

Method for Repeated Evaluation of Benzene Uptake in Rats and Mice During a Six Hour Inhalation Period

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Benzene uptake measurements and respiratory minute volume (RMV) were assessed in rats and mice during a 6-hour inhalation exposure to 300 ppm (v/v) of benzene vapor. The values for benzene uptake and RMV were used to compare predictions of inhaled dose in both species. The theoretical prediction assumed an unchanging RMV. The next prediction allowed adjustments for changes in RMV during exposure. The final prediction addressed benzene uptake directly. The uptake characteristics of benzene in both species were different from the theoretical prediction, from the prediction adjusted for changing RMV and from each other. The greatest difference was seen in rats where the observed benzene dose was only 28% of the theoretical value. The experiment was not designed to elucidate the causes for the difference between the predictions, nor was the data sufficient to suggest whether the rat or the mouse was a suitable model of benzene uptake in humans.

Introduction

Animal studies often are used to establish various relationships between chemical exposure and animal response. In studies which use inhalation exposures, a "concentration-response" relationship, rather than a "dose-response" relationship, is most often established.¹ This is because the concentration of the chemical is measurable more readily in the exposure chamber (and the workplace) than is the actual amount of the chemical entering the exposed subject.

The principle that an exposure effect (E) is proportional to the exposure concentration (C) multiplied by the exposure duration (T),

$$E \propto CT,$$

is frequently used to evaluate animal response to inhaled chemicals. One need only incorporate a chemical uptake parameter, such as respiratory minute volume (RMV), into the CT relationship to determine an expected maximum inhaled dose for a given exposure period.²

One difficulty with application of the CT relationship is that many chemicals have been shown to alter respiratory performance during inhalation exposure.³ If airborne chemicals alter RMV, they also would affect the rate of uptake of the chemical during exposure. Further, if the uptake of an airborne chemical also can be affected by processes other than respiratory performance (e.g., alterations in blood/gas transfer, etc.), the actual inhaled dose from a given period could be overestimated considerably by a straightforward CT relationship.

Long and Hatch⁽³⁾ and Rinehart and Hatch⁴ have demonstrated a method of measuring RMV using diethyl ether. Utilizing this method, net uptake measurements of diethyl ether (for RMV) and benzene (for the test chemical) were performed and compared to assess: (1) whether or not net benzene or ether uptake changed during a 6-hr inhalation

exposure; and (2) if such changes were observed, whether or not the rates of benzene and ether uptake were influenced in a similar manner.

Experimental

Chemicals for Testing

Commercial grade benzene, supplied by the Chemical Manufacturers Association, and diethyl ether (Mallinkrodt) were used for the study.

Inhalation Exposure to Benzene

Benzene was flash evaporated at 35°C in a three-neck, round-bottom flask. Glass tubing was used to deliver the benzene into the flash evaporator and to conduct the benzene vapor-laden air into the exposure chamber. Vapor-laden air was drawn into a one-cubic meter, stainless steel and glass exposure chamber, where it was diluted with room air to reach the desired exposure concentration (300 ppm).

The exposure chambers were operated dynamically at an airflow rate of 205 L/min. A vented glove box provided access to the chamber.

The chamber atmosphere was monitored continuously during the exposure with a MIRAN® 1A Ambient Air Analyzer. Infrared absorbance readings were recorded and compared to a calibration curve to determine the exposure concentration.

Exposure and Testing Regimen

Inhalation exposure to benzene vapor was continuous for 6 hr. Measurements of net benzene uptake and RMV were performed prior to benzene exposure after 1 hr, 3 hr and 6 hr of benzene exposure. The time that the animals spent in uptake measurements (approximately 20 min per measurement) was not included in the total exposure time.

TABLE I

Estimation of Inhaled Benzene Dose in Rats and Mice

Interval (hour)	Uptake Rates of Benzene and Ether—Mean Values		Benzene	
	U-Benzene (mL/min)	Pre-exposed (percent)	U-Ether (mL/min)	Pre-exposed (percent)
Rats				
0	152	100	145	100
1	50 ^A	33	123 ^A	85
3	31 ^A	22	113 ^A	78
6	14 ^A	9	96 ^A	66
Mice				
0	188	100	189	100
1	118 ^A	65	180	96
3	138 ^A	76	156 ^A	84
6	146 ^{A,B}	81	130 ^A	69

^AStatistically significantly lower than pre-exposed value ($p < 0.01$).

^BStatistically significantly higher than corresponding ether value ($p < 0.01$).

Animals for Testing

The test animals consisted of five male, Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass. — 214g to 218g), and 25 male, SCH:ARS (ICRF) Swiss Albino mice (ARS Sprague-Dawley, Madison, Wis. — 25g to 30g).

Husbandry

The test animals were housed (rats, individually; mice, grouped) in elevated stainless steel, wire mesh cages. Except during the benzene exposure periods the animals were given pelleted feed (Purina Laboratory Chow 5001) and tap water *ad libitum*. Rats and mice at seven weeks of age were exposed to benzene on separate days.

Net Uptake Measurements

Single rats, or groups of five mice, were individually removed from the benzene exposure chamber for net uptake measurements. The animals were placed in a closed-circuit recirculating air system of approximately 810 mL volume. The system then was charged with a predetermined amount of benzene or diethyl ether (for RMV) to approximate an initial 300 ppm concentration. Air samples taken every 30 sec during a 3-min period to measure concentration of benzene or ether remaining in the closed loop system. A decrease in benzene or ether concentration was presumed to be due to net uptake by the rat or group of mice. Benzene measurements preceded ether measurements to limit benzene losses by minimizing the time between chamber exposure and the benzene uptake measurement.

Each rat or group of mice served as its own control in order to monitor animal performance throughout the benzene period. When the measurements were completed each rat or group of mice was returned to the benzene chamber. Measurements were made to determine benzene and ether losses on the walls of the empty, closed-circuit system as well as the extent of benzene losses on the fur of the animals. Ether concentrations in the closed loop were measured using an infrared detector (Mine Safety Appliances LIRA 303). Samples for benzene determination were taken with a 1-mL gas sampling loop (Valco Instruments) and analyzed by gas chromatograph (Tracor, Model 550, using Carbopack C column with 0.2% Carbowax as the stationary phase), with the use of a flame ionization detector.

When all animals had been tested for all three exposure intervals (0 to 1 hr; 1 hr to 3 hr; and 3 hr to 6 hr), the "Inhaled Dose" was calculated with the following equation:⁽¹⁾

$$D(\text{mg/kg}) = (K \times C \times T)(\text{mg}) / \text{Body Weight (kg)}$$

where "D" was the dose of benzene inhaled during a particular exposure interval.

TABLE II
Estimation of Inhaled Benzene Dose in Rats and Mice
Cumulative Inhaled Benzene Dose — Mean Values

Mean Inhaled Benzene Dose, ^A Calculated Using:				
Elapsed Exposure Time (minutes)	Theory (mg/kg) ^B	Changing RMV (Ether Uptake) (mg/kg) ^B	Changing Benzene Uptake (mg/kg) ^B	Benzene Dose Observed/Expected (percent)
Rats ^{C,D}				
60	38.6	35.8	27.0	70
180	116.3	99.1	48.8	42
360	233.0	183.3	65.6	28
Mice ^{C,E}				
60	89.0	88.3	72.7	81
180	269.8	248.3	194.4	72
360	539.6	449.6	395.4	73

^ASee text for equations.

^BBody Weight: rats = 0.215 kg/rat; mice = 0.125 kg/group.

^CPopulation sizes: 5 individual rats; 5 groups of 5 mice.

^DBenzene concentration: 300 ppm = 0.00096 mg/ml.

^EBenzene concentration: 310 ppm = 0.00099 mg/ml.

uptake rate; "K" was an uptake rate factor (in mL/min) for a specific rat or group of mice during a specific exposure interval (e.g., 1 hr to 3 hr, etc.); "C" was the exposure concentration (ppm/mL); and "T" was the duration of the exposure interval (min).

The value of "K" depended upon the inhaled dose model used. When the theoretical model was calculated, "K" was taken as equal to the pre-exposure value for ether uptake rate (= RMV). When the theoretical model, influenced by changing RMV was calculated, "K" was an average ether uptake rate during a specific interval (e.g., 1 hr to 3 hr). When the theoretical model, influenced by benzene uptake was calculated, "K" was the average uptake rate for benzene during a specific interval.

Statistical Analysis

Differences in benzene uptake rates and RMV between preexposed and exposure intervals were analyzed by a Randomized Block Design⁽⁶⁾ with the animals as blocks and the time periods as treatments. Duncan's Multiple Range Test⁷ was used to determine which time periods (exposed) differed from control (preexposed).

Differences between benzene uptake rates and RMV, at each interval, were analyzed by a Paired Difference T-test⁽⁶⁾ using the animals as pairing elements and ether and benzene as treatments. Statistical analyses were performed separately for rats and mice at each time period (preexposure; 1-hr exposure; 3-hr exposure; and 6-hr exposure).

Results

Rats — Benzene Exposure and Uptake Measurements

The treated rats receive exposure to a mean benzene concentration of 300 ppm (s.d. = 10 ppm) during the 6-hr dosing period. Vapor uptake data for the rats are summarized in Table I.

Prior to benzene exposure, the rats exhibited a mean net uptake rate for benzene of 152 mL/min, and a mean RMV of 145 mL/min. The pretest uptake rates of benzene and ether (for RMV) had no significant, statistical difference.

After 1, 3 and 6 hr of continuous benzene exposure, the mean net uptake rates for benzene had decreased with statistical significance to 33, 22 and 9% of the pretest value. The mean RMV values at these same intervals had decreased with statistical significance to 85, 78 and 66% of the pretest value, and had statistical significant difference from the corresponding benzene uptake values.

The mean, cumulative, inhaled benzene dose for the rats, calculated by each of the three prediction models, is presented in Table II. The observed benzene inhaled dose was only 28% of the theoretical inhaled dose, and 36% of the dose which was adjusted for changing RMV.

Mice — Benzene Exposure and Uptake Measurements

The mice received exposure to a mean chamber concentration of 310 ppm (s.d. = 10 ppm) during the 6-hr dosing period. Vapor uptake data for mice are summarized

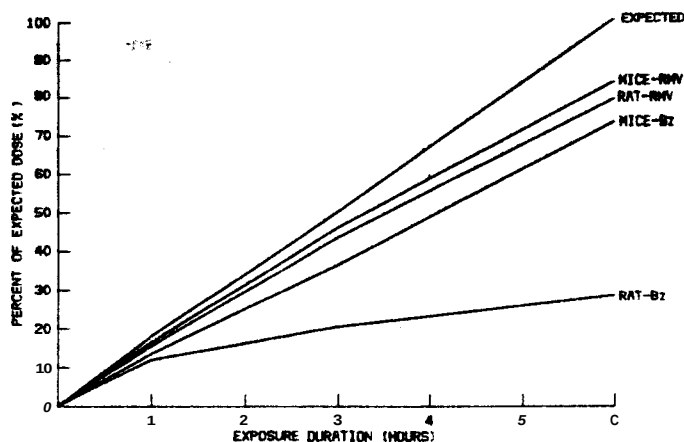


Figure 1 — Cumulative inhaled benzene dose in rats and mice, as influenced by theory, changing RMV, and changing benzene uptake rate. All values are normalized as percentages of the total expected dose.

Prior to benzene exposure, the mice exhibited a mean net uptake rate for benzene of 188 mL/min, and a mean RMV of 189 mL/min. The pretest uptake rates for benzene and ether (for RMV) had no significant statistical difference.

After 1 hr of exposure, the mean net uptake rate for benzene decreased with statistical significance to 65% of the mean pretest value in the mice, but it did not continue to decline during the remainder of the exposure. After 3 and 6 hrs of exposure, the mean benzene uptake rates were more stable, at 76 and 81% of the pretest value, respectively. The RMV values for these same intervals were decreased with statistical significance to 96, 84 and 69% of the pretest value, respectively, but were statistically different from corresponding benzene uptake rates only at the 1-hr (lower) and 6-hr (higher) intervals.

The mean, cumulative, inhaled benzene dose for the mice, calculated by each of the three prediction models, is presented in Table II. The observed benzene inhaled dose was 73% of the theoretical inhaled dose, and 89% of the prediction which was adjusted for changing RMV.

Figure 1 is a graphic representation of the build-up of inhaled dose over time, as predicted by each of the three models: theory; theory - adjusted for changing RMV; and theory - adjusted for benzene uptake rate. The values plotted are normalized as percentages of the dose expected by the unadjusted theoretical model. It was clear from the figure that the inhaled doses of rats and mice, as adjusted for changing RMV, were lower than the unadjusted theoretical dose, and that the inhaled doses, as adjusted for benzene uptake rate, were lower still.

Discussion

Benzene was selected for the study because of its wide industrial use and interest. There have been many investigations into the toxicity of benzene⁽⁷⁻¹¹⁾ Thus, there was interest to determine whether or not benzene uptake was constant or variable during inhalation exposure. The 300 ppm exposure level was selected because it was a level which could be experienced during an accidental worker exposure, and yet

was below the level at which one would expect to observe overt signs of narcotic effects.⁽¹²⁾

Theoretical methods used to predict inhaled dose require the incorporation of an appropriate rate factor (volume per unit time). The present study demonstrated that the most appropriate factor was the one related to benzene, the test material. The method used to determine the rate factor for this study was simple and enabled the determination of benzene uptake and RMV at rest, during exposure, without causing undue distress to the animals.⁽⁴⁾

The mean pre-exposed RMV values for rats and mice compared well to published values,⁽¹³⁾ which suggested that the uptake method accurately might monitor changes in the RMV during exposure. It was not known, however, if exposure to benzene would alter the relationship between RMV and diethyl ether uptake; it was assumed that it would not, since benzene is not reported to have toxic effects on the lung, especially during the time course of the exposure in the study.⁽⁷⁻¹¹⁾

The results from this study presented other points to consider. The cumulative inhaled doses plotted in Figure 1 clearly demonstrated a dramatic difference in benzene uptake characteristics between rats and mice. The findings in the rat were similar to those reported by Anderson *et al.*,⁽¹⁴⁾ using 1,1-dichloroethylene. They reported that the uptake curve was biphasic, possessing a rapid phase that was essentially complete within the first 60-80 minutes. This appeared to represent tissue equilibration and was independent of concentration level. The second phase, a slower uptake rate, appeared to be related to metabolism.⁽¹⁴⁾

The findings in the mouse suggested that the first phase of benzene uptake may have been completed within a shorter time frame than in the rat. A comparison of the ratio of RMV to animal volume suggested that the ratio for the mouse was approximately double that for the rat. Thus, the uptake rate curve measured for mice in this study may have represented the second, slower phase observed in Anderson's experiments.⁽¹⁴⁾ The greater inhaled dose in mice, on a mg, kg basis, might similarly be attributed to the higher rate of metabolism in the mouse, when compared to the

Depression of RMV during the course of dosing was apparent, but there was no clear correlation between RMV and benzene uptake rate in either rats or mice. Thus, the reduction of benzene uptake during exposure must have been related to some other process.

It also was reported that the benzene uptake in the mice was greater than the corresponding ether uptake rate (for RMV), after 6 hr of benzene dosing. While the difference was statistically significant, it was not considered to have been biologically significant, being related more to analytical variability than to animal response. If anything, ether uptake would be expected to be higher than benzene because of the greater water solubility of ether. Furthermore, ether uptake has previously been shown to provide a good estimate of RMV.

In summary, this study demonstrated that predicting inhaled dose from measured uptake rates of a test material

gave a much different picture than either an unadjusted or RMV-adjusted, model. The data presented in this study were not sufficient, however, to elucidate the factors responsible for alterations in benzene uptake, nor were they sufficient to determine whether the mouse of the present study was a suitable model for benzene uptake in humans.

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References

1. MacFarland, H.N.: *Respiratory Toxicology*. In *Environmental Toxicology*, vol. 7, pp. 121-155. New York: Academic Press, 1976.
2. Alarie, Y.: Irritation Properties of Airborne Materials in the Upper Respiratory Tract. *Arch. Environ. Health* 13: 1-10 (1966).
3. Long, J.E. and T.F. Hatch: A Method of Assessing Physiological Impairment Produced by Low-Level Exposure to Pulmonary Irritants. *Am. Ind. Hyg. Assoc. J.* 22:6-11 (1961).
4. Rinehart, W.E. and T. F. Hatch: Concentration-Time Product (CT) as an Expression of Dose in Sublethal Exposure to Phosgene. *Am. Ind. Hyg. Assoc. J.* 25:545-553 (1964).
5. Snedecor, G.W. and W.G. Cochran: *Statistical Methods*, 8th ed. Ames, Iowa: Iowa State University Press, 1973. pp. 109; 299-312.
6. Duncan, D.B.: Multiple Range and Multiple F Tests. *Biometrics* 11:1-42 (1955).
7. Snyder, R. and J.J. Kocsis: Current Concepts of Acute Toxicity. *CRC Crit. Rev. Toxicol.* 3(3):265-288 (1977).
8. Rysch, G.M., B.K.J. Leong and S. Leskin: Benzene Intoxication. *J. Toxicol. Environ. Health Suppl.* 2:23-36 (1977).
9. Leong, B.K.J.: Experimental Benzene Intoxication. *J. Toxicol. Environ. Health Suppl.* 2:45-61 (1977).
10. Bergman, K.: Whole-Body Autoradiography and Tracer Techniques in Distribution and Elimination of Some Organic Solvents. *Scand. J. Work Environ. Health* 1:1-263 (1979).
11. Nielsen, G.D. and Y. Alarie: Sensory Irritation, Pulmonary Irritation, and Respiratory Stimulation by Airborne Benzene and Alkylbenzenes: Prediction of Safe Inhalation Exposure Levels and Correlation with Their Toxicodynamic Properties. *Toxicol. Appl. Pharm.* 65:459-471 (1982).
12. Carpenter, C.P., C.B. Schaffer, C.S. Weil and H.F. Jr.: Studies on the Inhalation of 1,3-Butadiene: A Comparison of Its Narcotic Effect with Benzol, Toluene, Styrene, and a Note on the Elimination of Styrene in Human. *J. Ind. Hyg. Toxicol.* 28:69-78 (1944).
13. Dittmer, D. and R.M. Grebe, eds.: *The Handbook of Inhalation Toxicology*. Philadelphia: W.B. Saunders (1958). pp. 41-42.
14. Anderson, M.E., M.L. Gargas, R.A. Jones and L.L. Jr.: The Use of Inhalation Techniques to Assess the Kinetic Constants of 1,1-Dichloroethylene Metabolism. *Toxicol. Appl. Pharm.* 47:395-409 (1979).
15. Anderson, M.E., M.L. Gargas, R.A. Jones and L.L. Jr.: Determination of the Kinetic Constants for Metabolism of Inhaled Toxicants In Vivo Using Gas Uptake Methods. *Toxicol. Appl. Pharm.* 54:100-116 (1980).
16. Gonsaun, L.M., C. Witner, J.J. Kocsis and R. Snyder: Benzene Metabolism in Mouse Liver Microsomes. *Toxicol. Appl. Pharm.* 26:398-406 (1973).

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