

Dermal *In Vitro* Penetration of Methiocarb, Paclobutrazol, and Pirimicarb: Effect of Nonylphenoethoxylate and Protective Gloves

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Dermal exposure has become the major route of human occupational exposure to pesticides. Detergents are used as part of formulated pesticide products and are known to change the barrier properties of human skin *in vitro*. However, studies on the influence of detergents as well as protective glove materials on dermal penetration of pesticides are scarce. In an experiment using *in vitro* static diffusion cells mounted with human skin, we evaluated the effect of nonylphenoethoxylate on dermal penetration of three extensively used pesticides—methiocarb, paclobutrazol, and pirimicarb—and the protection against dermal penetration offered by protective gloves made of latex or nitrile. There was a general tendency, though not statistically significant for all pesticides, for nonylphenoethoxylate to decrease the percutaneous penetration of the three pesticides. The nitrile generally offered better protection against percutaneous penetration of pesticides than did latex, but the degree of protection decreased over time and depended on the pesticides used.

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Pesticides are used extensively in horticulture. When they are used repeatedly over long periods of time and personal protective equipment is not used to prevent skin contact, dermal exposure constitutes the major route of human occupational exposure to pesticides. Estimates of dermal exposure and percutaneous penetration of pesticides have therefore become a critical part of risk assessment.

Studies of percutaneous penetration of pesticides have been published from only a few studies of human volunteers. Most such evaluations are based on *in vivo* animal models or *in vitro* skin penetration models. Despite a European Community directive (91/414) prescribing the testing of active ingredients as well as commercial formulations, most studies have reported on percutaneous penetration of the active ingredient only, thus failing to acknowledge that solvents as well as detergents are part of the formulated product and may change penetration characteristics (1). Nonylphenoethoxylate (NPE) and similar polyethoxylates have been widely used as detergents in pesticide formulations (2). NPE is not known to cause irritation or allergy in humans, but is known to change the barrier properties of human skin *in vitro* (2,3). Thus, NPE recently has been demonstrated to facilitate and enhance the dermal *in vitro* penetration of tritiated water by 60% (4). However, published information on the influence of detergents on dermal penetration of active ingredients is scarce, although some recent studies have addressed permeation of commercial formulations of pesticides while mainly focusing on aspects relating to effects of different solvents (5–7).

Gloves are often prescribed for the mixing, loading, and spraying of pesticides, and

field data demonstrate the need to use safety procedures when handling pesticides or plant cultivars recently treated with pesticides (8). The protective abilities of glove materials against pesticides are often evaluated in experimental models using the glove material as the only barrier membrane, and penetration rates and breakthrough times of active ingredients as well as commercial formulations are reported (6,7,9,10). In general, natural rubber shows the least resistance to permeation, whereas nitrile and similar materials demonstrate the highest resistance (11–13). Data from more complete *in vitro* systems involving glove material situated on top of a skin membrane for prolonged exposure periods are seldom available. However, it may be important to measure dermal penetration with glove material present because large reservoirs of pesticide may reside in the glove membrane for more than 24 hr eliciting potential dermal exposure long after the primary exposure has ceased (14).

We report here on the influence of a widely used detergent, nonylphenoethoxylate, on dermal penetration of two extensively used fungicides (methiocarb, pirimicarb) and a growth retardant (paclobutrazol). Dermal penetration data from studies conducted with and without protective gloves made of latex or nitrile are also reported.

Materials and Methods

Chemicals Pirimicarb [2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate; CAS no. 23103-98-2; molecular weight (MW), 238 g/mol] and methiocarb (4-methylthio-3,5-xylyl methylcarbamate; CAS no. 2032-65-7; MW, 225 g/mol) were obtained as reference materials (Augsburg,

Ehrenstorfer, Germany). Paclobutrazol [(2*RS*,3*RS*)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1*H*-1,2,4-triazol-1-yl) pentan-3-ol; CAS no. 76738-62-0; MW, 294 g/mol] was obtained as reference material (Institute of Organic Industrial Chemistry, Warsaw, Poland). Nonylphenoethoxylate (12 ether groups) was obtained from Promochem GmbH (Wesel, Germany). Phosphate buffer (Na₂HPO₄ · 2H₂O; Merck; Darmstadt, Germany) was used as receptor medium (0.05M; pH = 7.4) and water was purified with a Milli-Q system from Millipore Corp. (Bedford, MA, USA). Acetonitril and methanol Lichrosolv (BDH, Poole, England) were used for HPLC analysis.

Glove materials. Samples of protective gloves (back-of-hand region) made of latex (Conform+ powder free) or nitrile (TNT powder free) from Ansell Edmont (Aalst, Belgium) were mounted on top of the skin membranes in experiments with penetration through glove material and skin.

Skin membranes. Abdominal skin from human cadavers was obtained from 27 Caucasian women without skin disease and with a median age of 77 years (range, 54–94 years). Samples were stored at –20°C for a period not exceeding 1 month and allowed to thaw at room temperature before use. The skin was cleaned and subcutaneous fat removed before mounting in the static diffusion cells. It should be noted that the use of full thickness skin in the static cell may slightly overestimate *in vivo* lag times because the system will not maintain perfusion.

Experimental model. As described previously (3,4), *in vitro* static diffusion cells adapted from Southwell et al. (15) were mounted with the skin on a metal grid and placed on a water bath (32°C) with individual magnetic stirring. The median diffusion area was 2.04 cm²/cell. Before experiments, the epidermal site was exposed to ambient laboratory conditions and the dermis bathed in phosphate buffer for 18 hr. Further, we

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evaluated the barrier integrity by capacitance measurements (Lutron DM-9023; Acer AB, Stockholm, Sweden) and excluded cells with a capacitance above 55 nF. During experiments, the phosphate buffer in receptor and donor chambers was exchanged with 50% ethanol solutions, and both chambers were covered with Parafilm to prevent evaporation. We applied pesticides as 50% ethanol solutions to the donor chamber in a total volume of 600 μ L. Samples were taken from the receptor chamber at appropriate intervals and replaced with fresh receptor fluid to maintain infinite sink conditions.

Pesticide analysis. We quantified pesticide concentrations in the receptor medium by HPLC. We injected a 50 μ L aliquot of the receptor medium into a LiChrospher RP-18, 125-4 column using a Kontron 360 autosampler (Kontron Instruments SpA, Milan, Italy). Pirimicarb was eluted with a mobile phase consisting of methanol:water (70:30), whereas paclobutrazol and methiocarb were eluted with acetonitrile:water (50:50). A flow rate of 1 mL/min provided by a Kontron HPLC 420 pump was used for both eluents. Detection was performed by a Kontron 430 UV-detector. Quantification was based on the peak area of the compound related to a standard curve. All data calculations were performed using Kontron MT-450 software for personal computers.

Data processing and statistics. Data on percutaneous penetration are presented as means of the concentrations of pesticides (millimoles) in the receptor chamber. Because a dilution factor is introduced every time a sample is removed from the receptor chamber and replaced by fresh receptor, we corrected all data for this dilution. We used Student's *t*-test for statistical comparisons between groups.

Ethics. The study was approved by the regional ethical review committee.

Results

With increasing exposure time, an increasing amount of pesticide penetrated the skin barrier in all experiments regardless of the presence of NPE in the donor phase (Figure 1). The increase was almost linear for pirimicarb and paclobutrazol, with significant increases at 48 hr as well as 72 hr. For methiocarb, however, there was no significant increase in penetrated pesticide at 72 hr compared to methiocarb concentration in the receptor fluid at 48 hr (Figure 1). At 24 hr, significantly more methiocarb than pirimicarb and paclobutrazol had penetrated the skin (Figure 1). The 5–8 times higher concentration of pesticide in the receptor phase after methiocarb exposure than after exposure to pirimicarb or paclobutrazol indicated a shorter lag time and/or considerably faster

penetration rate. The difference between penetrated amounts of pesticides remained throughout the experimental period of 72 hr, although the difference became smaller. Pirimicarb and paclobutrazol had comparable penetration characteristics (Figure 1).

The different percutaneous penetration characteristics among the three pesticides were not affected by concomitant exposure to the detergent NPE (Figure 1). In all experiments NPE reduced the dermal penetration of pesticide, although statistical significance was not obtained for methiocarb and was obtained for pirimicarb only at the 72-hr sampling time (Figure 1). NPE reduced the percutaneous penetration by 40–50% for pirimicarb and paclobutrazol, whereas the percutaneous penetration of methiocarb was reduced to a lesser extent. NPE and the pesticides were present in equimolar concentrations of 0.2 mM in the donor phase. Reducing the paclobutrazol concentration by 50% and thereby causing a ratio of 2 between detergent and pesticide did not quantitatively change the effect

observed on percutaneous penetration (Figure 1). Increasing the concentration of NPE in the donor phase to 0.8 mM and thereby increasing the ratio between pirimicarb and NPE to 4 caused a further and statistically significant decrease in the amount of pirimicarb penetrating the dermal barrier (Table 1). Increasing the ratio between detergent and pesticide to 10 did not cause a further decrease in percutaneous penetration of pirimicarb (Table 1).

The latex and nitrile materials both significantly reduced the percutaneous penetration of all three pesticides (Figure 2–4). The latex material reduced the dermal penetration of pesticides by 50% throughout the observation period of 45 hr (Figure 2–4). Nitrile provided a total protection against percutaneous penetration of paclobutrazol during 45 hr continuous exposure (Figure 2). The nitrile material also offered > 90% protection against pirimicarb, but only for 18 hr (Figure 3). Thereafter, the protective ability of nitrile to pirimicarb approached that of latex, but still offered a significant protection against

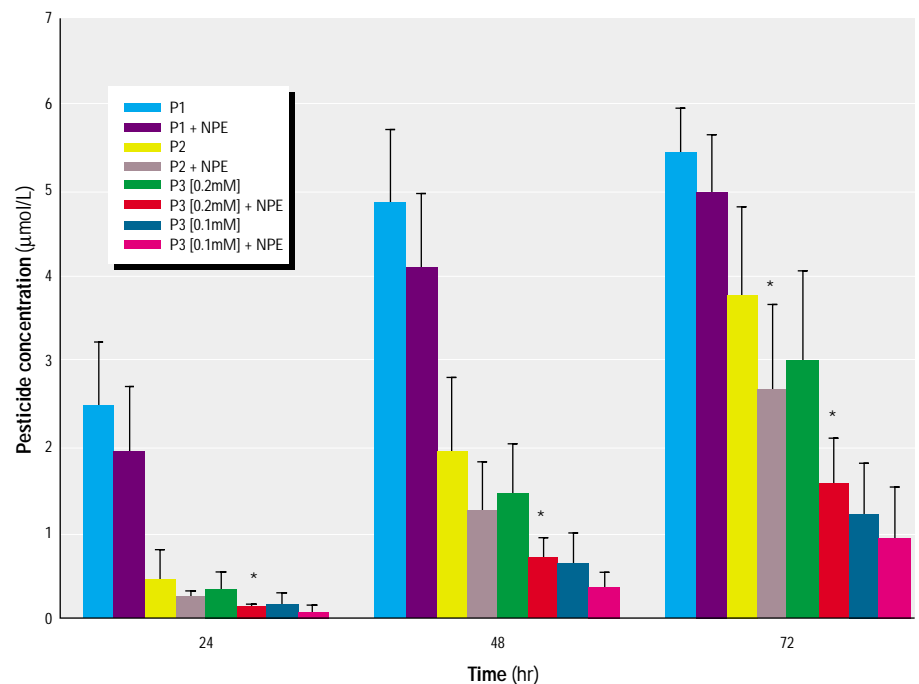


Figure 1. Percutaneous penetration of methiocarb (P1, 0.2 mM), pirimicarb (P2, 0.2 mM), and paclobutrazol (P3, 0.1 mM and 0.2 mM) during 72 hr continuous exposure ($n = 9$ cells per data point) to the pesticide alone or with NPE (0.2 mM). Results are given as mean pesticide concentration in the receptor phase + SD.

*Significantly different from group without NPE ($p < 0.05$; two-tailed).

Table 1. Influence of increasing concentrations of NPE on dermal *in vitro* penetration of pirimicarb (0.2 mM in donor chamber).

NPE (mM)	No.	Pirimicarb (μ M), mean + SD		
		24 hr	48 hr	72 hr
0.2	14	0.24 + 0.08 ^a	1.11 + 0.52 ^a	2.52 + 1.13 ^a
0.8	6	0.10 + 0.05 ^b	0.46 + 0.17 ^b	0.95 + 0.41 ^b
2.0	5	0.13 + 0.10 ^{ab}	0.58 + 0.26 ^b	1.23 + 0.50 ^b

Results are given in micromoles pirimicarb in the receptor chamber after 24, 48, and 72 hr and stated as mean + SD. Different letters indicate significant differences between groups ($p < 0.05$; two-tailed).

dermal exposure and percutaneous penetration (Figure 3). Addition of NPE to the donor phase changed the amounts of pesticide penetrating the glove material and skin only insignificantly (Figure 2–4).

Discussion

Calculation of penetration rates and direct comparison among experiments are based on the assumption that the receptor and donor

phases act as infinite sinks. Thus, the concentration in the donor phase should always be considerably higher than in the receptor phase to avoid affecting the free diffusion between the two compartments. For methiocarb, this was not the case at exposure times longer than 48 hr, as concentrations in the donor and receptor chambers approach the calculated equilibrium concentrations. Therefore, we did not see an increase in

methiocarb concentration between 48 and 72 hr equivalent to the increases observed with pirimicarb and paclobutrazol. The kinetic background is a shorter lag time and a faster penetration rate for methiocarb as compared to the other two pesticides (16).

In vitro studies have demonstrated that detergents may change the barrier properties of human skin (17,18). Thus, both anionic and nonionic detergents generally will increase the percutaneous penetration of tritiated water from a donor chamber to a receptor chamber, thus indicating an increased potential for exchange between the two compartments (3). Detergents are often added to commercial pesticide products to solubilize the active ingredients. Detergents used in pesticides may also increase the delivery of the active ingredient in the plant by increasing the percutaneous penetration through the surface membranes of the plant. NPE and other ethoxylated alcohols are used extensively as solubilizers in different pesticide formulations, and NPE has been demonstrated *in vitro* to increase permeation of tritiated water through human skin by 60% (4). The present study, however, demonstrates that NPE at equimolar concentrations as the active ingredient may decrease the percutaneous penetration of all three pesticides tested, although statistical significance was not obtained for methiocarb nor for pirimicarb at the initial sampling times. Moreover, the dermal penetration of pirimicarb could be lowered further by increasing the ratio between detergent and pesticide concentrations. These observations on only three pesticides may have more general implications because they cover a broad range of solubilities ranging from 0.027 g/L (methiocarb and paclobutrazol) to 2.7 g/L (pirimicarb) and a corresponding range of $\log P_{ow}$ values from 3.3 to 1.7, respectively. To the extent that these *in vitro* experiments are valid in the *in vivo* situation, our results, though not reaching statistical significance for all pesticides at all sampling times, indicate that NPE with respect to human occupational absorption of active ingredients may be an acceptable solubilizer. However, the potential toxicity of NPE itself should not be disregarded; NPE or its metabolites are suggested to act as xenoestrogens (19). The findings in the present study using NPE as model detergent calls for further evaluations with a broader range of detergents including anionic as well as nonionic detergents.

The present experiments on pesticide penetration through a membrane of latex or nitrile and a human skin barrier do not allow direct discrimination between effects on penetration through the glove materials or through the skin. However, comparison with the data from studies on skin alone may

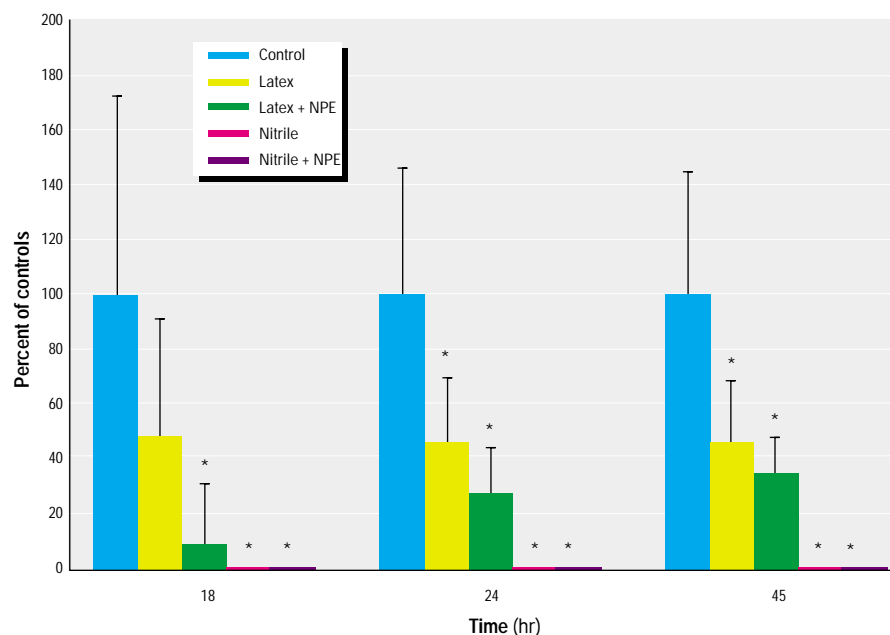


Figure 2. Effect of latex and nitrile on the percutaneous penetration of paclobutrazol (0.2 mM) observed during 45 hr continuous exposure ($n = 9$ cells per data point) to the pesticide alone or with NPE (0.2 mM). Results are given as percent of controls (no latex, no nitrile, no NPE) and stated as mean + SD.

*Significantly different from control ($p < 0.05$; two-tailed).

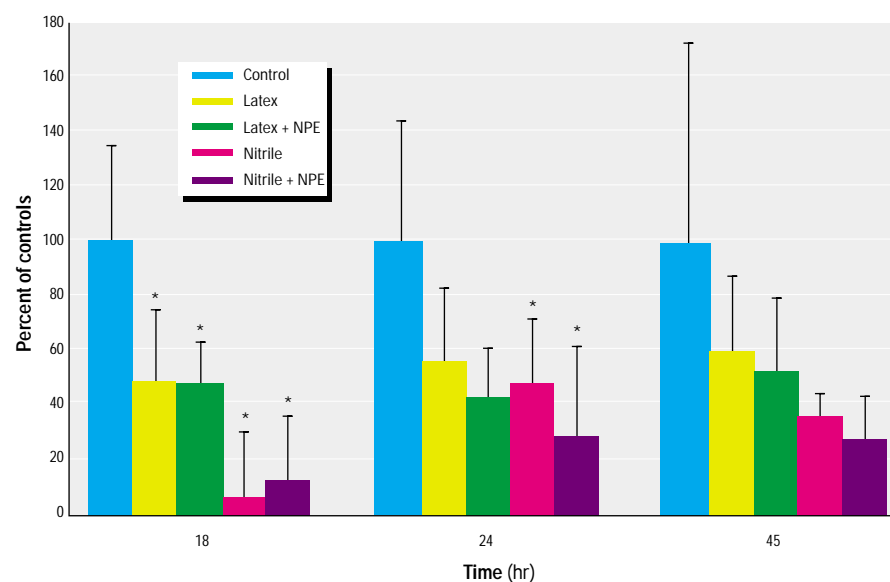


Figure 3. Effect of latex and nitrile on the percutaneous penetration of pirimicarb (0.2 mM) observed during 45 hr continuous exposure ($n = 9$ cells per data point) to the pesticide alone or with NPE (0.2 mM). Results are given in percent of controls (no latex, no nitrile, no NPE) and stated as mean + SD.

*Significantly different from control ($p < 0.05$; two-tailed).

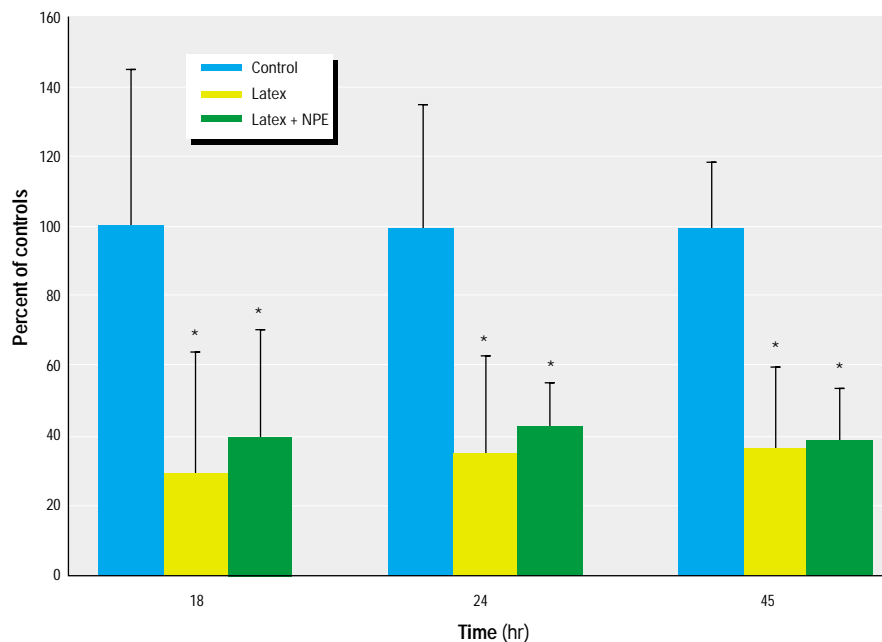


Figure 4. Effect of latex on the percutaneous penetration of methiocarb (0.2 mM) observed during 45 hours continuous exposure ($n = 9$ cells per data point) to the pesticide alone or with NPE (0.2 mM). Results are given in percent of controls (no latex, no NPE) and stated as mean + SD. *Significantly different from control ($p < 0.05$; two-tailed).

be used for this purpose. Thus, despite being merely indicative, the present data suggest that NPE cannot penetrate the glove barrier to a degree sufficient to exert the same effect as in experiments without a glove barrier.

In accordance with the literature, both glove materials showed resistance against pesticide penetration. That nitrile protected against penetration of pirimicarb for only 18 hr as opposed to the total protection by nitrile against paclobutrazol indicates the importance of the chemical characteristics of pesticides that affect glove materials differently. Thus, the complete protection against penetration of one pesticide may not occur for another. Further, penetration characteristics of a given glove material may change over time, as demonstrated for nitrile after pirimicarb exposure. This finding stresses the

importance of replacing gloves regularly during a work day. Another important observation was that not all glove materials give the same degree of protection against pesticides, and intervals for replacement of gloves are expected to depend on glove material. Therefore, inclusion of glove matrix in the *in vitro* dermal absorption model may be necessary to obtain accurate predictions of worker exposure where protective gloves are used.

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