

Dose dependent effects of inhaled ethylene oxide on spermatogenesis in rats

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Abstract

Male Wistar rats were exposed to ethylene oxide (EO) at concentrations of 50, 100, or 250 ppm for six hours a day, on five days a week for 13 weeks. Dose effect relations of inhaled EO on spermatogenesis were evaluated from testicular and epididymal weights, histopathological changes and lactate dehydrogenase X (LDH X) activity in the testis, and sperm counts and sperm head abnormalities in the epididymis. At 250 ppm, a decrease in epididymal weights, slight degenerations in the seminiferous tubules, decreased sperm counts, and increased numbers of abnormal sperm heads in the tail of the epididymis were found; these were not seen at lower doses. When the abnormal sperm heads were classified into immature types and teratic types, the number of immature heads increased only at 250 ppm. On the other hand, the teratic type had increased at doses of 50 and 100 ppm EO when compared with the control group. Hence, subchronic inhalation of EO at low concentrations affects spermatogenesis in rats.

Ethylene oxide (EO) is a highly reactive alkylating agent widely used in chemical syntheses and sterilisation and fumigation.¹ With respect to the effects on spermatogenesis and male reproduction, dominant lethal mutations,^{2,3} testicular atrophy,^{4,5} and changes in sperm morphology⁶ have been reported as a result of acute or subchronic EO inhalation at 200 to 1000 ppm. The effects of EO at lower doses have not been studied. We investigated the dose dependent effects of EO on testicular and epididymal weights, histology of the testis, sperm counts, and sperm head morphology after subchronic inhalation of EO at 50 to 250 ppm in rats.

Materials and methods

Male Wistar rats about seven weeks of age were randomly divided into four groups, and were acclimatised to the animal facility for one week before the start of exposure. The exposure system was the same as that described in our previous report.⁵ The food intake of control and lower dose groups was restricted according to the intake of the highest dose group to minimise differences due to nutrition. The rats in the exposed groups inhaled 50, 100, or 250 ppm of EO for six hours a day five days a week from Monday to Friday for 13 weeks. The rats in the control group were exposed to clean air in an identical system. Six rats were used in each exposed group and 12 in the control group. The concentrations of EO were repeatedly monitored by a gas chromatograph equipped with a flame ionisation detector.

The rats were killed using an overdose of ether about 40 hours after the last exposure and the testes and epididymides were removed and weighed. One testis from each pair was fixed in Bouin's solution, embedded in paraffin, and stained with periodic acid Schiff reagent (PAS) and Gill's haematoxylin for light microscopical examination.⁵ The other testis was used for the measurement of lactate dehydrogenase X (LDH X) activity in the cytosol fraction. The decapsulated testis was homogenised in four volumes of 1.15% KCl solution with a Potter-Elvehjem homogeniser. The homogenate was centrifuged at 105 000 *g* for 60 minutes, and LDH X activity was measured in the supernatant by the method described by Meistrich *et al.*⁷ using α -ketovalerate, a specific substrate for this isozyme. Protein content of the cytosol fraction was determined by the procedure of Lowry *et al.*⁸

One epididymis from each pair was divided into two portions (head and tail plus body) and sperm numbers in these portions were counted by the method described in our previous report.⁹ The appropriate part of the epididymis was homogenised in a 90 ml saline triton merthiolate solution (STM solution; 0.15 M NaCl, 0.05% (v/v) triton X-100, and merthiolate to give 0.25 M thimerosal) with a semimicro Waring blender for 1.5 minutes. Sperm present in the homogenates were counted with a haemocytometer. The tail of the other epididymis was opened by a razor and the sperm were squeezed into a 0.1 M sodium phosphate buffer, pH 7.2. The

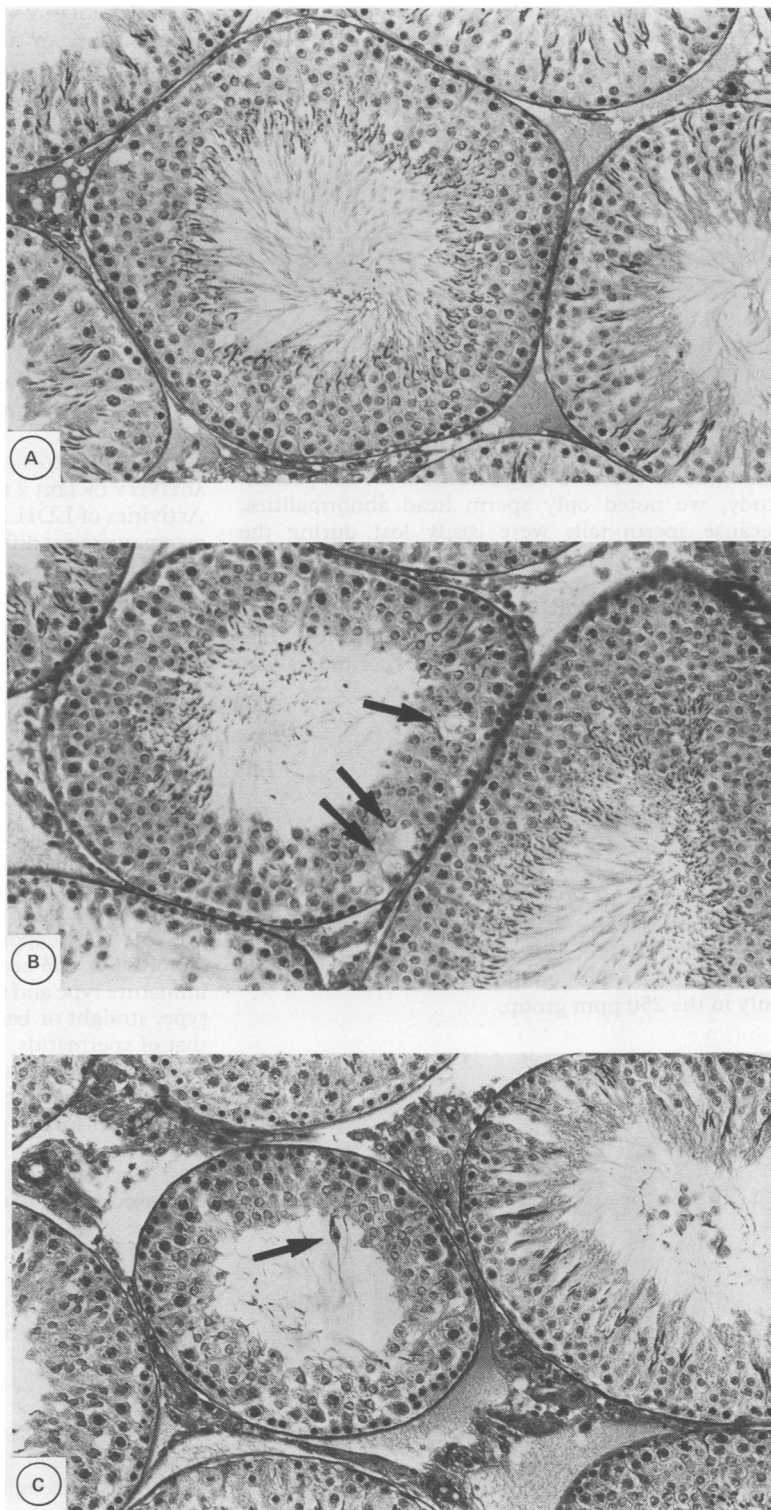
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Figure 1 (A) Control testis: germ cells are regularly arranged in the seminiferous tubules ($\times 50$); (B) testis exposed to 250 ppm EO: in some seminiferous tubules decreased numbers of spermatids accompanied by vacuolation (arrows) are seen ($\times 50$); (C) testis exposed to 250 ppm EO: seminiferous tubules focally reduced in diameter. A few degenerated maturation phase spermatids (arrow) are seen in the lumen ($\times 50$).



Effects of ethylene oxide on body, testicular, and epididymal weights

Concentration of EO (ppm)	Body weight (g; mean (SD))	Testicular weight (g; mean (SD))	Epididymal weight (g; mean (SD))
0 (n = 12)	462.7 (33.3)	3.73 (0.27)	1.32 (0.11)
50 (n = 6)	489.7 (20.5)	3.33 (0.26)	1.35 (0.09)
100 (n = 6)	467.2 (31.9)	3.40 (0.23)	1.26 (0.08)
250 (n = 6)	443.3 (37.3)	3.60 (0.39)	1.06 (0.11)*

*Significantly different from control; $p < 0.01$.

samples were washed in the same buffer three times and fixed with 30% ethanol. After fixation, a smear was made on a glass slide coated with meshcement (Nisshin EM Co Ltd, Tokyo) and stained with Gill's haematoxylin. For each smear 1000 sperm were examined at 400 fold magnification. In the present study, we noted only sperm head abnormalities, because sperm tails were easily lost during the preparation.

Data were analysed for mean and standard deviation (SD), compared by analyses of variance, and then analysed by Duncan's multiple range test. The level taken for significance in all experiments was $p < 0.05$.

Results

BODY AND ORGAN WEIGHTS

As a result of food restriction of the control and low dose groups, no difference was found in body weights after 13 weeks of exposure to EO among the four groups (table). The testicular weights in the exposed groups did not decrease when compared with the control group. The epididymal weights decreased only in the 250 ppm group.

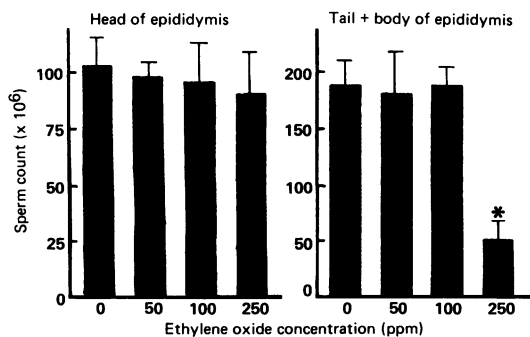


Figure 2 Effects of different concentrations of EO on sperm counts in the head and in the tail plus body of the epididymis. All results are expressed as means + SD ($n = 6$ for treatment groups; $n = 12$ for control group). * Significantly different from control, $p < 0.001$.

HISTOPATHOLOGY

Examination of testicular sections with a light microscope, showed that all types of germ cells were regularly arranged in the seminiferous tubules in the control group (fig 1A), and in the 50 and 100 ppm exposed groups. We could not find any appreciable morphological alterations among these groups. In the 250 ppm group, some seminiferous tubules had a reduced diameter (fig 1C), focal vacuolation of the seminiferous epithelium, and germ cell loss (fig 1B). A few degenerated maturation phase spermatids were seen in the lumen of seminiferous tubules (fig 1C). These abnormalities were not seen in the other groups, where the tubules appeared normal and well preserved. There were no changes in the interstitial area in the treatment groups compared with the control.

ACTIVITY OF LDH X IN THE TESTIS

Activities of LDH X in the cytosol in all the exposed groups were not different from the control group. The mean activities were 48.1 (SD 4.3) nmol/min/mg protein in the control group, 48.7 (SD 6.7) in the 50 ppm group, 48.5 (SD 4.0) in the 100 ppm group, and 53.4 (SD 3.6) in the 250 ppm group.

SPERM COUNT IN THE EPIDIDYMISS

The sperm counts in the head of the epididymis in all the exposed groups were similar to the control group but in the tail plus body of the epididymis the sperm count was lower in the 250 ppm exposed group (fig 2).

SPERM HEAD ABNORMALITY

The sperm head abnormality rate increased only in the 250 ppm exposed group (fig 3). The shape of abnormal sperm heads was classified into two groups, immature type and teratic type (fig 4). The immature type, straight or banana-like, had a shape similar to that of spermatids. This may be due to their release from the seminiferous tubules before they are completely mature. The teratic type of sperm heads, such as those with amorphous or pycnomorphous shape, was not found in the control seminiferous tubules. The rate of the immature type of sperm heads increased in the 250 ppm group, whereas it did not increase in the other exposed groups. On the other hand, the rate of the teratic type of sperm heads was significantly greater in the exposed groups compared with the control group but did not show a dose response relation (fig 3).

Discussion

Ethylene oxide is a small water soluble compound (molecular weight 44). Such a molecule is thought to pass easily through the blood testis barrier¹⁰ and directly attack the germ cells. Some studies have

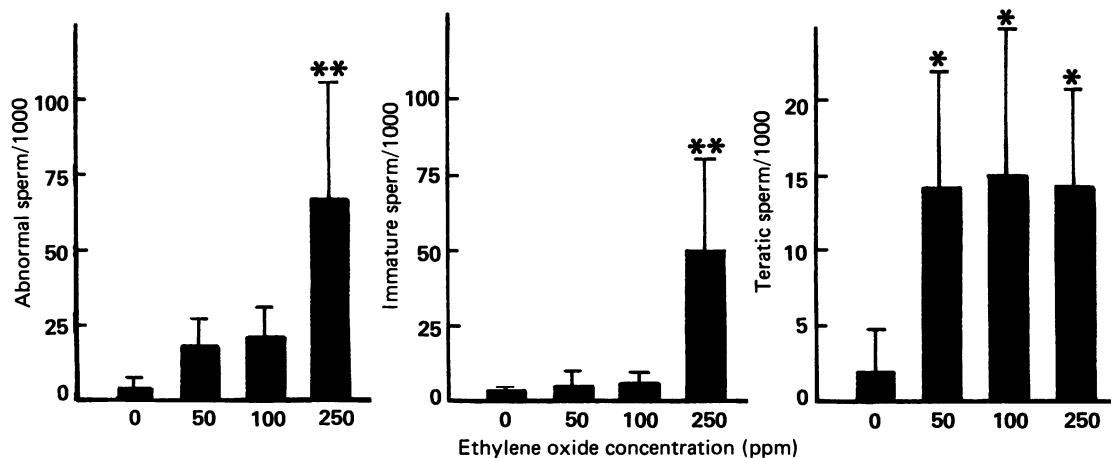


Figure 3 Effects of different concentrations of EO on the rate of sperm head abnormalities. All results are expressed as means + SD. The number of animals in each group is as fig 2. * $p < 0.05$, ** $p < 0.01$; significantly different from control.

shown that DNA and the protein of germ cells are alkylated after exposure to EO,¹¹ and EO induced various types of mutations. It is also an anti-spermatogenic agent. Recently, we reported that treatment with EO induced testicular atrophy, degeneration of the seminiferous tubules, and alterations in glutathione metabolism in the testis⁵ under the same conditions as our model of EO-induced neuropathy.¹² A few studies on the effects of EO on spermatogenesis and the male reproductive system besides ours have been reported, but the minimum dose to induce these effects remained to be elucidated.

Although moderate food restriction produced only little evidence of tubular degeneration,¹³ food restriction of more than 50% increased the number of abnormal sperm heads.¹⁴ In our present study, the same volume of food was given to all the groups to minimise the effects due to food intake.

Subchronic inhalation of EO at 250 ppm induced a

decrease in epididymal weight, morphological changes in the testis, and a decrease in the number of sperm in the tail of the epididymis, but at 50 or 100 ppm these effects were not seen. The sperm count in the epididymis is known to be one of the most sensitive tests for evaluating spermatogenesis,¹⁵ as it gives the result of all the stages of meiosis, spermiogenesis, and transition in the epididymis.

The fraction of total abnormal sperm heads in the epididymis increased only in the group exposed to 250 ppm EO, but when abnormal sperm heads were classified into immature and teratic types, the rate of teratic types increased in all treated groups but not in relation to the concentration of EO. The rate of immature types of sperm heads increased only in the 250 ppm group. These data suggest that the two types of abnormal sperm heads were made by different mechanisms. The immature germ cells are often sloughed from the seminiferous tubules by

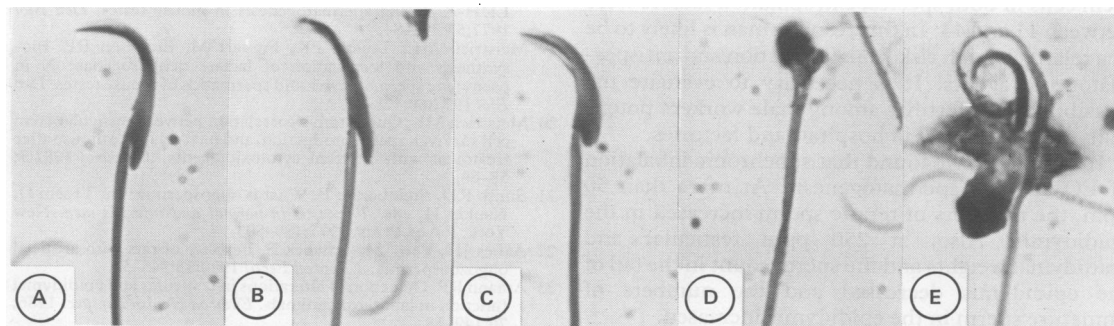


Figure 4 The shape of the heads of rat sperm. (A) normal head; (B) straight head; (C) banana-like head; (D) amorphous head; (E) pycnomorphous head. B and C are classified as immature sperm heads and D and E as teratic sperm heads.

agents which affect spermatogenesis. This type of damage is thought to be the result of alterations in Sertoli cell function.⁵ The increase in the immature type of sperm heads might result not only from a direct effect on the germ cells but also from alterations of the Sertoli cells by EO. As an increase in the abnormal sperms induced infertility,¹⁶ and EO at 50 ppm had already affected spermatogenesis, it could also have affected fertility. A sperm head morphology test is also used as an in vitro test for mutagenic potential,¹⁷ and exposure to EO at a dose as low as 50 ppm may also increase the risk of mutation.

The specific isozyme of the LDH in the testis is LDH X, and its activity can be detected only in the germ cells that are more mature than the mid-pachytene spermatocyte stage.^{18,19} Activity of LDH X has often been used as a marker of germ cell maturation.²⁰ In our present study, no alterations in activity in the testis were found after exposure to EO despite the morphological changes in the testis and decreased number of sperm in the epididymis in the 250 ppm group. The method of measurement of LDH X may not be sensitive enough, however, to detect a minor change.

It is difficult to extrapolate the results of the present study to man. The number of sperm in a human ejaculate is usually only two to fourfold more than the number of sperm at which fertility is significantly reduced,²¹ but in rats epididymal sperm counts can be reduced as much as 90% without significant loss of fertility.^{20,22} Men have a much smaller relative size of the testis, the lowest rate of daily sperm production per gram testis, and lower percentages of progressively motile sperm and morphologically normal sperm in semen than any of the animal models studied.²³ Meistrich and Samuels calculated extrapolation factors, which were the ratios of the dose to cause a certain effect in the mouse to that necessary to bring about the same effect in man using radiation data.²⁴ Values of between 2.6 and seven were obtained at the time when the maximum effects occur. If sperm counts were performed, however, at times at which the sperm develop from irradiated stem cells in both species, extrapolation factors were between 11 and 44. In this respect, man is likely to be at a relatively high risk from irradiation and antispermatic agents. It is necessary to evaluate the possibility of infertility among male workers potentially exposed to EO in hospitals and factories.

In summary, we found that subchronic inhalation of EO affected spermatogenesis. At more than 50 ppm, the numbers of teratic sperm increased in the epididymis. Also, at 250 ppm testicular and epididymal weights and the sperm count in the tail of the epididymis decreased and the numbers of immature sperm in the epididymis increased.

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