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Toxicological Studies of Certain Alkylated Benzenes and Benzene

Experiments on Laboratory Animals

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I. Introduction

Alkylated benzenes, like benzene, are important to the chemical industry because of their many uses both as solvents and as chemical raw materials. Their utilization, either alone or in combination with other materials, may be as solvents for colored ink in printing, for dyes in the coloring of cloth, and in lacquers and paints. Other uses include linoleum manufacture, the impregnation of many materials, such as paper and silk, and the manufacture of brake linings. As chemical raw materials or intermediates, the alkylated benzenes have proved useful in the manufacture of explosives, agricultural pesticides, plastics, synthetic detergents, conditioners, antioxidants, and medicinals.

The extensive use of the alkylated benzenes in industry has created the need for toxicological information which would permit a comparison of their toxicities and the evaluation of the hazards associated with their use. The experimental work presented here was undertaken in order to obtain such information. The data were collected over a 17-year period.

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The literature on benzene and on some alkylated benzenes has been reviewed by Browning,[8] by the Manufacturing Chemists' Association,* by the American Petroleum Institute in published toxicological reviews,† and by von Oettingen.[22] Boundy and Boyer [7] have reported on the toxicity of styrene.

Browning [8] states that benzene is an acute and chronic poison. Acutely it is an anesthetic agent, producing severe or even fatal depression of the central nervous system. The chronic effect is that of injury to the hemapoietic system. The acute and chronic effects are two distinct phenomena and should not be confused.

Browning [8] stresses that, while toluene has a definite narcotic effect, it does not cause any apparent injury to the hemapoietic system, such as that produced by benzene. Animal experiments have indicated that toluene is more toxic acutely than benzene, but it appears to be less toxic chronically. This is in substantial agreement with the views expressed by von Oettingen.[22]

The exact degree of toxicity of xylene is still uncertain. According to Browning [8] most workers have believed it to be more toxic acutely than benzene and less toxic than toluene. Some workers feel, however, that it is more toxic than either. Human experience has shown that in its chronic effects xylene appears to be less toxic than benzene. No definite injury to the hemapoietic system has been attributed to it.

The chronic toxicity of ethyl benzene has not been reported according to Browning.[8] Animal experiments have been reported by Yant and associates [24] which indicate that

* References 6 and 20.

† References 5, 11, 19, 21, and 23.

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this material has an acute vapor toxicity of the same order as that of benzene.

The review on isopropl benzene of the American Petroleum Institute [11] points out that it is regarded by most workers as having a low degree of hazard under the conditions encountered in industry. However, in sufficient concentration it is a nervous system depressant similar in action to benzene. No chronic experiments on isopropyl benzene have been reported, but it is postulated that cumulative effects are possible because of its slow rate of elimination.

Monomeric styrene in high vapor concentrations has been shown by Spencer and associates [17] to be capable of producing central nervous system depression. Animal experiments have demonstrated that no serious chronic effects may be expected from concentrations which can be tolerated by man. It is apparently metabolized rapidly within the body.

Rylova [16] claims that the minimum concentration of styrene that causes an apparent effect upon the central nervous system of rabbits is 0.25 to 2 mg/liter (55 to 435 ppm). Repeated three-hour exposures daily to 1 mg/liter (217 ppm) caused some loss of coordination in young rats. α-Methyl styrene showed central nervous system effects in rabbits at 0.06 to 1 mg/liter (12 to 207 ppm).

All the materials have been reported to cause skin irritation, which has been postulated to be due in part to their fat solvent properties and in part to the effect of the chemical. Varying degrees of eye irritation have been reported for some of these materials.‡ Absorption through the skin has been accepted or assumed.§ However, recently Conca and Maltagliati [10] reported that benzene was not absorbed in man after immersion for 25 to 35 minutes of the hands and forearms. Their conclusions were based on urine sulfate determinations and tests for benzene in the expired air.

II. Compounds Investigated

Pertinent chemical and physical properties of the materials studied are listed in

‡ References 9 and 15.
§ References 5, 6, 8, 11, 21-23.

TABLE 1.—*Some Chemical and Physical Properties of Materials Investigated*

Material	Synonym	Boiling Point, C	Specific Gravity At 20 C	Purity in Per Cent	Per Cent of Isomers
Benzene	Benzol	80.0	0.88	99.98	----
Toluene	Toluol; methyl benzene	110.6	0.87	94.4+	----
Xylene	Xylol; dimethyl benzene	135-145	0.86-0.87	95	19 *o* 52 *m* 24 *p*
Ethyl benzene	--------	136.2	0.86	98+	----
Diethyl benzene	--------	180-184	0.88	99+	25 *o* 40 *m* 35 *p*
Isopropyl benzene	Cumene	152.4	0.86	98+	----
Styrene	Vinyl benzene	144.7-145.1	0.90	99+	----
α-Methyl styrene	--------	165.4	0.91	98.6-99.1	----
Vinyl toluene	x-methyl styrene	171.0	0.90	98+	55-70 *m* 30-45 *p*

Table 1. Purity, where given, was obtained by ultraviolet or infrared analysis.

III. Experimental Procedures

A. *Source, Feeding, and Selection of Animals.*—The white rats used in these studies were raised in this laboratory from a stock obtained from the Wistar Institute of Anatomy and Biology in 1938. They were maintained on a modified Sherman stock diet consisting of the following ingredients:

Whole wheat, freshly ground ..	55% by weight
Dried whole milk	25% by weight
Dried meat residue (or dried extracted liver)	12% by weight
Dried brewer's yeast	5% by weight
Calcium carbonate	1% by weight
Iodized table salt	2% by weight

The albino guinea pigs and rabbits were of a heterogeneous stock raised in this laboratory. They were fed a commercial diet ‖ supplemented by alfalfa hay; in addition, the guinea pigs received fresh cabbage.

The rhesus monkeys were imported animals held under observation in the laboratory several months before use. They were fed a variable diet of commercial laboratory chow,¶ peanuts, fruits, and vegetables.

Healthy rats of 175 to 250 gm. weight were selected for the single dose oral experiments.

The rats used for the repeated oral feedings and vapor exposures were divided according to body weight, sex, and number into well-matched groups about two months after weaning. Five rats were caged together in wire-mesh-bottomed cages.

The rabbits, guinea pigs, and monkeys used in the repeated vapor experiments were selected carefully on the basis of general health, growth, body weight, and appearance after a preliminary period of observation. These animals were divided into matched groups as to number, sex, and age.

All the animals used in the repeated oral feedings and the vapor exposures were weighed at regular intervals during the studies. Frequent observations were made of their general appearance and behavior. Records were kept of food consumption and mortality. Growth curves were drawn on each group. Those animals failing in health were killed when moribund and examined. Surviving animals were killed 18 to 22 hours after their last exposure and examined for evidence of organic injury.

The gross appearance of the lungs, heart, liver, kidneys, spleen, and testes was observed, and these organs were weighed. In addition, portions of the adrenals, pancreas, and femoral bone marrow, as well as those tissues mentioned above, were preserved in formalin and saved for preparation of hematoxylin-and-eosin-stained sections.

Oxalated blood was obtained from selected animals of each group at time of autopsy for determination of urea nitrogen by the diacetyl monoxime method.[4]

Bone marrow counts were made on selected animals of each group during both the repeated oral and vapor-exposure experiments. The number of nucleated cells per cubic millimeter of femoral bone marrow was estimated by the method described by Farrar,[22] using a red blood cell diluting pipette and 1% acetic acid as the diluting fluid. The total cell count was determined in a similar manner using Hayem's solution.[24]

When applicable, the Fisher *t*-test[28] was used in comparing the mean values of the final body and organ weights obtained on the exposed and unexposed animals. Probability values (P) of less than 0.05 were interpreted as indicating a significant difference.

The criteria considered in judging the toxic effects on the test animals in both the repeated oral and vapor experiments were growth, mortality, appearance and behavior, hematological findings, terminal concentration of urea nitrogen in the blood, final average organ and body weights, histopathological findings, and bone marrow counts.

B. *Oral Administration.*—1. Single Dose: The toxicity of benzene and some alkylated benzenes was determined on young adult white rats. The materials were introduced into the stomach by means of a stomach tube (8FS 16 in. all-rubber catheter), either as the undiluted material or as an olive-oil or corn-oil solution emulsified with a 5%-10% aqueous solution of acacia (gum arabic). The total volume administered was never greater than 7 cc. All the surviving rats were observed until recovery was assured (usually about two weeks).

2. Repeated Doses: Matched groups of 10 female rats each were given single doses of benzene, toluene, ethyl benzene, isopropyl benzene, or styrene daily by stomach tube, five days a week for six months. The material was fed as an olive-oil solution, as described in the previous section. The total volume administered daily was never greater than 2 to 3 ml. A group of 20 rats served as controls and were fed doses of 2.5 ml. of olive oil emulsified in the acacia

‖ This was Purina rabbit chow (complete ration) produced by the Ralston Purina Company, St. Louis.

¶ This was Purina laboratory chow.

solution on the same schedule as the experimental groups. The olive-oil solutions were made up at one time in sufficient quantity to last for about one month.

Hematological examinations were made on selected animals of each group at varying intervals usually after 20, 40, 80, and 130 doses. These consisted of the determination of total erythrocytes and leucocytes, hemoglobin content, as well as a differential white blood cell count.

C. *Skin Contact—Irritation and Absorption.*—The effects of benzene and some alkylated benzenes upon the skin were studied on white rabbits. The method as described by Adams and associates[1] was used, except that with some of the materials the applications were decreased in number. The materials were tested undiluted. Routinely 10 to 20 applications were made to the ear and a like number bandaged onto the shaved abdomen over a period of two to four weeks. The animals were observed daily and were weighed weekly. A significant loss of weight or signs of abnormal behavior were interpreted as indicative of absorption through the skin in acutely toxic amounts.

D. *Eye Contact.*—The effect of each of the materials tested upon the rabbit's eye was ascertained by introducing two drops of liquid onto the right eyeball. Visual observations of irritation and corneal injury (both internal and external) were made upon the treated eye at the following times after treatment: three minutes; one hour; and one, two, and seven days. A 5% water solution of fluorescein dye was used to stain and render visible the external injury of the cornea in all observations after the first (three minutes).

E. *Vapor Inhalation.*—1. Repeated Exposures to Laboratory Animals: Benzene, ethyl benzene, styrene, α-methyl styrene, and vinyl toluene were introduced into the exposure chamber by vaporizing into the in-flowing air at a constant rate a measured amount of the liquid under test. The vapor concentration within the exposure chamber was established using a sensitive analytical procedure and adjusted to the desired level, when possible, with the animals in the chamber and the chamber fan going to provide adequate atmospheric circulation. Analysis during the experiments demonstrated that the vapor concentrations were held uniformly within 10% of the desired concentration.

Two types of chambers were used. The large rectangular chambers were made of sheet metal and had a capacity of about 1700 liters and were equipped with a 30 in. circulating fan. The smaller chambers were fashioned of glass and were of about 160 liter capacity. These are described more fully elsewhere.[10]

Matched groups of 10 to 25 male and female rats and 5 to 10 guinea pigs and of 1 to 2 rabbits and 1 to 2 monkeys of either or both sexes were employed as experimental animals. "Air-exposed" and/or "unexposed" controls were used, depending upon the material. "Air-exposed" controls were subjected to repeated exposures of room air in a chamber, while the "unexposed" controls were maintained in a routine manner in the animal quarters. Routinely the "air-exposed" and test animals were placed in the exposure chambers seven to eight hours daily, five days a week.

2. Odor and Irritation Experiments—Human Subjects: Calculated amounts of styrene, α-methyl styrene, or vinyl toluene were introduced into the atmosphere of an enclosed, tightly sealed room. Utilizing sensitive analytical procedures, the concentration within the room was determined. Then human subjects entered the room quickly and noted their reactions with respect to odor, eye irritation, and nasal irritation.

IV. Results of Experiments

A. *Oral Administration.*—1. Single Dose: The results of the data obtained on rats following the administration of single doses of the various alkylated benzenes and benzene are summarized in Table 2.

TABLE 2.—*Summary of the Results of the Acute Oral Experiments*

Material	Sex	Animals Used, No.	Approx. $L.D._{50}$ Gm./Kg.
Benzene	Male	25	5.6
Toluene	Both	26	7.0
Xylene	Male	20	4.3
Ethyl benzene	Both	57	3.5
Diethyl benzene	Female	16	1.2
Isopropyl benzene	Male	20	1.4
Styrene	Both	57	5.0
α-Methyl styrene	Male	20	4.9
Vinyl toluene	Male	42	4.0

It is evident that the alkylated benzenes are low in acute oral toxicity, the approximate L. $D._{50}$ values ranging from 1.2 to 7.0 gm. per kilogram of body weight.

When the rats were autopsied, slight liver changes and, in some instances, some kidney

TABLE 3.—*Summary of Repeated Oral Feeding to Female Rats*

Material	Dose, Mg./Kg./Day	Feedings, No.	Experimental Period, Days	Effects*
Benzene	1	132	187	No effect
	10	132	187	Very slight leucopenia
	50	132	187	Leucopenia and erythrocytopenia
	100	132	187	Leucopenia and erythrocytopenia
Toluene	118	138	193	No effect
	354	138	193	No effect
	590	138	193	No effect
Ethyl benzene	13.6	130	182	No effect
	136	130	182	No effect
	408	130	182	Lw+ and Kw+; Lp+ and Kp+
	680	130	182	Lw+ and Kw+; Lp+ and Kp+
Isopropyl benzene	154	139	194	No effect
	462	139	194	Kw+
	769	139	194	Kw++
tyrene	66.7	132	185	No effect
	133	132	185	No effect
	400	132	185	G+; Lw+ and Kw+
	667	132	185	G++; Lw++ and Kw+

*L=liver
K=kidney
G=growth depression
w=average weight
p=histopathology
+=slight effect
++=moderate effect

involvement of questionable significance were observed. Isopropyl benzene appeared to cause some irritation to the stomach and intestine.

2. Repeated Doses: The results of repeated feeding by intubation of benzene, toluene, ethyl benzene, isopropyl benzene, and styrene are given in Table 3.

TABLE 4.—*Irritation and Injury to Rabbits' Skin Caused by Repeated Application of Undiluted Material*

Material	Irritation*	Necrosis†
Benzene	Slight to moderate	Moderate
Toluene	Slight to moderate	Slight
Xylene	Moderate to marked	Moderate
Ethyl benzene	Moderate	Moderate
Diethyl benzene	Moderate	Slight
Isopropyl benzene	Moderate	Slight
Styrene	Moderate	Slight
α-Methyl styrene	Moderate to marked	Slight
Vinyl toluene	Slight to moderate	Moderate

* The degrees of irritation are:
slight=perceptible erythema only
moderate=definite erythema
marked=cherry-red erythema similar to that caused by a good sunburn

† The degrees of necrosis are:
slight=development of a thin layer of devitalized tissue which resulted in exfoliation
moderate=development of edema and superficial necrosis; this resulted in a "chapped" appearance and exfoliation of large patches of skin

The results show that benzene is 100 times more toxic than the other materials by this route. Toluene is the least toxic, with a "no effect" level greater than 590 mg. per kilogram of body weight per day. Ethyl benzene, isopropyl benzene, and styrene are intermediate in degree of toxicity.

It is significant that benzene was the only material that affected the hemapoietic system. Of the other materials, only ethyl benzene caused histopathological changes. These were slight in nature and were characterized by cloudy swelling of the parenchymal cells of the liver and cloudy swelling of the tubular epithelium in the kidney. The rest of the materials tested caused significant increases in the average weights of the liver and/or kidney only.

B. *Skin Contact—Irritation and Absorption.*—Table 4 is a summary of the results obtained from skin contact to rabbits of benzene and some alkylated benzenes.

TABLE 5.—*Irritation and Injury to the Eyes of Rabbits Caused by Contact with Undiluted Materials*

Material	Eye Response	
	Conjunctival Irritation*	Corneal Injury†
Benzene	Moderate	Very slight, transient
Toluene	Slight	None
Xylene	Slight	Very slight, transient
Ethyl benzene	Slight	None
Diethyl benzene	Slight	None
Isopropyl benzene	Slight	None
Styrene	Moderate	Slight, transient
α-Methyl styrene	Slight	None
Vinyl toluene	Slight	None

*The degrees of irritation were defined as follows:
slight=perceptible irritation of the conjunctival membranes
moderate=inflammation and slight swelling of the eyelids

†The degrees of corneal injury were defined as follows:
very slight=questionable or just perceptible superficial necrosis in very small areas of the cornea
slight=perceptible superficial necrosis involving less then 50% of the cornea
moderate=definite superficial necrosis over more than 50% of the cornea

It is evident that all these materials cause a similar type of response when in contact with the rabbit's skin. This is characterized by more or less reddening, accompanied by varying degrees of exfoliation. Benzene, xylene, ethyl benzene, and vinyl toluene caused blistering, particularly when confined.

As judged by the gross appearance, behavior, and body weight of rabbits during the skin-irritation tests, there was no indication that any of these materials were absorbed through the skin in acutely toxic amounts.

C. *Eye Contact.*—The responses of rabbits' eyes to benzene and the alkylated benzenes are summarized in Table 5.

D. *Vapor Inhalation.*—1. Repeated Exposures to Laboratory Animals: Rats, rabbits, guinea pigs, and monkeys, in some cases, were exposed for seven to eight hours per day, five days a week for up to six months

TABLE 6.—*Results of Repeated Vapor Inhalation Studies on Animals Exposed to Benzene*

Animal Species	Average Vapor Concentrations, Ppm	Mg./L.	Sex	7-Hr. Exposures, No.	Duration of Experiment, Days	Effects*						
Rat	9,400	30	M	1-10	1-19	Nar++;	G+++;		Lp±	Bp+		M+++
	6,600	21	M	70	93	Nar++;	G++;	Tw++	Lp+	Sp+	Bp+++	M+++
	4,400	14	M	28	38	Nar++;	G+++;			Sp+	Bp+ Blp+	M+
	2,200	7	M	133	212	Nar+;	G+;	Sw+		Sp+	Bp+ Blp+	
	88	0.28	Both	136	204			Sw+			Blp+	
Guinea pig	88	0.28	M	193	269		G+;	Sw+	Tw+		Bp+	Blp+
	88	0.28	M	23	32			Kw+				Blp+
Rabbit	80	0.26	M	175	243				Kp+	Tp+		Blp+

*Nar=narcosis
G=growth depression
w=weight
p=histopathology
L=liver
K=kidney
B=bone marrow
S=spleen
T=testes
Bl=blood
M=mortality

The intensity of response is noted as follows:
±=questionable
+=slight
++=moderate
+++=severe

TABLE 7.—*Results of Repeated Vapor Inhalation Studies on Animals Exposed to Ethyl Benzene*

Animal Species	Average Vapor Concentrations, Ppm	Mg./L.	Sex	7-Hr. Exposures, No.	Duration of Experiments, Days	Effects*				
Rat	2,200	9.5	M	103	144	G++;	Lw+;	Kw++;	Lp+;	Kp+
	1,250	5.4	Both	138	214	G±;	Lw+;	Kw+;	Lp+;	Kp+
	600	2.6	Both	130	186		Lw+;	Kw+		
	400	1.7	Both	130	186		Lw+;	Kw+		
Guinea pig	1,250	5.4	F	138	214	G+				
	600	2.6	Both	130	186		Lw+			
	400	1.7	Both	130	186			No effect		
Rabbit	1,250	5.4	F	138	214					
	600	2.6	Both	130	186				Tp+	
	400	1.7	Both	130	186			No effect		
Rhesus monkey	600	2.6	Both	130	186		Lw+;		Tp+	
	400	1.7	F	130	186			No effect		

*G=growth depression
w=weight
p=histopathology
L=liver
K=kidney
T=testes

The intensity of response is noted as follows:
±=questionable
+=slight
++=moderate

to benzene, ethyl benzene, styrene, α-methyl styrene, and vinyl toluene. The data are given in Tables 6, 7, 8, 9, and 10 and are summarized in Table 11.

It is evident that benzene, when given by repeated inhalation, was the most toxic material of those tested by a factor of 5 to 8. Furthermore, benzene, in addition to causing the effects typical of this group of materials, caused a leucopenia. None of the alkylated benzenes tested by repeated vapor inhalation caused this effect upon the cellular elements of the blood.

The data for benzene, as given in Table 6, show that at the 80 to 88 ppm level indications of damage characterized by leucopenia, increase in average weights of the spleen and testes, and histopathological changes in the bone marrow and testes were found in the rat, guinea pig, and/or the

TABLE 8.—*Results of Repeated Vapor Inhalation Studies on Animals Exposed to Styrene*

Animal Species	Average Vapor Concentrations, Ppm	Mg./L.	Sex	7-Hr. Exposures, No.	Duration of Experiment, Days	Effects*
Rat	2,000	9.3	Both	105	148	Eye and nasal irritation G++
	1,300	6.0-6.3	Both	139	214	Eye and nasal irritation
Guinea pig	2,000	9.3	Both	98	148	Eye and nasal irritation G++
	1,300	6.0-6.3	Both	139	214	Eye and nasal irritation G+
	650	3.0	Both	130	189	No effect
Rabbit	2,000	9.3	Both	126	148	No effect
	1,300	6.0-6.3	Both	264	360	No effect
Rhesus monkey	1,300	6.0-6.3	Both	264	360	No effect

*G=growth depression
+=slight
++=moderate

TABLE 9.—*Results of Repeated Vapor Inhalation on Animals Exposed to α-Methyl Styrene*

Animal Species	Average Vapor Concentrations, Ppm	Mg./L.	Sex	7-Hr. Exposures, No.	Duration of Experiment, Days	Effects*			
Rat	3,000	14.49	Both	3-4	3-4				M+++
	800	3.86	Both	28	38	G+;	Lw+;	Kw+	
	600	2.90	Both	149	212		Lw+;	Kw+	
	200	0.97	Both	139	197			No effect	
Guinea pig	3,000	14.49	Both	3-4	3-4				M+++
	800	3.86	Both	27	38	G+;	Lw+;	Kw+	
	600	2.90	Both	144	212		Lw+		
	200	0.97	Both	139	197			No effect	
Rabbit	600	2.90	Both	152	212	G+;			M+
	200	0.97	Both	139	197			No effect	
Rhesus monkey	600	2.90	F	149	212			No effect	
	200	0.97	Both	139	197			No effect	

*G=growth depression
w=weight
p=histopathology
L=liver
K=kidney
M=mortality

The intensity of response is noted as follows:
+=slight
++=moderate
+++=severe

rabbit. This indicates that this level should not be considered as that of minimal effects and that the level safe for repeated vapor exposure of human subjects is well below 80 ppm.

From Table 7 it can be seen that the repeated inhalation of approximately 400 ppm of ethyl benzene caused only a slight increase in average liver and kidney weights in the rats. The guinea pigs, rabbits, and monkeys were not affected, as judged by any of the criteria considered. It would seem probable, therefore, that this level should be considered to be that level which causes at most only a minimal effect in any of the animals tested.

At higher concentrations histopathological examination demonstrated effects on liver, kidney, and testes. These may be char-

TABLE 10.—*Results of Repeated Vapor Inhalation on Animals Exposed to Vinyl Toluene*

Animal Species	Average Vapor Concentrations, Ppm	Mg./L.	Sex	7-Hr. Exposures, No.	Duration of Experiment, Days	Effects*				
Rat	1,350	6.52	Both	92-100	139	G++;		Lw++	Lp±	M++
	1,130	5.43	Both	92-100	139	G++;		Lw±	Lp±	
	580	2.80	Both	92-100	139			No effect		
Guinea pig	1,350	6.52	Both	92-100	139	G+;	Kw±	Lw±	Lp+	
	1,130	5.43	Both	92-100	139	G+;	Kw±		Lp±	
	580	2.80	Both	92-100	139			No effect		
Rabbit	1,350	6.52	Both	92-100	139		Kw±		Lp±	
	1,130	5.43	Both	92-100	139		Kw±			
	580	2.80	Both	92-100	139			No effect		
Rhesus monkey	1,350	6.52	F	92-100	139			No effect		
	1,130	5.43	F	92-100	139			No effect		
	580	2.80	F	92-100	139			No effect		

*G=growth depression
w=weight
p=histopathology
L=liver
K=kidney
M=mortality

The intensity of response is noted as follows:
±=questionable
+=slight
++=moderate

TABLE 11.—*Comparison of Results of Repeated Vapor Inhalation Studies on Animals Exposed to Benzene and Some Alkylated Benzenes*

Material	Animal Species Tested	No Effect Level, Ppm	Beginning Effect Level, Ppm	Effects*
Benzene	Rat Guinea pig Rabbit	Not found: well below 80	88	Leucopenia G+ Kw+ Sw+ Tw+ Tp+
Ethyl benzene	Rat Guinea pig Rabbit	Not found: probably about 200	400	Lw+ Kw+
Styrene	Rat Guinea pig Rabbit	650	1,300	G+ Eye and nasal irritation
α-Methyl styrene	Rat Guinea pig Monkey	200	600	G+ Lw+ Kw+ M+
Vinyl toluene	Rat Guinea pig Monkey	580	1,130	G+ to ++ Lw± Kw± Lp±

*G=growth depression
K=kidney
S=spleen
T=testes

B=bone marrow
w=weight
p=histopathology

The intensity of response is noted as follows:
±=questionable
+=slight
++=moderate

acterized by cloudy swelling in the liver, by cloudy swelling of the tubular epithelium in the kidney, and by degeneration of the germinal epithelium in the testes.

From Table 10 it can be seen that at 1350 and 1130 ppm vinyl toluene caused liver histopathology which was characterized by fatty degeneration in the midzonal and central cells of the liver lobule.

2. Odor and Irritation Experiments—Human Subjects: The results of the limited experimental studies on styrene, α-methyl styrene, and vinyl toluene employing human subjects are summarized in Table 12.

It is evident that the "comfort" level of exposure to humans is well below the "no effect" level as determined by animal experiments.

V. Comment

A. *Nature of Physiological Responses.*—It is evident from both the repeated oral and vapor inhalation experiments that benzene at low levels affects the hemapoietic system of rats, rabbits, and guinea pigs, resulting in leucopenia. This effect was not shown by any of the other alkylated benzenes studied, emphasizing a very important

TABLE 12.—*Results of Limited Human Exposures*

Type and Degree of Response	Styrene, Ppm	α-Methyl Styrene, Ppm	Vinyl Toluene, Ppm
Very strong odor; strong eye and nasal irritation	600 or more	600 or more	400 or more
Objectionably strong odor	200 to 400	200	300
Strong odor but tolerated without excessive discomfort	100	100	200
Detectable odor but no irritation	60	50	50
Odor not detectable	Less than 10	Less than 10	Less than 10
"No effect" concentration as determined by animal experiments	650	200	580

difference of toxic effect. This finding is in accord with those of the majority of the published investigations, as summarized by Browning.[8]

In addition to its effect upon the hemapoietic system, benzene also produced evidence of ill effects characterized by increased average weights of spleen and kidney and degeneration of seminiferous tubules of the testes. The alkylated benzenes produced increases in the average weights of the kidney and/or the liver as well as histopathologic changes in these organs at higher concentrations; however, it should be pointed out that benzene produced toxic effects at a lower concentration than the other materials. Benzene was found to be markedly more toxic by repeated inhalation and ingestion than any of the alkylated benzenes. These findings agree with the conclusions of Henderson and Haggard, as quoted by Browning[8]: "In the literature the relative toxicity of benzene and toluene is uncertain. Probably there are no great differences in (acute) toxicity . . . Toluene is less active than benzene in causing chronic poisoning."

The repeated oral data on toluene, ethyl benzene, isopropyl benzene, and styrene demonstrate that the latter three materials are similar in degree of toxicity, whereas toluene was found to have a lower "no effect" level.

The similarity in degree and type of toxicity is also seen in the repeated vapor inhalation studies. The data show that with ethyl benzene, styrene, α-methyl styrene, and vinyl toluene there is only a threefold difference in "no effect" level of toxicity between the one most toxic and the one least toxic.

The results of studies upon skin and eyes of benzene and the alkylated benzenes show that, except for minor differences, all these materials are similar in degree and type of effect. Although the data presented show that absorption was not apparent, various reviews # indicate that absorption of toxic amounts may occur where exposures involve large areas of skin for prolonged periods of time. This conclusion is based, for the most part, on observed human experience. While this may be true, it would seem that, if such gross exposure to the skin occurs, the likelihood that toxic amounts of vapors would be inhaled is appreciable. Thus, it may be questioned whether the effects attributed to absorption through the skin were, in fact, caused by skin contact.[5] Furthermore, the significance of absorption through the skin in practical applications would seem to be minimal. This view is supported by the recent work of Conca and Maltagliati[10] who were unable to detect signs of benzene absorption.

B. *Handling Precautions.*—The precautionary measures for handling toluene, xylene, styrene, and, to a slighter degree, isopropyl benzene are well described in the literature.* It is proposed that the other alkylated benzenes considered herein be handled while observing the same precautions. This proposal is based upon the similarity of toxic action of these alkylated benzenes.

Benzene, because of its greater toxicity and the seriousness of the consequences of overexposure, requires more stringent handling precautions than the alkylated benzenes, particularly as they relate to vapor inhalation.†

C. *Industrial Hygiene Standards.*—The philosophy of Industrial Hygiene Standards has been discussed in a previous publication[2] from this laboratory. The Industrial Hygiene Standard may be defined as that concentration below which practically all analytical results must fall when air samples are taken intermittently in representative work locations throughout the whole workday. Allowance for short exposures to higher concentrations is made by means of the personal judgment of the qualified industrial hygienist.

References 5, 6, 8, 11, 21-23.

* References 11 and 19-23.

† References 5 and 6.

Threshold Limit Values for 1955,[3] suggested by the American Conference of Governmental Industrial Hygienists, are as follows: benzene, 35 ppm; toluene, 200 ppm; xylene, 200 ppm; ethyl benzene, 200 ppm, and styrene, 200 ppm.

In view of the results of toxicological studies on animals, together with the results of experiments with human subjects, it is probable that vapor concentrations of styrene, α-methyl styrene, and vinyl toluene, which will voluntarily be tolerated by most persons, will not cause adverse systemic effects. Because the vapors of these materials are definitely disagreeable at concentrations well below the levels capable of causing adverse systemic effects, there is little, if any, need for an Industrial Hygiene Standard based upon a concentration not likely to cause toxic effects. However, if such a standard is established, it is believed that a value of 400 ppm for styrene and vinyl toluene and 200 ppm for α-methyl styrene would be appropriate. In order to avoid complaints of disagreeable odor intensities and eye and nose irritation, it may be necessary to maintain the concentration of styrene, α-methyl styrene, and vinyl toluene at 100 ppm or less.

VI. Summary and Conclusions

Studies on rats have shown that benzene, toluene, xylene, ethyl benzene, diethyl benzene, isopropyl benzene, styrene, α-methyl styrene, and vinyl toluene have low acute oral toxicities. The calculated L. D_{50} values fall between 1.2 and 7.0 gm. per kilogram of body weight, with diethyl benzene being the most and toluene being the least toxic.

Experiments on rabbits have demonstrated that benzene and the above-named alkylated benzenes caused slight to moderate irritation of the skin and were not absorbed through the skin in acutely toxic amounts.

These materials were shown to cause slight to moderate irritation and only transient corneal injury when tested on the eyes of rabbits.

Female rats fed 1 mg. per kilogram per day of benzene, 590 mg. per kilogram per day of toluene, 136 mg. per kilogram per day of ethyl benzene, 154 mg. per kilogram per day of isopropyl benzene, or 133 mg. per kilogram per day of styrene by intubation for periods up to six months exhibited no evidence of ill effects as determined by gross appearance, growth, periodic blood counts, analysis for blood urea N, average final body and organ weights, histopathological examinations, and bone marrow counts.

Groups of rats, guinea pigs, rabbits, and monkeys were subjected to repeated seven- to eight-hour daily vapor exposures, five days a week for periods of up to six months. Styrene at 650 ppm, α-methyl styrene at 200 ppm, and vinyl toluene at 580 ppm were without ill effects, as judged by the criteria listed for the repeated oral-feeding studies. Benzene was shown to cause mild leucopenia and splenic and testicular degeneration in the test animals when exposed repeatedly to 80 to 88 ppm. Ethyl benzene at 400 ppm caused a slight increase in the average weight of the kidneys and livers of rats.

Experimental data are given to show that benzene was the only material that affected the hemapoietic system.

Industrial Hygiene Standards are discussed and defined. Based upon toxicity, it is believed that values of 400 ppm for styrene and vinyl toluene and 200 ppm for α-methyl styrene would be appropriate.

Experiments and experience with human subjects have shown that vapor concentrations suggested above are distinctly disagreeable to most persons. In order to avoid complaints of disagreeable odor or eye and nasal irritation, it may be necessary to maintain the vapor concentrations of styrene, α-methyl styrene, and vinyl toluene at 100 ppm or less.

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