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In Vivo Percutaneous Absorption Studies of Volatile Organic Solvents in Hairless Mice II. Toluene, Ethylbenzene and Aniline*

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Percutaneous absorption studies were conducted with three single-ring, radiolabeled aromatic solvents (benzene derivatives) using a recently described direct method for studying volatile chemicals in hairless mice. Total absorption, determined from the sums of radioactivity found in the excreta, expired breath and carcass, was 2·1%, 3·4% and 4·7% of the nominal dose for toluene, ethylbenzene and aniline, respectively. Breath decay curves indicated that absorption of toluene and ethylbenzene was complete by 15 min after application and that by this time the excretion rate of aniline exceeded the absorption rate. Evaporation rates were used to derive estimated contact times, and these in turn were used in conjunction with the absorbed doses to estimate percutaneous absorption rates. Equivalent dermal exposures ($cm^2 \cdot min$) that would yield body burdens equivalent to those expected following 8-h inhalations at existing US permissible exposure limits during light work were calculated. The data indicate that dermal absorption of these compounds could approach or exceed that from inhalation under some work conditions. Correlations between absorption and various physical properties were evaluated using Spearman's correlation coefficients. The physical properties evaluated included volatility, solubility, octanol/water partition coefficients and melting points. For this limited series of benzene derivatives, two measures of volatility, i.e. vapor pressure and boiling point, were the only physical properties significantly correlated with percutaneous absorption.

INTRODUCTION

In many workplace situations, workers' skin is exposed to chemicals such that the potential for percutaneous absorption and systemic toxicity exists. Regulations of the US Occupational Safety and Health Administration designate 139 substances for prevention or reduction of skin absorption in addition to the requirements for preventing skin contact with those compounds regulated as carcinogens.[1] In other countries, an additional 125 or more substances are considered to present a significant hazard from skin absorption.[2] In all cases, there are only qualitative notations and the literature contains relatively few studies designed to evaluate the importance of percutaneous absorption so that appropriate protection can be provided to the worker. The industrial hygienist can observe the worker and determine exposed skin surface area and frequency and duration of exposure or, by applying newer technology that is being developed, measure the total contamination of the skin.[3,4] If the absorption rate for the substance is known, then an estimate of the absorbed dose can be made and compared to the probable dose that the worker would acquire by inhalation at a given exposure concentration.[5]

This report describes percutaneous absorption studies in hairless mice that culminate in estimated absorption rates for benzene and its derivatives (toluene, ethylbenzene and aniline). Criteria for selection of the latter three compounds were:

(i) all were singly-substituted, volatile benzene derivatives that were liquids at room temperature;
(ii) percutaneous absorption data in humans were available for comparison with the hairless mouse data[6–10];
(iii) occupational exposure limits based on inhalation were available for each compound.

Criterion (iii) allowed comparisons of estimated systemic body burdens resulting from inhalation and dermal contact, which was important because of the growing concern about the percutaneous absorption of chemicals by workers and the need for methods to evaluate risk due to percutaneous absorption. Data from a prior study with benzene are included for comparison.[11]

EXPERIMENTAL

An *in vivo* method for measuring directly the percutaneous absorption of volatile chemicals using hairless mice was described recently[11,12] The method used a stainless-steel skin-depot device, which when glued to the backs of the mice served to capture for quantitation

* Mention of trade names and companies does not constitute an endorsement.

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the test chemical normally lost from the treatment site by evaporation. This property permitted the use of metabolism cages and thus a direct determination of absorption from the levels of radioactivity found in expired breath, excreta and carcass. Benzene was initially used as a model compound to test the method, and the results of these studies indicated that the model could be used for estimating the *in vivo* dermal absorption of volatile compounds under some occupational situations.[11] Since details of the model have been presented elsewhere,[11,12] only brief descriptions of the methods used for the treatment of the animals and for the collection and analysis of the various samples are provided.

Test compounds

The following uniformly [^{14}C]-ring-labeled compounds were purchased: [^{14}C]-toluene (New England Nuclear, Boston, MA; specific activity 60 mCi mmol^{-1}); [^{14}C]-ethylbenzene (Pathfinder Laboratories, St. Louis, MO; specific activity 33.47 mCi mmol^{-1}) and [^{14}C]-aniline (Amersham Corporation, Arlington Heights, IL; specific activity 101 mCi mmol^{-1}). Radiochemical purities for the above compounds were reported by the suppliers to be > 96% for aniline and > 98% for toluene and ethylbenzene. Reagent-grade non-labeled toluene, ethylbenzene and aniline solvents were used to dilute the appropriate radiochemicals. The amounts of isotope in nominal 5-μl doses were: toluene (2.13 μCi), ethylbenzene (2.48 μCi) and aniline (4.36 μCi); this resulted in nominal radioactive doses to a 25-g mouse of ca. 85, 100 and 174 μCi kg^{-1}, respectively.

Animals

Male albino hairless mice (HRS/J) (Jackson Laboratories, Bar Harbor, ME), weighing 23–32 g, were housed singly in shoe-box plastic cages with sawdust bedding and maintained on a 12-h light/dark cycle at 23 ± 2.5°C and 55 ± 15% relative humidity. Water and food (Purina Rodent Chow, St. Louis, MO) were available *ad libitum*. Mice were not used if blemishes were observed in the treatment area. Two mice were used for each experiment. A total of six experiments were conducted for each compound. Data from one mouse in the ethylbenzene study were excluded because the data suggested mechanical failure of the system and data from two mice in the aniline study were excluded because of unacceptably low total recovery (67%) in one and loss of breath samples in the other.

Treatment procedure

About 5–10 min prior to treatment, stainless-steel skin-depots circumscribing a skin area of ca. 0.8 cm^2 were glued (cyanoacrylate) to the backs (approximately mid-thoracic) of mice anesthetized with 50% CO_2 in air. A skin-depot consisted of an outer stainless-steel casing and an inner stainless-steel wire-mesh basket that contained 100–150 mg of a solid sorbent to collect evaporated vapor, a teflon cap with a single 1/32-inch hole in the center of the cap and a 21-gauge 'guide needle' to facilitate dosing.

For each compound of interest, a solid sorbent/solvent combination was selected on the basis of sampling and analytical procedures recommended by the US National Institute for Occupational Safety and Health.[13,14] For toluene and ethylbenzene, coconut-derived activated charcoal (20/40 mesh) served as the solid sorbent and non-labeled toluene was used to desorb the radioactivity from the charcoal. For aniline, silica gel was the solid sorbent and 95% ethanol was the desorbing agent. Preliminary tests using 5 μl of radiolabeled compound showed that adsorption and desorption efficiencies for each sorbent/solvent combination were > 98%.

Dermal application of ca. 5 μl of treatment solution was accomplished by inserting the blunted needle of a 10-μl microsyringe (Hamilton Model No. 701, Reno, NV) through the bore of the 21-gauge 'guide needle' until the tip of the microsyringe needle was just above the surface of the skin but below the solid sorbent. After expelling the contents of the microsyringe, the syringe needle and 'guide needle' were carefully removed from the skin-depot and the 'guide needle' was placed immediately into a vial containing scintillation fluid to remove any compound that may have adhered to it.

For each experiment, determination of radioactivity in the 5-μl nominal dose was accomplished by injecting 5-μl volumes of the test substance into each of three 10-ml volumetric flasks containing a suitable solvent. Triplicate aliquots (0.1 ml) were removed from each flask to determine the average total [^{14}C] content in each flask. The calculated mean for the three flasks was considered to be the nominal radioactive dose. Some radioactivity, and thus compound, remained in the guide needle (Table 1) and was subtracted from the nominal radioactive dose to determine the amount of compound applied to the skin: 3.17–4.99 μl (Table 2). The treated animals, with skin-depots attached, were placed into glass metabolism cages within 5 s of dosing.

Table 1. Distribution of radioactivity into various sampling compartments following dermal application of (^{14}C]-radiolabeled aromatic solvents

Compound	Percentage of nominal dose recovered[a]			
	Absorbed[b]	Skin-depot	Guide needle	Total recovered[a]
Benzene[d] (*N*=7)	0.9 ± 0.3	79.5 ± 3	10.4 ± 3	90.8 ± 2
Toluene (*N*=12)	2.1 ± 0.5	83.8 ± 2	9.0 ± 2	95.0 ± 1
Ethylbenzene (*N*=11)	3.4 ± 1.0	86.7 ± 1	5.0 ± 1	95.2 ± 1
Aniline (*N*=10)	4.7 ± 1.0	81.8 ± 3	8.3 ± 3	94.9 ± 1

[a] Values are means ± SEM.
[b] Absorbed = sum of the mean percentages of nominal dose recovered in excreta, carcass, skin application site and expired breath.
[c] Total recovery includes that recovered from a wipe of the skin area: aniline, 0.3%; ethylbenzene, 0.03%; and < 0.01% for toluene and benzene.
[d] From Ref. 11.

Table 2. Amounts of benzene, toluene, ethylbenzene and aniline applied to the skin, estimated contact times and calculated absorption rates (data include means ± SD (m) and ranges (r))

		Amount applied (μl)	Amount appplied (mg)	Amount absorbed (μg)	Estimated contact time (s)	Absorption rate (μg cm^{-2} min^{-1})
Benzene[a]	m	4.48	3.94	39	52	56
Toluene	m	4.49 ± 0.29	3.89 ± 0.25	89.67 ± 70.83	133 ± 8.76	49 ± 37.85
	r	3.94 - 4.98	3.65 - 4.31	12 - 206	116 - 148	8 - 115
Ethylbenzene	m	4.75 ± 0.22	4.10 ± 0.19	148.55 ± 127.80	294 ± 13.79	37 ± 31.49
	r	4.39 - 4.89	3.79 -4.30	6 - 457	273 - 309	2 - 114
Aniline	m	4.58 ± 0.55	4.68 ± 0.57	223.1 ± 178.33	7206 ± 872	2.26 ± 1.69
	r	3.17 - 4.99	3.24 - 5.10	49 - 587	4985 - 7846	0.74 - 5.61

[a] Data on benzene are derived from Ref. 11.

Sample collection and analysis

At the end of 4 h, the animals were killed in their cages by the introduction of carbon dioxide. Collected for analyses were: the skin-depot for measurement of evaporated test substance; a wipe of the treated skin site for unabsorbed chemical; the skin treatment site for bound chemical; the carcass minus the skin treatment area; the feces; the urine and the cage wash. In addition, as described by Susten *et al.*,[11] expired breath samples were collected on charcoal during the periods 0–15, 15–30, 30–60, 60–120, 120–180 and 180–240 min after the mice were placed in the metabolism cages. Carbon dioxide in expired breath was not collected, since preliminary tests showed that these ring-labeled compounds were not metabolized to $^{14}CO_2$ during the 4-h test. To estimate breath decay curves, the data were transformed to a log scale, a linear least squares equation was fit and the data were transformed back to the original scale. A more complex equation, such as that for a two-compartment model, could not be applied because of the limited number of time points. The treated skin area and the carcass were digested separately in known volumes of 1 N sodium hydroxide maintained at 60°C for at least 18 h. Aliquots of the digests were oxidized in a Packard Tricarb Oxidizer (Packard Instrument Company, Downers Grove, IL). Radioactivity was measured in all samples using a Beckman LS 8100 liquid scintillation spectrometer. Each sample was corrected for quenching, using an automatic external standardization procedure.[15] The total percentage of the nominal radioactive dose absorbed (Table 1) was determined by summing the percentages recovered in the excreta, carcass, expired breath and the skin application site fractions.

Physical constants data

The octanol/water partition coefficients presented in Table 3 were determined by adding 2 μl of test compound to a glass tube containing a mixture of 5 ml of distilled water and 5 ml of octanol. At the end of 24 h, the mixture was centrifuged at low speed to insure separation of the phases. Radioactivity in each solvent was determined and a ratio (partition coefficient) was also determined. Values in Table 3 for molecular weight, solubility, melting point, boiling point and vapor pressure were taken from the literature.[16–19] Vapor pressure at 30°C, the approximate surface skin temperature of the mice, was used. Evaporation rates were taken from the literature (benzene),[20] calculated from published relative evaporation rates (toluene and ethyl benzene)[21] or calculated from the vapor pressure and molecular weight (aniline).[22] Contact times during which absorption could occur (time it would take to evaporate the applied dose of material) were estimated for each substance. The applied doses (Table 2), converted to

Table 3. Physical constants and percutaneous absorption rate data

Compound	Octanol/water partition coefficient[a]	Molecular weight[b]	Solubility at 20°C (g l^{-1})	Melting point[b] (°C)	Boiling point[b] (°C)	Vapor pressure at 30°C[b]	Evaporation rate[c] (μg cm^{-2} s^{-1})	Absorption rate (μg cm^{-2} min^{-1})
Benzene	166	78	1.8	6	80	119	94.5	56
Toluene	499	92	0.5	−95	111	37	36.5	49
Ethylbenzene	221	106	0.2	−95	136	13	21.8	37
Aniline	8	93	35	− 6.3	185	0.9	0.8	2.3

[a] Determined as described in the text.
[b] From Refs 16–19.
[c] From Refs 20–22.

μg, were divided by the exposed surface area (0.8 cm^2) and the evaporation rates (Table 3). Relationships between the physical constants and the dermal absorption values were assessed using Spearman's correlation coefficient (r). Probabilities (P) of ≤ 0.05 were considered to be significant.

RESULTS

Data from the previously reported study on benzene[11] are included below for purposes of comparison with the present data. Since the data for benzene in Table 2 were calculated from the average values presented previously, ranges and standard errors are not included.

Percentages (mean ± SEM) of the nominal radioactive dose recovered in various compartments are shown in Table 1. Total recovery was > 90% in all cases, with the largest fractions appearing in the skin-depot (which includes the solid sorbent and the stainless-steel device). This would be expected because of the freedom of the compounds to evaporate into the solid sorbent. About 5–10% of the nominal radioactive dose was found in the guide needle, resulting in the following average amounts of compound applied to the skin: 3.94 mg for benzene, 3.89 mg for toluene, 4.10 mg for ethylbenzene and 4.68 mg for aniline (Table 2). Of these amounts, 39 μg (0.99%) of benzene, 90 μg (2.31%) of toluene, 148 μg (3.61%) of ethylbenzene and 223 μg (4.76%) of aniline were absorbed. It is obvious that the more volatile compounds, which evaporate sooner (Table 3) and have a shorter contact time in which absorption can take place, have smaller percentages of the applied dose absorbed.

Distribution of radioactivity into the various fractions comprising the 'absorbed radioactive dose' is shown in Table 4. The data show that those compounds absorbed to the greatest extent were distributed in greater percentages of the absorbed dose into non-volatile (excreta, carcass) compartments. This is to be expected, because the more volatile compounds will tend to be excreted in the expired breath more rapidly. The percentage of the absorbed dose collected in expired breath during 4 h after application was related to the vapor pressure of the compounds:

$$y = 8.09 + 0.28x \quad (r = 0.98)$$

where y = the percentage of absorbed dose recovered in expired breath during 4 h and
x = the vapor pressure (in mmHg) at 30°C.

The cumulative percentages of the absorbed doses found in the expired breath over time are shown in Fig. 1. For benzene, 11.2% of the absorbed dose was collected in expired breath during the first 15 min following application; for toluene, ca. 13.4% was collected during this time; for ethylbenzene and aniline, 9.3% and 1.0%, respectively, were absorbed during this time. Although data on the relationship between blood concentrations and exhaled breath concentrations of ethylbenzene and aniline were not found in the literature, it is generally known that exhaled breath concentrations of absorbed substances are proportional to blood concentrations, as reported for benzene[23] and toluene.[23,24] Thus, the data suggest that maximum blood concentrations were obtained some time during the first 15 min after application.

The concentrations of toluene, ethylbenzene and aniline in expired breath of individual mice are shown in Fig. 2. Data from one mouse in the ethylbenzene experiment are not included because that mouse absorbed such a large dose that the data obscured the general trend. For most mice, the excretion rate was fastest during the first 15 min. However, for four mice treated with toluene and five treated with ethylbenzene the breath excretion rate was faster during the second or third collection period, which is consistent with the observation of delayed absorption of toluene by human subjects reported by Sato and Nakajima.[25]

The expired breath decay curves (Fig. 3) all show a decreasing trend with time, which is consistent with a

Table 4. Percentage distribution of the absorbed dose following dermal application of [^{14}C]-labeled aromatic solvents

Compound	Percentage of absorbed dose[a]			
	Carcass[b]	Application site[b]	Expired breath	Excreta[c]
Benzene[d] (N=7)	22.6 ± 6	4.7 ± 2	40.1 ± 11	32.7 ± 7
Toluene (N=12)	15.4 ± 2	11.0 ± 3	20.5 ± 5	53.1 ±6
Ethylbenzene (N=11)	15.5 ± 2	4.5 ± 1	14.3 ± 6	65.6 ±5
Aniline (N=10)	43.4 ± 7	15.7 ± 3	4.5 ± 1	36.5 ± 9

[a] Values are means ± SEM.
[b] Tissues digested under vacuum in 1 N NaOH at 60°C. Values include the non-volatile and any trapped volatile fractions of the tissue digests.
[c] Excreta = sums of the means of percentages of applied dose recovered in urine and feces.
[d] From Ref. 11.

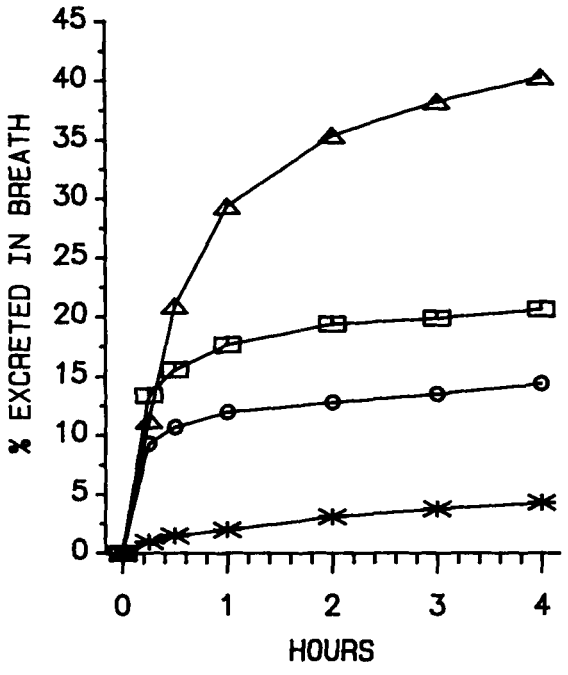

Figure 1. Cumulated percentages of absorbed dose recovered in breath following dermal application of undiluted [^{14}C)-labeled solvent: (△) benzene; (□) toluene; (○) ethylbenzene; (*) aniline.

model where the kinetics are dominated by the excretion rate after 15 min. Consistent with the short contact times estimated for benzene, toluene and ethylbenzene, absorption clearly had ceased at 15 min. Aniline had a longer estimated contact time; however, its decay curve had characteristics similar to those of the other compounds, suggesting a rapid initial absorption rate, after which the excretion rate exceeded the absorption rate. For all four compounds, there is an initial rapid decay followed by a more gradual one, suggesting a two-compartment model. By contrast, three-compartment models were described for benzene and toluene following inhalation exposure.[23] The first element of the three-compartment model, clearance of inhaled gas from the lungs, clearly does not apply to a percutaneous study.

Correlations between the percentage of the applied radioactive dose absorbed and the physical properties (Table 3) were significant for the two indices of volatility—vapor pressure and boiling point. Percutaneous absorption was inversely related to vapor pressure and positively related to boiling point, which is consistent with the relationship of evaporation rate to these parameters. This suggests that the evaporation rate could be used for evaluating percutaneous absorption of volatile chemicals.

Estimates of the contact times during which absorption could occur were ca. 1 min for benzene, 2 min for toluene, 5 min for ethylbenzene and 120 min for aniline (Table 2). These contact times are supportive of both the amounts absorbed and the expired breath data. The estimated contact time of 1 min for benzene is also consistent with the previously reported observation that all of the benzene recoverable in the skin-depot charcoal was present at 1.5 min, the first time at which it was measured after application.[12] The amounts absorbed were divided by the estimated contact times and the exposed surface area of 0.8 cm^2 and multiplied by 60 s min^{-1} to obtain absorption rates of 56, 49, 37 and 2.3 μg cm^{-2} min^{-1} for benzene, toluene, ethylbenzene and aniline, respectively (Table 2).

DISCUSSION

Bronaugh *et al.*[26] studied the permeability of pig, rat, hairless mouse and human skins *in vitro*, and also summarized their results and those of others as the ratio of animal to human permeability for each chemical. For hairless mice, ratios for eight of nine chemicals were between 0.5 and 2.0. On this basis, the next best species was the pig, with eight of thirteen ratios in this range. Thus, it would appear that the hairless mouse has potential as a useful animal model for conducting experiments that can be extrapolated to humans.

There have been only a few reports in the literature on percutaneous absorption of the subject compounds by human subjects. In early experiments, a known amount of the material was applied to a known area of the forearm under a watch glass and the amount of the compound that disappeared after 10–15 min was determined. Using this disappearance technique, quite rapid absorption rates were reported for toluene and ethylbenzene, ranging from 233 to 383 μg cm^{-2} min^{-1} for the former[18,19] and from 366 to 550 μg cm^{-2} min^{-1} for the latter.[7,9] In these reports the methodology was not well described, and evaporative losses during application or recovery of the unabsorbed material may have been a source of error. There were no independent measures of absorption such as analysis of blood and exhaled breath concentrations or urinary excretion of metabolites, to substantiate the rapidity of these rates compared to the rates found in hairless mice.

No other reports of percutaneous absorption of ethylbenzene were found in the literature. However, other studies with toluene, even though absorption rates cannot be estimated easily from the data, suggest much lower values. For example, the maximum concentration found in expired breath following immersion of both of their hands (ca. 900 cm^2 of exposed surface) in toluene for 10 min was 4.5 ppm (16 μg ℓ$^{-1}$),[27] compared to 20 ppm (71 μg l^{-1}) following a 30-min exposure to toluene by inhalation at 100 ppm.[24] This latter value (70 μg l^{-1}) was obtained when 48 mg of toluene had been absorbed. These data suggest that < 48 mg of toluene were absorbed from the 10-min immersion, an amount that is substantially less than the 2700 mg that would have been absorbed had an absorption rate of the order of 300 μg cm^{-2} min^{-1} been operating.

Similarly supportive of a slower absorption rate are concentrations of toluene in blood found in experiments in which one hand was immersed in toluene. A 5-min immersion yielded a concentration in venous blood of 45 μg l^{-1},[28] and a 30-min immersion yielded 180 μg l^{-1},[25] compared to ca. 400 μg l^{-1} when 48 mg of toluene had been absorbed by inhalation.[24] Had an absorption rate of 300 μg cm^{-2} min^{-1} been operating, 675 mg and 4050 mg of toluene would have been

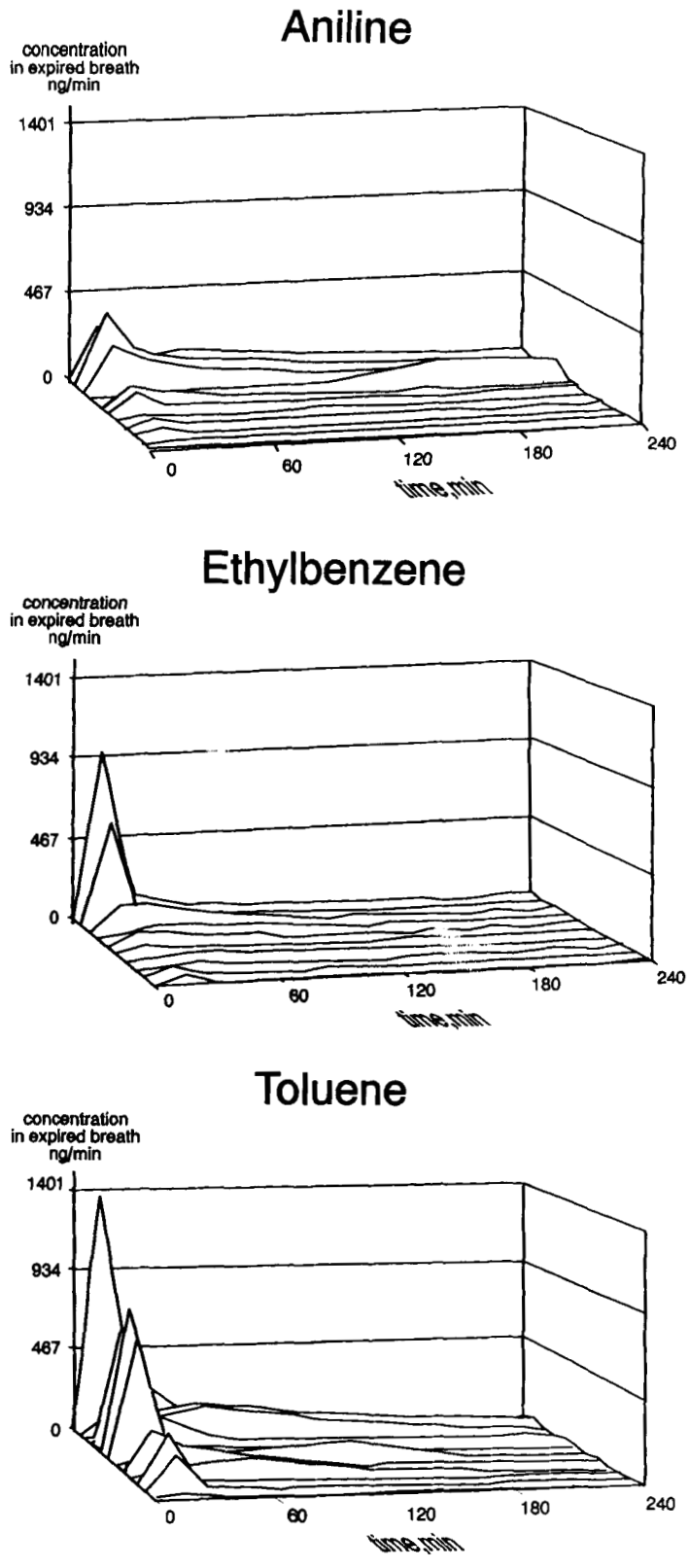

Figure 2. Breath excretion rates (ng min^{-1}) for individual mice during consecutive time periods following dermal application of toluene, ethylbenzene and aniline.

absorbed by the 5-min and 30-min immersion, respectively. Thus, it appears that the absorption rate for toluene found in the hairless mouse is much nearer a practical value for evaluating workplace exposures than the older value reported for human subjects. However, additional studies on both the hairless mouse and human subjects are needed to refine the estimate.

Hanke *et al.*[29] reported studies on percutaneous absorption of benzene by measuring its disappearance from the application site in one group of human

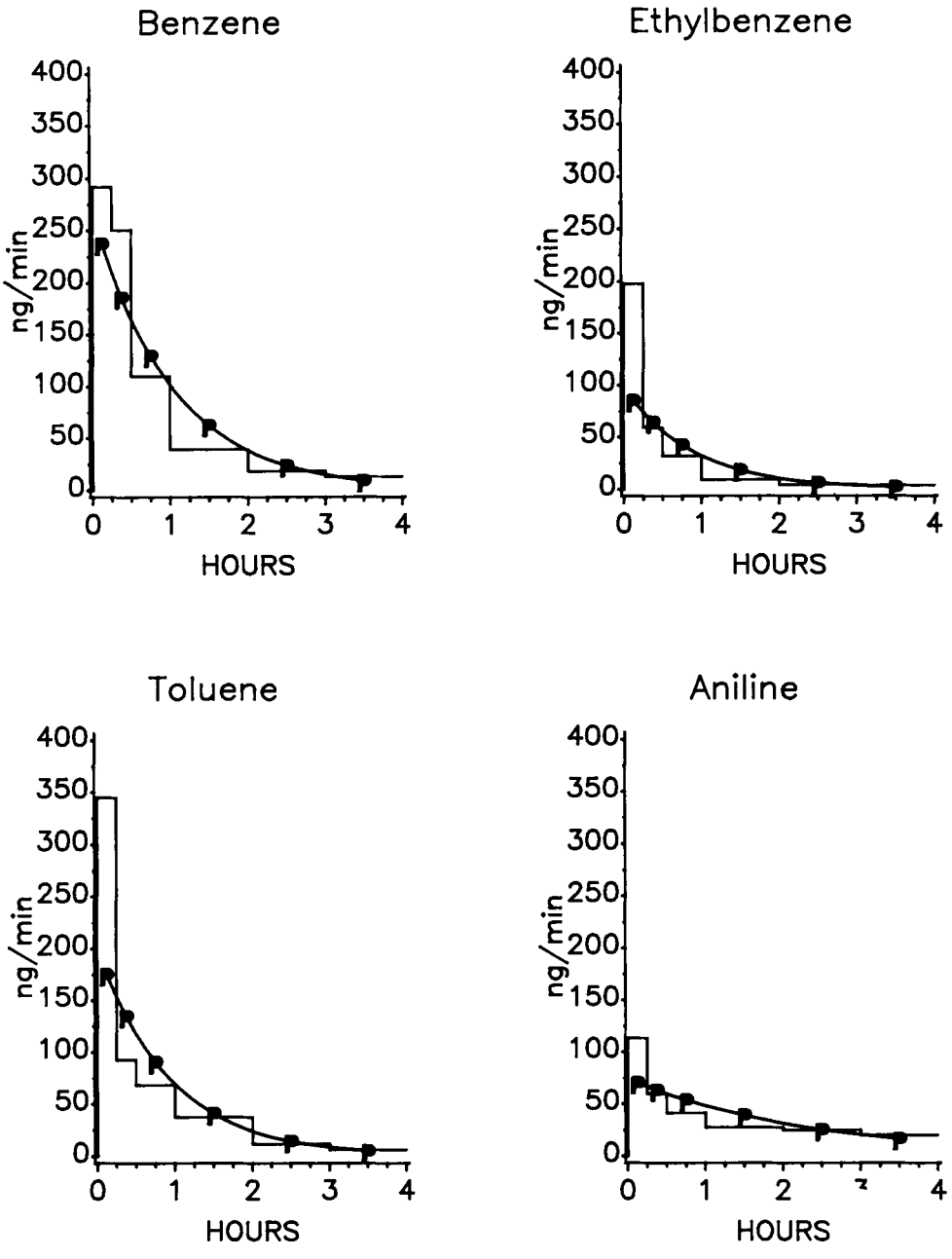

Figure 3. Average breath excretion rates (ng min^{-1}) during consecutive time periods (histogram) and estimated instantaneous excretion rates (P) following dermal application of benzene, toluene, ethylbenzene and aniline.

subjects and by analysis of excreted phenol in another group of subjects. In their report, the methodology is well described, evaporative losses should have been minimal and the skin should have been wet with benzene for the duration of the 75–120 min used to calculate the absorption rates. By the disappearance technique, these investigators found an absorption rate for benzene of 6.7 μg cm^{-2} min^{-1}, and from the excretion of phenol they estimated an absorption rate of 4 μg cm^{-2} min^{-1}. The slower rate that they obtained from phenol in urine may result, in part, from the investigators' assumption, derived from inhalation experiments, that 30% of the absorbed benzene was excreted as phenol. Under percutaneous absorption conditions, with no benzene in inhaled air, a relatively greater proportion might be excreted in the breath, as suggested by Sato and Nakajima[25] and by the hairless-mouse data. Nomiyama and Nomiyama[30] estimated that people excreted 17% of an inhaled dose of benzene in the breath, compared to at least 40% by hairless mice following percutaneous absorption. The percutaneous absorption rate obtained in hairless mice is about 10 times faster than the rate reported for humans. As suggested by data on methanol[31] and aniline,[10] the more rapid rate obtained in hairless mice over a brief time from a single application may not be maintained throughout the longer contact times used by Hanke *et al.*[29]

For aniline, the reported values range from 3 to 77 μg cm^{-2} min^{-1}.[6,10] Although the techniques varied, in both reports 0.25 mg of aniline was applied to a 25 cm^2 area of the forearm and covered to prevent

evaporation. The more rapid absorption rates were found with 30-min exposures and with 1-h exposures. The slower rate, which was found in 5-h exposures, is comparable to the value found in hairless mice with an estimated exposure time of 2 h. In the 30-min exposures, absorption rates were determined from the amount of aniline that disappeared from the amount applied. In the other experiments, the amount of aniline absorbed was determined from the excretion of *p*-aminophenol in urine. Perhaps because of the low volatility of aniline and slow excretion in breath, the experiments give similar results. Thus, an average absorption rate for prolonged skin contact with aniline of 2–3 $\mu g\ cm^{-2}\ min^{-1}$ seems reasonable. However, additional research is needed on the time-course of absorption because the apparently more rapid initial rate is relevant to designing guidelines for protecting the worker from undue absorption.

Estimated amounts of the four compounds that would be absorbed from inhalation at selected exposure concentrations are presented in Table 5, along with the product of exposed skin surface area and exposure time that would result in the same absorbed dose if the absorption rates determined from the hairless mouse applied. The selected exposure concentrations are the respective current US Federal limits for time-weighted average, 8-h exposures.[1] The allowable daily doses were calculated by assuming an 8-h air intake of 10 m^3 and pulmonary retentions of 60% for benzene, toluene and ethylbenzene and 90% for aniline.[32] The equivalent dermal exposure for each compound was calculated by dividing the allowable daily dose by the dermal absorption rate. For example a person at light work breathing 10 m^3 of air containing 375 mg m^{-3} of toluene would inhale 3750 mg and would retain about 60%, or 2250 mg, of the allowable daily dose. Converting this dose to μg and dividing by the absorption rate (49 $\mu g\ cm^{-2}\ min^{-1}$) gives an estimate of 46 000 $cm^2 \cdot$ min of dermal exposure as being equivalent to an 8-h inhalation exposure at the current US Federal exposure limit. Assuming that this equivalency holds for all combinations of exposed surface areas and exposure times, then any combination, e.g. 1000 cm^2 and 46 min, or 100 cm^2 and 460 min, that yields a product of 46 000 $cm^2 \cdot$ min would give an equivalent dose.

Assuming that the absorption rates determined in this study are applicable, the data in Table 5 can be used to evaluate workplace situations. The amounts of compound absorbed following dermal contact with undiluted ethylbenzene or toluene could be important in operations where one or two hands (500–1000 cm^2) were frequently (at 5–10 min intervals) wet by the solvents, or if a large splash should saturate the clothing. For example, dipping one hand in a container of toluene might be equivalent to applying 4 $\mu g\ cm^{-2}$ of toluene to the skin.[5] This is approximately the application rate used on the hairless mice, so the contact time would be about the same, or ca. 2 min. If the hand was wet in this manner once every 10 min during a working day (480 min), the total contact time would be 48 dips × 2 min per dip, or 96 min. The surface area of one hand might be 500 cm^2. Combining the estimated contact time (96 min) and exposed surface area (500 cm^2) yields 48 000 $cm^2 \cdot$ min, which is greater than the equivalency factor tabulated in Table 5. Percutaneous absorption of liquid aniline may be considerable because of its slow evaporation rate. For example, absorption over a 2-h period, the estimated contact time, from an area of ca. 330 cm^2 wet with aniline would provide the equivalent allowable dose, suggesting the use of protective gloves and washing contaminated skin as soon as possible. Dermal exposure to benzene, even when it is present only as a contaminant in a solvent system, may contribute substantially to the overall body burden of benzene when compared to potential absorption that might occur via inhalation.[11]

Finally, with respect to relationships between chemical/physical characteristics and percutaneous absorption, the results of this study clearly show that, for this small group of benzene derivatives, percutaneous absorption under conditions that simulate a single brief contact to intact skin is significantly correlated with each of two indices of volatility, i.e. vapor pressure and boiling point. The more volatile compounds have a more rapid absorption rate, but because they evaporate from the skin more rapidly, the total amount

Table 5. Dermal exposures to liquid benzene, toluene, ethylbenzene and aniline equivalent to selected inhalation exposures

Compound	Selected[a] exposure concentration (mg m^{-3})	Allowable[b] daily dose (mg)	Dermal[c] absorption rate ($\mu g\ cm^{-2}\ min^{-1}$)	Equivalent[d] dermal exposure ($cm^2 \cdot$ min)
Benzene	0.32	2	56	34
Toluene	375	2250	49	45900
Ethylbenzene	435	2610	37	70500
Aniline	10	90	2.3	39800

[a] From Ref. 1.
[b] Assumes an 8-h air intake of 10 m^3 and pulmonary retentions of 60% for benzene, toluene and ethylbenzene and 90% for aniline. Calculated value for benzene was 1.92.
[c] From Table 2. Value for aniline used in the calculations was 2.26.
[d] Calculated by dividing allowable daily dose (μg) by dermal absorption rate. Values are rounded.

absorbed per application is less. The more volatile compounds were also excreted more rapidly in the exhaled breath and accumulated to a lesser extent in the tissue and excreta. Since substances are absorbed through the skin into venous blood and pass through the lungs prior to being distributed to the tissues, future research needs to address the issue of the net absorption rate, e.g. absorption through the skin minus pulmonary excretion. The hairless-mouse data suggest this approach but, because of the long interval between breath samples, could not contribute to such an evaluation.

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