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To cite this article: Jerry L. Campbell , Mary Alice Smith , Mark A. Eiteman , Phillip L. Williams & Mark F. Boeniger (2000) Comparison of Solvents for Removing Pesticides from Skin Using an In Vitro Porcine Model, AIHAJ - American Industrial Hygiene Association, 61:1, 82-88

To link to this article: <https://doi.org/10.1080/15298660008984519>



Published online: 04 Jun 2010.



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# Comparison of Solvents for Removing Pesticides from Skin Using an In Vitro Porcine Model

This study compared four solvents (1-propanol, polyethylene glycol [avg. MW 400], 10% Ivory<sup>®</sup> Liquid and water, and D-TAM<sup>®</sup>) for their ability to remove selected pesticides from an in vitro porcine skin model using a solvent-moistened wipe. Wipes were performed 90 min after pesticide was applied to the skin. The four pesticides selected (glyphosate, alachlor, methyl parathion, and trifluralin) were chosen because of their differences in water solubility. This study also determined whether pretreatment of skin with a solvent prior to pesticide application would either increase or decrease recovery of the pesticide. Recovery efficiencies for all solvents and pesticides were affected by the amount of contaminant on the skin. Although pesticide recoveries from all four solvents were similar (range: 45–57%), on average 1-propanol had significantly higher recoveries, followed by soap and water. There was no significant difference between polyethylene glycol, and D-TAM. When skin was pretreated with any of the four solvents before pesticide application, the recoveries of the more water soluble compounds, glyphosate and alachlor, decreased. When pretreatment with solvent preceded application of trifluralin, the pesticide with the lowest water solubility, recoveries increased. 1-Propanol or soap and water were more effective in removing pesticides from skin than polyethylene glycol or D-TAM, but the amount of pesticide recovered from skin was affected by the chemical characteristics of the pesticide (such as water solubility) and the amount of pesticide originally on the skin. This study provides information useful to the interpretation of skin wipe sample results collected in field studies.

**Keywords:** dermal contamination, in vitro, pesticide, skin, wipe test

**D**ermal contamination by pesticides can be a major route of exposure resulting in subsequent absorption of pesticides among agricultural workers.<sup>(1)</sup> Solvents (including those used in handwashing) may increase penetration of pesticides due to absorption of the solvent, delipidization of the skin, or irritation of the skin. Hatanaka et al.<sup>(2)</sup> have shown that in certain situations ethanol can increase the penetration of both lipophilic and hydrophilic compounds. This enhanced penetration may increase the health risk to the worker and decrease the accuracy of the contamination assessment. An ideal wipe or handwash solution for assessing worker dermal contamination would accurately remove both hydrophilic and lipophilic pesticides without severely affecting

the skin. Geno et al.<sup>(3)</sup> suggested that mild aqueous surfactants might have these characteristics.

Several methods are currently being used to determine the amount of chemicals contaminating the skin of agricultural workers. The U.S. Environmental Protection Agency<sup>(4)</sup> estimates body surface contamination using dermal patches and recommends determining hand contamination with cotton glove dosimeters, a solvent handwash, or skin wipe methods.<sup>(5,6)</sup> The solvent-moistened wipe or swab has been evaluated by Wester and co-workers<sup>(7,8)</sup> to decontaminate skin exposed to pesticides and polychlorinated biphenyls, and by Geno et al.<sup>(3)</sup> to assess pesticide levels on hands. McArthur<sup>(9)</sup> published a thorough review of these methods including dosimeter patches, coveralls, dosimeter gloves, and

This project was supported by a CDC/NIOSH contract and the University of Georgia's Agricultural Experiment Station.

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**TABLE I. Solubilities, Formulations, and Suppliers of the Formulations**

Pesticide	Solubility in Water (mg/L)	Formulation	Supplier
Glyphosate	12,000	Roundup®	Monsanto Co.
Alachlor	240	Lasso®	Monsanto Co.
Methyl parathion	55	Clean Crop®	Platte Chemical Co.
Trifluralin	0.3	Treflan®	DowElanco

hand rinses. Advantages of skin wipe sampling are that samples can be quickly and easily taken directly from the skin.

The purpose of this study was to compare four solvents for their ability to remove selected agricultural pesticides from an in vitro porcine skin model. Polyethylene glycol (PEG, avg. MW 400), 1-propanol, soap and water, and D-TAM® (a commercial decontamination product containing propylene glycol and surfactants) were selected. These solvents were compared using a standard wipe technique to determine the recovery of pesticides applied to porcine skin. The four pesticides were chosen because of their differences in water solubility and because of their wide use in agriculture. Furthermore, to determine whether application of solvent affects subsequent wipe recoveries, pesticide recoveries from skin that was previously treated with solvent and skin that was not previously treated with solvent were compared.

The chemical properties of each solvent used in this study are different. 1-Propanol is a highly water soluble, low molecular weight solvent that can solubilize many organic compounds. The octanol-water partition coefficient for 1-propanol is 0.3, making it slightly lipophilic. PEG-400 does not penetrate the skin readily and has low toxicity in humans.<sup>(10)</sup> The terminal hydroxyl groups make PEG water soluble, and the alkyl chain allows solubilization of organic compounds. As shown by Ruddy and Hadzija,<sup>(11)</sup> PEG contains a range of molecular weight molecules and can solubilize both hydrophilic and hydrophobic compounds. Similar to 1-propanol, PEG-400 is more hydrophilic than lipophilic. D-TAM is a mixture of surfactants in propylene glycol. The product is designed for decontamination of skin and has properties similar to that of PEG and soap and water.

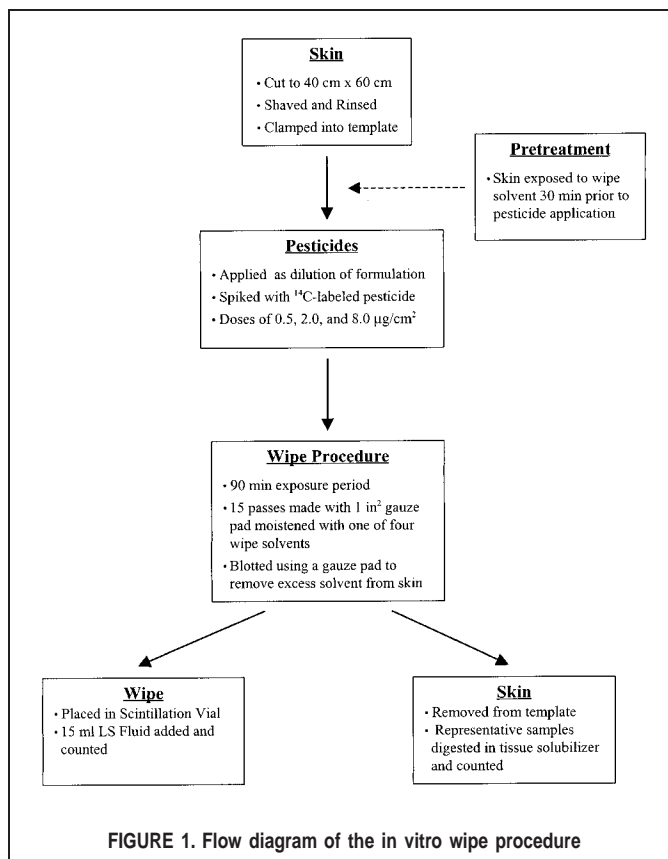
## METHODS

### Pesticides and Solvents

Pesticides were chosen based on their range of solubilities in water, and are listed with their formulations and suppliers in Table I. All tables and figures show pesticides in descending order of water solubility. Each formulation was spiked with the appropriate <sup>14</sup>C-labeled pesticide (0.1 μCi/80 μL). Monsanto Co. donated <sup>14</sup>C-alachlor and <sup>14</sup>C-methyl parathion and DowElanco donated <sup>14</sup>C-trifluralin. <sup>14</sup>C-glyphosate was purchased from Amersham Corporation (Arlington Heights, Ill.). The wipe solutions tested were 1-propanol (Fisher Scientific, Pittsburgh, Pa.), PEG (MW 400, Fisher Scientific), 10% soap and water (Ivory® Liquid, Procter & Gamble, Cincinnati, Ohio), and D-TAM (Colormetric Laboratories Inc., Des Plaines, Ill.).

### Tissue

A flow diagram of the procedure is shown in Figure 1. Dorsal porcine skin, obtained from a local slaughter house and returned to the laboratory on ice within 45 minutes, was trimmed to 40



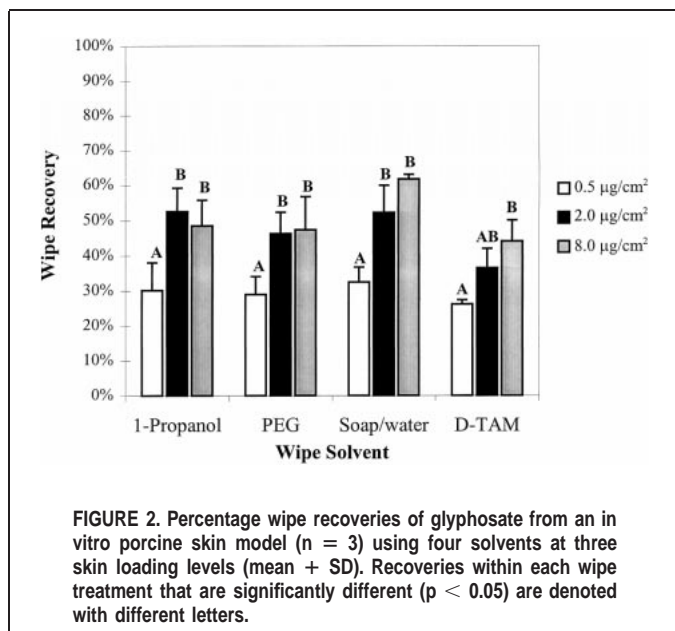
**FIGURE 1. Flow diagram of the in vitro wipe procedure**

cm × 60 cm, rinsed with water and soap, and shaved with a razor. The skin was visually inspected and if any deterioration had occurred, the skin was not used. To provide a consistent working surface, two pieces of 0.25-inch plexiglass (37 cm × 54 cm) were used to clamp the skin in place. A layer of gauze, moistened with a saline solution, was placed between the skin and the bottom piece of plexiglass to maintain moisture. The top piece of plexiglass served as a template with 28 cells (diameter of 4.37 cm) of 25 cm<sup>2</sup> plexiglass removed, and the template was arranged in 7 columns with 4 cells per column. The template had an outside border of 3 cm with 2 cm between cells or sampling areas. For samples, one skin loading level of at least two pesticides was used on each skin.

To get an estimate of variation among the nine skins used in the study, a control wipe of 1-propanol and alachlor (2.0 μg/cm<sup>2</sup>) was completed on each skin. The mean ± two standard deviations for the nine control wipes was 57.7% ± 5.4% and the coefficient of variation was 4.7%. Results for each individual skin were considered usable if the control wipe was within two standard deviations of the average of all control wipes (52.3 – 63.1%). All porcine skins tested were usable by this criterion.

### Treatments

Formulations were applied to the skin in two drops (40 μL each) and spread over the skin surface with the tip of a closed-end glass rod. An initial experiment was performed to determine the amount of pesticide retained by the glass rod. The test was performed by spreading 80 μL (final skin loading level of 2.0 μg/mL) of a pesticide on the skin surface. After the pesticide was spread, the tip of the glass rod was wiped with an alcohol wipe to remove the pesticide. The amount of the <sup>14</sup>C-labeled pesticide on the wipe was determined following the same procedure as that of



the skin wipe samples. Each pesticide was tested in triplicate. The amount retained by the glass rod ranged from 0.03 to 0.5% of the total amount of pesticide applied to the skin.

To determine whether the amount of pesticide applied to the skin affected the amount of pesticide recovered, three different quantities were applied to the skin. Skin loadings of 0.5, 2.0, and 8.0 µg/cm<sup>2</sup> were used, which are within the range of values (0.024 to 12.3 µg/cm<sup>2</sup>) reported by Fenske and Lu<sup>(12)</sup> for hand-washing. The pesticide sample was allowed to dry for 90 min.

For skin samples receiving solvent pretreatment, 0.5 mL of wipe solution was spread over the sample area. Excess solvent was removed by gently blotting with a piece of cotton gauze, and the pesticide was applied 30 min later.

### Wipe Procedures

After the pesticide had dried for 90 min, sample areas were wiped with a 6.25-cm<sup>2</sup> cotton gauze moistened with 0.5 mL of 1-propanol, PEG, 10% soap and water, or D-TAM. With each gauze wipe, 15 passes were made using the same wiping pattern for all samples. Each gauze was placed in a 20-mL glass scintillation vial along with a second gauze, which had been used to blot any excess solvent left on the skin after the 15 passes. After adding 15 mL of liquid scintillation fluid (Scintiverse®, Fisher Scientific), the wipes were sonicated overnight and counted in triplicate for 5 min with liquid scintillation counting (Beckman LS5801, Beckman Instruments Inc., Fullerton, Calif.). Counting efficiency for <sup>14</sup>C was 98%.

After completing the wipe procedure, skin sampling areas were removed with a scalpel and kept frozen at -20°C until further analysis. To determine the amount of radiolabeled pesticide remaining on the skin, representative tissue samples were solubilized in Soluene-350® (1 mL/g tissue, Packard Instrument Co., Downers Grove, Ill.). Once samples were completely solubilized, 1 mL was placed in a 20-mL scintillation vial with 15 mL of Insta-Fluor® (Packard Instrument Co.) liquid scintillation fluid. The final volume (mL) of solubilizer and dissolved tissue was multiplied by the amount of isotope determined in 1 mL to obtain the total amount of <sup>14</sup>C left on the skin. Each sample was counted in triplicate for 5 min.

### Calculations

Wipe recoveries were determined based on the total amount of isotope (<sup>14</sup>C) applied to the skin surface. Recoveries were calculated by dividing the amount of <sup>14</sup>C on the wipe by the total amount of <sup>14</sup>C applied to the skin. To account for all pesticide, a mass balance was completed for one skin sample from each pesticide/loading/solvent by combining the amount of isotope recovered from the wipe with the amount of isotope remaining on the skin. This quantity was compared with the total amount of isotope initially applied to the skin.

### Statistical Methods

Each solvent, pesticide, and skin loading combination was repeated three times. Significant differences between wipe recoveries for the three skin loading levels of pesticides and wipe treatments were determined by analysis of variance (STATA, Stata Corp., College Station, Tex.). Significant differences between wipe recoveries with and without pretreatment of solvent were determined using an unpaired t-test (STATA).

## RESULTS

All four solvents were compared for their ability to recover pesticides from an in vitro porcine skin model. The amount of pesticide applied to the skin had a significant effect on the amount of pesticide recovered from the skin.

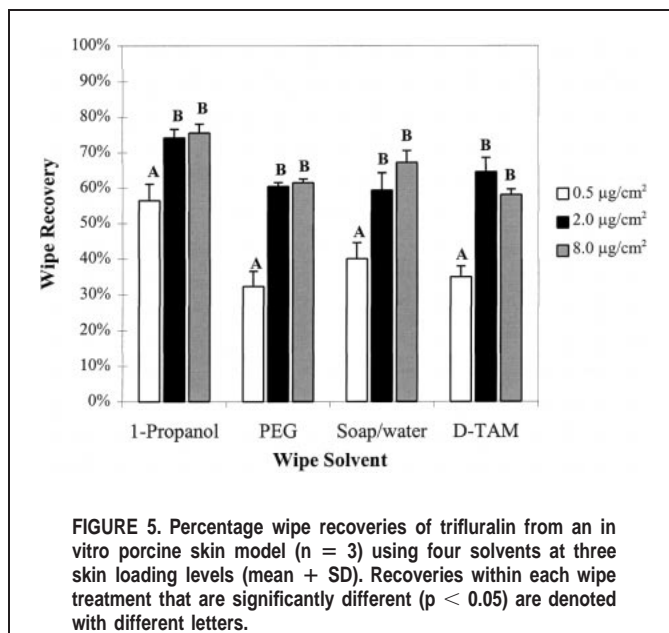
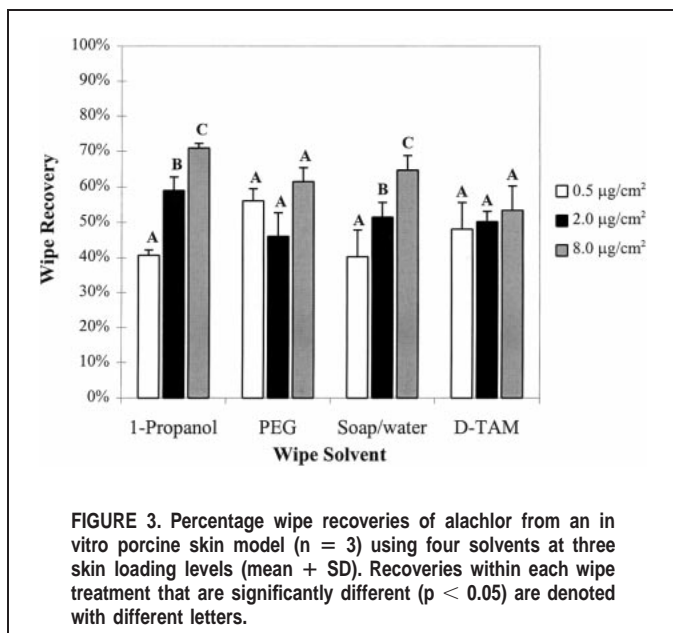
### Wipe Recoveries

1-Propanol, PEG, and soap and water wipe solvents gave similar recovery patterns for the pesticide glyphosate (Figure 2). These solvents had significantly higher (p < 0.05) recoveries when glyphosate was applied at the higher concentrations, 2.0 or 8.0 µg/cm<sup>2</sup>, compared with 0.5 µg/cm<sup>2</sup>. There was no significant difference (p < 0.05) in recoveries of glyphosate between the two higher skin loadings for any solvent tested. Recoveries of glyphosate using D-TAM® had a slightly different skin loading dependent pattern where the only significant difference (p < 0.05) was between the 0.5 µg/cm<sup>2</sup> and the 8.0 µg/cm<sup>2</sup> skin loadings of glyphosate.

For alachlor (Figure 3), there was a similar pattern of wipe recoveries for 1-propanol and soap and water. When 1-propanol or soap and water was used as a wipe solvent, the recoveries of alachlor for the three skin loadings showed a skin loading-dependent pattern in wipe recoveries, which was significantly different (p < 0.05) at each skin loading. Using PEG or D-TAM, wipe recoveries were not dependent on the amount of alachlor applied (p < 0.05).

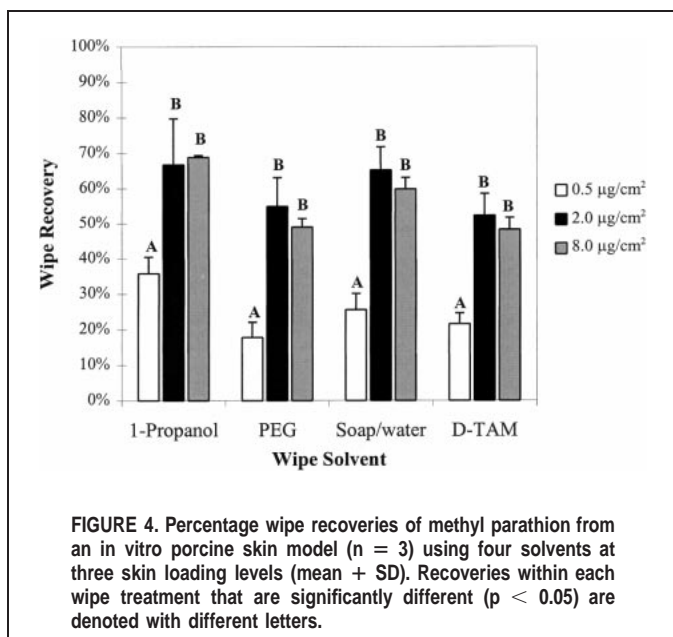
The recovery pattern for methyl parathion was similar for all wipe solvents (Figure 4). When 1-propanol, PEG, soap and water, or D-TAM were used to recover methyl parathion, there was a significant difference (p < 0.05) between the lowest skin loading and the two highest skin loadings. However, there were no significant differences (p < 0.05) between recovery of the 2.0 and 8.0 µg/cm<sup>2</sup> of methyl parathion for any wipe solvent, which may indicate a threshold skin loading level beyond which recoveries are about equal.

The pattern of wipe recovery of trifluralin (Figure 5) was similar to that of methyl parathion. The recoveries of the 0.5 µg/cm<sup>2</sup> skin loadings were significantly lower (p < 0.05) than the recoveries of the two highest skin loadings for all solvents. As with methyl parathion, there were no significant differences (p < 0.05)



between recoveries of the two higher skin loadings of trifluralin with any of the wipe treatments.

Table II shows the average recovery of the three skin loadings for each wipe treatment and pesticide. The overall recovery of all pesticides for each solvent is also shown illustrating the effectiveness of using one solvent for different pesticides. The overall recovery of the four pesticides with 1-propanol was significantly higher (p < 0.05) than recoveries with all other solvents. Soap and water recovered significantly more (p < 0.05) of the four pesticides than either PEG or D-TAM. For glyphosate, alachlor, and methyl parathion, there were no significant differences (p < 0.05) among wipe recoveries of the four solvents. On average, the greatest amount of trifluralin was recovered using 1-propanol moistened wipes. However, the recovery of trifluralin using 1-propanol was not significantly different (p < 0.05) from the recovery with soap and water.



### Wipe Recoveries with Pretreatment

Three pesticides were selected for the pretreatment study based on their water solubilities. Because glyphosate and trifluralin are highly polar and nonpolar pesticides, respectively, they were included in the pretreatment studies. Alachlor and methyl parathion are both intermediate in solubility, and alachlor was chosen for further study as a matter of convenience. Recoveries after pretreatment are shown in Figures 6–8, and statistical comparisons were between pretreatment recoveries and the previous recoveries done without pretreatment (Figures 1–4).

For glyphosate, wipe recoveries were reduced after pretreatment for all wipe solvents (Figure 6). Specifically, 1-propanol pretreatment significantly (p < 0.05) reduced the recovery of glyphosate at 2.0 µg/cm². Recoveries of glyphosate at 0.5 and 8.0 µg/cm² were significantly lower (p < 0.05) when soap and water was used as a pretreatment, whereas the only recovery significantly lower for D-TAM (p < 0.05) was at the 0.5 µg/cm² skin loading.

**TABLE II.** Average and Overall Solvent Wipe Recoveries for the Four Pesticides with Each Wipe Treatment

Pesticide	Wipe Treatment (Percentage Average Wipe Recovery ± SD)			
	1-Propanol (n = 9)	PEG (n = 9)	Soap and Water (n = 9)	D-TAM (n = 9)
Glyphosate	44 (±12)	41 (±11)	49 (±14)	36 (±9)
Alachlor	57 (±13)	55 (±8)	52 (±12)	51 (±6)
Methyl parathion	57 (±17)	41 (±18)	50 (±19)	41 (±15)
Trifluralin	69 <sup>A</sup> (±10)	51 <sup>B</sup> (±15)	56 <sup>AB</sup> (±13)	53 <sup>B</sup> (±14)
Overall recovery (n = 36)	57 <sup>A</sup> (±16)	47 <sup>C</sup> (±14)	52 <sup>B</sup> (±14)	45 <sup>C</sup> (±13)

Note: Recoveries that are significantly different (p < 0.05) are denoted by different superscript letters.

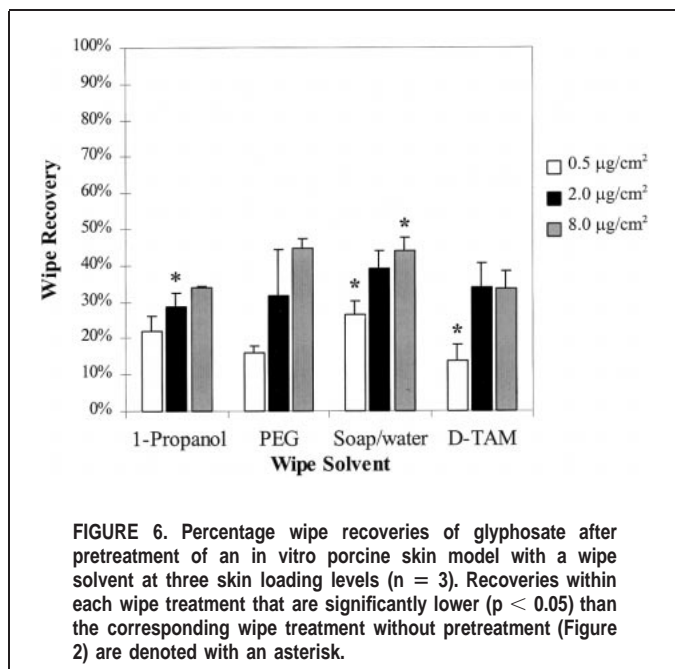


FIGURE 6. Percentage wipe recoveries of glyphosate after pretreatment of an in vitro porcine skin model with a wipe solvent at three skin loading levels ( $n = 3$ ). Recoveries within each wipe treatment that are significantly lower ( $p < 0.05$ ) than the corresponding wipe treatment without pretreatment (Figure 2) are denoted with an asterisk.

For PEG, there was no significant difference ( $p < 0.05$ ) between recoveries with and without pretreatment.

Pretreating the skin with solvents affected wipe recoveries of alachlor (Figure 7) more than glyphosate. Using 1-propanol as a pretreatment, recovery of all three skin loadings were significantly lower ( $p < 0.05$ ) than the corresponding recoveries without pretreatment. Using PEG as a pretreatment significantly reduced ( $p < 0.05$ ) wipe recoveries of alachlor for the 0.5 and 8.0 µg/cm<sup>2</sup> skin loadings, whereas using soap and water as a pretreatment significantly reduced ( $p < 0.05$ ) wipe recoveries of alachlor for the 2.0 and 8.0 µg/cm<sup>2</sup> samples. With D-TAM, only recovery of the 8.0 µg/cm<sup>2</sup> skin loading was significantly reduced ( $p < 0.05$ ) from the recovery without pretreatment for alachlor.

Pretreatment of the skin with the four solvents had an effect on wipe recoveries of trifluralin (Figure 8) that was different from

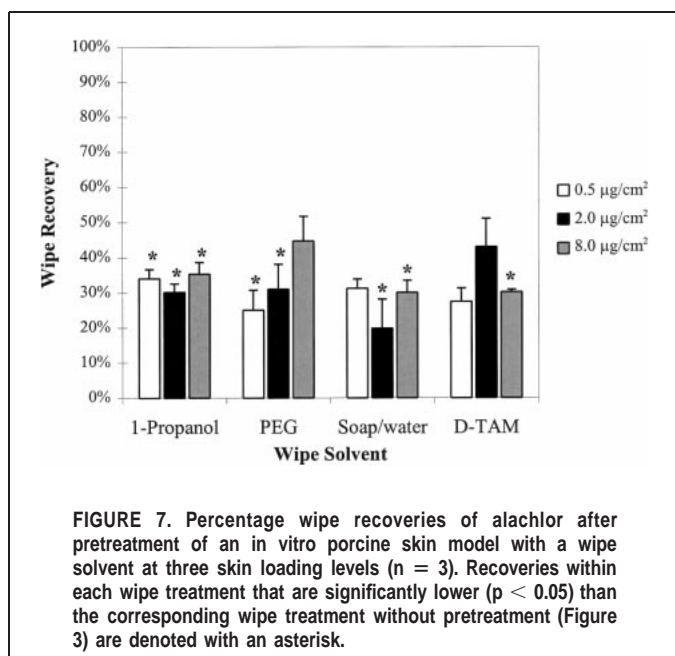


FIGURE 7. Percentage wipe recoveries of alachlor after pretreatment of an in vitro porcine skin model with a wipe solvent at three skin loading levels ( $n = 3$ ). Recoveries within each wipe treatment that are significantly lower ( $p < 0.05$ ) than the corresponding wipe treatment without pretreatment (Figure 3) are denoted with an asterisk.

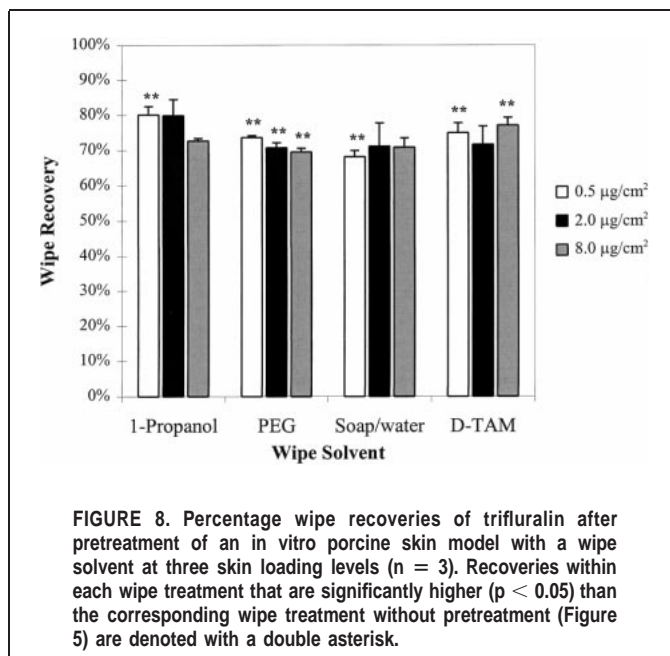


FIGURE 8. Percentage wipe recoveries of trifluralin after pretreatment of an in vitro porcine skin model with a wipe solvent at three skin loading levels ( $n = 3$ ). Recoveries within each wipe treatment that are significantly higher ( $p < 0.05$ ) than the corresponding wipe treatment without pretreatment (Figure 5) are denoted with a double asterisk.

that of the other pesticides. In general, recoveries of trifluralin after pretreatment were the same as or higher than recoveries without solvent pretreatment. Pretreatment with 1-propanol and soap and water gave recoveries that were significantly greater ( $p < 0.05$ ) than those without pretreatment for 0.5 µg/cm<sup>2</sup>. When PEG was used as a pretreatment, subsequent wipe recoveries of trifluralin were significantly greater ( $p < 0.05$ ) than those without pretreatment for all three quantities applied. With D-TAM, pretreatment recoveries were significantly greater ( $p < 0.05$ ) for 0.5 and 8.0 µg/cm<sup>2</sup> when compared with recoveries without pretreatment.

### Mass Balance

To account for all applied radiolabeled pesticide, a subset of skin samples including all pesticides and solvents tested (48 samples) were digested and counted. For these samples the quantity recovered with the wipe was added to the quantity remaining on the skin, and these totals were compared with the amount of radiolabeled pesticide applied to skin. Overall, total recoveries for the four pesticides were between  $95 \pm 5\%$  and  $102 \pm 1\%$  demonstrating that little or no pesticide was lost during the procedure.

## DISCUSSION

The purpose of this study was to determine which of four selected solvents was most effective for recovering both hydrophilic and lipophilic pesticides from an in vitro porcine skin model. Experimental studies of percutaneous absorption of compounds indicate that binding or diffusion of the compound into the stratum corneum occurs during the first 30 to 60 min.<sup>(7)</sup> To allow for maximum binding to the stratum corneum, a 90-min delay was used before sampling. Ninety minutes represents a reasonable minimal time that might elapse before gathering samples from agricultural workers (i.e., sampling of workers could be done during breaks that normally occur about every 2 hours). Wipe recoveries for alachlor and glyphosate from dorsal porcine skin after 90 min were lower than those reported by Geno et al.<sup>(3)</sup> on human skin that was wiped immediately after loading of the pesticide.

This lower recovery is likely due to skin permeation. Because agricultural workers are not likely to be tested immediately after contamination, the 90-min delay is more realistic to field conditions. Also, more variation may occur under field conditions due to the difficulty in constantly collecting samples in the same way (i.e., 15 swipes with gauze wipe).

The study compared recoveries using four solvent mixtures (i.e., PEG, D-TAM, 10% soap and water, and 1-propanol). For three of the four pesticides used in this project, no single wipe solvent consistently removed more pesticide than the others removed. However, for the most lipid soluble pesticide examined in this study, trifluralin, 1-propanol consistently recovered more of the pesticide than PEG or D-TAM.

Alcoholic solvents may have the propensity to increase the absorbed dose of compounds. Hatanaka et al.<sup>(2)</sup> used ethanol to enhance the absorption of both hydrophilic and lipophilic compounds. To determine whether the solvents themselves alter subsequent recovery of pesticide, the porcine skin was pretreated with the appropriate solvent prior to pesticide application. Application of 1-propanol or soap and water to skin prior to pesticide application reduced the amount of glyphosate and alachlor recovered. These wiping solvents may remove surface lipids from the skin and increase partitioning of the more hydrophilic pesticides.<sup>(2,13,14)</sup> Pretreatment of the skin with PEG or D-TAM did not reduce recoveries as much as the other two solvents. Although not a direct measurement, this observation suggests that PEG and D-TAM affect skin properties less than alcohol and soap and water.<sup>(1)</sup>

For the most lipophilic pesticide, trifluralin, pretreatment appeared to have an enhancing effect on recoveries with all wipe solvents tested. 1-Propanol pretreatment might decrease the amount of surface lipids on the skin or disrupt the intercellular organization of the lipids, which would reduce partitioning to the skin by highly lipophilic compounds.<sup>(15,16)</sup> Geno et al.<sup>(3)</sup> found increasing recoveries with daily application of pyrethrin—a very lipophilic pesticide—followed by isopropanol wiping of the same skin site.<sup>(3)</sup> Full recovery of skin lipids after organic solvent exposure apparently can exceed 24 hours.<sup>(17)</sup> On the other hand, skin pretreatment with PEG has been shown to reduce absorption of lipophilic compounds, presumably because the compounds preferentially partition into PEG.<sup>(18–20)</sup> The net effect of each of these processes would be to increase recovery of trifluralin. Based on results from the pretreatment experiment, wipe solvents used in this study can increase or decrease the amount of pesticide recovered to varying extents.

Previously, there has been no study directly comparing several solvents for their ability to recover pesticides with a wide range of solubilities. This study has shown that solubilities of both solvents and pesticides affect the amount of pesticide recovered from skin and suggests that although there was a difference in the amount of pesticide recovered for any solvent (45 to 57%), no single solvent appears to be better at removing all pesticides. Because of the wide range in chemical solubility of pesticides, solvents matching the contaminants should be selected to achieve maximum removal. On the other hand, solvents that are used to sample skin must not be harmful or increase subsequent absorption of chemicals. These attributes apply to skin sampling for contaminant documentation, but can be applied equally to wash decontamination requirements.

## CONCLUSIONS

An in vitro porcine skin model was used to determine the efficiency of using a solvent-moistened wipe to remove pesticides

from skin. In field conditions recovery efficiencies might be altered by uncontrollable factors including ambient temperature, humidity, or skin condition of the worker being tested. However, these factors can be controlled in laboratory conditions using an in vitro system. There was little variation in the porcine skin model due to source of the skin.

In most cases, the specific wipe solvent used did not greatly affect recoveries of the four pesticides studied. Overall, the four solvents recovered approximately 50% of the pesticide applied to the skin after a 90-min contact period. The most variable results were found for 1-propanol, although on average 1-propanol recovered the greatest amount of all pesticides examined in this study.

Pretreatment with any of the four wipe solvents affected subsequent wipe recoveries, suggesting that the solvent itself affected absorption of the pesticides. Pretreatment with any of the solvents increased the amount of trifluralin recovered, whereas pretreatment decreased recovery of both glyphosate and alachlor. Future research should focus on the correlation of wipe recoveries to duration of exposure and exposure level. By determining this relationship, it would then be possible to relate the amount of pesticide recovered from the wipe to the amount of pesticide originally on the skin surface.

## ACKNOWLEDGMENTS

The authors would like to thank Danny Morrison and Jimmy Woods for providing the porcine skin. We would also like to thank Monsanto Co. for donating Lasso®, DowElanco for donating Treflan®, and the University of Georgia Agricultural Experiment Station (Tifton, Ga.) for donating Clean Crop®.

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