Benzene fumes

pp 531-539
Journal of Toxicology & Environmental Health
Vol. 1 - No. 3 - Jan. 1976

ergism in

secticides
drocarbon

ty of the

, 27, 1975
, 24, 1975

RESPIRATORY RETENTION OF INHALED TOLUENE AND BENZENE IN THE DOG

John L. Egle, Jr., Bethe J. Gochberg

Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia

This study deals with the inhalation of toluene and benzene found in the vapor phase of cigarette smoke. Determined in this study were the uptake of each substance by the total respiratory tract and by the upper and lower portions under varying conditions of ventilatory rate, tidal volume, and concentration inhaled. Retention by the total tract of toluene fell within the range of 91–94% at all ventilatory rates seen, indicating no relationship between rate and retention. With benzene, total tract retention varied from 88 to 68%, with an inverse relationship between retention and rate apparent. This relationship was seen in all types of experiments conducted with benzene. Upper tract retention revealed an uptake of about 89% for both one- and two-way experiments with toluene. In the benzene experiments retention varied from 80 to 61% and from 81 to 63% in the one- and two-way procedures, respectively. Mean lower tract retention was 90% with toluene at all ventilatory rates considered. Benzene retention varied from 74 to 61% as the rate increased from 6 to 18 inhalations/min.

INTRODUCTION

Various components in the vapor phase of cigarette smoke have been shown to be toxic agents. The respiratory retention of four aldehydes, acetone, ammonia, isoprene, and methanol has been studied in the dog, as has the respiratory uptake of acetaldehyde in man (Egle, 1970, 1972a, b, 1973; Egle and Gochberg, 1975). Two additional components are benzene and toluene, with a concentration in the vapor phase of cigarette smoke reported to be 6.1 μg/40 ml pull (47 ppm) and 9.7 μg/40 ml puff (64 ppm), respectively (Newsome et al., 1965). This concentration of benzene is higher than the maximum allowable concentration (MAC) or 25 ppm.

Both benzene and toluene vapor have been studied with respect to their ability to cause chromosomal aberrations. It was shown that in an experiment conducted with three groups of men divided into those who inhaled only benzene, those who inhaled only toluene, and a standard control group, there was a significant increase in chromosomal aberrations seen in those who had been exposed to the benzene vapor (Forni et al., 1971). It has also been reported that exposure to toluene fumes impaired the

This study was aided by a grant from the American Medical Association Education and Research Foundation.

Requests for reprints should be sent to John L. Egle, Jr., Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23298.

Journal of Toxicology and Environmental Health, 1:531–538, 1976
Copyright © 1976 by Hemisphere Publishing Corporation

DBZ0030087

excretion of uric acid in men (Taher, 1973). Since Forni has shown that similar concentrations of benzene can cause chromosomal aberrations possibly leading to leukemia and toluene, while not as well studied, is also a toxic substance, it appears important to study the retention of these compounds by the respiratory tract.

The present investigation deals with the retention of toluene and benzene vapor in the respiratory tract of the dog, utilizing concentrations similar to those encountered in the vapor phase of cigarette smoke. The specific goals of this study were to determine the uptake of both benzene and toluene by the entire respiratory tract and by upper and lower portions under varying conditions of concentration inhaled and ventilatory rate.

METHODS

For all experiments in this study, mongrel dogs between 12 and 22 kg of both sexes were used. They were anesthetized with pentobarbital sodium intravenously (30 mg/kg). The animals were then placed in a supine position and allowed to breathe spontaneously for two of the procedures (total and lower tract) from a 13.5-liter recording respirometer for 1–2 min. The vapor inhaled in this series of experiments was generated by passing air through pure benzene or pure toluene at room temperature. In all cases, this concentrated vapor was transferred to the respirometer and diluted to obtain the necessary concentration. The exhalations were collected in a 5-liter rubber bag; the flow was directed by a two-way valve. Air samples that contained the vapors under investigation were withdrawn from the respirometer and collecting bag by 50-ml siliconized glass syringes. The samples were then bubbled through graduated cylinders containing 19 ml of the solvent compound being used. All samples during this study were analyzed via a Beckman G. C. 72-5 gas chromatograph. A Carbowax 6-ft column was used in these determinations. The column temperature for benzene was 110°C and for toluene 130°C. Sensitivity ranges for benzene and toluene were 100 and 10 K, respectively. Aliquot size for both vapors remained constant at 0.1 μl injected with a 1 μl Hamilton glass syringe with a Chaney adapter. The gas flow remained as in previous studies carrier gas (helium) 90 ml/min, breathing air 300 ml/min, and hydrogen 48 ml/min. The pressure of the carrier gas was 30 psi.

The types of experiments conducted can be divided into two broad categories: those in which the animals were allowed to inhale spontaneously from the respirometer and those in which we regulated rate (ventilations/minute) and volume inhaled. For the total tract and lower tract experiments, the animals inhaled via the nose from the respirometer and exhaled into a collecting bag. The procedure for total tract involved placing over the animal's mouth and nose a tightly fitting mask connected to a two-way valve; this arrangement ensured that inhalations came from the respirometer

DBZ0030088

and exhalations were captured in the collecting bag. The lower tract experiments were similar except that a 12-in. curved endotracheal tube was passed through the dog's trachea to limit the exposure of the vapor to the lower region of the respiratory tract.

Two types of experiments involved manual manipulation of the respirometer to expose the upper respiratory tract. These two types of upper respiratory tract experiments involved severing the trachea just above the bifurcation and attaching cannules to both of the severed ends. Cannulation of the lower (proximal) end allowed the animal to ventilate spontaneously. A mask was used to allow vapor to pass from the nose to the location of the other cannula at the lower end of the trachea. In the one-way, upper tract experiments the vapor passed into a collecting bag attached to the cannula at the end of the trachea. Two-way experiments involved the same surgical procedure and method except for the use of a 2-liter plastic syringe. This syringe was attached to the cannula at the end of the trachea with the vapor being drawn down and into the syringe, then returned upward and expelled through the nose, finally being collected in the bag.

RESULTS

All results presented here have been calculated to account for the various experimental dead space involved. The final percent uptake was determined by using the amount inhaled or exposed to the tissues and the amount recovered. Tabulated results are expressed as summaries of all animals within experimental designs since retention values within these groups of individual dogs were quite similar. At least six animals were used in each type of experiment, with several dogs being used chronically. Unless otherwise stipulated, the inhaled concentration of the compounds studied was ranged from 0.4 to 0.6 μg/ml.

Total Tract Retention

The retention of toluene and benzene by the total respiratory tract of the dog at ventilatory rates between 5 and 40 is summarized in Table 1. The retention of toluene fell within the narrow range of 91–94% with no evidence of a rate–retention relationship. With benzene, at a similar ventilatory rate range, the total tract retention varied from 88 to 68%. With this compound there appeared to be a definite inverse relationship between rate and retention. This trend was to continue throughout the benzene investigation.

Lower Tract Retention

In these experiments where only the lower tract was exposed to the benzene and toluene via an endotracheal tube, the retention values

DBZ0030089

TABLE 1. Total Tract Retention

Ventilatory rate			Percent uptake
Average	Range	No.	(mean ± SE)
	Toluene		
6	5–8	9	91.2 ± 1.39
10	9–11	12	94.0 ± 1.37
13	12–14	8	92.7 ± 1.20
15	15–17	7	90.6 ± 0.95
19	18–21	7	94.0 ± 0.90
30	22–40	10	92.7 ± 0.55
	Benzene		
7	5–8	7	88.3 ± 1.07
10	9–11	7	82.6 ± 1.52
13	12–14	13	77.5 ± 1.34
16	15–17	8	70.9 ± 0.64
19	18–21	7	68.7 ± 1.02
24	22–40	6	60.2 ± 0.96

are summarized in Table 2. The mean uptake at varying ventilatory rates was between 89 and 92% for toluene and 75–61% for benzene. Again it was evident that there existed an inverse relationship between benzene retention and rate. No such relationship was found with the toluene.

TABLE 2. Lower Tract Retention

Ventilatory rate			Percent uptake
Average	Range	No.	(mean ± SE)
	Toluene		
7	5–8	12	90.6 ± 0.84
10	9–11	10	89.4 ± 1.13
13	12–14	9	93.0 ± 1.05
16	15–17	9	90.4 ± 0.72
19	18–21	7	91.9 ± 1.72
36	22–40	11	91.5 ± 0.86
	Benzene		
6	5–8	11	76.7 ± 1.23
10	9–11	10	74.3 ± 0.74
12	12–14	14	73.5 ± 1.37
15	15–17	10	71.0 ± 1.06
21	18–21	12	67.5 ± 0.56
42	22–40	15	61.1 ± 1.84

DBZ0030090

Upper Tract Retention

As previously discussed, these experiments were divided into two types. The one-way experiments do not simulate any type of normal ventilation. The purpose of these experiments was to measure the reduction in concentration of the inhaled vapor between the nose and the bronchioles. Information of this type is of value in designing and interpreting experiments that involve nasal inhalation and studies that deal with effects on lower tract tissue using isolated organ or *in vitro* systems.

One-way upper tract experiments can be seen in Table 3. In these experiments retention of toluene was between 87 and 90%, with a mean of 89.1% regardless of ventilatory rate. The retention for benzene varied from 80 to 61%, again with the highest retention at the lowest ventilatory rate.

Two-way upper tract retention of toluene fell within the narrow range of 88–89%; no relationship was seen between rate and retention. With benzene, however, retention varied inversely with rate, declining from 81 to 63% as the rate increased from 6 to 18. The results for this type of experiment are summarized in Table 4.

Effect of Concentration

These experiments, summarized in Table 5, were designed to determine the effect, if any, of the concentration inhaled on the retention values. Their design is the same as for total tract exposures, except the concentration of the compound inhaled is lower and higher than the average concentration used (0.4–0.6 μg/ml). To minimize any

TABLE 3. Upper Tract One-Way Retention

Ventilatory rate			Percent uptake
Average	Range	No.	(mean ± SE)
	Toluene		
6	5–8	8	87.0 ± 1.61
9	9–11	8	89.5 ± 1.11
12	12–14	8	87.7 ± 0.86
15	15–17	8	90.3 ± 1.28
18	18–21	8	88.6 ± 0.92
	Benzene		
6	5–8	8	78.5 ± 1.18
9	9–11	9	73.2 ± 1.43
12	12–14	9	70.2 ± 0.74
15	15–17	9	63.7 ± 1.32
18	18–21	9	60.9 ± 1.56

DBZ0030091

TABLE 4. Upper Tract Two-Way Retention

Ventilatory rate			Percent uptake
Average	Range	No.	(mean ± SE)
	Toluene		
6	5–8	8	89.9 ± 2.24
9	9–11	8	89.0 ± 0.79
12	12–14	9	87.6 ± 1.32
15	15–17	8	88.6 ± 1.28
18	18–21	8	89.5 ± 0.88
	Benzene		
6	5–8	9	81.7 ± 1.52
9	9–11	7	74.3 ± 0.91
12	12–14	9	72.4 ± 1.38
15	15–17	9	70.1 ± 1.21
18	18–21	8	65.4 ± 0.94

possible rate effect that might become evident, only experiments that fell in the rate range of 10–19 were used. The mean of the low concentration range used was 0.37 μg/ml for both toluene and benzene, and the mean high concentration was 0.82 μg/ml for toluene and 0.93 μg/ml for benzene. The change in the concentration inhaled appeared to have no influence on retention of either compound.

Effect of Tidal Volume

The tidal volume experiments (Table 6) were carried out to observe if there was any relationship between the tidal volume of an animal and the amount retained within its respiratory system. Again a narrow range of ventilatory rates was used, 13–18 inhalations/min for toluene and 12–18

TABLE 5. Effect of Concentration on Retention

Concentration (μg/ml)		Average		Percent uptake
Average	Range	rate	No.	(mean ± SE)
		Toluene		
0.37	0.29–0.42	18	26	91.4 ± 1.10
0.51	0.45–0.56	15	18	92.4 ± 0.92
0.82	0.74–0.87	16	20	89.7 ± 0.96
		Benzene		
0.37	0.28–0.44	14	19	71.3 ± 1.07
0.52	0.46–0.56	15	23	70.6 ± 1.22
0.92	0.86–0.95	15	22	72.7 ± 1.30

DBZ0030092

TABLE 6. Effect of Tidal Volume

Tidal volume (ml)				
Average	Range	No.	Average rate	Percent uptake (mean ± SE)
		Toluene		
114	100–125	16	15	88 ± 1.02
140	126–150	22	14	86 ± 1.07
158	151–175	18	15	89 ± 0.96
189	176–200	20	16	88 ± 0.79
227	201–250	16	14	85 ± 0.85
		Benzene		
116	100–125	15	14	71 ± 0.89
143	126–150	22	16	69 ± 2.04
160	151–175	17	13	68 ± 1.27
181	176–200	19	14	70 ± 0.90
209	201–250	16	15	70 ± 1.06

inhalations/min for benzene. As tidal volume varied from 100 to 250 ml, there appeared to be no relationship between tidal volume and retention for toluene or benzene.

COMMENTS

Benzene has long been known for its ability to produce chromosomal aberrations. Kissling (1971) has found that subcutaneous injections of benzene disrupted the normal synthesis of both DNA and RNA. Recently, it has also become evident that chronic benzene exposure could lead to acute leukemia (Aksoy, 1972). Aksoy also reported an increase in aplastic anemia due to chronic benzene exposure in a working environment with a benzene concentration that varied between 150 and 210 ppm during working hours. Lange and his associates (1973a, b) published two reports dealing with workers exposed to benzene and toluene. The first dealt with the change in serum immunoglobulin levels; the second, with leukocyte agglutination in workers exposed to these compounds. The changes produced by benzene especially are significant enough to make one consider substituting an alternate compound for health and safety reasons alone. Toluene, although not yet proved to be as dangerous as benzene, is also capable of causing severe damage to man especially when "sniffed." Taher (1973) reported a significant correlation between toluene "sniffing" and renal tubular acidosis.

It is quite evident that retention in all types of experiments was markedly higher with toluene than benzene. With benzene, but not with toluene, there was an inverse relationship between retention and rate. The retention of toluene remained in the high 80 and mid 90% range throughout

DBZ0030093

the study, whereas benzene varied 20% within each experimental group with the inhalation rate being more significant than the type of experiment or the concentration inhaled.

REFERENCES

Aksoy, M. 1972. Acute leukemia due to chronic exposure to benzene. *Am. J. Med.* 52:160–166.

Egle, J. L., Jr. 1970. Retention of inhaled acetylaldehyde in man. *J. Pharmacol. Exp. Ther.* 174:14–19.

Egle, J. L., Jr. 1972a. Retention of inhaled acetaldehyde in the dog. *Arch. Environ. Health* 24:354–357.

Egle, J. L., Jr. 1972b. Retention of inhaled formaldehyde, propionaldehyde and acrolein in the dog. *Arch. Environ. Health* 25:119–124.

Egle, J. L., Jr. 1973. Retention of inhaled acetone and ammonia in the dog. *Am. Ind. Hyg. Assoc. J.* 34:533–539.

Egle, J. L., Jr. and Gochberg, B. J. 1975. Retention of inhaled isoprene and methanol in the dog. *Am. Ind. Hyg. Assoc. J.* 36:369–373.

Forni, A., Pacifico, E. and Limonta, A. 1971. Chromosome studies in workers exposed to benzene or toluene or both. *Arch. Environ. Health* 22:373–378.

Kissling, M. 1971. Chromosome aberrations in benzene exposure intoxication. *Helv. Med. Acta* 36:59–66.

Lange, A., Smolik, R., Zatonski, W. and Glazman, H. 1973a. Serum immunoglobulin levels in workers exposed to benzene, toluene and xylene. *Int. Arch. Arbeitsmed.* 31:37–44.

Lange, A., Smolik, R., Zatonski, W. and Glazman, H. 1973b. Leukocyte agglutination in workers exposed to benzene, toluene and xylene. *Int. Arch. Arbeitsmed.* 31:67–74.

Newsome, J. R., Norman, V. and Keith, C. H. 1965. Vapor phase analysis of tobacco smoke. *Tobacco Sci.* 9:102–110.

Taher, A. 1973. Toluene sniffing and renal acidosis. *Am. Occup. Hyg. J.* 15:193–201.

Received March 20, 1975
Accepted June 12, 1975

In
Science
of com
N. Am
been te
formula
mutagen
the sem
product
Since th
chemical
available
carcinoger

In ge
assay have
organisms,
the aroma
in some e
lack of a
experiment
mutagenicit

There
during the
urine for s
complete sy
the addition
myriad of a
form do the
How rapidly
additional qu
humans.

Copyright © 1976

DBZ0030094