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Genetic Effects of 2-Methoxyethanol and Bis(2-methoxyethyl)ether[1]

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Genetic Effects of 2-Methoxyethanol and Bis(2-methoxyethyl)ether. McGREGOR, D. B., WILLINS, M. J., McDONALD, P., HOLMSTRÖM, M., McDONALD, D., AND NIEMEIER, R. W. (1983). *Toxicol. Appl. Pharmacol.* **70**, 303–316. 2-Methoxyethanol and bis(2-methoxyethyl)ether were subjected to the following assays for genetic toxicity: Ames' test, unscheduled DNA synthesis (UDS) assay in human embryo fibroblasts, sex-linked recessive lethal (SLRL) test in Drosophila, dominant lethal test in male rats, bone marrow metaphase analysis in male and female rats, and the sperm abnormality test in mice. *In vivo* test animals were exposed to atmospheric concentrations of 25 or 500 ppm 2-methoxyethanol and 250 or 1000 ppm bis(2-methoxyethyl)ether. Point mutations in Ames' test and UDS in fibroblasts were not increased by either compound, while the SLRL test gave ambiguous results which deserve further investigation. Chromosomal aberration frequencies were not increased in rat bone marrow, but there was evidence from the dominant lethal tests that both compounds have profound effects upon male rat fertility during the meiotic phase. Pregnancy frequency was greatly reduced and preimplantation losses were large. In addition, there was evidence of postimplantation losses. Sperm abnormalities were increased in mice exposed to both compounds, but particularly bis(2-methoxyethyl)ether. These effects on male reproductive cells were confined to the higher concentrations of both compounds. It was concluded that the weak mutagenic and particularly the strong antifertility effects described here are important for the safety evaluation of these ethylene glycol ethers.

2-Methoxyethanol (CAS No. 109-86-4) is the lowest member of the series of monoalkylethers of ethylene glycol. These compounds, which bear the common trade name Cellosolves, are extensively used as solvents. Bis(2-methoxyethyl)ether (CAS No. 111-96-6) or diglyme is the condensation product of two 2-methoxyethanol molecules and is also a solvent which is particularly useful in the reaction medium for Grignard and similar syntheses.

2-Methoxyethanol exposure occurs commonly in the chemical, printing, publishing, paper, electronics, mining, and furniture industries. The diverse uses of this chemical include the following: (1) anti-icing additive in military jet aircraft, (2) paint stripping (along with dichloromethane, a known mutagen), (3) solvent for varnishes, wood stains, and enamels, (4) solvent for low-viscosity cellulose acetate, natural resins, some synthetic resins, and some alcohol-soluble dyes. It can also be found in nail varnishes and was used in some acne preparations, prior to the 1979 FDA recall of benzoyl peroxide-containing acne preparations in which 2-methoxyethanol was used as the solvent. Methoxyethanol is readily absorbed through the skin, most high level exposures of man apparently involving skin contact (Ohi and Wegman, 1978).

[1] Mention of company names or products does not constitute endorsement by the National Institute for Occupational Safety and Health.

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No published data concerning either the pharmacology or toxicology of bis(2-methoxyethyl)ether have come to our attention but the toxicology of 2-methoxyethanol has been reviewed recently (Rowe and Wolf, 1982). Of particular relevance to the present study, 2-methoxyethanol has been shown to induce testicular damage, in dogs and rabbits, manifest as desquamation of the spermatic epithelium and the formation of variable numbers of spermatid giant cells (Wiley *et al.*, 1938). More recent studies with mice (Nagano *et al.*, 1979) support these findings.

Ethylene glycol at oral doses up to 4000 mg/kg for 4 weeks had no significant effect upon testicular weight, whereas 2-methoxyethanol at only 250 mg/kg did reduce significantly testicular weight. In this latter group, spermatozoa and spermatids were seen in small numbers only in some of the tubules and there was a clear reduction in spermatocytes. The degenerative changes seen were even more obvious in higher dose groups, so that, in the 2000 mg/kg group, only Sertoli's cells were observed in the tubules, no germ cells whatsoever being present. Such testicular atrophy was not noticed in mice dosed with ethylene glycol. 2-Methoxyethanol also has embryotoxic effects, when given orally to mice, even at dose levels to the pregnant mice as low as 31 mg/kg. Effects seem to be mainly upon skeletal development, only mild toxicity being observed in the dams (Nagano *et al.*, 1981).

Recent work on the metabolism of ethylene glycol ethers has indicated two main pathways: a secondary alcohol such as 1-methoxy-2-propanol can be *O*-demethylated, whereas a primary alcohol such as 2-methoxyethanol can be oxidized by alcohol and aldehyde dehydrogenases to the corresponding acid (Miller *et al.*, 1983). Approximately 50 to 60% of an oral dose of 2-methoxyethanol to male rats appears as methoxyacetic acid in the urine within 48 hr. Miller *et al.* (1982) also demonstrated the remarkable similarities in the toxicity profiles of 2-methoxyethanol and methoxyacetic acid. This metabolic pathway should be considered, therefore, when examining the mutagenic potential of 2-methoxyethanol.

The work described in this paper involves the testing of 2-methoxyethanol and bis(2-methoxyethyl)ether in various systems for mutagenic potential in order that the risks associated with human exposure to these compounds can be better evaluated. The atmospheric concentrations used were chosen on the basis of current U.S. OSHA Permissible Exposure Levels and published toxicity values by the National Institute for Occupational Safety and Health (NIOSH).

METHODS

Chemicals. 2-Methoxyethanol (Batch No. 10632), bis(2-methoxyethyl)ether (Batch No. 21150), and methoxyacetic acid (Batch No. 35060) were obtained from Aldrich Chemical Company (Milwaukee, Wisc.). These substances were clear, colorless liquids which were stored at ambient temperature in the dark until used. Bis(2-methoxyethyl)ether was stored under nitrogen.

4-Nitroquinoline-*N*-oxide was obtained from ICN K & K Laboratories, New York; 2-aminoanthracene was obtained from Aldrich Chemical Company; and ethyl methanesulfonate (EMS) was obtained from Koch–Light Laboratories, Colnbrook, Bucks, England. Aroclor 1254 was purchased from Monsanto, 6-[^{3}H]thymidine (21 Ci/mmol) was obtained from the Radiochemical Centre, Amersham, England.

Bacteria. Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 were donated by Professor B. Ames, University of California.

Mammalian cells. Human embryonic intestinal fibroblasts, designated Flow 11,000 cells, at passages 12 to 35, were obtained from Flow Laboratories, Irvine, Scotland.

Drosophila. Oregon K (OrK) wild-type flies were divided arbitrarily into two stock lines (designated A and B) before the experiments were initiated. The Müller-5 (M-5) flies had the *basc* balancer X-chromosome, ln(1)SC^{S1L} SC^{8R} + S SC^{S1} SC^{8}w^{aB}. These flies have been maintained within the Institute of Animal Genetics, University of Edinburgh for many years.

Rodents. CD rats (a remotely derived Sprague–Dawley strain) were obtained from Charles River (U.K.), while B6C3F$_1$ hybrid mice were obtained from Charles River (U.S.A.).

Bacterial mutation tests. The methods used were as described by Ames *et al.* (1975) in which incubations were conducted both in the presence and absence of an adult, male rat liver postmitochondrial supernatant fluid and NADPH-generating system (S-9 mix). The only important deviation from Ames' method was a liquid preincubation step of 30 min duration at 37°C before the addition of

agar and pouring on to the selective medium plates. Also, in one experiment, alcohol metabolism was mediated by yeast βNAD$^+$-dependent alcohol dehydrogenase. Data recorded were for triplicate plates.

Unscheduled DNA synthesis tests. Cells were routinely cultured in Dulbecco's modification of Eagle's minimal essential medium (DMEM), 10% heat-inactivated fetal calf serum (FCS), 2 mM glutamine, and 10 μg gentamycin/ml. Prior to an experiment, cells were harvested, 2 ml (5×10^4 cells/ml) transferred to 35-mm tissue culture-grade Petri dishes containing three sterile coverslips, and incubated for 72 hr. At the end of this period, the medium was replaced with 2 ml arginine-deficient DMEM, 5% FCS. This medium was changed (2 ml) after 24 hr and incubation continued for 48 hr. At this stage, hydroxyurea and 6-[^{3}H]thymidine were added to all dishes to give final concentrations of 2.5 mM and 10 μCi/ml, respectively. Incubations were conducted with the test compounds both in the presence and absence of S-9 mix for 3 hr at 37°C, after which Kodak AR-10 stripping film was used to coat the fixed cultures, following the procedures recommended by Rogers (1973). Giemsa-stained autoradiographs were examined and grain counts made on 50 nuclei per coverslip. Data recorded were mean nuclear grain counts and standard deviations for 150 nuclei.

Atmosphere generation and analysis in animal tests. The test atmospheres were produced by bubbling dry, oxygen-free nitrogen (BOC Limited) through 2-methoxyethanol or bis(2-methoxyethyl)ether contained in a Drechsel bottle immersed in a temperature controlled water bath at 37 or 50°C, respectively. The nitrogen/vapor mixture so generated was diluted with filtered, conditioned compressed air and passed into the rodent exposure chambers. These were 1.5-m^3 capacity stainless-steel and glass chambers in which individually caged rats or mice were confined to a single tier of cages occupying 0.5 m^3. The chamber was ventilated at a rate of 12 air changes per hour. Drosophila were exposed to test atmospheres drawn off the 1.5-m^3 chambers at a rate of 5 liters/min and passed through a modified (with steel mesh screens) Drechsel bottle in which they were kept for the exposure period (Done and McGregor, 1981).

Atmospheres in the chambers were analyzed by infrared spectroscopy with Miran-1A Gas Analyzers (Foxboro/Wilks Incorporated). Samples of chamber air were pumped continuously through the instruments and the chamber concentrations were automatically recorded. Instrument calibration was performed by a closed-loop calibration system. Known volumes of test compound were sequentially injected into the gas analyzer with a Hamilton glass microsyringe. After each injection, the absorbence reading was allowed to stabilize as indicated on the chart recording. The analytical conditions were as shown in Table 1.

Exposure procedure. Rodents were exposed to the test atmospheres for 7 hr/day for either 1 or 5 days, as required. Drosophila were exposed to 2-methoxyethanol for either 1 hr (25 ppm) or 15 min (500 ppm) and to bis(2-methoxyethyl)ether for 2.75 hr (250 ppm). In the positive control groups, EMS was administered orally to all animals. Multiple-dosed rodents received 100 mg EMS/kg/day for 5 days, while single-dosed rats received 250 mg EMS/kg. Drosophila were given 0.4% EMS in 5% sucrose (v/v) for a single 5-hr period.

Cytogenetic analysis of rat bone marrow cells. Groups of 10 male and 10 female rats were exposed to the test compounds or filtered air for either 1 or 5 days. After their last exposure period, animals were injected ip with 3 mg colchicine/kg 2 hr prior to death. The 1-day-exposed groups were killed at one of three sampling times, namely, 6, 24, and 48 hr after the end of exposure. The 5-day-exposed groups were killed 6 hr after the end of exposure. Giemsa-stained slides were labeled with numbers not correlated with the animal numbers; therefore, all assessments of metaphases were "blind." Where possible, 50 metaphases per rat were scored.

Dominant lethal testing in male rats. Groups of 10 male, adult rats were exposed to the test substances for 7 hr/day on 5 consecutive days, then serially mated at weekly intervals for 10 weeks to untreated, virgin females in the ratio 1 male:2 females. The female rats were killed and examined 17 days after they were first caged with the males. Dominant lethality was assessed according to the method of Bateman (1977).

Sperm abnormality test in mice. Groups of 10 male,

TABLE 1

INSTRUMENT SETTINGS ON A MIRAN-1A GAS ANALYZER FOR THE INFRARED ABSORPTION ANALYSIS OF 2-METHOXYETHANOL AND BIS(2-METHOXYETHYL)ETHER

	2-Methoxyethanol		Bis(2-methoxyethyl)ether	
Instrument settings	25 ppm	500 ppm	250 ppm	1000 ppm
Wavelength	8.8 μm	8.8 μm	9.7 μm	9.7 μm
Pathlength	8.25 m	0.75 m	20.25 m	3.75 m
Absorbence range	0.25 Å	0.25 Å	1.0 Å	1.0 Å
Slit width	0.5 mm	0.5 mm	1.0 mm	1.0 mm
Meter response	1	1	4	1

adult mice were exposed to the test substances for 7 hr/day on 5 consecutive days (or 4 days in the case of the 1000 ppm bis(2-methoxyethyl)ether group), maintained without further treatment for 35 days, then killed by neck dislocation. Assessment techniques and criteria used were as described by Wyrobeck and Bruce (1975). The following types of sperm were not scored: (1) separated tails and heads; (2) clumps of sperm; (3) sperm orientated so that the hook could not be seen; (4) sperm partially masked by any remaining stain droplets.

Sex-linked recessive lethal test in Drosophila melanogaster. Three-day-old male OrK flies from stocks A and B were exposed for the required time, which was established in an initial toxicity test. In this initial test, survivors were counted 24 hr after exposure. From these, four males were picked and mated with four virgin females which were allowed to lay their eggs on medium darkened with charcoal for 24 hr, then removed and the number of eggs counted. After a further 24 hr, the eggs remaining unhatched was counted. These results were compared with those from untreated flies. In the recessive lethal test, each treated male was given a number which was retained throughout the brood analysis and by his progeny through the F_2 and, where appropriate, the F_3 generation. Any clusters of mutants could, therefore, be readily identified. Treated males were mated individually to virgin Müller-5 (M-5) females, in the ratio 1 male:2 females, on the morning following the exposure day. Each male was remated to two more virgin females 3 days and, again, 8 days after the first mating. All matings ceased on Day 11. The three broods obtained in this way ensured sperm treated at all stages of spermatogenesis were tested. Emergence of F_1 generation flies from the pupae began about 10 days after mating.

Pairings for the F_2 generation were set up 1 to 4 days later by brother–sister mating. Assessment of effects in the F_3 generation was undertaken in the same way as for the F_2 generation. Experiments were normally scored 11 to 14 days after setting up the F_2 or F_3 generation crosses. Vials were examined and scored as nonlethal if two or more wild-type males (red eyes) were seen. Vials in which there were no wild-type males, but eight or more Müller-5 males were present, were checked for the presence of heterozygous (M-5/OrK) females and scored as recessive lethals if these were present. If a vial could not be unambiguously scored, then it was returned to the incubation room to be rescored the following day, when more flies had hatched. Vials containing no F_2 generation flies were scored as sterile.

Result evaluation. (a) Bacterial mutation test. At least a doubling of the concurrent control frequency was required for a suspect positive result, except for *S. typhimurium* TA 100, where a 1.5-fold increase was accepted as positive. (b) Unscheduled DNA synthesis test. Dosed groups were compared with the concurrent control by Student's *t* test. (c) Body weights. The statistical analysis consisted of the application of a general linear model with a normal error. This evaluation provided analysis of variance and individual *t* comparisons. (d) Cytogenetics test. Data were transformed with the Freeman–Tukey transformation for proportions, then a one-sided Student's *t* test applied to these values. Rats, not spreads, formed the basis for statistical analysis. This analysis was performed on cells with any type of abnormality and on cells with abnormalities other than gaps alone. (e) Dominant lethal test. Each female was regarded as an independent replicate and the vehicle control, low- and high-dose groups were analyzed together, the positive control being analyzed separately. Variates analyzed were (i) corpora lutea graviditatis, (ii) total implantations, (iii) live implantations, (iv) live implantations + late deaths, (v) early deaths, Freeman–Tukey Poisson transformation, and (vi) early deaths, Freeman–Tukey binomial transformation. The proportion of females with one or more, or two or more early deaths was calculated, after which treatment and control groups were compared by the chi-square test. The number of pregnant females per number of mated females was computed and the chi-square test again applied. (f) Sperm abnormality test. Data were transformed with the Freeman–Tukey transformation for proportions, then a one-sided Student's *t* test applied to these values. The analysis was performed on the total number of abnormal cells and on each of the categories a–e. (g) Sex-linked recessive lethal test. In this test, true mutation frequencies can be determined only within certain limits because integral numbers are recorded (Würgler *et al.*, 1977). Mutation frequencies calculated strongly depend upon the sizes of the test groups studies (i.e., the individual broods), which were relatively small. Hence, it was considered that, in place of a test for statistical significance, it would be better to look for a reproducible increase in the frequency of lethals over the historical control value of about 0.1%. Control values accumulated for breeding stocks A and B over a 1.5-year period were as follows.

F_2 Generation.

	Stock A			Stock B			
	Brood			Brood			
	1	2	3	1	2	3	Total
No. of tests	9	9	9	9	9	9	54
No. of gametes	5319	5309	5339	5264	5088	4713	31026
% Lethals	0.12	0.04	0.09	0.11	0.03	0.00	0.07

Against this background, the criteria for result assessment were: (i) a compound giving frequencies below 0.5% in duplicate experiments was considered nonmutagenic; (ii) a compound giving frequencies over 1.0% in the same brood in duplicate experiments was classified as a mutagen; and (iii) a compound giving frequencies between 0.5 and 1.0% was a suspect mutagen deserving further study.

RESULTS

Bacterial mutagenicity tests. There was no evidence of mutagenicity in *S. typhimurium* in the standard Ames' plate incorporation assay of the preincubation modification at dose levels of up to 33 mg 2-methoxyethanol per plate (volume ca. 20 ml) or 94 mg bis(2-methoxyethyl)ether per plate. 2-Methoxyethanol was also tested in the presence of equimolar quantities of βNAD^+ and 150 units of yeast alcohol dehydrogenase. At dose levels greater than about 125 μg per plate, 2-methoxyethanol metabolites were toxic to the bacteria. At lower dose levels, no mutagenic effect was observed. It was concluded, therefore, that 2-methoxyethanol and methoxyacetic acid are not mutagenic in bacteria.

Unscheduled DNA synthesis test. There was no indication of increased UDS in cells exposed for 3 hr to concentrations of 2-methoxyethanol up to nearly 10 mg/ml or bis(2-methoxyethyl)ether up to 19 mg/ml.

Clinical observations on rats and mice. (a) 2-Methoxyethanol. No clinical signs of toxicity were observed other than failure to increase body weight during the 5-day exposure period in male rats in the 500 ppm groups of both the dominant lethal and the cytogenetic test, whereas air control group rats increased in weight from 326 ± 20 g to 338 ± 23 g and 301 ± 12 g to 313 ± 17 g, respectively ($p < 0.01$). (b) Bis(2-methoxyethyl)ether. Male mice exposed to 1000 ppm atmospheres showed reductions in body weight from 23.3 ± 1.1 g to 21.4 ± 1.3 g in 3 days ($p < 0.001$), whereas male and female rat body weight were unaffected. All multiply exposed mammals in the 1000 ppm atmosphere groups were subdued and unresponsive to audio stimuli during exposure. Mice were particularly affected and became ataxic. Four of ten mice died on the fourth exposure day, so, the six survivors were not dosed on the fifth day. The cause of death was not investigated.

Cytogenetic analysis of rat bone marrow cells. In neither the 5-day nor the single exposure test was there any good evidence for the induction of chromosomal damage, other than in the positive control groups. The only significant increases in aberrant cell frequency occurred in low-dose groups 6 hr following a single exposure to the test material. In female rats exposed to 25 ppm 2-methoxyethanol, chromatid breaks were rather common ($t = 2.290, p < 0.01$), while in male rats exposed to 250 ppm bis(2-methoxyethyl)ether, there was a small increase in the frequency of the total aberrations ($t = 2.216, p < 0.05$). These elevations were restricted to a single sex in each case and were not reproduced at the higher dose levels. It was concluded, therefore, that neither compound was a clastogen.

Dominant lethal test in male rats. (a) 2-Methoxyethanol. Pregnancy frequencies were satisfactory in the filtered air control group and the 25 ppm exposure group in all weeks, in the 500 ppm dose group in assessment Weeks 1 through 3 and 9 and 10 and the positive control group in Weeks 5 through 10. Pregnancy frequencies were reduced in the 500 ppm exposure group in Weeks 4 through 8 (Table 2). Total implantations per pregnancy were normal in the control and 25 ppm exposure groups, but reduced in the 500 ppm exposure group in Weeks 3 through 8, particularly in Weeks 5 and 6. EMS treatment also reduced total implantation numbers in Weeks 1 through 4. When implantation sites were not observed, developed corpora lutea also were not seen, indicating that implantation had not occurred. Hence, preimplantation losses were evident in the 500 ppm exposure group in Weeks 3 through 7, particularly in Weeks 5 and 6. Early deaths were increased only in Week 8, if the Freeman–Tukey Poisson transformation model was adopted, but according to the Freeman–Tukey binomial transformation model, significant increases in early deaths should be recorded

TABLE 2

DOMINANT LETHAL TEST IN MALE RATS OF 2-METHOXYETHANOL AND BIS(2-METHOXYETHYL)ETHER ADMINISTERED 7 hr/day ON 5 CONSECUTIVE DAYS

Assessment week from dosing	Air control (0 ppm)	2-Methoxyethanol 25 ppm	2-Methoxyethanol 500 ppm	EMS 5 × 100 mg/kg	Air control (0 ppm)	Bis(2-methoxyethyl)ether 250 ppm	Bis(2-methoxyethyl)ether 1,000 ppm	EMS 5 × 100 mg/kg
				Pregnancy frequency (females with implantations)				
1	17/20 85%	14/20 70%	20/20 100%	10/20 50%	18/20 90%	19/20 95%	15/20 75%	15/20 75%
2	17/20 85%	19/20 95%	18/20 90%	2/20 10%	19/20 95%	18/20 90%	19/20 95%	1/20 5%
3	20/20 100%	20/20 100%	15/20 75%	4/20 20%	18/20 90%	18/19 95%	16/20 80%	3/20 15%
4	13/20 65%	16/20 80%	6/20 30%	12/20 60%	19/20 95%	19/20 95%	10/20 50%	17/20 85%
5	19/20 95%	19/20 95%	3/20 15%	16/20 80%	19/20 95%	18/20 90%	2/19 11%	18/20 90%
6	19/20 95%	20/20 100%	0/20 0%	18/20 90%	20/20 100%	20/20 100%	2/20 10%	18/19 95%
7	20/20 100%	18/20 90%	2/20 10%	18/20 90%	19/20 95%	20/20 100%	2/20 10%	18/20 90%
8	20/20 100%	19/19 100%	8/18 44%	17/20 89%	19/20 95%	20/20 100%	8/20 40%	19/20 95%
9	19/20 95%	18/20 90%	15/20 75%	18/20 90%	19/20 95%	19/20 95%	10/20 50%	20/20 100%
10	19/19 100%	19/19 100%	19/20 95%	20/20 100%	18/20 90%	19/20 95%	17/20 85%	20/20 100%
				Total Number of corpora lutea per pregnancy				
1	12.7 ± 0.39[a]	12.1 ± 0.43	11.8 ± 0.36	8.6 ± 0.58**	12.7 ± 0.54	12.5 ± 0.53	12.6 ± 0.59	10.8 ± 0.76
2	11.9 ± 0.49	12.6 ± 0.47	11.9 ± 0.48	3.5 ± 0.50**	13.3 ± 0.50	13.2 ± 0.52	13.3 ± 0.50	5.0 ± 0.00
3	12.5 ± 0.75	13.1 ± 0.75	9.3 ± 0.87**	3.0 ± 0.71**	14.8 ± 0.79	13.8 ± 0.79	12.4 ± 0.84*	1.0 ± 0.58**
4	11.9 ± 0.87	12.3 ± 0.78	8.0 ± 1.28**	9.0 ± 1.10**	12.2 ± 0.54	12.5 ± 0.54	11.1 ± 0.74	8.6 ± 0.97**
5	12.1 ± 0.29	12.4 ± 0.29	1.3 ± 0.74***	11.2 ± 0.83	13.8 ± 0.55	12.6 ± 0.57	12.5 ± 1.71	12.6 ± 0.77
6	13.3 ± 0.42	13.6 ± 0.41		12.6 ± 0.62	13.4 ± 0.55	12.3 ± 0.55	7.0 ± 1.75**	13.0 ± 0.54
7	11.8 ± 0.45	12.3 ± 0.49	8.5 ± 1.41**	11.6 ± 0.49	12.2 ± 0.34	11.8 ± 0.33	2.5 ± 1.04***	11.4 ± 0.84
8	13.0 ± 0.49	12.2 ± 0.50	10.9 ± 0.78	12.0 ± 0.60	11.8 ± 0.62	11.9 ± 0.60	11.9 ± 0.96	11.8 ± 0.50
9	12.1 ± 0.46	12.8 ± 0.47	12.1 ± 0.52	11.8 ± 0.43	13.7 ± 0.57	13.2 ± 0.57	12.0 ± 0.79	13.6 ± 0.55
10	12.5 ± 0.58	12.8 ± 0.58	10.3 ± 0.58	12.9 ± 0.69	12.9 ± 0.51	12.3 ± 0.50	12.6 ± 0.52	13.6 ± 0.63
				Total implantations per pregnancy				
1	12.8 ± 0.52	11.1 ± 0.57	11.5 ± 0.48	6.4 ± 0.78***	13.0 ± 0.63	12.0 ± 0.61	13.4 ± 0.61	7.8 ± 0.86***
2	11.6 ± 0.65	12.2 ± 0.62	11.1 ± 0.63	2.0 ± 1.00	13.6 ± 0.54	12.4 ± 0.55	13.7 ± 0.54	1.0 ± 0.00
3	12.5 ± 0.74	13.0 ± 0.74	8.3 ± 0.85**	2.3 ± 0.48	13.8 ± 0.78	12.5 ± 0.78	12.4 ± 0.82	1.0 ± 0.00**
4	11.6 ± 1.01	12.1 ± 0.91	6.0 ± 1.48**	8.3 ± 1.29**	12.4 ± 0.60	12.3 ± 0.60	9.9 ± 0.82*	8.2 ± 1.06**
5	11.7 ± 0.37	12.1 ± 0.37	1.0 ± 0.92***	11.6 ± 0.88	13.5 ± 0.53	13.1 ± 0.55	11.0 ± 1.64	11.9 ± 0.74
6	13.3 ± 0.51	12.8 ± 0.50	0.0 ± 0.00***	11.9 ± 0.95	13.0 ± 0.47	13.6 ± 0.47	2.5 ± 1.49***	12.5 ± 0.60
7	12.1 ± 0.57	12.6 ± 0.60	7.0 ± 1.81**	12.3 ± 0.67	12.0 ± 0.31	11.7 ± 0.31	2.0 ± 0.97***	11.4 ± 0.72
8	13.3 ± 0.68	12.6 ± 0.69	8.9 ± 1.07**	11.9 ± 0.95	12.1 ± 0.68	12.3 ± 0.67	11.8 ± 1.06	12.4 ± 0.50
9	12.8 ± 0.57	12.9 ± 0.59	12.1 ± 0.64	11.8 ± 0.54	11.6 ± 0.63	12.4 ± 0.63	10.4 ± 0.87	12.8 ± 0.64
10	12.4 ± 0.64	13.3 ± 0.64	10.2 ± 0.64	12.5 ± 0.72	12.3 ± 0.50	12.1 ± 0.49	11.5 ± 0.52	12.5 ± 0.82

				Sum of live implantations and late deaths per pregnancy				
1	11.9 ± 0.53	11.0 ± 0.59	11.1 ± 0.49	6.1 ± 0.89**	12.5 ± 0.69	11.6 ± 0.67	12.4 ± 0.75	1.2 ± 0.63***
2	11.1 ± 0.70	11.8 ± 0.66	10.8 ± 0.68	0.0 ± 0.00	13.2 ± 0.56	12.1 ± 0.57	13.1 ± 0.56	0.0 ± 0.00
3	12.3 ± 0.74	12.6 ± 0.74	7.8 ± 0.86**	1.8 ± 0.63**	13.3 ± 0.78	11.6 ± 0.78	11.6 ± 0.82	0.3 ± 0.33**
4	11.6 ± 0.99	12.1 ± 0.89	6.0 ± 1.46***	8.3 ± 1.29**	11.9 ± 0.64	11.8 ± 0.64	9.5 ± 0.88*	4.7 ± 0.71***
5	11.0 ± 0.39	11.6 ± 0.39	1.0 ± 0.99***	11.3 ± 0.87	13.3 ± 0.54	12.4 ± 0.55	9.5 ± 1.65*	11.3 ± 0.71*
6	12.7 ± 0.52	12.3 ± 0.51	0.0 ± 0.00***	11.1 ± 0.87	12.3 ± 0.46	13.3 ± 0.46	1.5 ± 1.47***	12.0 ± 0.62
7	11.5 ± 0.54	11.3 ± 0.57	6.5 ± 1.71**	11.4 ± 0.61	11.3 ± 0.33	11.5 ± 0.32	2.0 ± 1.01***	10.9 ± 0.74
8	13.0 ± 0.69	12.2 ± 0.71	8.0 ± 1.10**	10.8 ± 0.84	11.5 ± 0.72	11.6 ± 0.70	11.0 ± 1.11	11.9 ± 0.52
9	11.7 ± 0.65	11.8 ± 0.66	11.0 ± 0.73	11.2 ± 0.54	11.1 ± 0.62	12.0 ± 0.62	10.0 ± 0.85	12.0 ± 0.60
10	12.0 ± 0.65	12.7 ± 0.65	10.0 ± 0.65	12.0 ± 0.74	12.3 ± 0.48	12.2 ± 0.47	11.6 ± 0.50	12.6 ± 0.77
				Early death frequency, Freeman–Tukey Poisson transformation				
1	1.80 ± 0.217	1.20 ± 0.239	1.43 ± 0.200	1.36 ± 0.244	1.63 ± 0.209	1.52 ± 0.204	2.00 ± 0.229	5.08 ± 0.426***
2	1.62 ± 0.208	1.39 ± 0.197	1.40 ± 0.202	3.07 ± 0.659***	1.52 ± 0.186	1.43 ± 0.192	1.86 ± 0.186	2.41 ± 0.000
3	1.32 ± 0.179	1.50 ± 0.179	1.61 ± 0.207	1.71 ± 0.408	1.67 ± 0.262	1.96 ± 0.262	1.63 ± 0.278	1.94 ± 0.471
4	1.00 ± 0.067	1.09 ± 0.061	1.00 ± 0.099	1.00 ± 0.000	1.64 ± 0.196	1.60 ± 0.196	1.50 ± 0.269	3.82 ± 0.299***
5	1.85 ± 0.217	1.51 ± 0.217	1.00 ± 0.545	1.40 ± 0.183	1.30 ± 0.188	1.82 ± 0.193	2.78 ± 0.579*	1.70 ± 0.230
6	1.70 ± 0.187	1.64 ± 0.182	0.00 ± 0.000	2.03 ± 0.210	1.85 ± 0.187	1.50 ± 0.187	2.07 ± 0.591	1.63 ± 0.198
7	1.65 ± 0.272	2.11 ± 0.286*	1.71 ± 0.859	2.05 ± 0.228	1.79 ± 0.169	1.28 ± 0.164	1.00 ± 0.519	1.63 ± 0.198
8	1.35 ± 0.180	1.56 ± 0.184	2.05 ± 0.284*	2.20 ± 0.250	1.67 ± 0.207	1.78 ± 0.201	1.89 ± 0.318	1.60 ± 0.191
9	2.04 ± 0.294	2.04 ± 0.302	2.02 ± 0.331	1.83 ± 0.210	1.55 ± 0.191	1.63 ± 0.191	1.50 ± 0.264	1.99 ± 0.200
10	1.49 ± 0.167	1.75 ± 0.167	1.19 ± 0.167	1.63 ± 0.188	1.36 ± 0.219	1.96 ± 0.213	1.71 ± 0.225	1.57 ± 0.183
				Early death frequency, Freeman–Tukey binomial transformation				
1	0.495 ± 0.0613	0.360 ± 0.0676	0.417 ± 0.0565	0.653 ± 0.2080	0.457 ± 0.0643	0.449 ± 0.0626	0.559 ± 0.0705	2.386 ± 0.1646***
2	0.476 ± 0.0715	0.401 ± 0.0676	0.443 ± 0.0694	2.468 ± 0.1342***	0.415 ± 0.0520	0.401 ± 0.0535	0.494 ± 0.0520	2.334 ± 0.0000
3	0.371 ± 0.0792	0.408 ± 0.792	0.706 ± 0.0915**	1.195 ± 0.4181***	0.443 ± 0.0738	0.572 ± 0.0738	0.478 ± 0.0782	1.818 ± 0.5263
4	0.287 ± 0.0360	0.318 ± 0.324	0.546 ± 0.0529**	0.407 ± 0.0576**	0.465 ± 0.0636	0.454 ± 0.0636	0.507 ± 0.0877	1.431 ± 0.0825***
5	0.539 ± 0.0627	0.424 ± 0.0627	0.785 ± 0.1578**	0.427 ± 0.0565	0.351 ± 0.0514	0.496 ± 0.0528	0.837 ± 0.1585**	0.487 ± 0.0632
6	0.469 ± 0.0518	0.445 ± 0.0505	0.000 ± 0.0000	0.586 ± 0.0517	0.512 ± 0.0698	0.394 ± 0.0698	1.516 ± 0.2208***	0.461 ± 0.0559
7	0.464 ± 0.0891	0.580 ± 0.0940	1.302 ± 0.2819***	0.573 ± 0.0591	0.513 ± 0.0487	0.363 ± 0.0474*	0.654 ± 0.1500	0.497 ± 0.0623
8	0.365 ± 0.0817	0.431 ± 0.0838	0.926 ± 0.1291**	0.634 ± 0.0606	0.472 ± 0.0705	0.499 ± 0.0687	0.704 ± 0.1086	0.452 ± 0.0548
9	0.568 ± 0.0860	0.576 ± 0.0884	0.584 ± 0.0968	0.530 ± 0.0638	0.449 ± 0.0590	0.454 ± 0.0590	0.503 ± 0.0813	0.547 ± 0.0518
10	0.417 ± 0.0516	0.475 ± 0.0516	0.406 ± 0.0516	0.491 ± 0.0755	0.380 ± 0.0599	0.532 ± 0.0584	0.478 ± 0.0617	0.463 ± 0.0713

[a] Mean ± SE.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

in Weeks 3, 4, 5, 7, and 8. EMS induced a large increase in early deaths in Week 2. These observations were supported by an earlier experiment which was abandoned because the atmospheric concentrations were outside preset limits. However, 5 mating weeks were permitted before curtailment of the experiment, the results of which are given in Table 3. No adverse effects were observed in the 25 ppm atmosphere group, whereas, pregnancy frequency was reduced in Weeks 3 to 5 after exposure to 500 ppm 2-methoxyethanol. Also, total implantations per pregnancy and the sum of live implantations and late deaths per pregnancy were reduced in Weeks 3 and 5 (but not 4). The proportion of early deaths was increased to 15.2% in Week 3, but three of the five early deaths occurred in a single pregnancy. (b) Bis(2-methoxyethyl)ether. Pregnancy frequencies were satisfactory in the filtered air control and the 250 ppm exposure group in all weeks, in Weeks 1 through 3 and 10 of the 1000 ppm exposure group and in Weeks 1 and 4 through 10 of the EMS-treated group. Large reductions in pregnancy frequency occurred in the 1000 ppm exposure

TABLE 3

CURTAILED DOMINANT LETHAL TEST IN MALE RATS OF 2-METHOXYETHANOL ADMINISTERED 7 hr/day ON 5 CONSECUTIVE DAYS

Assessment week from dosing	Air control (0 ppm)	2-Methoxyethanol 25 ppm	2-Methoxyethanol 500 ppm	EMS 5 × 100 mg/kg
	Pregnancy frequency (females with implantations)			
1	17/20 85%	18/20 90%	14/20 70%	15/20 75%
2	16/20 80%	16/20 80%	18/20 90%	9/20 45%
3	19/20 95%	20/20 100%	5/20 25%	7/20 35%
4	19/20 95%	17/20 85%	2/20 10%	15/19 79%
5	18/20 90%	16/20 80%	0/20 0%	18/20 90%
	Total implantations per pregnancy			
1	12.2	13.6	13.7	9.9
2	12.5	12.9	11.2	1.6
3	13.7	12.4	6.6	1.3
4	14.2	14.8	12.0	7.9
5	13.9	13.3	0	12.6
	Sum of live implantations and late deaths per pregnancy			
1	12.2	13.3	12.9	3.2
2	12.1	12.1	10.9	0
3	13.2	12.1	5.6	0
4	13.3	14.3	11.5	5.9
5	13.9	13.2	0	12.6
	Early deaths as a proportion of total implantations			
1	0/208 0.0%	4/244 1.6%	11/192 5.7%	100/148 67.6%
2	7/200 3.5%	13/207 6.3%	4/201 2.0%	14/14 100.0%
3	11/216 4.2%	7/248 2.8%	5/33 15.2%	9/9 100.0%
4	17/269 6.3%	8/251 3.2%	1/24 4.2%	30/119 25.2%
5	0/251 0.0%	3/212 1.4%	— —	0/227 0.0%

group in Weeks 4 through 9, but particularly in Weeks 5 through 7, when frequencies were only about 10%. Recovery from the influence of bis(2-methoxyethyl)ether was complete in Week 10. EMS treatment also drastically reduced pregnancy frequency in Weeks 2 and 3. Total implantations per pregnancy were normal in the air control and 250 ppm exposure group in all weeks, in Weeks 1 through 3, 5 and 8 through 10 of the 1000 ppm exposure group and in Weeks 5 through 10 of the EMS-treated group. In the 1000 ppm exposure group, there was a small reduction in the number of implantations in Week 4, but the reductions in Weeks 6 and 7 were particularly large and significant ($p < 0.01$). EMS effects were seen in Weeks 1 through 4 of this experiment. Preimplantation losses, manifest as reductions in corpora lutea graviditatis per pregnancy, were obvious in Weeks 6 and 7 of the 1000 ppm exposure group. A small, statistically significant reduction also was seen in Week 3 of this group. Analysis of the frequencies of early deaths per pregnancy, following the Freeman–Tukey Poisson transformation model, indicated a significant increase in Week 5 of the 1000 ppm exposure group ($p < 0.05$). A similar analysis, but assuming a binomial model, indicated significant increases in early deaths per pregnancy in the 1000 ppm exposure group in Weeks 5 ($p < 0.01$) and 6 ($p < 0.001$). In Week 7 of the 250 ppm exposure group, there was a significant decrease in early death frequency. EMS treatment induced increases in early deaths in Weeks 1 to 4.

Sperm abnormality test in mice. (a) 2-Methoxyethanol. The overall frequency of abnormal sperm was significantly increased ($p < 0.05$) from 5.2% in the air control to 9.4% in the 500 ppm exposure group (Table 4). Most of the increase was due to two abnormality categories: the banana-shaped head (B) and the amorphous head (C), which were increased in B, from 0.64 to 1.30% and significantly, in C ($p < 0.01$), from 2.02% to 5.11%. It was concluded the 2-methoxyethanol did induce sperm abnormalities. EMS treatment was without any observable effect in this experiment. (b) Bis(2-methoxyethyl)ether. While no increases in frequencies of abnormal sperm categories were observed after exposure to 250 ppm atmospheres, there were large responses to the 1000 ppm atmospheres. All categories of abnormalities were involved to some extent, but the greatest response was in the incidence of amorphous heads (C) which were increased from 2.18% in the air control to 20.87% in the 1000 ppm exposure group ($p < 0.001$). It was concluded that bis(2-methoxyethyl)ether does increase the frequency of abnormal sperm. Some increases were also observed after EMS treatment, the largest increase being in the folded tail (D) category.

Sex-linked recessive lethal test in Drosophila. (a) 2-Methoxyethanol. The two breeding stocks of flies (A and B) were exposed to 25 ppm for 1 hr of 500 ppm for 15 min, the maximum tolerated doses as established in the preliminary tests. In the first F_2 generation brood, which covers Days 1 to 3 of the spermatogenesis cycle, and the F_3 generation there was no evidence for recessive lethal induction (Table 5). The results of the second F_2 generation brood, covering Days 3 to 8 of spermatogenesis, and the third brood, covering Days 8 to 11, were conflicting for the two stocks of flies. At 500 ppm, Stock A produced 3/386 (0.78%) recessive lethals, all of them in Brood 3, whereas Stock B gave 2/457 (0.44%) recessive lethals, both of them in Brood 2. Thus, although the frequencies were relatively high, 2-methoxyethanol could not be considered responsible. (b) Bis(2-methoxyethyl)ether. Preliminary toxicity tests indicated a maximum dose to be used of 250 ppm for 2.75 hr. Fertility was not impaired by this treatment. In the air control groups, there were three F_2 generation lethals in Stock A flies and two in Stock B flies (Table 6). All of the Stock A lethals occurred in Brood 1, giving a frequency of 3/571 (0.53%), while the Stock B lethals were distributed as one in Brood 1 (0.18%) and one in Brood 2 (0.18%). In the bis(2-methoxyethyl)ether-exposed flies, there were six lethals in Stock A and one in Stock B. The

TABLE 4

SPERM ABNORMALITY TEST IN B6C3F$_1$ MICE EXPOSED TO 2-METHOXYETHANOL OR BIS(2-METHOXYETHYL)ETHER

Substance	Dose	Normal cells	Abnormal cells[a]						Percentage abnormal cells[a]					
			A	B	C	D	E	Total	A	B	C	D	E	Total
Air	—	4264	9	29	91	94	13	236	0.20	0.64	2.02	2.09	0.29	5.24
2-Methoxy-ethanol	25 ppm, 7 hr/day × 5	8683	10	31	116	119	41	317	0.11	0.34	1.29	1.32	0.46	3.52
	500 ppm, 7 hr/day × 5	9062	10	130	511	250	37	938	0.10	1.30	5.11**	2.50	0.37	9.38*
Ethyl methane-sulfonate	100 mg/kg/day × 5	8655	14	27	116	155	33	345	0.16	0.30	1.29	1.72	0.37	3.83
Air	—	9486	31	28	218	77	160	514	0.31	0.28	2.18	0.77	1.60	5.14
Bis(2-methoxy-ethyl)ether	250 ppm, 7 hr/day × 5	8576	18	25	190	46	145	425	0.20	0.28	2.11	0.51	1.61	4.71
	1,000 ppm, 7 hr/day × 4	4736	118	121	1461	239	325	2264	1.68***	1.73***	20.87***	3.41***	4.64***	32.30***
Ethyl methane-sulfonate	200 mg/kg day × 5	8271	22	25	338	145	199	729	0.24	0.28	3.76	1.60*	2.21	8.10

[a] See Methods for definitions: A, hook up-turned or elongated; B, banana-shaped head; C, amorphous head; D, hair pin or tight coil tail; E, miscellaneous (e.g., multiple tails, double head, twisted neck, filamentous, or enlarged midpiece).

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$.

TABLE 5

SEX-LINKED RECESSIVE LETHAL TEST IN 3-DAY-OLD DROSOPHILA EXPOSED TO 2-METHOXYETHANOL

	Stock A							Stock B					
	25 ppm, 1 hr			500 ppm, 15 min			0.4% EMS, 5 hr	25 ppm, 1 hr			500 ppm, 15 min		
Brood No.:	1	2	3	1	2	3	1	1	2	3	1	2	3
No. of F_1 vials	96	52	50	100	97	56	50	95	89	47	100	93	46
No. of sterile F_1 vials	67	26	43	40	23	27	0	15	42	36	22	64	32
No. of F_2 vials	307	379	137	428	502	420	50	524	492	221	409	477	77
No. of F_2 vials scored	284	365	132	421	481	386	95	512	478	203	364	457	71
No. of F_2 vials with lethals	0	0	2	0	0	3	18	1	4	0	0	2	0
Frequency of F_2 lethals	0	0	1.5%	0	0	0.71%	18.9%	0.19%	0.81%	0	0	0.42%	0
No. of F_3 vials	—	—	—	—	—	322	—	403	406	190	372	259	67
No. of F_3 vials scored	—	—	—	—	—	307	—	378	390	177	360	250	61
No. of F_3 vials with lethals	—	—	—	—	—	0	—	1	0	0	0	0	0
Frequency of F_3 lethals	—	—	—	—	—	0	—	0.24%	0	0	0	0	0

TABLE 6

SEX-LINKED RECESSIVE LETHAL TEST IN 3-DAY DROSOPHILA EXPOSED TO BIS(2-METHOXYETHYL)ETHER

	Stock A							Stock B					
	Air control			250 ppm, 2.75 hr			0.4% EMS, 5 hr	Air control			250 ppm, 2.75 hr		
Brood No.:	1	2	3	1	2	3	1	1	2	3	1	2	3
No. of F_1 vials	100	100	88	72	69	62	67	97	91	70	83	77	70
No. of sterile F_1 vials	19	34	28	14	13	15	2	25	20	27	9	17	11
No. of F_2 vials	609	600	600	602	599	600	195	600	599	556	616	600	598
No. of F_2 vials scored	571	527	575	539	520	490	179	560	544	534	556	504	543
No. of F_2 vials with lethals	3	0	0	1	1	4	36	1	1	0	0	0	1
Frequency of F_2 lethals	0.52%	0	0	0.18%	0.19%	0.81%	20.10%	0.18%	0.18%	0	0	0	0.18%
No. of F_3 vials	—	—	500	—	496	400	—	—	—	—	498	—	—
No. of F_3 vials scored	—	—	490	—	471	391	—	—	—	—	484	—	—
No. of F_3 vials with lethals	—	—	0	—	0	0	—	—	—	—	6	—	—
Frequency of F_3 lethals	—	—	0	—	0	0	—	—	—	—	1.24%	—	—

Stock A lethals were distributed as one in Brood 1 (1/539, 0.18%), one in Brood 2 (1/520, 0.19%), and four in Brood 3 (4/490, 0.81%). The single Stock B lethal occurred in Brood 3 (1/543, 0.18%). So, the high Stock A, Brood 3 result was not repeated in Stock B flies. It is difficult to interpret this result because the concurrent air control also showed an unusually high frequency of recessive lethals, so, bis(2-methoxyethyl)ether should be reinvestigated in the SLRL test. In the F_3 generation, there were no lethals in the air control group, while, in the bis(2-methoxyethyl)ether-exposed group, there were 6/484 (1.24%) lethals in Brood 1 and none in either Brood 2 or 3. This high frequency was a result of two clusters; four of the six lethals were derived from one exposed male and the remaining two were also derived from a single male. Mapping experiments showed that the lethals from the former of these males all mapped in the same part of the chromosome, indicating that these four lethals were not independent events.

DISCUSSION

The use of Ames' test, the UDS assay, and the bone marrow cytogenetic test failed to demonstrate any genotoxic effects of either 2-methoxyethanol or its condensation product, bis(2-methoxyethyl)ether. Antifertility was the most obvious result from the high concentration exposure groups of the dominant lethal tests in which pregnancy frequencies were low in Weeks 4 through 8 (2-methoxyethanol) and Weeks 4 through 9 [bis(2-methoxyethyl)ether]. The antifertility effect observed in the completed test was supported by the results of the curtailed dominant lethal test, reported here, and dominant lethal studies in which rats were exposed to 300 ppm 2-methoxyethanol 6 hr/day, 5 days/week for 13 weeks (Rao *et al.*, 1983). In this prolonged exposure experiment there were no successful matings from male rats exposed to 300 ppm, whereas there were no adverse effects upon reproduction following exposure to 30 or 100 ppm 2-methoxyethanol. Of those females which were pregnant in the current experiments, implantations were greatly reduced in Weeks 5 and 6 (2-methoxyethanol) and Weeks 6 and 7 (bis(2-methoxyethyl)ether]. The frequencies of mature corpora lutea were also reduced at these times. These results with the two compounds are very similar and the small differences in timing of the effects may be inconsequential.

There is some doubt whether preimplantation losses have any significance in the assessment of dominant lethal mutation. While a highly potent mutagen might induce complete preimplantation loss (Kratochvilova, 1978), it has been pointed out that a reduction in total implantations can be due to either preimplantation dominant lethals or unfertilized eggs due to low numbers of sperm (Bateman, 1977). The only way of distinguishing between these possibilities is to examine the shed ova and determine whether or not they have been fertilized. This determination cannot be done at the same time as a dominant lethal test. Therefore, the low corpora lutea graviditatis counts and the low numbers of implantations are not conclusive evidence of dominant lethal mutation.

The postimplantation losses apparent in the high-dose groups of rats exposed to 2-methoxyethanol or bis(2-methoxyethyl)ether occurred mainly in those assessment weeks where total implantations were very low. The coincidence of these features creates interpretative problems. Studies of large quantities of control group data from CD-1 mouse dominant lethal tests have shown that, in untreated mice, there are proportionally more early deaths in females with low numbers of implantations (Anderson *et al.*, 1981). The same appears to be true of CD rats in this laboratory. In 16 experiments, the mean percentage early deaths in untreated rats fell from 50% in pregnancies with a single implantation to about 7% when there were more than five implantations (unpublished data). Therefore, the high proportion of early deaths in Week 5 (2-methoxyethanol) and Week 6 [bis(2-methoxy-

ethyl)ether] could be partly explained by the low total implantation frequency. However, there were significant increases in early death frequency in some assessment weeks where total implantations were at reasonably high levels. This was the case, for 2-methoxyethanol, in Weeks 7 and 8 (binomial model) or Week 7 (Poisson model) and, for bis(2-methoxyethyl)ether, in Week 5 (binomial and Poisson models). Thus, for each compound there was good evidence for a postimplantation dominant lethal effect in one assessment week which was independent of the statistical model used.

The timing of the damage strongly suggests that the male reproductive cells most susceptible to damage are the meiotic cells, the spermatocytes (Roosen-Runge, 1977). By chance, this is also the cell maturation phase sampled in the mouse sperm abnormality tests where sperm head abnormalities were increased in the high dose groups of both compounds. Besides any general suppression of sperm maturation induced by these compounds, the increased frequencies of abnormal sperm from exposed spermatocytes probably would have contributed to the antifertility effects.

Metabolic studies have indicated that methoxyacetic acid might be the active metabolite generated from 2-methoxyethanol (Miller *et al.*, 1982). Both of these compounds were inactive in Ames' test. Also, this suggested involvement of methoxyacetic acid should be examined in some detail because bis(2-methoxyethyl)ether, which is not an alcohol, seems to have effects on male reproductive capacity similar to 2-methoxyethanol. All of these ethers (including methoxyacetic acid) are susceptible to peroxide formation and participation in radical reactions. Studies are required, therefore, upon the histopathology of bis(2-methoxyethyl)ether; they are also required in the Drosophila SLRL test to clarify the ambiguities encountered with this compound and 2-methoxyethanol. However, the general conclusions which can be reached are that the results from previously reported studies and the present investigation strongly suggest that effects upon male fertility and embryonic development are of much greater importance than genetic effects when setting tolerable limits. Those male reproductive effects which were seen occurred only at the high concentrations which also induced body weight changes.

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