FUNDAMENTAL AND APPLIED TOXICOLOGY 18, 30–39 (1992)

Dermal Absorption of Dilute Aqueous Chloroform, Trichloroethylene, and Tetrachloroethylene in Hairless Guinea Pigs

KENNETH T. BOGEN, BILL W. COLSTON, JR., AND LESIA K. MACHICAO[1]

Environmental Sciences Division, Lawrence Livermore National Laboratory, University of California, Livermore, California 94550

Received April 1, 1991; accepted July 2, 1991

Dermal Absorption of Dilute Aqueous Chloroform, Trichloroethylene, and Tetrachloroethylene in Hairless Guinea Pigs. BOGEN, K. T., COLSTON, B. W., JR., AND MACHICAO, L. K. (1992). *Fundam. Appl. Toxicol.* 18, 30–39.

Percutaneous absorption was measured in female hairless guinea pigs dermally exposed for 70 min to very dilute (—10 to 100 ppb) aqueous solutions of ^{14}C-labeled chloroform (CF), trichloroethylene (TCE), or tetrachloroethylene (PCE) in an airtight glass chamber containing no headspace. Similar experiments were conducted using aqueous solutions of TCE at 100,000 ppb. Dermal uptake was estimated by comparing the rate of radiolabel loss from chamber water in systems with and without experimental animals. After each low-concentration dermal-uptake experiment, radiolabel in total urine and feces excreted postexposure was measured and expressed as a fraction of corresponding estimated dermal uptake. For each of the compounds studied, the mean value of these fractions did not differ significantly from that obtained using animals injected with a known dose of that compound, indicating that our experimental system yielded accurate dermal-uptake estimates. The mean permeability coefficients obtained range from 0.13 cm/hr (CF) to 0.37 cm/hr (PCE); those obtained using low- vs high-concentration TCE are not significantly different. The value for CF is very close to one we calculate here from recently published data on CF uptake in human volunteers dermally exposed to aqueous CF while showering with normal tap water. Our results suggest that dermal absorption may be an important route of human exposure to chlorinated volatile organic compounds in domestic water supplies. © 1992 Society of Toxicology.

In vitro methods have been used to study the penetration through human skin of a range of dilute aqueous compounds, including steroids, alcohols, phenols, glycol ethers, and pesticides (e.g., Scheuplein *et al.*, 1969; Scheuplein and Blank, 1971, 1973; Roberts *et al.*, 1977; Wester and Maibach, 1989). However, due to the limitations associated with extrapolating *in vitro* data to estimate absorbed dose for regulatory purposes, there is growing interest in *in vivo* methods as a preferred basis for human dose estimation, particularly for lipophilic compounds (Franklin *et al.*, 1989; Maibach and Wester, 1989).

The extent and kinetics of dermal uptake of volatile chlorinated organic compounds (VCOCs) have been studied in humans exposed to undiluted liquid solvents by thumb immersion (Stewart and Dodd, 1964; Hake and Stewart, 1977), and similar studies have been conducted using live shaved guinea pigs and hairless mice (Wahlberg, 1976; Kronevi *et al.*, 1979; Susten *et al.*, 1986). *In vivo* dermal absorption of aqueous solutions of the pesticide dinoseb has been measured recently using the rhesus monkey (Wester and Maibach, 1989). More recently, elevated levels of chloroform (CF) in blood have been found in people swimming in continuously chlorinated indoor pools containing water with CF at 210 to 2580 parts per billion (ppb), compared to nonswimmers; CF concentrations in water, but also in air directly over the water, were significantly correlated with CF concentrations in swimmers' blood (Aggazzotti *et al.*, 1990). In a more controlled study, Jo *et al.* (1990a,b) examined dermal absorption of dilute aqueous CF in humans by measuring CF concentration in the breath of volunteers following showers with and without rubber wet suits using normal tap water at approximately 40°C containing CF at approximately 5 to 35 ppb.

With the exception of the recent study by Jo *et al.* (1990a,b), dermal absorption of extremely dilute aqueous VCOCs in humans or animals has not been studied. However, related studies using dilute aqueous solutions of the nonchlorinated aromatic compounds toluene, styrene, xylene, ethylbenzene, and benzene have been performed (Dutkiewicz and Tyras, 1967, 1968a,b). In these studies, a volunteer's entire hand was immersed for 1 hr in a 1-liter beaker containing the test compound in dilute aqueous solution ranging from 66.5 to 600 mg/liter at 23 to 25°C; uptake was determined by measuring the difference between initial and final concentrations. Brown *et al.* (1984) estimated dermal permeability constants from the data of Dutkiewicz and Tyras (1967, 1968a) on styrene, toluene, and ethylbenzene

[1] Present address: School of Veterinary Medicine, University of California at Davis, Davis, CA 95616.

0272-0590/92 $3.00

and used these estimates to extrapolate dermal uptake of these compounds in humans bathing in aqueous concentrations as low as four orders of magnitude below those at which Dutkiewicz and Tyras made their empirical determinations of dermal permeability.

The present study was designed specifically to measure dermal uptake of very dilute aqueous ^{14}C-radiolabeled chloroform, trichloroethlyene (TCE), and tetrachloroethylene (PCE) in hairless guinea pigs dermally exposed over a majority of their surface area. These compounds are all suspected human carcinogens found in groundwater throughout the United States in the parts per billion range (U.S EPA, 1985a,c, 1986,1987; Cothern *et al.,* 1986; Bogen *et al.,* 1988). The hairless guinea pig was chosen as the animal model because guinea pig skin has been shown to provide a fair approximation of human skin for the purpose of quantifying epicutaneous permeability, particularly in comparison with that of other laboratory rodents (Scheuplein and Blank, 1971; Wester and Noonan, 1980; Wester and Maibach, 1983; Maibach and Wester, 1989). Hairless rodents, and hairless guinea pigs in particular, have been shown to have dermal characteristics closer to those of human skin than normally haired varieties (Kao et *al.,* 1988; Wade et al., 1989).

We used a specially fabricated air-tight bathing chamber containing no headspace, in which sedated animals could be vertically immersed below the shoulders for a period of approximately 1 hr. Dermal uptake was estimated by comparing the rate of radiolabel loss from chamber water in systems with and without experimental animals. The relationship between these estimates and the uptakes that actually took place during the dermal experiments was examined using data on radiolabel excretion from dermally exposed animals versus "positive-control" animals injected with known doses of the labeled compounds. For each compound studied, total radiolabel excreted in postexposure urine and feces was expressed as a fraction of either estimated dermal uptake or known injected dose. An absence of statistically significant difference between the average fractions obtained from the dermal and the positive-control studies for each compound was taken as evidence that our experimental system measured dermal uptake of that compound. Without such a finding, additional experiments involving immediate postexposure sacrifice and measurement of whole-body radiolabel might have been appropriate. However, we did obtain such findings for all three compounds studied (see Results). Furthermore, if we had taken immediate, postexposure whole-body measurements for each of the time points used in our study, they would not necessarily have reflected true uptake because, e.g., of potential postexposure volatilization of initially bound compound from skin. Therefore, we did not undertake such additional measures in this study.

As explained below (under Methods), the permeability constants we derived were presumed to be independent of aqueous concentration within our low-level concentration range of interest. To provide a reasonable demonstration of this independence, we investigated percutaneous absorption of TCE at approximately 100,000 ppb (0.01%) in water—a concentration 10^3- to 10^4-fold higher than those used in our low-concentration TCE experiments. We note that even a concentration as high as 100,000 ppb falls within the low-concentration range in which concentration independence of the permeability coefficient is expected for a compound capable (at still higher concentrations) of inducing skin damage that might result in coefficient values proportional to concentration (Roberts *et* al., 1977). Similar experiments were not conducted for CF and PCE in the present study.

METHODS

Test chemicals. The test chemicals used were: [^{14}C]CF (2.0 mCi/mmol, >98% purity, ICN Biomedicals, Inc., Irvine, CA); TCE (>99.99% purity, Burdick & Jackson Laboratories, Inc., Muskegon, MI); [1,2-^{14}C]TCE (4.1 mCi/mmol, >99% purity, Sigma Chemical Co., St. Louis, MO); and [1,2-^{14}C]PCE (2.7 mCi/mmol, >98% purity, Sigma Chemical Co.). Radiolabeled compounds were obtained as sealed ampules; each ampule was crushed into a 5-ml beaker of methanol held in ice to yield an initial, continuously refrigerated stock of labeled sample (LS) with activities of 21.1 μCi/ml (CF), 27.6 μCi/ml (TCE), and 63.7 μCi/ml (PCE).

Animals. Sixteen female Crl:IAF(HA)BR hairless (but euthymic) guinea pigs (Charles River Laboratories, Chicago, IL), 10 to 40 weeks old (~400 to 700 g), were used in a total of 36 dermal exposure (DE) and/or positive-control (PC) experiments. In no case was an animal reused before radioactivity measured in urine and feces remained at background levels (see Radiolabel measurement), which in all cases was at least 3 weeks after prior use. Animals were reused because the experimental conditions, involving very small, infrequent doses of compounds known to be fairly rapidly metabolized by rodents to mostly excreted products (U.S. EPA, 1985a,c, 1986, 1987), were not considered to significantly affect the dermal-absorption properties studied. Food and water were available ad libitum except as noted below.

Anesthesia Sedation was required to ensure that the animals used did not disturb the DE apparatus during experiments. Sterile sodium pentobarbital (PB) at 50 mg/ml (Nembutal, Abbott Laboratories, North Chicago, IL), diluted in an equal volume of sterile 0.9% NaCl in H_2O, was used to achieve deep and continuous sedation during DE-chamber preparation and aqueous exposure. The doses used were 30 mg PB/kg body wt administered intraperitoneally 30 min prior to exposure plus 15 mg/kg administered subcutaneously between the left shoulder and the back of the neck 35 min into the exposure period. In some DE experiments, increases of 5 to 20% above these target doses were necessary to achieve deep sedation. Food and water were withheld 24 hr prior to sedation. Animals recovered within 30 to 60 min after dosing, but generally remained in light sleep for an additional 30 to 120 min. The relatively low dose levels and the ≤3-week interdose period used were selected to minimize the recovery time after sedation, while minimizing the chance for PB-induced hepatic metabolism in light of the relatively small and nonchronic nature of the PB dose used (see Conney, 1967).

Dermal exposure dumber. A glass dermal exposure chamber (DEC), illustrated in Fig. 1, was fabricated (height, 15.0 cm; inner diameter, 8.4 cm), allowing easy attachment of a water- and air-tight diaphragm by means of a flexible rubber O-ring. The diaphragms used (one per experiment) consisted of a 12-cm square of latex rubber sheeting (thickness, 0.64 ± 0.08 mm; McMaster–Carr, Los Angeles, CA) into the center of which was cut a circular hole (diameter, 2.9 cm). The diaphragms were backed by strips of aluminum/polyester adhesive tape (8.9-μm aluminum foil attached to 51-μm polyester film backed with 38-μm pressure-sensitive acrylic adhesive;

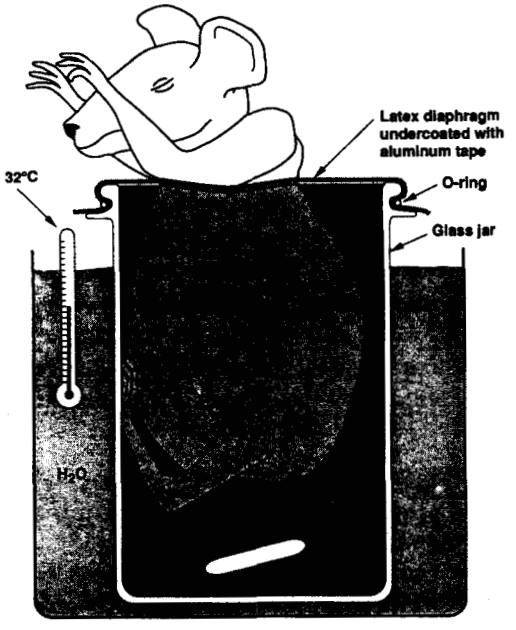

FIG. 1. Dermal exposure chamber for guinea pigs (capacity: 0.86 liter with no animal present). The sedated animal is placed through a precut hole in the latex diaphragm, which is then undercoated with aluminum-faced tape. Once attached to the chamber, the diaphragm maintains an air- and water-tight seal around the animal allowing aqueous dermal exposure with no airspace.

stock No. A-25, Lamart Corp., Clifton, NJ), one to two layers deep, affixed to the circular area of latex to be enclosed by the O-ring. This formed an aluminum tape undercoating (approximately 9 cm in diameter) with a central circular hole which, when stretched, fit snugly around the guinea pig chest yet allowed regular breathing.

Dermal exposure experiments. A total of 16 "low-concentration" DE experiments were conducted using animals dermally exposed to dilute aqueous concentrations ranging from approximately 20 to 110 ppb CF, TCE, and PCE (hereafter referred to as Experiments CF:D1–D6, TCE:D1–D5, and PCE:D1–D5, respectively). Five additional "high-concentration" experiments (hereafter referred to as Experiments TCE:D6–D10) involved dermal exposure to 100,000 ppb aqueous TCE. Prior to each DE experiment, from 40 to 100 μl LS was diluted in 1.0 ml doubledistilled water (DDW); this "input solution" (IS) was injected into the DEC prior to each experimental exposure.

All DEC and diaphragm materials and the stir bar were weighed dry prior to each experiment. In DE experiments, the latex diaphragm was placed around the upper chest of the animal just below the front legs and shoulders, with the edge of the latex curving upward at the point of dermal contact. Sections of aluminum tape were then attached to the underside of the diaphragm as close to the chest of the animal as possible, exposing the minimum possible latex surface area to the fluid placed subsequently into the DEC. A teflon-coated magnetic stir bar was placed into the DEC, which was then filled to two-thirds capacity with DDW (or, in Experiments TCE:D6–10, with a freshly prepared 100,000-ppb solution of TCE in DDW), preheated to 32°C, and placed under a hood. The animal was then quickly lowered into the DEC, the diaphragm was affixed to the chamber with one or two rubber O-rings, and any residual air bubble in the DEC was removed by syringe through the diaphragm. The DEC was weighed at this time to allow the determination of its initial fluid capacity, V. Chamber water was continuously stirred and maintained at an average temperature of 32°C (±2°C range) throughout each test period.

Prior to the beginning of a test period (hereafter referred to as t_0), samples were taken of DDW (0.5 ml, to serve as "blank controls"), of the fluid contained in the sealed DEC (0.5 ml, through the diaphragm to serve as "chamber controls"), and of IS (20 μl in Experiments TCE:D1–D5 and corresponding control experiments, and 50 to 100 μl in all other DE and corresponding control experiments). At t_0, 0.5 ml of IS was injected by syringe into the DEC through the diaphragm, so that at t_0 the DEC fluid volume, V_0, was approximately equal to V. Samples of DEC fluid were then withdrawn by syringe through a distant location in the diaphragm at 10, 15, 20, 30, 40, 50, 60, and 70 min after t_0. (Hereafter, t_n shall denote n min after t_0.) All transdiaphragm samples were taken using a 500-μl gas-sampling syringe with a teflon-tipped plunger (Series C; Dynatech Precision Sampling Corp., Baton Rouge, LA), which was always triple rinsed with DDW prior to each use. A separate 500-μl syringe was used for injecting IS into the DEC at t_0. After the last fluid sample was taken at t_0 + 70 min, the guinea pig was removed from the DEC, immediately patted completely *dry* using tissue paper, and then placed in a metabolism *cage* subject to negative *air* flow (to ensure that the animals breathed air practically free of radiolabel). The total exposure duration, T_e (min), in each DE experiment was actually 72 min (or 75 and 85 min in TCE:D2 and TCE:D1, respectively) because of the time required to complete the final DEC-fluid sample and the removal of the animal from the DEC. A single DEC was used for all experiments. After each experiment, the DEC was rinsed with methanol, triple rinsed with DDW, and air dried (resulting in background radioactivity levels upon subsequent sampling—see Radiolabel measurement).

Chamber control experiments. A total of 20 chamber-control (CC) experiments corresponding to the low- and high-concentration experiments with CF, TCE, and PCE were conducted. These CC experiments will hereafter be referred to as Experiments CF:C1–C5 (a set of CC experiments corresponding to CF:D1–D6), TCE:C1–C5 (corresponding to TCE:D1–D5), TCE:C6–C10 (corresponding to TCE:D6–D10), and PCE:C1–C5 (corresponding to PCE:D1–D5). In all CC experiments, in place of an animal in the DEC, a glass beaker 4 to 5 cm in diameter was inserted into the diaphragm hole to a depth of approximately 0.5 cm (the minimum depth required to attain a secure seal). Thus, in CC experiments the animal surface area was replaced by 60 to 65 cm^2 of glass surface similar to that of the DEC. All CC-experiment durations were extended to 190 min (except TCE:C1–C3, which lasted 70 min) to enable the detection of potentially significant loss rates, but all other procedures used (i.e., sampling, temperature maintenance, etc.) were identical to the corresponding DE procedures described above.

Positive control experiments. A total of 15 PC experiments (Experiments CF:P1–P5, TCE:P1–P5, and PCE:P1–P5) were conducted using very small doses of the test compounds. In all PC experiments, animals were sedated twice as in the DEC experiments and, within 15 min of the second sedative administration, were administered LS dissolved in corn oil (0.5 ml total volume) as a single subcutaneous (sc) dose in the animal's midback region slightly to the right of center [except in Experiments TCE:P1–P2, in which a single intramuscular (im) dose dissolved in methanol (0.2 ml total volume) in the back of animal's left thigh was used]. In each of the PC experiments, an estimate of injected dose was obtained from separate samples of the dosing solution used, taken prior and subsequent to animal injection.

Collection of urine and feces. All urine and feces produced subsequent to exposure in all PC and low-concentration DE experiments were collected for 2 to 4 weeks; samples were obtained for analysis at approximately 3, 15, and 24 hr postexposure, and thereafter approximately five times per week, until measured activity did not differ significantly from background, at which time *cage* rinses were performed. Total urine volumes and fecal output mass were recorded for each animal during postexposure observation.

Radiolabel measurement. All samples taken of "blank" DDW, IS, t_0 chambercontrol fluid, DEC fluid, urine (two 1-ml volumes/sample), diaphragm material (six 1-cm^2 sections each of latex and of aluminum tape per experiment), and PC-dosing vehicle and vehicle + dose were placed directly in 15 ml of Universol (ICN Biomedicals, Inc.) liquid scintillation cocktail for quantification of ^{14}C in counts per minute (cpm) in a Tri-Carb

4530 scintillation counter (Packard Instrument Co., Downers Grove, IL). All radiolabel measures in cpm were converted to equivalent disintegrations per minute (dpm) using a calibration curve for ^{14}C, based on external standards supplied by Packard Instrument Co., that was applied to all scintillation measures. Counting efficiencies (100% x dpm/cpm) for all samples of DDW or DEC water, urine, and feces/methanol (see below) had ranges of 93–94, 85–91, and 80–85%, respectively. Net dpm (ndpm) for samples were obtained by subtracting background dpm measured in appropriate control samples (typically 25 to 30 dpm). For each CC and DE experiment, measured ndpm in samples of IS, DEC water, and diaphragm materials taken as described above were used to estimate the total ndpm injected into the DEC at t_0 (hereafter referred to as I_0), the total ndpm present in DEC water at t_{10} (hereafter referred to as L_{10}), and the total ndpm present at T_e in the DEC diaphragm latex plus aluminum tape (hereafter referred to as D_e).

Fecal radioactivity was assayed as follows: 10 fecal pellets were selected at random from each weighed fecal collection, weighed, and then crushed by mortar and pestle to a fine powder which was then washed slowly with 20 ml methanol over course filter paper (used to remove debris—nearly all of the pellet mass was typically washed into the rinsate/suspension). To estimate the equivalent ndpm for each total fecal collection, the mean ndpm of two 1.0-ml aliquots of the stirred rinsate was normalized to reflect the total (20-ml) sample volume and corresponding total fecal weight; fecal samples from nonexposed animals were used to establish background. Similarly, measured ndpm in urine samples were normalized to each collected total volume, and urine collected from nonexposed animals was used to establish background for urine. Urinary and fecal ndpm were added to total recovered net cage-rinse activities to obtain total measured ndpm excreted postexposure.

Estimation of dermal surface areas. The animals used in the DE experiments were not terminated immediately after exposure to allow for ongoing metabolism studies. Therefore, exposed dermal surface area A (in cm^2) was estimated by interpolation from the allometric relation $A = aW^b$, where W = body weight in grams and where a and b were parameters estimated by log–log least-squares regression of experimentally determined surface areas of 10 female hairless guinea pigs weighing 360 to 760 g. The measured areas were selected to correspond as closely as possible to areas exposed in the DE experiments. Each surface-area measurement was performed immediately after termination by weighing a cutout tracing of the dissected skin on preweighed graph paper.

Data analysis. Radiolabel loss during all CC and DE experiments was expressed as percentage of label remaining in sampled DEC fluid compared to that first measured (at t_{10}), where the latter amount was assigned the value of 100%. Observed rates R (in percentage/hr) of relative net radiolabel loss over time from sampled DEC fluid in DE experiments were anticipated, following Scheuplein and Blank (1971, 1973), to reflect first-order loss in accordance with Fick's law, which states that, as a limiting approximation for dilute solutions, the flux or permeation rate, J_s (mg/cm^2-hr), of solute passively diffusing across a given area equals the concentration difference, ΔC_s (mg/ml), across that tissue multiplied by a permeability constant, k_p (ml/cm^2-hr), assuming ideal mixing far from equilibrium. (Note that k_p used here and below is expressed in the unit of milliliters of solution cleared of solute by dermal uptake per centimeter squared of dermal surface area exposed to the solution per hour of exposure, and that this unit is equivalent to, yet in the present context more meaningful than, the more commonly used unit of cm/hr.) Accordingly, data on relative radiolabel loss over time from sampled test chamber water were analyzed using simple linear regression to model the initial, approximately linear portion of anticipated exponential decay (observed loss was generally 30% or less in all DE experiments—see Results). Approximate linearity of radiolabel loss was also assumed for all CC experiments (in which observed loss was much less than that in the DE experiments—see Results). Thus, each set of CC or DE data on relative radiolabel loss from DEC fluid over the duration of the experiment was analyzed to obtain a corresponding value of R (% loss/hr), the negative of the slope obtained from linear regression on that data.

For all DE experiments, corresponding k_p values were estimated under the assumption that linear net radiolabel loss from the DEC was due entirely to corresponding linear uptake into exposed animals during dermal exposure; that is,

$$k_p = \frac{(V_0 - 2.0\ \text{ml})(R - R_0)}{100\%\ A} \quad (1)$$

where $(V_0 - 2.0\ \text{ml})$ represents the approximate average volume of DEC water during exposure (accounting for withdrawn sample volumes) and where R_0 is the average rate of relative radiolabel loss for (i.e., the negative of the linear slope fit to) the corresponding set of pooled CC data. For each low-concentration DE experiment, absorbed dermal dose was then estimated [under the same assumptions used for Eq. (1)] as

$$\text{Dose (in } \mu\text{g)} = \frac{k_p T_e C_{10} A}{60\ \text{min/hr}} \cdot \frac{9.95 \times 10^{-4}\ \mu\text{g}}{\text{ml ppb@32°C}} \times \left(1 + \frac{R\ 10\ \text{min}}{100\%\ 60\ \text{min/hr}}\right), \quad (2)$$

in which C_{10} is the calculated aqueous concentration of test compound (in ppb) in DEC fluid sampled at t_{10} (based on the corresponding measured value of L_{10}). Such estimated doses, in turn, were used with Corresponding radiolabel-excretion data to obtain the average fraction of estimated dermal dose excreted as urinary and fecal metabolites. The latter fraction was then compared to the average fraction obtained in corresponding PC experiments in order to test the assumptions that underlay the application of Eqs. (1) and (2).

For each CC and DE experiment, the recovery of input I_0 in DEC fluid was examined by estimating a hypothetical number, L_0, of ndpm in DEC fluid at t_0 corresponding to the measured value L_{10}, under the assumptions of instantaneous and ideal mixing at t_0 and of linear radiolabel loss between t_0 and t_{10}, as follows:

$$L_0 = L_{10}\left(1 + \frac{R\ 10\ \text{min}}{100\%\ 60\ \text{min/hr}}\right). \quad (3)$$

The value of L_0 obtained was then expressed as a percentage of I_0. Similarly, for each of these experiments, the number (hereafter denoted D_{72}) of ndpm in the DEC diaphragm (total in aluminum tape and latex) at t_{72} (a time picked to allow a standardized comparison of uptake into diaphragm material in CC and DE experiments) was estimated as

$$D_{72} = D_e(72\ \text{min})/T_e \quad (4)$$

and expressed as a percentage of L_0.

Unless otherwise specified, reported means are all given ± the coefficient of variation expressed as 100% × [SD]/[mean] (hereafter abbreviated as CVW). Reported significance levels (p values) are all for two-tailed t tests, except for means comparisons involving samples of unqeual variance (based on an F test with $p \leq 0.05$), in which cases the reported p values (denoted by p^*) refer to two-tailed Wilcoxon rank-sum tests. Reference to statistical nonsignificance without a reported value of p or p^* means that a value greater than 0.05 pertains.

RESULTS

The 10 measured dermal surface areas (in cm^2), fit to the function aW^b, yielded best-fit parameter values (and 95% confidence limits) of $a = 24.5$ (8.31 to 72.3) and $b = 0.394$ (0.221 to 0.568) ($r^2 = 0.77$, $p = 0.00078$).

For all CC and DE experiments combined, radioactivity in samples taken at t_{10} from exposure-chamber water after

radiolabel input at t_0 averaged 30 (and ranged 9 to 120) times above the background level for DDW. For each of the compounds studied, data on animal weights, estimated surface areas, DEC-water volumes, measured values of C_{10} and R, and estimated values of R_0 and k_p (calculated as explained under Methods) are given in Table 1. Data on relative radiolabel losses from the DEC observed during CC and corresponding DE experiments, from which values for R and R_0 were estimated, are shown with corresponding linear fits for the PCE experiments in Fig. 2. For all compounds, as indicated in Table 1, the values R_0 obtained for pooled CC data are all greater than zero (but not significantly so in the case of the pooled CC data for CF). When compared to these R_0 values, the loss rates R from corresponding DE experi-

TABLE 1
Percutaneous Absorption of Aqueous TCE, CF, and PCE in Hairless Guinea Pigs[a]

Experiment	Animal weight, W (g)	Exposed surface area, A (cm^2)	Initial water volume, V_0 (ml)	Concentration at time = 10 min, C_{10} (ppb)	Radiolabel loss rate,[b] R (%/hr)	Permeability constant,[c] k_p (ml/cm^2-hr)
TCE:C1–C5	—	—	794.9 to 812.9	15 to 84	1.6 ± 18%	—
TCE:D1	473	278	449.9	87	17[d]	0.25
TCE:D2	492	283	478.6	110	17[d]	0.26
TCE:D3	423	266	509.4	58	16[d]	0.27
TCE:D4	535	292	488.0	20	13[d]	0.19
TCE:D5	488	282	520.7	19	12[d]	0.19
						0.23 ± 17%
TCE:C6–C10	—	—	778.8 to 844.4	≈100,000	2.3 ± 17%	
TCEM	386	257	449.9	≈100,000	13[e]	0.24
TCE:D7	451	273	478.6	≈100,000	9.2[e]	0.16
TCE:D8	368	252	509.4	≈100,000	9.1[e]	0.21
TCE:D9	411	263	488.0	≈100,000	12[e]	0.23
TCEDIO	385	257	520.7	≈100,000	23[e]	0.47
						0.21 ± 58%
CFC1–c5	—	—	797.9 to 836.4	22 to 44	0.078 ± 590%	—
CFDI	604	306	428.1	52	9.1[f]	0.13
CF:D2	623	310	426.5	24	12[f]	0.16
CFD3	654	316	387.5	44	15[f]	0.18
CFD4	576	301	470.7	34	6.1[g]	0.094
CF:D5	514	288	482.4	19	8.3[f]	0.14
CF:D6	481	280	500.3	19	4.5[f]	0.079
						0.13 ± 29%
PCE:C1–C5	—	—	768.2 to 815.2	23 to 150	1.3 ± 18%	—
PCEDI	375	254	577.7	27	23[h]	0.49
PCE:D2	420	266	543.8	51	19[h]	0.36
PCE:D3	489	282	495.3	64	19[h]	0.31
PCE:D4	490	282	525.1	56	21[h]	0.37
PCE:D5	458	275	530.4	56	19[h]	0.34
						0.37 ± 18%

[a] TCE, trichloroethylene; CF, chloroform; PCE, tetrachloroethylene. See Methods section for description of notation for column variables and (if applicable)their calculation.

[b] For each set of chambercontrol experiments, the value listed is the estimated loss rate, R_0 (± CV%), for the pooled control data (see Methods).

[c] Below each set of related values is the corresponding arithmetic mean (± CV%).

[d] Significantly greater than the corresponding loss rate for pooled control data (by analyses of covariance, $p < 10^{-6}$).

[e] Significantly greater than the corresponding loss rate for pooled control data (by analyses of covariance, $10^{-9} < p < 0.022$).
[Significantly greater than the corresponding loss rate for pooled control data (by analsyes of covariance; $10^{-4} < p < 0.020$).

[g] Not significantly different from the corresponding loss rate for pooled control data (by analysis of covariance; $p = 0.085$ for the CF:D4, $p = 0.21$ for CF:D6).

[h] Significantly greater than the corresponding loss rate for pooled control data (by anlayses of covariance, $p < 10^{-12}$).

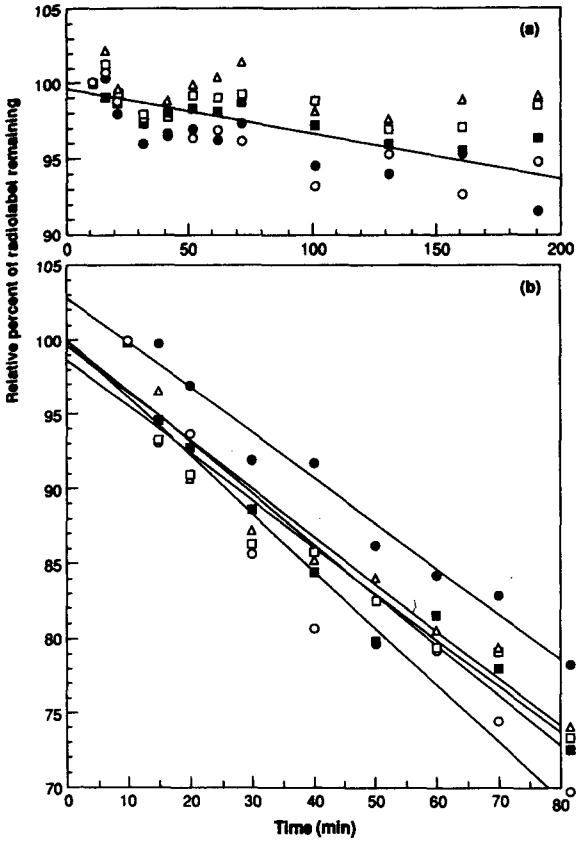

FIG. 2. Loss of radiolabeled tetrachloroethylene (PCE) from chamber water compared to that measured 10 min after input (PCE concentrations: 23 to 64 ppb). (a) Five control experiments (no animal): data points from each experiment at each sampling time are shown with a linear fit to the pooled data. (b) Five dermal exposure experiments (animal present): data points from each experiment at each sampling time are shown with corresponding linear fits (all slopes significantly less than that of pooled control data—see Table 1).

ments are all significantly greater, except in the case of two values obtained from DE data for CF. Table 1 shows that the mean k_p values obtained from the four DE data sets are in the following compound-related order: CF < TCE < PCE. Notably, the mean k_p value for the low-concentration TCE experiments (TCE:D1-D5) does not differ significantly ($p^* = 0.22$) from that based on the high-concentration experiments (TCE:D6-D10). The mean k_p value for CF is significantly less than that for TCE:D1-D5 ($p = 0.0019$) and for TCE:D1-D10 ($p^* = 0.011$), while that for PCE is significantly greater than that for TCE:D1-D5 ($p = 0.0039$) and for TCE:D1-D10 ($p = 0.0042$). The k_p values obtained from the three lowconcentration experiments are most consistent (CV% range: 17 to 29%); the k_p values obtained from the high-concentration TCE experiments are the most variable (CV% = 58%).

Measured radioactivity in urine samples from all animals exposed to TCE, PCE, and CF initially exceeded background urine levels by average factors (and corresponding factor ranges) of approximately 150 (1.5 to 480), 5 (2 to 8), and 2 (1 to 9), respectively, and in almost all cases exceeded the corresponding net dpm ratio for initial fecal/methanol samples (which typically was approximately 1 to 2).

Table 2 presents a comparison of radiolabel-excretion data from low-concentration DE and PC experiments. This table shows that, for each of the three compounds tested, the average excreted percentage of estimated dermal dose in the DE experiments (based on information fiom Table 1, as explained under Methods) does not differ significantly from the average excreted percentage of the injected dose in the corresponding PC experiments. The time taken to excrete 95% of the total radiolabel measured in urine and feces, however, was significantly different in DE and corresponding PC experiments for TCE (about twofold less) and PCE (about sixfold greater).

Analysis of data on recovery of radiolabel in DEC water and diaphragm materials in all CC and DE experiments revealed no statistically significant difference between any CC and corresponding DE experiment in the average recovery of radiolabel injected into the DEC based on the first withdrawn DEC-fluid sample taken at t_{10}. These recoveries, expressed as $100\% \times L_0/I_0$ (see Methods), were not significantly less than 100%, except in the case of Experiments TCE:D1-D5 (93%, $p = 0.023$) and for pooled Experiments TCE:C1-C5 + TCE:D1-D5 (91%, $p = 0.0035$). Differences between all CC and corresponding DE experiments in the recovery of radiolabel in DEC diaphragms at t_{72}, expressed as $100\% \times D_{72}/L_0$ (see Methods section), though significantly different in all studies except those involving low-concentratien TCE, exhibited no consistent pattern and reflected mean levels of diaphragm radiolabel uptake that were in all cases small ($\leq 1\%$) in relation to L_0.

DISCUSSION

Interpretation of DEC Results

Our analysis of surface-area measurements provides a reasonable basis for interpolation of the exposed surface areas of the medium-sized hairless guinea pigs used in our DE experiments. It is interesting, however, that the best-fit empirical allometric exponent of body weight we obtained (0.39 ± 19%)is significantly lower ($p < 0.05$) than the values of 0.67 or 0.75 typically used for interspecies extrapolation of surface areas (Schmidt-Nielsen, 1984).

The comparison of data and linear fits, combined with the results concerning R and R_0 summarized in Table 1 and discussed above, supports the conclusions that relative radiolabel losses from the DEC in DE and CC experiments were approximately linear over time, that the rates of such loss in DE experiments were greater than those observed in corresponding CC experiments, and that the k_p values estimated from the difference between these DE and corresponding CC loss rates are reasonably consistent for each of

TABLE 2
Excretion of Radiolabel in Urine and Feces by Hairless Guinea Pigs Exposed to TCE, CF, or PCE[a]

Experiment	Animal weight (g)	Exposure regimen	Administered dose (μg)	Excreted percentage of administered dose (% ± CV%)	Time to excrete 95% of metabolized dose (days ± CV%)
TCE:D1		Dermal	8.7	64	11
TCE:D2		Dermal	10	50	6
TCE:D3		Dermal	5.2	58	7
TCE:D4		Dermal	1.4	86	14
TCE:D5		Dermal	1.2	40	—
				59 ± 30%[b]	8.6 ± 44%[c]
TCEPI	467	Intramuscular	41	49	8
TCE:P2	550	Intramuscular	20	47	17
TCE:P3	469	Subcutaneous	2.6	76	22
TCE:P4	436	Subcutaneous	2.7	76	21
TCE:P5	561	Subcutaneous	2.7	77	22
				65 ± 24%[b]	18 ± 33%[c]
CF:D1		Dermal	2.4	12	2.5
CF:D2		Dermal	1.5	15	11
CFD3		Dermal	3.1	1.4	1.5
CF:D4		Dermal	1.2	9.3	6
CFDS		Dermal	0.91	0	—
CFD6		Dermal	0.50	0	—
				6.3 ± 110%[d]	5.2 ± 82%[e]
CFPI	646	Subcutaneous	3.3	6.1	8
CF:P2	618	Subcutaneous	3.3	5.5	8
CFP3	653	Subcutaneous	3.3	3.4	1
CF:P4	582	Subcutaneous	3.3	9.7	4
CF:P5	532	Subcutaneous	3.3	25	5.5
				9.9 ± 88%[d]	5.3 ± 56%[e]
PCED1		Dermal	4.2	22	8.5
XED2		Dermal	6.0	17	8
PCE:D3		Dermal	6.9	8.9	4.5
PCE:D4		Dermal	7.0	18	7
PCE:D5		Dermal	6.5	4.8	2
				14 ± 50%[f]	6.0 ± 50%[g]
PCE:P1	716	Subcutaneous	3.7	4.5	1
PCE:P2	686	Subcutaneous	3.7	17	1
PCE:P3	613	Subcutaneous	3.7	23	1
PCE:P4	648	Subcutaneous	3.7	15	1
PCE:P5	532	Subcutaneous	3.6	8.5	2
				14 ± 53%	1 ± 40%[g]

[a] TCE, trichloroethylene; CF, chloroform; PCE, tetrachloroethylene. Below each set of related values listed is the corresponding arithmetic mean (±CV%). See Methods section for explanation of experiment designations, positive-control and dermal exposure protocols, procedure used to estimate administered dose to animals in dermal exposure experiments based on information from Table 1, and CV%. Time until 95% excretion was estimated to the nearest half day. Weights of animals used in dermal exposure experiments are listed in Table 1.

[b] Not significantly different ($p = 0.60$).

[c] Significantly different ($p = 0.018$).

[d,e,f] Not significantly different ($p \geq 0.45$).

[g] Significantly different ($p^* = 0.012$).

the three VCOCs tested. The results of our PC experiments (Table 2) address the question of whether the k_p values we obtained actually reflect radiolabel losses from DECs due to percutaneous absorption, rather than loss by some other process(es). Multicompartment, physiologically based pharmacokinetic models for VCOCs predict that the metabolized fraction of a very small administered VCOC dose that is primarily metabolized in liver—such as the dose received in any of our PC and low-concentration DE experiments—is likely to be similar whether that dose is administered by sc or im injection or by dermal absorption (Bogen, 1988). Therefore, the consistencies between DE and corresponding PC data on excreted-radiolabel recovery for all three VCOCs tested (Table 2), and between DE and corresponding CC data on radiolabel recovery discussed under Results, jointly provide clear evidence that dermal uptake accounted for the decline of radiolabel in DEC fluid over time in the DE experiments over that observed in corresponding CC experiments.

Although differences were observed in the *kinetics* of radiolabel excretion between DE and corresponding PC experiments using TCE and PCE (Table 2), they are understandable in light of the different exposure routes involved and do not detract from our general conclusion from these DE/PC comparisons that our DE and CC results were used successfully to measure *in vivo* uptake of the tested compounds through guinea pig skin. The data obtained on recovery of radiolabel in DEC diaphragms (see Results section) indicate that such uptake, where it occurred in significant amounts, could be at most only a very small source of error in these measurements.

The average k_p value obtained for TCE at a relatively high aqueous concentration (~100,000 ppb) was not found to differ significantly from that obtained at concentrations three to four orders of magnitude lower. This invariance indicates that dermal uptake of TCE is strictly proportional to its concentration over a very large range, in accordance with Fick's law (see Methods section), and that similar "linearity" in dermal uptake over time is likely for closely related VCOCs, such as CF and PCE.

Compared to k_p values obtained for various compounds in aqueous solution using human dermal tissues *in vitro*, the mean k_p values we obtained are far (~10^3-fold) greater than those obtained for pure water (Scheuplein and Blank, 1973; Blank *et al.*, 1984), somewhat greater than those obtained for aliphatic alcohols as large as decanol (Scheuplein and Blank, 1973), similar to those obtained for 2-ethoxyethanol and a series of phenolic compounds (Scheuplein and Blank, 1971; Roberts *et al.*, 1977), and roughly two- to three-fold less than those obtained for some nonchlorinated aromatic hydrocarbons in *in vivo* studies (Dutkiewicz and Tyras, 1968a,b).

Although permeability constants were not calculated in the study of Jo *et al.* (1990a,b) on dermal absorption of aqueous CF by showering volunteers, their data may be used to estimate an average effective value of k_p for skin exposed to CF in shower water, and this value may be compared to the one we obtained using hairless guinea pigs. Consistently significant differences measured in CF concentration in exhaled breath following 10-min showers by persons with and without wet suits (intended to prevent dermal but not respiratory uptake) were used by Jo *et* al. as the basis of an estimate that dermal uptake from showering constituted a p proximately 90% of that due to inhalation alone. Presently, we assume this figure of 90% is correct for the purpose of calculating a value of k_p reflecting the results of the Jo *et al.* study. From Table IV of that study, we calculate that the mean air concentration of CF (in μg/liter) in the breathing zone of shower stalls used was 0.66% of the corresponding concentration (in μg/liter) of CF in the water used for each shower. Respiratory uptake of CF is thought to be driven by the alveolar ventilation rate, which we assume for a reference 70-kg adult is 378 liter/hr (Bogen, 1990). This reference adult has a dermal surface area of approximately 18,000 cm^2 (ICRP, 1975), about 80% of which we assume (following, e.g., Brown *et al.*, 1984) was effectively immersed continuously during showering. From these stated assumptions, the data of Jo *et al.* (1990a,b) imply the average, effective k_p value of

$$k_p = \frac{(0.90)(378{,}000\ \text{ml/hr})(0.0066)}{(0.80)(18{,}000\ \text{cm}^2)} \approx 0.16\ \text{ml/cm}^2\text{-hr},$$

which is very close to the mean k_p value of 0.13 ml/cm^2-hr we obtained for CF using hairless guinea pigs (Tahle 1). It may be that the total dermal CF dose was underestimated in the Jo *et al.* study, because its design may not have properly accounted for potential dermal-compartment-induced delay in the release of dermally absorbed CF into blood subject to equilibration with exhaled air. Roberts *et* al. (1977), for example, showed that linear diffusion of phenolic compounds through human epidermis *in vitro* at 25°C, yielding k_p values close to those obtained here for CF (Table 1), occurred only after significant lag periods ranging from 30 to 80 min. But such lag periods observed in *in vitro* studies may overestimate corresponding *in vivo* lag periods, particularly at elevated temperatures typical of water used in showering that may facilitate dermal penetration by, e.g., stimulating blood perfusion, opening pores, etc. (Loomis, 1980). Thus, some of the CF dermally absorbed by showerers in the Jo *et al.* study may not have been reflected in the first breath samples taken (5 min) after showering ended, and these samples were the only ones used in that study to calculate the relative contribution of dermal dose.

Implications for Human-Exposure Assessment

Health risks associated with exposure to water containing potentially carcinogenic VCOCs have been a continuing

regulatory concern (Crouch et al., 1983; U.S. EPA, 1984; Andelman, 1985; Cothern et *al.*, 1986; U.S. EPA, 1988). When first considered quantitatively, dermal VCOC absorption from domestic water appeared to constitute a significant uptake pathway compared to ingestion or respiration (Beech, 1980; Brown et *al.*, 1984). Integrated assessments considering multiple exposure pathways have subsequently lead to the conclusions that dermal absorption of particular VCOCs from domestic water supplies may constitute a substantial (Shehata, 1985; Bogen *et al.*, 1988; CDHS, 1988; McKone, 1989; Brown and Hattis, 1989) or minor (U.S. EPA, 1984; Cothern et *al.*, 1986; Brown and Hattis, 1989) fraction of corresponding total uptake from all routes.

As discussed above, the hairless guinea pig may provide a reasonable model for human percutaneous absorption of dilute aqueous VCOCs such as CF, TCE, and PCE. To the extent that this is true, the results we report here may be useful in assessing potential human exposure to these compounds. The k_p values we obtained for these compounds for hairless guinea pigs, if applicable to humans, show that dermal absorption may be an important route of human exposure to these compounds from all water-related sources. For example, the average k_p values we obtained for CF, TCE, and PCE—applied to a reference 70-kg human with 18,000 cm^2 of dermal surface area 80% immersed during a 20-min bath—imply dermal uptakes equal to the amount of those compounds present in approximately 0.6, 1, and 2 liters, respectively, of the water used for bathing. For comparison, a dermal-pharmacokinetic model recently developed by Brown and Hattis (1989) predicts that, under a similar bathing scenario, a reference adult would dermally absorb a dose of TCE or PCE equal to the amount of those compounds present in approximately 0.1 to 1 liter of that water.

ACKNOWLEDGMENTS

We are very grateful to Dr. J. P. Knezovich, Ms. D. J. Bishop, Mr. B. F. Brunckhorst, and Mr. S. M. Rose for their helpful suggestions/assistance. This work was performed under the auspices of the U.S. Department of Energy at Lawrence Livermore National Laboratory under Contract W-7405-ENG-48, with funds from the U.S. Air Force, H. G. Aerospace Medical Research Laboratory, Toxic Hazards Division (AF/DOE AML/86-20); the California Department of Health Services (MOU-3, 87-T0102); and the U.S. Environmental Protection Agency Office of Research and Development (DW89934205).

REFERENCES

Aggazzotti, G., Fantuzzi, G., Tartoni, P. L., and Predieri, G. (1990). Plasma chloroform concentrations in swimmers using indoor swimming pools. *Arch. Envir. Health* **45,** 175–179.

Andelman, J. B. (1985). Human exposures to volatile halogenated organic chemicals in indoor and outdoor air. *Environ. Health Perspect.* **62,** 313–318.

Beech, A. J. (1980). Estimated worst case trihalomethane body burden of a child using a swimming pool. *Med. Hypotheses* **6,** 303–307.

Blank, I. H., Moloney, J., III, Emslie, A. G., Simon, I., and Apt, C. (1984). The diffusion of water across the *stratum comeum* as a function of its water content. *Invest. Dermatol.* 82, 188–194.

Bogen, K. T. (1988). Pharmacokinetics for regulatory risk analysis: The case of trichloroethylene. *Regul. Toxicol. Pharmacol.* **8,** 447–466.

Bogen, K. T. (1990). Risk extrapolation for chlorinated methanes as promoters vs initiators of multistage carcinogenesis. *Fundam. Appl. Toxicol.* **15,** 536–557.

Bogen, K. T., Hall, L. C., Perry, L., Fish, R., McKone, T. E., Dowd, P., Patton, S. E., and Mallon, B. (1988). *Health Risk Assessment of Trichloroethylene in California Drinking Water,* report prepared for the California Public Health Foundation (UCRL-21007). Lawrence Livermore National Laboratory, Livermore, CA.

Brown, H. S., Bishop, D. R., and Rowan, Carol A. (1984). The role of skin absorption as a route of exposure for volatile organic compounds (VOCs) in drinking water. Am. J. *Public Health* **74,** 479–484.

Brown, H. S., and Hattis, D. (1989). The role of skin absorption as a route of exposure to volatile organic compounds in household tap water A simulated kinetic approach. *J. Am. Coll. Toxicol.* 8,839–851.

California Department of Health Services (CDHS) (1988). Notice of proposed rulemaking: Maximum contaminant level for tetrachloroethylene (PCE) in drinking water. R-75-87, Sacramento, CA.

Conney, A. H. (1967). Pharmacological implications of microsomal enzyme induction. *Pharmacol. Rev.* 19,317–366.

Cothern, R. C., Coniglio, W. A., and Marcus, W. L. (1986). Estimating risk to human health: Trichloroethylene in drinking water is used as the example. *Environ. Sci. Technol.* **20,** 111–116.

Crouch, E. A. C., Wilson, R., and Zeise, L. (1983). The risks of drinking water. *Water Resourc. Res.* 19, 1359–1375.

Dutkiewin, T., and Tyras, H. (1967). A study of the skin absorption of ethylbenzene in man. *Br. J. Ind. Med.* 24,330–332.

Dutkiewin, T., and Tyras, H. (1968a). Skin absorption of toluene, styrene, and xylene by man. Br. J. *Ind. Med.* 25,243.

Dutkiewicz, T., and Tyras, H. (1968b). The absorption of benzene through the skin of man. *Zes. Nauk. Bromat. Chem. Toksykol.* 1,159. [in Polish]. As reported in: Baranowska-Dutkiewicz, B. (1982). Skin absorption of aniline from aqueous solutions in man. *Toxicol. Lett.* 10,367–372.

Frankin, C. A., Somers, D. A., and Chu, I. (1989). Use of percutaneous absorption data in risk assessment. J. *Am. Coll. Toxicol.* **8,** 815–827.

Hake, C. L., and Stewart, R. D. (1977). Human exposure to tetrachloroethylene: Inhalation and skin contact. *Environ. Health Perspect.* 21, 231–238.

International Commission on Radiological Protection (ICRP) (1975). *Report of the Task Group on Reference Man,* ICRP No. 23. Permagon Press, New York.

Jo, W. K., Weisel, C. P., and Lioy, P. J. (1990a). Routes of chloroform exposure and body burden from showering with chlorinated tap water. *Risk Anal.* 10,575–580.

Jo, W. K., Weisel, C. P., and Lioy, P. J. (1990b). Chloroform exposure and the health risk associated with multiple uses of chlorinated tap water. *Risk Anal.* **10,** 581–585.

Kao, J., Hall, J., and Helman, G. (1988). *In vitro* percutaneous absorption in mouse skin: Influence of skin appendages. *Toxicol. Appl. Pharmacol.* **94,** 93–103.

Kronevi, T., Wahlberg, J., and Holmberg, B. (1979). Histopathology of skin, liver, and kidney after epicutaneous administration of five industrial solvents to guinea pigs. *Environ. Res.* 19, 56–69.

Loomis, T. A. (1980). Skin as a portal of entry for systemic effects. In *Current Concepts in Cutaneous Toxicity* (V. A. Drill and P. Lazar, Eds.), pp. 153–169. Academic Press, New York.

A

Maibach, H. I., and Wester, R. C. (1989). Percutaneous absorption: In *vivo* methods in humans and animals. J. *Am. Coll. Toxicol.* 8, 803–813.

McKone, T. E. (1989). Human exposure models. *Toxicol. Lett.* 49, 321–339.

Roberts, M. S., Anderson, R. A., and Swarbrick, J. (1977). Permeability of human epidermis to phenolic compounds. *Pharm. Pharmacol.* 29, 677–683.

Scheuplein, R. J., Blank, I. H., Brauner, G. J., and MacFarlane, D. J. (1969). Percutaneous absorption of steroids. Invest. *Dermatol.* 54, 63–70.

Scheuplein, R. J., and Blank, I. H. (1971). Permeability of the skin. *Physiol. Rev.* 51, 702–747.

Scheuplein, R. J., and Blank, I. H. (1973). Mechanism of percutaneous absorption. IV. Penetration of nonelectrolytes (alcohols) from aqueous solutions and from pure liquids. J. *Invest. Dermatol.* 60, 286–296.

Schmidt-Nielsen, K. (1984). *Scaling: Why Is Animal Size so Important?* pp. 78–82. Cambridge Univ. Press, London/New York.

Shehata, A. T. (1985). A multi-route exposure assessment of chemically contaminated drinking water. *Toxicol. Ind. Health.* 1,277–298.

Stewart, R. D., and Dodd, H. C. (1964). Absorption of carbon tetrachloride, trichloroethylene, tetrachloroethylene, methylene chloride, and 1,1,1-trichloroethane through human skin. *J. Am. Ind. Hyg. Assoc.* 25,439–446.

Susten, A. S., Dames, B. L., and Niemeier, R. W. (1986). *In vivo* percutaneous absorption studies of volatile solvents in hairless mice. I. Description of a skin-depot. J. *Appl. Toxicol.* 6, 43–46.

U.S. Environmental Protection Agency (EPA) (1984). *Techniques for the Assessment of the Carcinogenic Risk to the U.S. Population Due to Exposure from Selected Volatile Organic Compounds from Drinking Water Via the Ingestion. Inhalation and Dermal Routes, NTIS* Publ. No. PB84-213941. Office of Drinking Water, Washington, DC.

U.S. Environmental Protection Agency (EPA) (1985a). *Health Assessment Document for Chloroform.* EPA/600/8-84/004F. U.S. EPA, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Washington, DC.

U.S. Environmental Protection Agency (EPA) (1985b). *Health Assessment Document for Trichloroethylene.* EPA/600/8-82-006F. U.S. EPA, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Washington, DC.

U.S. Environmental Protection Agency (EPA) (1985c). *Health Assessment Document for Tetrachloroethylene* (Perchloroethylene). EPA/600/8-82-005FA. U.S. EPA, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Washington, DC.

U.S. Environmental Protection Agency (EPA) (1986). *Addendum to the Health Assessment Document for Tetrachloroethylene (Perchloroethylene), Updated Carcinogenicity Assessment for Tetrachloroethylene (Perchloroethylene, PERC, PCE), (Review Draft).* EPA/600/8-82-005FA. U.S. EPA, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Washington, DC.

U.S. Environmental Protection Agency (EPA) (1987). *Addendum to the Health Assessment Document for Trichloroethylene: Updated Carcinogenicity Assessment for Trichloroethylene (External Review Draf).* EPA-600/8-82-006F. U.S. EPA, Environmental Criteria and Assessment Office, Washington, DC.

U.S. Environmental Protection Agency (EPA) (1988). *Superfund Exposure Assessment Manual.* EPA/540/1-88-001, pp. 121–133. U.S. EPA, Office of Remedial Response, Washington, DC.

Wade, J. V., Mershon, M. M., Mitcheltree, L. W., and Woodard, C. L. (1989). The hairless guinea pig model and vesicant vapor exposures for bioassay purposes. In *Proceedings of the 1989 Medical Defense Bioscience Review, August 15–17, 1989.* (U.S. Army Medical Research Institute of Chemical Defense, U.S. Army Medical Research and Development Command, Ed.), pp. 569–575. Fort Detrick, Frederick, MD.

Wahlberg, J. E. (1976). Percutaneous toxicity of solvents: A comparative investigation in the guinea pig with benzene, toluene and 1,1,2-trichloroethane. *Ann. Occup. Hyg.* 19, 115–119.

Wester, R. C., and Noonan, P. K. (1980). Relevance of animal models for percutaneous absorption. *Int. J. Pharm.* 7, 99–110.

Wester, R. C., and Maibach, H. I. (1983). Cutaneous pharmacokinetics: 10 steps to percutaneous absorption. *Drug Metab. Rev.* 14, 169–205.

Wester, R. C., and Maibach, H. I. (1989). Human skin binding and absorption of contaminants from ground and surface water during swimming and bathing. J. *Am. Coll. Toxicol.* 8, 853–859.