

THE ACUTE TOXICITY OF VAPORS OF CERTAIN SOLVENTS CONTAINING APPRECIABLE AMOUNTS OF BENZENE AND TOLUENE*

J. L. SVIRBELY, R. C. DUNN, AND W. F. VON OETTINGEN

From the Division of Industrial Hygiene, National Institute of Health, Bethesda, Maryland

THE literature concerning the toxicity and potential dangers of aromatic hydrocarbons has been reviewed repeatedly in recent years (1, 2, 3, 4). Many of these hydrocarbons are employed in industry, either as individual solvents or in the form of complex mixtures or blends. The toxicity of the more common solvents such as benzene, toluene, and xylene is known with a fair degree of accuracy but little is known concerning the possible effects of exposure of the blends. In view of the increasingly extensive use of these mixed solvents in industry as substitutes for toluene, an investigation of two well-known blends was undertaken. These solvents were chosen because they contained a high percentage of benzene and because it was claimed that the toxicity of these products was of the same general order of magnitude as that of toluene and much below that of benzene. The present report covers the first part of this study. It is concerned with the effects of single, moderately long exposures of mice to various concentrations of these solvents and of toluene and benzene.

MATERIALS INVESTIGATED

The solvents studied were: Benzene, Baker's, C. P., 99.5 per cent; a 60 per cent benzene and 40 per cent toluene mixture (or solution) by weight; toluene (Barrett) containing not more than 0.01 per cent benzene, and two commercial blends which have been designated as solvent X and solvent Y.

The solvents were obtained from the manufacturers and were used without further attempt at purification. Analyses of the mixed solvents obtained from different sources are listed in Table 1.

Inspection of the data in Table 1 indicates that there was close agreement between the analysis of the solvent as furnished by the manufacturer compared to that found in our division by chemical and physical methods. It must be emphasized

that mixed solvents are not always of constant composition. For this reason, blends used in industry at the present time may not necessarily show the same analysis at all times. The proportions of the major components in the blends listed in the table are valid only for the solvents obtained at the time of analyses.

METHOD

Concentrations for exposure were obtained by the use of the apparatus described by Werner et al. (6) excepting that in those experiments where mixed solvents were used, it was necessary to use a dropping bottle in place of the bubblers. The solvent was dropped at a constant rate on a gauze in an enclosed flask which was immersed in a constant temperature water bath. All air used was first passed through a flowmeter into a flask and the vapor-air mixture was then passed through the mixing tube into the exposure chamber.

Values for concentrations were calculated in milligrams per liter by dividing the weight of the solvent vaporized at the end of exposure by the amount of air used. These values were in close agreement with the vapor concentration in the exposure chamber as measured by the Rayleigh-Jeans Interference Refractometer. Readings of the interferometer indicated that even distribution of vapor-air mixtures was obtained throughout the chamber and that maximal concentration was generally attained within 45 minutes. By means of frequent interferometric determinations (half-hour intervals) any fluctuations in concentration could be readjusted by controlling the rate of flow of the solvent. Mixtures of benzene and toluene have been satisfactorily analyzed by a photometric method (5). Application of this method to determine the vapor concentration of the solvents was not successful due to errors in sampling, the condensation of the vapor-air mixtures on walls of the glass apparatus, etc. This photometric method was also more time-consuming than the interferometric measurements and for this reason it was discontinued.

Calibration curves for the interferometer have

* Received for publication May 27, 1943. A paper presented at the annual meeting of the American Industrial Hygiene Association, May, 1943.

been drawn by plotting the scale readings against the concentration of the vaporized solvents, as shown in Figure 1. With some of the higher concentrations, the scale readings were beyond the range of the instrument. A modified method for reading the interferometer in these cases was used. The procedure involved the change in refractivity of vapor-air samples at the usual and interchanged positions of the sampling compartments. The values for concentration obtained by this modified

weighed approximately 20 grams they were exposed in groups of 16 or more for seven-hour periods.

RESULTS

Mice exposed to vapors of the solvents studied generally showed the following progressive symptoms—restlessness, muscular twitching, an S-shaped curve in the tail, changes in respiration, incoordination, and evidence of narcotic effect.

TABLE 1
ANALYSIS OF BLENDS INVESTIGATED

ANALYSIS	BENZENE PER CENT	TOLUENE PER CENT	XYLENE PER CENT	ALIPHATIC HYDROCARBONS PER CENT	MOLECULAR WEIGHT*
Solvent X					
Labeled percentage					
Manufacturer.....	60.0	33.3	3 to 4	3 to 4	—
Determined percentage					
a. Physical†.....	62.0	30	4	4 (heptane probably)	84.3 (calc.)
b. Chemical‡.....	60.9	26.6 (toluene and xylene)		12.5	83.7 {84.6 82.8
Solvent Y					
Labeled percentage					
Manufacturer.....	60 to 52	35 to 34	4 to 3	3 to 11	—
Determined percentage					
a. Physical†.....	50	45	5	—	85.8 (calc.)
b. Chemical‡.....	49.0	35.3 (toluene and xylene)		15.7	87.0 {86.5 87.5

* The molecular weight has been determined by Mr. D. E. Rushing, Associate Chemist of this Division.

† The infra-red and ultraviolet absorption spectra of the solvent agreed well with the spectra of a synthetic material of this composition. The experiments were conducted by Mr. C. Z. Nawrocki and Dr. M. B. Hall, formerly connected with the Washington Biophysical Institute in this Division.

‡ Dr. H. D. Baernstein, Biochemist of this Division, analyzed samples of the solvents by a colorimetric (photometric) method. Details of the procedure of this method have recently been published (5).

method agreed well with the calculated experimental concentrations.

As indicated in Table 2, the analysis of vapors may be made directly from the calibration curves if the scale readings are noted. This procedure is applicable when no refractivity data is available and compares favorably with the method involving the calibration formula of the interferometer and the refractivities of the substances being considered.

White Swiss mice, raised at the National Institute of Health and fed a basal diet of Ralston's Purina Dog Chow, were used. When they

Convulsions and the S-shaped position of the tail were frequently noted at or above the M.L.C. and the early occurrence of these symptoms seemed to depend on the benzene content of the solvent. In mice, twitches and tremors at the beginning of exposure was usually more pronounced below, at or above the lethal level and appeared to depend on the benzene content of the solvent. Animals which were more readily narcotized recovered as a rule more promptly than those which were narcotized slowly. Below the lethal level recovery was about equally rapid with all solvents, while at or above the lethal concentration the delayed

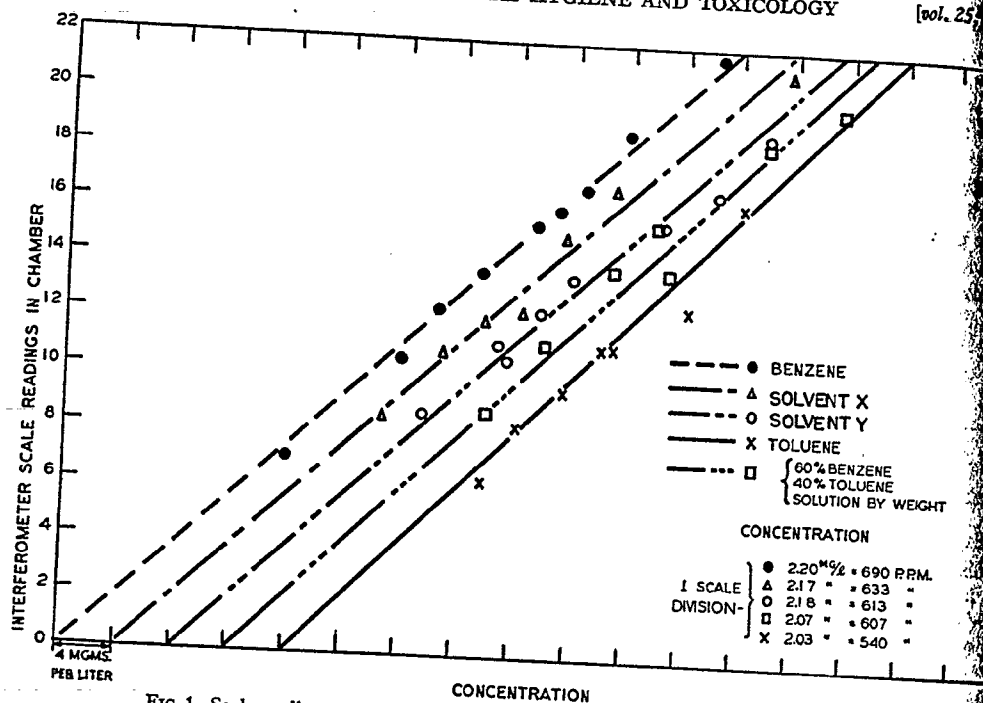


FIG. 1. Scale readings of the interferometer at known concentrations of the solvents.

TABLE 2
VALUES FOR CONCENTRATION AS OBTAINED BY INTERFEROMETER

SOLVENT	INDEX OF REFRACTION* (GASEOUS STATE)	SCALE READINGS OF INTERFEROMETER FOR 1,000 P.P.M. AT 26°C.	
		Calculated from index of refraction	Obtained from Figure 1
Benzene	1.001823	1.51	1.45
X	1.0020188 (from manufacturer's labeled percentage)	1.71	
	1.001945 (from physicist's analysis of blend)	1.65	1.62
Y	Difficult to calculate from variable manufacturer's labeled percentage of blend	—	
	1.001971 (from physicist's analysis of blend)	1.66	1.63
60% benzene + 40% toluene mixture	1.0019282	1.58	1.65
Toluene	1.002086†		
Xylene	1.002415	1.77	1.85
Heptane	1.0023		

* The index of refraction of the blends was calculated from the index of refraction of the components times its percentage.

† Calculated from index of refraction of liquid toluene by Lorenz-Lorentz formula.

60%
40%
mi

Toluene

* Ti
† Ba
‡ De

recove:
conten
noted

TABLE 3
CUMULATIVE MORTALITY RATES AT DIFFERENT INTERVALS AFTER 7-HOUR EXPOSURES TO VAPORS OF BENZENE AND RELATED SOLVENTS

SOLVENT	NUMBER OF MICE	CONCENTRATION		PERCENTAGE OF MICE DEAD						
		mg./l.	p.p.m.†	Hours*			Weeks*			
				8	36	48	1	2	3	4
Benzene	18	46.5	14,600	100‡						
	20	39.6	12,430	75						
	18	36.8	11,540	89						
	20	34.9	10,950	45						
	16	33.3	10,450	50						
	16	32.2	10,200	75					56.3	56.3
	20	29.6	9,280	35						
	18	26.6	8,330	16.7				22.2		27.8
	18	23.9	7,490	11.1						16.7
	18	15.9	4,980	0						
X	18	47.5	13,830	100‡						
	16	35.01	10,250	87.6						
	18	31.4	9,180	72.3		87.6				
	16	28.75	8,390	62.5						
	18	25.9	7,560	44.5						
	16	22.9	6,680	18.7						
	16	18.72	5,460	0						
Y	16	42.09	11,830	93.7						
	16	38.38	10,780	87.6		93.7				
	16	34.43	9,680	87.6			93.7			
	16	27.95	7,860	81.3		93.7				
	16	26.12	7,340	75						
	16	23.42	6,580	25				43.7		
	16	22.8	6,410	56.2				68.7		
	16	17.57	4,940	6.25						
60% benzene + 40% toluene mixture	16	43.34	12,670	100						
	16	37.8	11,050	75		93.7	100			
	17	31.09	9,080	100‡						
	16	30.30	8,840	62.5		75	87.6			
	16	26.56	7,760	50		56.2			62.5	
	17	21.77	6,350	29.4		37.5	47.1		58.8	
	16	17.78	5,190	12.5				25.0		31.2
Toluene	16	32.1	8,520	87.5	100					
	20	28.44	7,550	75	100					
	19	23.05	6,120	57.9	84.2					
	19	22.17	5,880	63.2	84.2					
	16	19.43	5,160	75	100					
	16	16.29	4,320	50	93.8		100			
	19	13.80	3,660	10.5	42.0		57.9			

* Time figure from beginning of exposure. * Entries made after 36 hours only when additional deaths occurred.

† Based on assumption that 1 gm. molecular volume occupies 24.45 liters at 25° C. and 760 mm. pressure.

‡ Deaths noted at or near end of 6 hours of exposure.

recovery from narcosis depended on the toluene content of the solvent. Many of the symptoms noted with benzene and toluene had previously

been observed with different species of animals in inhalation experiments by others (7, 8, 9, 10).

From the data presented in Table 3 it is ap-

parent that, with nearly all concentrations, the larger part of the mortality occurred within 8 hours after starting exposure. Dyspnea, muscular weakness and delayed deaths were frequently found in those mice exposed to a high percentage of toluene in the solvent-air mixture.

DISCUSSION

The data in Table 3 indicate the time as well as percentage of death with various concentrations of vaporized solvents. The mortality within the eight hours was distributed fairly evenly between

calculated including all deaths up to 48 hours and are shown in Tables 4 and 5.

The evaluation of the relative toxicities of the solvents was obtained by inspection of the curves in Figure 2 and the data in Table 3. The methods used in calculating the comparative toxicities of the solvents were:

a) M.L.C. (minimum lethal concentration or that which will kill 50 per cent of the animals) representing all deaths observed in a stated interval. In this method a sigmoid curve is drawn through a number of points obtained by plotting

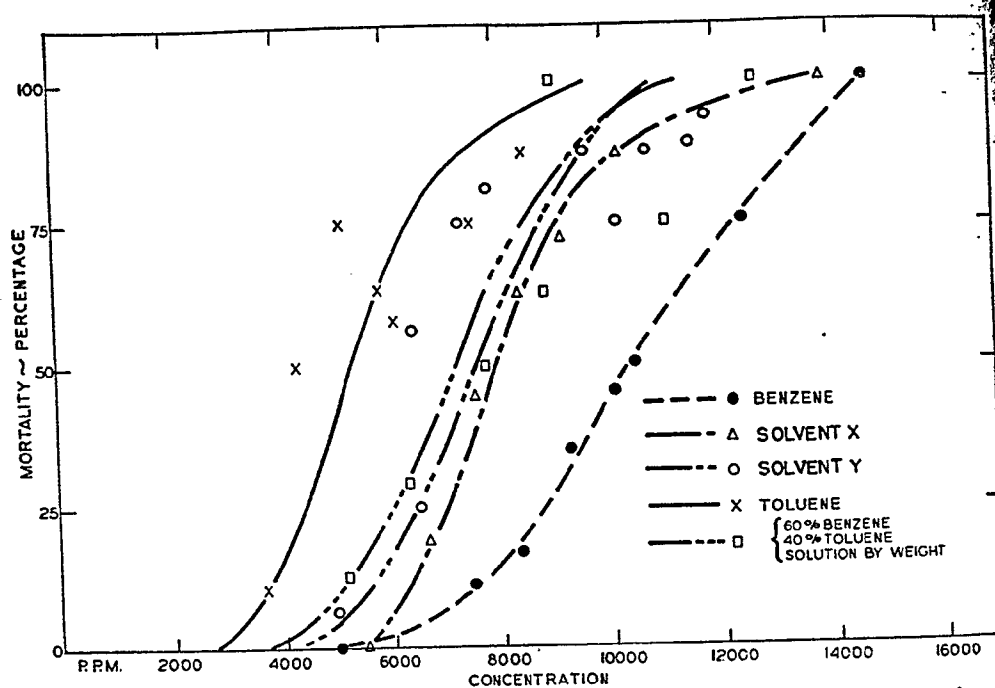


FIG. 2. Curves relating mortality for 7-hour exposure plus 1 hour for observation to vapor concentration expressed as parts per million.

2 and 8 hours. It is apparent that most of the deaths occurred within one hour after terminating exposures. The number of animals dying after this period is small, therefore a comparison of the solvents as to delayed deaths is difficult. However, it is important to note that with the exception of solvent X some deaths occurred within one to four weeks after exposure. In the case of toluene as well as the mixture of benzene and toluene, there were an appreciable number of deaths between 8 and 48 hours after starting exposures. In these instances data for LD 50 have also been

mortality against the concentration. That point in the curve which represents 50 per cent mortality is taken as the M.L.C. The interpolation of a sigmoid curve through a number of points which may be widely scattered occasionally presents difficulties.

b) L.D. 50 (lethal dose killing 50 per cent of the animals) is the term of a statistical method which is coming into general use and offers excellent possibilities for the evaluation of toxicity studies. For complete details, several papers by Bliss (11, 12, 13) are available. The method has been applied in

determining derivatives

The compounds obtained by analysis and listed in Table 3 have been found to have relative toxicity regarding the most toxic blends are solvents. The species of a city and naphthalene (7, 10) for mice

Tc

sc

Benzene.....
X.....
Y.....
60% benzene
40% toluene
8 hr.....
48 hr.....
Toluene
8 hr.....
36 hr.....

liter (7980 ml)
liter (14.1 ml)
peritoneal
indicated to
compared to

In Table 3 "rectified" series of Table 3 of 19 in 20 coefficient of logarithm of coefficient of definition, "coefficient" is the given population the logarithm

determining the toxicology of some glycols and derivatives by Calvery, et al (14).

The concentrations for M.L.C. and L.D. 50 obtained by inspection of the curves in Figure 2 and analysis of the data by statistical methods are listed in Table 4. Surprisingly good agreement has been found between the two methods. The relative toxicities of the solvents indicate that with regard to the acute toxicity in mice, toluene is the most toxic, benzene the least toxic and that the blends are of similar toxicity between these two solvents. Inhalation experiments with different species of animals had indicated the greater toxicity and narcotic action of toluene as compared to benzene (7, 9, 10). The minimal fatal concentration for mice has been reported as 30 to 35 mg. per

Although valuable data can be obtained by such acute toxicity studies, information concerning the toxic effects of solvents by repeated exposures to concentrations appreciably lower than the lethal dose is much more important from the industrial health viewpoint. Experiments to obtain such information at various concentrations are under way. Since these chronic studies with different species of animals require a long time to complete, it was thought advisable to publish the data obtained by the acute toxicity studies in mice.

PATHOLOGY

Paraffin sections were made from the lungs, liver, kidney, spleen, and heart of animals dying during exposure. Sections were routinely stained

TABLE 4
TOXICITY OF BENZENE AND RELATED SOLVENTS IN MICE IN 7-HOUR EXPOSURE PERIOD PLUS
1 HOUR FOR OBSERVATION

SOLVENT	MINIMUM LETHAL CONCENTRATION		LIMITS OF ERROR			LIMITS OF ERROR			PER CENT DEVIATION BETWEEN M.L.C. AND L.D. 50
			L.D. 50	Upper	Lower	L.D. 50	Upper	Lower	
	mg./l.	p.p.m.	mg./l.	mg./l.	mg./l.	p.p.m.	p.p.m.	p.p.m.	
Benzene.....	33.1	10,400	31.79	34.57	29.23	9,980	10,860	9,180	+4.0
X.....	27.2	7,980	27.36	28.95	25.86	8,000	8,460	8,000	-0.45
Y.....	26.9	7,580	22.95	25.95	20.30	6,460	7,310	5,720	+14.7
60% benzene + 40% toluene mixture									
8 hr.....	24.58	7,200	25.86	28.70	23.30	7,560	8,400	6,820	-5.1
48 hr.....	—	—	23.97	25.98	22.12	7,000	7,600	6,470	—
Toluene									
8 hr.....	19.90	5,300	19.96	21.45	18.64	5,320	5,710	4,960	-0.3
36 hr.....	—	—	17.20	17.42	16.97	4,580	4,640	4,520	—

liter (7980 to 9320 ppm.) for toluene and 45 mg. per liter (14,130 p.p.m.) for benzene (8). Intraperitoneal injections of solvents in rats likewise indicated the greater acute toxicity of toluene as compared with benzene (15).

In Table 5 the constants for the equations of the "rectified" dosage-mortality curves obtained by the series of transformations from the original data of Table 3 are given. The fiducial limits for odds of 19 in 20 have been calculated for the regression coefficient as well as the L.D. 50's expressed as the logarithm and as the actual dose. The regression coefficient is a measure of the variability, since by definition, "s", the reciprocal of the regression coefficient is the standard deviation of the response of a given population to a drug or poison in terms of the logarithm of the dose.

by Lillie's (16) eosin-polychrome methylene blue method. Sections from the spleens and livers were also stained by ferrocyanide to demonstrate the presence or absence of iron-bearing pigment. A total of 581 histological sections from 60 mice were studied.

No conspicuous pathological findings were observed, due to the shortness of the exposure period. However, some changes were noted that could be attributed to materials used.

A slight to moderate congestion of the alveolar capillaries was present in all animals. It was more frequently accompanied by extravasation of red blood cells into the alveolar spaces in the mice exposed to benzene. In contrast, hemorrhages were found in only a limited number of animals exposed to solvent X, solvent Y, toluene and a mixture of

60 per cent benzene and 40 per cent toluene. Pulmonary edema was present in only 1 mouse in the 5 groups of experiments. There was no other quantitative difference in the degree of lung pathology in the animals exposed to various substances, regardless of concentration, material, or time of death.

Evidence of kidney irritation, consisting of casts and granular basophilic debris in the lumina of the tubules, was more marked, both in degree and number of animals, in those exposed to solvent X, solvent Y, toluene and the toluene-benzene mixture than in those exposed to benzene alone. Congestion was inconspicuous in practically all of the mice. Relatively little or often no damage was

changes which could be attributed to the exposures were as follows:

1. Pulmonary irritation, shown by moderate congestion and some extravasation of red blood cells into the alveolar spaces was most marked in the animals exposed to benzene.

2. Renal irritation, evident in the presence of casts and granular debris in the lumina of the tubules, was more prominent in the animals exposed to the substances containing toluene.

3. In the spleen, follicular phagocytosis of nuclear material, indicative of cellular destruction was observed in all but 1 of the mice, regardless of the solvent to which they were exposed or the duration of exposure. Hence, this finding does not

TABLE 5
CONSTANTS FOR EQUATION $Y = \bar{y} + b(x - \bar{x})^*$

SOLVENT	$\bar{x}$	$\bar{y}$	b	$\pm 1.96 \times$ S.E.	LOG DOSE	$\pm 1.96 \times$ S.E.	L.D. 50 mg./l.	LIMITS OF ERROR	
								Upper mg./l.	Lower mg./l.
Benzene.....	1.520	5.085	4.814	3.26	1.502	0.036	31.79	34.57	29.23
X.....	1.441	5.048	11.468	3.595	1.437	0.024	27.36	28.95	25.86
Y.....	1.404	5.213	4.953	1.446	1.361	0.053	22.95	25.95	20.30
60% benzene + 40% toluene mixture									
8 hrs.....	1.456	5.334	7.612	2.398	1.413	0.045	25.86	28.70	23.30
48 hrs.....	1.430	5.446	8.887	2.673	1.380	0.035	23.97	25.98	22.12
Toluene									
8 hrs.....	1.328	5.247	8.865	4.683	1.301	0.030	19.96	21.45	18.64
36 hrs.....									
1st approx.....	1.308	6.165	4.002	2.311	1.017	0.184	10.39	6.81	15.86
2nd approx.....	1.254	6.567	0.8236	1.889	1.235	0.006	17.20	16.97	17.42

* $\bar{Y}$, expected probit; $\bar{y}$, weighted mean probit; b, regression coefficient; x , log dose $\approx$ L. D. 50 when $Y = 5$; $\bar{x}$, weighted mean log dose.

found in the kidneys of the mice exposed to benzene. In the kidneys, as in the lungs, concentration and duration of exposure produced no evident quantitative difference in the degree of pathology.

With the exception of traces of hemosiderin and very slight congestion of the sinusoids no changes were observed in the liver.

In the spleen, fragments of nuclear material were noted in the follicles in significant amounts throughout the entire series of animals. Hemosiderin, indicative of red blood cell destruction, was negligible. Other changes in the spleen appeared to be of no importance.

The heart showed no changes in any of the mice. To summarize, the significant pathological

appear significant. However, hemosiderin, which would indicate red blood cell destruction, was present only in negligible quantities.

The lack of prominent pathological changes appears to be due to the short duration of the exposures and the resulting early deaths of the animals.

SUMMARY AND CONCLUSIONS

1. Narcosis and dyspnea were the usual signs of toxic action of the solvents for mice. Immediate deaths, suggestive of respiratory failure, characterized the solvents. Delayed deaths were observed in those solvents containing appreciable amounts of toluene.

2. The comparative acute toxicity of the solvents

for mice, were: Toluene - benzene - (7,200 p.p.m.) solvent X 2 (10,400 p.p.m.) approxima. From the most other blend two solven
3. It m

- (1) VON G. dang A cri Hea
- (2) SCHWA. reflexe zene
- (3) KILLIC. vesti to th 307,
- (4) LEHMA. hygie Sprir
- (5) BAERNE. of be Ind.
- (6) WERNE. AND v of va lene g
- (7) LEHMAN. CHOW toxic spirits 1, 191
- (8) LAZARE. differe Pharm

for mice, as illustrated by the M.L.C. in mg./l. were: Toluene 19.90 (5,300 p.p.m.), 60 per cent benzene + 40 per cent toluene mixture 24.58 (7,200 p.p.m.), solvent Y 26.9 (7,580 p.p.m.), solvent X 27.2 (7,980 p.p.m.), and benzene 33.1 (10,400 p.p.m.). The L.D. 50 for these solvents approximated the values obtained for the M.L.C. From the data obtained, toluene was found to be the most toxic, benzene the least toxic, and the other blends were of similar toxicity between these two solvents.

3. It must be emphasized that the order of

toxicity of the solvents was obtained with acute toxicity studies in mice and that it is quite possible that with chronic repeated exposure the order of toxicity may follow a different sequence.

4. Single seven-hour exposures of mice to vapors of benzene and its homologues produced lung, kidney, and splenic changes. Negligible quantities of hemosiderin, indicative of red blood cell destruction, were demonstrated histologically. The lack of prominent pathological changes appears to be due to the short duration of exposures and resulting early deaths of the animals.

REFERENCES

- (1) VON OETTINGEN, W. F.: Toxicity and potential dangers of aliphatic and aromatic hydrocarbons. A critical review of the literature. U. S. Public Health Bull. No. 255, 1940.
- (2) SCHWARTZ, E., AND TELEKY, L.: Some facts and reflections on the problem of poisoning by benzene and its homologs. *THIS J.*, 23: 1, 1941.
- (3) KILLICK, E. M., AND SCHILLING, R. S. F.: An investigation into the effects of continued exposure to the vapors of volatile solvents. *THIS J.*, 24: 307, 1942.
- (4) LEHMAN, H. B., AND FLURY, F.: Toxicology and hygiene of industrial solvents, Berlin, J. Springer, 1938.
- (5) BAERNSTEIN, H. D.: Photometric determination of benzene, toluene and their nitro derivatives. *Ind. Eng. Chem., Anal. Ed.*, 15: 251, 1943.
- (6) WERNER, H. W., MITCHELL, J. L., MILLER, J. W. AND VON OETTINGEN, W. F.: The acute toxicity of vapors of several monoalkyl ethers of ethylene glycol. *THIS J.*, 25: 157, 1943.
- (7) LEHMANN, K. B., WEISSENBERG, R., VON WOJCIECHOWSKI, A., LUIG, AND GUNDERMANN: On the toxicity of benzene, toluene and xylene, mineral spirits and solvent naphtha. *Arch. f. Hyg.*, 75: 1, 1912.
- (8) LAZAREW, W. W.: On the toxicity of vapors of different hydrocarbons. *Arch. exptl. Path. Pharmacol.*, 143: 223, 1929.
- (9) ENGELHARDT, W. E., AND ESTLER, W.: Studies on the acute narcotic action of aliphatic and aromatic hydrocarbons. *Arch. f. Hyg.*, 114: 249, 1935.
- (10) ESTLER, W.: Studies on the acute narcotic action of aliphatic and aromatic hydrocarbons. *Arch. f. Hyg.*, 114: 261, 1935.
- (11) BLISS, C. I.: The calculation of the dosage mortality curve. *Ann. appl. Biol.*, 22: 134, 1935. The comparison of the dosage mortality data. *Ibid.* 22: 307, 1935.
- (12) BLISS, C. I.: The determination of the dosage-mortality curve from small numbers. *Quart. J. Pharm. Pharmacol.*, 11: 192, 1938.
- (13) BLISS, C. I.: The methods of probits. *Science*, 79: 38, 1934. The methods of probits—a correction. *Ibid.* 79: 409, 1934.
- (14) LANG, E. P., CALVERY, H. O., MORRIS, H. J., AND WOODWARD, G.: The toxicology of some glycols and derivatives. *THIS J.*, 21: 173, 1939.
- (15) WINSLOW, C. E. A.: Summary of the National Safety Council study of benzene poisoning. *THIS J.*, 9: 60, 1927.
- (16) LILLIE, R. D.: Romanowsky staining with buffered solutions. III. Extension of the method to Romanowsky stains in general. *Stain Technology*, 16: 1, 1941.