Comparison of the In Vivo and In Vitro Percutaneous Absorption of a Lipophilic Molecule (Cypermethrin, a Pyrethroid Insecticide)

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The absorption of a pyrethroid insecticide, cypermethrin, through rat skin has been measured both in vitro and in vivo. Cypermethrin did not penetrate in vitro through whole skin but did penetrate epidermal membranes. The in vitro absorption was influenced by the choice of receptor fluid in the glass diffusion cell. There was good agreement between in vivo and in vitro data using 50% aqueous ethanol, 6% Volpo 20, or total calf serum receptor fluids. Rat epidermal membranes in vitro were more than 20 times more permeable to cypermethrin than human epidermal membranes, indicating that cypermethrin would be less readily absorbed in humans than in the rat.

The percentage of the applied dose absorbed after 8-h contact was measured and, separately, the profile of the absorption over 24 h. Parallel in vitro studies measured cypermethrin absorption through excised rat and human cadaver skin (using both whole skin and epidermal membranes). To facilitate analysis and allow a more accurate assessment of absorption, [14C]cypermethrin was added to the formulation.

In these studies, we have been primarily concerned with the definition of the amount of chemicals that might be absorbed in a specific time period and produce general, rather than local effects. We have therefore considered a chemical to be absorbed when it has actually diffused through the epidermis.

M any pesticides during manufacture and use have the potential to contact skin and be absorbed. Consequently, part of the necessary safety evaluation involves an assessment of percutaneous absorption rate.

Often, assessments involve the application of the chemical to the intact skin of a living animal (typically a rat) and a determination of the extent of absorption made by examination of body fluids and tissues. Such methods require substantial technical skill, much time, and the use of live animals [1]. Confident extrapolation of the derived data to humans is often questionable as the permeability of human skin has been shown to be less permeable than animal skin to a wide range of chemicals and no ideal animal model has been reported [2–5]. The monkey has been shown to have similar permeability to human skin for a small range of well-absorbed nitroaromatic compounds [6], but with more poorly absorbed molecules significant differences might be found.

During the past 20 years, the factors governing the percutaneous absorption of chemicals have been studied using in vitro methods [7–9]. The rationale for this approach is that the rate of absorption is governed by diffusion through the dead, outer layer of the epidermis, the stratum corneum [8], and that there is no active transport [10]. There have been many publications describing the utilization of this technique with many different types of chemicals [11–13]; however, there are fewer publications demonstrating that in vitro results are in agreement with in vivo results [6,14–16].

We have measured the in vivo percutaneous absorption of the pesticide cypermethrin after application to rat skin, formulated in a typical formulation. The formulation was applied in small, finite amounts as might contact a spray operator under field application conditions (personal communication from Mr. G. Chester, ICI plc, 1985).

The MATERIALS AND METHODS

Radiochemicals (±) α-Cyano-3-phenoxymethyl (±) cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate (trivial name cypermethrin) was supplied by ICI plc, Plant Protection Division, Fernhurst, Sussex, U.K. as [14C]cycloprop-label led cis-cypermethrin (1.9 GBq mmol⁻¹) and [14C]cycloprop-labeled trans-cypermethrin (2.0 GBq mmol⁻¹). Both isomers were purified by high-performance liquid chromatography to a radiochemical purity of >97% as determined by thin-layer chromatography. The isomers were mixed to give a cis-cypermethrin to trans-cypermethrin ratio of 50:2:49.8. Two cypermethrin dosing solutions were prepared; a concentrate formulation of 359 g/liter and an oil based spray dilution of 29 g/liter. The dosing solutions were monitored for stability throughout the study and at all times the radiochemical purity was >97%.

Tritiated water was supplied by Amersham International, Buckinghamshire, U.K. and was diluted for use to 0.1 MBq ml⁻¹.

In Vitro Studies Human abdominal skin was obtained from autopsy and the subcutaneous fat removed. Human epidermal sheets were separated from full-thickness skin by immersing in water at 60°C for 45 s and gently peeling away the epidermis [17]. Excised skin from the dorsolateral region of 4- to 5-week-old rats (Alpk/AP strain, Wistar derived) was used. Rat epidermal sheets were prepared by soaking in 2 M NaBr solution before...
gently peeling away the epidermis [17]. Membranes that were not used immediately were wrapped in aluminium foil and stored at 4°C for up to 2 weeks.

Both whole skin and epidermal membranes were mounted in horizontal glass diffusion cells and exposed to ambient conditions at 25°C and 50% relative humidity, and the epidermal surface left exposed to the atmosphere. Test chemicals were then applied to the intact epidermal membranes on the next day.

Prior to the application of the formulations the integrity of each membrane was assessed by determining the permeability to triphenyl phosphine oxide (Sartorius IKA, Belmont, U.K.) fitted with a 10S probe. The samples were centrifuged at 1000 g for 10 min in an IEC Centra-7R (Damon/IEC (U.K.) Ltd, Dunstable, Bedfordshire, U.K.) and duplicate 0.2-ml aliquots mixed with 10 μL of Dimilum-30 (Packard) in glass scintillation vials (Packard) and the radioactivity determined by liquid scintillation counting.

Fecal samples were suspended in methanol and homogenized using an Ultra-Turrax homogenizer (Sartorius IKA, Belmont, Surrey, U.K.) fitted with a 10S probe. The samples were centrifuged at 1000 g for 10 min in an IEC Centra-7R (Damon/IEC (U.K.) Ltd, Dunstable, Bedfordshire, U.K.) and duplicate 0.2-ml aliquots mixed with 10 μL of Optiphase “MP” and taken for liquid scintillation counting. The fecal residue was dried and ground to a fine powder using a mortar and pestle.

The individual carcasses were mixed with twice their weight of rat chow and minced to a fine homogenous powder using a Hobart E4522 mincer (Hobart Manufacturing Co Ltd, London, U.K.).

Blood, liver, kidney, ground fecal residue, and carcass were prepared for radiochemical analysis using a Packard Tri-carb 306 sample oxidizer (Packard). The resulting CO2 was absorbed in 8 ml of Optiphase I (Fisons plc) and admixed with 12 ml of Optiphase “S” scintillant (Fisons plc). Aliquots of 14C/cypermethrin containing 40,000 dpm were used to determine the overall combustion efficiency.

All sample liquid scintillation counting was done on a BETA-matic II counter with the appropriate quench correction data loaded into the memory of the machine.

RESULTS

Following the in vivo dermal application of the concentrate formulation to the rat, 1.0% (SEM 0.4%; n = 3; equivalent to 10.9 ± 4.0 μg cm−2 cypermethrin) of the total recovered dose was absorbed during the 8-h exposure time.

From the parallel in vitro studies no cypermethrin was absorbed (limit of determination <0.1% of the recovered dose) from either the concentrate or spray dilution, during the 8-h application exposure of whole human or rat skin (Table I). When rat epidermal membranes were used, with 50% aqueous ethanol, 6% Volpo 20, or 20% fetal calf serum as the receptor fluids, absorption of cypermethrin from both the concentrate and spray dilution formulations was detected. However, absorption was detected only through human epidermal membranes with 50% aqueous ethanol as the receptor fluid (Table I).

Figure 2 shows the profile of cypermethrin absorption from the in vitro (using 50% aqueous ethanol and 6% Volpo 20 as the receptor fluids) and in vivo time course study. The in vivo absorption profile was very similar to the in vitro profiles. During the initial 12 h there was no significant difference between the in vivo and in vitro data, however, at 24 h the in vitro experiment
Table I. In Vitro Absorption of Cypermethrin From Concentrate and Spray Dilution Formulations Through Whole Skin (Human and Rat) and Epidermal Membranes (Human and Rat) Into Different Receptor Fluids

<table>
<thead>
<tr>
<th>Formulation Application</th>
<th>Human Whole Skin</th>
<th>Human Epidermis</th>
<th>Rat Whole Skin</th>
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</thead>
<tbody>
<tr>
<td>Concentrate (µg)</td>
<td>3</td>
<td>5</td>
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<td>5</td>
</tr>
<tr>
<td>Spray Dilution (µg)</td>
<td>0.5</td>
<td>1.0</td>
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</tr>
<tr>
<td>Total Cypermethrin Applied (µg)</td>
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<td>1.7</td>
<td>2.1</td>
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<tr>
<td>% Dose Absorbed After 8 Hours</td>
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<td>Whole Skin</td>
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<td>Rat Whole Skin</td>
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<td></td>
<td>2.7 ± 0.4%</td>
<td>1.0 ± 0.2%</td>
<td>3.2 ± 0.4%</td>
<td>1.0 ± 0.2%</td>
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Key Receptors: 1. Ethanol 2. 6% Volpo 20 in saline 3. Physiological saline 4. 0.9% saline 5. Standard Error 6. Not detectable

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Figure 2. Profile of the time course of [14C]-cypermethrin absorption measured in vivo (triangles) and in vitro using 50% aqueous ethanol (square) and 6% Volpo 20 in saline (circles) as the receptor fluids.

DISCUSSION

The results from the in vivo studies on the percutaneous absorption of cypermethrin form the basis for comparison with the in vitro results. The doses selected for application to the skin were small and representative of the estimated amounts which could contact workers in normal practice. Many in vitro studies have previously been criticized, as the amounts applied to the skin were excessive compared with the practical in vivo situation; this was not the case in this study. By precisely matching the applications in vivo and in vitro any differences detected could not be ascribed to the application regime.

We have considered the chemical as percutaneously absorbed once it has reached the systemic circulation, which is located beneath the dermal-epidermal junction. Chemical that was still on, or in, the skin was not considered as absorbed at the termination of the study. In vivo only 1.0% of the applied cypermethrin was absorbed in 8 h following application of the concentrate formulation. Although precise comparisons cannot be made due to differences in doses and vehicles, this is low when compared to other pesticides that have been studied in vivo through human [19] and animal skins [1, 20, 21].

The epidermal membranes used in these experiments are different from skin slices which have been used by other workers [6] as they do not contain any dermis [17], a barrier to lipophilic molecules in vitro but not in vivo. The receptor fluids were selected both to have a range of solubility properties and to reflect normal body fluids (saline and fetal calf serum); similar receptor fluids have previously been used [22].

Previous percutaneous absorption studies with lipophilic molecules have shown that in vitro the aqueous dermis can act as a significant, additional, artificial barrier [8]: penetrating molecules enter the systemic circulation in vivo at the top of the dermis and do not have to traverse its full thickness. Other in vitro studies [22] with lipophilic molecules have reported poor agreement compared with in vivo human data [19] rather than the good agreement achieved with more water-soluble molecules. The in vivo results indicated lower absorption for the lipophilic molecules than expected and the results were probably influenced by the presence of the dermis. As anticipated, the dermis influenced
absorption of cypermethrin in vitro, during the 8-h exposure time, through both human and rat whole skin (Table I). When epidermal membranes were used, however, we were able to quantify the absorption of cypermethrin.

The influence of the dermis as an extra barrier to the absorption of lipophilic molecules has also been reported in in vivo studies with human skin transplanted onto athymic mouse skin [23]. Good agreement was seen between the permeability of the transplanted human skin and published human in vivo data [19] for water-soluble chemicals but poorer agreement was seen with the more lipophilic molecules. In the grafted skin, the dermis was abnormally thick and poorly vascularized and might have acted as an additional barrier to the lipophilic molecules.

The necessity of a suitable receptor fluid in vitro percutaneous absorption assessments has been reported [22] and we have confirmed that the various receptor fluids chosen for our study influenced the absorption of cypermethrin. The highest absorption was measured with 50% aqueous ethanol as the receptor fluid and, although absorption measured with the other two receptor fluids with lipid-soluble properties (6% Volpo 20 and 20% fetal calf serum) was very similar, no absorption was detected with the other receptor fluids. These results reinforce the view that the choice of a receptor fluid in which the penetrating molecule is highly soluble is necessary for successful in vitro measurements. Otherwise, solubility in the receptor phase, rather than diffusion through the epidermis, will be the rate-limiting step.

It is apparent with the concentrate formulation, that there was a similar magnitude of absorption measured in the in vivo experiments and the in vitro experiments (when appropriate skin membrane and suitable receptor fluid were used). The results indicate slightly greater absorption in vitro than in vivo. The absorption measured in vivo was very low and even with the use of a radiolabeled molecule there are significant analytic difficulties in obtaining determinations from tissues.

Based on these data, the in vitro absorption of cypermethrin with time was measured from the spray dilution formulation through rat epidermal membrane and 50% aqueous ethanol and 6% Volpo 20 as the receptor fluids. Fetal calf serum was not suitable for use over long time periods. The results were compared to data from an equivalent in vivo study (Fig 2). The absorption was greater with the 50% aqueous ethanol receptor fluid than with the 6% Volpo 20 in saline, although the absorption profiles were similar. The in vivo profile showed a good correlation with the in vitro profiles, using both receptor fluids up to 12 h. At 24 h there was still good agreement between the in vivo data and the in vitro data in the 50% aqueous ethanol receptor fluid, but the results with the 6% Volpo 20 receptor fluid underestimated the in vivo absorption of cypermethrin. A similar in vitro underestimation of in vivo absorption, though with a similar absorption profile, has been seen with other lipophilic molecules when 6% Volpo 20 in saline was used as the receptor fluid [22].

These studies have confirmed the marked species differences in percutaneous absorption that can occur. Any assessment of the percutaneous absorption of cypermethrin, using rat skin, will lead to an overestimation of potential human absorption. We have shown [2] that not only the rat, but other common laboratory animals, are poor models for human skin permeability, particularly with slowly absorbed chemicals. However, the in vitro technique provides a method for quantifying the difference in the permeability properties of human and animal skin. The only data that allow a quantitation of the difference in the permeability of human and rat skin to cypermethrin are those using the spray dilution, epidermal membranes, and 50% aqueous ethanol receptor fluid. In these experiments, human skin was 24 times less permeable to cypermethrin than rat skin. With the other receptor fluids used, if it is assumed that 0.1% was actually absorbed, these experiments indicate that rat skin is 17–32 times more permeable than human skin.

This study has shown that an understanding of the technical difficulties and limitations of the in vitro percutaneous absorption technique allows experiments to be done which give data in good agreement with in vivo data. Once the influence of the type of membrane and receptor fluid are understood, data can be obtained for a wide range of molecules with different physicochemical properties. In vitro assessments are less demanding than in vivo experiments, and further understanding of the in vitro technique should promote its wider acceptance and might reduce the need for many in vivo studies.

REFERENCES


