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STUDIES ON THE INHALATION OF 1:3-BUTADIENE; WITH A COMPARISON OF ITS NARCOTIC EFFECT WITH BENZOL, TOLUOL, AND STYRESE, AND A NOTE ON THE ELIMINATION OF STYRENE BY THE HUMAN*

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THE extensive use of butadiene in the manufacture of synthetic rubber has stimulated the investigation of its physiological properties in view of the notable lack of information on the subject. Von Oettingen, in reviewing the literature on this hydrocarbon, gives references to its narcotic action upon mice, rabbits, and man (1). The statement is made that in humans the inhalation of one per cent vapors of butadiene for 5 minutes caused no other effects on the blood pressure or respiration than a quickening of the pulse, together with a slight feeling of prickling and dryness in the mouth and nose. No other published articles pertaining to the effect of butadiene on animals or man have been noted.

1:3-Butadiene, C_4H_6, is an unsaturated hydrocarbon, which at room temperature is a colorless gas with an aromatic odor. Its molecular weight is 54.088 and it boils at −4.7°C. at 760 mm. of mercury. The explosive limits in air are 2.0 to 11.5 per cent by volume (2).

This paper reports upon daily exposure of animals to butadiene for 8 months, determination of the distribution coefficient of butadiene between blood and air, a comparison of the narcotic action of butadiene, benzol, and toluol upon rabbits, daily anesthetization of rabbits with butadiene, and investigation of the psycho-motor responses of humans to butadiene, toluol, and styrene. Full protocols appeared in a mimeographed report to the Rubber Reserve Co.

The butadiene used was obtained from the Carbide and Carbon Chemicals Corporation. The first 100-lb. cylinder was delivered in June, 1942, and represented butadiene reclaimed from the cracking of butane. Seven additional cylinders containing 800 lbs. of the same grade of butadiene were received in August, 1942. When the present investigation had been nearly completed butadiene made commercially by other processes became available. No toxicological study of other samples was undertaken, however, because it is judged that the results set forth here apply equally to butadienes suitable for polymerization, no matter what their source. This conclusion is sound because of the very high purity required and the absence of impurities of probable high physiological activity.

DETERMINATION OF BUTADIENE IN AIR AND BLOOD BY THE IODINE PENTOXIDE METHOD

For a quantitative study of the physiological actions of butadiene, there was needed a sensitive, simple and accurate method for the determination of small amounts of this gas in blood and air. After several failures, a basis for such a method was found in the fact that, at a temperature of

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about 200°C., iodine pentoxide completely oxidizes butadiene with the liberation of 2.2 molecules of iodine for each molecule of butadiene. By collecting the liberated iodine and titrating it with sodium thiosulfate, the amount of butadiene acting upon the pentoxide may be determined. The procedure does not differ essentially from that used in the estimation of ethyl ether (3). While a variety of organic vapors will react with iodine pentoxide at high temperatures, the procedure is admirably suited for the quantitative determination of any one of them, provided, as in this case, others are known to be absent.

Samples of butadiene-air mixtures were collected over mercury either in small gas-sampling tubes or in gas burettes. Blood samples were taken with a syringe and hypodermic needle and immediately transferred to a 125 ml. Erlenmeyer flask fitted with a rubber stopper partially perforated so that the needle could be forced through it readily but which would automatically seal upon removal of the needle. This stopper was also fitted with inlet and outlet arms occluded by screw clamps so that it could be placed in the iodine pentoxide train without any loss of butadiene A mechanical shaker, to agitate the Erlenmeyer flask containing the blood sample, was found to be necessary in order to remove completely the gas contained in the blood. Comparative tests indicated that approximately 20 per cent of the butadiene content of the blood remained entrapped in it where the mechanical shaker was not used.

To check the validity of this analytical method, known amounts of butadiene were taken directly from the storage cylinder. With a gas sampling tube of 5.975 ml. capacity the average recovery was 99.5 per cent on 3 trials. Five other trials with a very thin-walled sampling tube of 2.925 ml. capacity yielded recoveries which averaged 96.28 per cent The latter tests were influenced unduly by changes attributable to variation in room temperature and heat transfer from handling this very sensitive sampling tube.

Repeated Vapor Exposures of Animals

Four identical chambers of 546-liter capacity constructed of masonite and coated with a Bakelite varnish were used. The airflow through each of the chambers ranged from 100 to 190 liters per minute, which resulted in 10 to 20 complete changes of air per hour. Butadiene was metered directly from a cylinder through a reducing valve and capillary flowmeter, and added to the dilution air being drawn into the chamber from the room. The highest concentration used for repeated exposure approximated one-third of the lower explosive limit. It is thought that prolonged human exposure in industry cannot exceed this value because of necessary precautions against fire.

A total of 164 animals inhaled butadiene vapors 7½ houn per day, 6 days a week for 8 months. Groups of 24 albino rats, 12 guinea pigs, 4 rabbits, and one dog inhaled flowing butadiene-air mixtures averaging 600, 2300, and 6700 ppm., with controls exposed in a similar chamber to air alone. Sexes were equally distributed, except for the 4 dogs which were all females. The maximum number of exposures given any animal was 202 within a period of 8 months.

Preliminary weight studies and blood counts mere made as a basis for selecting normal healthy stock. The diet was Purina Rabbit Chow Entire Ration for the guinea pigs and rabbits with kale as an adjunct 3 times a week. The rats and dogs mere fed on Purina Dog Chow with fresh bone marrow and liver similarly provided. Fresh water was available at all times while animals were not being exposed to butadiene. The exposures of the control animals paralleled those of the test animals in every way except that no butadiene was added to the air stream passing through their chamber.

During the course of the exposures the concentration in each of the chambers was estimated daily by means of a Zeiss 50 cm. portable interferometer. one scale division of which was found equivalent to 21.1 ppm. of butadiene. The iodine pentoxide method described above was applied at intervals to check the optical instrument. Each cylinder was analyzed for butadiene, acetylenes, and aldehyde at the beginning and near the end of its useful life. Weekly weight records were kept for all animals and body-length measurements on rats were made monthly. At the termination of exposure, kidney and liver weights of rats were determined. Monthly blood counts were made on all animals. In addition, mean corpuscular volume, coagulation time, and reticulocyte counts were determined for the dogs and rats. Records were kept of all progeny and fertility was calculated for the rats. Urinalyses and blood chemistry studies were made upon sacrifice of animals during or at the termination of the exposures. The eyes of the rabbits and dogs were examined previous to, as well as during and after, the exposures. Tissues were taken at death or

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Growth and General Health

It is inevitable that some extraneous infections will occur in any large group of animals kept for long periods of time and this study has proved to no exception. About 8 per cent of the animals studied were omitted from consideration because of such infections. In view of the fact that a total of 180 animals, including 16 first filial generation rats, was used on the chronic vapor studies, there not an unduly high incidence of infection.

There were no deaths among exposed animals could be attributed to the effect of butadiene. The animals showed no sign of anorexia nor did their vigor or activity visibly diminish during the period they were exposed to the vapors except insofar as they developed extraneous infections. The rats received their first exposure at the of 68 days. At this time the average weights of the 4 groups were practically identical. At the termination of the exposures the weights were 90.5, 86.3, and 81.2 per cent of the controls for the 600, 2300, and 6700 ppm. concentration groups, respectively. These data show that the exposures had a definite influence on the growth of rats, which was roughly proportional to the concentration of butadiene. The final weights of the surviving male guinea pigs *indicated* the same trend. Too few rabbits were used to make comparisons on the basis of growth, and rabbits are notoriously erratic in this respect at best. The 4 female dogs fluctuated in weight from week to week and, when sacrificed, they were heavier than at the start by 44, 111, 8.0, and 14.7 per cent, respectively, for the control, 600, 2300, and 6700 ppm. concentrations.

The final ratio of body length to body weight for the 600, 2300, and 6700 ppm. rats was 4.3, 8.7, and 14.8 per cent less than that of the controls. However, comparison with the ranges published for normal albino rats of the Wistar strain (4) indicates that all groups can be considered normal during the entire exposure period.

Before determining liver and kidney weights the animals were killed by section of the cord and were allowed to bleed completely. This treatment has caused the liver weights to average only about 60 per cent of those reported by Donaldson for normal male rats not exsanguinated (4). However, comparison with the data on the control group makes the results useful. The liver weights for the 600 and 2300 ppm. group are slightly lower than those of the controls for a given body weight, but those of the 6700 ppm. rats were normal. In view of the fact that the highest concentration compares favorably with the controls, it is our opinion that the uncertainty inherent in the method is responsible for the lower values in the 600 and 6700 ppm. concentrations and that no significant effect on liver weight was found.

Using the findings of Donaldson (4) on kidney weight, all groups were normal for their particular body weight, in this respect. The kidney weights were proportional to those of the controls, which was to be expected as no kidney pathology was apparent in any uninfected rat.

Blood Cytology

No blood dyscrasias resulted from exposure to butadiene in any species or at any concentration. The routine procedure included monthly hemoglobin determinations, erythrocyte, leucocyte, and differential counts. In addition, on dogs and rats the clotting time, reticulocyte count, and the mean corpuscular volume were determined. There was no progressive increase or decrease in any of the values. Shifts in the blood picture were transient and not indicative of any effect from exposure.

Fertility

The question as to whether or not the fertility of the rats was depressed because of exposure to butadiene is debatable depending on the interpretation given to the concept of fertility. If the criterion of fertility is taken as the number of litters per female conceived in a given *time* period, then it is evident that the 6700 and 2300 ppm. groups were definitely affected by the exposure. Even when the barren females are disregarded this inference is true, for the number of litters per female on this basis was 3.3, 2.72, 2.5, and 2.62, respectively, for the 0, 600, 2300, and 6700 ppm. groups.

However, a recent work on the rat (5) points out: "Fertility is at its maximum when rats are from 100–300 days of age. At this time the average number of young in a litter should be *above* the norm (six per litter)." This statement might be interpreted as indicating that *the* concept of fertility includes litter-size as well as litter frequency. If such is the case, butadiene at 600 ppm. increased fertility because these females produced 84 pups per litter. The 2300 and 6700 ppm. groups aver-

aged 7.9 and 7.8 pups per litter, which is equivalent to the control group and well above the so-called normal litter size.

The first filial generation rats which were allowed to live and were exposed with their parents were represented by only 2 females and 2 males in each concentration group and therefore did not furnish sufficient data to calculate fertility. All groups produced progeny, with the control and the 600 ppm. group responsible for about 3 times as many as the 2300 and 6700 ppm. groups.

The evidence obtained from guinea pigs and rabbits further substantiates the argument that fertility was not impaired by butadiene as all guinea pig groups were about equivalent in number of progeny. Guinea pigs produced 16, 13, 10, and 13 pups, respectively, for the control, 600, 2300, and 6700 ppm. groups. In the case of the rabbits the 600 and 2300 ppm. groups had no litters, whereas the control and 6700 ppm. groups had 24 and 27 pups, respectively. The dogs were all females, but they were not bred during the exposure period. Embryos found at autopsy were considered as having been born, in all the above data. No evidence of degeneration was seen in any of the testicular sections examined histologically. It is entirely possible that any apparent effect on fertility was caused by the hereditary characteristics of the individual animals concerned, which arbitrarily limited fecundity. This would seem to be more logical in view of the evidence cited, than to attribute damage or alteration in function to the effect of butadiene inhalations.

Blood and Urine Chemistry

Blood chemistry studies revealed no abnormal values for urea nitrogen, bilirubin, or glucose during and following the full course of 202 exposures to butadiene. Routine urinalyses for albumin, sugar, and casts likewise yielded no abnormal values.

Pathology

The ophthalmoscopic examination of the eyes of the dogs and rabbits disclosed no signs of injury during the course of the exposures. In order to verify this finding, whole eyes were enucleated when the animals were sacrificed at the termination of the exposure period and histopathologic studies made. The cornea, sclera, and ciliary body were interpreted as normal. None of the sections showed any indication of degeneration of the retinal ganglion cells, and the other retinal layers displayed no abnormality. Sections of the optic nerve with adjacent retina stained with osmic acid showed no degeneration of myelin sheaths.

No pathology whatever was noted in the adrenal, heart, kidney, skeletal muscle, pancreas, spleen, testis or ovary of either the exposed animals or the controls.

The livers of 68 per cent of the 6700 ppm. group revealed light cloudy swelling. As this condition occurred in only 10 per cent of the control group, it was probably a response to butadiene. However, in all cases, with the exception of 2 rabbits, the cloudy swelling was quite light and therefore of little significance, for this phenomenon may occur as a physiological response to any slight stimulus. Congestion of the liver occurred sporadically among the 4 groups, but as it has no particular relationship to concentration it is regarded as of no significance. Fatty infiltration occurred in the livers of 3 guinea pigs, 2 from the 2300 ppm. and one from the 600 ppm. group. This finding cannot be explained, but it is not sufficiently consistent to be noteworthy, and no other species showed this effect.

Lung congestion occurred quite uniformly in all groups with the exception of the 2300 ppm. animals which had an incidence only one-half as great as the others. The fact that the controls show a higher percentage of this involvement than any other group discounts its being the result of exposure to butadiene.

Narcotic Properties of Butadiene, Benzol, and Toluol for Rabbits

Rabbits were anesthetized with butadiene in order to determine its effect on the body reflexes during the induction period and until death ensued, and the butadiene content of the blood during anesthesia. This operation also compared the narcotic properties of butadiene with those of benzol and toluol.

Butadiene was metered from the storage cylinder through a calibrated flowmeter and diluted with house air metered through a second flowmeter. The butadiene and air were mixed in a 4-liter bottle and conducted to a 6-inch hollow rubber bail fitted with inlet and outlet ports. A hole about 1½ inches in diameter was cut in the ball to admit the rabbit's nose and mouth but not the eyes which were to be examined for reflex actions produced by anesthesia. The entire set-up was constructed of glass

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Excitation, running movem chewing.....
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Late and marked dilation
Death.....

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and rubber tubing to avoid explosion which might result from static electrical discharges if metal parts were used. The work of others (1) indicated that 25 per cent by volume (250,000 ppm. or 550 mgm./liter) mixture of butadiene with oxygen loss of Labyrinth and motor reflexes, with rapid recovery of the rabbits after removal from exposure. This concentration in air was found to be suitable for our purposes since it re- in the death of the majority of rabbits exposed within 25 to 35 minutes and therefore made possible the completion of all necessary analytical work on the day of exposure.

TABLE 1

SYMPTOMS OR REFLEXES OF 29 RABBITS UNDERGOING ANESTHESIA WITH 25 PER CENT BUTADIENE IN AIR

SYMPTOM OR REFLEX	AVERAGE TIME OF OCCURRENCE (MINUTES)
Light anesthesia, relaxed	1.6
Loss of pupillary reflex to strong light	3.8
Early dilation of the pupil	3.9
Excitation, running movements, tremors, chewing	4.6
Loss of blink reflex to tactual stimulus	6.5
Dry râles, laryngeal, tracheal or bronchial	7.1
Involuntary blinking	11.4
Late and marked dilation of the pupil	11.4
Death	23.0

The average times observed for the occurrence of the various reflexes and symptoms of 2.75 kg. rabbits undergoing anesthesia with 25 per cent by volume of butadiene in air are listed in Table 1. No attempt was made to draw samples every few minutes to correlate blood concentration with reflex actions because of the short survival period and the difficulties of such rapid manipulations on rabbits. In about 9 minutes the butadiene content of the blood in the femoral artery was 0.26 mgm. per ml., while the concentration in the femoral vein was 0.18 mgm. per ml.

It is notable that one rabbit survived 34 daily inductions into deep anesthesia with 20–25 per cent butadiene in air without damage to the lungs. This rabbit, which weighed 2350 gm. at the start of the experiment, *gained* 702 gm. during the 34 consecutive days on which butadiene was administered. Tissue studies revealed that the lung was normal, as were liver and kidney.

A mechanically driven syringe was used to deliver benzol at a constant rate into a vaporizer. through which metered dilution air was supplied. The vapor-air mixture was then led into a 4-liter mixing chamber and thence to the rubber-ball inhalator previously described. Trials demonstrated that a concentration of 45,000 ppm. (144.0 mgm./liter) was necessary to produce death in a 30-minute exposure, which is roughly ½ that of the concentration of butadiene necessary to kill in the same time.

TABLE 2

SYMPTOMS OR REFLEXES OF 10 RABBITS UNDERGOING ANESTHESIA WITH 3.5 TO 45 PER CENT BENZOL VAPOR IN AIR

SYMPTOM OR REFLEX	AVERAGE TIME OF OCCURRENCE (MINUTES)
Light anesthesia, relaxed	3.7
Excitation, running movements, tremors, chewing	5.0
Loss of pupillary reflex to strong light	6.5
Loss of blink reflex to tactual stimulus	11.4
Pupillary contraction	12.0
Involuntary blinking	15.6
Death	36.2

TABLE 3

SYMPTOMS OR REFLEXES OF 6 RABBITS UNDERGOING ANESTHESIA WITH 4.5 TO 7 PER CENT TOLUOL VAPOR IN AIR

SYMPTOMS OR REFLEX	AVERAGE TIME OF OCCURRENCE (MINUTES)
Light anesthesia, relaxed	2.9
Pupillary contraction	9.5
Dry râles, laryngeal, tracheal or bronchial	11.0
Loss of blink reflex to tactual stimulus	14.8
Excitation, running movements, tremors, chewing	16.1
Death	49.0

Six rabbits were exposed to the above concentration, and arterial and venous blood samples were collected. Death took place before equilibrium was established; therefore no distribution coefficient was calculated for benzol. The benzol content of the blood was determined by a modification of the colorimetric method of Schrenk and associates (6), using a photoelectric colorimeter. The

average value in terms oi mgm./ml. of benzol in the arterial blood from the femoral artery after 15 minutes of inhalation was 0.1843, for venous blood from the femoral vein 0.1607, while at 22.5 minutes the concentration in the abdominal aorta was 0.2754 and in the postcaval vein 0.1558. Death ensued on the average in 36 minutes with the range covering 22.5 minutes to 71 minutes. This finding indicates the wide difference in the tolerance that individual rabbits have for benzol narcosis. Reflex actions and symptoms were variable in time of occurrence. The results appear in Table 2.

The same apparatus used for producing benzol-*air* mixtures was employed for toluol. A concentration of about 55,000 ppm. (249.2 mgm./liter) was found to be lethal for rabbits in about 40 minutes over a range of 24 to 62 minutes. Wide differences in response were noted. Blood samples were analyzed for toluol by a modification of the microcolorimetric method of Yant and co-workers (7). The arterial blood concentration in mgm./ml. of blood averaged 0.093 at 13 minutes and 0.104 at 33 minutes. Venous concentrations were 0.054 at 14.6 minutes and 0.086 at 30 minutes. The time of occurrence of symptoms is shown in Table 3.

Daily Anesthetization

Further evidence of the lack of permanent injury from butadiene may be gleaned from the experiments on daily induction into fourth stage anesthesia. Two rabbits received 15 consecutive daily inductions in a flowing vapor-air stream and one rabbit 34, with 200,000 to 250,000 ppm. of butadiene. It required less than 2 minutes for loss of motor and labyrinth reflexes to occur at this high concentration, but the administration of butadiene was continued until wide dilation of the pupil was seen and stentorian breathing and râles were heard with a stethoscope. These last symptoms occurred within 8 to 10 minutes. Recovery of balance and muscular control was achieved within 2 minutes after removal from butadiene. No pathology could be detected in any of these rabbits. Weight gains were not adversely affected and the animals maintained their excellent condition. Three other rabbits received 21 daily anesthetizations followed by a 15-day observation period. These gained weight normally and had no complications. On the basis of the findings on these 6 rabbits therefore it is apparent that repeated anesthesia with butadiene had neither deleterious effect on general condition nor on visceral organs.

There is no intention on our part to suggest butadiene for consideration as a safe material for general anesthesia, as there is not complete muscular relaxation even in the fourth stage. Furthermore, death ensues rapidly and abruptly when the animal is kept in deep anesthesia ior any length of time. No concentration other than 20–25 per cent by volume was used for this phase of the work and a complete and thorough study of its narcotic activity was thought unessential. However, judging from the rapidity with which rabbits recover from deep narcosis, it seems reasonable to suppose that, if humans can be removed from accidental exposure to anesthetic concentrations while their respiration and heart action are still strong, they will quickly return to normalcy without ill effects.

Distribution Coefficients of Butadiene

The dstribution coefficient of butadiene between water and air was found to be 0.25 by equilibrating a known volume of water with pure butadiene vapor in a bath at 37.5° C. and determining by analysis the butadiene content of the water using the iodine pentoxide method. This figure was confirmed by bubbling pure butadiene for 2 to 3 houn through a series of 4 tubes immersed in a water bath at 37.5°C. Five ml. samples of water were then withdrawn and analyzed with results completely in accord with those of the first method.

The distribution coefficient between blood and air, *in vitro*, was estimated similarly. Five ml. samples of oxalated rabbit blood obtained by cardiac puncture were equilibrated by mechanical shaking with pure butadiene during a-20-minute interval at 37.5°C. in order to saturate it at atmospheric pressure. At the end of this period the excess butadiene was expelled from the equilibration vessel by displacement with mercury. The tube, then containing only blood saturated with butadiene, was placed in the iodine pentoxide analysis train when washed dry air was drawn through it at 0.2 liter/minute for one-half hour while it was mechanically shaken. An additional half hour of aeration with continued shaking revealed no more butadiene in the blood. The results of 5 analyses yielded an average distribution coefficient of 0.603 (range 0.555–0.662) between oxalated rabbit blood and air at 37.5°C. and 760 mm. pressure.

The average *in vivo* distribution coefficient between blood and air in the rabbit, corrected for the prevailing barometric pressure and temperature of the day on which the exp... was found to be 0.654 (range figure is based on 19 arteri lected from 10 individual ra... of 25 per cent butadiene in ples were from the femora abdominal aorta, and 3 from before death. The average at which the femoral arter was about 10 minutes, whi tricular samples were take about 17 minutes. Death u 23 minutes. Attempts we from the femorals first and toneum was opened and th tered, or, if the latter colla was punctured after openi...

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The Psycho-motor Response of Humans to Butadiene, Toluol and Styrene

In an attempt to evaluate the effect on the psycho-motor response of humans inhaling various concentrations of butadiene, toluol, and styrene, tapping rate and steadiness tests were performed before and during exposures of two male subjects. Pulse rate and blood pressure were determined and subjective symptoms mere reported. Urinary hippuric acid excretion was followed after the toluol and styrene exposures; this topic is discussed separately.

The influence on tapping rate was observed by means of a long paper kymograph fitted with 2 ink writing pens. One pen recorded the taps and a second pen marked off each 6-second interval by deviation from the straight base line. In this manner, the number of taps in each 6-second interval could be accurately determined. The first tapping board was arranged so that contact was made on a copper sheet, 4.5 x 6 in., with a wooden stop, 0.5 x 0.5 x 6 in., placed in the center of the plate. Contact was made with a metal finger stall which closed the circuit and deflected the writing point. Tests made in 2000 and 4000 ppm. butadiene and 200 ppm. toluol were done with this arrangement. Obtaining no significant deviations from normal tapping rate while inhaling these amcentrations, the tests were made more strenuous by cutting the original 4.5 x 6 in. sheet into 2 parts and separating them a distance of about 12.5 inches. A similar strip or barrier was placed between them to prevent sliding the stylus from one plate to the other. A metal stylus in a wooden handle was used to make contact instead of the metal finger stall. Then, too, the kymograph speed was reduced so that a complete circuit of the paper was accomplished in 3 minutes instead of the previous one minute, thus increasing the fatigue factor. By effecting these changes it was anticipated that any disturbance of the psychomotor response by the vapor inhalations would be more readily detected.

In evaluating the results of the tapping test, the number of taps in each 6-second interval of the 3-minute test period were enumerated. The lowest, highest, and mean scores for these 6-second intervals were recorded, and the coefficient of variation was calculated according to the following formulae:

$$\text{Standard deviation} = \sqrt{\frac{\Sigma d^2}{n-1}} \text{ and}$$

$$\text{Coefficient of variation} = \frac{\text{standard deviation}}{\text{mean}},$$

where d is the deviation of the number of taps in each 6-second interval from the mean, and n is the total number of 6-second intervals in the test period. The coefficient of variation expresses numerically the magnitude of the fluctuation in tapping-rate and is therefore an indication of erratic performance.

The steadiness test was performed by the subject holding at arm's length for a period of 3 minutes a 7/32-inch wire in a 1/4-inch hole drilled in a strip of copper. The contacts of the wire with the periphery of the hole were recorded on the same kymograph used for the tapping studies. The percentage of time the wire was actually in contact with the side of the hole was determined by measuring the width of each deviation mark on the record. Steadiness and speed of reaction are better estimated by this method than by the simple summation of the total number of actual contacts.

The initial steadiness test each day was made prior to establishing the gas concentration because of the initial eye irritation caused by some of the materials. All the human exposures were performed in a 4000 cu. ft. room, in which fans were arranged to produce rapid and thorough mixing of

the air. Butadiene was bled directly from a cylinder, toluol was automatically metered into a heated dish to evaporate the fluid, and styrene was evaporated at room temperature from large wicks in an air stream. Vapor concentrations were followed with the interferometer and controlled manually.

Blood pressure was taken by auscultation, using a sphygmomanometer after the subject had rested 5 minutes or more. These readii as well as pulse rates mere too variable to be of any signifi-

TABLE 4

ABBREVIATED PROTOCOL OF OBSERVATIONS DURING HUMAN EXPOSURE *TO* VAPORS

CONCENTRATION AND MATERIAL	HOURS EXPOSED*	TAPPING RATE		STEADINESS
		Minimum rate in any 6 sec. as percentage of day's normal	Maximum coefficient of variation	Maximum time in contact as percentage of day's normal
2000 ppm. butadiene	7	(1)	(1)	(1)
4000 ppm. butadiene	6	(1)	(1)	266
8000 ppm. butadiene	8	100	0.087	136
200 ppm. toluol	7	(1)	(1)	138
400 ppm. toluol.	7	93	0.091	176
600 ppm. toluol	8	80	0.073	155
800 ppm. toluol	8	96	0.103	717
800 ppm. styrene	4	86	0.078	630

(1) Tests too brief to be reliable.

* Approximately in the middle of the test period for butadiene and toluol the subjects left the exposure for a one hour lunch interval. The table lists the total time vapors were actually inhaled.

cance as far as could be determined by physicians consulted.

Listed below are the subjective symptoms reported by our 2 subjects. Table 4 gives the most aberrant values obtained with the psycho-motor tests for each concentration. Great importance is not attached to the tapping rate data of this table, which are included only to show the range of results obtained. The steadiness data, however, appear significant after the threshold concentration discussed below is passed.

SUBJECTIVE SYMPTOMS

2000 and 4000 ppm. butadiene: slight smarting of the eyes and difficulty in focusing on instrument scales.

8000 ppm. butadiene: no subjective symptoms reported, probably because of preoccupation with the control of the concentration.

200 ppm. toluol: transitory mild throat and eye irritation; slight exhilaration.

400 ppm. toluol: mild eye imtation, lacrimation when performing the steadiness test, lassitude, quickly passing nausea, and hilarity.

600 ppm. toluol: lassitude, hilarity, verbosity, boisterousness; *after* termination of exposure, anorexia and listlessness.

800 ppm. toluol: metallic taste, transitory headache, extreme lassitude, scotomata, verbosity, inebriation, slight nausea.

800 ppm. styrene: immediate eye and throat irritation, increased nasal mucus secretion, pronounced and persistent metallic taste, listlessness, drowsiness, impairment of balance; after termination of exposure, slight muscular weakness and unsteadiness, accompanied by inertia and depression.

It should be emphasized that, after the subjects had endured a single exposure to butadiene or toluol, they became much less aware of subjective symptoms on repeating the exposure at the same or a higher concentration. Other workers (8) have found a pronounced improvement in tapping rates near the end of *the* day and exceptional performances during evening trials. This factor tends to offset the effect produced on the subject by the inhalation of vapors which would be *expected* to have their greatest and most marked effect at the end of the day.

No subjective symptoms were reported during the 8000 ppm. butadiene exposure. Probably, because of slight anxiety concerning the possibility of an explosion from local high concentration at the butadiene cylinder, both subjects thought they felt particularly alert and capable of meeting emergencies. On the other hand, this condition may be ascribable to the increased feeling of confidence that often precedes light inebriation from vapor exposure.

From the results it is concluded that, while the symptoms produced by butadiene were not progressively more severe with increasing concentration in the range tried, the opposite was tme for toluol. Styrene is in a class by itself, its effect being depressant rather than stimulant, as is the case with toluol. Butadiene in the concentrations inhaled was innocuous. The results on the tapping test as a means of evaluating psycho-motor response proved to be inconclusive with the mate-

jals tested at these concentr
steadiness was apparent in
ppm. styrene and 800 ppm.
ppm. butadiene showed thk
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ppm. concentrations o
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performed poorly. The lo
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at 800 ppm. and was not
even at 8000 ppm. In the
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The subject of psychomo
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METABOLIC

A search for possible me
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being inducted once each
with 20–25 per cent butyl
rabbits near the conclus
peated exposure to 6700 p
Urine from both group
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acid (10), aldehydes (11),
by periodic acid (12). T
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glyoxal, glycollic aldehyd

rials tested at these concentrations. Marked unsteadiness was apparent in both subjects in 800 ppm. styrene and 800 ppm. toluol. Four thousand ppm. butadiene showed this same effect to a less extent, but results at 8000 ppm. butadiene were approximately normal. The lower concentrations of butadiene and toluol showed little or no effect as indicated by the steadiness test.

On the basis of symptoms produced, the two human subjects concur in the opinion that 800 ppm. styrene was most objectionable, that 800, 600, 400, and 200 ppm. toluol came next in that order, and that 8000 ppm. butadiene had an effect no greater than 200 ppm. toluol. The 2000 and 4000 ppm. concentrations of butadiene are objectionable only from the point of view of odor, which is soon forgotten. The fact that where the concentration of butadiene was 8000 ppm. much better records on steadiness tests were achieved than where inhaling 4000 ppm. indicates that both subjects were probably physically or mentally below par on the day of the 4000 ppm. test and therefore performed poorly. The lower concentrations of toluol likewise did not influence steadiness in proportion to the concentration, in either subject. This observation leads to the belief that there is a threshold concentration below which the results of the steadiness and tapping tests will tend to vary widely. This threshold concentration for the steadiness test was reached for styrene and toluol at 800 ppm. and was not attained for butadiene even at 8000 ppm. In the tapping test this concentration apparently was not reached during exposure to any of the materials tested.

The subject of psychomotor effects from solvent vapors deserves further study as a means for judging the action on humans of exposure to low concentrations of vapors.

Metabolic Products

A search for possible metabolic products of butadiene was made in the urine of rabbits which were being inducted once each day into deep anesthesia with 20–25 per cent butadiene and in the urine of rabbits near the conclusion of the 8-month repeated exposure to 6700 ppm. butadiene. Urine from both groups of rabbits was investigated for the presence of formic acid (9), oxalic acid (10), aldehydes (11), and materials oxidizable by periodic acid (12). The latter test would show the presence of significant amounts of 1,2-glycols, glyoxal, glycollic aldehyde, pyruvic aldehyde, actoin, biacetyl and erythritol which conceivably might be metabolites formed from butadiene. No appreciable differences were found between the animals exposed to butadiene and the unexposed controls. Because of these negative results similar studies following human exposures were omitted.

The hippuric acid excretion of humans exposed to toluol has been shown to rise considerably during the 24-hour period in which the exposure is made (13). Similar results following oral administration of styrene to rabbits have been reported (14). Twenty-four-hour urine samples were collected from both human subjects during toluol and styrene exposure. Hippuric acid excretion increased with the severity of the exposure to toluol. The greatest excretion was 7 gm./24 hours above the subject's normal as a result of 8 hours exposure to 800 ppm. Likewise, although the data are meager, styrene caused a considerable rise in hippuric acid excretion. Four hours exposure to 800 ppm. caused an added excretion of 32 gm. in one subject.

Previous work on monomeric styrene (14) has revealed that neutral sulfur increases in rabbits which have received styrene by mouth or by inhalation. Therefore, since the opportunity presented itself to make such determinations upon humans inhaling a known concentration of styrene, sulfur partitions were performed on the 24-hour urine samples collected during and after the exposure. Neutral sulfur for one subject increased 48 per cent above his normal level, but the increase for the other was only 17 per cent. These results are not conclusive, but it was considered unwise to subject humans to further exposures in order to prove this minor point.

Summary and Conclusions

1. Butadiene in concentrations of 600, 2300, and 6700 ppm. caused no significant progressive injury to small animals during an 8-month exposure period, 6 days a week, 7½ hours per day, although the highest concentration retarded growth slightly and caused light cloudy swelling in some livers. Data on fertility, blood cytology, body weight and length, organ weights, blood chemistry, urinalyses, and micropathology are discussed in support of this statement.

2. The iodine pentoxide method of analysis has been adapted to the determination of butadiene in air and in blood.

3. The distribution coefficient for butadiene between blood and air was found to be 0.603 *in vitro*

and 0.645 *in vivo*. This substantial agreement indicates that the passage of butadiene irom inspired air into the blood is a process of simple diffusion of the gas irom the alveoli and simple solution in blood.

4. Anesthetization experiments on rabbits were carried out to compare the symptoms, reflexes, and blood levels of butadiene, benzol, and toluol. The narcotic action of butadiene was less than that of the other two.

5. Investigation of the urine of rabbits after chronic and acute exposure failed to reveal the presence of any metabolic products of butadiene. The increase in urinary hippuric add excretion in humans following exposure to toluol was verified, and suggestive information obtained along the same line following styrene inhalation was noted.

6. Two human subjects exposed for 4- to 8-hour periods *agree* that 800 ppm. styrene is the most objectionable vapor they used. 800, 600, 400, 200 ppm. toluol are next in that order, and 8000 ppm. butadiene had an effect no greater than 200 ppm. oi toluol. On the basis of psycho-motor effect, which may indicate a tendency to carelessness and accidents, 8000 ppm. butadiene is no worse than 200 ppm. toluol.

7. Butadiene is practically innocuous aside from its narcotic effect at very high concentrations and has little if any cumulative action. Provided the lower explosive limit is not *exceeded,* it is that the health of anyone inhaling its vapors will be impaired, although many may find its odor objectionable. Humans accidentally anesthetized by extremely high concentrations should return quickly to normal if they are rescued while respiration and heart action *are* still strong. Deep anesthesia, however, is likely to terminate fatally in a short time.

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OTHER OBSERVATIONS O

HAS become quite generally reco both acute and chronic lung ch occur when welders are exposed ntrated fumes. The acute rea studied at length in the past, es fume fevers, and will not he ed consideration here. It is ges which have been recognized m about which there still appear t their proper evaluation. Their chest roentgenograms has been est to the author since 1935. when irst group of welders showing th will be recalled from our origina the lung changes as seen on ches consisted of discrete nodular s distributed throughout both ling somewhat the appearance sis. The borders of these nu generally more sharply defined, h silicotic nodules and the hilum minent. Histologic sections fro tem material of one of these cases ment deposits in the lymphatics blood vessels and it was conclud collections were responsible for th. ows seen on the X-ray films. osits had resulted from the use rodes on very confined jobs i ure of gasoline and milk tank. as emphasized that the iron e no reaction of any kind and us tissue proliferation was ent e pathologic findings have thus med in this country, but K an clinician, appears to be in c in his 1941 report, which inclu tem study. Enzer (1) now ha post-mortem studies with the he will publish soon. In his

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