Shah *et al*., 1987a). However, the permeability coefficients calculated for the herbicides tested in this study increased as the dilution decreased by a factor of four, indicating that penetration becomes more efficient with increasing concentration.

In vitro mass transfer studies of these three herbicides across Caco2 cell monolayers (a model for small intestine absorption) demonstrated similar relative levels of penetration, i.e., alachlor > atrazine > trifluralin. Interestingly, it was found that because trifluralin is very hydrophobic, it partitioned into the monolayer at a high rate, but tended to remain there. [Brand, unpublished results]. We hypothesized that this same phenomenon would occur in the skin's stratum corneum. Tape-stripping experiments, therefore, were used to determine herbicide distribution within the stratum corneum. This allowed examination of whether differences in transdermal transport are due

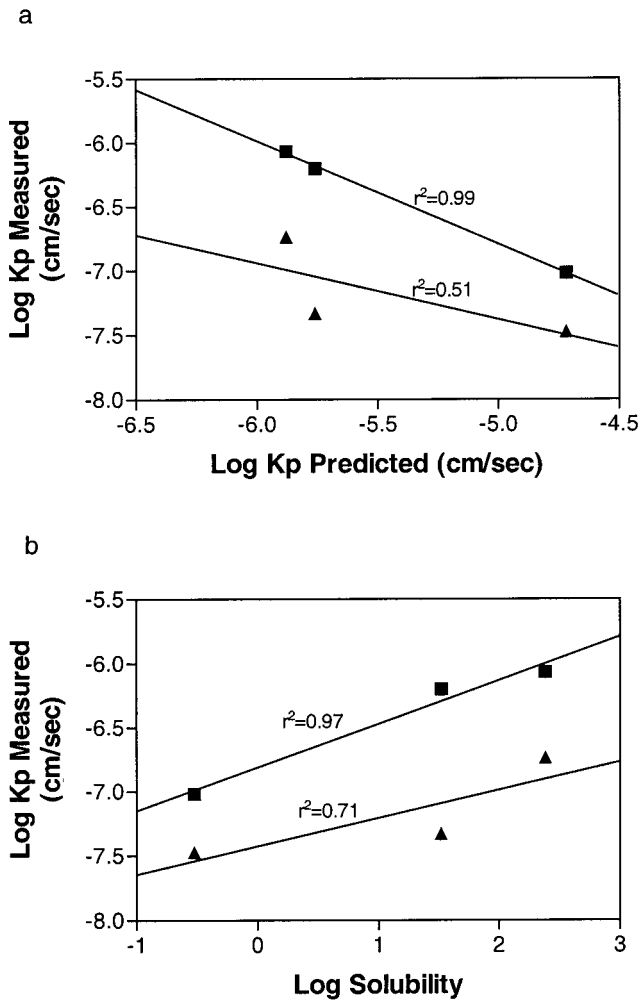

FIG. 2. Measured log Kp as a function of (a) log Kp predicted using equation 1; (b) log water solubility for both pure (triangles) and commercial formulation (squares) diluted 1:40.

to molecules remaining in the lipid bilayers of the stratum corneum, as opposed to not even entering the stratum corneum. The fact that Treflan entered and remained in the stratum corneum at a greater rate than either Aatrex or Lasso is consistent with the *in vitro* Caco2 cell monolayer data and demonstrates a reservoir effect within the stratum corneum.

Several models have been developed to predict transdermal penetration based on physical properties of the molecule (Bunge and Cleek, 1995; Kirchner *et al*., 1997; Potts and Guy, 1992, 1995). Many of these are based on a database containing the physiochemical properties of over 90 compounds, with molecular weights ranging from 18 to 750 and log Kow from –3 to +6 (Flynn, 1990). The models incorporate physical properties such as donor concentration, molecular weight, molar volume, octanol/water partition coefficient, and hydrogen-bonding capabilities for predictions. The 1992 Potts and Guy model was selected for use in this study because the required information (log Kow and molecular weight) were readily available for the herbicides tested. This model was used on both commercially formulated and pure herbicides to see which sets of data would be more accurate on a theoretical basis. Additionally, the penetration as a function of water solubility was examined because alachlor is approximately nine times more water soluble than atrazine, but is only two times more soluble in octanol. Interestingly, each method was better at predicting the penetration of commercial formulations than the pure compounds.

The studies combining two herbicides demonstrate that commercial formulations of herbicides with different solubilities can lead to alterations in penetration of the individual herbicides. Further experiments showed that trifluralin strongly inhibited flux of both Aatrex and Lasso. The presence of Treflan, however, increased penetration of Aatrex. This indicates that the nonherbicide ingredients in Treflan are increasing penetration at a greater rate than trifluralin is inhibiting. A series of experiments has been performed to examine herbicide penetration in well-defined mixtures of solvents. Different combinations of solvents altered penetration for a given test compound (Baynes *et al*., 1996). A study examining the percutaneous absorption of parathion and its metabolites administered alone or in combination found that the rate of absorption in mixed compounds was significantly different from the penetration by a single compound. These results demonstrated that the absorption data from a single parent compound or metabolite alone were not adequate to determine absorption from a mixture (Chang *et al*., 1994). In another study, absorption interactions between alachlor and atrazine in a commercial formulation that combines the two (Lariat) was determined (Bucks *et al*., 1989). No interactions were found between the transdermal penetration of these two compounds. We found that Aatrex inhibited Lasso penetration. This would be consistent with the hypothesis that the nonherbicide ingredients cause interactions.

TABLE 3
Percutaneous Penetration of Herbicide Combinations

Formulation	Peak penetration	Cumulative penetration	Log Kp (cm/h)
Aatrex* alone	$56.7 \pm 7.30^{a,b}$	$335.92 \pm 30.03^{f,g}$	-6.20 ± 0.11^{l}
Aatrex* + Lasso	66.51 ± 6.11	343.55 ± 48.28	-6.01 ± 0.06
Aatrex* + Treflan	$102.6 \pm 19.41^{a,c}$	$703.61 \pm 177.78^{f,h}$	$-5.75 \pm 0.14^{l,m}$
Aatrex* + Trifluralin	$6.35 \pm 1.23^{b,c}$	$42.90 \pm 6.75^{g,h}$	-6.99 ± 0.06^{M}
Lasso* alone	$61.48 \pm 7.89^{d,e}$	$510.83 \pm 59.47^{i,j,k}$	$-6.07 \pm 0.05^{n,\circ}$
Lasso* + Aatrex	37.32 ± 5.84	299.77 ± 40.05^{i}	-6.27 ± 0.06
Lasso* + Treflan	20.96 ± 3.85^{d}	220.27 ± 33.00^{j}	-6.53 ± 0.09^{n}
Lasso* + trifluralin	9.03 ± 1.37^{e}	89.94 ± 18.69^{k}	$-7.01 \pm 0.14^{\circ}$
Treflan* alone	11.39 ± 3.08	50.73 ± 7.56^{e}	-7.02 ± 0.08
Treflan* + Aatrex	4.33 ± 0.73	43.67 ± 20.02	-7.08 ± 0.17
Treflan* + Lasso	3.31 ± 0.44	19.80 ± 2.84^{e}	-7.34 ± 0.08

Note. Peak penetration given in μg/cm2/h; cumulative penetration given in μg/cm2. Cells with the same letter are significantly different from each other at the following levels: $^{a}p < 0.05$, $^{b}p < 0.05$, $^{c}p < 0.001$, $^{d}p < 0.01$, $^{e}p < 0.01$, $^{f}p < 0.05$, $^{g}p < 0.01$, $^{h}p < 0.001$, $^{i}p < 0.05$, $^{j}p < 0.05$, $^{k}p < 0.01$, $^{l}p < 0.01$, $^{m}p < 0.001$, $^{n}p < 0.01$, $^{\circ}p < 0.001$. *Herbicide that was radiolabeled during combination experiments.

There is evidence that multiple pesticides can interact and form synergistic responses, inducing biological responses far greater than would be expected. Mixtures of organophosphate insecticides and fungicides demonstrated potentiation of protein synthesis in neuroblastoma cells (Marinovich *et al*., 1996). Synergistic effects were also found between the tumor promoter phorbol-12-myristate-13-acetate (PMA) and the pesticide aldrin in inhibiting metabolic cooperation in V79 lung fibroblast cells (Mills *et al*., 1991). Furthermore, exposure to a nontoxic dose of chlordecone resulted in a 67-fold increase in death from an ordinarily inconsequential dose of carbon tetrachloride (Mehendale, 1998). A cause of Gulf War Syndrome was hypothesized to be interactions of multiple protective chemicals, which were not seen when the individual compounds were tested (Wester *et al*., 1996). Clearly, to understand the metabolic consequences of pesticides, they must be studied in combinations, as they are applied.

Overall, this manuscript demonstrates that inert ingredients can greatly affect the penetration of both hydrophilic and hydrophobic herbicides. Concentration, formulation, and the presence of other herbicides can also influence absorption. These complications should be taken into account when performing *in vitro* hazard prediction for agricultural workers exposed to herbicides.

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