THE TOXICITY OF ETHYLENE OXIDE.

By Squadron-Leader W. J. GREAVES WALKER, R.A.F., M.S.
and Surgeon-Commander C. E. GREESON, R.N.

This paper gives some account and results of work conducted on the properties of ethylene oxide, as this compound has been recommended as an insecticide and vermin destroyer, and what is of more importance, as a fumigant for foodstuffs. The concentration recommended is in the nature of 2 lb. per 1000 c. ft. (or 1 part in 60) for 24 hours. It has also been mentioned as a possible agent for use as a lachrymator in war.

Ethylene oxide is at low temperatures a colourless mobile fluid boiling at 10·5° C. It has a specific gravity of 0·887 at 7° C. and a molecular weight of 44. The empirical formula is C_2H_4O, and the structural $\begin{matrix} CH_2 \\ CH_2 \end{matrix}\!\!>\!O$.

It is miscible with water in all proportions. The sample examined was obtained from British Drug Houses in 25 grm. phials.

The compound was examined by us as follows:

On man. Inhalation of the vapour. Vapour effect on the skin. Fluid effect on the skin.

On animals. The effect of splashes in the eye. Administration by intravenous injection. Administration by inhalation both in single and repeated exposures. Feeding with food contaminated by vapour and by spray. Estimation of the blood urea content in an animal given a fatal dose by intravenous injection.

On Man.

Inhalation of the vapour.

A concentration of 1 : 400 ethylene oxide was put up in a small chamber and breathed by four subjects. It had a not unpleasant acetic acid-like smell and was slightly irritant to the nasal passages, but produced no after-effects. Breathed in a concentration of 1 : 80, the compound has a definite irritating effect on the nasal passages in 10 sec. There was no lachrymation.

Effect on the skin.

The liquid placed on the skin of the forearm boils and at once evaporates leaving no mark and producing no irritation or erythema. When a short test-tube containing a plug of cotton-wool soaked in this compound was inverted over the skin of the forearm and held in position for 15 min. there was no irritation or any after-effect.

https://doi.org/10.1017/S0022172400018143 Published online by Cambridge University Press

On Animals.

Effect of the compound when splashed into the eye of a rabbit.

Experiments showed that a sharp conjunctivitis with blepharitis followed the introduction of a drop of ethylene oxide into the eyes of rabbits, but the condition subsided rapidly and the eyes were in all cases normal or practically so in 4 days' time. Treatment by irrigation with saline after the instillation appeared to have no hastening effect on recovery, though doing no harm. There was no permanent damage to the eyes.

Administration to rabbits by intravenous injection.

As a result of a series of experiments carried out to ascertain the fatal dose of ethylene oxide when thus administered to rabbits, it was shown that an amount equivalent to 175 mg. per kg. body weight was fatal in 6 hours, whereas a dose of 146 mg. per kg. body weight produced no symptoms beyond diarrhoea and muscular weakness with dislike of movement. The train of symptoms following the injection of a fatal dose was as follows:

Diarrhoea was an early symptom. After a period of 4 hours, during which the animal seemed normal, muscular effort produced a fine tremor of the limbs, the stance was wide and feet were splayed with the head drooping until it finally rested on the floor. Respiration was regular but somewhat hurried. Nystagmus was present and there were incoordinated movements of limbs and jaws passing to convulsive movements with marked head retraction and rigidity of the muscles of the limbs. Between these convulsive movements the animal lay helpless and unable to hold up its head or support its body on the legs, while all muscles were lax and toneless.

An injection of 133 mg. per kg. body weight was given and repeated after 20 hours. Following the first injection the animal showed wide stance and splayed feet with fine muscular tremors following an effort to move. The second dose was followed by death during the night. Again, in another experiment, a quantity equivalent to 85 mg. per kg. body weight was injected into a rabbit, and the dose was repeated after an interval of 48 hours and again in a further 48 hours. After the first dose the animal showed nothing abnormal; after the second there was a convulsive seizure as previously described but the animal recovered; after the third dose there were further seizures and death resulted during the night following injection.

The findings at autopsy in all these animals were those of congestion of all organs with catarrhal changes in the kidney tubules and small but numerous submucous haemorrhages in the stomach. Apart from generalised congestion the lungs showed no damage. It seems then that this substance is a poison of some potency administered in this way, and further that elimination from the system is not rapid, while the poison tends to show a cumulative effect.

https://doi.org/10.1017/S0022172400018143 Published online by Cambridge University Press

Administration of the compound by inhalation.

Ethylene oxide has been recommended for use as a fumigant in the proportion of 2 lb. per 1000 c. ft. of space (*i.e.* a concentration of 1 : 60) for a duration of 24 hours.

It is shown in these experiments that an exposure to 1 : 80 for 30 min. will cause a fatal result to mice, rats and guinea-pigs, each animal showing post-mortem marked damage throughout the body.

Concentrations varying from 1 : 80 to 1 : 150 for single exposures and from 1 : 80 to 1 : 800 in repeated exposures were used in this series of experiments. It was found that with a concentration of 1:80 for a 30 min. exposure all mice (3) were dead in $2\frac{3}{4}$ hours, one having a discharge of blood-stained mucous from the nose; the rats (3) were all dead in 6 hours, having become markedly dyspnoeic in $3\frac{3}{4}$ hours; the guinea-pigs had blood-stained mucous coming from the nose and mouth and showed marked dyspnoea; two were so ill that 7 hours after exposure they were destroyed (by $CHCl_3$), but the third survived; all the rabbits showed normal behaviour beyond perhaps a slight weakness of the hind limbs. All surviving animals were killed 3 days after exposure.

At autopsy the general picture was as described in the paragraph dealing with injection, but the lungs were more markedly congested and in some cases showed an acute pneumonic condition. It is of interest to note in this series of experiments that the death-rate varies with the size of the animal; even in the same species the larger animal would appear to be more resistant than the smaller, while guinea-pigs are more resistant than rats, and rats than mice. This was never contradicted throughout the work done on ethylene oxide. Further, it seems that, though ethylene oxide produces an acute pneumonic state in the lungs, death is due to paralysis of the respiratory centre before this lung condition becomes fatal.

Further experiments were done with concentrations of 1 : 100, which for 30 min. exposure was fatal to rats and mice, and 1:150, a concentration which proved fatal to mice only, the effects on the other animals being in proportion to those noted for 1 : 80. All these were single exposures.

To satisfy ourselves that a smaller concentration if often repeated might prove to be dangerous, a further group of experiments with varying concentrations and exposures was carried out. These are given in Table I.

Post-mortem findings on these animals showed congestion and thickening of the alveolar walls of the lungs with desquamation of epithelium of the bronchial mucous membrane and pus in the bronchi. The liver showed small changes, while the kidneys showed congestion and in one or two cases swelling of the convoluted tubules but no obvious damage. Diarrhoea was a feature of the animals in the last experiment.

The series of experiments with a concentration of 1:800 is of interest—it shows that this concentration is harmless when breathed for 40 min.

https://doi.org/10.1017/S0022172400018143 Published online by Cambridge University Press

once a day or even twice a day, and for as many as 17 exposures once a day or 11 twice a day—at these points the experiments were discontinued, as it was considered that further exposures would yield no practical information. But this concentration, when breathed for 5 hours even with an interval of $\frac{1}{2}$ hour in the middle of exposure, will kill mice and may kill rats, and further, the damage shown at post-mortem is undoubtedly more severe in the smaller animals, while the biggest show little sign of any effect.

Table I.

Species	Concentration	Length of each exposure (min.)	No. of exposures	Total minutes exposed	Reaction of animals
3 rabbits 3 g.-pigs 3 rats	1 : 250	30	5 daily	150	Very little effect noted beyond salivation during exposure
3 mice	1 : 250	30	5 daily	150	One died after first exposure
1 rabbit 1 g.-pig 1 rat 1 mouse	1 : 800	40	17 daily	680	Nil
1 rabbit 2 g.-pigs 2 rats 2 mice	1 : 800	40	11 twice daily	440	Nil
2 rabbits	1 : 800	150	2	300	Diarrhoea
2 g.-pigs	1 : 800	150	30 min.	300	Loss of power in limbs. Diarrhoea
2 rats	1 : 800	150	interval between	300	1 died. 1 very ill. Diarrhoea and loss of power
2 mice	1 : 800	150		300	Both died

Feeding with vapour-contaminated food.

In these experiments rats were used and starved for 48 hours before being fed once daily on the materials chosen. These were thin slices of bread and butter, thin slices of bread and margarine, thin slices of boiled fat bacon, thin slices of cheese, bread and milk, oatmeal and bread only. These were placed in a desiccator (made airtight by using a vaselined joint) for 20–24 hours (48 in the first experiment), together with a watch-glass containing a suitable amount of ethylene oxide (about 1 c.c.). The food so treated was fed at once to the starved rats. Fresh clean water was given in all cages. The food débris was removed from each cage after 6 hours and the animals were kept warm by a radiator to compensate for loss of bedding.

The results are recorded in Table II.

After the fifth feed (or third in the second series) all rats were killed by $CHCl_3$ for post-mortem examination, and the findings are noted against each animal. It is obvious, we think, that enough ethylene oxide has been retained by the foodstuffs to render them a danger to health at least. No class of foodstuff has escaped suspicion entirely.

https://doi.org/10.1017/S0022172400018143 Published online by Cambridge University Press

https://doi.org/10.1017/S0022172400018143 Published online by Cambridge University Press

Table II. *Feeding experiments with food contaminated with ethylene oxide vapour.*

Rat no.	Foodstuff	Observations on successive feeds: 1st feed	2nd feed	3rd feed	4th feed	5th feed	Post-mortem findings
3	Bacon	Refused for 6 hours	Ate at once. Diarrhoea after 2 hours	Diarrhoea	Diarrhoea	Diarrhoea	Slight congestion of kidney and catarrhal changes, stomach normal. Intestines—fluid contents
6	Bread and margarine	Ate freely	Normal	Normal	Normal	Normal	Lungs slightly congested. Liver shows necrotic changes at periphery of lobules
54		Dislike but fed. Diarrhoea	Animal quiet. Loose stools	Stools formed. Animal quiet	—	—	Normal
55		Dislike but fed. Diarrhoea	Animal not well. Loose stools	Animal ill. Loose stools	—	—	Stomach congested and tendency to erosion
7	Bread and butter	Ate freely. Diarrhoea marked	Diarrhoea	Normal	Normal	Normal	Kidney shows catarrhal inflammation of patchy distribution in convoluted tubules
58		Ate after few moments	Quiet. Sneezing	Stools normal	—	—	Normal
59		Ate after few moments	Very quiet. Diarrhoea. Animal sneezing continuously	Stools normal, still sneezing	—	—	Normal
5	Cheese	Refused 2 hours	Fed at once	Normal	Normal	Normal	Stomach shows two small petechial haemorrhages. Liver and kidney congested
10	Bread and milk	—	—	Ate freely. Diarrhoea	Diarrhoea	Loose stools	Slight catarrhal inflammation in kidney. Other organs normal
60		Fed after some delay	Stools formed	Normal	—	—	Normal
61		Fed after some delay	Stools formed	Normal	—	—	Normal
56	Oatmeal	Animal spent some time picking food over	Diarrhoea present	Loose stools	—	—	Lungs congested. Stomach congested with tendency to erosion
57		Animal spent some time picking food over	Diarrhoea present	Loose stools	—	—	Stomach congested
53	Bread	Animal fed	Normal	Loose stools	—	—	Intestines are congested
52		Animal fed	Loose stools	Diarrhoea	—	—	Intestines are congested

Feeding with spray-contaminated food.

The articles were as before but they were sprayed with the ethylene oxide, removed to the air for 5 min. and given to rats again starved for 48 hours before the first feed. Feeds were given daily. No other inducement was used to make the animals take the contaminated foodstuffs. Seven rats out of a total of sixteen were killed by the food, the details being recorded in Table III.

It seems reasonable to assume that ethylene oxide when sprayed on to foodstuffs is a highly dangerous substance. The survivors were killed by $CHCl_3$ 24 hours after the last feed.

Results are most positive, particularly in the lungs where congestion was marked, while the stomachs showed in six cases a sharp degree of congestion with submucous haemorrhages in five of them. The other organs showed changes similar to those observed in the last experiments.

Estimation of the blood urea content in an animal given a fatal dose of ethylene oxide intravenously.

This was done to ascertain if the damage to the kidney by this substance was sufficient to be the cause of death. The method of estimation employed was that recommended for small quantities of blood as described by Joyce Patterson (1925)[1]. The results showed a small drop in the urea content from a normal of 42 mg. per 100 c.c. of blood to 33 mg. per 100 c.c. The two specimens were taken one before injection and the other 4 hours after injection when symptoms were well established.

Conclusions.

On the evidence of these experiments, it appears that ethylene oxide is by no means a harmless compound. From a practical point of view the results of experiments on inhalation and feeding of food contaminated with this substance by spray and vapour seem to hold the chief interest.

Ethylene oxide has been recommended[2] for use at the rate of 2 lb. per 1000 c. ft. or 1 part in 60. It is stated that no disagreeable odour was discernible immediately after removal of foodstuffs from such an atmosphere. With this statement we cannot agree. Rats showed a very decided appreciation of the presence of a foreign odour on all vapour-contaminated food offered to them—so much so that in spite of having been starved for 48 hours—they refused to eat for some time until, as seems likely, the ethylene oxide had largely disappeared.

[1] *Biochem. Journ.* **19**, 601–603.

[2] R. T. Cotton and R. C. Roark (16–19. iv. 1928), "Ethylene Oxide as a Fumigant," paper read before the Division of Agriculture and Food Chemistry at the 75th Meeting of the American Chemical Society, St Louis, Ma.

https://doi.org/10.1017/S0022172400018143 Published online by Cambridge University Press

https://doi.org/10.1017/S0022172400018143 Published online by Cambridge University Press

Table III. *Feeding experiments with food contaminated with ethylene oxide by spray.*

Rat no.	Foodstuff	Observations on successive feeds: 1st feed	2nd feed	3rd feed	4th feed	5th feed	Post-mortem notes
11	Bread	Ate at once—showed dislike with irritation of nose	*Dead*	—	—	—	Liver and kidney marked congestion. Lungs heavily congested. Catarrhal changes in kidney
45		Do.	*Dead*	—	—	—	Punctate haemorrhages stomach. Liver markedly congested and lungs and small intestines congested
46		Do.	Stools loose. Animal is ill	Animal better	Normal	—	No obvious lesions
12	Bread and margarine	Do.	Diarrhoea marked	Showed marked dislike	Do.	Normal	Patchy congestion lung. Kidney dark and congested
43		Do.	Diarrhoea present	—	—	—	Congestion of lungs and liver marked. Punctate haemorrhages. Stomach with congestion and of intestines
44		Do.	*Dead*	—	—	—	Severe inflammation. Stomach with haemorrhages. Liver slightly congested. Lungs normal
13	Bread and butter	Do.	*Dead*	—	—	—	Lungs heavily congested. All organs congested. Streaky haemorrhagic patches in stomach
41		Do.	*Dead*	—	—	—	Lungs congested. Stomach slightly congested. Liver dark
42		Do.	Animal ill. Diarrhoea present	Animal very ill and disinclined to move or feed	*Dead*	—	Stomach congested. Spleen congested. Other organs no gross lesion
14	Bacon	Ate after refusing for some hours	Diarrhoea marked	Stools loose	Normal	Normal	Patchy congested lung but little beyond mild congestion of other organs
15	Bread and milk	Ate at once but showed dislike and irritation of the nose	Do.	Do.	Do.	Do.	Stomach slight inflammation of mucous membrane and patchy congestion lung
4		Do.	*Dead*	—	—	—	Lungs marked congestion. Liver and kidney dark and congested. Numerous submucous haemorrhages stomach
49		Do.	Diarrhoea marked	—	—	—	No obvious lesions
50		Do.	Do.	—	—	—	Lungs slightly congested. Stomach and intestines generally so
47	Oatmeal	Ate after some delay but more freely later	Loose stools	Normal	Normal	—	No obvious lesions
48		Do.	Do.	Do.	Do.	—	No obvious lesions

Koelsch and Lederer (1930)[1] append the following remarks to their paper on the use of ethylene oxide as a vermin destroyer:

"From consideration of our results, it appears necessary to take proper precautions against breathing the vapour, although not to the extent necessary with the more highly toxic HCN derivatives. It is never permissible to regard ethylene oxide as practically non-poisonous."

With this conclusion we agree, but would suggest that precautions need to be as thorough against this substance as with any dangerous gas, since we cannot agree with "degrees of precaution" when dealing with a known toxic agent. Indeed, if this substance is to be used or produced in any quantities, we think that all individuals engaged with it should be thoroughly protected and warned of its toxic properties to avoid unfortunate accidents.

[1] *Zbl. f. Gewerbehyg. u. Unfallverhütung* (17), **9**, 264–66.

(*MS. received for publication* 6. II. 1932.—Ed.)

https://doi.org/10.1017/S0022172400018143 Published online by Cambridge University Press