Effect of Humidity and Occlusion on the Percutaneous Absorption of Parathion *in Vitro*

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INTRODUCTION

Many studies have been conducted to measure the effect of hydration (2–4) and occlusion (5) on percutaneous absorption of various compounds *in vivo* and *in vitro,* but little has been done in the past to study the effect of humidity on absorption of chemicals. It is widely accepted that fresh excised skin retains the barrier properties of the living integument. Therefore, permeability of skin may be assessed through various *in vitro* methods, these involving different types of diffusion cells mounted with excised skin (6,7). The purpose of the studies reported here was to determine the effect of different relative humidities (RH) on the percutaneous absorption of parathion, compare the results under hydration (%RH = 100) and occlusive conditions, and confirm the utility of a flow-through diffusion cell system. In these studies, experimental conditions were well controlled, and only relative humidity varied among experiments. The organophosphate pesticide parathion [(*O,O*-diethyl)-*O*-(4-nitrophenyl) phosphorothioate] was used as a model compound.

MATERIALS AND METHODS

Radiolabeled parathion, ^{14}C ring-2,6 (sp act, 12.4 mCi/mmol), was purchased from Sigma Chemical Company (St. Louis, MO). Nonradiolabeled parathion was obtained from Chem Service Inc. (West Chester, PA). The purity of the radiolabeled and nonradiolabeled compounds was determined by high-pressure liquid chromatography and thin-layer chromatography to be more than 98%. Ethyl alcohol (Aaper Alcohol and Chemical Co., Shelbyville, KY), sodium chloride (Baxter Healthcare Corporation, Deerfield, IL), and double-deionized distilled water were employed for dilution and washing.

The membranes used in these studies were full-thickness skin sections taken from the dorsal surfaces of weanling, female, Yorkshire pigs weighing approximately 20 kg. Hair was clipped from the back at least 24 hr prior to the experiments. Skin was carefully excised and dermatomed to a thickness of 500 μm using a Padgett dermatome (Padgett Dermatone division of K. C. Assemblage Co., Kansas City, MO). Circular skin sections were produced with a 16-mm steel punch biopsy and placed epidermal side up into a diffusion cell. Teflon flow-through diffusion cells (Crown Glass Co., Somerville, NJ) with an available exposure surface area of 0.32 cm^2 were employed (7). After the cells were assembled with the freshly excised pig skin between the top cap and the cell body, air bubbles were carefully removed from the receptor compartment (0.13 ml) of the cell, and the assembled cells were then mounted into a constant-temperature aluminum arm holder, with the temperature maintained by a Brinkmann constant-temperature circulator (Brinkmann Inc., Westbury, NY). The dermal side of the skin of each diffusion cell was bathed with perfusate at 4 ml/hr by a multichannel peristaltic cassette pump (Monostat, New York, NY). The standard perfusion medium used in these experiments contained a Kreb's–Ringer bicarbonate buffer, glucose, and 4.5% bovine serum albumin. Bovine serum albumin was added to facilitate partitioning of the penetrant from the dermis into the perfusate and to mimic *in vivo* conditions more closely. Medium was oxygenated with 95% O_2 and 5% CO_2 and the pH was kept between 7.35 and 7.45 during the experimental period. Air temperature and humidity were maintained at standard conditions (i.e., air temperature = 37°C, perfusate temperature = 37°C, relative humidity = 60%, flow rate = 4 ml/hr) or adjusted to experimental conditions (i.e., %RH = 20, 70, 80, 85, or 90) with an air temperature and humidity control unit (Diamond Research, Raleigh, NC). Hydration (%RH = 100) was achieved by adding 200 μl of physiological saline to the surface of the skin and maintaining the highest possible air humidity (%RH ≈ 98). Occlusion was achieved using parafilm (American National Can, Greenwich, CT) to cover each cap opening. An equilibration period of 30 min in the perfusion chamber (1) was provided before the topical application of parathion. The overall procedures were designed to maintain essentially fixed environmental conditions during each experiment. After 30 min of equilibration, one of the following doses (4, 40, or 400 μg/cm^2) of parathion was applied to the skin in 10 μl of ethanol vehicle. Perfusate was collected in scintillation vials at 1-hr intervals using an automatic fraction collector. Thus, total flux of radiolabel coming through the skin over time was measured. Perfusate samples were combusted in an open-flame tissue oxidizer (Packard Oxidizer Model 306, Packard Instrument Co., Downers Grove, IL), and the total penetration of parathion was determined by scintillation spectroscopy (LKB-Wallac 1219 Rackbeta liquid scintillation counter, Wallac Co., Turke, Finland).

Absorption was defined as radioactivity appearing in the perfusate. No attempt was made to assess parathion metabolites in the perfusate; only the effect of humidity on transdermal flux was assessed. An 8-hr period was selected as the standard experimental length, since previous studies in our laboratory using these conditions demonstrated that porcine skin sections under these conditions were uniformly viable at the end of an 8-hr experiment and 8 hr was the optimal point to assess the effect of environmental conditions on parathion absorption (1).

Four or five replicates were conducted for each dose–humidity combination. The data were plotted as cumulative total counts (8 hr) collected in the receptor compartment versus relative humidity. Statistical differences between

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treatments were analyzed by the General Linear Models (GLM) procedure (SAS Institute Inc. Cary, NC) and multiple comparison tests were performed using the Student t test (least significant difference at the 5% level of significance) to control for Type I error.

RESULTS

Flow-through cell results suggested that the absolute absorption of parathion was increased with dose (0.30–1.10 μg/8 hr at 4 μg/cm^2 vs. 1.80–8.08 μg/8 hr at 400 μg/cm^2), but absorption efficiency (% applied dose absorbed) was decreased with higher doses. The absorption efficiency ranged from 7.48–27.43% (4 μg/cm^2) to 0.45–2.02% (400 μg/cm^2).

Humidity had a significant effect on the rate and efficiency of parathion absorption. Under the fully hydrated conditions (%RH = 100), parathion penetration was significantly increased ($P < 0.05$), approximately fourfold, over the value under the standard conditions at all three doses (Table I). Higher relative humidity (%RH = 90 and 85) also significantly increased parathion penetration, twofold, during the 8-hr experimental period. A %RH = 80 tended to increase parathion penetration at 40- and 40-μg doses ($P < 0.10$). At %RH = 70 or 20, there was no significant effect compared to the control condition (%RH = 60) (Table I). With the occlusion condition, penetration increased 3.4-fold over the value under the standard conditions ($P < 0.05$) at the 40-μg dose, while penetration increased 2.2- and 2.4-fold at the 4- and 400-μg doses, respectively ($P < 0.05$). Furthermore, parathion penetration was not significantly different between occlusion and higher-humidity conditions (%RH = 85 and 90), but occlusive parathion penetration was lower than under the fully hydrated condition ($P < 0.05$). These effects are best illustrated in Fig. 1.

Table I. Humidity Effect on Passive Percutaneous Absorption of Parathion *in Vitro* (μg/cm^2/8 hr)[a]

	Parathion		
	4 μg/cm^2	40 μg/cm^2	400 μg/cm^2
%RH			
60	7.69	1.91	0.46
	(0.31)[b]	(0.76)	(1.84)
20	7.48	1.51	0.45
	(0.30)	(0.60)	(1.80)
70	9.91	2.07	0.46
	(0.36)	(1.08)	(1.84)
80	8.91	2.86**	0.68**
	(0.36)	(1.14)	(2.72)
85	12.89*	3.77*	1.03*
	(0.52)	(1.51)	(4.12)
90	16.91*	5.25*	1.18*
	(0.68)	(2.10)	(4.72)
100	27.43*	7.72*	2.02*
	(1.10)	(3.10)	(8.08)
Occlusion	17.46*	3.03*	1.04*
	(0.70)	(1.21)	(4.16)

[a] n = 4–5/dose–treatment combination.
[b] In parentheses, % of applied dose/8 hr.
* Significantly different from RH = 60% condition ($P < 0.05$).
** Significantly different from RH = 60% condition ($P < 0.10$).

DISCUSSION

Previous results from our laboratory (1) demonstrated that increasing humidity was the most consistent factor in increasing parathion percutaneous absorption, compared to other parameters investigated (i.e., air temperature, perfusate temperature, perfusate flow rate, and perfusate composition). The present study clear demonstrates a humidity threshold above which absorption significantly increases.

Wurster and Kramer's (2) data showed a ninefold hydration effect for ethylene glycol salicylate, increasing two- to threefold for more hydrophobic methyl and ethyl salicylate esters *in vivo*. Behl *et al.* (3) also indicated that prolonged immersion of hairless mouse skin in saline was accompanied by alteration in permeability of butanol hexanol and heptanol but not methanol or ethanol. In the present hydration studies, parathion penetration increased about four times over the value under standard conditions (%RH = 60) at all three doses, which agrees with previous investigators. Also, in the complete hydration situation (%RH = 100), since the amount of parathion applied may be considered to be an infinite dose, the relatively small amount that was absorbed during each hour interval may not significantly change the surface concentration.

The physically hydrated and softened stratum corneum and the thermal gradients between the skin surfaces would be expected to be greater in a saline immersion than in an air exposure at the same temperature because of the higher heat conductivity of water. These factors, in addition to fully hydrating stratum corneum, may also increase parathion thermodynamic activity, solubility, and skin partition coefficient, all of which would further increase penetration. Hydration effects on chemical absorption may be seen with swimming, bathing, or other immersion conditions. This contact of skin with water may not represent normal conditions, and most hydration conditions may dilute or wash out chemicals from the skin surface. Therefore, the relationship among the effects of hydration of the stratum corneum, the magnitude of penetration, and the toxic potential needs more careful examination.

Under normal conditions the stratum corneum will receive moisture from the lower layers of the skin and from the sweat glands. The stratum corneum also may receive water from the environment, as well as losing water to the environment by evaporation into the surrounding atmosphere. The rate of water loss from the skin surface can be much more rapid than that which reaches the stratum corneum. Therefore, in a certain period, environmental factors may be a more important mechanism for the control of the water content of the stratum corneum that diffusion from the underlying tissues (8–10). The most important effect of higher humidity is apparently to increase the rate of hydration of the stratum corneum layers and change the absorption of water into the intercellular lipid domains. Hydration may facilitate partitioning of the penetrating compound from the stratum corneum to the viable epidermis (11), thus increasing penetration.

In the present studies, a relative humidity higher than 85% significantly increased absorption at all doses ($P < 0.05$). A relative humidity of 80% tended to increase parathion penetration at 40- and 400-μg doses ($P < 0.10$). No

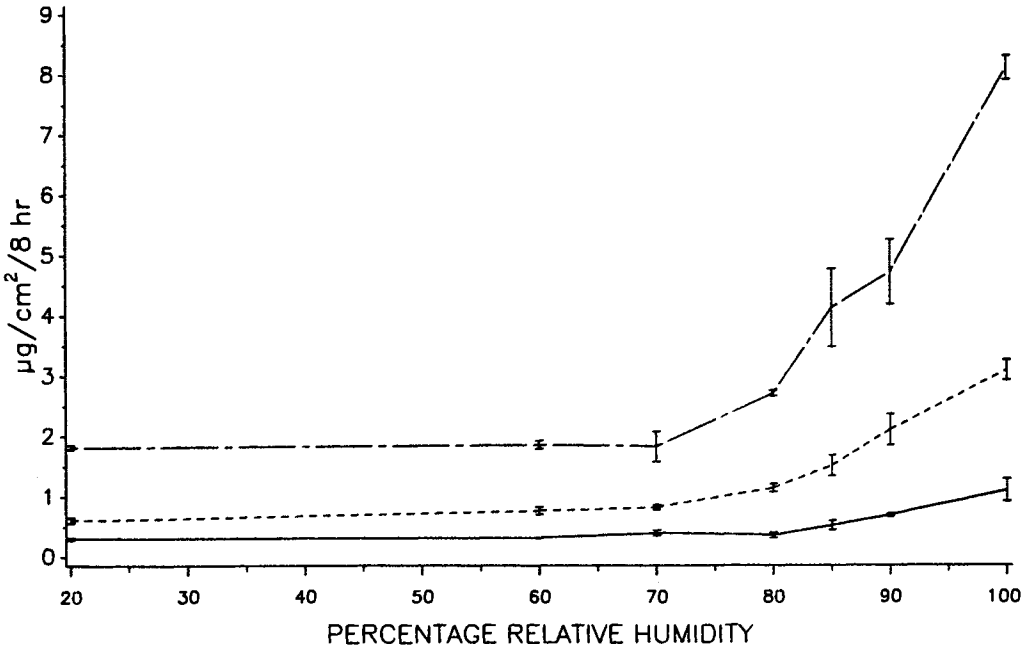

Fig. 1. Humidity effect on passive percutaneous absorption of parathion *in vitro*. Dose: (——) 4, (----) 40, and (- —) 400 μg/cm².

effect was seen when the relative humidity was from 20 to 70%. These results suggest that a very high humidity significantly increases parathion percutaneous absorption, with a threshold occurring at about 80% relative humidity. Furthermore, this absorption profile of parathion at different relative humidities (Fig. 1) is consistent with the reported stratum corneum water content at different environmental humidities (8,9) and confirms the previous theory that the water content of the stratum corneum correlates with penetration. Although most investigators assume that this hydration change affects only polar compounds, at higher humidities, the penetration of nonpolar compounds such as parathion also increases. This result suggest that the hydrated condition of the skin may facilitate the penetration of nonpolar compounds by other mechanisms. Alternatively, the humidity may change the vapor pressure of parathion, thereby modulating its absorption.

One of the most widely used methods to increase percutaneous absorption of topically applied therapeutic agents is the use of occlusive plastic films. With this technique, absorption of some substances can be increased by 2- to 10-fold (5,12,13), in addition to forming a long-lasting reservoir within the stratum corneum. When the stratum corneum becomes hydrated under an occlusive condition, the tissue changes from one containing little water (5–15%) to one with as much as 50% water (14), therefore modifying the absorption profile. Also, occlusion may increase the surface temperature of skin, may cause vasodilation or irritation *in vivo*, and can decrease evaporation of the applied chemical and/or its solvent, thus increasing absorption.

In our studies, parathion penetration showed no significant differences between occlusion and higher humidity (%RH = 85 and 90), but parathion penetration with occlusion was lower than with full hydration ($P < 0.05$). Since we postulated that changes in skin permeability occurred through altering the relative humidity, this result implies that the humidity in occlusion was similar to 85 to 90% humidity but lower than 100%, suggesting that the primary effect of occlusion on enhancing penetration is due to its local effect of increasing humidity under the patch. Fritsch and Stoughton (12) noted a similar observation with acetylsalicylic acid. Also, in the field, at higher air temperatures, there is more sweating and hence a higher relative humidity under clothing. Since organophosphate insecticides cannot be readily removed from contaminated clothing, this combination could increase the potential of dermal toxicity from parathion.

The ultimate goal of an *in vitro* transdermal penetration study is to understand and predict the absorption of a xenobiotic from the skin surface into the systemic circulation of a living animal. Because of the significant threshold effect observed, quantitative humidity data are necessary for construction of theoretical absorption models. The test conditions must reflect realistic conditions of the environment. Thus, in order to evaluate the health hazard of workers exposed to pesticides, the influence of relative humidity conditions found in diffusion-cell work needs to be taken into account in designing percutaneous absorption experiments and in making exposure and risk assessments.

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