THE TOXICITY OF METHYLAL

BY

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Methylal, $\text{CH}_2 \begin{matrix} \diagup \text{OCH}_3 \\ \diagdown \text{OCH}_3 \end{matrix}$, known also as formal, dimethoxymethane, formaldehyde dimethyl acetal and methylene dimethyl ether, the simplest of the group of acetals, can be prepared very simply by the interaction of formaldehyde and methanol catalyzed by dry hydrogen chloride or even by calcium chloride. It is a clear, thin, mobile liquid, lighter than water, and soluble in water to the extent of one part in three of water. Methylal boils at 42.3°C ., melts at -105°C ., has a density of d_{4}^{20} of 0.8608 and a refractive index of N_D^{18} 1.3589. Until recently, methylal was a substance of little commercial importance. However, it is now available on a tonnage basis, and its characteristics, particularly its excellent solvent properties, have proved of industrial interest. It has also been found useful in the plastics and perfume industries, as well as serving as a special fuel.

Methylal, in common with many other volatile organic substances, has at various times been suggested as an inhalation anaesthetic (Krafft-Ebing, 1888; Dujardin-Beaumetz, 1889; Langgaard, 1888), and as early as 1888 attempts were made to use it for this purpose. However, interest waned for many years, and it was not until much later that Bacq and Dallemagne (1943) made a serious investigation of the anaesthetic action of methylal. Using dogs as experimental animals, these investigators made a comparative study of the influence of both methylal and ether on the respiratory and circulatory systems of dogs, and found methylal as an anaesthetic to be less toxic than ether. Subsequent use of methylal as an anaesthetic in some 22 surgical operations by Bach and Dallemagne (1943) indicated that methylal could be safely used for this purpose, and that the after effects were

less than with either chloroform or ether. However, it produced anaesthesia more slowly than ether, and its effect was more transitory.

Apart from these few investigations with regard to its anaesthetic action, no investigation of the toxicity of methylal has been reported.

Experimental Procedure

Guinea-pigs and white Swiss mice from the stock bred at the National Institutes of Health were used as experimental animals. Mature guinea-pigs were used as test animals. The weights of the mice used varied between 15 and 20 g. Approximately equal numbers of males and females were employed in the following experiments.

Apparatus.—The equipment used for inhalation experiments consisted of three 34-l. glass chambers into which diluted methylal vapour was conducted (Fig. 1). Air from the laboratory supply line passed through a pressure regulator valve (A, Fig. 2) in order to buffer any changes within the supply line. The air flow through the flowmeter (C) could be controlled readily by adjusting the needle valves (B). The flowmeters were in turn calibrated to give the calculated concentration within the exposure chambers. Air at the adjusted constant rate of flow was bubbled through a flask (D) containing methylal. This flask was immersed in a constant temperature bath maintained at 22.2°C . The methylal-laden air was then mixed with a metered stream of diluting air, and the mixture then passed through a dry gas meter (E) which had previously been calibrated against a wet gas meter.

Method of Analysis.—Air samples were taken from the sampling outlet (G). Diluted (1:100) hydrochloric acid was then added to the flask, and this was allowed to stand overnight in order to permit complete absorption of the methylal by the reagent. The solution was then diluted to 100 ml. with water, and 10 ml. of the diluted solution was transferred to a 100 ml. Nessler tube and made up to 100 ml. with water. Schiff's Reagent (5 ml.) was added to the Nessler tube, and the colour was

FIG. 1.—Photograph of apparatus.

FIG. 2.—Diagrammatic arrangement of apparatus.

allowed to develop for at least 10 minutes. The colour was then compared with standards prepared under identical conditions.

Subcutaneous Injection.—The subcutaneous injection of methylal in guinea-pigs was painful at the time of injection, but no apparent discomfort occurred after the injection was completed. Only mild sedative effects and slight impairment of the righting reflex occurred in amounts up to 3 ml. per kg. At levels of 3.5 to 5 ml. per kg. methylal produced anaesthesia within five to 10 minutes, and this effect lasted from three to five hours. At higher levels, anaesthesia was even more immediate, and death occurred following a gradual decrease in rate and depth of respiration.

In guinea-pigs receiving the subcutaneous injection of methylal there was no ill effect apart from a local tissue reaction at the site of the injection. Within two weeks, scab formation progressed to local tissue necrosis and extensive ulceration of 1 to 1½ in. in diameter. This then healed with no further complication.

Acute Toxicity.—Guinea-pigs exposed to very high concentrations of methylal exhibited symptoms of eye and respiratory tract irritation as well as gross incoordination. The irritation was evidenced by vomiting, squinting, lacrimation, sneezing, coughing, and nasal discharge. Death occurred within two to three hours. At measured concentrations slightly above 15% (153,594 p.p.m.), anaesthesia occurred in 20 minutes, and death in one and a half to two and a half hours, and was immediately preceded by slow and irregular respiration. Meyer and Gottlieb-Billroth (1921) established the limiting concentration of methylal for complete narcosis with recovery at 2.8% with an exposure interval of 30 minutes, as compared with a value of 0.44% for chloroform. Meyer and Hemmi (1935) consider methylal to be very similar to ether with reference to its narcotic properties.

Median Lethal Concentration.—Mice in groups of 10 were subjected to varying concentrations of methylal in the exposure chambers, and the death rates were noted for 24 hours following exposure. Where fatalities occurred, these were almost without exception during the exposure period. Eighty per cent of the deaths occurred within the last hour of exposure. With only one exception, all animals surviving the seven-hour exposure period continued to live and thrive.

Within the first 15 minutes of exposure and at the approximate LD_{50} concentration, signs of respiratory tract irritation appeared. Within an hour the mice became excited and progressively more ataxic. In the

second hour they were unsteady to the point of being incapable of remaining upright, the fur was ruffled, and the eyes were irritated. Within the third hour, anaesthesia was reached and maintained until the end of the seven-hour period. During anaesthesia the only change noted was in the character of respiration which became progressively more convulsive and irregular. Where death did not occur during exposure, the mice almost invariably survived on removal from the chamber. Approximately two hours of breathing fresh air were required to restore the animals to full consciousness and coordinated control of their extremities. The results of this series of experiments are indicated in Fig. 3, in which each point represents a group of 10 mice. It will be noted that the LD_{50} value is approximately at the concentration of 57 mg. of methylal per l., or 18,354 parts per million. This value is somewhat lower than the value of 28,025 parts per million found by Meyer and Gottlieb-Billroth (1921) to produce complete narcosis. However, the LD_{50} exposure data are based upon continuous exposures of seven hours' duration.

Chronic Exposure.—Large groups of mice were exposed for a seven-hour period daily to methylal vapour over an extended period of time in order to determine whether any pathological changes would result from the inhalation of this substance. Food and water were withheld during the exposure. The control mice were subjected to similar crowded conditions endured by the exposed animals. Three groups of mice were exposed in a different manner.

Series A.—A group of 50 mice received 15 seven-hour exposures to a concentration of methylal averaging 35.1 mg./l. At this concentration methylal proved only mildly irritating. A lack of coordination was apparent only after three or four hours of exposure, and only

FIG. 3.—Lethal concentration values for methylal.

slight anaesthesia occurred. Recovery was usually complete one hour after removal from the chamber. There were six deaths (all but one of which occurred during exposure) over the 22-day period.

Series B.—A group of 20 mice were given 13 exposures of seven hours each over a 17-day period with methylal averaging 42 mg./l. Irritation was more evident, the anaesthesia time was shorter and anaesthesia deeper, and recovery was slower. The curve (Fig. 4) is somewhat steeper than would be anticipated owing to the difficulty of maintaining a gas concentration at exactly 42 mg./l.

Series C.—A group of 45 mice was subjected to repeated exposures to the LD₅₀ value, but the death rate was slightly over 50% for the first two exposures, leaving too few survivors for significant observation.

In Fig. 4 the sharp distinction between the various concentrations is clearly evident. In general the weights of the test animals (particularly at the 35.1 mg./l. concentration) showed no significant variation from those of the controls. Red, white, and differential cell counts and haemoglobin determination on test and control mice revealed no significant variations.

In view of the threshold value of 11,300 p.p.m. of series A and the threshold anaesthesia value of 28,000 p.p.m. reported by Meyer and Gottlieb-Billroth (1921), and by Bacq and Dallemagne (1943), it would appear that one-tenth of this value or 1,000 parts per million would be a safe working concentration.

Metabolism of Methylal.—Experimental investigation did not indicate that the path of methylal excretion was other than that of ordinary respiration. In view of Keiser's (1931) investigation of the metabolism of methanol, tests for formaldehyde and formic acid were made of the vitreous humor and urine. However, there was no indication of the presence of these substances in any case. It is possible, therefore, in view of the stability of methylal in neutral or alkaline media, that methylal is not hydrolyzed at the pH of body tissue fluids.

Pathology

The tissues from 14 exposed guinea-pigs, 84 exposed mice, and 30 control mice not exposed to methylal were saved for microscopic study and fixed in formaldehyde. Routine paraffin sections were stained with azure eosinate and Masson's trichrome stain and were examined for haemosiderin after treatment with acidulated potassium ferrocyanide. The eyes were usually enucleated before decalcifying the skulls with formic acid. Frozen

FIG. 4.—Survival following chronic inhalation exposure to methylal.

sections of the heart, liver, and kidney were stained for fat with oil red (Lillie, 1948).

Five guinea-pigs received a single two to eight-hour inhalation exposure to methylal (over 250 mg./l.). One each died at two and 64 hours, and three were killed at 16, 20, and 74 hours after the beginning of the exposure. The animal that died at two hours showed no significant changes; the other four had moderate to severe fatty degeneration of the liver and kidney, and an extensive bronchopneumonia with polymorphonuclear leucocytes predominating in the alveolar exudate. In addition, two showed moderate fatty degeneration of the myocardium. Three other guinea-pigs killed 23 hours after three successive seven-hour daily inhalation exposures to methylal showed similar changes in the lungs, liver, and kidneys.

Six guinea-pigs were given four to five daily inhalation exposures to methylal (51 to 140 mg./l.), usually seven hours on successive days. They were killed for study about 24 hours after the beginning of the last exposure. Four showed no significant changes, one showed a small area of pneumonia, and one showed a small focus of necrosis in the adrenal cortex.

Six mice were studied at three to 72 hours after beginning a three-hour exposure to 122 mg./l. of methylal, and 25 at six to 154 hours after a six-hour exposure to 88 mg./l. of methylal. Significant changes were found only among 16 mice that were killed at six to 54 hours after beginning the exposure; eight had slight pulmonary congestion, three had pulmonary oedema, and four had slight fatty changes in the liver and kidney.

Fifty-three mice were exposed to methylal seven hours daily except Saturday and Sunday. Four of nine that died after four to 11 exposures to methylal (36 to 87 mg./l.) had a bronchopneumonia and four had slight fatty changes in the liver or kidney. Six mice were killed 40 days after the last of 13 exposures to methylal (34 to 54 mg./l.); four had slight to moderate splenic haemosiderosis, three had pneumonia, and one showed slight fatty changes in the kidney. Twenty of 38 mice were killed 20 hours after the fifteenth exposure to methylal (28 to 45 mg./l.); two each had bronchopneumonia, pulmonary oedema, and pulmonary haemorrhages, and one had slight fatty changes in the kidney. Of the remaining 18 mice, five each were killed at 23 and 30 days, and four each at 44 and 72 days after the last exposure. Eleven of the 18 had slight haemosiderosis of the spleen, and two had slight fatty changes in the kidney.

Pneumonia and other incidental lesions were more common in the exposed mice than in those not exposed, but the incidence of such lesions may vary markedly in different groups of mice. The fatty changes and haemosiderosis are considered non-specific changes; similar changes have been found after exposure to various toxic agents (Highman and Heppel, 1946).

No changes were found in the optic nerve or retina attributable to the methylal. About 10% of both control and exposed mice had rodless retinas, an interesting congenital condition described previously in other strains (Jaffé, 1931). It is characterized by the absence of rods and cones and the outer nuclear and reticular layers of the retina.

One exposed animal showed a slight infiltration of the cornea and the anterior chamber mainly by polymorphonuclear leucocytes, and another showed a similar infiltration in the cornea beneath a large sub-epithelial bleb containing some basophilic material. These and other minor corneal changes, seen occasionally in both control and exposed mice, are probably incidental lesions.

Summary

Animals exposed to highly toxic concentrations of methylal often developed severe fatty changes in the liver, kidney, and heart, and inflammatory changes in the lungs. Low concentrations generally produced no significant pathologic changes.

The LD₅₀ value of methylal for male laboratory animals was found to be 57 mg./l. (18,354 parts per million). The threshold appears to be about 11,300 parts per million. It would appear that a reasonable safe working concentration for workers continuously exposed to methylal vapour for an eight-hour working day would be 1,000 parts per million.

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