

Further Studies on the Toxicology of the Glycol Ethers with Emphasis on Rapid Screening and Hazard Assessment

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The discovery that ethylene glycol monomethyl ether (EGME) could affect the testis and the developing fetus in laboratory animals prompted further work to understand the effect of EGME and to examine additional glycol ethers to see if they showed EGME's reprotoxicity.

Propylene glycol monomethyl ether (PGME) was shown not to cause testicular atrophy or to affect the development of rats at 600 ppm by inhalation, whereas EGME caused testicular atrophy at 300 ppm and showed teratogenic potential at 100 ppm.

Diethylene glycol monomethyl ether (diEGME) was found to show no teratogenic potential when administered subcutaneously in rats at up to 1000 μ L/kg, whereas EGME had effects at 40 μ L/kg.

EGME has been shown to cause effects on the testis in rats after a single exposure to 600 ppm or above for 4 hr. The effects can be seen as little as 24 hr after exposure. Ethylene glycol monoethyl ether (EGEE) also causes a reduction in testicular weight following a single exposure to saturated vapor for 3 hr (17 mg/l), but ethylene glycol monoisopropyl ether (EGPE) at 15 mg/l and ethylene glycol monobutyl ether (EGBE) at 4 mg/l showed no effect on the testis.

Introduction

The reproductive toxicology of glycol ethers has been the subject of much concern in recent years. This concern was initiated by a report (1) describing testicular atrophy in mice dosed with ethylene glycol monomethyl ether (EGME) and ethylene glycol monoethyl ether (EGEE). Moreover, both EGME (2) and EGEE (3) have been shown to cause fetotoxicity and/or teratogenicity in laboratory animals. These observations lead to a general reappraisal of the glycol ethers as a class and this paper describes some of the work which was carried out within Imperial Chemical Industries PLC in order to determine the reproductive toxicology of several members of the glycol ethers group which were of proven or potential commercial importance. None of the studies was conducted to full classical protocols, but each one was designed to answer a specific question to aid in either product development or in the development of safe handling. In retrospect, this series of experiments can be seen as a way in which toxicology can provide useful data even though the studies are not carried out to a regulatory protocol.

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Comparative Reproductive Toxicology by Inhalation in Rats of Ethylene Glycol Monomethyl (EGME) and Propylene Glycol Monomethyl Ether (PGME)

This study was carried out at a time when the effects of EGME upon both the testes by inhalation (4) and its effects of the fetus (2) were known. However, there were no data available on the toxicology of PGME, and this study was designed to assess whether PGME was capable of causing the same effects on reproductive toxicology as EGME. It was therefore important to use EGME as a positive control for both the testicular effects and the teratogenic effects. The study was designed in two parts: effect on testis and hematology and determination of teratogenic potential.

Effect on Testis and Hematology

Ten male rats of the Alpk/Ap (Wistar-derived) strain were exposed to the following concentrations: 100 ppm EGME, 300 ppm EGME, or 200 ppm PGME or 600 ppm PGME for 6 hr/day for 10 consecutive days in 3.4 m³ inhalation chambers (5). During the exposure period

the animals were periodically weighed and observed for changes in their clinical condition or other behavioral changes. On the day after the 10th exposure the animals were killed by exposure to Fluothane. Blood samples were taken for hematological assessment and the testis weighed and removed and processed, along with the thymus, into slides for subsequent histopathological evaluation.

The body weight gain and absolute weights of the rats exposed to 300 ppm EGME were significantly reduced; however, 100 ppm EGME and 200 or 600 ppm PGME had no effect on body weight. None of the treatments caused any changes in clinical observations. At postmortem there were no effects at 100 ppm EGME or at 200 and 600 ppm PGME, however, the thymus and the testes of the rats exposed to 300 ppm EGME were reduced in size (Fig. 1). In the blood samples taken at postmortem 300 ppm of EGME was shown to cause a reduction in total white blood cells, the number of red blood cells and the hemoglobin concentration of the red blood cells. There were no effects at 100 ppm EGME or with either concentration of PGME. Histological examination of the testis revealed a few seminiferous tubules with disordered spermatogenesis with a similar incidence in the controls and in rats exposed to 100 ppm EGME, 200 ppm PGME or 600 PGME. Pronounced tubular atrophy was seen in the testis from all the rats exposed to 300 ppm EGME, where 70 to 100% of the seminiferous tubules were affected.

Determination of Teratogenic Potential

In order to provide a rapid assessment of the effect of PGME and EGME on the developing embryo when rats

were exposed by inhalation, an adaptation of the procedure described by Chernoff and Kavlock (6) was employed. Pregnant female rats, approximately 11 to 13 weeks of age, of the Alpk/Ap (Wistar-derived) strain were used. Groups of 20 rats per group were exposed to air, 100 or 300 ppm EGME and 200 or 600 ppm of PGME for 6 hr/day on days 6 to 17 (inclusive of gestation, day of mating being day 0). After the last exposure period the rats were removed from the exposure chamber and housed singly. The rats were allowed to deliver their litters which were observed for 3 days. The number of live and dead pups in each litter and the weight of each litter were determined on days 1 and 3 postpartum. Any rats not producing litters by day 24 of gestation were killed, and a gross postmortem examination was carried out to determine the status of pregnancy. All the rats were pregnant: all the control rats and all the rats exposed to PGME produced litters. Exposure to 300 ppm EGME caused a significant reduction in body weight gain throughout the study, and none of these rats produced litters. Only 9 of the 20 rats exposed to 100 ppm produced litters and in these rats the gestation period was significantly increased to a mean value of 23.6 days from a control value of 22 days. The comparative litter data are shown in Table 1. There was a significant reduction in the proportion of live pups initially and the proportion surviving for 3 days postpartum in the litters of dams exposed to 100 ppm EGME. The weight of the pups in this group on day 1 was similar to the controls, although they had gained significantly less weight by day 3. All the pups were normal externally. There was no evidence of any effect in either of the groups exposed to PGME.

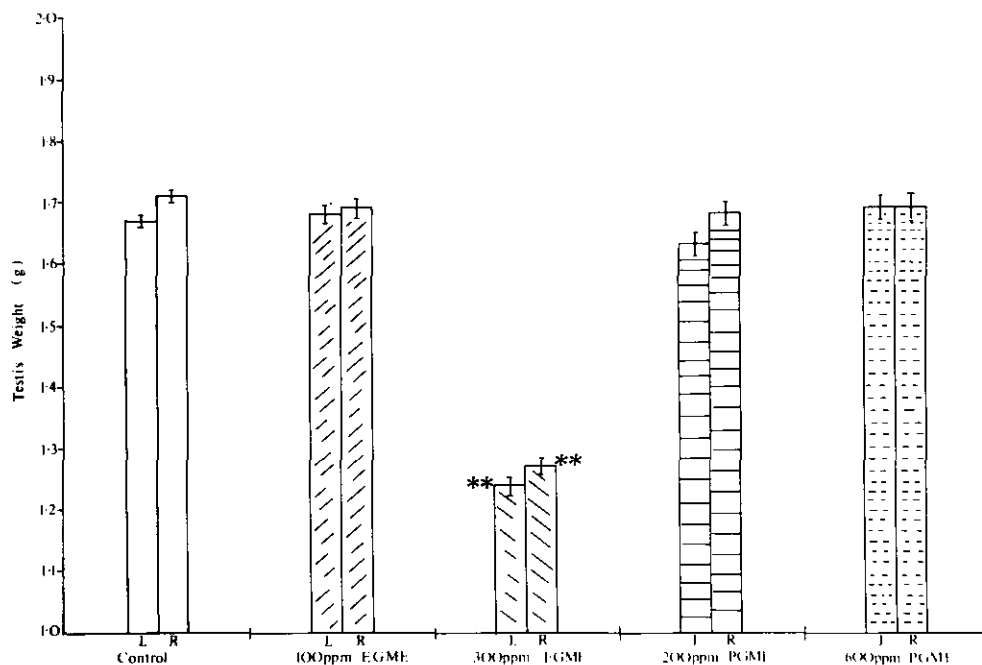


FIGURE 1. Effect on testis weights of the inhalation of EGME and PGME for 10 consecutive days in male rats. Asterisks denote significantly different from controls: (**) $p < 0.01$; (*) $p < 0.05$.

Table 1. Comparative effect of EGME and PGME by inhalation on pregnant rats: litter data.

Treatment	Group mean no. of live and dead pups on day 1 (n)	Proportion of live pups on day 1	Proportion of pups surviving to day 3	Group mean per litter, day 1 (n)	Live pup weight on day 3, g (n)
Control	11.5 \pm 3.0 (20)	226/229 (98.7%)	223/226 (98.7%)	6.0 \pm 0.5 (20)	7.2 \pm 0.8 (20)
100 ppm EGME	3.3 \pm 2.4 (9) [†]	23/30 ^{††} (76.7%)	16/23 [†] (69.6%)	6.1 \pm 0.6 (7)	6.3 \pm 1.1 (4)
300 ppm EGME	—	—	—	—	—
200 ppm PGME	11.7 \pm 2.0 (20)	231/233 (99.1%)	226/231 (97.8%)	6.0 \pm 0.4 (20)	7.1 \pm 0.7 (20)
600 ppm PGME	11.7 \pm 2.6 (20)	228/233 (97.9%)	227/228 (99.6%)	6.0 \pm 0.4 (20)	7.0 \pm 0.7 (20)

[†]Statistically significantly different from the control group at the 1% level (Fisher's exact test, two-sided).

^{††}Statistically significantly different from the control group at the 1% level (*t*-test, two-sided).

Conclusions

This experiment demonstrated that 300 ppm of EGME caused hematological changes, testicular atrophy and thymic atrophy in male rats and complete embryo lethality in pregnant female rats.^A 100 ppm level caused fetal mortality, fetotoxicity and the impairment of postnatal development. This spectrum of effects has been defined as describing teratogenic potential (6). In contrast, neither 200 nor 600 ppm of PGME caused any effects in either males or pregnant females. The results of this preliminary study therefore indicate clearly that PGME does not share the spectrum of activity shown by EGME upon the reproductive process at significant dose levels. The study has been described previously (7).

Teratogenic Potential of Diethylene Glycol Monomethyl Ether (diEGME) in the Postnatal Development Test in Rats

Few or no data existed at the time this study was carried out on the effect of diethylene glycol ethers on the developing embryo. This study was carried out to assess the effect of diethylene glycol monomethyl ether (diEGME) on the rat fetus in order to determine whether diEGME showed the same spectrum of activity as EGME, which would have meant reconsideration of the way in which the compound is handled. The postnatal development test (6) was used, as EGME had already been shown to be positive in this test by inhalation. As diEGME is relatively nonvolatile, human exposure is more likely to be by dermal contact; consequently diEGME was administered by the subcutaneous route as this represents the worst case, assuming the complete breakdown of the barrier function of the skin. Pregnant female Alpk/Ap (Wistar-derived) rats 11 to 13 weeks of age were mated and the day on which spermatozoa were detected was taken as day 0 of pregnancy. Groups of 15 rats were used and they were injected subcutaneously with 2 mL of sterile water containing the appropriate compounds on days 6 to 20 of gestation inclusive. The groups were controls,

250 μ L/kg diEGME, 500 μ L/kg diEGME, 1000 μ L/kg diEGME, 40 μ L/kg EGME, 250 μ L/kg EGME. The rats were observed daily for changes in clinical condition and weighed on day 1 and on each day between day 6 and 20. Each rat was allowed to litter and the offspring weighed and the number of dead and live pups were recorded on days 1 and 4 postpartum. The female rats and the pups were then killed. All animals which failed to produce a litter by day 24 of pregnancy were killed and the status of pregnancy determined.

No abnormalities in clinical condition were seen in any of the rats treated with diEGME; however, some of the rats treated with 250 μ L/kg EGME showed piloerection and vaginal bleeding. The body weight gain of these rats treated with 250 μ L/kg EGME was significantly reduced compared with controls. One control animal did not produce a litter and was found not to be pregnant. None of the rats treated with 250 μ L/kg EGME produced litters, although they were all found to have been pregnant.

The litter data are shown in Table 2, where it will be seen that 40 μ L/kg of EGME reduced the number of litters as well as the number of live pups per litter and the percentage of pups surviving for 4 days. EGME therefore clearly showed its fetotoxic effect in this study. diEGME, on the other hand, caused no maternal toxicity and little or no effect on the development of the young at 250 or 500 μ L/kg. There was a slight, but not statistically significant, decrease in the survivability in the offspring of the rats treated with 1000 μ L/kg, and this may be indicative of a slight fetotoxic effect at this high dose level. However, diEGME certainly does not share the marked fetotoxicity shown by EGME, and, as the route of administration was chosen to maximize the possible effects of diEGME, it is unlikely that dermal exposure to diEGME in humans would present a significant risk. These data were reported previously (8).

Effect on Route of Administration on Teratogenic Potential of EGME

EGME has been shown to cause testicular atrophy and/or fetotoxicity by a variety of routes such as

Table 2. Effect of subcutaneous diEGME and EGME in pregnant rats: litter data.

	Control	diEGME 250 μ L/kg	diEGME 500 μ L/kg	diEGME 1000 μ L/kg	EGME 40 μ L/kg	EGME 250 μ L/kg
No. pregnant	14/15	15/15	15/15	15/15	15/15	15/15
No. of litters	14	15	15	15	7	0
No. of live pups per litter (\pm SD)	11.7 \pm 2.7	12.0 \pm 1.9	10.9 \pm 3.1	10.5 \pm 3.6	3.7 \pm 4.2 [†]	—
Pup weight at day 1, g (\pm SD)	5.9 \pm 0.6	6.0 \pm 0.5	6.3 [*] \pm 0.4	6.2 \pm 0.5	6.1 \pm .5	—
Pup weight at day 5, g (\pm SD)	8.5 \pm 1.1	8.8 \pm 1.2	9.3 \pm 1.1	8.6 \pm 1.1	10.4 \pm 0.7 [*]	—
Weight gain per pup, g (\pm SD)	2.5 \pm 0.8	2.8 \pm 0.8	3.0 \pm 1.0	2.4 \pm 0.9	3.6 \pm 0.2	—
No. live pups per litter day 5 (\pm SD)	11.2 \pm 2.9	11.3 \pm 1.9	10.6 \pm 2.5	9.1 \pm 3.3	2.1 \pm 4.5 [†]	—
Surviving on day 5, %	95.2	91.4	89.9	85.6	46.9	—
No. of litters with deaths day 1-5	4/14	6/15	8/15	10/15	6/7	—
No. of litters with more than one death day 1-5	2/15	2/15	2/15	7/15	4/7	—

^{*}Statistically significantly different from the control group means at 95% level Student *t* test.

[†]Statistically significantly different from the control group means at 99% level, Student *t* test, two-sided.

Table 3. Effect of route of administration on teratogenic potential of EGME.

Treatment and route	Number pregnant	Number of litters	Mean litter size, day 0	Mean pup weight, day 0, g	Mean litter size, day 4	Survival, %	Mean pup weight, day 4, g	% Weight gain (pups)
10 mL/kg saline IP	15	12	7.6	5.3	5.4	64	8.8	43
200 mg/kg EGME IP	15	—	—	—	—	—	—	—
10 mL/kg saline PO	15	15	10.7	6.1	10.7	99	9.0	48
200 mg/kg EGME PO	15	0	—	—	—	—	—	—
2 mL/kg saline SC	15	15	10.8	6.0	10.5	91	9.25	52
40 mg/kg EGME SC	15	7	4.6 [*]	6.0	2.1 [*]	23 [*]	10.7	31
250 mg/kg EGME SC	15	0	—	—	—	—	—	—
2 mL/kg saline dermally	15	15	13.2	5.6	12.7	96	9.2	59
40 mg/kg EGME dermally	15	15	12.7	5.9	12.3	97	9.4	98
Control	20	20	11.5	6.0	11.5	99	7.0	17
100 ppm EGME, inhalation	20	9	3.3 [*]	6.2	4.0 [*]	49 [*]	6.5	— 15 [*]
300 ppm EGME, inhalation	20	0	—	—	—	—	—	—

^{*}Statistically significantly different from control (*p* < 0.05).

inhalation (4) and ingestion (2). The work reported here forms a comparison of the results of using EGME as a positive control in several studies to determine the teratogenic potential of a variety of compounds. EGME has been given by the following routes during the course of these studies: intraperitoneally, orally, subcutaneously, dermally and by inhalation. The results are shown in Table 3. It will be seen that 200 to 250 mg/kg of EGME caused complete loss of litters by the intraperitoneal route, by the oral route, and when given subcutaneously; 300 ppm of EGME by inhalation, which corresponds approximately to a dose of 350 mg/kg assuming 100% retention, caused a similar result. At lower doses, EGME causes a loss of approximately half the litters, with reduced litter size and survivability in the pups. This is shown by 40 mg/kg EGME subcutaneously and 100 ppm EGME by inhalation (which corresponds to an approximate dose of 115 mg/kg assuming 100% retention). Interestingly, a similar dose administered dermally, i.e., 40 mg/kg EGME, had no effect on the developing fetus. This result indicates that the skin has some barrier function with regard to EGME.

Effect of Ethylene Glycol Monoisopropyl Ether (EGPE) on the Testis and Blood of Rats

Groups of 10 male Alpk/Ap (Wistar-derived) rats were exposed to air, 300 or 1000 ppm of EGPE for 6 hr/day for 5 days of week 1 and for 4 days of week 2. On the day following the last exposure, the rats were killed, blood samples were taken, and the testes were weighed.

Rats in the 1000 ppm EGPE group showed marked hematuria on the first day of exposure; subsequently, no more hematuria was seen. The skin of these rats was pale in appearance up to and including day 4; subsequently their clinical condition appeared comparable to the controls. The body weight gains of the rat in the 1000 ppm EGPE group were statistically significantly lower than the controls, while the rats in the 300 ppm groups had weight gains similar to the control group. At postmortem the appearance and weights of the testis from both the 1000 and 300 ppm groups were similar to the controls.

At 1000 ppm, statistically significant reductions were seen in hemoglobin concentration, red blood cell count and mean corpuscular hemoglobin concentrations. In addition, mean cell volumes and mean cell hemoglobin were statistically significantly higher in the 1000 ppm group. These changes are consistent with the initial hematuria observed in these rats. There were no changes in the white blood cell counts, and apart from a slight increase in mean cell volume there were no hematological effects in the rats exposed to 300 ppm EGPE. This study indicates that EGPE behaves in a similar manner to ethylene glycol monobutyl ether (EGBE) (9), i.e., the major effect appears to be hemolysis, followed by replacement with resistant cells which are younger and larger. There was no suggestion of the typical response seen with EGME of testicular atrophy and leukocytopenia.

Effect of a Single Inhalation Exposure to Ethylene Glycol Monoalkyl Ethers (EGME, EGEE, EGPE, EGBE)

Ethylene glycol monomethyl ether (EGME) has been shown to cause testicular atrophy following 90 days exposure (10) and 10 days exposure (4,7). In these studies, 300 ppm (= 960 mg/m³) caused testicular atrophy. The work reported here was designed to assess whether a single very high exposure to EGME would also cause testicular atrophy. In order to determine whether there is a clear structure activity relationship, ethylene glycol monoethyl (EGEE), monoisopropyl (EGPE) and monobutyl (EGBE) ethers were also examined in the same experiment. Male 250 g Alpk/AP (Wistar-derived) rats were exposed for 3 or 4 hr to the saturated vapor generated by blowing air through the relevant test material contained in a sintered glass bubbler. The undiluted vapor was led into 1 L glass exposure chambers, each containing five rats housed individually. The atmosphere concentrations were estimated by dividing the weight loss from the bubbler by the total airflow. The rats were observed during expo-

sure and throughout a subsequent 14-day observation period. On day 15 the animals were killed by over exposure to halothane and subjected to gross macroscopic postmortem examination, which included the weighing of the testes. The results are shown in Table 4.

Both EGME and EGEE caused reductions in testis weight when compared with the controls on day 15, EGME causing an approximately 50% reduction and EGEE a 20% reduction. Neither EGPE nor EGBE caused a reduction in testes weight. Clinical observations also revealed another sign associated with the glycol ethers, hematuria in the animals treated with EGEE, EGPE or EGBE. EGME did not cause hematuria. These limited data indicate that both EGME and EGEE can cause testicular atrophy after a single, albeit, very high, exposure by inhalation. However, even the saturated vapor from EGPE and EGBE did not cause testicular atrophy.

Effect of a Single Exposure to EGME on the Testis of Male Rats

The previous study had shown that EGME is capable of causing testicular weight loss after a single exposure to a saturated vapor. These studies were designed to determine the dose-response relationship of the effect of the single exposure on the testes, to determine the histological effect, and to determine the time course of the onset of testicular damage.

In the first study, groups of 20 male Alpk/AP rats were exposed to 5000, 2500, 1250, 625, 300 or 150 ppm EGME. A further group of rats was exposed to air only. Following exposure which took place in 3.4 m³ whole body exposure chambers, they were returned to their holding cages for 13 days, and 14 days after treatment, were killed with an overdose of halothane. At autopsy the left and right testis were weighed separately and stored in Bouin's fixative for subsequent processing and microscopic examination. The caudal epididymides were weighed together and preserved for processing and microscopic examination. The accessory glands, seminal vesicle and ventral prostate were weighed and later examined histopathologically.

There was a dose-related fall in body weight gain as shown by the body weight immediately before postmortem. These were accompanied by a dose-related fall in testes weight which was significant down to 1250 ppm (Fig. 2). There were reductions in the caudal epididymidal weight and an increase in the accessory gland weight at 5000 ppm of EGME. Histological examination of the testes revealed severe bilateral tubular atrophy with disordered spermatogenesis in rats exposed to 5000 ppm EGME for 4 hr, with many tubules showing only stem cells and Sertoli cells. Similar, though less marked changes, were seen in rats exposed to 2500 and 1250 ppm EGME. Although testes weight was not reduced at 625 ppm EGME, maturing spermatids showed evidence of damage.

Table 4. Comparative data on effect of exposure to saturated vapor of glycol ethers on testis weights at day 15.

Compound and concentration	Testis weight, % of body weight		Hematuria (Present or absent)
	3-hr exposure	Control	
EGME, 24 mg/L (7500 ppm)	0.49 ± 0.08*	1.06 ± 0.06	—
EGEE, 17 mg/L (4500 ppm)	0.78 ± 0.07*	1.06 ± 0.06	+
EGPE, 15 mg/L (3500 ppm)	0.95 ± 0.09 ^a	0.98 ± 0.10	+
EGBE, 4 mg/L (800 ppm)	0.95 ± 0.06	0.93 ± 0.05	+

^a4-hr exposure.

*Statistically different from control (Student's *t*-test, *p* < 0.05).

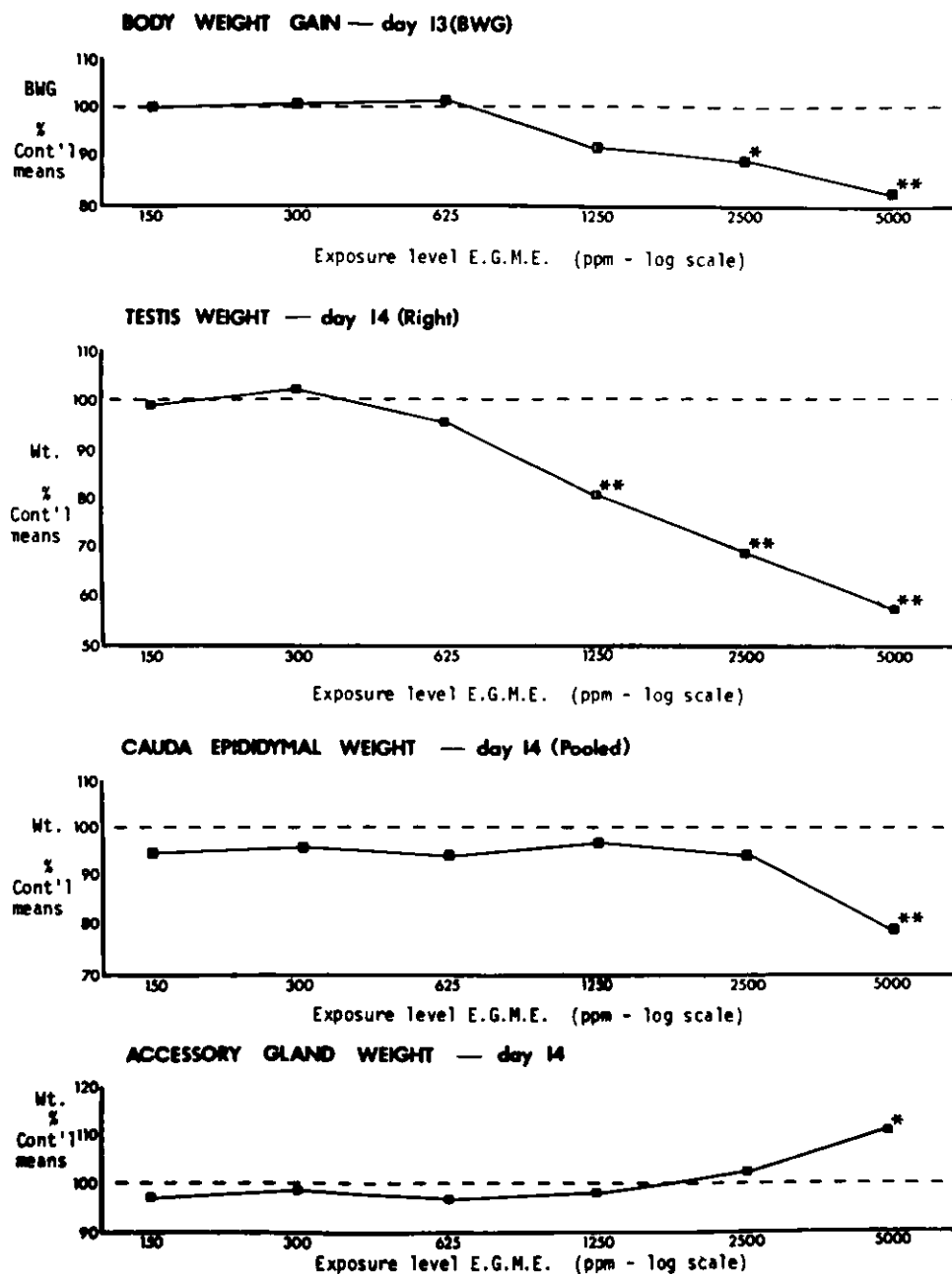


FIGURE 2. Effect on rat testis weight of a single exposure of EGME. Significance as in Figure 1.

In the second study, groups of 90, 8-week-old Alpk/AP rats were exposed to either 2500 or 1000 ppm EGME for a single 4-hr period. A further group of 90 rats was sham-exposed to air. Ten rats per group were killed on each of days 1, 2, 3, 4, 5, 8, 10, 15 and 19 following exposure. Individual body weights were recorded on the day before exposure and subsequently on days 4, 8, 15 and 19. Testes, epididymides and accessory gland weights were recorded at autopsy. Samples of these tissues were preserved in Bouin's fixative prior to examination. The results are shown in Figure 3.

In the second study, testis weight reduction was seen

in exposed rats 48 hr after exposure to both 2500 and 1000 ppm, and testes weights were lower than control values in both the exposed groups at subsequent time points. Histological examination of the testes revealed damage to the germinal epithelium 24 hr after exposure with primary spermatocytes as the target cells for EGME. Recovery was not evident at day 19 following exposure to 2500 ppm EGME, and the germinal epithelium remained disordered. Electron microscopy revealed changes in the Sertoli cells, with cytoplasmic retraction and swollen mitochondria evident 4 days after exposure to EGME. There was a biphasic response in

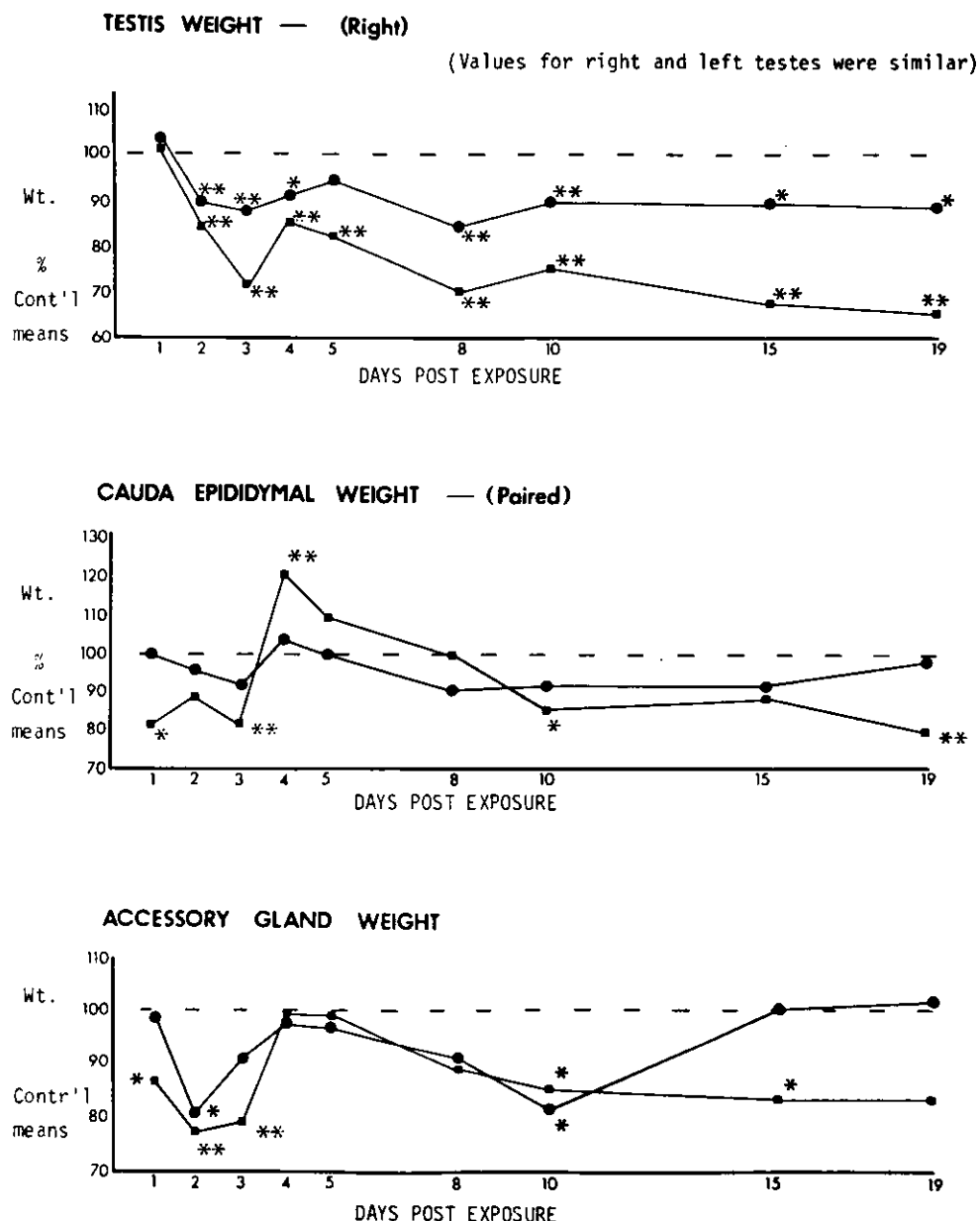


FIGURE 3. Time course of the effect of a single exposure of EGME in male rats. Significance as in Figure 1.

exposed animals with regard to epididymal weight. The increased weight at day 4 probably reflects the passage through the epididymides at this point of large numbers of immature germ cells shed from the testes following the initial insult. Subsequently, epididymal weights were reduced when compared to control values, again reflecting the situation in the testes where there was a marked reduction in the number of spermatozoa produced following exposure. EGME would appear to be exerting its effect primarily on rapidly dividing cells, with primary spermatocytes being affected initially. Subsequently, Sertoli cells showed signs of damage and this may account for the resulting marked loss of immature germ cells.

These studies indicate that even a relatively short exposure to EGME vapor can caused marked testicular atrophy although at high concentrations. Even at concentrations comparable with those causing testicular atrophy over a 90-day period (i.e., 300 ppm versus 625 ppm), there were subtle effects on spermatogenesis highlighting the need for further study of the effect of EGME on the testes. The results were reported previously (11).

General Conclusion

The work reported in this paper demonstrates very clearly the role of toxicology examinations which do not

fall clearly into the type of testing currently done for regulatory purposes. Both the testicular effect of EGME and the effect on the developing fetus can be seen in relatively short term and/or inexpensive experiments. The effect of EGME on the testis has been shown after 10 days exposure or indeed after a single exposure. This has enabled the effect of other glycol ethers to be examined very quickly. These data were in many cases generated before the effects of the other glycol ethers in regulatory type studies were available and where this has occurred these studies have accurately predicted the effect in the regulatory studies. Thus, EGME has been shown to affect the testis (10). EGME on the other hand, does not appear to affect the testis in a similar manner (9). It is therefore reasonable to assume that EGPE which did not affect the testes weights after either 10 days exposure at 1000 ppm or 4 hr exposure to a saturated vapor would not cause testicular atrophy in a longer regulatory study.

The effect of EGME upon the developing embryo is dramatically revealed using the test for teratogenic potential (6).

At higher levels EGME causes complete resorption of all embryos and at lower levels all three of the factors suggested by Chernoff and Kavlock (6) affected, i.e., numbers of litters, numbers of pups, their survivability to day 4. Again, these results are in agreement with the results of full teratology studies which have been carried out with EGME. EGME has been shown to be fetotoxic and teratogenic in the rabbit (10) and in the mouse (2). This activity of EGME gives added confidence to the results found with PGME and with diEGME in this test. Even though EGME has such a dramatic effect, neither PGME nor diEGME caused any significant changes in the parameters listed by Chernoff and Kavlock (6). It is important to remember, however, that in both the studies reported here the dose of test compound was not so high that there was severe maternal toxicity. It is important when trying to interpret the results of this type of study that extravagantly high doses are not used. These studies give a fair amount of confidence in predicting that neither diEGME nor PGME will be significantly teratogenic.

Taken overall, these studies indicate that even with a limited resource in terms of time and availability of experimental facilities, significant knowledge can be

gained on the toxicology of a group of compounds, provided that a good positive control substance, such as EGME, is available.

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