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SKIN PENETRATION AND LAG TIMES OF NEAT AND AQUEOUS DIETHYL PHTHALATE, 1,2-DICHLOROETHANE AND NAPHTHALENE

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Cutaneous exposures to occupational chemicals may cause toxic effects. For any chemical, the potential for systemic toxicity from dermal exposure depends on its ability to penetrate the skin. Most laboratory studies measure chemical penetration from an aqueous solution through isolated human or laboratory animal skin, although most exposures are not from pure aqueous solutions. The US EPA Interagency Testing Committee (ITC) mandated by the Toxic Substances Control Act, has required industry to measure the in vitro penetration of 34 chemicals in their pure or neat form (if liquid). The goal of the present study was to measure skin permeability and lag time for three neat chemicals of industrial importance, representing the general types of chemicals to be studied by the ITC (nonvolatile liquids, volatile liquids, and solids), and to examine interlaboratory variation from these studies. Steady state fluxes and lag times of diethyl phthalate (DEP, slightly volatile), 1,2-dichloroethane (DCE, highly volatile), and naphthalene (NAP, solid) were studied in two different laboratories using different analytical methods. One lab also measured fluxes and lag times from saturated aqueous vehicle. Static diffusion cells, dermatomed hairless guinea pig skin, and gas chromatography were used to measure skin penetration. In the two laboratories, the steady state fluxes (mean $\pm$ SD; $\mu g\ cm^{-2} hour^{-1}$) of DEP applied neat were: 11.8 ± 4.1 and 23.9 ± 7.0; fluxes of DCE (neat) were 6280 ± 1380 and 3842 ± 712; fluxes of NAP from powder were 30.4 ± 2.0 and 7.5 ± 4.7. Compared with neat fluxes measured in the same laboratory, flux from saturated aqueous solution was higher with DEP (1.9×) but lower with DCE (0.17×) and NAP (0.45×). The three chemicals studied including a dry powder, demonstrate the potential for significant dermal penetration.

Keywords: Diffusion; Hairless guinea pig; Maximum flux; Permeability; Skin absorption

INTRODUCTION

Dermal exposures to toxic chemicals may cause adverse local and systemic effects. The most common measurement of a chemical's capacity to penetrate the skin from a particular vehicle is the permeability coefficient. Particularly in industrial

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settings, many dermal exposures are to neat chemicals. However, most skin permeability measurements have been made with the chemical of interest dissolved in an aqueous vehicle. These have been compiled in several extensive databases of permeability coefficients (1–3). While skin absorption rates of the neat chemical form have been reported, for example, for primary alcohols (4) glycol ethers (5,6), polycyclic aromatic hydrocarbons (7) and commercial solvents (8), no extensive data sets exist for permeability measurements from neat chemicals.

From thermodynamic principles, it is expected that the steady-state flux from a saturated aqueous solution and from a neat liquid should be identical if there are no alterations of the barrier properties of the skin (9–11). Thus, the neat chemical skin permeability would be given by the aqueous vehicle permeability times the ratio of aqueous solubility (S_w) to density (12):

$$neat\ K_p = aqueous\ K_p \times \frac{S_w}{Density} \tag{1}$$

However, owing to the significant potential for barrier alteration (including corneocyte swelling and lipid bilayer disruption by water; delipidization and corrosion by solvents and corrosive substances), extrapolation of permeability from aqueous solutions to the pure chemical requires a great deal of caution. For example, Barry et al. (11) showed that the flux ratios for 4 of 5 organic chemicals tested were 2.5 to 7 times greater with the pure liquid than with the saturated aqueous solution. If neat chemical and saturated aqueous fluxes are not similar, then the use of Eq. (1) is not justified.

Recently, the US Environmental Protection Agency has finalized requirements for in vitro skin absorption rate testing of certain industrial chemicals (12). Under the Toxic Substances Control Act, the EPA has been empowered to require this testing by manufacturers and processors of 34 chemicals that were selected based on high-volume production and a paucity of dermal absorption data. Liquid chemicals are to be tested in their neat form, while ambient-temperature solids are to be dissolved in water or isopropyl myristate. The measurements will be made in a variety of different laboratories following a detailed published protocol (13). The Occupational Safety and Health Administration will ostensibly use the data to evaluate the need for "skin designations" for these chemicals.

Two recent studies examined the variability in the measurement of in vitro permeation parameters among different laboratories. Chilcott et al. (14) attempted to eliminate the sources of variability rising from the use of skin by comparing the measurement using a standardized procedure of the permeation of one compound through silicone membranes. The coefficient of variance (CV, SD/mean) among the 18 participating laboratories was 35%. Van de Sandt et al. (15) compared in vitro skin absorption predictions of 3 compounds measured in 10 participating laboratories following a detailed protocol. The CV was higher, ranging from 64% to 119%.

In this study, we measured skin absorption rates and lag times for three neat chemicals representative of volatile liquids, non-volatile liquids, and solids (Table 1). Two of the chemicals (1,2-dichloroethane and naphthalene) are among

Table 1 Some properties of studied chemicals

Chemical	Abbreviation	CAS#	ρ	MW	Log K_{ow}	S_w	S_b (HFF)	S_b (JNM)
Diethyl phthalate	DEP	84-66-2	1.118	222.2	2.47	1,000	825	920
Naphthalene	NAP	91-20-3	1.162	128.2	3.30	30	24	4.8
1,2-Dichloroethane	DCE	107-06-2	1.235	99.0	1.48	8,600	5,347	87,000

CAS#: Chemical Abstract Society reference number; μ: density (g/cm^3); MW: molecular weight; log K_{ow}: base 10 logarithm of the octanol-water partition coefficient; S_w: water solubility ($\mu g/mL$ at 20–25°C). Data derived from different sources and summarized in Hazardous Substances Data Bank (http://toxnet.nlm.nih.gov). S_b: measured solubility in buffer ($\mu g/ML$) at 22°C (HFF), 20°C (JNM).

the 34 chemicals designated by the EPA for dermal absorption testing, and one (diethyl phthalate) has been removed from the originally designated list. To address questions of inter-laboratory validation, measurements were independently made in two laboratories using the same chemicals. While both labs used standard in vitro techniques, they were allowed to develop their own methods based on individual preferences and equipment. The justification was that this process reflects reality in that permeability databases have been collected retrospectively from disparate labs employing a range of techniques. Hairless guinea pig skin was used as a conservative surrogate for human skin and because the skin could be reliably obtained from the same source. In one lab, comparisons were made between saturated aqueous solutions and the neat chemicals.

Methods

Parallel experiments were independently performed in two laboratories under the supervision of HFF and JNM. Information and discussions regarding procedures were shared, but both labs were free to develop methods based on available equipment and individual preferences. Results were not shared until all experiments and analyses were concluded.

Chemicals and materials

HFF. $NaHCO_3$, gentamicin sulfate, Hepes, 1,2-dichloroethane 99.8% (DCE), diethyl phthalate 99.5% (DEP), naphthalene (99.7%) (NAP) and HPLC grade methanol (99.9%), were purchased from Sigma-Aldrich (St. Louis, MO). NAP in methanol standard (5,000 mg/mL) and SPME fibers were purchased from Supelco (Sigma-Aldrich). Hanks's Balanced Salt Solution (HBSS) was purchased from Gibco-Invitrogen Corporation (Carlsbad, CA). HPLC grade water, used for all aqueous solutions, was purchased from Fisher Scientific (Fairlawn, NJ).

JNM. DCE (99.5%), DEP (99.5%), and NAP (99%) were obtained from Sigma-Aldrich. Hexane (95%) was obtained from EM Science. HBSS was obtained from Cambrex (East Rutherford, NJ). A 4 L SKC quality sample bag was obtained from Sigma-Aldrich (St. Louis, MO).

Buffer

HFF. The buffer was composed of 5.96 g of Hepes, 0.32 g of $NaHCO_3$, and 50 mg of gentamicin sulfate added to 1000 mL of HBSS. The solution was brought to pH to 7.4 at 32°C by titration with NaOH. The buffer was degassed by heating to 40°C and stirring under laboratory vacuum for ~15 minutes.

JNM. Hanks' Balanced Salt Solution (HBSS modified, JRH Biosciences, Lenexa KS) was used for buffer. HBSS was degassed by stirring over night under lab vacuum.

Calibration standards and saturated solutions

HFF. For each chemical, calibration solutions and aqueous solutions were made fresh prior to each experiment. For DEP and DCE, saturated solutions were made by adding an excess of the chemical to buffer, vortexing for ≈24 hours at room temperature, then centrifuging at 4,000 rpm for 30 minutes. For NAP, crystals of NAP were added to buffer and vortexed for ≈24 hours at room temperature. Care was taken to pipette saturated buffer containing no visible crystals.

DEP stock solution of 100 mg in 100 mL methanol was made and stored at 4°C. Calibration standards were made by serial dilution in buffer.

DCE calibration solutions were made by adding 20 μL of DCE into16 mL buffer in a zero-head space vial, which had been tared on an analytic balance. The vial was capped and the weight of DCE was recorded. Serial dilutions were made from this solution.

NAP calibration solutions were made by serial dilution of NAP standard in buffer. NAP crystals were ground with a mortar and pestle, then sieved through a 100-mesh sieve and stored in a desiccator. Sieved NAP was used as donor for the neat NAP experiments. Due to the difficulty in making saturated NAP solution (the solid did not sediment with centrifugation); diluting NAP made aqueous NAP donor solution was made by diluting NAP with buffer (25 μg/mL).

JNM. DEP standards were made fresh each day by serial dilution of DEP in hexane.

DCE standard curves were generated each day using a 4 L SKC quality sample bag. The bag was used after cleaning 10 times using air/vacuum pump to make sure no trace of any chemical remained from previous use. A calculated volume of air was pushed in the bag and measured by Wet Test Gas Meter attached to a compressed air source. A calculated volume of the chemical tested was injected in the bag, which was heated using a hair dryer, until the entire chemical was evaporated. The bag was then set-aside for 30 minutes so the concentration of the chemical could equilibrate. Ten μL HBSS were pipetted in each of 5 empty headspace vials. The vials were capped and injected with 5 different known volumes of the bag air, one in each of the vials after removing the same volume of the vial's air using a gas-tight syringe (the syringe was cleaned by heat and vacuum before being used).

NAP was ground under the hood using a coffee grinder, and then sifted through 80 mesh metal sieve. Fresh standard curves were generated each day from serial dilutions of NAP in absolute ethanol.

Hairless Guinea pig skin

Male hairless guinea pigs (HGP) of the strain Crl:IAF(HA)-hrBr were obtained from Charles River Laboratories (Wilmington, MA). All studies proceeded under approved institutional animal use protocols.

HFF. HGP's (500–670 g) were euthanized with CO_2 on the day of the experiment. Dorsal skin was removed and underlying muscle and fat was dissected free. The skin was dermatomed at 315 μm setting using a Padgett Dermatome Model B (Integra Lifesciences Corp. Plainsboro, NJ). Skin disks (3/4'') were cut using a stainless steel punch, weighed, and placed on the warmed (37°C) diffusion cell receptor chambers which were pre-filled with buffer.

JNM. HGP's (350–650 g) were sacrificed by CO_2 asphyxiation. A cardboard circle the diameter of the outside edge of the diffusion cell was used as a template to mark a circle on the midscapular area of the guinea pigs back with a waterproof marker, (16,17). The skin from the back was gently excised with scissors and blunt dissection. The skin was pinned stratum corneum side up, on a 5×30 centimeters oak board and dermatomed to 350 μm with a Padgett Dermatome Model B. The skin was trimmed with scissors to match the size of the circular mark, weighed and placed on the diffusion cell receptor chamber that was previously filled with buffer and prewarmed.

Diffusion cells

HFF. Franz static water-jacketed cells with 5 mL receptor volume, 9 millimeter diameter opening (0.636 centimeter^2 skin exposure area) (Permegear, Bethlehem, PA) were used. The temperature in the receptor compartments was maintained at 37°C using a recirculating bath, resulting in donor compartment temperatures of 32°C. Receptor compartments were stirred at ~1000 rpm.

JNM. Jacketed Franz static cells with 8 mL receptor volume and 11.28 mm diameter opening (1 cm^2 skin exposure area) (Permegear) were used. Temperature in the receptor compartments was maintained at 38°C using a recirculating bath, which kept skin surface temperature at 32°C. Receptor compartments were stirred at ~800 rpm.

Diffusion cell experiments

HFF. From each HGP ($n = 8$ per chemical), 6 pre-weighed skin discs were mounted onto the diffusion cells, which were pre-filled with buffer and warmed. Three cells were dosed with neat chemical, and three cells were dosed with saturated solutions in buffer.

Before adding the donor and then at specified time points, samples were removed from the receptor compartments and replaced with the same volume of fresh buffer. Gas-tight Hamilton syringes fitted to Chaney adaptors (Hamilton Company, Reno NV) were used to remove samples and to replenish buffer. For DEP and DCE, 500 μL of neat or saturated chemical were placed in donor compartments which were then sealed with parafilm. For NAP, sufficient powder to cover the skin surface, or 500 μL of saturated NAP, was placed in the donor compartments, which

were then sealed. Aqueous donor solutions were replaced every hour (DEP and NAP) or 15 minutes (DCE).

The sequences of sampling were as follows. For DEP and NAP, samples (0.5–1.5 mL) were removed from the receptor at 0, 0.5, 1, 2, 3, 4, and 5 hours and placed in 2 mL autosampler vials. For DCE, samples (100 μL) were removed at 0, 10, 20, 30, 45, 60, and 90 minutes and placed in 10 mL headspace autosampler vials.

JNM. Receptor compartments were filled with 8 mL HBSS. Six dermatomed guinea pig skin circles (two from each guinea pig) were placed on the six-receptor compartments. One milliliter of the liquid chemicals or 500 mg of NAP were placed on five of the skin/membrane surfaces and sealed with a glass stopper. Ten to twenty microliter samples of the receptor solution were removed at 15–30 minutes intervals, for up to 4–6 hours (depending on rate of penetration). Receptor solution (500 μL) was replaced with fresh HBSS only in the diethyl phthalate experiment using a gas-tight syringe. For all chemicals the flux experiment was repeated on at least 2 different days.

The solubility of each chemical in HBSS was determined to assure that was not a limiting factor in the flux experiment.

Sample Preparation

HFF. Automated solid-phase microextraction (SPME) was used to prepare both DEP and NAP aqueous samples for GC analysis. Details have been presented elsewhere (18). Briefly, 85 μm polyacrylate fibers (Supelco) were used for both analytes. Automation was implemented with a CombiPal autosampler (CTC Analytics, Zwingen, Switzerland). Direct immersion of the fiber in the sample was used. For DEP, extraction time was 45 minutes, at 40°C with agitation (250 rpm). For NAP, extraction time was 30 minutes at 40°C with 250 rpm agitation.

For DCE, automated static headspace sampling was implemented with the CombiPal. The sample was incubated at 40°C for 5 minutes with agitation (250 rpm). 100 μL of the headspace gas was then directly injected into the GC (split ratio 10) using a 1 mL headspace syringe that was preheated to 42°C.

JMN. Automated headspace sampling of DCE and NAP were implemented using an Agilent 7694 headspace sampler. The headspace conditions were chosen differently for each chemical while developing each method and are summarized in Table 2.

DEP was analyzed using automated liquid injection (Agilent 7683) following liquid-liquid extraction. One milliliter of hexane was added to each vial to extract DEP from the 0.5 mL receptor sample. The vials were shaken for 30 minutes, and then allowed to settle for 15 minutes. Five hundred μL of the upper hexane layer was removed into a different vial and subjected to analysis by direct automatic injection of 0.2 μL onto the GC.

Instrumentation

HFF. Chemical concentrations quantified by gas chromatographic (GC) analysis using a Varian CP-3800 gas chromatograph with electronic flow control (Varian Inc., Walnut Creek, CA). The GC was equipped with a Combipal autosampler (CTC Analytics, Zwingen, Switzerland). The column was 5% diphenyl, 95% dimethyl

Table 2 Headspace conditions for chemical analysis (JNM)

	Chemical	
Parameter	DCE	NAP
Oven temperature	100	80
Loop temperature	110	95
Transfer line temperature	115	110
Loop volume	1	1
GC cycle time	15	9
Vial equilibration time	5	2
Pressurization time	0.1	0.1
Loop fill time	0.5	0.5
Loop equilibration time	0	0
Injection time	1	0.75

Parameters are for Agilent 7694 headspace sampler. Temperature units are °C; volumes are mL, and times are minutes.

polysiloxane, 30 m long, 0.25 mm inner diameter, with 0.25 μm film thickness (Restek, Bellefonte, PA). A model 1177 split-splitless injector and flame ionization detector (FID) was used for DEP and NAP. Nitrogen was used as both carrier and make-up gas at constant flow rates of 1 mL/minute and 25 mL/minute respectively. The split ratio was programmed: initial split ratio, 10; at 0.01 minute, splitless; at 5 minutes, split ratio 100; at 10 minutes, split ratio 10. For DEP the injector was set at 270°C isothermal and the detector at 300°C. The oven was programmed from 60°C with 5 minutes hold time, then ramped to 280°C at 15°C/minute, and held for 5 minutes (Total GC time = 24.7 minutes). For NAP the injector was set at 270°C isothermal and the detector at 300°C. The oven was programmed from 60°C with 5 minutes hold time, then ramped to 180 at 15°C/minute, then ramped to 250 at 30°C/minutes (Total GC time = 18.3 minutes).

A model 1079 injector and electron capture detector was used for DCE. Split ratio was set to 10. The detector temperature was 250°C. The oven was programmed from 30°C with 1 minutes hold time, then ramped to 60 at 8°C/minute, then ramped to 150 at 50°C/minute (Total GC time = 6.55 minutes).

JNM. Gas chromatographic analytical methods were developed and optimized for each chemical. An Agilent 6890 N gas chromatograph equipped with a 7694-headspace sampler or a 7683 automated liquid sampler was used. The column used for all three chemicals was an HP-5MS (Agilent), nominal length of 30 m, nominal diameter of 250 μm and nominal film thickness of 0.25 μm. A constant flow mode was used, with initial flow of 1.0 mL/minute, nominal initial pressure of 9.38 psi, and an average velocity of 37 cm/sec. Detection was accomplished with a 5973 quadrupole mass spectrometer in scan mode or with a G2397A micro electron capture detector (DCE).

Data Analysis

HFF. For each HGP, mass accumulation results for all skin discs were averaged for each treatment. Permeability coefficients (k_p) and lag times (τ) were

calculated by nonlinear regression (SigmaPlot 2004, SPSS) of the following equation (19) through these averages (18):

$$Q(t) = k_p C t - k_p C \tau - \frac{12 k_p C \tau}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left(\frac{-n^2 \pi^2 t}{6\tau}\right) \quad (2)$$

Here, $Q(t)$ is the cumulative amount of chemical penetrated per unit area of skin at time t; C is the donor concentration. There are 2 unknowns to be estimated: k_p and τ. The equation was truncated to 7 terms in the series. For neat liquid chemicals, C was taken to be the density of the chemical. Maximum steady-state flux was calculated by multiplying k_p by C for neat chemicals, or by the saturation concentration for aqueous donor. Only those experiments for which the coefficient of determination exceeded 0.99 were included in the results.

Because the contact of a solid chemical with the skin surface at a micro level is unknown, the donor concentration of solids is not well defined and the calculation of a k_p is not valid. (20,21). Therefore, for naphthalene, we report only flux values and lag times.

JNM. Fluxes were estimated from the slopes of the cumulative absorption plots over time. Lag times were estimated as the intercept of the steady state portion of the flux curves with the time axis. Individual fluxes were calculated from each diffusion cell and the average and standard deviation were calculated for the group. The permeability coefficient was estimated by dividing the flux by the density of chemical. Graphing and statistical analysis were done using Prism (GraphPad Prism 4).

RESULTS

Figure 1 displays mass accumulation curves measured in both labs of the three chemicals in their pure form and from aqueous donors. Shown are individual experimental values for each sample time point (open circles) from each animal, and the means and standard deviations of the experiments (slightly offset in time for clarity). The continuous line is a best-fit regression of Equation (2) with the mean data.

Results from both labs are summarized in Table 3. Skin permeability coefficients, maximum steady-state fluxes, and lag times are given for both neat chemical and aqueous solution donors.

Maximum steady-state fluxes for the three chemicals spanned an approximately 500-fold range in both labs, however the order from lowest to highest differed in the two labs. For HFF, the order was DEP $<$ NAP $\ll$ DCE; while for JNM, the order was NAP $<$ DEP $\ll$ DCE. Differences in measured fluxes or permeabilities between the two labs ranged from 1.6 $\times$ (DCE) to 4.0 $\times$ (NAP).

Differences in lag times measured by the 2 labs differed by <3 times for all three chemicals. Differences were found in the measurement of steady-state fluxes from the neat compounds compared with those from aqueous donor solutions. For DEP, steady-state flux from the aqueous donor was 1.9 $\times$ higher than from the neat compound. For NAP and DCE, steady-state flux from the neat compound was higher than from the aqueous (2.2 $\times$ for NAP, 5.8 $\times$ for DCE).

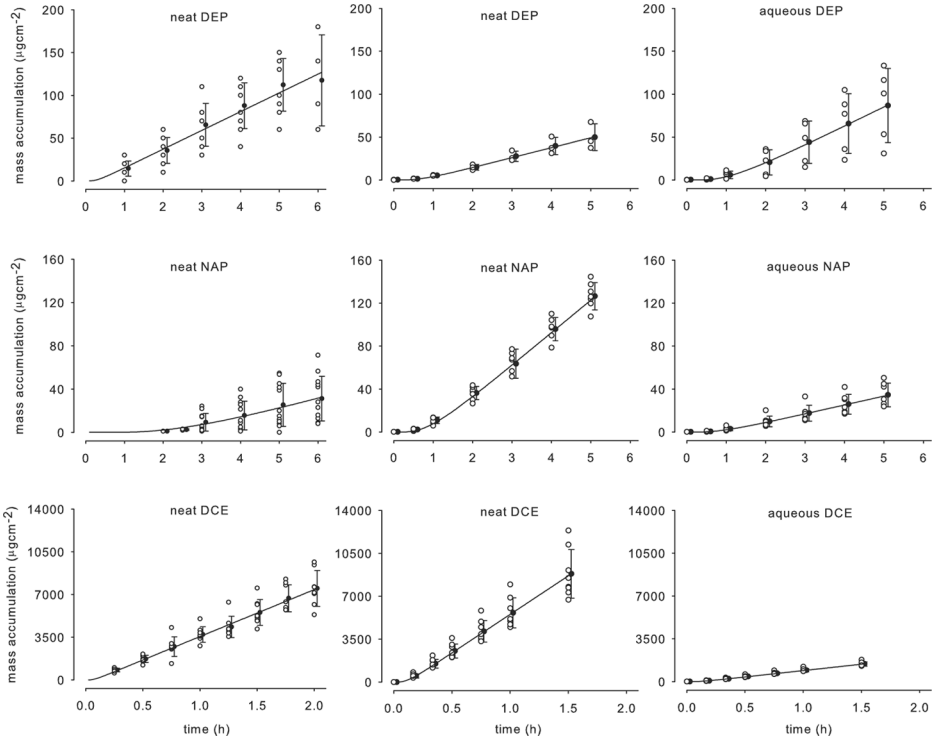

Figure 1 Mass accumulation curves for three chemicals. First column displays data from infinite dose neat chemical exposure from the JNM lab; middle column displays the same from the HFF lab; right column displays accumulation data from saturated aqueous vehicle from the HFF lab. Individual experiments are shown from both labs (open circles) along with means (closed circles) and standard deviations displaced slightly in time for clarity. Solid curves are best-fit regressions with Equation 2. DEP: diethyl phthalate; NAP: naphthalene; DCE: 1,2-dichloroethane.

There were no notable differences in lag time measurements between neat and aqueous vehicles for 2 compounds, NAP and DCE. For DEP, t_{lag} for the neat chemical was approximately 1/2 that for the aqueous solution.

Coefficients of variance (CV, SD/mean) of neat compound flux measurements varied among the chemicals and between the two labs. The CV's were: 7% (NAP), 22% (DCE), 35% (DEP) for HFF; and 19% (DCE), 29% (DEP), and 63% (NAP) for JNM. Flux measurements from aqueous donors exhibited similar ranges of variance. The highest CV of aqueous donor chemicals was exhibited by DEP (44%). Generally, lag time measurements exhibited wider variances than flux measurements (range: 26–225% for all measurements from both labs).

DISCUSSION

Dermal exposure assessments of environmental and occupational toxins rely on estimates of their potential for dermal penetration. In-vitro diffusion cell

Table 3 Results of in vitro skin penetration experiments. Reported values are means ± standard deviations for the reported numbers (n) of hairless Guinea pigs

	Neat						Aqueous		
	k_p (cm/h)		SS flux ($\mu g h^{-1} cm^{-2}$)		t_{lag} (h)		k_p (cm/h)	SS flux ($\mu g h^{-1} cm^{-2}$)	t_{lag} (h)
Chemical	HFF	JNM	HFF	JNM	HFF	JNM	HFF	HFF	HFF
DEP	$1.05 \times 10^{-5} \pm 3.71 \times 10^{-6}$ (n = 3)	$2.14 \times 10^{-5} \pm 6.28 \times 10^{-6}$ (n = 9)	11.77 ± 4.14 (n = 3)	23.92 ± 7.02 (n = 9)	0.64 ± 0.31 (n = 3)	0.41 ± 0.35 (n = 9)	$2.75 \times 10^{-2} \pm 1.12 \times 10^{-2}$ (n = 5)	22.74 ± 9.32 (n = 5)	1.28 ± 0.56 (n = 5)
NAP	–	–	30.39 ± 2.03 (n = 8)	7.52 ± 4.68 (n = 12)	0.82 ± 0.22 (n = 8)	2.02 ± 0.94 (n = 12)	$4.78 \times 10^{-1} \pm 1.49 \times 10^{-1}$ (n = 8)	13.61 ± 4.54 (n = 8)	0.97 ± 0.39 (n = 8)
DCE	$5.01 \times 10^{-3} \pm 1.10 \times 10^{-3}$ (n = 8)	$3.07 \times 10^{-3} \pm 5.69 \times 10^{-4}$ (n = 8)	6280 ± 1380 (n = 8)	3842 ± 712 (n = 8)	0.11 ± 0.04 (n = 8)	0.04 ± 0.09 (n = 8)	$2.01 \times 10^{-1} \pm 3.39 \times 10^{-2}$ (n = 8)	1076 ± 178 (n = 8)	0.11 ± 0.03 (n = 8)

k_p: skin permeability coefficient; SS flux: steady state flux; t_{lag}: lag time; DEP: diethyl phthalate; NAP: naphthalene; DCE: 1,2-dichloroethane. HFF: lab of HF Frasch; JNM: Lab of JN McDougal.

experiments are a simple means of obtaining this data. One widely recognized issue in these types of experiments is the variability arising from the measurements, both within one laboratory (22–24) and among different laboratories (14,15). The latter issue is a particularly important consideration because databases have been compiled from a variety of sources, and in light of the EPA's proposal to collect skin permeation data from different manufacturers and processors (13).

Several attempts have been made to minimize this variability through the promulgation of standardized guidelines for in vitro skin absorption studies. Examples include the Organization for Economic Co-operation and Development (OECD)'s guideline for in vitro skin absorption testing (25) and the US EPA's rule for in vitro dermal absorption rate testing (13). To address questions of variance, a group of 10 participating laboratories measured absorption rates of 3 chemicals (15). Despite following a detailed protocol in accordance with the OECD guidelines, the intra-laboratory CV ranged from 6% to 111%, while among laboratories the CV ranged from 64% to 119%. Chilcott et al. (14) eliminated the sources of variance arising from the use of skin by measuring permeability through silicone rubber membranes. Still, the inter-laboratory CV among 18 participating laboratories was 35%, with a 4-fold difference between the lowest and highest measurements. With these data in mind, the results obtained herein and reported in Table 3 are within the expected range for both intra- and inter-laboratory variability. However, it is not possible to quantitatively compare the variance from only two labs with that obtained from these multi-lab studies. It is possible that the differences between the two labs could have been reduced with attempts to standardize protocols, lab reagents, and methods. Instead it was decided at the outset to allow both labs to develop their own methods based on previous experience, preferences and available equipment. It was thought that this approach might more realistically reflect the disparate approaches of the skin permeation research community.

To our knowledge, the only published in vitro skin permeation data for these three chemicals are two studies on DEP. Scott et al. (26, 27) reported a steady-state flux of the neat chemical of 12.8 $\mu g cm^{-2} h^{-1}$ for human epidermis and 414 $\mu g cm^{-2} h^{-1}$ for rat epidermis. These compare with our measurements of 11.8 and 23.9 $\mu g cm^{-2} h^{-1}$ for hairless guinea pig. Frasch and Barbero (18) estimated a maximum steady-state flux of 16 $\mu g cm^{-2} h^{-1}$, extrapolated from an approximately half-saturated aqueous vehicle. These values are in general agreement, and indicate that hairless guinea pig skin more comparable than rat skin to human skin, at least for DEP.

Little work has been done on the comparison of neat chemical penetration compared with that from an aqueous vehicle. The maximum steady-state flux of a compound through skin will occur when the thermodynamic activity approaches one. Therefore one would expect that maximum penetration rates would be the same from the neat chemical as from the saturated aqueous solution (12). However, owing to the significant potential for vehicle effects on barrier properties, it could be a mistake to make this assumption. Some examples of vehicle effects include the dehydration effect of alcohols, delipidization by organic solvents, and resolvation from displaced bound water by such products as DMSO (28). Water itself is a well-known permeation enhancer, presumably through delamination of lipid lamellae and the resultant creation of high permeability voids.

In these experiments, the maximum measured flux of saturated aqueous DEP was $\sim 2\times$ greater than that from neat DEP (Table 3). (We consider only comparisons made within the one lab that measured both conditions in simultaneous, paired experiments.) DEP is widely used as a vehicle for fragrance and cosmetic ingredients (29) and is not likely to damage skin. We hypothesize that the increased penetration rate from saturated buffer is a result of the enhancement effects of water.

On the other hand, maximum flux of neat DCE was nearly $6\times$ greater than from the saturated aqueous buffer. DCE is a low molecular weight volatile solvent. It is possible that this compound dissolves and extracts stratum corneum lipids, thus creating low resistance diffusion pathways.

Very little work has been done on the dermal penetration of powdered solids. Despite the fact that dermal contact with solids does occur in environmental and industrial settings, the US EPA has dictated for their in vitro skin absorption rate testing of industrial chemicals (13), that solids will be dissolved in a vehicle of water or isopropyl myristate. The rationale for this decision is not given. McCarley and Bunge (30) found that the penetration rate of cyanophenol through silicone rubber membranes was almost the same from powders and from saturated aqueous solutions. More recently Romonchuk and Bunge (31) found much smaller absorption rates of two chemicals from powder than from saturated aqueous donor. Clearly, more work needs to be done to understand these processes.

The contact of a powder with skin depends on the size and distribution of the particles. For solids with low vapor pressure, the contact area between the particle and membrane surface is where mass transfer occurs. For skin, it is also likely that solubilization occurs in the thin layer of sweat and sebum on the surface of stratum corneum. The situation is more complicated for chemicals such as naphthalene with higher vapor pressure. A localized region of high vapor near the skin also creates a driving force for diffusion. It would thus be quite difficult to predict the concentration of naphthalene at the surface of the skin. Because this concentration is unknown and likely to vary over the surface, it is not possible to specify a permeability coefficient for solid powders. Therefore, we report only steady-state fluxes for this compound. The maximum measured penetration rate of solid NAP powder was $\sim 2\times$ greater than the rate from a saturated aqueous solution. Naphthalene is a strong skin irritant (32); therefore it seems reasonable to speculate that NAP powder may corrode the skin and increase its permeation rate.

In conclusion, we have measured skin permeation variables for 3 compounds of industrial importance. All three demonstrate the potential for significant dermal penetration. This is true even for a solid chemical that is applied in powdered form. The results provide further evidence that dermal absorption measurements from a variety of labs will exhibit rather significant variance. Finally, the results illustrate some of the potential difficulties that may arise when comparing flux measurements from different vehicles.

DISCLAIMER

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

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