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Percutaneous Penetration of Benzene in Hairless Mice: An Estimate of Dermal Absorption During Tire-Building Operations

Allan S. Susten, PhD, Barbara L. Dames, BS, JeAnne R. Burg, PhD, and Richard W. Niemeier, PhD

Repeated skin contact with solvents containing as much as 0.5% benzene is common in workers building regular bias passenger tires. To estimate the amount of benzene absorbed through the skin of these workers, a series of in vivo studies was conducted in hairless mice. Percutaneous absorption, following single dermal applications of ^{14}C-benzene contained in rubber solvent at a concentration of 0.5% (v/v), was calculated directly from the sums of radioactivity found in excreta, expired breath, and the carcass. Data from the study, together with observations made during tire-building operations, suggest that a worker could absorb 4–8 mg of benzene daily through the skin. This compares to 14 mg per day via inhalation at the NIOSH recommended standard of 1 ppm. Thus dermal absorption could contribute from 20–40% of the total benzene dose of these workers.

Key words: benzene, solvents, occupational hazards, tire industry, percutaneous absorption, petroleum naphtha

INTRODUCTION

Employees engaged in manual tire-building operations have repeated exposures of the skin to petroleum naphthas (rubber solvent) which contain trace amounts of benzene, a known carcinogen. Although the concentration of benzene in rubber solvent may vary from facility to facility, the Rubber Manufacturers Association has indicated that the maximum concentration of benzene rarely exceeds 0.5% [Ryan, 1983]. Since the use of gloves during this operation may represent a safety hazard and interfere with crafting good-quality tires, exposure to these solvents is often considered unavoidable. Therefore, information on the amount of benzene that may

Department of Health and Human Services, U.S. Public Health Service Centers for Disease Control, National Institute for Occupational Safety and Health, Division of Biomedical and Behavioral Science, Experimental Toxicology Branch, Cincinnati, OH.

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Address reprint requests to Allan S. Susten, Dept. of Health and Human Services, U.S. Public Health Service Centers for Disease Control, Natl. Inst. for Occupational Safety and Health, Division of Biomedical and Behavioral Science, Experimental Toxicology Branch, 4676 Columbia Pkwy, Cincinnati, OH 45226.

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be absorbed through the skin of workers is required for accurate assessment of risk. Indeed, the question of how much benzene is absorbed by exposure of the skin to liquid solvents containing benzene is one of the outstanding issues concerning the Occupational Safety and Health Administration (OSHA) during its deliberations on the development of a permanent standard for benzene [Auchter, 1983].

The percutaneous absorption of undiluted benzene has been studied, in vivo, in animals [Maibach and Anjo, 1981; Boman et al, 1982; Franz, 1983; Susten et al, 1984a] and in man [Cesaro, 1946; Conca and Maltagliati, 1955; Hanke et al, 1961; Maibach, 1980a,b; Franz, 1983]. However, only Maibach and Anjo [1981] have reported on benzene absorption through the skin following the dermal application of a rubber solvent mixture containing trace amounts (0.35%) of benzene.

This report summarizes a series of studies, conducted at the request of OSHA, which measured the amount of benzene absorbed through the skin of hairless mice following the application of either undiluted benzene or rubber solvent containing 0.5% benzene, (v/v). The method used in these studies incorporated a skin-depot designed to capture test substances which evaporated from the skin application site, and a glass metabolism cage system for the capture of urine, feces, and expired breath. Total absorption was determined from the sum of radioactivity found in excreta, expired breath, skin application site, and the animal carcass. The 0.5% concentration of benzene in the rubber solvent used in these studies represents the upper limit of contamination commonly found in solvents used in tire-building operations in the United States [Ryan, 1983]. In addition to these skin absorption experiments, a visit to a tire manufacturer was conducted in order to observe tire-building operations. This visit provided information on work practices regarding exposure to rubber solvent.

MATERIALS AND METHODS

Treatment Solutions

Rubber solvent used as a tackifier for tire-building and repair operations was obtained from the Goodrich Tire Company, Ft. Wayne, Indiana. Analysis of the rubber solvent by GC/MS showed the rubber solvent to be composed largely of C4-C7 aliphatic hydrocarbons and a small percentage of aromatics including benzene (0.09%), toluene (5.7%), and xylenes (0.4%).

Ampoules containing 2.5 mCi of ^{14}C-benzene (uniformly ring labeled, specific activity 100.5 mCi/mmol, lot numbers C6 and C7), were purchased from the Amersham Corporation, Arlington Heights, Illinois. The radiochemical and chemical purities of the labeled benzene were greater than 99% according to the manufacturer. Prior to use, the contents of the ampoules were diluted to 5 mL with either reagent grade benzene (Baker Resi-Analyzed Baker Chemical Company, Phillipsburg, New Jersey) or rubber solvent.

The resulting ^{14}C-labeled solutions, each containing approximately 0.5 mCi/ml were (1) an undiluted benzene treatment solution and (2) a rubber solvent "stock" solution containing 0.14% benzene by volume. A sufficient quantity of the ^{14}C-benzene treatment solution was then added to a portion of the radiolabeled rubber solvent "stock" solution to produce a treatment solution containing 0.5% benzene (v/v). Levels of radioactivity are expressed here on the basis of 5 uL since that was the volume of the treatment solutions applied. The ^{14}C-benzene and ^{14}C-rubber

solvent treatment solutions contained approximately 3.18 μCi and 2.56 μCi per 5 uL, respectively. These levels of radioactivity represent the averages determined at the time of skin application for each experiment. When applied to a 25-gm mouse, this is equivalent to approximately 127 μCi/kg for the undiluted benzene and 102 μCi/kg for the rubber solvent. The radioactive dose levels were selected when preliminary studies indicated that the amount of ^{14}C recovered in the carcass following the topical administration of ^{14}C-benzene at a dose level of 10 μCi/kg did not produce count rates sufficiently over background (greater than twice) to be considered accurate.

Animals

Male albino hairless mice (HRS/J) were purchased from the Jackson Laboratories, Bar Harbor, Maine. Upon arrival, they were placed in individual shoe-box plastic cages with sawdust bedding and quarantined for at least 1 week. The mice were maintained in environmentally controlled facilities with free access to Purina Mouse Chow and tap water. Each was identified by an individual ear tag to facilitate randomization and record keeping. At the time of their use the mice were weighed (23–32 gm) and visually inspected for skin damage. Mice were not used in the study if blemishes were observed in the treatment area. For each experiment, two of the mice were randomly selected. A total of ten experiments were conducted, four with undiluted benzene and six with rubber solvent.

The choice of the hairless mouse was based on five factors: (a) prior removal of hair is unnecessary; (b) compared to those of heavily haired animals, the size and density of hair follicles are more like those of humans [Bronaugh et al, 1982]; (c) relatively good agreement for skin permeabilities (within an order of magnitude) to a variety of compounds has been shown, in vitro, between hairless mouse skin and human skin [Bronaugh et al, 1982; Stoughton, 1975]; (d) the relatively inexpensive cost and ease of handling of mice, compared to larger animals such as the pig or monkey; and (e) the ability to use larger numbers of animals to increase the power of the experiments.

Skin-Depot Attachment

Five to ten minutes prior to the dermal application of benzene or rubber solvent, a stainless steel skin-depot (Fig. 1) containing approximately 100 mg of activated charcoal was glued to the mid-dorsal skin over the thoracic region of the spinal cord using cyanoacrylate adhesive (Duro brand, Super-Glue, Loctite Corporation, Cleveland, Ohio). To facilitate the attachment, the mice were anesthetized with 50% carbon dioxide in air. Attachment of the skin-depot was accomplished by placing the mice in a prone position on a flat surface with their hindlegs and forelimbs positioned to the side. The skin-depot, with its guide-needle in place (Fig. 1) and with glue applied to the rim, was carefully placed on the skin and held firmly to prevent it from sliding or moving from the original site of contact. Although attachments were normally complete within 1 minute, the mice were held immobile or anesthetized for an additional 3–5 minutes to secure the skin-depot's attachment. During this 3–5-minute period, the seal was inspected for gaps and additional glue was added where necessary.

The purpose of the skin-depot used in these studies was to circumscribe a defined skin area of approximately 0.8 cm^2, capture any test substance that evaporated from the skin, prevent ^{14}C-labeled flaked or shed skin from contaminating urine and feces, and to prevent the evaporated test material from contaminating the expired

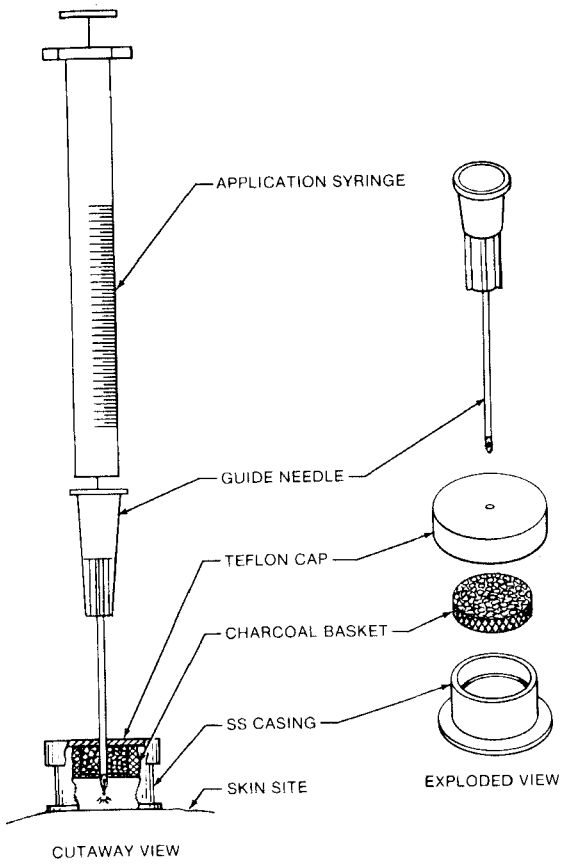

Fig. 1. Diagram of skin-depot showing components (exploded view) and method for administering test substance to the treatment site (cutaway view).

breath. The capacity of the skin-depot to capture and hold 5 μL of test material had been tested previously under ideal conditions with benzene. In that study, the procedures used were the same as those described above except that the mice were killed with carbon dioxide just prior to the administration of labeled benzene, placed into metabolism cages, and handled as described below. No radioactivity was found in the charcoal tubes normally used to capture expired breath while more than 90% of the label was recovered in the skin-depots [Susten et al, 1984b]. Thus, under ideal conditions, the charcoal in the skin-depot was found to be capable of adsorbing all vaporized test material. These results were not surprising given the demonstrated adsorptive capacity of the charcoal, about 7 mg of benzene when used for air sampling at a rate of 0.2 L/minute [Eller, 1984]. The 7 mg capacity is in excess of the 4.4 mg (5 μL) dose of benzene used in these experiments.

Treatment Procedure

Dermal application of 5 μL of treatment solution was accomplished by inserting a 10 μL GC syringe (Hamilton Model No. 701) containing 5 μL of the test substance through the bore of a 21 gauge guide needle until the blunted tip of the syringe needle touched the skin. The syringe was then withdrawn slightly (Fig. 1) and, using a

solvent flush technique (a technique which utilizes a layer of air and unlabeled solvent in the syringe directly above the treatment solution), the contents of the syringe were expelled onto the skin. The syringe and the guide needle were then carefully withdrawn from the skin-depot and the treated mouse was quickly placed into a glass metabolism cage (Bellacour Co., New York). The guide needle was placed immediately into scintillation fluid for determination of any radioactivity that adhered to the needle.

The radioactivity delivered for each experiment was determined by injecting 5 μL of the test chemical into each of three 10 mL volumetric flasks containing toluene. Triplicate 0.1 mL aliquots were taken from each flask to determine the average total ^{14}C in each flask. The mean for the three flasks was used as the applied radioactive dose.

For each experiment, two glass metabolism cage units were mounted in parallel. Air, prefiltered to remove organic chemicals, moisture, and carbon-dioxide, was drawn through the system at a rate of 200–225 mL/minute. In-line charcoal sampling tubes (100/50, Catalog No. 226-01, SKC Inc., Eighty-Four, Pennsylvania) were used to capture expired breath at 0–15, 15–30, 30–60, 60–120, 120–180, and 180–240 minutes after the test materials were applied to the skin. Negative pressure inside the cages was maintained at 1.8–2.0 inches of water. The animals were not restrained during their 4-hour residence in the cage. Food and water were not provided.

Experience had shown us that, during experiments, areas of detachment between the skin-depot and the skin could occur, usually between 55 to 150 minutes after the animals were placed into the metabolism cages. We were therefore concerned that, should one of these gaps progress to the point that the treated area became uncovered prior to the test material's being absorbed into the animal or adsorbed to the charcoal, erroneously elevated levels of ^{14}C might occur in the expired breath fraction. This could also result in falsely decreased levels of radioactivity in the carcass, urine, and skin-depot. Thus, skin-depot attachments were observed throughout the experiment. If a gap progressed into the treated area, the skin-depot was then pulled off, removed from the metabolism cage, and placed in toluene to recover the radioactivity. The data for that mouse were carefully scrutinized for deviations.

Factors which would tend to obviate the concern about seal breakage are the small volumes of test substance applied, the volatility of the benzene and rubber solvent, the affinity of the charcoal for the benzene, and the rapidity with which benzene is absorbed through the skin. Tests in our laboratories have shown that capture of benzene by the charcoal in skin-depots attached to the backs of mice was maximal at 1.5 minutes after the chemical was applied and that the level of adsorbed benzene remained unchanged for at least 2.5 hours (duration of the test) [Susten et al, 1984b].

Sample Collection and Analysis

At the termination of each experiment, the mice were killed by introducing carbon dioxide into the cages. Skin-depots were removed intact with the cap on and were placed into 25 mL of toluene for desorption of the labeled benzene from the charcoal. Triplicate 1 mL aliquots were taken from the toluene solution and placed into scintillation vials containing Permafluor V scintillation fluid (Packard Instrument Company) for analyses.

The skin application sites were dissected from the carcass and placed individually into gas washing bottles, as were the carcasses. Known volumes of 1 N sodium hydroxide had been added to these washing bottles. Digestion of the samples was accomplished with constant stirring under vacuum at 55°C, for approximately 18 hours. Any labeled materials released during the digestion process were trapped using in-line charcoal sampling tubes (400/200, Catalog No. 226-09, SKC Inc., Eighty-four, Pennsylvania). The charcoal contained in the front and back sections of these tubes, as well as the tubes used to capture expired breath, was desorbed separately; aliquots of the desorption fluid were processed in the same manner as those from the skin-depot. Since the efficiency of the desorption process was found to exceed 98%, a correction factor for incomplete desorption of ^{14}C from the skin-depots and charcoal tubes was not utilized, ie, desorption was considered to be 100% efficient.

Urine and feces were collected from their respective cage reservoirs. Fecal pellets and weighed aliquots of the skin application site and carcass digests were oxidized in a Packard Tricarb Oxidizer using Carbosorb (Packard Instrument Company) as a trapping agent. The urine was diluted to 5 mL with a 50/50 methanol/water mixture. To determine the residual radioactivity remaining on the wire-mesh screen and the urine/feces separator, a small quantity of the methanol/water mixture was used to wash the cages. This wash was collected and brought to a total volume of 10 mL. Triplicate 1 mL aliquots of diluted urine and cage washings were pipetted directly into scintillation vials containing 10 mL Permafluor V and 3 mL Carbosorb.

Radioactivity was measured in all samples by a Beckman LS 8100 Liquid Scintillation Spectrometer. Each sample was corrected for quenching, using an automatic external standardization procedure based on the H number concept [Horrocks, 1977].

Statistical Analysis

For each mouse, the percent of the applied dose recovered was determined for the skin-depot, the guide needle, and the fractions that constituted the absorbed dose (excreta, expired breath, skin application site, and the carcass); when summed, these values represented total recovery (Table I). Distribution of the absorbed ^{14}C into the various tissue categories was also evaluated (Table II). For each variable, the mean, standard error, and range were calculated. The two treatment groups were compared on each calculated variable using a t-test.

TABLE I. Distribution of Radioactivity Into Various Sampling Compartments Following the Dermal Application of ^{14}C-Benzene and ^{14}C-Benzene Contained in Rubber Solvent at a Concentration of 0.5% (v/v)*

Test materials	Means of percents of applied dose recovered[a]			
	Absorbed[a]	Skin depot	Guide needle	Total recovered
Undiluted benzene (N = 7)	0.89 ± 0.3 (0.13–2.12)	79.5 ± 3.6 (66.6–96.3)	10.4 ± 2.5 (0.06–19.1)	90.8 ± 1.8 (82.3–98.4)
Rubber solvent (N = 12)	0.88 ± 0.2 (0.16–2.12)	85.0 ± 2.8 (68.2–97.9)	7.8 ± 2.2 (0.02–23.3)	93.7 ± 0.8 (89.8–99.5)

*Values are mean ± SE. Range is in parentheses.

[a]Absorbed, percent of dose applied which was recovered in excreta, carcass, skin application site, and expired breath.

TABLE II. Percentage Distribution of the Absorbed Dose (^{14}C) Into Various Fractions Following the Dermal Application of ^{14}C-Benzene and ^{14}C-Benzene Contained in Rubber Solvent at a Concentration 0.5%*

Test material	Percents of absorbed dose in various fractions			
	Carcass	Application site	Expired air	Excreta[a]
Undiluted benzene (N = 7)	22.6 ± 6.4 (3.9–45.8)	4.7 ± 1.7 (0.28–13.3)	40.1 ± 10.6 (14.8–85.2)	32.7 ± 6.5 (9.67–64.6)
Rubber solvent (N = 12)	21.9 ± 5.7 (3.4–80)	7.2 ± 1.9 (0–21)	33.9 ± 6.1 (13.8–81.6)	35.0 ± 4.7 (0.9–56.2)

*Values are mean ± SE. Range is in parentheses.
[a]Excreta, the sums of the means of the percents absorbed for urine, cage washings, and feces.

RESULTS

The data from one mouse in the benzene experiments were discarded because of excessively high absorption of ^{14}C radioactivity (5%) and low recovery of radiolabel in the skin-depot (62%), perhaps indicative of an early break in the seal or leakage through the charcoal. In addition to the discarded animal, problems with adherence of the cells to skin developed in seven of the 12 rubber solvent experiments and two of the remaining seven benzene experiments. As noted above, the data were carefully examined for increases in total absorbed, in the percentages of the dose found in expired air, urine, feces, and cage washings, and for decreases in percent of dose present in the skin-depot, carcass, and skin application sites. No consistent or substantial differences were found in these values in mice with intact attachments compared to those in which the attachments did not last 4 hours. Because of the volatility of benzene, it is probable that the evaporation and subsequent adsorption of the benzene to the charcoal of the skin-depot was complete within 2–5 minutes of application. We therefore used all data with the single exception of the one benzene-treated animal noted above.

Table I summarizes the percentages of applied dose recovered in the various sample categories. Less than 1% of the applied dose was found to be absorbed in each treatment group (0.89 and 0.88% for benzene and rubber solvent, respectively). Total recoveries of radioactive label were greater than 90% (Table I). No significant differences were observed between treatment groups for any of the sample categories.

Radioactivity remaining in the guide needle was not available for dermal absorption. Therefore, the percentages reported for the absorbed fraction in Table I, which were based on applied dose, were not representative of the absorption potential of test materials which actually contact the skin. For that reason, the absorbed fraction was also calculated on the basis of available dose (applied dose minus the percentage of the applied dose remaining in the guide needle). The percentages (mean ± SE) of available dose which were absorbed for the benzene and rubber solvent experiments were 0.98 ± 0.3 and 0.97 ± 0.2, respectively. Thus, absorption, at least in terms of either percent of applied dose or percent of available dose was not affected by concentrations of benzene in the material applied.

Table II summarizes the distribution of absorbed ^{14}C among the fractions which constitute the absorbed benzene. The relative distributions were similar for the benzene and rubber solvent treatment groups. In each case, most of the absorbed radioactivity was found in expired breath and excreta, together about 70%. About

22% was found in the carcass and less than 8% of the absorbed dose was recovered in the skin removed from the application site.

The percentages of the total ^{14}C recovered in expired breath at the various sampling intervals are shown in Figure 2. While the percentages found during the 30–60-minute sampling interval were significantly greater ($p < 0.05$) for the benzene experiments than for the rubber solvent experiments, this difference is not considered to be a biologically significant finding. The fact that peak recoveries (30–40% of the ^{14}C in breath) occurred in each case within the first 15 minutes is indicative of the rapid absorption of benzene through the skin. Because of benzene's volatility and the ease in which it adsorbs to charcoal, it is likely that the percutaneous penetration of the chemical was also completed within this time period.

DISCUSSION

The results of these studies in hairless mice under conditions which simulate a brief contact show that undiluted benzene, as well as benzene contained in rubber solvent, is absorbed quickly and that it is absorbed to the extent of approximately 1% of the applied dose which was available for absorption.

Observations made at a tire-building operation showed that a worker may build between 150 and 170 bias ply passenger tires, in a period of 6 to 8 hours. To assure tackiness of the rubber, workers applied rubber solvent using various kinds of applicators, some of which had handles. Usually the solvent was applied once to each side of the uncured tire during the process. Often the workers used the right hand to apply solvent to the right side of the tire core and the left hand was used for the left side; thus, both hands were exposed during this operation. The contact time of the hands with the applicators was approximately 3–5 seconds. Inspection of the hands of

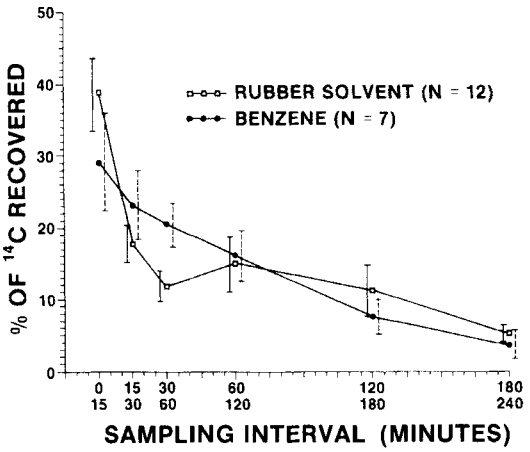

Fig. 2. Percentages of total ^{14}C recovered in breath at various sampling intervals following the dermal application of ^{14}C-benzene and ^{14}C-benzene contained in rubber solvent at a concentration of 0.5% (v/v). The points show means ± S.E. The difference between the benzene and rubber solvent was significant ($p < 0.05$) only at the 30-to-60-minute sampling interval (t-test).

workers engaged in this operation showed an area of about 75 cm^2 of the palmar surfaces of the fingers of both hands as well as the callus portion of the palms to be dry, cracked, and fissured. These skin injuries, however, were not universal and in part were dependent on the technique of the individual craftsman.

The results from the skin absorption study and the observations of the workers were used together to estimate the amount of benzene absorbed by exposure to rubber solvent using the following assumptions: (1) a worker will in the course of an 8-hour day have 150 contacts with the rubber solvent; (2) the total surface area involved amounts to 150 cm^2 (75 cm^2 per hand) and includes the palmar surfaces of the fingers of both hands and the callus portion of the palms; (3) each cm^2 area of skin will be covered by approximately 6.25 μL of the solvent; and (4) absorption of benzene from a rubber solvent containing 0.5% benzene can be expected to be approximately 1%. These calculations, shown in Table III, indicate that workers performing this operation might absorb 4 to 8 mg of benzene per day. This compares to an estimate of 14 mg per day that would be absorbed from inhalation by a worker exposed to benzene at the 1-ppm limit recommended by NIOSH [1976].

Since repeated dermal contact with rubber solvents occurs in the work place, estimates of dermal absorption based on studies using repeated application protocols are desired. One such estimate has been reported by Johnson [1979] and is discussed below in relation to the present estimate. Our methodology, however, which utilizes metabolism cages maintained under constant negative pressure for the capture of expired breath, does not lend itself to repeated application protocols. Consideration is being given to modifying the design of the skin-depot and collection systems to permit multiple application studies of volatile compounds.

The estimate by Johnson [1979] of the amount of benzene absorbed through the skin of workers exposed to rubber solvent was based on a series of studies conducted by Maibach [1979] for the Rubber Manufacturers Association. The protocol involved a total of ten separate applications (one application every 10 minutes over 2.5 hours) of a rubber solvent containing 0.35% benzene to the forearms of Rhesus monkeys. Benzene penetration was reported as 0.309% of the applied benzene dose [Maibach, 1979]. (These data were later reported as 0.43% Maibach and Anjo [1981]). From the data, Johnson calculated that approximately 0.4 to 0.9 mg of benzene would be

TABLE III. The Estimated Range of Benzene Dermally Absorbed Following Repeated Skin Contact With Rubber Solvent Containing 0.5% Benzene

Exposure conditions				
Benzene per unit[a] area of skin (mg/cm^2)	Surface area[b] exposed (cm^2)	Total No. of[c] daily exposures	Percent of available[d] benzene absorbed	Total absorbed (mg)
0.0275	150	150	0.97 ($\pm$0.19)	6.0[e] (3.7–8.4)

[a]Calculated as follows: volume (μL) per unit area (cm^2) of skin (6.25) × percent of benzene in rubber solvent (0.5 percent v/v) × specific gravity of benzene (0.88) = milligrams of benzene per unit area of skin.

[b]Assumes exposure of 1/3 of palmar surface of both hands; palmar surface assumed to be 225 cm^2.

[c]Based on daily quotas for workers making regular bias ply passenger tires.

[d]Based on the mean ($\pm$S.E.) of the percent of available dose absorbed for rubber solvent.

[e]Mean and calculated range at the 95% confidence limits.

absorbed through the skin during an 8-hour workday. His estimate was based on the following assumptions: (1) 88 μL of solvent will cover 2 square inches (12.9 cm^2) of skin (6.82 $\mu L/cm^2$)—an assumption based on data reported by B.F. Goodrich [1979]; (2) one-third of the palm of one hand (approximately 65 cm^2) will come in contact with the solvent 30 times a day; and (3) palmar absorption for benzene is 7.5 times greater than absorption through the forearm (0.309%) of the monkey. Had Johnson used 150 applications per day and two hands exposed, his estimates would have been 4 to 9 mg per day. Considering that different species and different methodologies were used, the estimate obtained by Johnson [1979] and the estimate obtained from the present study are in reasonable agreement.

The data used by Johnson [1979] and by us to obtain estimates of worker exposure were obtained from animals with apparently intact skin. Maibach and Anjo [1981] have reported that absorption of benzene applied to cellophane-stripped skin and skin repeatedly exposed to benzene was increased about fivefold. Since the hands of several of the workers we observed had damaged skin, their potential for benzene absorption could have been substantially greater than the estimated 4 to 9 mg per day.

When the data from the present study are compared (on the basis of percent of applied dose absorbed) to data derived from other animal species, the results suggest that dermal absorption of a single dose of undiluted benzene through the skin of the hairless mouse is some 5 to 7 times greater than in monkeys and about 10–18 times greater than in the minipig and human, respectively (Table IV). Similarly, the absorption of benzene from rubber solvent, reported as 0.88% in the present study, is likewise higher than the 0.08 and 0.65% reported by Maibach and Anjo [1981] following single applications of a rubber solvent containing approximately 0.35% benzene to the Rhesus monkey forearm and palm, respectively.

Although these comparisons are useful, one should be aware of the problems that exist in comparing data reported as percent of dose absorbed. For example, differences in specific dose (mg/cm^2), radioactive dose ($\mu Ci/kg$), percent of the total body surface area exposed, and duration of exposure can all influence the observed percent of applied dose absorbed.

The hairless mouse model used in these studies was also evaluated by comparing the ratios of absorption between different compounds under similar methods of study.

TABLE IV. Comparison of In Vivo Dermal Absorption of Liquid Benzene (% Applied Dose)

Species	Radioactive[a] dose ($\mu Ci/kg$)	Absorbed % of applied dose (Mean ± SD)[b]	Source
Hairless Mouse (back)	100.0	0.89 ± 0.65 (7)[b]	Susten et al [present study]
Monkey (forearm)	0.6	0.17 ± 0.14 (3)	Maibach and Anjo [1981]
Monkey (back)	13.3	0.14 ± 0.08 (6)	Franz [1983]
Human (palm)	0.1	0.13 ± 0.04 (4)	Maibach [1980b]
Mini-pig (back)	10.0	0.09 ± 0.04 (2)	Franz [1983]
Human (forearm)	0.06	0.07 ± 0.04 (4)	Maibach [1980a]
Human (back)	1.3	0.05 ± 0.05 (4)	Franz [1983]

[a]Weights (kg) were assumed to be as follows: hairless mouse, 0.025; Rhesus monkey, 7.5; mini-pig, 10; human, 80. Data calculated on the basis of information provided by the original reports.
[b]Values in parentheses are numbers of subjects.

Franz [1980], using excretion analysis procedures in monkeys, presented data showing that the absorption of toluene was about twice (2.2) that reported for benzene (0.44 vs. 0.20% of applied dose, respectively) (Table V). Approximately the same ratio (2.3) was calculated for the absorption of toluene in the hairless mouse as compared to the absorption of benzene in the same species (Table V). Similarly, if one compares the absorption of benzene and toluene across species (ie, hairless mouse vs. monkey), ratios of 4.5 and 4.7 are calculated for benzene and toluene, respectively (Table V). Thus similar patterns of absorption were observed in these species regardless of the experimental approach.

Some of the quantitative differences of absorption noted above may also be due to experimental approach. The indirect excretion analysis method of Feldman and Maibach [1965, 1970] measures total radioactivity appearing in excreta (urine and feces), usually over a 5-day period following dermal exposure. To calculate total amount of labeled compound absorbed, the method requires the use of a correction factor based on the amount of radioactivity appearing in the excreta following a parenteral administration of the test compound. However, the assumption required, ie, that the pharmacokinetics of parenterally administered compound are the same as dermally absorbed compound, may not always be valid, especially for compounds which are very slowly eliminated [Franz, 1975; Shah and Guthrie, 1983]. The excretion analysis method, as generally performed, does not provide data on absorption and elimination by other routes at time periods immediately following dermal application. This is especially critical for volatile liquids such as benzene and toluene which are apparently rapidly absorbed [Franz, 1983; Susten et al, 1984a,b] and eliminated to some extent in expired breath [Lauwerys, 1980]. The direct method described in this paper does not require any correction factor, and thus dermal absorption can be summed directly from levels of radioactivity in the carcass and expired breath as well as the excreta.

Two additional differences in methodology should also be noted. First, our system was designed to approach the uncovered rather than the occluded site condition, since occlusion has been reported to increase absorption as much as 10 times [Feldman and Maibach, 1965]. The exposure site was covered in our studies but, because of the adsorptive power of the charcoal and the aperture in the cap of the depot allowing equilibration to atmospheric pressure, it is unlikely that a delay in evaporation occurred as compared to still-air conditions. Thus we do not believe that there was an increase in absorption due to the use of the skin depot. Second, the radioactive dose (μCi/kg) used by us was in some cases more than 100 times greater

TABLE V. Ratios of Dermal Absorption for Benzene and Toluene

	Percent of dose absorbed		
Species	Benzene	Toluene	Ratio
	(A)	(B)	(B/A)
Monkey[a]	0.20	0.44	2.2
	(C)	(D)	(D/C)
Hairless mouse[c]	0.89[b]	2.06[c]	2.3
Ratio	C/A = 4.5	D/B = 4.7	

[a]Franz [1980], indirect method (excretion analysis).
[b]Susten et al [this study].
[c]Susten et al [1984a], direct method (measurement of expired breath, carcass, and excreta).

than those used by the other investigators, thus enabling an increase in accuracy and sensitivity. The radioactive dose may be an important factor particularly in excretion analysis studies, where rather small percentages of the dose are absorbed and even smaller amounts are excreted via the urine. The consequences of the latter would be very low count rates in the urine, often approximating the limits of detection, which could compromise the accuracy of the excretion data. Thus, we believe that the experimental model described in this paper is useful for directly evaluating the in vivo dermal absorption of volatile compounds.

SUMMARY

In summary, it is estimated that under the job conditions observed during tire building, a worker dermally exposed 150 times per day to rubber solvent containing 0.5% benzene (v/v) will absorb approximately 6 mg (4–8) of benzene. This represents a significant addition to the 14 mg of benzene which is estimated to be retained in the body following inhalation of benzene at a concentration of 1 ppm. The fact that the skin on the hands of tire builders is often cracked and fissured almost assures that the penetration of benzene contacting the skin will be rapid and greater than would be expected for intact skin. It should be noted that in many facilities the concentration of benzene contained in rubber solvent is much less than 0.5% and may be as low as 0.09% (v/v). If, however, absorption potential is not affected by concentration as the present data suggest (see Table I), then it can be estimated that exposure to solvents containing 0.1% benzene would result in approximately 1.2 mg of benzene being absorbed daily through the skin. Whether or not this would represent significant increased risk to health remains to be determined.

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