TERATOLOGY 35:321–328 (1987)

Relative Potency of Four Ethylene Glycol Ethers for Induction of Paw Malformations in the CD-1 Mouse

BRYAN D. HARDIN AND CAROL J. EISENMANN
Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, Cincinnati, Ohio 45226

ABSTRACT Time-mated CD-1 mice were orally dosed on gestation day 11 (plug = 0) with distilled water (control) or one of four glycol ethers at a dose of 4 mmol/kg: ethylene glycol monomethyl ether (EGME, 304 mg/kg), ethylene glycol dimethyl ether (EGdiME, 361 mg/kg), diethylene glycol dimethyl ether (diEGdiME, 537 mg/kg), triethylene glycol dimethyl ether (triEGdiME, 713 mg/kg). Fetuses were collected on gestation day 18, weighed, and examined for gross external malformations. Fetuses were cleared and stained to examine paws. There were no signs of treatment-related maternal toxicity, and intrauterine survival was unaffected by glycol ether treatments. Fetal body weights were significantly reduced only in litters treated with EGdiME. There was no treatment-related pattern of gross external malformations other than paw defects. Only triEGdiME failed to produce a significant incidence of malformations. Paw defects were present in 87.5% of EGME-treated litters (68.5% of fetuses), 86.7% of EGdiME-treated litters (33.8% of fetuses), and 77.8% of diEGdiME-treated litters (39.7% of fetuses). Hindpaw defects predominated over forepaw, and syndactyly was the most common malformation. The incidences of oligodactyly and short digits were also significantly increased. The similarity of malformations produced by these methyl-substituted glycol ethers is proposed to be attributable to in vivo conversion to a common teratogen, methoxyacetic acid.

The ethylene glycol ethers are an important and widely used class of solvents, a number of which exhibit developmental or testicular toxicity. Ethylene glycol monomethyl ether (EGME) is the simplest representative of this chemical family, and it is both a teratogen and testicular toxicant (reviewed by Hardin, '83). In vitro (Rawlings et al., '85; Yonemoto et al., '84) and in vivo (Brown et al., '84; Ritter et al., '85) studies indicate that EGME is not directly teratogenic. EGME is metabolized by the alcohol dehydrogenase system to methoxyacetic acid (MA) (Miller et al., '83), and that metabolism is a required activation step. Inhibition of alcohol dehydrogenase with 4-methylpyrazole protects against the teratogenic effects of EGME (Ritter et al., '85). It has also been suggested recently (Sleet et al., '86a,b) that further biotransformation of MA may be required.

Glymes are symmetrical ethylene glycol diethers. Aprotic solvents, they solvate cations and leave anions reactive. Diverse chemical and industrial applications include electrochemistry, polymer chemistry, fuel and lubricant additives, catalysts, and separation processes (Grant Chemical Co., '82). Although they lack a primary alcohol site (Fig. 1) for activation similar to that of EGME, the following dimethyl ether derivatives of ethylene glycol are also teratogenic: ethylene glycol dimethyl ether (EGdiME, monoglyme) (Uemura, '80); diethylene glycol dimethyl ether (diEGdiME, diglyme) (Price et al., '85); and triethylene glycol dimethyl ether (triEGdiME, triglyme) George et al., '85). The role of metabolic activation in the teratogenicity of these diethers has not been extensively studied, but the malformations observed are similar to those produced by EGME. Furthermore, it has been reported that diEGdiME is metabolized to both EGME and diEGME in male rats (Cheever et al.,

Received July 22, 1986; accepted October 28, 1986.

EGME	CH_3-O-CH_2CH_2-OH
EGdiME (Monoglyme)	CH_3-O-CH_2CH_2-O-CH_3
diEGdiME (Diglyme)	CH_3-O-CH_2CH_2-O-CH_2CH_2-O-CH_3
triEGdiME (Triglyme)	CH_3-O-CH_2CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_3

Fig. 1. Molecular structures of four glycol ethers studied.

'86) and pregnant mice (Daniel et al., '86). The latter metabolite, diEGME, is teratogenic in rats (Hardin et al., '86).

Horton et al. ('85) reported that a single 500 mg/kg oral dose of EGME, administered to pregnant CD-1 mice between gestation days (gd) 9 and 12, produced digit malformations, predominantly syndactyly and oligodactyly. The incidence of paw defects was greatest following treatment on gd 11, and effects were more extensive in forepaws than hindpaws. Treatment on gd 12 reduced the total response sharply and hindpaws were somewhat more sensitive. Using eight dose levels ranging from 100 to 500 mg/kg, a dose-response experiment was also conducted in animals treated on gd 11. Paw defects were not seen at 100 mg/kg but increased in a dose-related pattern from 175 to 350 mg/kg, at which dose the maximal response was achieved (100% of litters affected).

The purpose of this study was to investigate the pattern of malformations produced by single doses of dimethyl-substituted ethylene glycol ethers, and to relate the potency of those glycol ethers to that ot EGME. Paw anomalies served as an index of effect after single equimolar doses of EGME, EGdiME, diEGdiME, or triEGdiME were administered to pregnant CD-1 mice on gd 11.

MATERIALS AND METHODS

Chemicals

Glycol ethers were purchased from Aldrich Chemical Company[1] (Milwaukee, WI): EGME, CAS 109-86-4, catalog No. 10 989-4 lot 3623ME; EGdiME, CAS 110-71-4, catalog No. E2 740-8 lot 5311TD; diEGdiME, CAS 111-96-6, catalog No. 25 639-0 lot 0115LE; triEGdiME, CAS 112-49-2, catalog No. T5 980-5 lot 0919EH. Chemical purity was represented as 99$^+$% but was not confirmed. All four are miscible in water, which served as the vehicle.

Animals

Time-mated female CD-1 mice were obtained from Charles River Laboratories (Kingston, NY). Mice were shipped on gd 4 (day of plug = 0) and received on gd 5. Four weekly shipments of at least 40 animals were received, and each shipment was randomly divided into two groups, one to serve as a vehicle control and the other to receive one of the four glycol ethers. Throughout the experiment, mice were housed in groups of five in solid-bottom cages with absorbent bedding and with free access to food and water. Light was provided on a 12-hr light-dark cycle.

Methods

Treatments were administered by gavage in a standard volume of 10 ml/kg body weight. On gd 11 control mice were dosed with distilled water as the vehicle, and experimental mice received a glycol ether in distilled water at a dose of 4 mmol/kg body weight: EGME, 304 mg/kg; EGdiME, 361 mg/kg; diEGdiME, 537 mg/kg; triEGdiME, 713 mg/kg. On gd 18 mice were killed by carbon dioxide asphyxiation. Gross maternal body weights and gravid uterus weights were recorded. Live and dead implants were counted, and live fetuses were removed, weighed, and preserved in 70% ethanol for subsequent staining with alizarin red (Staples and Schnell, '64). Stained specimens were examined for paw malformations.

Statistical analysis

Adjusted maternal body weight (body weight at term minus gravid uterus weight), fetal body weight (average weights per-litter), and numbers of live and dead implants per litter for each glycol ether and concurrent control group were analyzed by analysis of variance. The incidence of fetal malformations (the number of litters with one or more fetuses affected) was evaluated by one-sided Fisher's exact test. All analyses were carried out by means of the Statistical Analysis System (SAS Institute Inc., Cary, NC). Throughout this report "significant" is understood to mean "statistically significant" ($p < 0.05$).

RESULTS

Litters were collected from control and treated groups in the early afternoon of gd 18. A total of eight dams (one each in EGME

[1] Mention of a company name or product does not constitute endorsement by the authors or the National Institute for Occupational Safety and Health.

TABLE 1. Litter data at equimolar doses (mg/kg) of glycol ethers

	EGME		EGdiME		diEGdiME		triEGdiME	
	0	304	0	361	0	537	0	713
Maternal body weight (DG18)								
Gross	57.3 ± 5.6	55.4 ± 5.4	48.2 ± 6.1	51.4 ± 4.8	53.4 ± 8.3	54.1 ± 3.6	53.5 ± 5.1	55.4 ± 7.8
N =	14	16	16	15	12[2]	17	14	16
Adjusted	36.7 ± 3.2	35.6 ± 1.6	32.2 ± 1.6	33.3 ± 2.0	33.9 ± 3.1	33.6 ± 2.0	34.1 ± 1.5	34.7 ± 2.6
N =	14	16	15[1]	14[1]	12[2]	17	13[1]	16
Implants per litter								
Live fetuses	11.5 ± 2.4	11.0 ± 2.9	8.8 ± 4.1	10.6 ± 2.4	10.9 ± 4.1	11.5 ± 2.0	11.1 ± 2.6	11.2 ± 3.3
Dead + resorbed	0.1 ± 0.4	0.4 ± 0.5	0	0.1 ± 0.3	0.3 ± 0.5	0.1 ± 0.2	0.1 ± 0.4	0.2 ± 0.6
N =	14	16	16	15	13	17	14	16
Fetal body weight (mean of litter means)								
	1.39 ± 0.05	1.40 ± 0.09	1.52 ± 0.13	1.32 ± 0.22*	1.43 ± 0.15	1.40 ± 0.20	1.45 ± 0.08	1.49 ± 0.10
N =	14	16	16	15	13	17	14	16

[1]1 Gravid uterus not weighed.
[2]1 Gross body weight not recorded.
*Differs from concurrent control ($p < 0.01$).

TABLE 2. Gross fetal observations at equimolar doses (mg/kg) of glycol ethers

		EGME		EGdiME		diEGdiME		triEGdiME	
		0	304	0	361	0	537	0	713
No. examined—litters (fetuses)		15 (169)	16 (176)	16 (140)	15 (158)	15 (164)	18 (201)	15 (164)	19 (205)
Malformations litters (fetuses)									
Open eye	No.	0	1 (1)	0	1 (1)	1 (1)	0	1 (1)	0
	%[1]	0	6.2 (0.6)	0	6.7 (0.5)	6.7 (0.6)	0	6.7 (0.2)	0
Exencephaly	No.	0	0	1 (1)	1 (1)	2 (4)	0	1 (1)	0
	%[1]	0	0	6.2 (0.5)	6.7 (0.5)	13.3 (1.3)	0	6.7 (0.2)	0
Paw defects	No.	2 (3)	14*** (112)	0	13*** (57)	0	14*** (81)	0	1 (1)
	%[1]	13.3 (1.7)	87.5 (68.5)	0	86.7 (33.8)	0	77.8 (39.7)	0	5.3 (.04)
Total	No.	2 (3)	14*** (112)	1 (1)	13*** (57)	2 (4)	14*** (81)	1 (1)	1 (1)
	%[1]	13.3 (1.7)	87.5 (68.5)	6.2 (0.5)	86.7 (33.8)	13.3 (1.3)	77.8 (39.7)	6.7 (0.2)	5.3 (0.4)

[1]% of fetuses = average % per litter.
***No. of litters affected differs from concurrent control ($p < 0.001$).

TABLE 3. Paw malformations by type and location at equimolar doses (mg/kg) of glycol ethers

		EGME		EGdiME		diEGdiME		triEGdiME	
		0	304	0	361	0	537	0	713
No. examined—litters (fetuses)		15 (169)	16 (176)	16 (140)	15 (158)	15 (164)	18 (201)	15 (164)	19 (205)
Paw defects—litters (fetuses)									
Polydactyly									
Forepaws	No.	0	5* (20)	0	1 (1)	0	0	0	0
	%[1]	0	31.2 (11.3)	0	6.7 (0.5)	0	0	0	0
Hindpaws	No.	1 (1)	1 (1)	0	1 (1)	0	0	0	1 (1)
	%[1]	6.7 (0.6)	6.2 (0.5)	0	6.7 (0.5)	0	0	0	5.3 (0.4)
Syndactyly									
Forepaws	No.	1 (2)	6 (9)	0	2 (2)	0	2 (3)	0	0
	%[1]	6.7 (1.1)	37.5 (5.1)	0	13.3 (1.8)	0	11.1 (1.4)	0	0
Hindpaws	No.	0	14*** (101)	0	11*** (48)	0	13*** (62)	0	0
	%[1]	0	87.5 (54.9)	0	73.3 (27.8)	0	72.2 (30.9)	0	0
Short Digits									
Forepaws	No.	0	8** (26)	0	2 (3)	0	2 (2)	0	0
	%[1]	0	50.0 (14.4)	0	13.3 (1.8)	0	11.1 (0.8)	0	0
Hindpaws	No.	0	12*** (27)	0	3 (5)	0	10*** (21)	0	0
	%[1]	0	75.0 (14.5)	0	20.0 (2.6)	0	55.6 (10.4)	0	0
Oligodactyly									
Forepaws	No.	0	6* (16)	0	4* (8)	0	3 (4)	0	0
	%[1]	0	37.5 (8.6)	0	26.7 (5.5)	0	16.7 (1.8)	0	0
Hindpaws	No.	0	9*** (33)	0	1 (1)	0	7** (7)	0	0
	%[1]	0	56.2 (18.3)	0	6.7 (0.5)	0	38.9 (3.2)	0	0
Total									
Forepaws	No.	1 (2)	10** (65)	0	7** (15)	0	5* (10)	0	0
	%[1]	6.7 (1.1)	62.5 (36.2)	0	46.7 (10.2)	0	27.8 (4.4)	0	0
Hindpaws	No.	1 (1)	14*** (111)	0	11*** (53)	0	13*** (77)	0	1 (1)
	%[1]	6.7 (0.6)	87.5 (60.1)	0	73.3 (30.4)	0	72.2 (38.0)	0	5.3 (0.4)

[1] % of fetuses = average % per litter.
* $p < 0.05$;
** $p < 0.01$;
*** $p < 0.001$;
No. of litters affected differs from concurrent control.

control, diEGdiME treated, and triEGdiME control; two in diEGdiME control; and three in triEGdiME treated groups) delivered all or part of their litters before sacrifice, and data for those dams and litters are omitted from Table 1. Pups from those litters were examined for external malformations and paw defects, and those data are included in Tables 2 and 3. There was no evidence of cannibalization in any of these litters.

At doses of 4 mmol/kg, none of the four glycol ethers affected adjusted maternal body weight at term (Table 1). Similarly, none of the glycol ethers was associated with reduced numbers of live fetuses or increased numbers of dead implantation sites. Only in litters treated with EGdiME did fetal body weight differ significantly from concurrent controls ($p < 0.01$), reflecting, at least in part, chemically induced fetal toxicity. Since that concurrent control group was exceptional in having the lowest number of live fetuses per litter and the highest average fetal weight, the four control groups were contrasted in an analysis of variance. There was no difference among control groups in litter size, but fetal body weights were significantly different ($p < 0.05$). A Duncan's multiple range test indicated that control fetal weights in the triEGdiME and EGdiME studies did not differ significantly, nor did the controls in EGME, diEGdiME, and triEGdiME studies. Thus, characteristics of the concurrent controls for EGdiME might have contributed to that study's difference in treated versus control fetal weight.

Gross fetal malformations seen at necropsy included open eye and exencephaly, but these did not appear to be treatment related (Table 2). Paw defects were rarely seen in control fetuses but occurred with significantly increased frequency in litters of mice treated with EGME, EGdiME, and diEGdiME. Only triEGdiME failed to induce digital defects. Essentially the same proportion of litters included at least one pup with a paw defect in EGME, EGdiME, and diEGdiME groups (in pairwise comparisons of litters affected in those three groups, there were no significant differences by two-sided Fisher's exact test). However, EGME affected a greater proportion of fetuses: average percent affected per litter was 68.5% (EGME), 33.8% (EGdiME), and 39.7% (diEGdiME) (Table 2).

The specific types of paw defects and their distribution in fore- and hindpaws are presented in Table 3. Polydactyly was a rare observation except in litters treated with EGME, where the frequency was significantly increased in forepaws. A prominent tendency toward treatment-related fusion and reduction of digits was seen in the incidence of syndactyly, short digits, and oligodactyly. Syndactyly was the most common digital defect observed, appearing with significantly increased frequency in hindpaws of EGME, EGdiME, and diEGdiME litters.

Short digits were not seen in any of 61 control litters (637 fetuses) or in 19 triEGdiME-treated litters but occurred in both fore- and hindpaws of EGME-, EGdiME-, and diEGdiME-treated litters. The frequency was significantly increased in both fore- and hindpaws of EGME litters but only in hindpaws of diEGdiME-treated litters. Similarly, oligodactyly was not observed in any control or triEGdiME litters, but appeared at a significant frequency in both fore- and hindpaws of EGME litters, forepaws of EGdiME litters, and hindpaws of diEGdiME litters.

DISCUSSION

A single 4 mmol/kg oral dose of EGME, EGdiME, diEGdiME, or triEGdiME on gd 11 did not produce signs of maternal toxicity in CD-1 mice. With paw defects as an index, EGME, EGdiME, and diEGdiME were teratogenic. All three glycol ethers affected about the same proportion of litters. However, EGME affected 68.5% of fetuses, while EGdiME affected only 33.8% and diEGdiME affected 39.7%. EGME also had the broadcast spectrum of response, producing a significant incidence of four types of paw defect: polydactyly, syndactyly, short digits, and oligodactyly. diEGdiME produced a significant incidence of all except polydactyly, while EGdiME significantly affected only syndactyly and oligodactyly. Treatment with triEGdiME did not elevate the frequency of paw defects. However, it cannot be concluded from this that 4 mmol/kg was a no effect dose for triEGdiME, since no effort was made to evaluate visceral defects and only paws were scored for skeletal defects.

The observations here are quite similar to those reported by Horton et al. ('85) following oral treatment of CD-1 mice with EGME. These investigators found that, at 500 mg (6.6 mmol)/kg, paw defects peaked with treatment on gd 11. Polydactyly was a rare observation, but syndactyly and oligodactyly affected both forepaws and hindpaws in 100% of litters treated on gd 11. In a dose-response

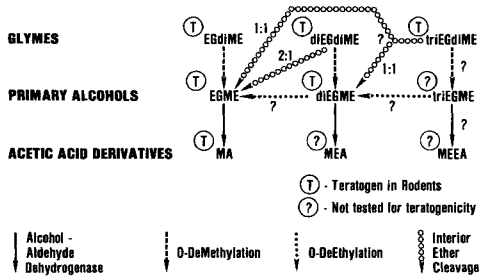

Fig. 2. Proposed metabolic pathways and relationships between the four glycol ethers studied.

study on gd 11, slightly over 75% of litters treated with 300 mg (3.94 mmol)/kg had forepaw defects and hindpaw defects were seen in slightly less than 75% of litters. In the present study, essentially the same EGME dose produced forepaw defects in 62% of litters and hindpaw defects in 88% of litters. Syndactyly was the most prominent defect, affecting hindpaws more than forepaws. Horton et al. ('85) bred mice in their own laboratory over a 2.5-hour interval in the morning, and designated the next 24 hours as day 0. In the present study, mice were mated overnight by the vendor and the following day was designated day 0. Timing of gestation was therefore more precise in the former study, and day 11 here probably corresponds to day 11.5–12.0 in that study. That shift in timing may account for hindpaws' being affected more than forepaws here, while Horton et al. ('85) reported a greater proportion of fetuses with forepaw defects on gd 11.

The teratogenicity of EGME is known to be dependent upon metabolism to MA (Brown et al., '84; Rawlings et al., '85; Ritter et al., '85; Yonemoto et al., '84). The similarity of the defects described in the present communication as produced by EGME, EGdiME, and diEGdiME, and by all four of these glycol ethers in conventional studies (George et al., '85; Nagano et al., '81; Price et al., '85; Uemura, '80) suggests that a common active teratogen may be involved. However, the diethers lack a primary alcohol site and therefore cannot be directly metabolized by the alcohol dehydrogenase system, which activates EGME.

Figure 2 presents a proposed scheme for the metabolism of the four glycol ethers examined in the present study which could account for the conversion of the three glymes to potentially teratogenic species. All of the glymes are presumed to be substrates for O-demethylation, producing a primary alcohol (EGME in the case of EGdiME). Each primary alcohol is known or presumed to be a substrate for alcohol/aldehyde dehydrogenase. Presently, there is no direct evidence for the existence of the possible O-deethylation pathway shown in Figure 2. The primary alcohols might also be subject to a second O-demethylation, producing ethylene, diethylene, or triethylene glycol (not shown in Fig. 2).

Of the final products, MA and MEA (methoxyethoxyacetic acid) are known, but only MA has been evaluated for teratogenicity. Based on in vitro studies of various other alkoxy acids (Rawlings et al., '85), it can be predicted that if MEA and MEEA (methoxyethoxyethoxyacetic acid) retain teratogenic potential, there will be very sharp reductions in potency as the total chain length increases. Additionally, recent studies (Sleet et al., '86a,b) suggest that MA requires still further metabolic conversion for teratogenicity, which may also influence the teratogenicity of MEA and MEEA.

Interior ether cleavage as proposed in Figure 2 has not been directly demonstrated but is inferred from the observations of MA and MEA excretion following treatment with diEGdiME (Cheever et al., '86; Daniel et al., '86). The existence of this pathway, even if less favored than O-demethylation, could explain the teratogenicity of diEGdiME and triEGdiME by the one-step production of EGME. In the case of diEGdiME the 2:1 yield of EGME also helps to explain why diEGdiME was approximately equivalent to EGdiME while triEGdiME was inactive in the present studies.

A similar figure, drawn for the corresponding ethyl-substituted glycol ethers, might also predict the relationships for that series. Of them, only EGEE and diEGEE have been evaluated for teratogenicity: EGEE is teratogenic in rats and rabbits (Andrew and Hardin, '84), while diEGEE was not teratogenic in inhalation (Nelson et al., '84) or skin painting studies (Hardin et al., '84). Generalizing the pattern for methyl-substituted glycol ethers suggests that the potential for developmental toxicity will be directly related to the ease of in vivo production of the simplest glycol ether in the series (EGME or EGEE).

In the present study, the distribution and variety of paw defects in litters and fetuses suggests a relative teratogenic potency of

EGME > EGdiME = diEGdiME >> tri-EGdiME (which did not produce paw malformations under the conditions of this study). This ranking assumes equal biological availability of each of these glycols followng oral dosing in water. Although equimolar gavage doses were used here, gastrointestinal uptake, distribution, metabolism, and excretion patterns have not been compared. Evaluation of these factors will be required to establish the basis for the differences in potency observed here. However, these results as well as previously published studies of glycol ether developmental toxicity are consistent with a proposed metabolic scheme that accounts for teratogenicity of the diethers in terms of in vivo conversion to EGME and MA.

LITERATURE CITED

Andrew, F.D., and B.D. Hardin (1984) Developmental effects after inhalation exposure of gravid rabbits and rats to ethylene glycol monoethyl ether. Environ. Health Perspect., *57*:13–24.

Brown, N.A., D. Holt, and M. Webb (1984) The teratogenicity of methoxyacetic acid in the rat. Toxicol. Lett., *22*:93–100.

Cheever, K.L., D.E. Richards, W.W. Weigel, J.B. Lal, A.M. Dinsmore, and F.B. Daniel (1986) Metabolism of a testicular toxin, bis (2-methoxyethyl) ether, in the rat. Toxicologist, *6*:32 (Abstract).

Daniel, F.B., C. Eisenmann, K.L. Cheever, D.E. Richards, and W.W. Weigel (1986) Metabolism of a reproductive toxin, bis (2-methoxyethyl) ether, in the pregnant mouse. Teratology, *33*:75C (Abstract).

George, J.D., C.J. Price, M.C. Marr, and C.A. Kimmel (1985) Teratogenicity of triethylene glycol dimethyl ether (TGDM) in mice. Teratology, *31*:53A (Abstract).

Grant Chemical Company (1982) Glymes—The Grant Family of Glycol Diethers. Technical Brochure, Grant Chemical Division, Ferro Corporation, Baton Rouge, LA, 16 pp.

Hardin, B.D. (1983) Reproductive toxicity of the glycol ethers. Toxicology, *27*:91–102.

Hardin, B.D., P.T. Goad, and J.R. Burg (1984) Developmental toxicity of four glycol ethers applied cutaneously to rats. Environ. Health Perspect., *57*:69–74.

Hardin, B.D., P.T. Goad, and J.R. Burg (1986) Developmental toxicity of diethylene glycol monomethyl ether (diEGME). Fund. Appl. Toxicol., *6*:430–439.

Horton, V.L., R.B. Sleet, J.A. John-Greene, and F. Welsch (1985) Development phase specific and dose-related teratogenic effects of ethylene glycol monomethyl ether in CD-1 mice. Toxicol. Appl. Pharmacol., *80*:108–118.

Miller, R.R., E.A. Herman, P.W. Langvardt, M.J. McKenna, and B.A. Schwetz (1983) Comparative metabolism and disposition of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in male rats. Toxicol. Appl. Pharmacol., *67*:229–237.

Nagano K., E. Nakayama, H. Oobayashi, T. Yamada, H. Adachi, T. Nishizawa, H. Ozawa, M. Nakaichi, H. Okuda, K. Minami, and K. Yamazaki (1981) Embryotoxic effects of ethylene glycol monomethyl ether in mice. Toxicology, *20*:335–343.

Nelson, B.K., J.V. Setzer, W.S. Brightwell, P.R. Mathinos, M.H. Kuczuk, T.E. Weaver, and P.T. Goad (1984) Comparative inhalation teratogenicity of four glycol ether solvents and an amino derivative in rats. Environ. Health Perspect., *57*:261–271.

Price, C.J., J.D. George, M.C. Marr, and C.A. Kimmel (1985) Teratogenicity of diethylene glycol dimethyl ether (DYME) in mice. Teratology, *31*:57A (Abstract).

Rawlings, S.J., D.E.G. Shuker, M. Webb, and N.A. Brown (1985) The teratogenic potential of alkoxy acids in post-implantation rat embryo culture—Structure-activity relationships. Toxicol. Lett., *28*:49–58.

Ritter, E.J., W.J. Scott, Jr., R.L. Randall, and J.M. Ritter (1985) Teratogenicity of dimethoxyethyl phthalate and its metabolites methoxyethanol and methoxyacetic acid in the rat. Teratology, *32*:25–31.

Sleet, R.B., J.A. Greene, and F. Welsch (1986a) Embryonal accumulation of methoxyacetic acid (MAA) persists with ethanol (ETOH) attenuation of methoxyethanol (ME) teratogenicity. Teratology, *33(3)*:70C (Abstract).

Sleet, R.B., J.A. Greene, and F. Welsch (1986b) Reduction of methoxyethanol (ME)- and methoxyacetic acid (MAA)-induced paw malformations in CD-1 mice by small endogenous carboxylic acids. Teratology, *33(3)*:95C (Abstract).

Staples, R.E., and V.L. Schnell (1964) Refinements in rapid clearing technic in the KOH-alizarin red S method for fetal bone. Stain Technol., *39*:61–62.

Uemura, K. (1980) (The teratogenic effects of ethylene glycol dimethyl ether on mouse.) Acta Obstet. Gynecol. Jpn., *32*:113–121. (in Japanese).

Yonemoto, J., N.A. Brown, and M. Webb (1984) Effects of dimethoxyethyl phthalate, monomethoxyethyl phthalate, 2-methoxyethanol and methoxyacetic acid on post implantation rat embryos in culture. Toxicol. Lett., *21*:97–102.