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Acute Oral Toxicity of Ethylene Glycol Monomethyl Ether and Diethylene Glycol Monomethyl Ether

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The glycol ethers are a class of chemicals exhibiting the polar properties of alcohols and the nonpolar properties of ethers, making them useful in a variety of industrial applications including the manufacture of plastics, as bases for inks, dyes and cleaners and as deicing agents. They are also used as chemical intermediates, as diluents for hydraulic brake fluid, and in water-base paints (Rowe and Wolf 1982). Ethylene glycol monomethyl ether (EGME) and diethylene glycol monomethyl ether (diEGME) are two of the most widely used representatives of glycol ethers. EGME, however, has been reported to have some toxic affects on animals. Nagano et al. (1984) reported that EGME (in drinking water for 18 days at a level of 2.5%) reduced body weight gain in mice. Testis atrophy and depression of white blood cell count were caused by the oral treatment of EGME at the dosage of 250 mg/kg b.w./day and 500 mg/kg b.w./day for 5 weeks, respectively. Foster et al. (1984) reported that oral administration of 250 mg/kg b.w./day of EGME for 7 days reduced relative testis weight in rats. These reports seem to indicate that the no observable effect level (NOEL) for these events is 50-100 mg/kg b.w./day. On the other hand, diEGME is not thought to cause reproductive toxicity or hematopoietic toxicity. Though Nagano et al. (1984) put 2.0% of diEGME into drinking water of mice for 25 days and Miller et al. (1985) exposed rats to 216 ppm of diEGME for 13 weeks, the results showed negative toxicity. However, their studies were performed at relatively low doses because diEGME has a low volatility at room temperature and is absorbed much more slowly than EGME through the human skin. Dugard et al. (1984) studied the absorption rate using the human abdominal epidermis in vitro system and reported that the skin absorption of EGME was 2.82 mg/cm^2/hr and

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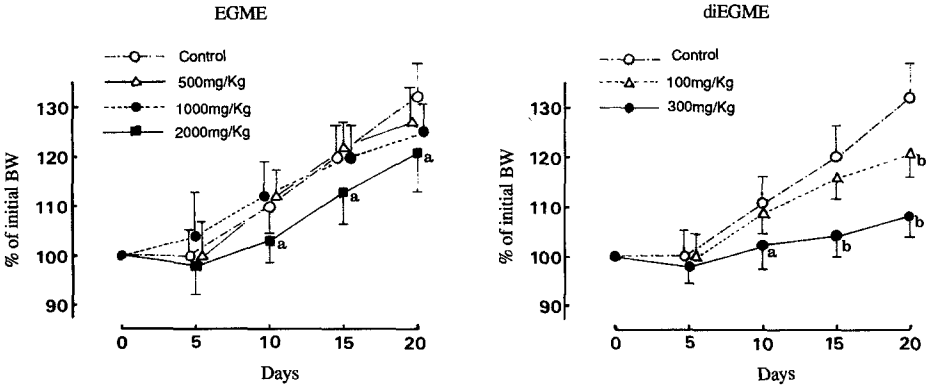

Figure 1. Effect of diEGME and EGME treatment on body weight growth pattern. Mean and S.D. are represented.
a: $p<0.05$ compared with control
b: $p<0.01$ compared with control

diEGME, 0.206 mg/cm^2/hr. Recently the use of diEGME is increasing as a replacemant chemical for EGME in dyes, cleaners, pesticides, jet aircraft fuels and so on (Hobson et al. 1986). Therefore, the intake of diEGME has become greater and greater. In this study, we administered relatively high doses of diEGME orally to rats and observed the changes of body and organ weights, comparing them with the toxicity of EGME.

MATERIALS AND METHODS

EGME (purity: more than 99%) and diEGME (purity: more than 98%) were purchased from Wako Chemical Co. These substances were administered to animals after distillation. Male adult Wistar rats weighing 220g were used. They were allowed free access to feed (CE-2, Clea Japan Inc.) and water, and were kept at 24°C on a 12-hr light/dark cycle for the duration of the study.
(a) Dose-response study. Animals were randomly assigned to six groups. Groups 1 to 3 received diEGME p.o. at dosages of 500, 1000, 2000 mg/kg b.w./day for 20 days. Groups 4 and 5 were administered EGME by the same route at dose levels of 100, 300 mg/kg b.w./day for 20 days. EGME and diEGME were diluted individually with water and dosages of 4 ml/kg of body weight were administered by gavage. Control animals in Group 6 were administered equivalent volumes of water.
(b) Time course study. Animals were administered 2000 mg/kg b.w. of diEGME, 300 mg/kg b.w. of EGME, or equivalent volumes of the water by gavage for 1, 2, 5 or 20 days.
Differences between mean values were evaluated using the Student's unpaired t-test.

Table 1. Effect of diEGME and EGME on the ralative organ weight of liver, kidney, spleen, testis, heart, lung and testis.

	Days of Study	N	Relative organ weight (organ weight / body weight x 1000) Liver	Kidney	Spleen	Thymus	Heart	Lung	Testis
Control	1	4	36.92 ± 0.57	8.81 ± 0.39	3.31 ± 0.26	3.15 ± 0.54	3.62 ± 0.11	5.43 ± 0.50	10.78 ± 0.91
	2	4	38.86 ± 0.63	8.48 ± 0.19	3.47 ± 0.19	2.95 ± 0.54	4.13 ± 0.32	5.91 ± 1.14	11.72 ± 1.21
	5	4	34.96 ± 2.10	8.06 ± 0.25	3.42 ± 0.27	2.36 ± 0.21	3.97 ± 0.20	6.05 ± 0.61	12.54 ± 0.70
	20	8	35.34 ± 4.31	8.22 ± 0.79	2.28 ± 0.23	2.08 ± 0.45	3.49 ± 0.32	4.89 ± 0.84	11.16 ± 0.69
diEGME	1	4	37.71 ± 0.58	8.36 ± 0.39	3.02 ± 0.33	2.30 ± 0.14*	3.80 ± 0.22	4.50 ± 0.64	11.17 ± 0.70
(2000mg/Kg/day)	2	4	37.63 ± 1.13	8.92 ± 0.18*	3.58 ± 0.21	2.92 ± 0.50	3.96 ± 0.16	5.59 ± 0.52	11.58 ± 0.55
	5	4	31.88 ± 0.52*	8.59 ± 0.42	2.54 ± 0.17**	1.49 ± 0.35*	3.81 ± 0.22	5.73 ± 0.42	10.52 ± 1.09*
	20	8	31.90 ± 0.85*	8.01 ± 0.72	2.33 ± 0.17	1.24 ± 0.24**	3.53 ± 0.25	5.53 ± 0.74	9.09 ± 2.33*
EGME	1	4	36.39 ± 0.55	8.80 ± 0.34	3.12 ± 0.17	2.49 ± 0.34	3.80 ± 0.22	5.48 ± 0.78	9.98 ± 0.78
(300mg/Kg/day)	2	4	31.23 ± 2.24**	7.68 ± 0.73	2.32 ± 0.25**	1.33 ± 0.60**	3.61 ± 0.05*	6.20 ± 1.30	9.52 ± 0.48*
	5	4	33.38 ± 3.47	7.39 ± 0.42*	1.93 ± 0.26**	0.48 ± 0.12**	3.82 ± 0.45	6.40 ± 1.52	8.63 ± 0.62**
	20	8	31.40 ± 1.94*	7.93 ± 0.34	2.40 ± 0.30	0.40 ± 0.18**	3.84 ± 0.26	5.38 ± 0.41	4.88 ± 0.61**

Mean and S.D. are represented.
* : $p<0.05$ compared with control
** : $p<0.01$ compared with control

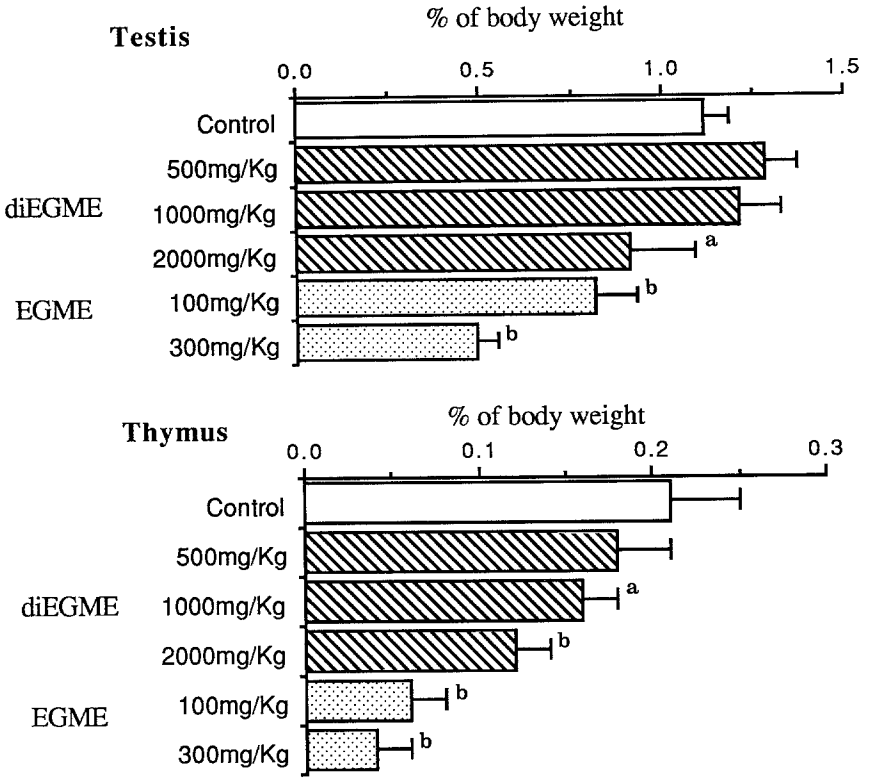

Figure 2. Testis and thymus weights of rats treated with diEGME and EGME for 20 days. Mean and S.D. are represented.
a: $p<0.05$ compared with control
b: $p<0.01$ compared with control

RESULTS AND DISCUSSION

Fig 1. shows the body weight growth pattern of rats that were orally administered diEGME (500, 1000, 2000 mg/kg b.w./day) and EGME (100, 300 mg/kg b.w./day). The animals treated with 2000 mg/kg b.w. of diEGME and 100, 300 mg/kg b.w. of EGME exhibited a statistically significant decrease in weight gain relative to control animals.
The effects of diEGME and EGME on the relative organ weight of liver, spleen, testis, heart, lung and testis are shown in Table 1. Daily administration of 2000 mg/kg b.w. of diEGME produced significant decreases in the liver, spleen, thymus and testis by day 5. The decreases in the weights of testis and thymus became more pronounced with the 20 day-treatment. By the 20th day, the testis weight was reduced to about 80% of control, and the thymus weight was reduced to 60% of control. However, the spleen weight of rats treated with diEGME for 20 days was the same as that of the

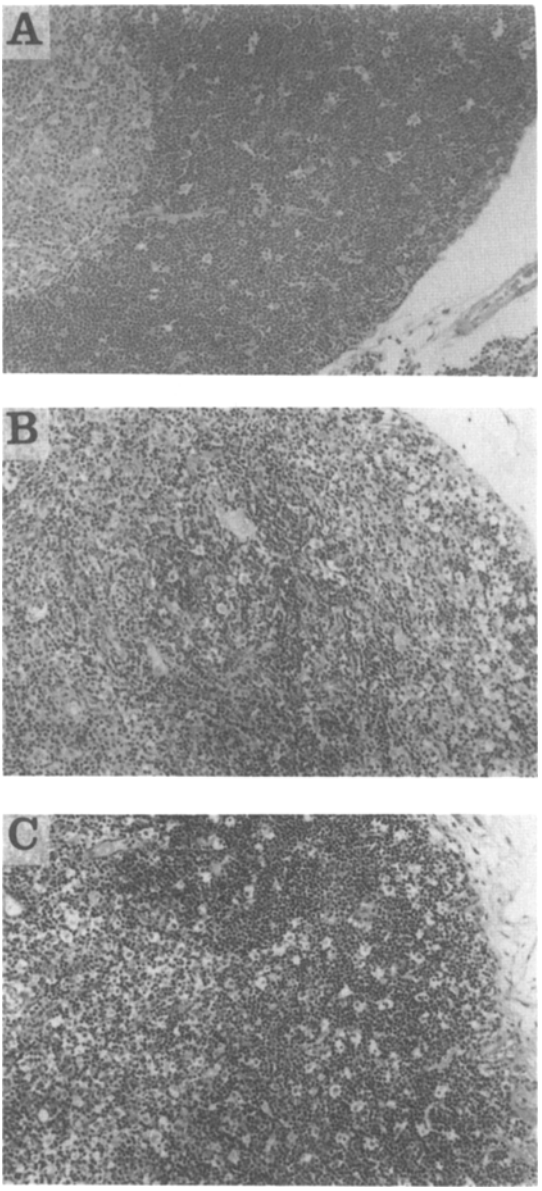

Figure 3. Sections of thymus from rats treated with water (as vehicle) for 5 days (A), 300 mg/kg/day EGME for 5 days (B) and 2000mg/kg/day diEGME for 5 days (C). (H.E. stain)

control group. On the other hand, diEGME (300 mg/Kg b.w./day) reduced the weights of liver, spleen, thymus, heart and testis significantly after only 2 days gavage. After 5 days, the thymus weight was reduced to one-fifth of control. Fig 2. shows the dose-response study regarding the effects of diEGME and EGME on testis and thymus weight. DiEGME (1000 mg/kg b.w./day for 20 days) reduced thymus weight to about 65% of control, however, 500 mg/kg b.w. of diEGME did not reduce the testis or thymus weight.

In this experiment, the authors demonstrated that diEGME reduced thymus and testis weight to the same extent as EGME did. However, the dose required to produce these results are quite different. The EGME dose used to reduce testis and thymus weight is thought to be less than 100 mg/kg b.w./day. On the other hand, the treatment with diEGME (500 mg/kg b.w./day x 20 days) did not cause a significant reduction of testis or thymus weight. From our data, the NOEL of diEGME is suspected to be in the neighborhood of 500 mg/kg b.w./day. The toxicity of EGME is related to its conversion to methoxyacetic acid, which is a major metabolite of EGME. DiEGME seems to convert to (2-methoxyethoxy)acetic acid, which has not been evaluated previously as a testicular toxicant (Cheever et al. 1989).

Fig.3 shows the sections of thymus obtained from rats administered 5 daily dosages of water, 300 mg/kg b.w. of EGME and 2000 mg/kg b.w. of diEGME. In the EGME-adminstered group, animals showed severe lymphocyte depletion in the cortex of the thymus. In the diEGME-adminstered group, the lymphocyte in the thymus cortex was also reduced. The depletion in diEGME-administered rats was not so severe as that in EGME-administered rats. Because a large population of thymocyte in the cortex is T-cells, it is suspected that the depletion of T-cells was caused by glycol ethers, and that glycol ethers affect the immune system. House et al. (1985) reported that 100mg/kg of EGME gavage for 10 days showed a 48% reduction of thymus weight. No significant alternation in immune function or host resistance to L. monocyteges, however, were observed in animals exposed to either EGME or methoxy acetic acid. In our experiments, 300 mg/kg b.w. EGME gavage for 5 days showed 80% reduction of thymus weight. Our results suggest that the effect of glycol ethers on the immune function should be reexamined.

Some long-term studies have shown that diEGME does not possess any toxicity (Nagano et al. 1984; Miller et al. 1985). Our results, however, indicated that a high dose administration of diEGME caused the same damages as its structural homologue, EGME. Our experiment also showed that the NOEL of diEGME is 500 mg/kg b.w./day.

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