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Percutaneous Penetration of Benzene and Benzene Contained in Solvents Used in the Rubber Industry

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ABSTRACT. Penetration of benzene through the skin of the rhesus monkey was determined using ^{14}C-benzene, and quantitating the labelled metabolites in urine. The modes of application and amounts of benzene that penetrated the skin (indicated in parentheses) are as follows: (1) a single, direct cutaneous application of liquid benzene (0.172 ± 0.139%); (2) a single application of benzene-containing [0.36%] solvent (0.0805 ± 0.0306%); (3) multiple washes with full-strength benzene (0.848 ± 0.0806%); (4) multiple washes with the benzene-containing [0.35%] solvent (0.431 ± 0.258%); (5) removal of the stratum corneum followed by application of full-strength benzene (0.909 ± 0.627%); and (6) application of benzene to the palmar surface (0.651 ± 0.482%). Until more complete human data becomes available, benzene penetration in the monkey may be used to estimate penetration in man, both for industrial hygiene purposes and general toxicological use.

ALTHOUGH BENZENE is a ubiquitous substance to which most of the population is exposed daily,[1] there has been little direct experimental data regarding benzene absorption through the skin. This is of practical importance since benzene is present, for example, in small amounts in gasoline (up to 2–3%) and in minute quantitics in water supplies.[2] Various amounts of benzene are present in solvents and mixtures used in manufacturing and other industrial processes; therefore, employees may have skin contact with solvents or other mixtures containing benzene in trace amounts, i.e., less than 0.5% by volume.

The data contained in studies conducted prior to 1962 investigating benzene absorption via the skin are summarized in the DISCUSSION section. Early data do not provide a sufficient factual basis for determining to what extent, if any, benzene is absorbed through skin—either at full strength (100%), or where present in a small amount in a solvent or other liquid mixture.

This report describes skin absorption of full-strength benzene and benzene which is present in trace amounts in solvents used in the rubber manufacturing industry. The purpose of the study was to determine the extent of benzene percutaneous penetration, if any, using full-strength benzene and a rubber solvent containing small amounts of benzene.

MATERIALS AND METHODS

The experimental details are the same as those described by Feldmann and Maibach.[3] Three rhesus monkeys were studied for each experimental variable. The rhesus monkey was selected for these experiments because it has been shown to be a reasonable model for percutaneous absorption where relevance to man is sought.[4] While percutaneous penetration is a complex process, it appears from data derived from research with compounds other than benzene that absorption in the rhesus monkey bears a marked similarity to absorption in man.

Parenteral administration of benzene. A 1.13 μg (0.755 μCi) dose of ^{14}C-benzene was dissolved in propylene glycol and injected into the thigh of each rhesus monkey.

Cutaneous administration of benzene. A 50 µl (43.95 mg) dose of benzene with an activity of 4.33 µCi was applied to a 12.9-cm^2 (3.41 mg/cm^2) area of the forearm or palm of each monkey and allowed to dry. The treated arm was kept isolated for 4 hr after application of benzene.

During all cutaneous applications of benzene the monkeys were transferred outside the vivarium to minimize inhalation and to preserve the controlled atmosphere of the laboratory; all monkeys wore respirators during the application. One to 3 days prior to benzene application, the forearms were lightly clipped with an electric clipper. It should be noted that a previous experiment with rhesus monkeys indicated that this procedure does not grossly alter penetration.[1]

All monkeys used in this study were trained to sit in metabolic chairs. The monkeys were sedated with ketamine hydrochloride and appropriately dosed (see above). They were kept in the metabolic chair and were fed food and fruit (each having a high water content) and water ad libidum.

Percutaneous administration of benzene in a rubber solvent. A petrolleum distillate mixture containing 0.36% benzene (15.7 µCi/50 µl dose) was preparea. The 50 µl dose was applied over a 12.9-cm^2 (123 µg/cm^2) area and was allowed to evaporate. The rubber solvent—a petrolleum distillate—was a mixture of aliphatic and aromatic hydrocarbons with a boiling point range of 104° to 266°F. The major components of this mixture are C_4–C_7 aliphatic hydrocarbons, including butane, isopentane, pentane, hexane, cyclopentane, and heptane. The aromatic portion of the mixture contains benzene, toluene, and xylenes as major components.

Palmar exposure to benzene in a rubber solvent. ^{14}C-benzene was dissolved in a rubber solvent containing 0.35% benzene to a concentration of 5.92 µCi/50 µl. The solution was made from a benzene lot with an activity of 54 mCi/mmol; 8.53 µg benzene was added to the solvent containing 158 µg of benzene. This addition, however, did not significantly increase the benzene concentration. Fifty ml of the solvent containing ^{14}C-labelled benzene were applied to 12.9 cm^2 (12.3 µg/cm^2) of the palm and allowed to dry.

Exposure of "damaged skin" to full-strength (100%) benzene. An area of the forearm skin was repeatedly stripped with cellophane tape until it glistened. This process removed the stratum corneum to simulate damaged skin. Fifty microliters of ^{14}C-labelled benzene (43.9 mg; radioactive dose of 4.25 µCi (4.31 mg/cm^2)] were applied to the stripped skin area and allowed to dry.

Multiple exposures to full-strength (100%) benzene. One hundred microliters of reagent grade benzene (unlabelled) were applied to a 12.9-cm^2 area of the monkey's forearm on 10 separate occasions at 15-min intervals during a 2.5-hr period. A total of 68.1 mg/cm^2 benzene was applied. Fifteen minutes following the last application, 50 µl of ^{14}C-labelled benzene [43.9 mg; radioactive dose of 4.94 µCi (3.41 mg/cm^2)] were applied to the same site and allowed to dry. The application of the ^{14}C-labelled benzene was always performed in a ventilated passage open to the outside air with the monkeys wearing respirators.

Multiple exposures to benzene in a rubber solvent. A rubber solvent containing 0.35% benzene was utilized, the characteristics of which are described above. ^{14}C-benzene was dissolved in the rubber solvent (0.35% benzene) to a concentration of 5.92 µCi/ml. This increased the initial amount of benzene (3.16 mg) by 8.52 µg, but not significantly (^{14}C-benzene activity = 54 mCi/mmol). The rubber solvent containing ^{14}C-labelled benzene was applied to the monkey's forearm in 100 µl (1.29 cm^2) doses on 10 separate occasions at 10-min intervals. A total of 5.92 µCi was applied (68.1 mg/cm^2 benzene). The application was done in a ventilated passage open to the air with the monkeys wearing respirators.

Urine and fecal analysis. Urine and feces were collected for assay of ^{14}C to determine the completeness of excretion and to identify the kinetics involved. Each aliquot of urine was oxidized with chromic acid; the ^{14}C-CO_2 released was captured and counted. The feces were diluted with water, aliquoted, and oxidated in a manner similar to that of urine.[3] Data analysis was performed on a programmable microcomputer.

RESULTS

Parenteral administration of benzene. The average excretion was 41.04 ± 9.41% of the injected dose, with individual values of 47.04%, 45.84%, and 30.1 8% (Table 1). Parenteral excretion data were utilized in preparing the correction figure for topical experiments, i.e., to correct to incomplete urinary excretion. No significant counts were found in the feces.

Cutaneous administration of benzene. The penetration values were 0.1 06%, 0.1 22%, and 0.288% of the applied benzene doses, with an average of 0.1 72 ± 0.1 39% [standard deviation (*SD*)] (Table 1). This utilized the correction factor of 2.44, as determined in the parenteral study of benzene. No significant counts were found in the feces. All standard deviation values include the uncertainty contribution of the parenteral injection.

Percutaneous administration of benzene in a rubber solvent. Using the parenteral correction factor of 2.44, the penetration was 0.0805 ± 0.0306% of the benzene applied dose, or 0.00029% benzene in relation to the solvent applied (Table 1).

Palmar exposure to benzene in a rubber solvent Using the parenteral correction factor of 2.44 to correct for incomplete excretion, the ^{14}C-benzene and metabolites detected in the urine was 0.651 ± 0.482% (*N* = 3) of the benzene portion (0.35%) of the applied dose, or 0.00208% benzene in relation to the solvent applied (Table 1).

Exposure of "damaged skin" to full-strength (100%) benzene. Penetration, corrected for incomplete excretion using the parenteral correction factor of 2.44, was 0.6023%, 1.3868%, and 0.7393% of the applied dose, or an average of 0.909 ± 0.627% of the applied dose (Table 1).

Multiple exposures to full-strength (100%) benzene. Urinary excretion of the ^{14}C-labelled benzene, corrected for incomplete excretion, was an average of 0.838 ± 0.806% (*N* = 3) of the applied dose (Table 1).

Multiple exposures to benzene in a rubber solvent. Penetration of the benzene portion of the applied dose, corrected for incomplete excretion, was 0.431 ± 0.258% (*N* = 3) of the benzene portion (0.35%) of the applied dose, or

Table 1.--Benzene Skin Penetration Data

Method of Exposure		Time (hr) 0-4	4-8	8-12	12-24	24-48	48-72	72-96	96-120	120-144	144-168	Total*
Intramuscular	PP†	31.2	5.12	2.02	1.93	0.752	0.321	0.170	0.098			41.2 ± 9.41
	PHP‡	7.82	1.28	0.505	0.161	0.031	0.134	0.00708	0.00408			
Benzene (0.36%) in solvent	PP	0.0343	0.0673	0.0593	0.0110	0.00814	0.00850	0.00744	0.00877			0.0805 ± 0.0185
	PHP	0.00855	0.00168	0.00148	0.000813	0.000339	0.000271	0.000310	0.000115			
Damaged skin exposed to full strength benzene	PP	0.182	0.260	0.0497	0.143	0.0585	0.0268	0.0440	0.0865			0.903 ± 0.627
	PHP	0.0454	0.0651	0.0124	0.0119	0.00244	0.00350	0.00133	0.00360			
		0-6	6-12		12-24	24-48	48-72	72-96	96-120	120-144	144-168	
Cutaneous administration of benzene	PP	0.0829	0.0062		0.0280	0.0220	0.0138	0.0112	0.00381			0.172 ± 0.139
	PHP	0.0138	0.00104		0.00233	0.000916	0.000575	0.000468	0.000409			
		0-4			4-24	24-48	48-72	72-96	96-120	120-144	144-168	
Multiple exposures to full-strength benzene	PP	0.0161			0.203	0.0213	0.0114	0.239	0.133	0.0611	0.0210	0.848 ± 0.806
	PHP	0.00403			0.0101	0.00255	0.00473	0.00996	0.00554	0.00255	0.000837	
						24-48	48-72	72-96	96-120			
Palmar exposure to benzene in rubber solvent	PP				0.116	0.238	0.0501	0.101	0.0416	0.0303	0.0229	0.651 ± 0.482
	PHP				0.00735	0.00949	0.00209	0.00423	0.00173	0.00126	0.000956	
Multiple exposures to benzene (0.35%) in rubber solvent	PP				0.164	0.0357	0.0440	0.0363	0.0294	0.100	0.0219	0.431 ± 0.258
	PHP				0.00682	0.00149	0.00183	0.00151	0.00123	0.00417	0.000914	

*The total is expressed as the mean ± standard deviation, which utilizes the correction factor of 2.44, as determined in the parenteral study of benzene (see RESULTS)
†PP = percent penetration.
‡PHP = percent per hour penetration.

.001[illegible]% benzene in relation to the solvent applied (Table).

DISCUSSION

Full strength ^{14}C-benzene was injected into rhesus monkeys and its presence was identified quantitatively and kinetically through analysis of the urine and feces. Radioactive benzene was applied to the forearm of the monkeys to quantitate absorption. Using the correction factor of 2.44 determined in the parenteral study the penetration was 0.172 ± 0.100% of the 100% benzene administered. ^{14}C-benzene, a component of a rubber solvent containing 0.36% benzene, was applied topically to the forearm of the monkeys. Using the parenteral correction factor of 2.44, the actual penetration was 0.085 ± 0.0306% of the benzene portion (0.36%) of the applied dose, or 0.00029% benzene in relation to the solvent applied. The 0.172% and 0.080% penetration figures suggest that when similar amounts of benzene are presented in diluted and concentrated forms, proportionally less benzene may be absorbed in the diluted form. In the multiple exposure experiment, 100 µl of full-strength (100%) benzene (unlabelled) was applied to the rhesus monkey's forearm on 10 occasions at 15-min intervals, followed by a 50 µl dose of ^{14}C-benzene. The penetration of that eleventh dose was 0.848%—approximately 4.5 times the 0.172% penetration reported in the single exposure study.

The single and multiple exposure results cannot be compared in a linear relationship, because in the multiple exposure experiment the first 10 doses were 100 µl each, rather than 50 µl. The larger doses were to approximate the effects of chronic exposure to full-strength benzene. It was thought that the use of 100 µl doses of full-strength benzene might have a more deleterious effect on the skin than 10 similar doses in 50 µl amounts.

The results of the stripped skin experiment using full-strength benzene suggest that the multiple exposure experiment may approximate benzene's chronic effects. After the stratum corneum layer of the forearm skin had been stripped using cellophane tape, the penetration was 0.909%, which is in a close range to the 0.848% actual penetration reported in the multiple exposure study.

In the multiple exposure experiment using rubber solvent labelled with ^{14}C-benzene, 10 separate 100 µl doses were applied at 10-min intervals. The penetration was 3.481% of the benzene portion (0.35%) of the applied dose, or 0.00108% benzene in relation to the solvent applied. This is approximately 4 times the single dose actual penetration (using a 50 µl dose rather than 100 µl doses) of 3.0805% of the benzene portion (0.36%) of the applied dose, or 0.00029% in relation to the solvent applied.

To approximate chronic exposure to benzene contained in rubber solvent, 100 µl doses, rather than 50 µl doses were used in the multiple-exposure experiment with a solvent. The 0.431% actual penetration of benzene computed in this multiple-exposure experiment is slightly more than half the 0.848% penetration in the multiple-exposure experiment using full-strength benzene. A similar relationship between penetration of full-strength benzene and benzene in rubber solvent was found in the single-exposure experiments reported previously. In the solvent experiment, however, the benzene penetration of all 10 doses was measured, while the full-strength benzene experiment measured only the penetration of the eleventh dose.

The actual penetration of ^{14}C-benzene in rubber solvent through the rhesus palm was 0.651%. This penetration was greater than the penetration through the rhesus forearm of 0.0805%, and indicates that at least in the rhesus monkey, the palm is probably a poorer barrier for benzene than the forearm. It is not possible to compare rhesus palmar absorption to absorption through the human palm since no literature is available on this subject. It may be questioned whether the relationship between rhesus forearm and palmar absorption of benzene will be relevant to man. Based on the limited data with compounds other than benzene in man, the absorption through the human palm was found to be approximately equal to absorption through the forearm.[5,6]

Adequate quantitative identification of systemically absorbed benzene requires that the chemical and its metabolites be isolated from biological fluids or excreted products. This task is complicated by the relatively simple nature of this chemical and the biochemical ubiquity of its compounds and metaboiites. Much of absorbed benzene is metabolized and the metabolite pool is derived from various sources in addition to the exogenous chemical.' Because of normal biological variability, it is difficult to quantitate the exogenous benzene source of such metabolites. Even with many of the highly specific and sensitive methods of modern analytical technology, this remains a problem. Radiochemical methods provide a means of selectively tracing the biochemical fate of radioisotopically labelled benzene because the labelled moieues are retained in metabolites and can be detected with great sensitivity and accuracy.

Evaluation of the older literature. The most pertinent older data is cited by Hanke et al.[8] The present comments are confined to questions of analytical methodology and to quantitative studies relevant to human absorption. This includes three publications appearing from 1946 to 1961.

Two reports[5,6] involving human subjects concluded that there was no percutaneous absorption of benzene. The analytical methodology then available limits the reliability of these studies. These methods were based upon a change in the sulfur ratio of total urinary sulfate, due to changes in the organic vs inorganic sulfate, that would result from sulfate conjugation of phenol formed metabolically from any absorbed benzene. Since the total sulfur ratio normally varied between 75% and 95%, and the amount of sulfate normally excreted in urine is relatively high (approximately 2,000 mg/L), this is not a reasonably sensitive method for measuring benzene absorption. The rncthod would probably not detect increases in urinary phenol excretion of the order of 10 mg/L.

Hsnke et al.[8] employed analytical methods sufficiently sensitive to detect benzene absorption, although not sufficiently precise to accurately quantitate that absorption. One method involved the direct assay of excreted phenol obtained by chemical hydrolysis of urinary phenol sulfates. The results indicate that urinary phenol was elevated in individuals dermally exposed to benzene (maximum value of 1.58 to 25.3 mg/L as compared to a maximum of 16.0

mg/L in nonexposed individuals). Large variations in experimental values occurred among individuals. Normal 24-hr urinary excretion of total phenol varied from less than 10 mg to more than 50 mg.[7] The authors acknowledged the problem of quantitation and discussed various methods of calculation and also arrived at the unjustified conclusion that "the results would have less error if physiologic excretion was calculated as the average of all four methods of calculation." The authors did not present statistical evaluation of their data, nor did they present sufficient data to allow application of statistical evaluations.

These data apply to continuous occluded exposure of a 35 to 45 cm^2 area of human forearm by 60 mg/cm^2 benzene for 1.25 to 2 hr. For these conditions the authors calculated an average absorption rate of 100% benzene of 0.24 $mg/cm^2 \cdot hr$. This calculation takes into account that not all of the benzene absorbed systemically appears in the urine as phenol. Their data, however, do not allow an estimation of the reliability of this figure. It is based on an estimated average "surplus" of approximately 5 mg of excreted phenol for exposed individuals above an estimated average level of approximately 5 mg for nonexposed individuals. Since values for nonexposed individuals were reported to vary between 3.0 and 16.0 with an average of 7.2 mg, it would seem that the calculated value of 0.24 $mg/cm^2 \cdot hr$ is subject to considerable error and may be substantially overestimated.

The same study employed an alternative method in which absorption was determined as the difference between the amounts of benzene recoverable by evaporation from the skin surface at the time of application and after 1.25 hr of continuous occluded exposure (17.6 mg applied to 8 cm^2). While this method is characterized as more "direct" chemically, it is actually less direct biologically in that it does not necessarily measure benzene reaching the systemic circulation. The unrecoverable benzene presumably consisted of that systemically absorbed plus that retained temporarily in the stratum corneum. Since a substantial portion of the unrecoverable benzene may not have been systemically absorbed during the 1.25-hr exposure period, the calculated "absorption rate" of 0.4 $mg/cm^2 \cdot hr$ is likely to be substantially overestimated.

In spite of the deficiencies cited and the limited precision, the similarity of the results obtained by the two independent methods used by Hanke et al.[8] is at least indicative of an absorption rate upper limit of less than 0.24 to 0.4 $mg/cm^2 \cdot hr$ for the severe conditions of exposure employed, These conditions involved continuous exposure by direct contact with 100% benzene and surface occlusion for 1.25 to 2 hr. Respecting the effect of occlusion, the occlusive method employed by Hanke et al. may have had the effect of increasing the reported absorption rate more than 10-fold.[9]

The presented percutaneous penetration data can be compared to levels of benzene absorbed by workers from airborne exposure. However, before these estimates are accepted for general use, we believe that confirmatory cutaneous human penetration experiments should be performed. Although the present experimental data are the most complete available, we believe that this should be interpreted in a tentative way until human data becomes available.

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