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Bis(2-Methoxyethyl) Ether: Metabolism and Embryonic Disposition of a Developmental Toxicant in the Pregnant CD-1 Mouse[1]

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Bis(2-Methoxyethyl) Ether: Metabolism and Embryonic Disposition of a Developmental Toxicant in the Pregnant CD-1 Mouse. DANIEL, F. B., CHEEVER, K. L., BEGLEY, K. B., RICHARDS, D. E., WEIGEL, W. W., AND EISENMANN C. J. (1991). *Fundam. Appl. Toxicol.* **16,** 567–575. An embryotoxic oral dose of bis(2-methoxyethyl) ether (DGDME), 3.73 mmol/kg body wt (500 mg/kg), administered on the 11th day of gestation to pregnant CD-1 mice was metabolized predominantly by *O*-demethylation to 2-(2-methoxyethoxy)ethanol with subsequent oxidation to (2-methoxyethoxy)acetic acid. Urinary excretion of this metabolite over 48 hr amounted to 63 ± 2% of the dose. A smaller percentage of the administered dose was metabolized at the central ether linkage to produce 2-methoxyethanol, which was further metabolized by alcohol dehydrogenase to methoxyacetic acid. Urinary excretion of methoxyacetic acid, a potent developmental toxicant, amounted to 28 ± 1% of the administered dose by 48 hr and was the second most prominent urinary metabolite. Unchanged DGDME and methoxyacetic acid were detected in the embryonic tissues from these animals, and embryos harvested after the initial 6-hr period showed detectable amounts of only methoxyacetic acid. The average amount of methoxyacetic acid per embryo was calculated to be 1.5 ± 1.0 μmol (5.9 mmol/kg body wt) at the 6-hr termination time. This finding suggests that the reported teratogenic effects of DGDME are due to methoxyacetic acid formed, either in the fetus or by hepatic metabolism in the dam with subsequent distribution to the embryonic tissue. These results suggest that such developmental toxicity may occur with structurally similar aprotic ethylene glycol ethers in which metabolic *O*-dearylation would yield 2-methoxyethanol. © 1991 Society of Toxicology.

Bis(2-methoxyethyl) ether (diethylene glycol dimethyl ether; DGDME; CAS Reg. No. 111-96-6)[4] shown in Fig. 1 is an aprotic member of the important group of industrial solvents collectively known as ethylene glycol ethers (NIOSH, 1983). DGDME is used in a variety of industrial products and possible exposure of workers to dermal and inhalation concentrations of the compound has long been recognized (Zavon, 1963). DGDME has been shown to be a reproductive toxicant in male and to be developmentally toxic in female rats (Plasterer *et al.*, 1985; Cheever *et al.*, 1989b;

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[4] Abbreviations used: DGDME, bis(2-methoxyethyl) ether; 2ME, 2-methoxyethanol; MA, methoxyacetic acid; MEAA, (2-methoxyethoxy)acetic acid; MEE, 2-(2-methoxyethoxy)ethanol.

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Lee *et al.*, 1989) and mice (McGregor *et al.*, 1983; Plasterer *et al.*, 1985; Price *et al.*, 1986). Recently, DGDME has been reported to produce developmental toxicity with teratogenicity in the mouse consisting of a significant incidence of three types of paw defects: syndactyly, short digits, and oligodactyly (Hardin and Eisenmann, 1987). Those investigators reported that qualitatively but not quantitatively similar paw defects were produced after a single oral 3.73 mmol/kg body wt dose of either DGDME or 2-methoxyethanol (2ME), administered to pregnant CD-1 mice on Day 11 of gestation. The toxicity reported for short-chain ethylene glycol ethers, such as 2ME or 2-ethoxyethanol (2EE) (Wiley *et al.*, 1938; Morris *et al.*, 1942; Miller *et al.*, 1981, 1982; Nagano *et al.*, 1981, 1984), is dependent on the sequential metabolism of these compounds by alcohol and aldehyde dehydrogenase enzymes with formation of the corresponding alkoxyacetic acids (Miller *et al.*, 1982, 1983; Cheever *et al.*, 1984; Moss *et al.*, 1985; Daniel *et al.*, 1986). The agent responsible for the reproductive toxicity produced by DGDME was thought to be similar to that of the short-chain glycol ethers (McGregor *et al.*, 1983), and other investigators suggested that MA formation could be responsible for DGDME developmental effects related to metabolite formation (Daniel *et al.*, 1986; Hardin and Eisenmann, 1987). The formation of such toxic metabolites, however, was previously a matter of conjecture because the compound does not have a free hydroxyl group and therefore would not be directly metabolized by alcohol dehydrogenase. Recently, Cheever and coworkers (1988, 1989a) demonstrated that testicular toxicity produced by DGDME in the rat can be explained by a P450-mediated oxidative cleavage of the central ether linkage. This biotransformation results in the formation of 2ME, a compound which Miller *et al.* (1983) showed is further metabolized to methoxyacetic acid (MA), a known reproductive toxicant (Miller *et al.*, 1982; Brown *et al.*, 1984; Ritter *et al.*, 1985).

The objective of the current study was to determine whether developmental toxicity in the pregnant CD-1 mouse, reported to occur after the oral administration of DGDME, can be explained by the metabolism or fetal distribution of DGDME in the mouse.

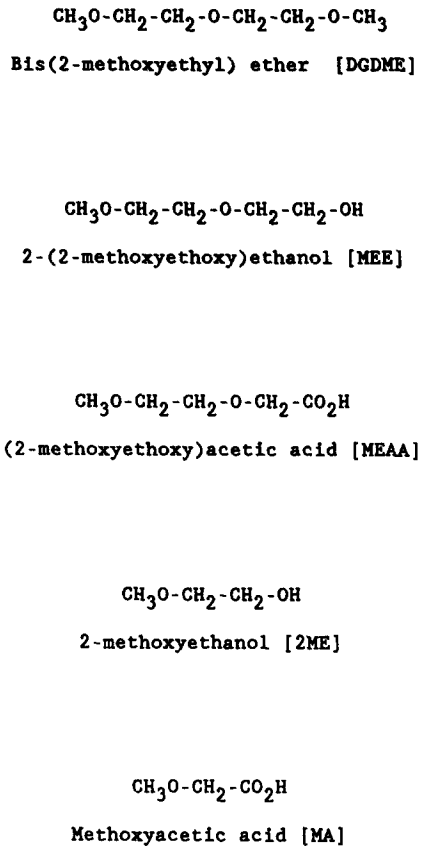

FIG. 1. Chemical structures.

MATERIALS AND METHODS

Chemicals. The test compound, DGDME (>99.5%), was obtained from the Fluka Chemical Corp. (Hauppauge, NY)[5] and [1,2-*ethylene*-^{14}C]DGDME (>99% radiochemical purity) was purchased from Pathfinder Laboratories, Inc. (St. Louis, MO). Using appropriate amounts of these two compounds a dosing solution of 0.373 mmol/ml DGDME in distilled water was prepared at a specific activity of 50.3 μCi/mmol. Commercially available reference compounds of the highest purity corresponding to possible

[5] Mention of company or product names is not to be considered an endorsement by the National Institute for Occupational Safety and Health.

metabolites were obtained. Diglycolic acid (>97%) was purchased from Fluka Chemical Corp. MA (99%), 2-(2-methoxyethoxy) ethanol [MEE] (99%), and 2ME (99%) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). The preparation of both (2-methoxyethoxy) acetic acid [MEAA] and *N*(methoxyacetyl)glycine methyl ester has been described previously (Cheever *et al.*, 1988).

Animals. Time-mated CD-1 Swiss mice, purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA), were received on Day 5 of gestation (observation of a vaginal plug taken as Day 0) and were maintained on a NIH-07 rat and mouse diet (Zeigler Brothers, Inc., Gardner, PA) and water *ad libitum*. The laboratory temperatures were maintained at 22 to 25°C, with the relative humidity ranging from 45 to 51% and a 12-hour light–dark cycle. The mice were kept 6/cage until Day 11 of gestation at which time the animals were randomized and treated.

Dosing. The pregnant mice were weighed (35.3 ± 1.9 g), and 60 animals were administered a single 3.73 mmol dose of DGDME/kg body wt (500 mg/kg body wt) by gavage at 9:00 AM of Day 11 of gestation. Doses were chosen to correspond with doses previously shown to result in developmental toxicity (Price *et al.*, 1987; Hardin and Eisenmann, 1987). Immediately after dosing the animals were placed, 3 per cage, into glass Roth-style metabolism cages. During the metabolism study the animals had free access to food and distilled water. Laboratory air, freed of organic vapors, carbon dioxide, and water vapor, was drawn by vacuum through the metabolism chambers at a rate of 200 ml/min. The pooled urine for each metabolism cage was collected until time of termination at 1, 3, 6, 24, and 48 hr after dosing and stored at −20°C until analysis.

Sample preparation. At 1, 3, 6, 24, and 48 hr after dosing, mice from each of four metabolism cages (12 mice per time point) were anesthetized with pentobarbital and terminated by exsanguination. Blood removed from the posterior vena cava was immediately transferred to an EDTA-containing Vacutainer. The uteri were excised, split longitudinally, and the yolk sacs were gently freed from the placentae. Embryos, weighing 0.06 ± 0.02, 0.07 ± 0.02, 0.08 ± 0.03, 0.12 ± 0.04, and 0.26 ± 0.06 g, were harvested at 1, 3, 6, 24, and 24 hr (n = 60 at each time point), respectively. The embryos were teased from the yolk sacs, rinsed with buffered saline (pH 7.4), and blotted to remove excess fluid. Five embryos from each dam (three from the middle of the uterus and one from each end) were placed, one per vial, into tared 7-ml scintillation vials and weighed. The embryos and duplicate 0.1-ml aliquots of each whole blood sample were digested separately at 60°C as described previously (Weigel *et al.*, 1978). A 0.5-ml aliquot of each digest was decolorized with 0.1 ml of 70% *t*-butyl hydroperoxide and neutralized with 0.1 ml of concentrated nitric acid prior to counting in 15 ml of Scinti-Verse II (Fisher Scientific Co., Fairlawn, NJ). Radioactivity in the samples was determined using a Model LS8100 scintillation counter (Beckman Instruments Co., Fullerton, CA). Counting efficiencies were calculated by the external standard method of Horrocks (1974). Any remaining blood from mice in each metabolism cage was combined and prepared for chromatographic analysis for quantification of DGDME or DGDME metabolites. Embryos (4/metabolism cage) from the same dams were homogenized directly with 2 ml acetonitrile/g of embryonic tissue. Subsequently, protein-free samples were prepared for analysis by high-performance liquid chromatography (HPLC) by passing blood or embryo homogenates through Centrifree Micropartition filters (Amicon Corp., Danvers, MA) by centrifugation at 1000*g*. The urine samples were thawed, adjusted to pH 3 with H_2SO_4, and passed through a 0.02-μm pore size Acrodisc filter (Gelman Sciences, Ann Arbor, MI) prior to analysis.

Metabolite analysis. DGDME and DGDME metabolites present in extracted embryos, filtered blood, or urine samples were separated using a Waters Model ALC/GPC 201 HPLC equipped with two Model 6000A pumps programmed by a Model 720 system controller (Waters Associates, Inc., Milford, MA). No detectable loss of radioactivity per milliliter was incurred by filtration. Each sample was injected separately onto the chromatographic column using a Waters WISP 710A. The radiolabeled components were chromatographed on a 50 cm × 9.0 mm i.d. stainless-steel column packed with 10-μm particle size Partisil 10 OD-2 (Whatman, Inc., Clifton, NJ) maintained at 30°C with a Waters Column Heater Module. The solvent program consisted of 15 min isocratic 1% acetic acid in water, then methanol:1% acetic acid increasing from 15 to 80% methanol using a linear gradient over a 40-min period, and finally methanol:1% acetic acid maintained at 80% methanol for 15 min. The flow rate was 1 ml/min throughout the chromatographic run. Radiolabeled components appearing in the effluent were detected and quantified using a Packard Tri-Carb RAM 7500 radioactivity detector (Packard Instrument Co., Inc., Downers Grove, IL). Individual radioactive fractions were collected for further analysis by gas chromatography–mass spectrometry (GC-MS) using an ISCO Model 2103 fraction collector (Lincoln, NE).

Mass spectral identification of tissue and urinary metabolites. Radioactive fractions of embryo, blood, or urine, collected from the HPLC column, were extracted continuously for 6 hr with methylene chloride and concentrated under a stream of nitrogen. The methyl esters of organic acids were prepared by reaction with *N,N*-dimethylformamide dimethyl acetal (Pierce Chemical Co., Rockford, IL) in accordance with the general method of Thenot *et al.* (1972). Mass spectrometry for metabolite identification used a Hewlett-Packard Model 5710A gas chromatograph, fitted with a 50 cm × 0.2 mm i.d. Ultra-1 (d_f = 0.33 μm) fused capillary column (Hewlett-Packard Co., Palo Alto, CA), coupled by a direct capillary interface to a Model 5890A quadrupole mass spectrometer (Hewlett-Packard Co., Palo Alto, CA). Helium was used as the carrier gas at a linear velocity of 38 cm/sec. Splitless injections were made onto the column with the oven temperature maintained at 10°C. The temperature was then programmed to 200°C at 8°C/min. Electron impact mass spectra were

acquired at an electron energy of 70 eV and at a source temperature of 200 ± 10°C. These spectra were stored and processed with a Model 59970 MS ChemStation (Hewlett-Packard Co.). Mass spectrometric identifications were made by comparison of the mass spectra of radiolabeled components with those of authentic compounds.

Statistical analysis. Statistical differences between group means were determined using one-way analysis of variance. Data were processed using the HP 98820A Statistical Library Revision B (Hewlett-Packard, Co., Fort Collins, CO), installed on an HP 9000 Model 310 ChemStation. The probability level of $p < 0.05$ was considered significant.

RESULTS

The elimination of DGDME from the pregnant mouse was primarily via the urine, with >97% of the administered dose excreted by that route within 48 hr. Typically, 10 radioactive peaks were detected on HPLC separation of urine fractions from these mice. After isolation of the radiolabeled fractions, listed in Table 1 by HPLC elution order, seven components were identified by comparison of GC retention times and GC-MS spectra with those of corresponding standards. The percentage of the various urinary metabolites in the pregnant CD-1 mouse was dependent on the time interval of urine collection after administration of DGDME. After an initial 3-hr period, only a small percentage of the blood or urinary ^{14}C was detected as the parent compound (peak X). The major urinary metabolite (peak VII) present at each of the times tested was identified as MEAA (Table 1). During the 0- to 3-hr interval, this metabolite accounted for 86 ± 1% of the urinary radioactivity excreted. The second most abundant urinary metabolite (peak V) was identified as MA and did not appear in the first hour. For the 1- to 3-hr period, MA accounted for 6 ± 1% of the eluted radioactivity. However, an increasing portion of the urinary radioactivity was determined to be MA for succeeding collection periods. In the 6- to 24-hr urine sample, the two acids, MEAA and MA, accounted for 71 ± 2% and 24 ± 2% of the total urinary radioactivity, respectively. During the 24- to 48-hr period, levels of 55 ± 5% for MEAA and 40 ± 5% for

TABLE 1

48-hr URINARY ^{14}C EXCRETION OF DGDME AND METABOLITES IN THE PREGNANT CD-1 MOUSE[a]

HPLC peak	Compound	Urine concentration (μmol)[b]				
		0–1 hr	1–3 hr	3–6 hr	6–24 hr	24–48 hr
I	—	ND[c,d]	0.1 ± 0.01	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
II	*N*-(Methoxyacetyl)glycine	ND	<0.1	0.6 ± 0.2	0.3 ± 0.1	1.2 ± 0.3
III	Diglycolic acid	ND	ND	<0.1	ND	ND
IV	—	<0.1	0.1 ± 0.0	1.6 ± 0.7	7.0 ± 1.6	2.8 ± 0.7
V	MA	ND	0.5 ± 0.2	7.5 ± 3.3	10.4 ± 4.5	17.9 ± 4.8
VI	2ME	ND	<0.1	0.4 ± 0.1	0.2 ± 0.1	0.7 ± 0.2
VII	MEAA	0.8 ± 0.3	6.8 ± 3.2	15.9 ± 6.9	30.7 ± 13.4	28.3 ± 7.6
VIII	—	ND	<0.1	0.1 ± 0.0	ND	0.1 ± 0.0
IX	MEE	0.1 ± 0.0	<0.1	ND	ND	ND
X	DGDME	ND	0.2 ± 0.1	ND	ND	ND

[a] Pregnant female CD-1 mice (35.3 ± 1.9 g) were administered 3.73 mmol/kg body wt (20 μCi/kg) of [1,2-*ethylene*-^{14}C]DGDME by gavage on Day 11 of gestation, and housed 3/cage in glass metabolism cages.

[b] HPLC separation was obtained using Partisil OD-2 and Nova-Pak C18 packed columns in series. Elution of radiolabeled components using 1% acetic acid in water for 15 min was followed by a 40-min linear gradient program from 15 to 80% methanol:1% acetic acid.

[c] Urinary values represent the cumulative umoles of DGDME and metabolites appearing in combined urine and cage washings for the sampling periods (mean ± SD), $n = 4$ groups per time interval). The urinary metabolites—I, IV, and VIII—were unidentified.

[d] Not detected.

TABLE 2

48-hr BLOOD LEVELS OF [^{14}C]DGDME AND METABOLITES IN THE PREGNANT CD-1 MOUSE[a]

HPLC peak	Compound	Blood concentration (nmol/mg) 1 hr	3 hr	6 hr	24 hr	48 hr
V	MA	0.4 ± 0.3[b]	2.3 ± 0.1*	3.4 ± 0.2*	1.4 ± 0.3*	0.2 ± 0.0*
VII	MEAA	<0.1	0.4 ± 0.1*	0.3 ± 0.1*	ND[c]	ND
VIII	—	0.1 ± 0.0	0.3 ± 0.0*	0.5 ± 0.0*	ND	ND
X	DGDME	7.7 ± 0.5	4.3 ± 0.1*	0.3 ± 0.0*	ND	ND

[a] Pregnant female CD-1 mice (31.75 g) were administered 3.73 mmol/kg body wt (20 μCi/kg) of [1,2-*ethylene*-^{14}C]DGDME by gavage on Day 11 of gestation, and housed three mice per cage in glass metabolism cages. Blood taken at termination by cardiac puncture was deproteinized by filtration prior to HPLC analysis.

[b] Values represent the amount of DGDME and metabolites as nmol/mg of pooled blood from three mice per metabolism cage at 1, 3, 6, 24, and 48 hr (mean ± SD, $n = 4$ groups per time interval). Metabolite VIII was not identified.

[c] Not detected.

* Statistically significant by one-way ANOVA comparison with corresponding 1-hr values ($p < 0.05$).

MA were detected. Other metabolites present in the mouse urine, diglycolic acid (peak III) and MEE (peak IX), together amounted to less than 1% of the administered dose. Additionally, detectable amounts of *N*-(methoxyacetyl)glycine (peak II) and 2ME (peak VI) were present in the urine but not in the blood. The peaks I, IV, and VIII (5 to 7%) remain unidentified. Initially, high levels of DGDME in the blood were detected that decreased rapidly during the first 3-hr period (Table 2). MA in the blood was the major peak for the 3- to 6-hr period and the only radiolabeled peak detected in the blood thereafter.

HPLC analysis of the radioactivity present in embryonic tissue collected at termination revealed a profile of ^{14}C-labeled metabolites similar to that noted for the blood (Table 3). After the initial 3-hr interval the major peak showed an elution time consistent with that of MA. This radioactive fraction was isolated, concentrated by extraction with methylene

TABLE 3

48-hr EMBRYONIC LEVELS OF [^{14}C]DGDME AND METABOLITES IN THE PREGNANT CD-1 MOUSE[a]

HPLC peak	Compound	Embryo concentration (nmol/mg) 1 hr	3 hr	6 hr	24 hr	48 hr
V	MA	0.8 ± 0.1[b]	3.5 ± 0.2*	5.9 ± 0.4*	2.1 ± 1.1*	0.3 ± 0.1*
VIII	—	<0.1	0.1 ± 0.0*	0.3 ± 0.1*	ND[c]	ND
X	DGDME	9.8 ± 0.6	4.6 ± 0.2*	0.3 ± 0.1*	ND	ND

[a] Pregnant female CD-1 mice (31.75 g) were administered 3.73 mmol/kg body wt (20 μCi/kg) of [1,2-*ethylene*-^{14}C]DGDME by gavage on Day 11 of gestation, and housed three per cage in glass metabolism cages. At termination, embryos were pooled by cage, homogenized, and deprotenized by filtration prior to HPLC analysis.

[b] Values represent the amount of DGDME metabolites as nmol/mg of embryo appearing in pooled embryos of three mice per metabolism cage at 1, 3, 6, 24, and 48 hr (mean ± SD), $n = 4$ groups per time interval). Metabolite VIII was not identified.

[c] Not detected.

* Statistically significant by one-way ANOVA comparison with corresponding 1-hr values ($p < 0.05$).

chloride, and derivatized to form the methyl ester. Subsequent GC-MS analysis of this compound revealed that both the GC retention time and mass spectrum were consistent with those determined for the methyl ester of authentic MA. The average amount of ^{14}C present in embryos taken at each time point after administration of DGDME to the dam was determined by scintillation counting, and the amount of MA was calculated. The highest levels, 1.5 ± 2.1 μmol (5.9 μmol/g embryo) for the average embryo, were detected at 6 hr after dosing. Significantly lower amounts of MA were detected in blood taken from the dam at that time point (3.4 μmol/g blood as the acid) than were present in embryonic tissue. The ratio of residual MA present in the embryo to that of the maternal blood was 1.6 ± 0.2 for the time points tested. MA was the only radiolabeled peak detected in either maternal blood or embryonic tissue after the initial 6-hr period following DGDME administration.

DISCUSSION

This study indicates that DGDME is rapidly metabolized in the pregnant CD-1 mouse and is excreted principally in the urine. Two urinary metabolites, MEAA amounting to 63 ± 2% of the administered dose and MA amounting to 28 ± 1% of the administered dose, constitute the major portion of the urinary products. This HPLC profile is similar to that reported previously for DGDME in the rat (Cheever *et al.*, 1988, 1989a). Studies conducted by McGregor and co-workers (1983) showed that rats exposed to both 2ME and DGDME developed reversible sterility during dominant lethal tests on those compounds. These investigators suggested that the toxicity of DGDME could result from central ether cleavage. The occurrence of dealkylation at ether bond sites has been reported by Hutson and Pickering (1971) who found cleavage of the central ether of orally administered 2-(isopropoxy) ethanol in dogs and rats. Lubet *et al.* (1985) further demonstrated the induction of cytochrome(s) P450 enzymes which were capable of *O*-dependentylation of 7-pentoxyphenoxazone. Previous studies in this laboratory have demonstrated that approximately 11% of orally administered 2-ethoxyethanol, a related ethylene glycol ether, undergo *O*-deethylation in the rat (Cheever *et al.*, 1984). Results of the current study indicate that a secondary enzymatic pathway may occur in addition to the major route of metabolism of DGDME. The major urinary metabolite, MEAA, results as a consequence of *O*-demethylation of DGDME to form MEE, which is subsequently oxidized by alcohol and aldehyde dehydrogenases. The second most abundant metabolite, MA, results from cleavage of DGDME at the central ether linkage. This initial cleavage is thought to result in the formation of 2ME, a compound previously shown to be a precursor of MA in laboratory rodents (Miller *et al.*, 1983; Moss *et al.*, 1985). A second possible source of MA would be the central ether cleavage of MEE, a reaction which could be expected to yield one equivalent of both 2ME and ethylene glycol. However, long-term MEE exposure of rats by the inhalation route produced no overt toxicity (Miller *et al.*, 1985). In a recent study of alkoxyethanols using Fisher 344 rats, Medinsky *et al.* (1990) observed that ethylene glycol was a major 24-hr urinary metabolite of 2ME at dosing levels achieved via drinking water that were calculated to range form approximately 0.16 to 1.4 mmol/kg body wt. Ethylene glycol could be further metabolized and may be the source of diglycolic acid, a minor metabolite (peak III) of DGDME observed for mice in this study and previously for Sprague–Dawley rats (Cheever *et al.*, 1988, 1989a).

The MA metabolite became detectable in the urine only after the first hour and appears to be excreted more slowly than MEAA since the percentage of urinary and tissue MA, relative to MEAA, increased with time. Recent work by Clarke *et al.* (1990) indicates that the half-life for MA elimination in the CD-1 mouse is approximately 8 hr. The only detectable radiolabeled compound present in either maternal blood or embryonic tissue fol-

lowing the initial 6-hr period of [^{14}C]DGDME administration was MA. However, the 24- to 48-hr pooled urine collected from the dams contained similar amounts of MA and MEAA. It is presently unclear whether the absence of MEAA from the embryonic tissue may be related to either a "placental barrier," to rapid elimination of that compound from the embryonic tissues and blood, or differing partition coefficients.

The testicular toxicity of DGDME has been attributed by Cheever and co-workers (1988) to its metabolism first to 2ME with subsequent formation of MA. This was supported by experiments which showed that DGDME induced testicular pathology in male Sprague–Dawley rats, but equimolar doses of either MEE or MEAA produced no corresponding toxicity. The teratogenic effects reported for DGDME, however, are not clear-cut. Mouse limb abnormalities were detected in embryos after administration of DGDME between Days 9 to 12 of gestation, with the maximum effects resulting from exposure on Day 11 (Hardin and Eisenmann, 1987) as are those of 2ME (Horton *et al.*, 1985). However, in contrast to male rats, the administration of MEE to pregnant CD-1 mice showed evidence of fetal toxicity resulting in resorption at doses as low as 5.7 mmol/kg/day (Schuler *et al.*, 1984). For pregnant Sprague–Dawley rats given 12.4 mmol/kg/day doses of MEE, the reported teratogenic effects of MEE consisted primarily of rib and cardiovascular malformations, but no paw abnormalities (Hardin *et al.*, 1986). In rats, digit malformations were reported to be a common defect for 2ME or MA *in vivo* as a single 2.5 mmol/kg dose on Day 10 of gestation (Brown *et al.*, 1984) or after 5 mmol/kg of 2ME on Day 12 of gestation (Scott *et al.*, 1987). Further, incubation of rat embryos *in vitro* at MA concentrations as low as 1 mmol produced significant malformations (Yonemoto *et al.*, 1984).

A single dose of 3.73 mmol DGDME/kg body wt, administered to pregnant mice under the conditions employed in the present study, produced paw malformations in the offspring (Hardin and Eisenmann, 1987) similar to those noted for the rat. The formation of MA, either by the dam or in the fetus, has been suggested as the responsible agent for such developmental effects of DGDME (Daniel *et al.*, 1986; Hardin and Eisenmann, 1987). In the current study, significant levels of MA were detected in embryonic tissue at the 3-, 6-, and 24-hr periods. The highest embryonic level of MA *in utero*, detected at 6 hr after dosing, was calculated to be 5.9 mmol/kg body wt. This appears to be an embryo-specific accumulation since the concentrations of MA measured in these embryos after administering a developmentally toxic dose of DGDME were similar to the MA dosing level reported to cause no detectable pathology in adult rats (Miller *et al.*, 1982). For mice, the administration of ^{14}C-labeled 2ME, a DGDME metabolite, at 0.19 mmol/kg resulted in unrestricted transplacental passage of the parent compound and/or metabolites (Sleet *et al.*, 1986). It was noted that ^{14}C in the embryos was always greater than that in the maternal blood, and it was suggested that ^{14}C-labeled 2ME was selectively incorporated into macromolecules by the liver and embryo. Recently, Welsch and co-workers (1990) reported that doses of 2ME on Days 7 and 8 of gestation caused exencephaly in 32% of mouse embryos. These investigators noted, however, that a single 3.3 mmol/kg dose of 2ME on Day 11, a protocol consistent with that used for DGDME in the current study, induced only paw malformations. Significantly, it was shown that both lesions were attenuated by concurrent dosing with acetate or serine, and was suggested that the presence of MA in the embryo during this period may interfere with biosynthesis of purines required for DNA and RNA (Welsch *et al.*, 1987, 1990).

The results of the present study indicate that the metabolism of DGDME in the pregnant mouse is similar to that reported previously for rats in that it proceeds primarily through *O*-demethylation with subsequent oxidation to form MEAA. Additionally, the aprotic glycol ether is subjected to enzymatic hydrolysis of the central ether bond with subsequent formation of MA. This alkoxyacetic acid is pres-

ent in mouse embryonic tissue at concentrations which exceed those of maternal blood, results similar to the embryo/blood distribution of radiolabeled 2ME (the precursor of MA) reported for pregnant mice (Sleet *et al.* 1986). The clearance of MA in the present study appears to confirm the 8-hr half-life reported for MA in CD-1 mice (Clarke *et al.* 1990). However, the half-lives of alkoxyacetic acids in human subjects have been reported to be 2.8-fold greater for ethoxyacetic acid (Groeseneken *et al.* 1986) and 9.6-fold greater for MA (Groeseneken *et al.* 1989). After administration of DGDME, measurable amounts of MA were present in the embryonic tissue throughout the 48-hr period of the present study, and, after 6 hr, MA was the only radiolabeled compound present in that tissue. These results suggest that the distribution of MA, a known developmental toxicant, may be responsible for the teratogenic effects of DGDME. The sensitive embryonic tissue may be at risk during organogenesis at DGDME exposure levels which result in no obvious parental toxicity. The possibility that embryotoxicity may occur with other aprotic glycol ethers capable of similar metabolic activation should be investigated.

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