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PERCUTANEOUS ABSORPTION OF DIAZINON IN HUMANS

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Abstract—Diazinon is an organophosphorus insecticide which, through general use, comes into contact with human skin. To investigate its percutaneous absorption, human volunteers were exposed for 24 hr to ^{14}C-labelled diazinon applied in acetone solution (2 μg/cm^2) to the forearm or abdomen, or in lanolin wool grease (1.47 μg/cm^2) to the abdomen. Complete void urine samples were collected daily for 7 days. Percutaneous absorption ranged from 2.87 $\pm$ 1.16% (mean $\pm$ SD, n = 6) to 3.85 $\pm$ 2.16% of the applied dose, and there were no statistically significant differences with regard to site or vehicle of application. In rhesus monkeys, over the 7 days after iv dosing (2.1 μCi [^{14}C]diazinon, 31.8 μg) a total of 55.8 $\pm$ 6.8% (n = 4) of the dose was excreted in the urine, and 22.6 $\pm$ 5.2% was eliminated in the faeces (78.4% total accountability). In *in vitro* percutaneous absorption studies with human abdominal skin, 14.1 $\pm$ 9.2% of the applied dose accumulated in the receptor fluid over 24 hr of exposure to 0.25 μg/cm^2 (acetone vehicle). The calculated mass absorbed was the same (0.035 μg/cm^2) for both *in vitro* and *in vivo* absorption through human skin.

INTRODUCTION

Percutaneous absorption is now a primary focal point for study in dermatotoxicology. It is now recognized that both local and systemic toxicity depends on a chemical penetrating the skin. There is a relationship between percutaneous absorption and toxicological activity. A local or systemic effect cannot occur unless the chemical has inherent toxicity and the chemical is able to overcome the barrier properties of skin and enter a biological system (local skin and/or systemic circulation) (Wester and Maibach,1983).

Diazinon is an organophosphorus insecticide which through ordinary use comes into contact with human skin. Therefore it is important to know its percutaneous absorption and disposition to help assess potential human health hazards. Our objectives were to determine the percutaneous absorption of [^{14}C]diazinon in humans, both *in vivo* and *in vitro* using human skin. We have also investigated the pharmacokinetic disposition of [^{14}C]diazinon in the rhesus monkey.

Since diazinon is used to control external parasites on sheep and has been found in lanolin, for the *in vivo* studies in humans wool grease was used as a vehicle along with the standard acetone vehicle. Skin application sites were the forearm and abdomen, both relevant skin exposure areas (Wester and Maibach, 1989).

MATERIALS AND METHODS

In vivo, humans

The study included six normal volunteer outpatients per group (total 18 patients). The patients were all males, aged 19–65, for whom approval (Human Use Committee) and informed consent had been obtained. The subjects were topically dosed with [^{14}C]diazinon (0.066 mCi/mg; Ciba Geigy, Basle, Switzerland) in acetone solution or lanolin wool grease. The [^{14}C]diazinon was ring labelled (no. 2 carbon on the pyrimidinyl ring). Solutions of 20 μg [^{14}C]diazinon in 50 μl acetone were dosed over a 10-cm^2 area of skin (2 μg/cm^2) on either the ventral forearm of the abdomen. A formulation of 14.7 μg [^{14}C]diazinon in 50 μl lanolin wool grease was dosed over a 10-cm^2 area of skin (1.47 μg/cm^2) on the abdomen area. The wool grease was prepared from fleeces from sheep kept on a property known to be free of pesticides. The wool was subsequently extracted with both hexane and then isopropanol so that the grease contained both polar and non-polar components, and should therefore have been representative of the lipid material that would be encountered by shearers. The skin sites of application were not covered/occluded. After 24 hr, the skin application sites were washed with 50% liquid Ivory soap (1:1, v/v) and water. Daily complete void urine samples were collected from volunteers and assayed for radioactivity content. Percutaneous absorption was determined from the urinary ^{14}C excretion. After the 7-day urine collection period the skin site of application was cellophane tape stripped (Transparent, 3M Co., St Paul, MN, USA) 10 times and the tape strips were assayed for residual ^{14}C content.

In vitro, human skin

Two separate donor skin sources with six replicates per experiment were used (Wester and Maibach,

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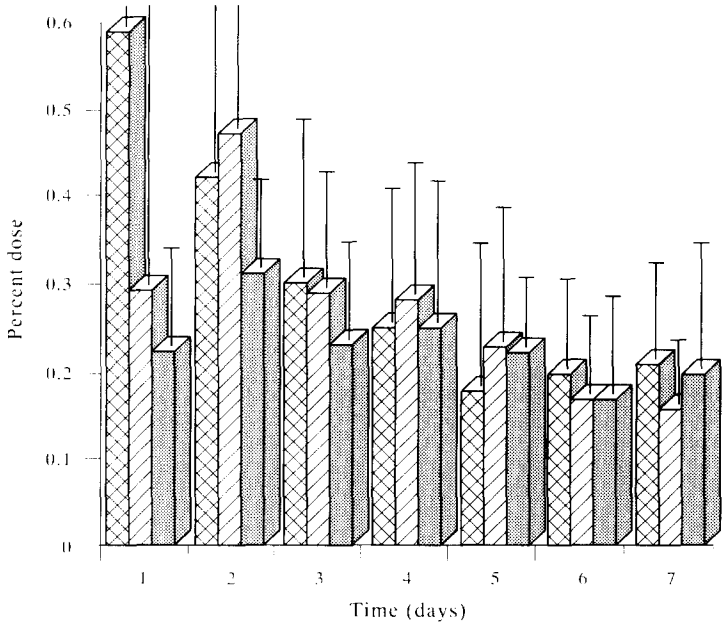

Fig. 1. Urinary ^{14}C excretion in human volunteers following skin application of [^{14}C]diazinon in acetone solution to the forearm (▩) or abdomen (▨) or in lanolin wool grease to the abdomen (■). Values are means for groups of six volunteers and range bars indicate the SD.

1989). Small cells were of the flow-through design with a 1-cm^2 surface area. Buffered saline at a flow rate of 1.25 ml/hr (1 reservoir volume) served as receptor fluid. Human cadaver skin was cut with a dermatome to 500 μm and stored at 4°C in Eagle's minimum essential medium to preserve skin viability. The skin was used within 5 days of collection. This preservation/use regimen follows that used by the human skin transplant bank (Hurst *et al.*, 1984) and in studies by Bronaugh *et al.* (1989).

^{14}C-labelled chemical in acetone vehicle was applied with a micropipette to the surface of the skin of the six cells. The dose was 0.25 μg/cm^2. At the end of a 24-hr period, the system was stopped. The residual fluid remaining in the cells was collected and analysed. The skin surface was washed once with 1 ml liquid soap (Joy; Procter and Gamble, Cincinnati, OH, USA) and twice with 1 ml distilled water, and the wash solutions were analysed by scintillation counting. The skin itself was completely solubilized in Soluene 350 (Packard Instruments, Downers Grove, IL, USA) and 80% acetic acid was added to neutralize the homogenate. The receptor phase samples from the permeation cells' residual fluid, the skin surface washes, the cotton balls, the glass apparatus and the skin itself were assayed for ^{14}C content by liquid scintillation counting.

In vivo, rhesus monkey

Percutaneous absorption of diazinon was determined from the following equation:

Percent dose absorbed = (urinary ^{14}C excretion following topical dosing/urinary ^{14}C excretion following iv dosing) × 100

The urinary ^{14}C excretion for topical application was determined in the human volunteers. An iv dose is 100% bioavailable (injected into body) and it is needed to account for diazinon, which would be eliminated by some other route (faeces) or retained in the body. This was done in the rhesus monkey, rather than human volunteers, to minimize the human ex-

Table 1. ^{14}C disposition following topical application of [^{14}C]diazinon to human volunteers

Site and vehicle of dosing	Parameter	^{14}C disposition (% of dose)*
Forearm, acetone	Urine (7 days)	2.2 ± 1.2
	Surface wash (24 hr)	0.5 ± 0.5
	Skin tape strip (day 7)	0.01 ± 0.01
Abdomen, acetone	Urine (7 days)	1.8 ± 1.1
	Surface wash (24 hr)	1.4 ± 2.9
	Skin tape strip (day 7)	0.01 ± 0.01
Abdomen, lanolin	Urine (7 days)	1.6 ± 0.6
	Surface wash (24 hr)	0.35 ± 0.42
	Skin tape strip (day 7)	0.04 ± 0.04

*Mean ± SD for six volunteers per group.

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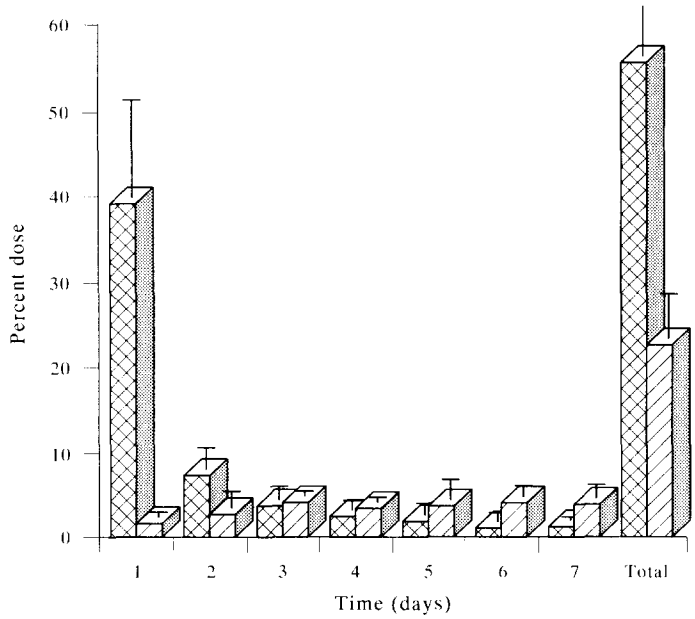

Fig. 2. Disposition of ^{14}C after iv administration of [^{14}C]diazinon to female rhesus monkeys. Levels of ^{14}C in the urine (▩) and faeces (▨) were monitored for 7 days. Values are means for four monkeys, and range bars indicate the SD.

posure to this pesticide (Wester *et al.*, 1992). Four female animals were dosed iv with 2.1 μCi [^{14}C]diazinon (31.8 μg) in propylene glycol and their urine was collected for 7 days to determine the extent of ^{14}C excretion.

Sample analysis

Urine samples were analysed in duplicate for ^{14}C. A 5-ml aliquot of each urine sample was assayed in 10 ml scintillation cocktail (Universal ES, Costa Mesa, CA, USA) with a Packard 4640 liquid scintillation spectrophotometer. The cotton balls from the washing of the site of application were individually counted in 16 ml scintillation cocktail with the liquid scintillation spectrophotometer.

Background control samples and test samples were counted in the Packard Model 4640 liquid scintillation counter (Packard Instruments). Control and test sample counts were transferred to a computer program (Appleworks/Apple IIE computer; Apple Computer Co., Mountain View, CA, USA) which subtracted × 1 background control samples and generated a spreadsheet with the data reported under Results.

Table 2. Percutaneous absorption of diazinon in humans

Skin site	Vehicle	Percutaneous absorption (% of dose)*
Forearm	Acetone	3.85 ± 2.16
Abdomen	Acetone	3.24 ± 1.94
Abdomen	Lanolin	2.87 ± 1.16

*Mean ± SD for six volunteers per group, calculated from human urinary ^{14}C disposition (Table 1) corrected for incomplete/other route excretion with the monkey urinary disposition after iv dosing.

RESULTS

Figure 1 shows the daily ^{14}C urinary excretion following topical [^{14}C]diazinon application to human volunteers. Diazinon applied in acetone vehicle to the forearm had the highest ^{14}C excretion at day 1 at 0.59 ± 0.42% (mean ± SD). The excretion level declined each day to a low of 0.21 ± 0.11% on day 7. In contrast, when diazinon was applied to the abdomen, whether in acetone solution or lanolin, the peak urinary ^{14}C excretion was on day 2. Table 1 summarizes the ^{14}C disposition. A total of 2.2 ± 1.2% ^{14}C was excreted in the urine after application of [^{14}C]diazinon to the forearm, and 1.8 ± 1.1 and 1.6 ± 0.6% ^{14}C was excreted after application to the abdomen in the acetone and lanolin vehicles, respectively. Levels of 0.2% radioactivity were still being excreted on day 7. The soap and water wash recovered little residual diazinon on the skin (0.35–1.4%), and the skin tape stripping recovered virtually no residual diazinon (0.01–0.04%).

Figure 2 shows the disposition of ^{14}C in urine and faeces following iv administration of [^{14}C]diazinon to rhesus monkeys. A total of 55.8 ± 6.8% of the dose was excreted in urine and 22.6 ± 5.2% was eliminated in the faeces during the 7 days of observation. Most of this radioactivity was excreted in the urine on day 1. Note that urinary ^{14}C excretion was complete, but that faecal ^{14}C excretion was steady and continuing on day 7.

Table 2 shows the *in vivo* percutaneous absorption of diazinon in human volunteers. These values are calculated from the human urinary ^{14}C disposition in Table 1 corrected for incomplete/other route excretion with the monkey urinary disposition after iv

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Table 3. *In vitro* percutaneous absorption of diazinon in human skin

Skin source*	Radioactivity (% of dose)† in Receptor fluid	Skin digest	Surface wash	Total
23 yr Male	8.5 ± 1.6	5.6 ± 1.4	48.3 ± 1.8	62.4 ± 2.2
56 yr Male	19.7 ± 10.4	4.8 ± 2.1	34.6 ± 14.2	59.1 ± 9.2
Average ...	14.1 ± 9.2	5.2 ± 1.7	41.4 ± 12.0	60.7 ± 6.6

*Abdominal skin with diazinon applied in acetone vehicle.
†Mean ± SD for six replicates per skin source.

dosing (55.8%). Percutaneous absorption of diazinon was 3.85 ± 2.16% from acetone solution on the forearm, 3.24 ± 1.94% from acetone solution on the abdomen, and 2.87 ± 1.16% from lanolin vehicle on the abdomen. There was no statistical difference ($P > 0.05$) in these absorption values.

Table 3 gives the *in vitro* percutaneous absorption through abdominal human skin for diazinon applied in acetone vehicle. Receptor fluid accumulation for skin source 1 was 8.5 ± 1.6% and 19.7 ± 10.4% for skin source 2, an overall average of 14.1 ± 9.2%. Skin digests accounted for approximately 5% of the dose, the surface wash accounted for approximately 40% of the dose, and overall dose accountability was about 60% of the administered dose.

DISCUSSION

Percutaneous absorption of diazinon in humans ranged from 2.87 ± 1.16% to 3.85 ± 2.16%. There was no statistical difference between levels of absorption from different sites of application (forearm or abdomen) or from different vehicles (acetone solution or lanolin wool grease). There are no literature reports on diazinon percutaneous absorption with which to compare our results.

Percutaneous absorption *in vitro* through human skin was 14.1 ± 9.2% of the applied dose. The *in vivo* dose was 2.0 $\mu g/cm^2$ and the *in vitro* dose was 0.25 $\mu g/cm^2$ The calculated mass (μg) absorbed was the same (0.035 $\mu g/cm^2$ skin surface area) for both *in vitro* and *in vivo* human skin absorption.

The excretion pattern of ^{14}C following iv administration of [^{14}C]diazinon to rhesus monkeys and following topical administration in humans suggests that some interesting pharmacokinetic parameters and/or metabolism may be occurring. With iv administration in the monkey, urinary ^{14}C disposition is rapid and complete by day 7. Faecal ^{14}C excretion increases up to day 3 and is fairly constant from days 3–7, inclusive. The overall dose recovery of 78.4% does not account for the total administered dose. Continual faecal ^{14}C excretion might suggest metabolism and enterohepatic recirculation (Wagner, 1975). The enterohepatic recirculation of diazinon would produce additional exposure of the liver to diazinon metabolite(s). This may be a link to the suggested porphyrogenic effect of diazinon (Nicholas and Collins, 1987).

In vivo, the soap and water wash of the skin surface at 24 hr and the tape strip of the skin at day 7 did not show any surface/upper skin layer residue. This suggests that diazinon is lost to clothing or to evaporation. Similar results have been shown to occur with isofenphos (Wester *et al.*, 1992). *In vitro* data accounted for only 5% of the dose in skin, suggesting no large skin depot mechanism. The *in vitro* diffusion cell has a glass cylinder around the dosing area, open at the top to air. The *in vitro* dose accountability was 60%. Some diazinon may have evaporated off the skin during the absorption study.

The *in vitro* and *in vivo* percutaneous absorption values for diazinon are in agreement with regard to the amount of potential systemic delivery. For compounds of higher lipophilicity, *in vitro* and *in vivo* data do not always agree. Highly lipophilic compounds are not soluble in the water receptor fluid and thus are not absorbed. Therefore, it is good to have *in vivo* confirmation of *in vitro* absorption to validate the *in vitro* system. Subsequent *in vitro* data can thus be used to define potential health hazard effects of diazinon.

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