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Absorption, Distribution, and Excretion of Ethylbenzene, Ethylcyclohexane, and Methylethylbenzene Isomers in Rats

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Toxicity studies on the petroleum hydrocarbon mixture "60 Solvent" have recently been reported by CARPENTER et al. (1975). Little information exists, however, on the metabolism and disposition of the principal components of this mixture, i.e. ethylbenzene (EB), ethylcyclohexane (ECH), and methylethylbenzene (MEB). The purpose of this study therefore, was to examine the absorption, distribution, and excretion of these hydrocarbons in rats.

MATERIALS AND METHODS

^{14}C-ring labeled EB, ECH, and MEB having specific activity of approximately 5 mCi/mmole were obtained from New England Nuclear, Inc. Unlabeled EB and ECH were obtained from Phillips Petroleum Co. and unlabeled MEB was obtained from Pfaltz & Bauer, Inc. The sample of MEB was a mixture of ortho, meta, and para isomers at a ratio of approximately 1:2:1 respectively. The stock solutions of radioactive EB, ECH, and MEB isomers used were diluted with corresponding nonradioactive samples to give 2 to 3 x 10^6 cpm (counts per min)/uL of solution. The ratio of isomers for the sample of MEB was same between labeled and unlabeled material.

A diagram of the closed cycle inhalation metabolism system used for these studies is illustrated in Figure 1. It consisted of a 6-L glass metabolism chamber (Delmar Scientific Laboratories), 0.25 in. Teflon® tubing and a sampling pump (Model A-1000, Science Pump Corp.) which was used to recirculate the chamber air.

Duplicate experiments were conducted with each of the three labeled hydrocarbons. Three male, Harlan-Wistar rats weighing 100-120 g were used in each exposure. Chamber air was recirculated at a rate of about 1 L/min. During each 6 h exposure, oxygen was introduced into the system at the rate of 3 mL/min/100 g of rat while CO_2 was removed from the chamber air with a 1:1 (w/w) mixture of Ascarite® and Chromosorb®. The chamber concentration of test chemical was maintained at about 1 mg/L. This was accomplished by charging the system intermittently with 5 to 10 uL of radiolabeled test chemical. The total amount of radiolabeled

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chemical dosed to the chamber during a 6 h exposure was about 2.00 x 10^8 cpm. The absorbed dose for each compound was determined by summing the quantity of test chemical required to maintain chamber concentration during the exposure period.

Chamber air was monitored by gas chromatography at 15 minute intervals during the first hour and at 30 minute intervals thereafter. The complete program for gas chromatographic analysis is furnished in Table 1. Calibration curves were constructed according to CARPENTER et al. (1975).

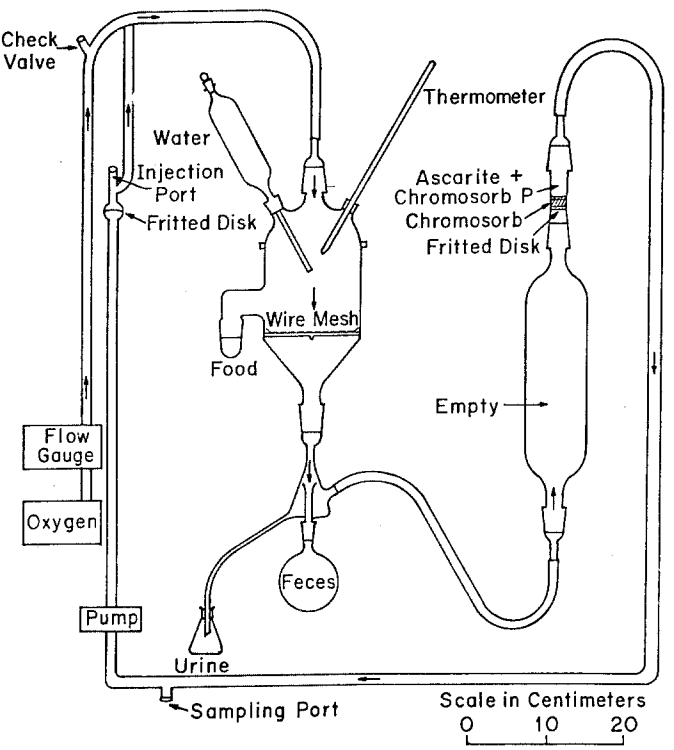

Figure 1. Inhalation Metabolism Cage for the Total and Separate Collection of CO_2, Urine, and Feces During Exposure

At the conclusion of each 6 h exposure, activated charcoal filters (Environmental Compliance Corp.), were inserted into the system to remove the test chemical from the chamber air. The animals were then transferred to individual Roth-type metabolism cages where they were housed for the remainder of the study. These chambers allowed for the separate collection of urine, feces, and CO_2; in addition, activated charcoal filters were used to trap volatile organics in the expired gases. Food and water were supplied ad libitum during the postexposure period.

Urine, cage washings, and feces were collected at 6, 24, and 48 hours and analyzed for radioactivity by liquid scintillation counting (KNAAK et al. 1965). Counting efficiency corrections were made based upon a urine quench curve prepared for this study.

Radioactivity in the respiratory CO_2 was determined by acidifying the Ascarite^R and Chromosorb^R mixture with concentrated HCl. The released $^{14}CO_2$ was passed through two absorption traps. Two mL of the trapping reagent, 20% ethanolamine in ethylene glycol monomethyl ether, were analyzed by liquid scintillation counting.

Radioactivity in the activated charcoal filters was determined by desorbing the organic material from the charcoal filters with carbon disulfide and then counting in a liquid scintillation counter.

The first study with EB was terminated after 72 h and all other studies 48 h after the start of an exposure. At the termination of an experiment the rats were anesthetized with ether and exsanguinated via heart puncture. The tissues listed in Table 2 (except blood) were removed and pooled according to tissue type. They were then homogenized in acetone, centrifuged and the supernatant removed for counting. The residues were resuspended in acetone, centrifuged and the supernatant was again removed for counting. The resulting residues were dried and combusted in duplicate using the same procedure as for fecal samples. Plasma was counted directly in liquid scintillation solution. Blood cells were combusted and analyzed for radioactivity using the procedure as for fecal samples. The radioactivity in the remaining carcasses was determined using the procedure of KNAAK et al. (1965).

RESULTS

The mean quantity of ^{14}C-EB, ^{14}C-ECH, and ^{14}C-MEB absorbed by 3 rats during a 6 h exposure was 48, 52, and 58 mg, respectively. The excretion of radioactivity was essentially complete for all 3 compounds within 24 h after the start of the exposure (see Figure 2). Table 2 shows the distribution of radioactivity in urine, feces, CO_2, expired gases and tissues for each compound. Total mean recoveries of 91%, 80%, and 76% were obtained for EB, ECH, and MEB respectively. The major route of excretion for each hydrocarbon was through the urine. Expired gases contained the next highest concentration of radioactivity while CO_2, feces and tissues contained only minor amounts. The quantity of MEB recovered in expired gases was between 10% and 20% of that obtained by this route for the other two compounds.

Table 3 shows the distributions of radioactivity as percentages of absorbed dose found in tissues. Mean total radioactivity remaining in the tissue after 48 h (72 h in the case of the first study with EB) was 0.2%, 0.2% and 0.3% for EB, ECH, and MEB respectively. In general, highest amounts were observed in carcasses, the

G-I tract and liver. Other tissues containing relatively high amounts of radioactivity were fat (EB and ECH) and plasma (MEB).

DISCUSSION

This study showed that EB, ECH, and MEB were absorbed by rats very efficiently during respiration when animals are exposed to the average chamber concentration of 1 mg/L of each hydrocarbons. Under the inhalation system employed, the sensitivity and the precision of procedures were satisfactory for all data (Table 2) except for the percent urinary recovery data of MEB. The urinary data for 2 studies of MEB were not satisfactory as indicated by the large standard deviations. The cause for this poor reproducibility of data is not known.

Assuming a respiratory rate of 100 ml/min for male rats weighing 100 g, 36 liters of air would be inhaled during a 6-hour exposure. If the rat is breathing air containing 1 mg/liter of EB, the rat would receive a total of 30 mg of EB. Because each rat absorbed a total of 16 mg of EB in a 6-hour period, this is equivalent to 44% absorption of the 36 mg EB inhaled. The 44% retention of EB by rats is very close to the finding by BARDODEJ and BARDODEJOVA (1970) who reported that in men inhaling 23-85 parts per million EB for 8-hour periods, 64% of EB was retained in the respiratory tract. The retention of other hydrocarbons ECH and MEB by rats in this study were similar to that of EB.

As far as the elimination of hydrocarbons in the exhaled breath are concerned, 8 and 11% of the absorbed EB (B.P. 136°C) and ECH, (B.P. 132°C) respectively, was eliminated during 42 hours after the inhalation exposure to ^{14}C-EB and ^{14}C-ECH for 6 hours in rats. However, elimination of MEB isomers (B.P. of 165, 161, and 162°C for o, m, and p isomers, respectively) in breath was 1% of the absorbed MEB. This is not surprising when B.P. for this hydrocarbon is concerned.

Comparable studies in female rats were not performed for EB, ECH, and MEB but based on the preliminary studies, there was no sex difference in the absorption, distribution, and excretion of EB in rats. Also, no sex difference was found in the EB metabolism based on the metabolic profiles of EB from urine of rats exposed to ^{14}C-EB as well as *in vitro* liver-generated metabolites of EB reported by CHIN *et al.* (1978).

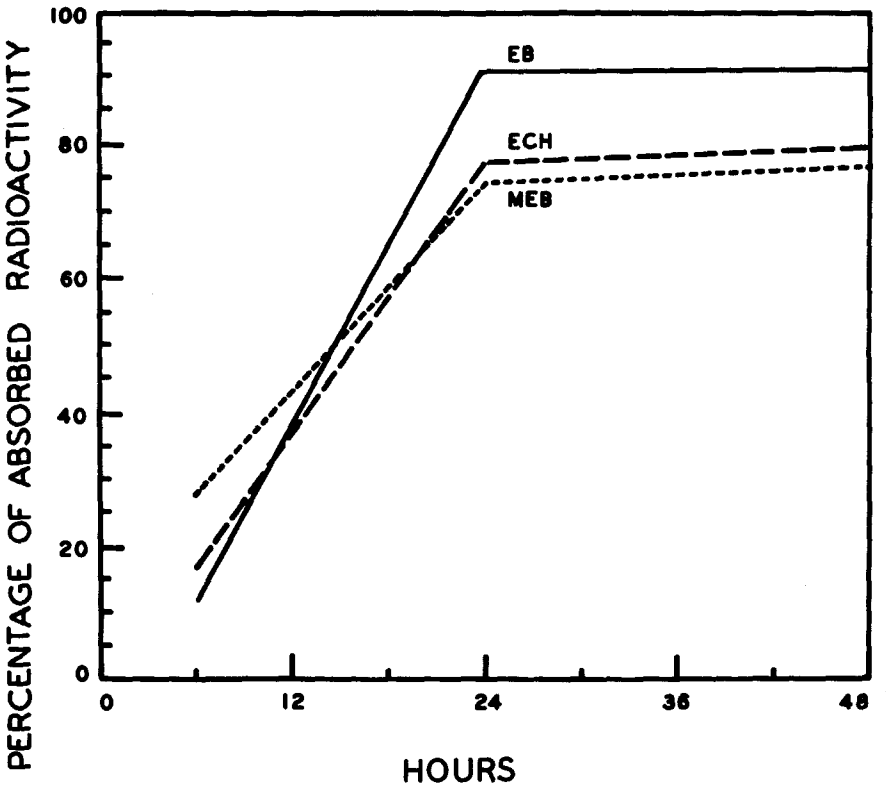

Figure 2. Cumulative Recovery of Radioactivity

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TABLE 1

Gas Chromatographic Conditions

Conditions	Ethylbenzene	Ethylcyclohexane	Methylethylbenzene
Chromatograph	F & M 609	Varian 2700	F & M 609
Column	25 Ft. 15% Apiezon M	11 Ft. 15% Apiezon M	25 Ft. 15% Apiezon M
Column Temp. (^{o}C)	150 Iso.	134	185
Injection Temp. (^{o}C)	285	200	285
Detector Temp. (^{o}C)	285	290	285
Carrier, Flow Rate (ml/min)	H_e,80	N_2,50	H_e,80
Hydrogen Flow Rate (ml/min)	60	36	60
Air Flow Rate (ml/min)	450	350	450
Sample Size (ml)	1	1	1
Retention Time (min)	8	2.5	9 & 10
Attenuation	1 x 32	10^{-10} x 32	1 x 64
Solvent for Standards	CS_2	CS_2	n-Hexadecane

TABLE 2

Disposition of Radioactivity During 42 Hours Following Inhalation Exposure to Radioactive EB, ECH, or MEB for 6 Hours in Rats[a]

Administered Compound	Urine[b]	Feces	CO_2	Expired Gases	Tissues	Total
EB	82.6 ± 6.6	0.7 ± 0.3	0.03 ± 0.01	8.2 ± 3.8	0.20 ± 0.05	91.7 ± 2.6
ECH	64.8 ± 1.6	3.9 ± 0.5	0.07 ± 0	11.2 ± 0.7	0.21 ± 0.01	80.2 ± 1.9
MEB	71.5 ± 25.9	2.7 ± 0.6	0.25 ± 0.3	1.3 ± 0.4	0.32 ± 0.05	76.1 ± 26.3

[a]Mean and standard deviations from two studies - three rats per study, expressed as percent of absorbed dose

[b]Include cage wash

TABLE 3

Radioactivity in Tissues 42 Hours After Inhalation Exposure to Radioactive EB, ECH, or MEB for 6 Hours[a]

Tissue	^{14}C-EB	^{14}C-ECH	^{14}C-MEB
Thyroid	<0.001	<0.001	0.001
Adrenal	<0.001	<0.001	<0.001
Plasma	0	0	0.010
Blood Cells	0.001	<0.001	0.006
Lung	0.006	0.002	0.002
Kidney	0.003	0.002	0.003
Spleen	<0.001	<0.001	<0.001
G-I Tract	0.008	0.064	0.102
Liver	0.014	0.008	0.068
Brain	<0.001	0.002	<0.001
Fat	0.007	0.003	0.006
Bone Marrow	<0.001	<0.001	<0.001
Carcass	0.160	0.120	0.120
Total	0.199	0.211	0.318

[a]Mean values for two studies - three rats per study, expressed as percent of absorbed dose

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