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Metabolism of Bis(2-methoxyethyl) Ether in the Adult Male Rat: Evaluation of the Principal Metabolite as a Testicular Toxicant[1]

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Metabolism of Bis(2-methoxyethyl) Ether in the Adult Male Rat: Evaluation of the Principal Metabolite as a Testicular Toxicant. CHEEVER, K. L., RICHARDS, D. E., WEIGEL, W. W., LAL, J. B., DINSMORE, A. M., AND DANIEL, F. B. (1988). *Toxicol. Appl. Pharmacol.* **94**, 150–159. The metabolism of the reproductive toxicant bis(2-methoxyethyl) ether was studied in male Sprague–Dawley rats, and the principal metabolite (2-methoxyethoxy)acetic acid and its metabolic precursor 2-(2-methoxyethoxy)ethanol were evaluated separately as testicular toxicants. For the metabolism study, rats were given single po doses of [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether at 5.1 or 0.051 mmol/kg body wt. Within 96 hr, approximately 86 to 90% of the radioactivity was excreted in the urine. Urinary metabolites were separated by high-performance liquid chromatography and isolated for characterization by gas chromatography–mass spectrometry. The principal urinary metabolite, accounting for 67.9 ± 3.3% of the administered high dose and 70.3 ± 1.3% of the low dose, was identified as (2-methoxyethoxy)acetic acid. A second metabolite, representing 6.2 ± 0.8% of the high dose and 5.8 ± 0.8% of the low dose, was identified as methoxyacetic acid, a previously recognized testicular toxicant. In the toxicity study, (2-methoxyethoxy)acetic acid and 2-(2-methoxyethoxy)ethanol were administered to rats at 5.1 mmol/kg body wt by gavage as single daily doses for as many as 20 consecutive days. The testes of rats killed 24 hr after the administration of even numbered doses showed no gross or microscopic abnormalities. These results are in contrast to the previously reported testicular atrophy evoked after as few as 8 daily doses of the parent compound, bis(2-methoxyethyl) ether, tested under the same experimental conditions. Thus, the testicular toxicity reported for bis(2-methoxyethyl) ether could be explained by the presence of a minor metabolite, methoxyacetic acid.

Bis(2-methoxyethyl) ether (diethylene glycol dimethyl ether; diglyme; CAS Reg. No. 111-96-6) is used as an industrial solvent and as a reaction medium for chemical synthesis. This solvent has recently been shown to be a reproductive toxicant in male rats (McGregor *et al.*, 1983; Cheever *et al.*, 1985) and in mice of both sexes (Plasterer *et al.*, 1985). McGregor and his co-workers noted that rats exposed to bis(2-methoxyethyl) ether in a subacute inhalation study developed reversible sterility and dominant lethal effects, the timing of which would indicate damage to the spermatocytes. These researchers showed that the adverse effects of this aprotic solvent are remarkably similar to those occurring in rats after exposure to 2-methoxyethanol, a related glycol ether solvent. Consequently, these investigators suggested that the toxicity of both bis(2-methoxyethyl) ether and 2-methoxyethanol

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may be due to the *in vivo* formation of same metabolite, methoxyacetic acid, a testicular toxicant that had been shown previously to be a metabolic product of 2-methoxyethanol (Miller *et al.*, 1983; Moss *et al.*, 1985). Testicular damage by 2-methoxyethanol itself has been demonstrated, and the early pachytene spermatocytes appear to be the initial site of action of this compound in mice (Nagano *et al.*, 1977, 1979) and in rats (Miller *et al.*, 1981; Foster *et al.*, 1983, 1984; Doe *et al.*, 1984; Chapin *et al.*, 1985a,b). However, the metabolite methoxyacetic acid, not the parent compound, has been shown to be the agent responsible for the observed testicular toxicity of 2-methoxyethanol (Miller *et al.*, 1983; Foster *et al.*, 1983, 1984; Moss *et al.*, 1985).

The objective of this study was to supplement the earlier work of McGregor *et al.* (1983) on the testicular toxicity of bis(2-methoxyethyl) ether by identifying the compound responsible for such toxicity.

METHODS

Chemicals and solutions. The test compound, bis(2-methoxyethyl) ether having a chemical purity of >99.5%, was purchased from Fluka Chemical Corp. (Hauppauge, NY).[2] [1,2-*ethylene*-^{14}C]Bis(2-methoxyethyl) ether, with a specific activity of 0.88 mCi/mmol and a radiochemical purity of greater than 99% by gas–liquid chromatography (GC), was synthesized by Pathfinder Laboratories, Inc. (St. Louis, MO). The radiochemical purity of this radioisotope was verified by high-performance liquid chromatography (HPLC) with radioactivity detection. Commercially available reference compounds corresponding to possible metabolites were obtained at the highest purity. Diglycolic acid (>97%) was purchased from Fluka Chemical Corp. Methoxyacetic acid (99%), 2-(2-methoxyethoxy)ethanol (99%), and 2-methoxyethanol (99%) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). (2-Methoxyethoxy)acetic acid was synthesized by reaction of the sodium salt of 2-methoxyethanol with monochloroacetic acid using the method of Palomaa and Siitonen (1930). The resulting thick oil was dissolved in distilled water, and the pH of the solution was adjusted to pH 14 by addition of 50% (w/w) NaOH solution. The solution was subsequently extracted with methylene chloride to remove any unreacted 2-methoxyethanol. The aqueous solution was then adjusted to pH 1 with concentrated HCl and reextracted with methylene chloride. This extract was concentrated under vacuum using a rotary evaporator (Heidolph, GmbH, West Germany). The chemical purity of the (2-methoxyethoxy)acetic acid (b.p. 121–122°C/4 mm uncorr.; lit., 121–122°C/4 mm, Palomaa and Siitonen (1930)) was found to be 98.2% by gas chromatographic analysis. *N*-Methoxyacetyl glycine methyl ester (b.p. 147.5°C/0.7 mm, uncorr.) was synthesized by reaction of methoxyacetyl chloride with glycine methyl ester using the method of Wolf and Niemann (1963).

A dosing solution was prepared for the metabolism study by dissolving appropriate amounts of [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether and bis(2-methoxyethyl) ether in distilled water to give a final concentration of 1.02 mmol/ml (sp act 29 μCi/mmol). A second dosing solution was prepared by diluting sufficient [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether in distilled water to give a final concentration of 0.0102 mmol/ml (sp act 980 μCi/mmol). Radioactivity was quantified by scintillation spectrometry.

For the toxicity studies, separate dosing solutions of 2-(2-methoxyethoxy)ethanol and (2-methoxyethoxy)acetic acid were prepared in distilled water to give each a final concentration of 1.02 mmol/ml.

Animals, doses, and collections. Male Sprague–Dawley (Crl:CD (SD)BR outbred), cesarean-derived rats, weighing 51 to 75 g, were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA). Immediately upon receipt, these animals were placed in quarantine and maintained on NIH-07 rat and mouse diet (Ziegler Brothers, Inc., Gardners, PA) and tap water *ad libitum*. During this period, a 12-hr light–dark schedule was maintained, with the light cycle beginning at 7:00 AM. Laboratory temperatures ranged from 22 to 24°C, and the relative humidity ranged from 45 to 51%. After 2 weeks, 10 rats, weighing 190–220 g, were transferred to separate Roth-type glass metabolism cages where they had free access to food (hard sticks of NIH-07 diet baked with raw egg binder) except for the 18-hr period immediately prior to treatment, when food was withheld. Distilled water was available at all times. Laboratory air, from which moisture, carbon dioxide, and organic vapors had been removed, was drawn by vacuum through the metabolism chambers at a rate of 500 ml/min. The rats were allowed 3 days to acclimate to the chambers prior to the administration of the test compound between 9 and 10 AM on the fourth day. To each rat, a single po dose of [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether at either 5.1 or 0.051 mmol/kg body wt was administered in a constant 5-ml volume of dosing solution/kg body wt. The radioactivity in the high and low doses amounted to 148 and 50 μCi/kg body wt, respectively.

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

Immediately following the administration of the individual doses, the rats were returned to their cleaned individual metabolism chambers for the collection of exhaled compounds, urine, and feces. The emergent air from each chamber was drawn by vacuum first through a sorbent air-sampling tube containing an 800-mg front section and a 200-mg back section of coconut-based charcoal (SKC, Inc., Eighty-Four, PA) to trap exhaled organic compounds and subsequently through an absorption tower filled with 500 ml of ethanolamine to trap expired carbon dioxide. The air-sampling tubes and the ethanolamine solutions were changed periodically as required to avoid loss of radioactivity. Urine was collected at 6, 12, 24, 48, 72, and 96 hr. Each urine sample was diluted to 10 or 25 ml and immediately passed through an Acrodisc filter having a 0.45-μm pore size (Gelman Sciences, Ann Arbor, MI). Feces were collected at 24, 48, 72, and 96 hr. The air-sampling tubes, urine, and feces were stored at −20°C until analyzed. The rats were killed by asphyxiation with carbon dioxide 96 hr after dosing. The cages were washed successively with water and methanol, and the washes from each cage were collected separately for ^{14}C analysis.

For use in the toxicity study, rats weighing 190–240 g, were randomly assigned to one of three groups of 50 animals each and were housed 5 per cage. All animals within a given group were administered by gavage a daily dose (5 ml/kg body wt) for up to 20 days of 2-(2-methoxyethoxy)ethanol or (2-methoxyethoxy)acetic acid at 5.1 mmol/kg body wt, a molar dose equivalent to that of bis(2-methoxyethyl) ether previously shown to produce testicular atrophy. During this period, no overt signs of toxicity or early deaths were noted. Animals in each treatment group were killed in groups of five at 2-day intervals on Days 3 through 21 for evaluation of any histopathological changes in the testes. At necropsy, animals were anesthetized with secobarbital sodium and killed by exsanguination. The testes were excised, weighed separately, and fixed in Bouin's solution for 24 hr. The testes were then rinsed with 70% ethanol and embedded in paraffin wax. The tissues were sectioned at 5 μm and stained with periodic acid–Schiff stain for demonstration of the spermatid acrosome or hematoxylin and eosin for general morphological examination by light microscopy.

Determination of radioactivity. Aliquots (100 μl) of the diluted urine samples were added to liquid scintillation vials containing 3 ml of methanol and 10 ml of scintillation medium (Scinti-Verse II, Fisher Scientific Co., Fairlawn, NJ) for the determination of ^{14}C. Aliquots (1 ml) of the cage washes were dissolved in 10 ml of the same scintillation medium. Front and back sections of charcoal from each air-sampling tube were desorbed in separate 5-ml quantities of bis(2-methoxyethyl) ether, a solvent found to desorb greater than 98% of [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether adsorbed in amounts typical of those collected from the emergent chamber air. A 1-ml aliquot of each solution was transferred to a liquid scintillation vial containing 10 ml of the scintillation medium. The carcass digests were homogenized separately with a Polytron Model PCU-1 homogenizer equipped with a PC-10 probe (Kinematica GmbH, Luzern, Switzerland), and 1-ml aliquots were analyzed in the scintillation medium. Aliquots (1-ml) of ethanolamine were prepared for scintillation counting in Permafluor V (Packard Instrument Co., Downers Grove, IL). Feces, while still frozen, were ground with an equal weight of microcrystalline cellulose (E. Merck, Darmstadt, West Germany) in a Model A10 microanalytical mill (Janke & Kunkel GmbH, West Germany) until a homogenous mixture was obtained. Weighed portions (0.5–1.0 g) of feces–cellulose mixtures were burned in a sample oxidizer (Tri-Carb Model B306, Packard Instrument Co.). The $^{14}CO_2$ from the combustion was absorbed in 6 ml of Carbo-Sorb (Packard Instrument Co.) and analyzed in 12 ml of Permafluor V.

The radioactivity in the samples was measured in a model LS8100 liquid scintillation spectrometer (Beckman Instruments Co., Fullerton, CA). Counting efficiencies were determined by the external standard method of Horrocks (1977).

Separation, quantification, and isolation of urinary metabolites. Prior to analyzing the urine samples by HPLC, suitable conditions for the separation of compounds representing possible metabolites of bis(2-methoxyethyl) ether were established. The HPLC system consisted of a Model ALC/GPC 201 liquid chromatograph equipped with two Model 6000A pumps controlled by a Model 720 system controller (Waters Associates, Inc., Milford, MA). Samples were introduced by a Waters WISP 710A automatic sample injector module, and eluted compounds were detected by a Model 1040A spectrophotometric detector (Hewlett–Packard Co., Palo Alto, CA) set at 210 nm or by a Tri-Carb RAM 7500 radioactivity monitor equipped with a 50-μl RAM 754 heterogeneous flow cell (Packard Instrument Co.). Better than base-line resolution for all compounds was achieved using a 50-cm-long × 9-mm-i.d. stainless-steel column packed with 10-μm Partisil-10 OD-2 (Whatman, Inc., Clifton, NJ) in series with a 15-cm-long × 3.9-mm-i.d. stainless-steel column packed with 5-μm spherical NOVA-PAK C18 (Waters Associates, Inc.). The column temperature was maintained at 30°C using a column heater module and an associated temperature control module (Waters Associates, Inc.). Radioactive urinary components were chromatographed with 1% acetic acid in water isocratically for 20 min, then with a methanol: 1% acetic acid solvent system programmed from 15 to 80% methanol using a linear gradient over a 40-min period, and finally with methanol:1% acetic acid (80:20) for 15 min. The flow rate was maintained at 1 ml/min.

Each urine sample was thawed, and a 3-ml portion was acidified to pH 2 with H_2SO_4. Aliquots (1 ml) of the acidified urine samples were injected into the HPLC, and ra-

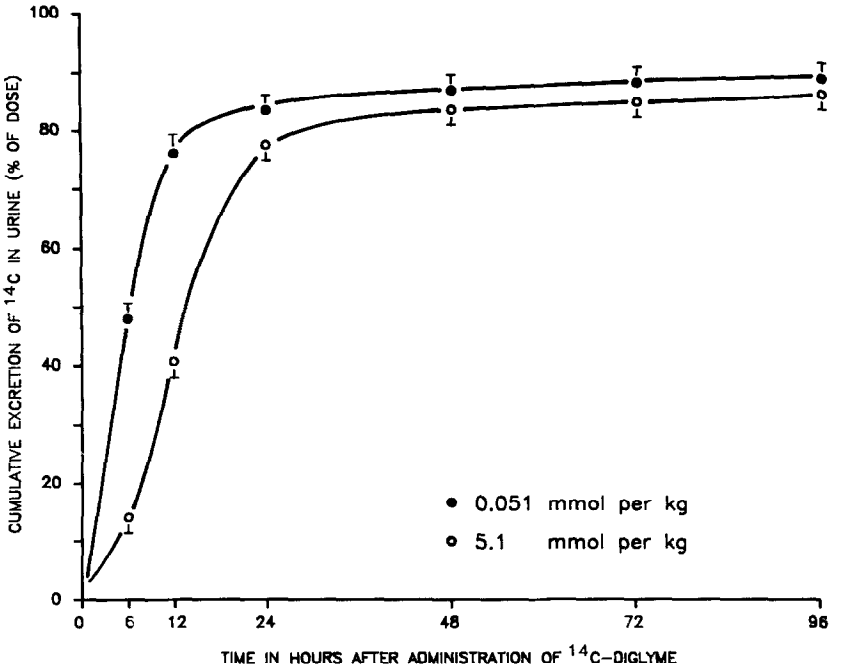

FIG. 1. Cumulative 96-hr urinary ^{14}C excretion after oral administration of radiolabeled bis(2-methoxyethyl) ether at 5.1 or 0.051 mmol/kg body wt in the adult male rat.

dioactive components were detected and quantified by the radioactivity monitor. The retention time of each radioactive component was recorded, and the radioactive fractions were collected separately using a Model 2103 fraction collector (ISCO, Lincoln, NE) for further characterization of the radioactive compounds.

Mass spectral identification of urinary metabolites. Each radioactive fraction of urine, collected from the HPLC column, was lyophilized using a Model 75035 freeze-dryer (Labconco Corp., Kansas City, MO), and the residue was redissolved in a small amount of methanol for analysis by gas chromatography–mass spectrometry (GC–MS). The methyl esters of organic acids were prepared by reaction with *N,N*-dimethylformamide dimethyl acetal (Methyl-8 concentrate, Pierce Chemical Co., Rockford, IL) in accordance with the general method of Thenot *et al.* (1972). A Model 5890A gas chromatograph (Hewlett-Packard Co., Avondale, PA), fitted with a 50-m × 0.2-mm-i.d. Ultra-1 (d_f = 0.33 μm) fused silica capillary column (Hewlett-Packard Co., Avondale, PA), was coupled by direct capillary interface with a Model 5970B quadrupole mass spectrometer (Hewlett-Packard Co., Palo Alto, CA). Helium was used as the carrier gas at a linear velocity of 38 cm/sec at 200°C. Splitless injections were made with the column at 10°C. This 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 a source temperature of 200°C and were stored and processed with a Model 59970 MS ChemStation (Hewlett-Packard Co., Palo Alto, CA). Mass spectrometric identifications were made by comparison of the mass spectra of the metabolites with those of authentic compounds.

Radioactive constituent of breath. Volatile organic compounds expired by two rats during the 24-hr period immediately following the administration of bis(2-methoxyethyl) ether at 5.1 mmol/kg body wt were collected using a single, charcoal air-sampling tube for each rat. The charcoal sections from these sampling tubes were combined and subsequently desorbed for 24 hr with 10 ml of methanol. Aliquots of the methanol solutions were analyzed by HPLC and GC–MS to characterize any radioactive component eliminated in the breath.

Statistical analysis. Statistical differences between group means were determined using one-way analysis of variance. The level of significance chosen was $p < 0.05$.

RESULTS

Excretion of Radioactivity

In rats given a single po dose of [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether, the elimi-

nation of the administered radioactivity occurred principally through the kidneys. The recovery of the administered ^{14}C directly from this route over 96 hr was 85.7 ± 1.7% for rats treated with the high dose (5.1 mmol/kg) and 88.8 ± 1.4% for the low dose (0.051 mmol/kg). When the amounts of ^{14}C in the cage washes—radioactivity considered to be from residual urine—were included, the total recoveries from the urinary route were 86.5 ± 1.6% for the high dose and 90.0 ± 1.2% for the low dose. The elimination of radioactivity in the urine was rapid with 77.5 ± 3.1% of the high dose and 83.1 ± 2.7% of the low dose excreted over the first 24 hr. Only an additional 9.0% of the high-dose ^{14}C and 6.9% of the low-dose ^{14}C were excreted during the ensuing 72 hr (Fig. 1). Respired $^{14}CO_2$ collected from rats receiving either 5.1 or 0.051 mmol/kg body wt doses of bis(2-methoxyethyl) ether was negligible, amounting to only 1.4 ± 0.1 and 3.6 ± 0.3% of the administered dose, respectively. Only trace amounts of ^{14}C were found in the breath as volatile organics, totaling 1.2 ± 0.1% of the administered high dose and 0.4 ± 0.1% of the low dose. Less than 5% of the administered ^{14}C was excreted in the feces. At 96 hr, the elimination of ^{14}C was nearly complete with less than 2.5% of the dose remaining in the carcasses. These results are summarized in Table 1.

TABLE 1

TOTAL 96-hr RECOVERY OF ^{14}C

Sample	% of administered bis(2-methoxyethyl) ether	
	Low dose[a]	High dose[b]
Total urinary	90.0 ± 1.2	86.5 ± 1.6
Urine	88.8 ± 1.4	85.7 ± 1.7
Cage wash	1.2 ± 0.3	0.8 ± 0.1
Carbon dioxide	3.6 ± 0.3	1.4 ± 0.1[c]
Volatile organic	0.4 ± 0.1	1.2 ± 0.1[c]
Feces	2.9 ± 0.6	4.6 ± 1.0[c]
Carcass	1.7 ± 0.2	2.2 ± 0.4
Total recovery	98.6 ± 0.8	95.9 ± 1.3

[a] Male Sprague–Dawley rats (190–220 g) received 0.051 mmol/kg body wt doses of [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether (sp act 980 μCi/mmol) (mean ± SE, $n = 5$).

[b] Male Sprague–Dawley rats (190–220 g) received 5.1 mmol/kg body wt doses of [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether (sp act 29 μCi/mmol) (mean ± SE, $n = 5$).

[c] Statistically significant at $p < 0.05$ by comparison with corresponding low-dose value.

Identification of Urinary Metabolites

Radioactive compounds present in each urine sample collected over the 96-hr period immediately following the administration of a single po dose of [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether at 5.1 or 0.051 mmol/kg were separated by HPLC and quantified by liquid scintillation spectrometry. An HPLC composite profile of radioactive components in the urine was constructed for each dose level. These qualitatively identical profiles contained 10 compounds, which were designated by their order of elution from the HPLC column as I through X. Each radioactive HPLC fraction was collected separately, lyophylized, and redissolved in methanol for further characterization by GC–MS. Metabolites tentatively identified as organic acids by their characteristic GC peak shape and by their mass spectra were rechromatographed as their methyl ester derivatives. The chromatographic retention times of the radioactive urinary components and those of certain corresponding authentic compounds are shown in Table 2. The identities of compounds II, III, V through VII, IX, and X were established by matching their GC retention times and mass spectra with those of authentic compounds. The electron impact mass spectra of the principal metabolite, (2-methoxyethoxy)acetic acid (Fig. 2), and methoxyacetic acid (Fig. 3) are shown along with spectra of corresponding reference compounds. The three remaining metabolites (I, IV, and VIII), representing a total of less than 3% of the administered dose, were not identified.

TABLE 2

HPLC AND GC COMPARISON OF BIS(2-METHOXYETHYL) ETHER URINARY METABOLITES AND CERTAIN REFERENCE COMPOUNDS

Compound	HPLC RT[a] (min)	GC RT[b] (min)
I	30.6 ± 0.1	—
II	32.2 ± 0.1	28.4
N-(Methoxyacetyl)glycine	32.2 ± 0.1	28.4
III	46.5 ± 0.1	25.1
Diglycolic acid	46.1 ± 0.1	25.1
IV	47.4 ± 0.1	—
V	48.5 ± 0.1	15.5
Methoxyacetic acid	48.2 ± 0.1	15.5
VI	50.1 ± 0.1	12.4
2-Methoxyethanol	50.4 ± 0.1	12.4
VII	54.2 ± 0.1	22.9
(2-Methoxyethoxy)acetic acid	54.3 ± 0.1	22.9
VIII	57.3 ± 0.1	—
IX	61.7 ± 0.1	20.2
2-(2-Methoxyethoxy)ethanol	61.7 ± 0.2	20.2
X	66.3 ± 0.0	20.6
Bis(2-methoxyethyl) ether	66.2 ± 0.0	20.6

[a] HPLC retention time was obtained using a 50-cm-long × 9.0-mm-i.d. stainless-steel column packed with 10-μm particle size Partisil OD-2 in series with a 15-cm-long × 3.9-mm-i.d. stainless-steel column packed with 5-μm spherical NOVA-PAK C18. Column temperature was maintained at 30°C. Elution was effected using 1% acetic acid for 20 min and was followed by a 40-min linear solvent gradient program from 15 to 80% methanol: 1% acetic acid (mean ± SE, $n = 5$).

[b] GC retention time was obtained using a 50-m-long × 0.20-mm-i.d. Ultra-1 ($d_f = 0.33$ μm) fused silica column. Oven temperature was programmed from 10 to 200°C at 8°C/min with 38.5 cm/sec helium linear velocity at 200°C. Carboxylic acids were analyzed as the corresponding methyl esters.

Although the HPLC composite profiles of urinary components from rats given the high and low doses were qualitatively identical, the percentages of certain components were related to the dose. Based on these percentages, the amounts of metabolites I and VIII and unchanged bis(2-methoxyethyl) ether excreted in the urine were significantly greater for the rats given the high dose, whereas the amount of compound IV was significantly lower as shown in Table 3.

Identification of the Radioactive Component in Expired Air

Volatile organic compounds collected from the breath were desorbed from the charcoal sampling tubes with methanol and analyzed by HPLC. The single radioactive component gave a retention time identical to that of authentic bis(2-methoxyethyl) ether. The identity of this compound was confirmed by matching the GC retention time and mass spectrum with those of bis(2-methoxyethyl) ether.

Histopathology

Examination of the testes of rats given as many as 20 consecutive daily doses of either the primary metabolite, (2-methoxyethoxy)acetic acid, or its metabolic precursor, 2-(2-methoxyethoxy)ethanol, at 5.1 mmol/kg body wt, indicated that no degenerative changes had occurred.

DISCUSSION

The results of this study indicate that the metabolism of bis(2-methoxyethyl) ether in the rat proceeds primarily through O-demethylation with subsequent oxidation to form (2-methoxyethoxy)acetic acid. Such results are consistent with the metabolism shown to occur in the rat with certain related glycol ether solvents. Miller and his co-workers (1983) reported that O-demethylation of two such compounds, 1-methoxy-2-propanol and 2-methoxyethanol, accounted for 63 and 12%, respectively, of their metabolism in the rat. A secondary metabolic pathway for bis(2-methoxyethyl) ether involves the enzy-

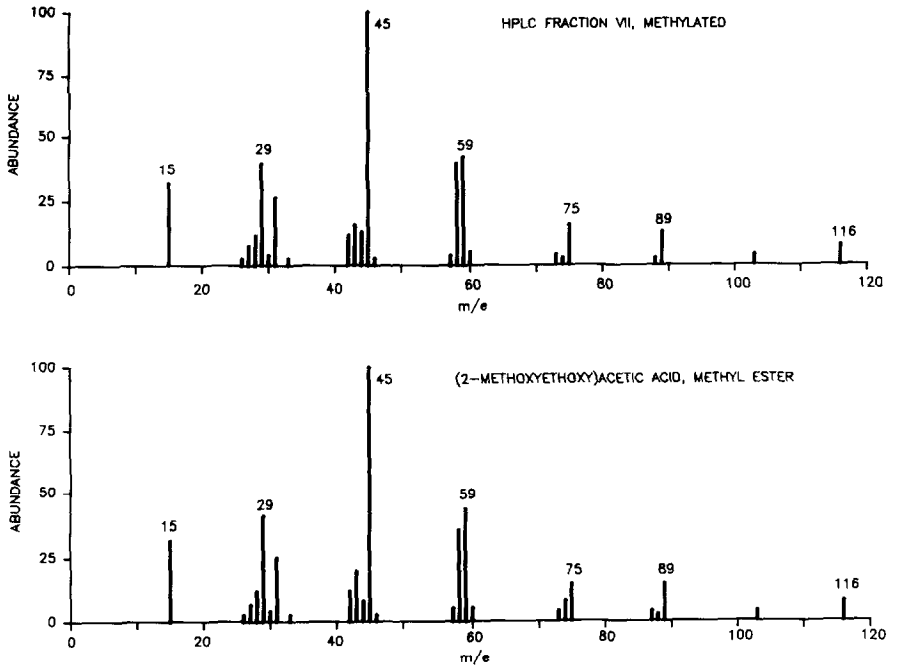

FIG. 2. Comparison of mass spectra of metabolite VII (top) and (2-methoxyethoxy)acetic acid (bottom) isolated from rat urine after administration of radiolabeled bis(2-methoxyethyl) ether at 5.1 or 0.051 mmol/kg body wt.

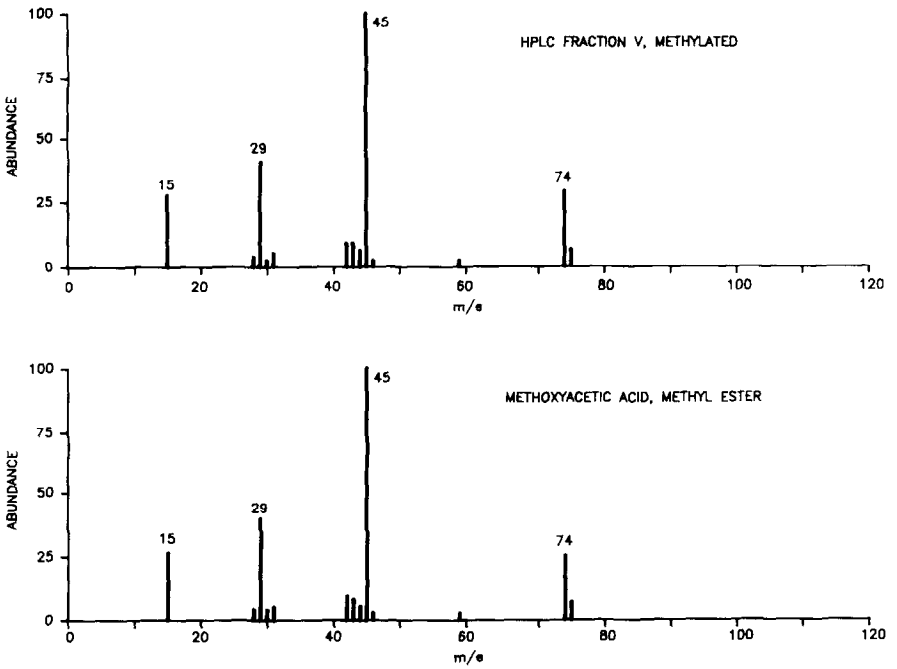

FIG. 3. Comparison of mass spectra of metabolite V (top) and methoxyacetic acid (bottom) isolated from rat urine after administration of radiolabeled bis(2-methoxyethyl) ether at 5.1 or 0.051 mmol/kg body wt.

TABLE 3

BIS(2-METHOXYETHYL) ETHER 96-hr URINARY METABOLIC PROFILE

Compound	Low dose[a]	High dose[b]
Metabolite I	<0.1[c]	0.3 ± 0.1[d]
N-(Methoxyacetyl)glycine	0.1 ± 0.1	0.3 ± 0.2
Diglycolic acid	6.7 ± 1.0	3.9 ± 1.0
Metabolite IV	2.5 ± 0.4	1.0 ± 0.3[d]
Methoxyacetic acid	5.8 ± 0.8	6.2 ± 0.8
2-Methoxyethanol	2.2 ± 1.1	0.8 ± 0.3
(2-Methoxyethoxy)acetic acid	70.3 ± 1.3	67.9 ± 3.3
Metabolite VIII	0.4 ± 0.1	1.2 ± 0.3[d]
2-(2-Methoxyethoxy)ethanol	0.3 ± 0.2	<0.1
Bis(2-methoxyethyl) ether	0.4 ± 0.1	1.8 ± 0.3[d]
Total urinary recovery	88.7 ± 1.6	83.4 ± 3.3

[a] Male Sprague–Dawley rats (190–220 g) received 0.051 mmol/kg body wt doses of [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether (sp act 980 μCi/mmol) (mean ± SE, $n = 5$).

[b] Male Sprague–Dawley rats (190–220 g) received 5.1 mmol/kg body wt doses of [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether (sp act 29 μCi/mmol) (mean ± SE, $n = 5$).

[c] Urinary 96-hr excretion of metabolites calculated as a percentage of dose.

[d] Statistically significant at $p < 0.05$ by comparison with corresponding low-dose metabolite value.

matic hydrolysis of the central ether bond to form 2-methoxyethanol which, in part, undergoes oxidation to yield methoxyacetic acid. Subsequent glycine conjugation of methoxyacetic acid is shown, a reaction suggested by Miller and his co-workers (1983). The possibility of such enzymatic hydrolysis of the central ether bond was suggested by McGregor *et al.* (1983), who evaluated the reproductive toxicity of this compound. That research group noted that the effects of bis(2-methoxyethyl) ether, an aprotic solvent, and those of 2-methoxyethanol, a primary alcohol, were remarkably similar and stated that methoxyacetic acid, a known testicular toxicant, may be a common metabolite of these two glycol ethers.

At both dosing levels used in this study, the major portion of the administered radioactivity was excreted in the urine. After the administration of these doses 0.4 to 1.8% of the urinary radioactivity was present as unchanged bis(2-methoxyethyl) ether. The metabolism of [1,2-*ethylene*-^{14}C]bis(2-methoxyethyl) ether in the rat is complex, as evidenced by the number of radiolabeled urinary components detected by HPLC. The urinary metabolites, (2-methoxyethoxy)acetic acid and methoxyacetic acid, together accounted for 76.1 to 74.1% of the radiolabel. Minor metabolites present in the urine represent other products of O-demethylation or hydrolysis of the central ether bond of bis(2-methoxyethyl) ether. These compounds were identified as diglycolic acid, 2-(2-methoxyethoxy)ethanol, 2-methoxyethanol, and *N*-(methoxyacetyl)glycine. *N*-(Methoxyacetyl)-glycine has previously been identified as a product of 2-methoxyethanol metabolism in the rat (Moss *et al.*, 1985). The precursor of diglycolic acid has not been established for mammals; however, Prochazka *et al.* (1966) noted that certain glycol ethers were utilized as the sole carbon source by TEG-5 bacteria, and suggested that O-demethylation of 2-(2-methoxyethoxy)ethanol by these bacteria in the formation of diethylene glycol is a possibility. Hrotmatka and Polesofsky (1962), using *Acetobacter suboxydans,* identified β-hydroxyethoxyacetic acid and diglycolic acid as products of diethylene glycol oxidation. Although these intermediates have not been identified for the rat, the presence of diglycolic acid in the urine strongly suggests that the same route of metabolism occurs. The central ether linkage of bis(2-methoxyethyl) ether is cleaved *in vivo* in the rat to the extent of at least 7.3% after administration at 5.1 mmol/kg body wt and 8.1% after administration at 0.051 mmol/kg body wt. Of the respective high and low doses, 0.8 and 2.2% are excreted as methoxyethanol and 6.2 and 5.8% are oxidized to methoxyacetic acid. Conjugation of methoxyacetic acid from the high dose proceeds to the extent of 0.3%. A small but statistically significant dose dependence in the metabolism of bis(2-methoxyethyl) ether is indicated by examination of the high- and low-dose metabolite profiles. At the high-dose level, a significantly greater percentage of the administered radioactivity was

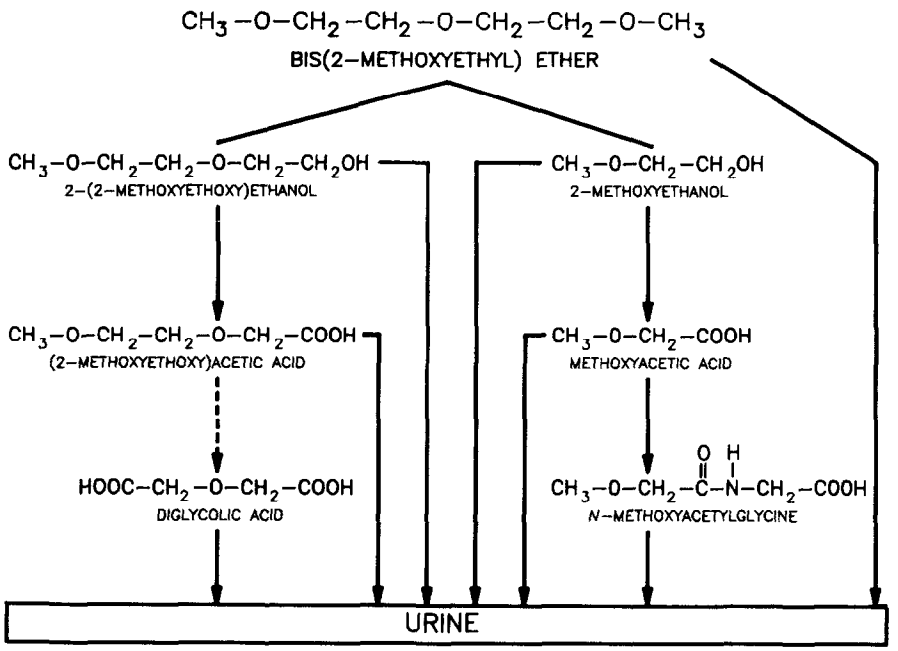

FIG. 4. Postulated pathway for the metabolism of bis(2-methoxyethyl) ether in the adult male rat.

excreted in the urine as metabolites I and VIII and unchanged bis(2-methoxyethyl) ether, whereas a lower percentage was excreted as metabolite IV. Based on the compounds identified in this study, a general scheme of metabolism is proposed for bis(2-methoxyethyl) ether (Fig. 4).

The principal metabolite, (2-methoxyethoxy)acetic acid, and its precursor, 2-(2-methoxyethoxy)ethanol, were shown not to cause changes in the testes of rats. These observations are in marked contrast with the degenerative changes which occurred in the testes of rats after treatment with as few as 8 daily doses of bis(2-methoxyethyl) ether at the same molar dose (Cheever *et al.*, 1985). However, the cumulative formation of methoxyacetic acid after dosing with 5.1 mmol/kg of bis(2-methoxyethyl) ether is consistent with the amount of that compound reported to cause testicular atrophy (Miller *et al.*, 1982). Although (2-methoxyethoxy)acetic acid has not been evaluated previously as a testicular toxicant, 2-(2-methoxyethoxy)ethanol given in the drinking water of mice (Nagano *et al.*, 1984) and by inhalation in the rat (Miller *et al.*, 1985) resulted in negative toxicity. This absence of testicular toxicity noted for both 2-(2-methoxyethoxy)ethanol and (2-methoxyethoxy)-acetic acid indicates that the toxicant is not derived from the primary O-demethylation pathway of metabolism and provides support for the proposed metabolism scheme.

Of the metabolites of bis(2-methoxyethyl) ether identified in the present study, only two, 2-methoxyethanol and methoxyacetic acid, have been reported to be potent testicular toxicants in rats (Miller *et al.*, 1981, 1982; Foster *et al.*, 1983). However, inhibition of the alcohol dehydrogenase-mediated metabolism of 2-methoxyethanol in rats by pretreatment with pyrazole, which blocked the formation of methoxyacetic acid, has been shown to eliminate the testicular toxicity of 2-methoxyethanol (Foster *et al.*, 1984). Thus, the reproductive toxicity reported for bis(2-methoxyethyl) ether can be accounted for by effects of the methoxyacetic acid formed.

REFERENCES

CHAPIN, R. E., DUTTON, S. L., ROSS, M. D., AND LAMB, J. C., IV (1985a). Effects of ethylene glycol monomethyl ether (EGME) on mating performance and epididymal sperm parameters in F344 rats. *Fundam. Appl. Toxicol.* **5,** 182–189.

CHAPIN, R. E., DUTTON, S. L., ROSS, M. D., SWAISGOOD, R. R., AND LAMB, J. C., IV (1985b). The recovery of the testis over 8 weeks after short-term dosing with ethylene glycol monomethyl ether: Histology, cell-specific enzymes, and rete testis fluid protein. *Fundam. Appl. Toxicol.* **5,** 515–525.

CHEEVER, K. L., PLOTNICK, H. B., RICHARDS, D. E., AND WEIGEL, W. W. (1984). Metabolism and excretion of 2-ethoxyethanol in the adult male rat. *Environ. Health Perspect.* **57,** 241–248.

CHEEVER, K. L., WEIGEL, W. W., RICHARDS, D. E., LAL, J. B., AND PLOTNICK, H. B. (1985). Testicular effects of bis(2-methoxyethyl)ether in the adult male rat: Equimolar dose comparison with 2-methoxyethanol and 2-ethoxyethanol. *Toxicologist* **5,** 1, 140.

DOE, J. E. (1984). Further studies on the toxicology of the glycol ethers with emphasis on rapid screening and hazard assessment. *Environ. Health Perspect.* **57,** 199–206.

DOE, J. E., SAMUELS, D. M., TINSTON, D. J., AND DE SILVA WICKRAMARATNE, G. A. (1983). Comparative aspects of the reproductive toxicology by inhalation in rats of ethylene glycol monomethyl ether and propylene glycol monomethyl ether. *Toxicol. Appl. Pharmacol.* **69,** 43–47.

FOSTER, P. M. D., CREASY, D. M., FOSTER, J. R., AND GRAY, T. J. B. (1984). Testicular toxicity produced by ethylene glycol monomethyl and monoethyl ethers in the rat. *Environ. Health Perspect.* **57,** 207–217.

FOSTER, P. M. D., CREASY, D. M., FOSTER, J. R., THOMAS, L. V., COOK, M. W., AND GANGOLLI, S. D. (1983). Testicular toxicity of ethylene glycol monomethyl and monoethyl ethers in the rat. *Toxicol. Appl. Pharmacol.* **69,** 385–399.

GRAY, T. J. B., MOSS, E. J., CREASY, D. M., AND GANGOLLI, S. D. (1985). Studies on the toxicity of some glycol ethers and alkoxyacetic acids in primary testicular cell cultures. *Toxicol. Appl. Pharmacol.* **79,** 490–501.

HORROCKS, D. L. (1974). *Applications of Liquid Scintillation Counting,* pp. 72–74. Academic Press, New York.

HROTMATKA, O., AND POLESOFSKY, W. (1962). Untersuchungen über die Essiggärung. VII. Über die Oxydation verchiedener primärer Alkohole und Glykole. *Enzymologia* **24,** 372–384.

MCGREGOR, D. B., WILLINS, M. J., MCDONALD, P., HOLMSTRÖM, M., MCDONALD, D., AND NIEMEIER, R. W. (1983). Genetic effects of 2-methoxyethanol and bis(2-methoxyethyl) ether. *Toxicol. Appl. Pharmacol.* **70,** 303–316.

MILLER, R. R., AYRES, J. A., CALHOUN, L. L., YOUNG, J. T., AND MCKENNA, M. J. (1981). Comparative short-term inhalation toxicity of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in rats and mice. *Toxicol. Appl. Pharmacol.* **61,** 368–377.

MILLER, R. R., CARREON, R. E., YOUNG, J. T., AND MCKENNA, M. J. (1982). Toxicity of methoxyacetic acid in rats. *Fundam. Appl. Toxicol.* **2,** 158–160.

MILLER, R. R., EISENBRANDT, D. L., GUSHOW, T. S., AND WEISS, S. K. (1985). Diethylene glycol monomethyl ether 13-week vapor inhalation toxicity study in rats. *Fundam. Appl. Toxicol.* **5,** 1174–1179.

MILLER, R. R., HERMANN, E. A., LANGVARDT, P. W., MCKENNA, M. J., AND SCHWETZ, B. A. (1983). Comparative metabolism and disposition of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in male rats. *Toxicol. Appl. Pharmacol.* **67,** 229–237.

MOSS, E. J., THOMAS, L. V., COOK, M. W., WALTERS, D. G., FOSTER, P. M. D., CREASY, D. M., AND GRAY, T. J. B. (1985). The role of metabolism in 2-methoxyethanol-induced testicular toxicity. *Toxicol. Appl. Pharmacol.* **79,** 480–489.

NAGANO, K., NAKAYAMA, E., ADACHI, H., AND YAMADA, T. (1977). [Testis disorders from Cellosolve derivatives.] *Rodo Eisei* **18,** 24–27.

NAGANO, K., NAKAYAMA, E., KOYANO, M., OOBAYASHI, H., ADACHI, H., AND YAMADA, T. (1979). [Testicular atrophy of mice induced by ethylene glycol mono alkyl ethers]. *Sangyo Igaku* **21,** 29–35.

NAGANO, K., NAKAYAMA, E., OOBAYASHI, H., NISHIZAWA, T., OKUDA, H., AND YAMAZAKI, K. (1984). Experimental studies on toxicity of ethylene glycol alkyl ethers in Japan. *Environ. Health Perspect.* **57,** 75–84.

PALOMAA, M. H., AND SIITONEN, T. A. (1930). Studien über äther-artige Verbindungen. III Polyäther-säuren vom Typus R. O. $[CH_2 \cdot CH_2 \cdot O]_n \cdot CH_2 \cdot COOH$. *Ber. Dtsch. Chem. Ges. B* **63,** 3117–3120.

PLASTERER, M. R., BRADSHAW, W. S., BOOTH, G. M., CARTER, M. W., SCHULER, R. L., AND HARDIN, B. D. (1985). Developmental toxicity of nine selected compounds following prenatal exposure in the mouse: Naphthalene, *p*-nitrophenol, sodium selenite, dimethyl phthalate, ethylenethiourea, and four glycol ether derivatives. *J. Toxicol. Environ. Health* **15,** 25–38.

PROCHAZKA, G. J., MAYBERRY, W. R., AND PAYNE, W. J. (1966). Model systems for studying energy yields from synthetic organic compounds. *Dev. Ind. Microbiol.* **8,** 167–178.

THENOT, J-P., HORNING, E. C., STAFFORD, M., AND HORNING, M. G. (1972). Fatty acid esterification with *N,N*-dimethylformamide dialkyl acetals for GC analysis. *Anal. Lett.* **5,** 217–223.

WOLF, J. P., III, AND NIEMANN, C. (1963). The α-chymotrypsin catalyzed hydrolysis of a series of acylated glycine methyl esters. *Biochemistry* **2,** 82–90.