Provisional Peer-Reviewed Toxicity Values for

2-Methoxyethanol
(CASRN 109-86-4)

and

2-Methoxyethanol Acetate
(CASRN 110-49-6)

Superfund Health Risk Technical Support Center
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Questions regarding the contents of this document may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300)

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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMD	benchmark dose
BMCL	benchmark concentration lower bound 95% confidence interval
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
POD	point of departure
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

**PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR
2-METHOXYETHANOL (CASRN 109-86-4) AND
2-METHOXYETHANOL ACETATE (CASRN 110-49-6)**

BACKGROUND

HISTORY

On December 5, 2003, the U.S. Environmental Protection Agency's (EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1) EPA's Integrated Risk Information System (IRIS)
2) Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in EPA's Superfund Program
3) Other (peer-reviewed) toxicity values, including
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR);
   - California Environmental Protection Agency (CalEPA) values; and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the EPA IRIS Program. All provisional toxicity values receive internal review by a panel of six EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

DISCLAIMERS

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility

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in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

QUESTIONS REGARDING PPRTVS

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

2-Methoxyethanol (2-ME; see Figure 1) is a clear, colorless liquid that is used mainly as a solvent and is miscible with water and other solvents. It is used as a solvent for many different purposes such as varnishes, dyes, and resins, and as an additive in airplane deicing solutions (ACGIH, 2006a). Similarly, 2-ME acetate (see Figure 2) is a colorless liquid used primarily as a solvent in painting, furniture finishing, printing, semiconductor and other electronics industries (ACGIH, 2006b). Both have high vapor pressures, so inhalation of the vapor is a likely route of exposure. However, data from occupational studies indicate that the skin is the major route of absorption in the workplace (Sparer et al., 1988; Piacitelli et al., 1990; Chang et al., 2004; Kezic et al., 1997).
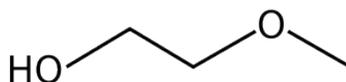
Figure 1. 2-ME Structure

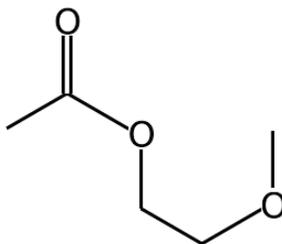2-Methoxyethanol
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Figure 2. 2-ME Acetate Structure

No RfD for 2-ME or 2-ME acetate is available on the IRIS (U.S. EPA, 2010a) database or in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2009). HEAST (U.S. EPA, 2010b) lists a subchronic RfD of 0.01 mg/kg-day and a chronic RfD of 0.001 mg/kg-day for 2-ME, based on route-to-route extrapolation from an inhalation NOAEL of 31 mg/m^3 for testicular effects in rabbits (Miller et al., 1983a), as originally derived in a Health and Environmental Effects Profile (HEEP) for 2-ME (U.S. EPA, 1986). HEAST (U.S. EPA, 2010b) also lists subchronic and chronic RfDs of 0.02 and 0.002 mg/kg-day, respectively, for 2-ME acetate derived in a HEEP for 2-ME acetate (U.S. EPA, 1987). These RfDs were derived by analogy to 2-ME based on in vitro evidence for metabolic hydrolysis of 2-ME acetate to 2-ME in nasal epithelium, lungs, liver, kidney, and blood of several animal species (Stott and McKenna, 1985). The adjustment across chemicals was made by multiplying the RfD derived for 2-ME (0.001 mg/kg-day) by the ratio of molecular weights for 2-ME and 2-ME acetate. In addition to the HEEPs for 2-ME (U.S. EPA, 1986) and 2-ME acetate (U.S. EPA, 1987), the Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994a) reports a Health Effects Assessment (HEA) for glycol ethers (U.S. EPA, 1984) that included 2-ME. HEA declined to derive oral toxicity values due to inadequate data.

IRIS (U.S. EPA, 2010a) reports a RfC for 2-ME of 0.02 mg/m^3 based on a NOAEL$_{HEC}$ of 17 mg/m^3 for testicular effects in rabbits (Miller et al., 1983a) (modified by a total UF of 1000, including a factor of 10 for extrapolation of subchronic effects data to chronic exposure, 10 for protection of sensitive human populations, and a combined factor of 10 for interspecies extrapolation [a dosimetric adjustment was used] and database deficiencies). The RfC assessment, verified in 1990, was not reported in any EPA source document. HEAST (U.S. EPA, 2010b) lists a subchronic RfC of 0.2 mg/m^3 for 2-ME based on the same endpoint. This assessment also was not attributed to any source document. CalEPA (2000a,b) derived chronic inhalation reference exposure levels of 0.06 mg/m^3 for 2-ME and 0.09 mg/m^3 for 2-ME acetate, extrapolated from Miller et al. (1983a) using an UF of 300. Occupational exposure limits for 2-ME include an American Conference of Governmental Industrial Hygienists (ACGIH, 2006a, 2010) threshold limit value-time weighted average (TLV-TWA) of 0.1 ppm (0.3 mg/m^3), a National Institute for Occupational Safety and Health (NIOSH, 2005) recommended exposure limit-TWA of 0.1 ppm (0.3 mg/m^3), and an Occupational Safety and Health Administration (OSHA, 2010) permissible exposure limit-TWA (PEL-TWA) of 25 ppm (80 mg/m^3). For 2-ME acetate, ACGIH (2010, 2006b) recommends a TLV-TWA of 0.1 ppm (0.5 mg/m^3) derived by analogy to 2-ME. The NIOSH (2005) recommended exposure limit is also 0.1 ppm, and the OSHA (2010) PEL-TWA is 25 ppm (120 mg/m^3).

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An Environmental Health Criteria Document (WHO, 1990) that includes both 2-ME and 2-ME acetate is available, but no toxicity values were derived for either chemical. The chronic toxicity and carcinogenicity of 2-ME and 2-ME acetate have not been assessed by ATSDR (2010), IARC (2010), or NTP (2010, 2005).

Literature searches were performed in December 2006 and May 2010 for 2-ME (CASRN 109-86-4) and in July 2009 and May 2010 for 2-ME acetate (CASRN 110-49-6). For both chemicals, literature searches included publications from the 1960s to the present for studies relevant to the derivation of toxicity values. Databases searched included MEDLINE, TOXLINE (with NTIS), BIOSIS, TSCATS/TSCATS2, CCRIS, DART, GENETOX, HSDB, RTECS, Chemical Abstracts, and Current Contents (6 months prior to search date for each chemical).

REVIEW OF PERTINENT DATA

Unless otherwise indicated, the statistical significance of the findings discussed below was determined by the study authors.

HUMAN STUDIES
Oral Exposure
Young and Woolner (1946) reported a case of a 44-year-old man who ingested an unknown but fatal amount (reported by the researchers to have been as much as one-half pint or 235 mL, purity unspecified) of a liquid thought to be 2-ME. Using the specific gravity of 965 mg/mL reported by the researchers for the ingested liquid, which matches the specific gravity for pure 2-ME and a body weight of 70 kg, the ingested dose may have been up to 3240 mg/kg. Findings at autopsy were mainly hemorrhagic gastritis and toxic changes to the liver and kidney.

Nitter-Hauge (1970) reported on two cases of accidental ingestion of ~100 mL of 2-ME of undisclosed purity. Using the density of 965 mg/mL for pure 2-ME and a body weight of 70 kg, the ingested dose was ~1379 mg/kg in each case. Within 8−18 hours after ingestion, both patients became confused and complained of general weakness and nausea. Both patients exhibited rapid, deep respiration, as well as metabolic acidosis. Both cases made an uneventful recovery.

No studies examining humans orally exposed to 2-ME acetate were located.

Inhalation Exposure
Human occupational studies, discussed below, have identified hematological and neurological effects in workers exposed to 2-ME, although it has not been conclusively demonstrated that 2-ME was responsible for the observed effects in any of these cases, because in most cases, workers were exposed to a variety of chemicals. However, hematological effects have been reported for 2-ME and 2-ME acetate in animals and in humans (Welch and Cullen, 1988; NIOSH, 1991; Larese et al., 1992; Shih et al., 2000, 2003).

Cohen (1984) reported a single case of a worker presenting with subjective, reversible neurological symptoms (apathy, fatigue, decreased appetite) and macrocytic anemia (an asymptomatic hematological effect). The report followed both skin and inhalation exposure to a mean airborne concentration of 35-ppm (110-mg/m^3) 2-ME for 1−1.5 years. The symptomatic findings were confounded by concurrent exposure to 1−5-ppm methyl ethyl ketone (2-butanone), a known neurotoxicant at higher exposures, and 4.2−12.8-ppm propylene glycol monomethyl ether (1-methoxypropanol).

Greenburg et al. (1938), Parsons and Parsons (1938), and Donley (1936) reported hematological and neurological effects in several workers exposed to 2-ME while making "fused" collar shirts. Neurological effects included encephalopathy, dizziness, fainting, headache, weakness, ataxia, psychopathic disturbances, and personality changes. Anemia, granulopenia, and respiratory tract irritation were found as well. The 2-ME content of the solvents to which these workers were exposed ranged from <3 (with <3% dimethyl phthalate and 74% isopropanol) to 33% (with 67% denatured ethanol). Limited air analyses (single 2−2.5-hour samples with pressing machines in operation and windows open or partially closed) in one plant with affected workers found air concentrations of 25−76 ppm (78−240 mg/m^3) for 2-ME and 70−215 ppm for ethanol. The researchers considered it likely that actual exposure concentrations were much higher due to worker habits (standing near dipping tanks, bending low over tanks when working) and frequent clogging of exhaust ducts. Affected workers had been exposed for ≥6 months. The central nervous system and hematological effects observed in this study have been observed as well following exposure to ethanol or isopropanol alone, thus confounding the possible association between 2-ME and the observed effects.

Case reports of five workers using 2-ME as a cleaning solvent for printing operations described central nervous system depression, memory loss, narcolepsy, language difficulties, and anemia following at least 7 months of exposure (Zavon, 1963). Experimental recreations of work activities provided exposure estimates of 61−3960 ppm (190−12,300 mg/m^3). Upon institution of strict industrial hygienic practices including improvement of work area ventilation, airborne 2-ME concentrations were reduced to ~20 ppm (62 mg/m^3). After exposure was reduced, neurological symptoms were resolved, and hematological parameters returned to normal in the case subjects.

A cross-sectional occupational exposure study (Cook et al., 1982) examined effects on two groups of exposed workers at a facility involved in the manufacture and packaging of 2-ME. Air sample measurements in the production area and packaging area, reported as 8-hour TWAs, were ≤0.42 ppm (≤1.3 mg/m^3, unspecified number of samples collected in January 1976, sampling times not reported) and 5.4−8.5 ppm (17−27 mg/m^3, 8-hour TWA calculated from an unspecified number of 2-hour samples collected from warehouse operators packaging 2-ME in July 1980), respectively. However, the workers were exposed to various other chemicals as well, including other ethylene glycol ethers; ethylene, propylene, and butylene oxides; chlorobenzenes; brake fluids; and various organic amines. Control workers came from manufacturing sites for alkanolamine and salicylic acid and may have been exposed to a variety of other chemicals. There were a total of 53 exposed and 44 control workers eligible for the study. The study group included 40 exposed workers and 25 controls, for which hematological evaluations were made. Subgroups of six exposed and nine control workers were used for fertility evaluations. Neurological effects were not evaluated. Duration of exposure was

estimated for each participant from work history. These data were used in the analysis but were not separately reported. Adjustments to the analysis were made for age and smoking status. There were no statistically significant differences between groups in hematological parameters, blood hormone concentrations, testicle size, sperm counts, or sperm motility. Study limitations, including incomplete exposure information, lack of neurological data, and confounding exposures among both exposed and control workers to other chemicals, preclude the identification of a NOAEL from this study.

One shipyard was the subject of several publications where there were attempts to relate 2-ME exposures with urinary methoxyacetic acid (MAA) and to health effects. Welch and Cullen (1988), McManus et al. (1989), Sparer et al. (1988), and Welch et al. (1988) reported studies of shipyard painters exposed to a wide variety of paint solvents including 2-ethoxyethanol and 2-ME. Mean 2-ME concentrations were 0.8 ± 1.0 ppm (2.5 ± 3.1 mg/m^3); MAA was not found in any urine samples. These studies of shipyard painters indicated a nonstatistically significant reduction in sperm parameters. However, the studies suffered from methodological difficulties and the presence of confounding exposures, precluding identification of a LOAEL or NOAEL.

Shih et al. (2000) reported hematological and spermatotoxic effects in a study of 53 copper-clad laminators in two semiconductor facilities. Control subjects were 121 laminate workers with indirect exposure to 2-ME. When the two plants were considered together, the hematological parameters (hemoglobin $p < 0.0001$), packed cell volumes (PCVs) ($p < 0.0001$), and erythrocyte count ($p < 0.001$) were statistically significantly lower in exposed males than in controls. Of the sperm parameters investigated, only sperm pH was statistically significantly depressed ($p < 0.005$). The authors noted a positive correlation of airborne 2-ME with erythrocyte counts ($p < 0.003$) and a positive correlation of urinary MAA with hemoglobin ($p < 0.001$), packed erythrocytes ($p < 0.001$), and erythrocyte count ($p < 0.001$). The authors concluded that at the current legal occupational exposure limit of 5 ppm (15 mg/m^3) in Taiwan, demonstrable hematological effects were seen, and there was a direct relationship between these effects and MAA in urine, as well as 2-ME in air.

In a follow-up study of hematological effects at one plant, Shih et al. (2003) monitored changes in hematological parameters initially, and at intervals of 3 and 6 months after the implementation of aggressive engineering controls. The weekly geometric mean (GM) 8-hour TWA 2-ME concentration for the exposed group before intervention was 9.62 ppm (geometric standard deviation [GSD] = 4.75; $n = 29$; range 0.75−320), or 29.8 ± 14.7 mg/m^3. Three months after intervention, the exposed group had a GM exposure of 2.34 ppm (GSD = 1.76; $n = 29$; range 0.2−10) or 7.3 ± 5.5 mg/m^3. Six months after intervention, the exposed group had a GM exposure of 0.34 ppm (GSD = 2.69; $n = 29$; range 0.1−3.5) or 1.1 ± 8.3 mg/m^3. Urinary MAA in specimens collected after the last work shift of the workweek showed a GM of 50.7-mg/g creatinine (GSD = 1.67; $n = 29$; range 24.3−139) at baseline. After the 3-month intervention, the GM for MAA in the exposed group fell to 19.7-mg/g creatinine (GSD = 2.09; $n = 29$; range 4.60−54.9). After the 6-month intervention, the GM for MAA in the exposed group continued to fall to 6.8-mg/g creatinine (GSD = 4.2; $n = 29$; range 0.95−25.2). As in the previous study, there were statistically significant changes in exposed male workers compared to controls in hemoglobin, packed erythrocytes, and erythrocyte count in males but not females. Airborne 2-ME at baseline before intervention was correlated with hematological changes in controls

($p < 0.001$). The correlation between MAA in urine at baseline and hematological effects in male controls was more pronounced with hemoglobin ($p = 0.005$), packed erythrocytes ($p < 0.001$), and erythrocytes ($p < 0.001$). Hematological changes from baseline to the reduced exposures at 6 months progressed with increases seen in hemoglobin ($p < 0.001$), packed erythrocytes ($p < 0.001$), and erythrocyte count ($p < 0.001$). The study confirmed that a GM 2-ME exposure of 0.34 ppm and a urinary MAA GM of 6.77 did not result in hematological changes. Shih et al. (2003) demonstrated an 8 hours per day, 5 days per week, NOAEL of 1.1 ± 8.3 mg/m^3 and LOAEL of 7.3 ± 5.5 mg/m^3 for reversible hematologic effects in humans.

No data were located for humans exposed to 2-ME acetate by inhalation exposure.

ANIMAL STUDIES
Oral Exposure
    Subchronic Studies
        Nagano et al. (1979) gave groups of five male JCL-ICR mice gavage doses of 62.5, 125, 250, 500, 1000, or 2000 mg/kg-day of 2-ME or 2-ME acetate (purity not reported), 5 days/week, for 5 weeks. A control group of 20 mice was used. Blood was collected after the final dose and analyzed for erythrocyte and leukocyte counts, PCV, and hemoglobin concentrations. Testes were excised, weighed, and subjected to histopathological examination. All endpoints were compared statistically.

        For 2-ME, Table 1 shows that statistically significantly reduced leukocyte counts (54−77% lower than controls) were observed at ≥500 mg/kg-day, while erythrocyte counts and hemoglobin concentrations were statistically significantly reduced (19−32 and 9−39% lower than controls, respectively) at 1000 mg/kg-day (Nagano et al., 1979). Absolute and relative vesicular and coagulating gland weights were statistically significantly lower than controls (37 and 24%, respectively) at 1000 mg/kg-day. Absolute and relative testes weights were statistically significantly lower than controls (44−81% and 45−80%, respectively) at ≥250 mg/kg-day. A clear reduction in spermatocytes was observed in the seminiferous tubules at 250 mg/kg-day (incidence data not reported). Seminiferous tubule atrophy was seen at 500 mg/kg-day, while complete absence of germ cells occurred at 1000 mg/kg-day (data not shown). This study identified a LOAEL of 250-mg/kg-day 2-ME, with an associated NOAEL of 125 mg/kg-day, for testicular effects in mice.

        For 2-ME acetate, Table 1 shows the statistically significantly reduced leukocyte counts (71−79% lower than controls) and hemoglobin concentrations (10% lower than controls) were observed at ≥1000 and 2000 mg/kg-day, respectively (Nagano et al., 1979). Absolute and relative vesicular and coagulating gland weights were statistically significantly lower than controls (35 and 32%, respectively) at 2000 mg/kg-day. Absolute and relative testes weights were statistically significantly lower than controls (63−76 and 64−75%, respectively) at ≥500 mg/kg-day. A clear reduction in spermatocytes was observed in the seminiferous tubules at 500 mg/kg-day (incidence data not reported). Tubule atrophy was seen at 1000 mg/kg-day, while complete absence of germ cells occurred at 2000 mg/kg-day. This study identified a 5 days/week, 5-week LOAEL of 500 mg/kg-day, with an associated NOAEL of 250 mg/kg-day, for testicular effects for 2-ME acetate exposure in mice.

Table 1. Selected Changes in Male Mice Treated with 2-Methoxyethanol or 2-Methoxyethanol Acetate by Gavage 5 Days/Week for 5 Weeks							
	Dose in mg/kg-day						
	Control	62.5	125	250	500	1000	2000
2-Methoxyethanol							
Number of animals examined	20	5	5	5	5	5	5
Hematology							
WBC (/mm^3)	3840 ± 1572[a]	3270 ± 1714	4250 ± 2100	3470 ± 2164	1770 ± 392[b]	1000 ± 221[b]	900
RBC (/mm^3)	764 ± 79	772 ± 31	694 ± 93	771 ± 93	760 ± 110	617 ± 43[b]	517
Hgb (g/dL)	12.7 ± 0.9	12.7 ± 0.5	12.8 ± 0.6	12.6 ± 0.1	12.4 ± 0.5	11.6 ± 1.0[b]	7.8
Organ weights							
Testes, absolute (mg)	291 ± 25	263 ± 32	300 ± 28	162 ± 37[b]	82 ± 13[b]	74 ± 8[b]	54
Testes, relative (%)	0.76 ± 0.08	0.68 ± 0.14	0.79 ± 0.10	0.42 ± 0.10[b]	0.22 ± 0.03[b]	0.10 ± 0.02[b]	0.15
Vesicular and coagulating glands, absolute (mg)	376 ± 59	354 ± 73	373 ± 30	361 ± 26	323 ± 66	237 ± 25[b]	258
Vesicular and coagulating glands, relative (%)	0.99 ± 0.16	0.95 ± 0.12	0.98 ± 0.06	0.94 ± 0.09	0.86 ± 0.17	0.75 ± 0.03[b]	0.77
2-Methoxyethanol Acetate							
Number of animals examined	20	5	5	5	5	5	5
Hematology							
WBC (/mm^3)	3840 ± 1572	3430 ± 849	3980 ± 990	3890 ± 1634	2940 ± 634	1030 ± 452[b]	750 ± 297[b]
Hgb (g/dL)	12.7 ± 0.9	12.3 ± 0.5	12.5 ± 0.4	12.8 ± 1.2	12.7 ± 1.3	12.0 ± 0.7	11.4 ± 0.4[c]
Organ weights							
Testes, absolute (mg)	291 ± 25	315 ± 22	270 ± 23	289 ± 56	106 ± 10[b]	73 ± 7[b]	69 ± 5[b]
Testes, relative (%)	0.76 ± 0.03	0.82 ± 0.03	0.68 ± 0.09	0.71 ± 0.12	0.27 ± 0.01[b]	0.19 ± 0.02[b]	0.19 ± 0.02[b]
Vesicular and coagulating glands, absolute (mg)	376 ± 59	369 ± 70	353 ± 53	331 ± 40	356 ± 86	328 ± 48	214 ± 26[b]
Vesicular and coagulating glands, relative (%)	0.99 ± 0.16	0.93 ± 0.17	0.89 ± 0.09	0.87 ± 0.13	0.90 ± 0.19	0.92 ± 0.15	0.67 ± 0.04[b]

[a]Mean ± SD.
[b]Significantly different from control at $p < 0.01$.
[c]Significantly different from control at $p < 0.05$.

Source: Nagano et al. (1979).

The National Toxicology Program (NTP, 1993) conducted 13-week drinking water toxicity studies in F344/N rats and B6C3F1 mice. In rats, 10 animals/sex/group were offered 0, 750-, 1500-, 3000-, 4500-, or 6000-ppm 2-ME (98% purity) in drinking water. Based on body weight and water consumption data, average doses were calculated by the study authors to be 0,

71, 165, 324, 715, or 806 mg/kg-day for males and 0, 70, 135, 297, 546, or 785 mg/kg-day for females, respectively. Rats were observed twice daily for general health. Weekly evaluations were made for clinical signs, water consumption, and body weights. Blood and urine samples were collected at Week 13. Observed hematological and clinical chemistry parameters included hematocrit, hemoglobin, and methemoglobin concentrations; total and nucleated erythrocyte, reticulocyte, platelet, and total and differential leukocyte counts; mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean cell hemoglobin concentration (MCHC); total bone marrow cellularity; and serum concentrations of urea nitrogen, creatinine, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase, and bile acids. Urinalysis included urine volume, specific gravity, and pH. At necropsy, ~38 tissues were collected from the control and high-dose groups and subjected to histopathological evaluation. Lower-dose groups were examined for histopathological changes of the bone marrow, ovary, preputial or clitoral gland, prostate gland, seminal vesicle, spleen, stomach, thymus, and uterus. Males in the ≤3000-ppm groups were examined for epididymal weights and measurement of spermatozoal parameters and sperm motility. Females in the ≤4500-ppm groups were examined for body weights, estrous cycle length, and vaginal cytology.

All rats in the 6000-ppm groups died by Week 5 (males) or 7 (females), with 8/10 males and 5/10 females in the 4500-ppm groups dying by the end of the study (NTP, 1993). No mortality was seen at ≤3000 ppm in rats of either sex. Clinical signs from observations of all rats, deemed by the study authors to be treatment-related, were tremors, diarrhea, emaciation, abnormal posture, pallor, tachypnea, hypoactivity, and comatose state (relevant doses and incidences not reported). Final mean body weights for males and females exposed to ≥1500 ppm (6000 ppm not examined due to early mortality) were statistically significantly ($p < 0.01$) decreased (10−56%) in a dose-related manner, relative to controls. Mean water consumption was decreased in male rats (11−22%) exposed to ≥3000 ppm and in female rats (13−15%) exposed to ≥1500 ppm; clinical signs of dehydration (altered posture and appearance) were seen in these groups. In males exposed to ≥750 ppm, dose-related anemia was evident at Week 3 of exposure, as determined by statistically significant decreases in hematocrit and hemoglobin (see Table 2). However, by Week 13, hemoglobin in the 750-ppm group, hematocrit in the 750- and 3000-ppm groups, and erythrocyte counts in the 750-, 1500-, and 3000-ppm groups had increased to levels that were not statistically significantly different from controls. Dose-related decreases also were observed in reticulocytes, platelets, leukocytes, segmented neutrophils, lymphocytes, and total bone marrow cellularity. MCV also exhibited dose-related decreases at all but the highest dose.

In females, Table 3 shows statistically significant anemic effects, including decreased hematocrit, hemoglobin, and erythrocytes generally were not seen in rats exposed to drinking water concentrations below 1500 ppm (NTP, 1993). Statistically significant dose-related decreases in leukocytes were seen at 750−1500 ppm. Statistically significant changes in clinical chemistry in males were transient, with dose-related reductions in albumin and alkaline phosphatase appearing at Weeks 1 or 3 in groups receiving ≥750 ppm (see Table 4). Bile acids were statistically significantly higher than controls at Week 3, with dose-related increases being observed at ≥750 ppm. However, concentrations of bile acids were similar to controls in all groups after 13 weeks. In females, statistically significant dose-related decreases in multiple clinical chemistry parameters were seen as early as Week 1 and through Week 13 in groups given ≥750 ppm (see Table 5).

Table 2. Selected Hematological Effects Observed in Male Rats Exposed to 2-Methoxyethanol in Drinking Water for 13 Weeks						
		Concentration in ppm (Dose in mg/kg-day)				
Parameter[a]	**Control**	**750 (71)**	**1500 (165)**	**3000 (324)**	**4500 (715)**	**6000 (806)**
Hematocrit (%)						
Week 1	46.1 ± 0.6^a	45.8 ± 0.5	46.2 ± 0.6	44.3 ± 0.4	45.4 ± 0.3	43.6 ± 0.5^b
Week 3	49.3 ± 0.6	45.6 ± 0.6^b	46.2 ± 0.6^b	41.6 ± 0.5^b	NT	NT
Week 13	48.1 ± 0.4	46.9 ± 0.6	45.4 ± 0.7^b	46.0 ± 0.8	31.6 ± 7.0^b	NT
Hgb (g/dL)						
Week 1	15.0 ± 0.1	14.7 ± 0.1	14.8 ± 0.2	14.3 ± 0.1^b	14.4 ± 0.2^b	13.9 ± 0.2^b
Week 3	16.0 ± 0.2	14.9 ± 0.2^b	14.9 ± 0.2^b	13.8 ± 0.1^b	NT	NT
Week 13	16.0 ± 0.2	15.5 ± 0.2	15.2 ± 0.2^b	14.9 ± 0.2^b	10.1 ± 1.9^b	NT
Erythrocytes ($10^6/\mu L$)						
Week 1	7.88 ± 0.12	7.88 ± 0.10	7.96 ± 0.12	7.60 ± 0.07	7.70 ± 0.11	7.44 ± 0.11
Week 3	8.80 ± 0.10	8.32 ± 0.14	8.47 ± 0.14	7.61 ± 0.10^b	NT	NT
Week 13	9.44 ± 0.11	9.40 ± 0.13	9.20 ± 0.13	9.08 ± 0.16	5.94 ± 1.24	NT
Reticulocytes ($10^6/\mu L$)						
Week 1	0.22 ± 0.03	0.27 ± 0.02	0.21 ± 0.02	0.12 ± 0.02	0.07 ± 0.01^b	0.05 ± 0.01^b
MCV (fL)						
Week 3	56.1 ± 0.3	54.9 ± 0.4^b	54.6 ± 0.2^c	54.8 ± 0.2^c	NT	NT
Week 13	50.9 ± 0.3	49.8 ± 0.5	49.1 ± 0.2^c	50.8 ± 0.2	53.0 ± 1.0	NT
Platelets ($10^3/\mu L$)						
Week 1	937.5 ± 31.3	864.8 ± 12.1^b	791.8 ± 13.0^c	492.5 ± 18.6^c	338.1 ± 21.0^c	276.2 ± 20.8^c
Week 3	797.7 ± 13.3	730 ± 16.5^c	568.7 ± 11.8^c	267.7 ± 7.9^c	NT	NT
Week 13	582.4 ± 12.1	612.8 ± 18.0	490.9 ± 13.5^c	401 ± 33.8^c	265 ± 53.5^c	NT
Leukocytes ($10^3/\mu L$)						
Week 1	7.87 ± 0.51	7.45 ± 0.45	7.05 ± 0.37	4.94 ± 0.29^c	3.37 ± 0.34^c	2.92 ± 0.22^c
Week 3	8.49 ± 0.40	7.68 ± 0.35	6.81 ± 0.46^c	4.81 ± 0.19^c	NT	NT
Week 13	7.49 ± 0.63	8.51 ± 0.73	6.47 ± 0.61	6.18 ± 0.54	1.80 ± 0.30^b	NT
Segmented neutrophils ($10^3/\mu L$)						
Week 1	0.96 ± 0.12	0.75 ± 0.08	1.04 ± 0.13	0.77 ± 0.12	0.51 ± 0.13^b	0.39 ± 0.05^c
Week 3	1.02 ± 0.06	1.07 ± 0.08	0.68 ± 0.08^c	0.63 ± 0.11^c	NT	NT
Week 13	1.26 ± 0.20	1.19 ± 0.14	1.06 ± 0.16	0.79 ± 0.07^b	0.25 ± 0.06^c	NT
Lymphocytes ($10^3/\mu L$)						
Week 1	6.97 ± 0.56	6.80 ± 0.38	5.71 ± 0.32	4.09 ± 0.18^c	2.78 ± 024^c	2.50 ± 0.21^c
Week 3	7.36 ± 0.43	6.47 ± 0.31	6.03 ± 0.41^b	4.14 ± 0.20^c	NT	NT
Week 13	6.09 ± 0.45	7.17 ± 0.61	5.32 ± 0.51	5.19 ± 0.45	1.51 ± 0.25^b	NT

Parameter[a]	Control	Concentration in ppm (Dose in mg/kg-day)				
		750 (71)	1500 (165)	3000 (324)	4500 (715)	6000 (806)
Total bone marrow cellularity (10^6/femur)						
Week 1	70.6 ± 2.7	NT	66.6 ± 3.2	53.5 ± 2.9[c]	32.7 ± 2.0[c]	25.5 ± 1.2[c]
Week 3	66.1 + 2.9	82.2 ± 3.6	75.1 ±3.9	53.2 ± 2.4	NT	NT
Week 13	66.0 ± 2.9	71.1 ± 3.0	58.4 ± 2.1	57.0 ± 2.2[b]	31.4 ± 12.2[c]	NT

Table 2. Selected Hematological Effects Observed in Male Rats Exposed to 2-Methoxyethanol in Drinking Water for 13 Weeks

[a]Mean ± standard error.
[b]Significantly different ($p < 0.05$) from controls by Dunn's or Shirley's test.
[c]Significantly different ($p < 0.01$) from controls by Dunn's or Shirley's test.

NT = not tested.

Source: NTP (1993).

Table 3. Selected Hematological Effects Observed in Female Rats Exposed to 2-Methoxyethanol in Drinking Water for 13 Weeks

Parameter	Control	Concentration in ppm (Dose in mg/kg-day)				
		750 (70)	1500 (135)	3000 (297)	4500 (546)	6000 (785)
Hematocrit (%)						
Week 1	46.8 ± 0.4^a	45.6 ± 0.7	44.8 ± 0.6^b	43.2 ± 0.5^c	43.7 ± 0.6^c	43.4 ± 0.7^c
Week 3	48.6 ± 0.6	48.4 ± 0.5	47.4 ± 0.6	43.1 ± 1.2^c	NT	NT
Week 13	44.5 ± 0.4	43.8 ± 0.4	42.2 ± 0.8^b	41.5 ± 0.5^c	40.7 ± 0.9^c	NT
Hgb (g/dL)						
Week 1	15.8 ± 0.1	15.4 ± 0.2	15.1 ± 0.2^b	14.5 ± 0.1^c	14.8 ± 0.2^c	14.9 ± 0.2^c
Week 3	16.0 ± 0.1	15.8 ± 0.1	15.8 ± 0.2	14.3 ± 0.2^c	NT	NT
Week 13	15.2 ± 0.1	14.8 ± 0.1^c	14.5 ± 0.2^c	13.7 ± 0.1^c	13.6 ± 0.3^c	NT
Erythrocytes ($10^6/\mu$L)						
Week 1	8.14 ± 0.09	7.94 ± 0.12	7.86 ± 0.12	7.43 ± 0.10^c	7.66 ± 0.15^c	7.57 ± 0.12^c
Week 3	8.73 ± 0.11	8.80 ± 0.12	8.85 ± 0.14	8.09 ± 0.22	NT	NT
Week 13	8.34 ± 0.09	8.30 ± 0.09	8.24 ± 0.13	8.13 ± 0.12	7.91 ± 0.18	NT
Reticulocytes ($10^6/\mu$L)						
Week 1	0.22 ± 0.02	0.15 ± 0.02^b	0.09 ± 0.00^c	0.05 ± 0.01^c	0.03 ± 0.00^c	0.03 ± 0.00^c
MCV (fL)						
Week 3	55.7 ± 0.3	55.1 ± 0.2	53.6 ± 0.3^c	53.2 ± 0.2^c	NT	NT
Week 13	53.3 ± 0.3	52.8 ± 0.2	51.3 ± 0.3^c	50.9 ± 0.3^c	51.6 ± 0.2^c	NT
MCH (pg)						
Week 13	18.3 ± 0.2	17.9 ± 0.1	17.6 ± 0.1^c	16.9 ± 0.2^c	17.3 ± 0.1^c	NT
Platelets ($10^3/\mu$L)						
Week 1	852.8 ± 19.7	775.3 ± 14.6^b	539.0 ± 12.9^c	261.6 ± 10.6^c	180.1 ± 22.3^c	159.9 ± 21.7^c
Week 3	861.4 ± 20.1	658.0 ± 11.3^c	531.1 ± 13.7^c	349.6 ± 20.7^c	NT	NT
Week 13	658.9 ± 24.3	650.6 ± 12.0	534.9 ± 25.4^c	400.7 ± 27.2^c	376.0 ± 32.0^c	NT
Leukocytes ($10^3/\mu$L)						
Week 1	9.24 ± 0.36	7.35 ± 0.35^c	5.80 ± 0.39^c	4.49 ± 0.23^c	3.51 ± 0.37^c	3.45 ± 0.30^c
Week 3	7.87 ± 0.56	7.48 ± 0.39	8.24 ± 0.61	5.36 ± 0.52^b	NT	NT
Week 13	7.14 ± 0.23	6.76 ± 0.18	5.74 ± 0.26^c	4.16 ± 0.45^c	4.62 ± 0.50^c	NT
Segmented neutrophils ($10^3/\mu$L)						
Week 1	1.07 ± 0.19	0.69 ± 0.07	0.75 ± 0.08	0.54 ± 0.06^c	0.42 ± 0.08^c	0.43 ± 0.10^c
Week 13	0.94 ± 0.14	0.97 ± 0.12	0.75 ± 0.06	0.48 ± 0.08^c	0.53 ± 0.15^b	NT
Lymphocytes ($10^3/\mu$L)						
Week 1	8.02 ± 0.31	6.55 ± 0.34^c	4.96 ± 0.36^c	3.91 ± 0.19^c	3.03 ± 0.33^c	2.95 ± 0.24^c
Week 13	6.08 ± 0.34	5.63 ± 0.12	4.80 ± 0.24^c	3.56 ± 0.43^c	4.00 ± 0.46^c	NT

Table 3. Selected Hematological Effects Observed in Female Rats Exposed to 2-Methoxyethanol in Drinking Water for 13 Weeks						
		Concentration in ppm (Dose in mg/kg-day)				
Parameter	Control	750 (70)	1500 (135)	3000 (297)	4500 (546)	6000 (785)
Total bone marrow cellularity (10^6/femur)						
Week 1	55.2 ± 2.4	NT	43.6 ± 2.0^c	25.9 ± 1.1^c	21.5 ± 1.4^c	19.9 ± 1.3^c
Week 3	46.2 ± 1.4	40.6 ± 1.8^b	34.7 ± 1.5^c	30.2 ± 2.7^c	NT	NT
Week 13	38.9 ± 1.7	45.5 ± 1.3	42.6 ± 1.8	33.0 ± 2.7	39.1 ± 2.2	NT

[a]Mean ± standard error.
[b]Significantly different ($p < 0.05$) from controls by Dunn's or Shirley's test.
[c]Significantly different ($p < 0.01$) from controls by Dunn's or Shirley's test.

NT = not tested.

Source: NTP (1993).

		Concentration in ppm (Dose in mg/kg-day)				
Parameter	**Control**	**750 (71)**	**1500 (165)**	**3000 (324)**	**4500 (715)**	**6000 (806)**
Total protein (g/dL)						
Week 13	6.6 ± 0.1^a	6.4 ± 0.1	6.2 ± 0.1^b	6.0 ± 0.1^b	5.0 ± 0.1^b	NT
Albumin (g/dL)						
Week 1	3.4 ± 0.0	3.4 ± 0.1	3.3 ± 0.1	3.2 ± 0.1^c	3.2 ± 0.1^c	3.0 ± 0.1^b
Week 3	3.7 ± 0.0	3.5 ± 0.1^c	3.6 ± 0.0^c	3.3 ± 0.1^b	NT	NT
Week 13	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.0	3.5 ± 0.0	2.8 ± 0.3^b	NT
Alkaline phosphatase (IU/L)						
Week 1	442 ± 8	401 ± 10^b	364 ± 10^b	321 ± 14^b	317 ± 18^b	308 ± 8^b
Week 3	271 ± 5	281 ± 10	238 ± 8^c	137 ± 5^b	NT	NT
Bile acids (μmol/L)						
Week 3	9.30 ± 1.56	11.44 ± 1.36^c	23.50 ± 4.66^b	33.78 ± 7.85^b	NT	NT

Table 4. Selected Clinical Chemistry Effects Observed in Male Rats Exposed to 2-Methoxyethanol in Drinking Water for 13 Weeks

[a]Mean ± standard error.
[b]Significantly different ($p < 0.01$) from controls by Dunn's or Shirley's test.
[c]Significantly different ($p < 0.05$) from controls by Dunn's or Shirley's test.

NT = not tested.

Source: NTP (1993).

Table 5. Selected Clinical Chemistry Effects Observed in Female Rats Exposed to 2-Methoxyethanol in Drinking Water for 13 Weeks

Parameter	Control	750 (70)	1500 (135)	3000 (297)	4500 (546)	6000 (785)
Urea nitrogen (mg/dL)						
Week 3	16.8 ± 0.4[a]	17.4 ± 0.7	20.3 ± 0.7[b]	23.2 ± 0.7[b]	NT	NT
Week 13	22.3 ± 1.4	19.2 ± 0.6[b]	19.0 ±1.1[b]	18.8 ± 1.1[c]	18.4 ± 1.9[c]	NT
Creatinine (mg/dL)						
Week 3	0.59 ± 0.02	0.57 ± 0.02	0.54 ± 0.02	0.52 ± 0.02[c]	NT	NT
Week 13	0.55 ± 0.02	0.51 ± 0.02	0.47 ± 0.02[b]	0.48 ± 0.04[b]	0.52 ± 0.04	NT
Total protein (g/dL)						
Week 1	6.1 ± 0.1	5.7 ± 0.1[b]	5.5 ± 0.1[b]	5.2 ± 0.1[b]	5.1 ± 0.1[b]	5.3 ± 0.1[b]
Week 3	6.0 ± 0.1	5.7 ± 0.1[c]	5.6 ± 0.1[c]	5.4 ± 0.1[b]	NT	NT
Week 13	6.6 ± 0.1	6.4 ± 0.1	6.1 ± 0.1[b]	5.9 ± 0.1[b]	5.8 ± 0.1[b]	NT
Albumin (g/dL)						
Week 1	3.4 ± 0.0	3.4 ± 0.1	3.2 ± 0.0[b]	3.1 ± 0.1[b]	3.0 ± 0.1[b]	3.1 ± 0.1[b]
Week 3	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.2 ± 0.1[b]	NT	NT
Week 13	3.79 ± 0.07	3.62 ± 0.07	3.62 ± .003	3.57 ± 0.08[c]	3.46 ± 0.09[b]	NT
Alkaline phosphatase (IU/L)						
Week 1	333 ± 7	285 ± 7[b]	257 ± 7[b]	251 ± 6[b]	227 ± 12[b]	242 ± 8[b]
Week 3	188 ± 5	175 ± 11	120 ± 5[b]	85 ± 4[b]	NT	NT
Week 13	192 ± 10	171 ± 10	157 ± 12[c]	155 ± 13[c]	137 ± 9[b]	NT
Bile acids (µmol/L)						
Week 1	6.20 ± 0.49	5.57 ± 0.57	8.88 ± 1.95	22.70 ± 4.16[b]	13.33 ± 2.46[c]	21.22 ± 3.84[b]
Week 3	11.75 ± 2.46	23.00 ± 5.21[c]	18.80 ± 2.93	31.80 ± 7.12[b]	NT	NT

[a]Mean ± standard error.
[b]Significantly different ($p < 0.01$) from controls by Dunn's or Shirley's test.
[c]Significantly different ($p < 0.05$) from controls by Dunn's or Shirley's test.

NT = not tested.

Source: NTP (1993).

Table 6 shows that statistically significant, dose-related decreases in relative thymus weights among female rats and absolute thymus weights in male and females were observed in all dose groups (NTP, 1993). The decreases from control were ~25% at 750 ppm, ranging up to ~75% at 4500 ppm. In male rats, testis weights showed a similar pattern, with statistically significant, dose-related decreases of 50−80% in absolute and relative weights at doses of 1500 to 4500 ppm. Weight changes in other organs (heart, lung, liver, and kidney) showed a pattern of decreases in absolute weights and increases in relative weights, primarily at ≥3000 ppm; the study authors attributed the findings in these organs to low body weights.

Table 6. Selected Organ Weights Measured in Rats Exposed To 2-Methoxyethanol in Drinking Water for 13 Weeks						
		Concentration in ppm (Dose in mg/kg-day)				
Parameter	**Sex**	**Control**	**750 (70−71)**	**1500 (135−165)**	**3000 (297−324)**	**4500 (546−715)**
Absolute right testis weight (g)	Male	1.398 ± 0.048^a	1.411 ± 0.019	0.603 ± 0.044^b	0.442 ± 0.032^b	0.254 ± 0.010^b
Relative right testis weight (g/g body weight)	Male	4.44 ± 0.15	4.81 ± 0.09	2.31 ± 0.14^b	2.07 ± 0.15^b	1.89 ± 0.20^c
Absolute thymus weight (g)	Male	$0.268 + 0.026$	0.198 ± 0.017^c	0.160 ± 0.016^b	0.095 ± 0.016^b	0.072 ± 0.005^b
Relative thymus weight (g/g body weight)	Male	0.85 ± 0.08	0.67 ± 0.05	0.61 ± 0.06	0.45 ± 0.07^b	0.53 ± 0.04
Absolute thymus weight (g)	Female	0.224 ± 0.010	0.180 ± 0.012^c	0.125 ± 0.010^b	0.084 ± 0.008^b	0.099 ± 0.011^b
Relative thymus weight (g/g body weight)	Female	1.19 ± 0.06	0.95 ± 0.06^b	0.74 ± 0.06^b	0.57 ± 0.06^b	0.66 ± 0.07^b

[a]Mean ± standard error
[b]Significantly different from controls ($p < 0.01$) by Shirley's test.
[c]Significantly different from controls ($p < 0.05$) by Shirley's test.

Source: NTP (1993).

Table 7 shows histopathological changes in the rat testes, which consisted of a dose-related degeneration of the germinal epithelium in the seminiferous tubules that increased in intensity from minimal at 750 ppm to marked at ≥3000 ppm (NTP, 1993). Chemical-related fibrosis of the splenic capsule was seen in male and female rats and was most prominent in animals in the 1500−4500-ppm groups. Only one (male) animal—dying by Week 5 in the 6000-ppm group—had time to develop splenic capsule fibrosis (see Table 7). The study authors associated other microscopic changes with decreased body weight gain or stress-related physiological illness. Spermatozoal measurements, including spermatid heads/g testis, sperm motility, and sperm concentration, were statistically significantly and markedly decreased for males exposed to 1500 or 3000 ppm (see Table 8). In females, mean estrous cycle length was not affected by dose. However, the incidence of rats with estrous cycles of ≥12 days increased with dose and was statistically significantly different from controls in the 3000-ppm group (see Table 8). This study identified a 13-week LOAEL of 750 ppm (70−71 mg/kg-day) for testicular lesions (degeneration of seminiferous tubules), reduced semen quality (reduced sperm concentration), and decreased thymus weights in male rats and decreased thymus weights in female rats; no NOAEL was identified in either gender.

			Concentration in ppm (Dose in mg/kg-day)				
Lesion	**Sex**	**Control**	**750 (70−71)**	**1500 (135−165)**	**3000 (297−324)**	**4500 (546−715)**	**6000 (785−806)**
Degeneration of seminiferous tubules	Male	0/10	7/10[a] (1.0)[b]	10/10[a] (2.6)	10/10[a] (4.0)	9/10[a] (4.0)	10/10[a] (4.0)
Splenic capsule fibrosis	Male	0/10	1/10 (1.0)	4/10[a] (1.5)	10/10[a] (2.2)	5/9[a] (1.2)	1/10 (1.0)
Splenic capsule fibrosis	Female	0/10	0/10	3/10 (1.0)	5/10[a] (1.2)	0/10	0/10

Table 7. Incidences of Selected Nonneoplastic Lesions Observed in Rats Exposed to 2-Methoxyethanol in Drinking Water for 13 Weeks

[a]Significantly different from controls ($p < 0.05$) by Fisher's exact test, calculated for this assessment.
[b]Parentheses indicate lesion severity averaged over all animals with lesion; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Source: NTP (1993).

Table 8. Reproductive Effects Observed in Rats Exposed to 2-Methoxyethanol in Drinking Water for 13 Weeks

			Concentration in ppm (Dose in mg/kg-day)			
Parameter	**Sex**	**Control**	**750 (70−71)**	**1500 (135−165)**	**3000 (297−324)**	**4500 (546−715)**
Spermatid heads (10^7/g testes)[a]	Male	9.14 ± 0.32	8.63 ± 0.33	1.79 ± 0.52[b]	0 ± 0[b]	NT
Spermatid heads (10^7/testes)[a]	Male	13.69 ± 0.63	12.84 ± 0.48	1.41 ± 0.50[b]	0 ± 0[b]	NT
Spermatid concentration (mean/10^{-4} mL suspension)[a]	Male	68.43 ± 3.17	64.20 ± 2.42	7.03 ± 2.51[b]	0 ± 0[b]	NT
Spermatozoal motility (%)[a]	Male	98.43 ± 0.15	97.49 ± 0.39	0 ± 0[b]	0 ± 0[b]	NT
Spermatozoal concentration (10^6/g caudal epididymal tissue)[a]	Male	755.4 ± 25.6	655.8 ± 14.1[c]	13.0 ± 3.4[b]	7.2 ± 2.2[b]	NT
Incidence of estrous cycle longer than 12 days	Female	1/10	NT	4/10	7/10[d]	3/5

[a]Mean ± standard error.
[b]Significantly different from controls ($p < 0.01$) by Shirley's test.
[c]Significantly different from controls ($p < 0.05$) by Shirley's test.
[d]Significantly different from controls ($p < 0.05$) by Fisher's exact test.

NT = not tested.

Source: NTP (1993).

Mortality was not observed in either sex at any dose (NTP, 1993). Terminal body weights of males exposed to 10,000 ppm and females exposed to 8000 or 10,000 ppm were statistically significantly ($p < 0.01$) lower than controls (25% for males, 10−20% with increasing dose for females). Water consumption was variable, but no treatment-related patterns were evident. Hematologic and clinical chemistry evaluations were not performed in mice. Males exposed to ≥8000 ppm showed statistically significantly ($p < 0.05$) increased relative weights of the heart (9−30%), kidney (25−40%), and lung (13−26%). Treated females showed increased relative weights of the hearts (21−29%) at ≥4000 ppm, kidneys (25−41%) at ≥8000 ppm, and lungs (20−30%) at ≥6000 ppm. The study authors attributed these increases in relative organ weights to low body weights. Absolute and relative testis weights were decreased (16−81%) in male mice exposed to ≥4000 ppm, while absolute and relative thymus weights were decreased in males (16−50%) exposed to ≥8000 ppm and in females (23−46%) exposed to 10,000 ppm (absolute only at 8000 ppm).

Upon histological examination, increased hematopoiesis was observed in the spleens of all groups of treated mice except males in the lowest-dose group; the severity was minimal in all groups (see Table 9) (NTP, 1993). Testicular degeneration was observed in males at 4000 ppm (3/10). However, because of the small number of rats per dose group, the increased incidence was not statistically significant until ≥6000 ppm, the concentration at which all rats exhibited this effect (see Table 9). In the adrenal gland of 100% of the female mice in all dose-groups, there was hypertrophy of the X-zone that increased in severity with dose from mild at 2000 ppm to moderate-to-marked at 10,000 ppm. Spermatozoal concentrations were statistically significantly lower than controls (reduced 26−79%) in males treated with ≥2000 ppm (see Table 10). Although sperm motility was statistically significantly decreased in the 2000-ppm males relative to controls, > 99% of sperm were judged to be motile. Thus, the statistically significant changes in sperm motility at 2000 ppm were not considered toxicologically significant. Female mice in the ≥6000-ppm groups differed significantly from controls in the relative frequency of time spent in estrous stages (see Table 10). The LOAEL in mice was 2000 ppm for decreased spermatozoal concentration and motility in male mice (295 mg/kg-day) and splenic hematopoiesis and adrenal hypertrophy in females (492 mg/kg-day); no NOAEL was identified.

The Mellon Institute (1962) studied the effects of dietary exposure to 2-ME (purity not reported) on DW albino rats. Groups of 10 rats/sex were exposed to 0, 0.01, 0.05, 0.25, or 1.25% of 2-ME in the diet for 3 months. Due to increased mortality, the 1.25%-groups were terminated early (Day 18 of exposure). Based on food consumption and body-weight data, the study authors calculated average daily doses of 7, 40, or 178 mg/kg-day for males and 8, 43, or 201 mg/kg-day for females in the 0.01-, 0.05-, and 0.25%-groups, respectively. Animals were weighed weekly, and food consumption was measured monthly. At the conclusion of exposure, animals were sacrificed, the liver and kidneys were weighed, and a gross examination of the organs was conducted. The bladders were examined for concretions, and sections of representative (unspecified) tissues were taken and fixed for examination.

Table 9. Incidences of Selected Nonneoplastic Lesions Observed in Mice Exposed to 2-Methoxyethanol in Drinking Water for 13 Weeks							
			Concentration in ppm (Dose in mg/kg-day)				
Lesion	**Sex**	**Control**	**2000 (295−492)**	**4000 (529−902)**	**6000 (765−1194)**	**8000 (992−1489)**	**10,000 (1367−1839)**
Splenic hematopoiesis	Male	0/10	0/10	10/10[a] (1.0)[b]	9/10[a] (1.0)	9/10[a] (1.0)	10/10[a] (1.1)
Testicular degeneration	Male	0/10	0/10	3/10 (1.0)	10/10[a] (3.0)	10/10[a] (4.0)	10/10[a] (4.0)
Splenic hematopoiesis	Female	0/10	5/10[a] (1.0)	10/10[a] (1.0)	8/10[a] (1.1)	9/10[a] (1.0)	10/10[a] (1.0)
Adrenal X-zone hypertrophy	Female	0/10	10/10[a] (2.1)	9/9[a] (2.9)	10/10[a] (3.1)	10/10[a] (3.7)	10/10[a] (3.6)

[a]Significantly different from controls ($p < 0.05$) by Fisher's exact test, performed for this assessment.
[b]Parentheses indicate lesion severity averaged over all animals with lesions; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Source: NTP (1993).

Table 10. Reproductive Effects Observed in Mice Exposed to 2-Methoxyethanol in Drinking Water for 13 Weeks					
			Concentration in ppm (Dose in mg/kg-day)		
Parameter	**Sex**	**Control**	**2000 (295)**	**4000 (529)**	**6000 (765)**
Spermatid heads (10^7/g testes)	Male	19.44 ± 0.63[a]	19.49 ± 0.69	16.79 ± 0.95[b]	1.49 ± 0.58[c]
Spermatid heads (10^7/testes)	Male	2.22 ± 0.08	2.21 ± 0.11	1.63 ± 0.11[c]	0.04 ± 0.01[c]
Spermatid concentration (mean/10^{-4} mL suspension)	Male	69.43 ± 2.67	69.18 ± 3.32	50.78 ± 3.29[c]	1.20 ± 0.46[c]
Spermatozoal motility (%)	Male	99.29 ± 0.07	99.06 ± 0.08[b]	98.93 ± 0.24	0 ± 0[c]
Spermatozoal concentration (10^6/g caudal epididymal tissue)	Male	1587.8 ± 69.0	1181.0 ± 56.3[c]	1077.4 ± 38.7[c]	335.9 ± 40.1[c]
		Control	**6000 (1194)**	**8000 (1489)**	**10,000 (1839)**
Estrous cycle length (days)	Female	4.60 ± 0.22	7.17 ± 0.83[b]	5.63 ± 0.47[b]	8.50 ± 1.50[b]

[a]Mean ± standard error.
[b]Significantly different from controls ($p < 0.05$) by Shirley's test.
[c]Significantly different from controls ($p < 0.01$) by Shirley's test.

Source: NTP (1993).

In the 1.25% (high-dose) group, three males and five females died during the first 3 weeks of dosing, body weights declined from preexposure levels (weights not reported), and food consumption was less than half of control consumption rates (Mellon Institute, 1962). Surviving animals in the high-dose group were sacrificed on Day 18 of dosing for tissue examination. Mortality also occurred in the 0.01% (low-dose) group (one male and one female dying on Days 19 and 84, respectively) and the 0.25%-group (two males and one female dying on Days 31−76). There were no deaths in the 0.05%-group. Deaths observed at 0.01 and 0.25% were not treatment related, as determined by the study authors, because gross examination of the dead animals from these groups revealed peritonitis and/or lung infection in all cases, while treatment-related mortality in the high-dose group was associated with starvation and did not involve the lungs. Mean body-weight gain was statistically significantly ($p < 0.05$) decreased relative to controls in the 0.05% (11% below controls) and 0.25% (34% below controls) groups for male rats and in the 0.25% (37% below controls) group of female rats. A transient decrease in weight gain was observed at 1 and 2 months in the 0.01% male rats, but this decrease was no longer statistically significant at study termination. Food consumption by male and female rats in the 0.25%-groups was statistically significantly decreased ($p < 0.05$) but not in rats at lower doses. No changes in liver or kidney weights or histopathology relative to controls were seen at exposure concentrations of ≤0.25% in either sex. In the 1.25% animals that were sacrificed early, a marked testicular atrophy was evident, but this effect was not seen in the lower-exposure groups. This study identified a 3-month NOAEL of 0.01% (7 mg/kg-day) and a LOAEL of 0.05% (40 mg/kg-day) for decreased body-weight gain in male rats.

Reproductive Studies
Foster et al. (1984, 1983) reported on the testicular effects of 2-ME in groups of 6 male Sprague-Dawley rats administered gavage doses of 0, 50, 100, 250, or 500 mg/kg-day for up to 11 days. To observe progression of effects on spermatocyte development, groups of six rats were sacrificed at 6 and 24 hours after the initial dosing, and additional groups that received repeated daily doses were sacrificed after 2, 4, 7, and 11 days. At sacrifice, the testes, seminal vesicles, prostate, and liver were removed, weighed, and subjected to histopathological examination. In a second study to determine the extent of recovery from testicular effects after cessation of treatments, groups of 24 rats were gavaged with 0 or 500 mg/kg-day for 4 days. Groups of six rats were sacrificed at 0, 2, 4, or 8 weeks after the last dosing and were examined as described above. Intergroup differences in organ weights were compared statistically. Incidence rates of histopathological lesions were not reported.

Body weights of treated groups reportedly did not differ statistically significantly from controls at any time point (data not shown) (Foster et al., 1984, 1983). Relative testes weights were statistically significantly decreased in the 500-mg/kg-day group starting on Day 2 and in the 250-mg/kg-day group starting on Day 7 (see Table 11). On Day 11, the reduction from controls was close to 50% in both dose groups. The relative weights of the prostate and seminal vesicles were not consistently statistically different from controls, but both tissues showed reductions of ~30% versus controls in the 500-mg/kg-day group on Day 11. Relative liver weights were decreased primarily in the 250- and 500-mg/kg-day groups for much of the study (reductions of 10−20%) but did not differ from controls on Day 11. No histological effects were observed at 50 mg/kg-day, but degeneration of pachytene spermatocytes was seen at ≥100 mg/kg-day at 24 hours. Continued dosing resulted in progressive depletion of spermatocytes, as well as maturation depletion of early spermatids. After 4 and 7 days at 500

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and 250 mg/kg-day, respectively, chromatin margination was seen in spermatid nuclei. By Day 11, no spermatids or late spermatocytes were found at ≥250 mg/kg-day. Because early spermatocyte degeneration was not consistently observed at 24 hours, an additional group of six rats was gavaged with 500 mg/kg-day and sacrificed at 16 hours. In these animals, mitochondrial swelling, vacuolation, and early nuclear chromatin condensation were observed in the spermatocytes. In the recovery study (see Table 12), reduction in relative testicular weights after 4 weeks of cessation from exposure was similar to that observed at the end of the dose-response study (see Table 11). However, by Week 8, the relative tissue weights had recovered to that of controls. This study identified a LOAEL of 100 mg/kg-day, with an associated NOAEL of 50 mg/kg-day, for spermatocyte degeneration in rats.

NTP (1990) investigated the reproductive and developmental effects of 2-ME in the drinking water of two generations of rats. In the first part of this study, 20 pairs (exposed groups) or 40 pairs (control group) of Sprague-Dawley rats were provided with drinking water containing 0, 0.01, 0.03, or 0.1% by volume (w/v) 2-ME (>99% pure) during a 6-week cohabitation period. During this period, the F0 rats typically delivered and weaned one F1 litter and remated for production of a second F1 litter. The investigators estimated doses of 0, 8.81, 23.56, or 75.77 mg/kg-day for males and 0, 12.65, 36.30, or 122.10 mg/kg-day for females.

In the second part of this study, control rats were cross-mated with rats provided with drinking water containing 0.03% 2-ME. The investigators estimated doses of 0 or 16.71 mg/kg-day for the males and 0 or 31.29 mg/kg-day for the females.

In the final part of this study, reproductive performance was studied in the second generation (F1) breeding pairs from the first part of the study provided with drinking water containing 0, 0.01, or 0.03% 2-ME. The investigators estimated doses of 0, 9.07, or 27.15 mg/kg-day for the males and 0, 14.96, or 40.78 mg/kg-day for the females. Experimental observations for all parts of the study (F0 and F1 generations) included clinical signs, body weights, organ weights (liver, kidney, ovary, seminal vesicles, testis, cauda, epididymis, prostate gland), drinking water consumption, semen quality, testicular histology (in controls, 0.01%- and 0.03%-groups), fertility ratio (litters/breeding pairs), litters/pair, live pups/litter, and pup viability, sex, and birth weights.

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Tissue	Dose in mg/kg-day				
	0 (*n* = 6)	50 (*n* = 6)	100 (*n* = 6)	250 (*n* = 6)	500 (*n* = 6)
Testes[a]					
6 Hours	100.0 ± 1.2[b]	101.6 ± 4.5	96.5 ± 2.4	102.8 ± 4.9	93.4 ± 3.3
24 Hours	100.0 ± 7.0	108.7 ± 4.3	122.3 ± 3.8[c]	99.7 ± 2.2	94.1 ± 2.6
Day 2	100.0 ± 3.4	99.6 ± 2.8	105.5 ± 2.9	96.5 ± 2.7	83.5 ± 6.5[d]
Day 4	100.0 ± 2.8	95.3 ± 1.8	88.3 ± 2.8	89.9 ± 4.5	76.8 ± 5.3[d]
Day 7	100.0 ± 3.9	105.3 ± 2.5	102.5 ± 3.2	81.9 ± 1.8[d]	62.4 ± 3.1[d]
Day 11	100.0 ± 2.8	96.5 ± 1.7	88.0 ± 3.4	54.7 ± 6.5[d]	52.1 ± 1.7[d]
Prostate					
6 Hours	100.0 ± 11.4	124.3 ± 8.6	114.3 ± 10.0	85.6 ± 4.4	93.3 ± 8.9
24 Hours	100.0 ± 11.1	101.4 ± 8.3	107.0 ± 9.7	92.8 ± 11.6	101.4 ± 14.5
Day 2	100.0 ± 5.5	88.0 ± 4.0	93.3 ± 9.3	92.3 ± 6.4	74.4 ± 6.4[c]
Day 4	100.0 ± 2.8	100.0 ± 6.9	94.4 ± 2.8	86.7 ± 14.7	94.7 ± 6.7
Day 7	100.0 ± 7.4	100.0 ± 5.9	95.6 ± 10.2	84.7 ± 6.9	83.3 ± 4.2
Day 11	100.0 ± 9.5	108.0 ± 7.2	96.7 ± 4.5	89.2 ± 9.5	68.9 ± 4.1[c]
Seminal vesicles					
6 Hours	100.0 ± 17.8	132.1 ± 14.2	139.3 ± 21.4	100.9 ± 10.2	85.0 ± 8.4
24 Hours	100.0 ± 12.8	105.1 ± 12.8	110.3 ± 5.1	97.4 ± 10.5	105.3 ± 5.2
Day 2	100.0 ± 8.8	111.8 ± 5.9	114.7 ± 11.8	82.8 ± 5.1	71.7 ± 16.2
Day 4	100.0 ± 6.0	76.0 ± 4.0	66.0 ± 6.0[c]	75.0 ± 6.8	93.2 ± 11.4
Day 7	100.0 ± 10.0	115.0 ± 7.5	102.5 ± 7.5	118.8 ± 21.7	66.7 ± 4.3
Day 11	100.0 ± 8.1	127.5 ± 16.4	107.1 ± 12.7	80.3 ± 4.0	71.1 ± 6.6
Liver					
6 Hours	100.0 ± 1.8	96.1 ± 2.0	94.5 ± 1.6	89.5 ± 3.8[c]	95.5 ± 1.3
24 Hours	100.0 ± 1.7	93.9 ± 0.9	89.4 ± 3.3[c]	90.3 ± 3.2[c]	88.1 ± 2.8[c]
Day 2	100.0 ± 3.3	101.0 ± 1.8	95.7 ± 2.5	93.8 ± 1.9	88.0 ± 3.7[c]
Day 4	100.0 ± 2.4	100.0 ± 1.6	91.8 ± 3.8	81.0 ± 1.0[d]	79.0 ± 2.4[d]
Day 7	100.0 ± 2.2	106.5 ± 2.5	99.7 ± 1.7	85.1 ± 1.2[d]	91.2 ± 2.5[c]
Day 11	100.0 ± 2.4	97.4 ± 2.4	92.7 ± 1.8	94.8 ± 4.7	101.4 ± 3.8

Table 11. Relative Organ Weights Observed in Male Rats Administered Gavage Doses of 2-Methoxyethanol for up to 11 Days

[a]g/100 g body weight.
[b]Mean ± standard error.
[c]Significantly different (*p* < 0.05) from controls by Dunnett's test.
[d]Significantly different (*p* < 0.01) from controls by Dunnett's test.

Sources: Foster et al. (1984, 1983).

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Table 12. Relative Organ Weights Observed in Male Rats Administered Gavage Doses of 2-Methoxyethanol for 4 Days Followed by Recovery of up to 8 Weeks		
Tissue	**Control ($n = 6$)**	**500 mg/kg-day ($n = 6$)**
Testes[a]		
0 Weeks	0.985 ± 0.028[b]	0.752 ± 0.031[c]
2 Weeks	0.473 ± 0.017	0.221 ± 0.01[c]
4 Weeks	0.448 ± 0.010	0.255 ± 0.019[c]
8 Weeks	0.690 ± 0.038	0.643 ± 0.037
Prostate		
0 Weeks	0.072 ± 0.002	0.073 ± 0.002
2 Weeks	0.077 ± 0.007	0.081 ± 0.006
4 Weeks	0.109 ± 0.009	0.080 ± 0.009
8 Weeks	0.114 ± 0.010	0.105 ± 0.007
Seminal vesicles		
0 Weeks	0.050 ± 0.003	0.031 ± 0.004[d]
2 Weeks	0.153 ± 0.010	0.127 ± 0.009
4 Weeks	0.205 ± 0.020	0.231 ± 0.007
8 Weeks	0.179 ± 0.036	0.289 ± 0.026[e]
Liver		
0 Weeks	4.821 ± 0.115	3.850 ± 0.061[c]
2 Weeks	4.800 ± 0.156	4.760 ± 0.067
4 Weeks	4.368 ± 0.171	4.062 ± 0.084
8 Weeks	3.675 ± 0.095	3.651 ± 0.069

[a]g/100 g body weight.
[b]Mean ± standard error.
[c]Significantly different ($p < 0.001$) from controls by Student's *t*-test.
[d]Significantly different ($p < 0.01$) from controls by Student's *t*-test.
[e]Significantly different ($p < 0.05$) from controls by Student's *t*-test.

Sources: Foster et al. (1984, 1983).

NTP (1990) observed profound effects on reproduction. Male rats exposed to 0.1% had decreased sperm concentrations and motility, and increased percentage of abnormal sperm (see Table 13). Only one litter was born to F0 rats treated with 0.1% 2-ME; F0 rats treated with 0.03% 2-ME showed statistically significant decreases in live pups/litter, pup viability, and pup body weights (see Table 14), as well as an increase in cumulative days to litter (see Table 15). There were no effects on reproduction in F0 rats provided drinking water containing 0.01%. In the cross-mated pairs (controls and 0.03% treated animals), there were no effects on mating or fertility. However, reproductive performance was affected in the mating of 0.03% males with control females, as shown by a statistically significant decrease in proportion of pups born alive.

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A similar, but nonsignificant, reduction in the same endpoint was seen in the mating of 0.03% females with control males. At necropsy, body weights were statistically significantly reduced 15% in the 0.1% males, and the weights of the individual organs were all statistically significantly reduced as well. In the 0.03% males, body weights were reduced only slightly from controls (3%, nonsignificant), but organ weights (adjusted for body weights by analysis of covariance) were statistically significantly reduced by 7−9% for liver, seminal vesicles, cauda, and epididymis. Necropsy body and organ weights were not reduced in the 0.01% males or in the F0 females at any dose. Testicular histology was comparable between control, 0.01-, and 0.03%-groups of F0 males.

Table 13. Sperm Parameters of F0 Male Rats Given 2-Methoxyethanol in Drinking Water				
Sperm Parameter	**Control (0)**	**0.01% (9 mg/kg-day)**	**0.03% (24 mg/kg-day)**	**0.1% (76 mg/kg-day)**
Sperm motility (%)	81.8 ± 1.8 (20)[a]	83.7 ± 2.3 (20)	84.5 ± 4.6[b] (20)	40.8 ± 10.4[b,c] (16)
Sperm concentration (10^6/g caudal tissue)	492.8 ± 24.5 (20)	465.8 ± 18.5 (20)	425.0 ± 28.9 (20)	186.3 ± 50.3[b] (20)
Abnormal Sperm (%)	1.01 ± 0.13 (20)	0.82 ± 0.12 (20)	1.04 ± 0.15[d] (19)	6.22 ± 1.76[e] (9)

[a]Mean ± standard error (*n*).
[b]Significantly different ($p < 0.05$) from controls.
[c]A few dead sperm were observed in both the right and left cauda in 4/20 rats.
[d]Few intact sperm observed in 1/20 rats.
[e]Few or no intact sperm observed in 11/20 rats.

Source: NTP (1990).

Table 14. Reproductive Effects in F0 Female Rats Given 2-Methoxyethanol in Drinking Water				
Reproduction Parameter	**Control (0)**	**0.01% (9−13 mg/kg-day)**	**0.03% (24−36 mg/kg-day)**	**0.1% (76−122 mg/kg-day)**
Fertility (number fertile/number cohabited)	38/38	20/20	16/18	1/20[a]
Litters/breeding pair	3.79 ± 0.19[b]	4.25 ± 0.30	3.88 ± 0.34	1.00 ± 0
Proportion of live pup births	0.95± 0.01	0.97 ± 0.01	0.74 ± 0.07[a]	0.89 ± 0
Proportion of pups surviving to PND 4 (males)	0.99 ± 0.01	0.99 ± 0.01	0.53 ± 0.11[a]	NT
Proportion of pups surviving to PND 4 (females)	0.97 ± 0.01	0.98 ± 0.01	0.69 ± 0.10[a]	NT
Pup body weight at PND 21 (g) (males)	51.93 ± 1.28	51.83 ± 0.88	42.83 ± 1.89[a]	NT
Pup body weight at PND 21 (g) (females)	49.79 ± 1.16	49.92 ± 1.07	40.59 ± 1.39[a]	NT

[a]Significantly different ($p < 0.05$) from controls.
[b]Mean ± standard error.

NT = not tested.

Source: NTP (1990).

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Table 15. Cumulative Days to Litter for F0 Rats Given 2-Methoxyethanol in Drinking Water

Treatment Group	Control (0)	0.01% (9−13 mg/kg-day)	0.03% (24−36 mg/kg-day)	0.1% (76−122 mg/kg-day)
Litter 1	27.3 ± 1.2^a	27.2 ± 1.6	35.4 ± 3.4^b	151.0 ± 0
Litter 2	53.5 ± 2.5	52.6 ± 3.5	69.8 ± 8.1^b	NT
Litter 3	113.0 ± 1.9	112.5 ± 0.3	121.3 ± 4.0	NT
Litter 4	138.6 ± 2.4	138.9 ± 2.1	149.1 ± 4.2^b	NT
Litter 5	157.7 ± 1.7	159.6 ± 1.2	160.0 ± 1.2	NT

[a]Mean ± standard error.
[b]Significantly different ($p < 0.05$) from controls.

NT = not tested.

Source: NTP (1990).

In the F1 matings, the proportion of live pups sired by the 0.03%-group males (95%) was statistically significantly less than controls (99%) (NTP, 1990). There were no effects on litter parameters in F1 rats provided the 0.01% concentration. Sperm concentration and motility were statistically significantly decreased among F1 male rats in the 0.01% group, and the percent of abnormal sperm was increased at 0.03% (see Table 16). Necropsy body and organ weights were statistically significantly reduced in the 0.03% F1 males and females but not the 0.01% F1 males or females. The incidence of testicular degeneration in the F1 males was 2/10 (minimal severity), 7/10 (minimal-mild severity), and 5/10 (minimal-moderate) in the control, 0.01-, and 0.03%-groups, respectively. The incidence in the 0.01%-group was statistically significantly higher than controls; though incidence also increased at 0.03%, the increase at this concentration did not quite achieve statistical significance. The observation of effects at lower doses in the F1 than in the F0 generation suggests that immature rats are more susceptible than mature rats to the reproductive effects of 2-ME. The 0.01% concentration, 9.07 mg/kg-day in the F1 males, is a LOAEL for testicular degeneration and reduced sperm density in male rats. A NOAEL in male rats was not identified.

NTP (1989, 1988a,b) performed two-generation reproductive assessment studies in three strains of mice (C57BL/6, CD-1, and C3H) using a continuous breeding protocol similar to that used for rats (NTP, 1990). Drinking water concentrations of 2-ME (purity >99%) used in the mouse studies were 0, 0.03, 0.1, or 0.3% by weight (w/v). The period of cohabitation was generally sufficient to allow delivery of five consecutive litters. Usually offspring from the fifth litter were used for evaluation of reproductive performance in the F1 generation. Male and female F1 mice were exposed to 0, 0.03, or 0.1% 2-ME in drinking water. Exposure began 7 days prior to being paired for 14 weeks of cohabitation. F1 animals were mated at 74 ± 10 days of age. They were weighed at weaning, first day of cohabitation, and weekly thereafter. Reproductive measurements included fertility index, number of litters/mating pair, proportion of pups born alive, and pup survival and body-weight gain to Day 21. At necropsy, liver, kidney, and right ovaries (females) or liver, kidney, seminal vesicle, right testis, right cauda, epididymis, and prostate gland (males) were weighed. F0 and F1 males were examined for semen quality and testicular histopathology.

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Table 16. Reproductive Effects in F1 Male Rats Given 2-Methoxyethanol in Drinking Water

Sperm Parameter	Control (0)	0.01% (9 mg/kg-day)	0.03% (27 mg/kg-day)
Sperm motility (%)	84.30 ± 1.34 (20)[a]	87.25 ± 1.48 (19)[b d]	77.87 ± 4.38 (20)[c d]
Sperm density (10^6/g caudal tissue)	640.56 ± 31.72 (20)	532.03 ± 39.06 (20)[d]	496.29 ± 52.62 (20)[d]
Abnormal sperm[a] (%)	0.53 ± 0.06 (20)	0.68 ± 0.09 (19)[b]	0.94 ± 0.10 (17)[c d]
Degeneration of seminiferous tubules	2/10	7/10[e]	5/10

[a]Mean ± standard error (*n*).
[b]No sperm observed in either the right or left cauda for 1/20 rats.
[c]No sperm observed in right cauda of 3/20 rats.
[d]Significantly different ($p < 0.05$) from controls.
[e]Significantly different ($p < 0.05$) from controls by Fisher's exact test (one-tailed), performed for this review.

Source: NTP (1990).

NTP (1989) estimated doses of 0, 64, 219, or 636 mg/kg-day for the male C3H mice and 0, 63, 235, or 645 mg/kg-day for the females, corresponding to drinking water concentrations of 0, 0.03, 0.1, or 0.3% 2-ME, respectively. A reduction in the fertility index was exhibited, with F0 mice receiving 0.3% 2-ME producing no litters (see Table 17). Mean necropsy body weights were statistically significantly reduced (by 7−8%), relative to controls, for the 0.3% males and females. These same males had statistically significantly lower testis (~50%), cauda (~13%), and epididymis (~25%) weights, compared to controls (with or without adjustment for body weights by analysis of covariance). Sperm density and motility were statistically significantly lowered, while the percentage of morphologically abnormal sperm was increased in the 0.3% males. Sperm motility was also statistically significantly decreased in the 0.1% males. Further, the number of pups born alive and the body weights of male pups on Postnatal Day (PND) 4 were statistically significantly reduced for the 0.1% F0 mice (see Table 17). F0 males in the 0.3%-group had statistically significantly higher incidences of seminiferous tubule degeneration than controls (see Table 17), with lesion severity ranging from moderate to severe (compared to minimal lesions in the controls). Treated males also exhibited an accumulation of sloughed cells and degeneration of ductal epithelium in the epididymis.

Table 17. Reproductive Effects Observed in Two Generations of Mice Exposed to 2-Methoxyethanol in Drinking Water					
Parameter	**Sex**	**Control (0)**	**0.03% (63−64 mg/kg-day)**	**0.1% (219−235 mg/kg-day)**	**0.3% (636−645 mg/kg-day)**
F0 mice					
Fertility index (No. fertile/No. cohabitated × 100)	Both	93%	89%	84%	0%[a]
Pups born alive (%)	Both	81%	78%	52%[a]	NT
Male pup body weight at Day 4 (g)[b]	Male	3.45 ± 0.13	3.57 ± 0.12	2.79 ± 0.02[a]	NT
Sperm motility (%)	Male	92%	93%	85%[a]	26%[a]
Sperm density (10^6 cells)[b]	Male	1298 ± 54[a]	1323 ± 63	1216 ± 86	518 ± 156[a]
Morphologically abnormal sperm (%)	Male	6%	7%	8%	50%
Seminiferous tubule degeneration	Male	19/30	NE	NE	30/30[a]
F1 mice					
Fertility index (No. fertile/No. cohabitated × 100)	Both	73%	87%	0%[a]	NT
Sperm density (10^6 cells)[b]	Male	1596 ± 128[a]	1520 ± 122	761 ± 322[a]	NT
Morphologically abnormal sperm (%)	Male	4%	4%	25%[a]	NT
Seminiferous tubule degeneration[c]	Male	6/11 (minimal)	10/15 (moderate)	4/4 (severe)	NT

[a]Significantly different from controls ($p < 0.05$).
[b]Mean ± standard error.
[c]Relative severity described in parentheses

NE = not examined; NT = not tested: no litters at this dose

Source: NTP (1989).

   F1 C3H mice exposed to 0.1% 2-ME produced no litters (see Table 17); no effects on 0.03% F1 litters or pups were observed (NTP, 1989). Mean necropsy body weights in F1 males from the 0.1%-group were 15% lower than in controls. This was not reported to be statistically significant by the researchers, although a two-tailed *t*-test conducted for this review showed a statistically significant difference from controls ($p = 0.016$). The absolute weights of reproductive organs were also decreased in the 0.1% males, including testis, cauda, seminal vesicles, and epididymis. After adjustment for body weights by analysis of covariance, only weights of the cauda and epididymis were statistically significantly reduced. Weights of the liver and kidney were increased after adjustment, but this was secondary to the decreases in body weights; absolute weights of these organs were slightly lower than in controls. Mean necropsy body weights in the 0.03% F1 males did not differ from controls, and there were no changes in absolute organ weights in this group. There was a statistically significant increase in adjusted kidney weights in the 0.03% F1 males, but the change from controls was small (+4.8%, <1 standard deviation [SD]) and was not accompanied by any corresponding effect on absolute kidney weights, suggesting that this was not a biologically relevant change. In F1 females at necropsy, body weights were statistically significantly reduced by 23% in the 0.1%-group, but

there was no difference from controls in the 0.03%-group. Organ weights in F1 females were reduced in the 0.1%-group but did not differ from controls after adjustment for body weights. F1 males in the 0.1%-group had statistically significantly decreased sperm counts and increased occurrences of morphologically abnormal sperm (see Table 17). Seminiferous tubule degeneration was observed in 55, 67, and 100% of the males examined in the control, 0.03-, and 0.1%-groups, respectively; although none of these changes achieved statistical significance because of the high incidence among controls, they appeared to show a dose-response trend (see Table 17). Interstitial cell hyperplasia was seen in treated animals but not in controls. The 0.03% dose (64 mg/kg-day) is a NOAEL, and the 0.1% dose (219 mg/kg-day) is a LOAEL for C3H mice based on testicular lesions, reduced sperm quality, fetal toxicity, and impaired fertility.

In the study of C57BL/6 mice, NTP (1988a) estimated doses of 0, 53, 170, or 505 mg/kg-day for the males and 0, 54, 174, or 543 mg/kg-day for the females, corresponding to drinking water concentrations of 0, 0.03, 0.1, and 0.3% 2-ME, respectively. Table 18 reports selected reproductive effects. Treatment of F0 mice with 0.3% 2-ME statistically significantly decreased the fertility index, resulting in no live pups. Treatment with 0.3% also extended gestation, with cumulative days to first and second litters of 57 and 71 days, respectively, compared to 26 and 54 days in controls (data not shown). In the 0.1%-group, the percentages of pups born alive and pup viability were statistically significantly decreased. Pup survival also was decreased at the low dose of 0.03%. Sperm motility and density were statistically significantly reduced in the 0.3%-group; the appearance of abnormal sperm was statistically significantly increased in both the 0.1- and 0.3%-groups (see Table 18). Table 19 shows that F0 males in the 0.3%-group had statistically significant decreases in body and organ weights at necropsy; weights of the testis (29%) and epididymis (11%) remained statistically significantly decreased after adjustment for body weights, by analysis of covariance. F0 females had no statistically significant changes in body or organ weights associated with treatment (see Table 19). Mild degeneration of the seminiferous tubules was seen in 57% of control F0 males, while the 0.3% males exhibited 100% moderate-to-severe occurrence of this lesion (see Table 18). Sloughed cell accumulation was also observed in treated males.

In F1 C57BL/6 mice, the fertility index was reduced in a dose-related manner, with no litters produced by the 0.1%-group (NTP, 1988a). Dose-related increases in morphologically abnormal sperm and decreases in sperm density were observed, including a statistically significant decrease in sperm density at 0.03% (see Table 18). Table 20 shows that, at necropsy, there were no differences from controls in F1 female or male body weights. In females, ovary weights (see Table 20) were statistically significantly reduced in the 0.1%-group (~50%), with or without adjustment for body weights, by analysis of covariance. In the males, the most notable organ-weight changes were decreases in prostate weights (~35%, with or without adjustment for body weights) in the 0.1%-group (see Table 19). Small decreases in seminal vesicle weights were found in both the 0.1% (15%) and 0.03% (6%) groups after adjustment for body weights. There was also an increase in adjusted kidney weights at 0.1% (see Table 20). Seminiferous tubule degeneration of minimal severity was observed in control and treated groups of F1 males. Seminiferous tubule degeneration occurred in 60% of controls, 70% of the 0.03%-group, and 100% of the 0.1%-group (not statistically significant [$p > 0.05$] by Fisher's exact test, calculated for this assessment) (see Table 18). The severity of the lesions was not different between groups. The low dose of 0.03% (53 mg/kg-day) is a LOAEL for mice based on reductions in sperm quality and pup viability. A NOAEL was not identified.

2-Methoxyethanol
                                        2-Methoxyethanol Acetate

Parameter	Sex	Control (0)	0.03% (53−54 mg/kg-day)	0.1% (170−174 mg/kg-day)	0.3% (505−543 mg/kg-day)
F0 mice					
Fertility index (number fertile/number cohabitated × 100)	Both	85%	93%	86%	25%[a]
Pups born alive (%)	Both	92%	91%	82%[a]	0%[a]
Male pup survival to Day 4 (%)	Male	85%	53%[a]	39%[a]	NT
Female pup survival to Day 4 (%)	Female	87%	61%	31%[a]	NT
Sperm motility (%)	Male	91%	92%	91%	61%[a]
Sperm density (10^6)[b]	Male	1847 ± 110[a]	1540 ± 91	1633 ± 56	1221 ± 101[a]
Morphologically abnormal sperm (%)	Male	30%	32%	38%[a]	96%[a]
Seminiferous tubule degeneration	Male	16/30	NE	NE	30/30[a]
F1 mice					
Fertility index (number fertile/number cohabitated × 100)	Both	70%	50%	0%[a]	NT
Pups born alive (%)	Both	94%	77%	NT	NT
Sperm density (10^6)[b]	Male	1776 ± 70	1590 ± 76[a]	1379 ± 372	NT
Morphologically abnormal sperm (%)	Male	26%	28%	44%[a]	NT
Seminiferous tubule degeneration	Male	12/20	14/20	6/6	NT

Table 18. Reproductive Effects Observed in Two Generations of Mice Exposed to 2-Methoxyethanol in Drinking Water

[a]Significantly different from controls ($p < 0.05$).
[b]Mean ± standard error.

NE = not examined; NT = not tested: no litters at this dose.

Source: NTP (1988a).

Table 19. Body and Organ Weights[a] in F0 Mice Following Treatment with 2-Methoxyethanol in Drinking Water				
	Dose (mg/kg-day)			
Parameter	0 (Control)	0.03% (53−54 mg/kg-day)	0.1% (170−174 mg/kg-day)	0.3% (505−543 mg/kg-day)
Females				
Body (g)	28.916 ± 0.359(27)[b]	30.091 ± 0.597(30)	28.912 ± 0.349(29)	28.102 ± 0.471(30)
Rt. Ovary (mg)	5.774 ± 0.215(27)	5.853 ± 0.295(30)	6.072 ± 0.357(29)	6.030 ± 0.236(30)
Liver (g)	1.591 ± 0.046(27)	1.607 ± 0.049(30)	1.634 ± 0.029(29)	1.543 ± 0.035(30)
Kidneys (g)[c]	0.410 ± 0.007(27)	0.418 ± 0.007(30)	0.420 ± 0.006(29)	0.410 ± 0.005(30)
Males				
Body (g)	33.178 ± 0.657(29)[b]	31.681 ± 0.647(29)	31.254 ± 0.645(30)	30.094 ± 0.693(28)[d]
Liver (g)	1.667 ± 0.036(29)	1.535 ± 0.035(29)[d]	1.689 ± 0.046(30)	1.590 ± 0.036(28)
Kidneys (g)[c]	0.517 ± 0.008(29)	0.490 ± 0.008(29)[d]	0.491 ± 0.009(30)	0.473 ± 0.010(28)[d]
Seminal Vesicles (g)	0.493 ± 0.013(29)	0.495 ± 0.013(29)	0.469 ± 0.015(30)	0.453 ± 0.012(28)[d]
R. Testis (g)	0.111 ± 0.002(29)	0.107 ± 0.002(29)	0.106 ± 0.002(30)	0.075 ± 0.004(28)[d]
R. Cauda (mg)	13.979 ± 0.343(29)	14.307 ± 0.992(29)	13.360 ± 0.358(30)	12.832 ± 0.410(28)[d]
R. Epididymis (mg)	41.617 ± 0.665(29)	40.428 ± 0.635(29)	39.220 ± 0.752(30)[d]	35.939 ± 0.764(28)[d]
Prostate Gland (mg)	14.903 ± 0.700(29)	15.324 ± 0.858(29)	13.003 ± 0.719(30)	15.789 ± 0.751(28)

[a]Mean weights ± standard error.
[b]Number of animals providing the data indicated in parenthesis.
[c]Kidneys were weighed with the adrenal glands attached.
[d]Significantly different ($p < 0.05$) from the control group.

Source: NTP (1988a)

Table 20. Body and Organ Weights[a] in F1 Mice Following Treatment with 2-Methoxyethanol in Drinking Water			
Treatment Group			
Parameter	0 (Control)	0.03% (53−54 mg/kg-day)	0.1% (170−174 mg/kg-day)
Females			
Body (g)	24.451 ± 0.240(20)[b]	24.803 ± 0.359(18)	23.990 ± 0.826(06)
Rt. Ovary (mg)	6.320 ± 0.383(20)	5.856 ± 0.329(18)	3.017 ± 0.347(06)[c]
Liver (g)	1.428 ± 0.033(20)	1.510 ± 0.053(18)	1.303 ± 0.087(06)
Kidneys (g)[d]	0.352 ± 0.005(20)	0.362 ± 0.007(18)	0.434 ± 0.068(06)
Males			
Body (g)	25.432 ± 0.320(20)[b]	26.111 ± 0.370(20)	25.632 ± 0.795(06)
Liver (g)	1. 326 ± 0.031(20)	1.408 ± 0.044(20)	1.395 ± 0.060(06)
Kidneys (g)[d]	0.393 ± 0.007(20)	0.423 ± 0.009(20)[c]	0.501 ± 0.046(06)[c]
Seminal Vesicles (g)	0.285 ± 0.006(20)	0.276 ± 0.006(20)	0.244 ± 0.014(06)[c]
R. Testis (g)	0.104 ± 0.002(20)	0.101 ± 0.003(20)	0.101 ± 0.004(06)
R. Cauda (mg)	9.575 ± 0.264(20)	9.830 ± 0.338(20)	9.333 ± 1.146(06)
R. Epididymis (mg)	31.530 ± 0.476(20)	31.230 ± 0.693(20)	35.650 ± 5.147(06)
Prostate Gland (mg)	11.905 ± 0.400(20)	11.570 ± 0.800(20)	7.900 ± 1.843(06)

[a]Mean weights ± standard error.
[b]Number of animals providing the data indicated in parenthesis.
[c]Significantly different ($p < 0.05$) from the control group.
[d]Kidneys were weighed with the adrenal glands attached.

Source: NTP (1988a)

2-Methoxyethanol
2-Methoxyethanol Acetate

Doses estimated for the CD-1 mice were 0, 60, 198, or 540 mg/kg-day for the males and 0, 58, 194, or 584 mg/kg-day for the females, corresponding to drinking water concentrations of 0, 0.03, 0.1, and 0.3% 2-ME, respectively (NTP, 1988b). Treatment with 0.3% in F0 mice resulted in a statistically significantly reduced fertility index, increased percentage of morphologically abnormal sperm, low percentage of pups born alive, and no survival of offspring to PND 4 (see Table 21). The cumulative days to litter were extended in the 0.3%-group, with cumulative littering of the first through fourth litters occurring at Gestation Days (GDs) 36, 75, 88, and 106 for the treated pairs, compared to GDs 23, 43, 65, and 86 for the controls. F0 mice treated with 0.1% exhibited statistically significantly decreased proportion of pups born alive and decreased pup survival to PND 4 (see Table 21). At necropsy, body weights did not differ statistically significantly from controls for males or females. Weights of the testis (16% lower), epididymis (13% lower), and cauda (16% lower) were statistically significantly reduced in the 0.3% males compared to controls (with or without adjustment for body weights by analysis of covariance). F0 males in the 0.3%-group had degeneration of the seminiferous tubules in 77% of animals, compared to 63% in controls (not statistically significant [$p > 0.05$] by Fisher's exact test, calculated for this assessment), including observations of the accumulation of sloughed cells and degeneration of ductal epithelium. Effects observed in the F1 generation of CD-1 mice treated with 0.1% include a statistically significant decrease in the fertility index and increase in incidence of seminiferous tubule degeneration (see Table 21). The proportion of pups born alive to F1 parents was statistically significantly decreased in both the 0.1- and 0.03%-groups. However, it should be noted that the 95% live birth rate to F1 mice in the 0.03%-group is slightly higher than the rate among F0 controls. Body weights of treated F1 mice at necropsy did not differ from controls for either sex. In males at 0.1%, weights of the cauda (20%) and epididymis (10%) were reduced, with or without adjustment for body weights. On the basis of a slight but statistically significant decrease in pups born alive, the low dose of 0.03% (60 mg/kg-day) is a LOAEL for F1 CD-1 mice. A NOAEL is not identified.

In all three strains of mice, the effects were more severe in the F1 generation compared with the F0 generation, suggesting that immature mice are more sensitive than older mice to the reproductive effects of 2-ME. Overall, these NTP (1989, 1988a,b) data suggest the lowest dose of 53 mg/kg-day in male C57BL/6 mice, corresponding with a 0.03% concentration in drinking water, is a LOAEL for statistically insignificant reproductive effects, including reduced survival of male offspring in F0 and reduced sperm quality in F1 males; a NOAEL was not identified.

2-Methoxyethanol
2-Methoxyethanol Acetate

Table 21. Reproductive Effects Observed in Two Generations of Mice Exposed to 2-Methoxyethanol in Drinking Water

Parameter	Sex	Control	0.03% (58−60 mg/kg-day)	0.1% (194−198 mg/kg-day)	0.3% (540−548 mg/kg-day)
F0 mice					
Fertility index (number fertile/number cohabitated × 100)	Both	100%	100%	100%	30%[a]
Pups born alive (%)	Both	93%	94%	87%[a]	14%[a]
Male pup survival to Day 4 (%)	Male	100%	92%	69%[a]	0%[a]
Female pup survival to Day 4 (%)	Female	94%	99%	72%[a]	0%[a]
Morphologically abnormal sperm (%)	Male	4%	5%	4%	18%[a]
Seminiferous tubule degeneration	Male	19/30	NE	NE	23/30
F1 mice					
Fertility index (number fertile/number cohabitated × 100)	Both	85%	80%	35%[a]	NT
Pups born alive (%)	Both	100%	95%[a]	90%[a]	NT
Seminiferous tubule degeneration	Male	6/20	11/20	13/20[a]	NT

[a]Significantly different from controls ($p < 0.05$).

NE = not examined; NT = not tested: no litters at this dose.

Source: NTP (1988b).

A 12-week study in male Dutch rabbits examined effects of 2-ME in drinking water. Foote et al. (1995) exposed groups of six male Dutch rabbits to 2-ME (purity not reported) in drinking water, resulting in daily doses of 0, 12.5, 25, 37.5, or 50 mg/kg-day, for 5 days/week, over a 12-week period. Semen was collected twice weekly for 12 weeks and analyzed by computer-assisted sperm analysis for both sperm morphology and motility. At the end of the 12-week exposure, animals still producing sufficient sperm were tested for fertility. Profound reductions in sperm production, including azospermia (absence of spermatozoa in the semen), were observed in five of the six animals in the 37.5- and 50-mg/kg-day groups (measured after 9 and 6 weeks of exposure, respectively). Table 22 shows decreases in other parameters of semen quality relative to control rabbits that were seen at Weeks 10 or 12 at these two highest doses. Semen quality parameters that decreased included average path velocity, track speed, straight line velocity, percent sperm motility, and progressive sperm motility at 50 mg/kg-day, and total number of sperm per ejaculate at 37.5 and 50 mg/kg-day.[1] Exposure to 25 mg/kg-day caused no microscopic changes in sperm morphology but statistically significantly altered several indices of semen quality, including the percentage of progressively motile sperm and the ratios of

[1]Foote et al. (1995) appears to have compared the Week-10 data for the 37.5-mg/kg-day dose with Week-12 data for controls. Because the control data at Week 10 were notably lower for all parameters except total sperm per ejaculate, only this parameter of sperm quality is significantly lower than the Week-10 controls at 37.5 mg/kg-day.

2-Methoxyethanol
2-Methoxyethanol Acetate

straight line velocity to average path velocity and track speed. No effects were seen in any parameters in rabbits exposed to 12.5 mg/kg-day. Sperm produced from rabbits after 12 weeks of exposure showed no reduction in the ability to fertilize ova in vitro. This study identified a NOAEL of 12.5 mg/kg-day and a LOAEL of 25 mg/kg-day for decreased semen quality of rabbits exposed for 12 weeks.

Table 22. Semen Quality in Male Dutch Rabbits Given 2-Methoxyethanol in Drinking Water, 5 Days/Week, for 12 Weeks										
	Dose (mg/kg-day)									
	Control			**12.5**		**25**		**37.5**		**50**	
Semen Quality Parameter	Week 1	Week 10[b]	Week 12	Week 1	Week 12	Week 1	Week 12	Week 1	Week 10	Week 1	Week 12
Average path velocity (μm/s)	112	97	114	114	109	114	99	127	101	119	112[a]
Track speed (μm/s)	138	121	144	140	135	142	131	150	125	146	133[a]
Straight line velocity (μm/s)	96	83	96	99	91	97	79	113	89	99	90
Ratio of straight line velocity/average path velocity	0.85	0.86	0.82	0.86	0.82	0.84	0.78[a]	0.88	0.87	0.82	0.80
Ratio of straight line velocity/average track speed	0.71	0.68	0.67	0.71	0.68	0.70	0.61[a]	0.76	0.71	0.69	0.69
Sperm motility (%)	83	74	79	82	71	81	76	87	67	81	40[a]
Progressive sperm motility (%)	51	40	49	52	41	49	36[a]	62	38	51	28[a]
Total sperm/ejaculate (10^6)	181	280	261	366	172	225	183	198	152[a]	254	18[a]

[a]Significantly different from control rabbits at Week 12, or at Week 10 for 37.5 mg/kg-day ($p < .05$). SDs were not reported.
[b]Control values at Week 10 are interpolated from Foote et al. (1995), Figure 2.

Source: Foote et al. (1995).

An identical exposure of male Dutch rabbits to 2-ME in the drinking water for 12 weeks was followed by testicular histological evaluation (Berndtson and Foote, 1997). Groups of six to seven sexually active, mature male Dutch rabbits were exposed to 0, 12.5, 25, 37.5, or 50 mg/kg-day of 2-ME in the drinking water, 5 days/week, for 12 weeks in two sequential replicates. Animals were weighed weekly. At the end of the treatment period, animals were sacrificed. Testes were removed and weighed; one testis was prepared for histological examination, and the other used for determination of spermatid number.

In the 50-mg/kg-day groups, five of seven animals showed a severe disruption of spermatogenesis (Berndtson and Foote, 1997). A dose-related increase in disrupted seminiferous tubules was seen at doses ≥37.5 mg/kg-day. Statistically significant ($p < 0.05$) reductions in the yield of round spermatids per old primary spermatocyte, per young primary spermatocytes, or per spermatogonium, as well as the mean numbers of elongated spermatids, were seen at doses of 37.5 and 50 mg/kg-day. At lower doses, reductions were seen but did not attain statistical significance. This study identified a NOAEL of 25 mg/kg-day and a LOAEL of 37.5 mg/kg-day

for effects on spermatogenesis in male rabbits. The higher NOAEL identified in this study (in which semen quality was not evaluated) relative to Foote et al. (1995) suggests that semen quality may be a more sensitive indicator of adverse effects in rabbits than histological evaluation.

Nagano et al. (1984) evaluated the reproductive tissues and hematology in male JCL-ICR mice, Syrian golden hamsters, and guinea pigs given gavage doses of 2-ME (purity not reported) 5 days/week for 5 weeks. At sacrifice, blood was collected for hematological evaluation (leukocyte counts). Testes, seminal vesicles, and the coagulating gland were removed, weighed, and subjected to histopathological examination.

The mice (five/group) were treated with 0, 62.5, 125, 250, 500, 1000, or 2000 mg/kg-day of 2-ME (Nagano et al., 1984). Data were presented in graphic, rather than tabular, form. Inspection of the graphical data figures revealed a dose-related decrease in relative testicular weights, which was markedly different from controls, in mice treated with ≥250 mg/kg-day. The investigators reported a dose-related atrophy of the seminiferous epithelium; but, dose-response data were not presented, and the threshold for this effect cannot be identified. Evaluation of the graphical data indicated that leukocyte counts in mice receiving ≥250 mg/kg-day decreased with increasing dose; however, no control data were shown for comparison. The limited data precluded identification of a NOAEL or a LOAEL.

Nagano et al. (1984) treated hamsters (four/group) with 0, 62.5, 125, 250, or 500 mg/kg-day of 2-ME. Dose-related reductions in relative testicular (7−80%) and combined seminal vesicle and coagulating gland (2−24%) weights, and leukocyte counts (9−19%) were observed. No data were reported for SDs from the means, precluding statistical comparisons between treatment groups. The limited data precluded identification of a NOAEL or LOAEL.

Guinea pigs (three/group) were treated with 0, 250, or 500 mg/kg-day (Nagano et al., 1984). Marked reductions in relative testicular weights (75−77%) and leukocyte counts (55−56%) were observed in treated groups. No data were reported for deviations from the means, precluding statistical comparisons between treatment groups. The limited data precluded identification of a NOAEL or LOAEL.

Chapin et al. (1985a) gave male F344 rats (20/group) daily gavage doses of 0-, 50-, 100-, or 200-mg/kg-day 2-ME (purity not reported) for 5 days. The males were then individually cohabitated with two females per week, for 8 weeks, after which they were housed singly for 8 more weeks. After this second 8-week period, they were again cohabitated with two females each for 5 days. Statistically significantly ($p < 0.05$) reduced fertility (approximately a 2-fold reduction in live fetuses/pregnant female, compared to controls) was seen in the 200-mg/kg-day group beginning at Week 4 and in the 100-mg/kg-day animals at Week 5 only. Separate groups of males (96/group/time point) were exposed similarly but were not allowed to mate. At weekly intervals, nine animals per group were subjected to bilateral efferent duct ligation. The following morning, animals were sacrificed, and the testis, epididymis, prostate, and seminal vesicle weights were recorded. A sample of sperm was taken from the distal cauda of the epididymis for analysis. No changes in weights of the seminal vesicles or prostate were seen at any dose or time. Evaluation of spermatogenesis revealed statistically significantly ($p < 0.05$) reduced numbers of total and motile sperm and increased numbers of morphologically abnormal sperm in the ≥100-mg/kg-day groups beginning at 3 weeks of exposure and in the 50-mg/kg-day group

during Week 5 only. Data were presented in graphical form; actual values for spermatogenesis were not reported. A LOAEL of 50 mg/kg-day was identified for transient alterations in sperm number and morphology in rats; no NOAEL was identified.

In a follow-up study (Chapin et al., 1985b), male F344 rats (nine/group) were given daily gavage doses of 0-, 50-, 100-, or 200-mg/kg-day 2-ME (99.5% pure) for 5 days and then observed for 8 weeks. Weekly testicular histology was performed. Biochemical activities of β-glucuronidase, acid phosphatase, sorbitol dehydrogenase, and lactate dehydrogenase in the testes were measured. A dose-related increase in histological alterations of the testes was seen, with the 200-mg/kg-day animals showing pronounced damage and cell death, the 100-mg/kg-day animals showing a high degree of alteration (appearance of numerous Giant cells and spermatid heads near the basement membrane, absence of round spermatids) but little cell death, and the 50-mg/kg-day animals showing a transient effect, lasting from Weeks 5−7, in spermatogenesis (testicular lesions were not compared statistically). 2-ME treatment also resulted in statistically significantly ($p > 0.05$) increased activities of β-glucuronidase and acid phosphatase and reduced activities of sorbitol dehydrogenase and lactate dehydrogenase in testicular homogenates. Data were presented in graphical form; no actual incidences of testicular histopathology or biochemical activities were reported. A LOAEL of 50 mg/kg-day was identified for testicular histopathology in this 5-day study; no NOAEL was identified.

Dodo et al. (2009) treated 10 female Sprague-Dawley rats per group with 0, 30, 100, or 300 mg/kg-day of 2-ME in drinking water for 2 or 4 weeks, to assess toxicity. Other groups of 10 female rats were treated similarly 2 weeks prior to mating through GD 6, to assess fertility. In the toxicity study, continuous diestrous and hypertrophy of the corpora lutea and other alterations in ovarian morphology were observed in rats treated with ≥ 100 mg/kg-day. "Minimal" irregularities in the estrous cycle were observed at the low dose of 30 mg/kg-day; estrous changes appeared to correlate well with histopathological changes in the ovaries. Adrenal weights were statistically significantly decreased at 300 mg/kg-day after 2 weeks, and at ≥30 mg/kg-day following 4 weeks treatment. In the female fertility study, continuous diestrous was observed at all doses. At 300 mg/kg-day, mating was postponed, and no rats became pregnant although 7/10 copulated. These data indicate a 4-week LOAEL of 30 mg/kg-day for estrous cycle alterations in female rats, with no NOAEL.

Developmental Studies

Scott et al. (1989) examined the maternal and developmental effects of repeated gavage doses of 2-ME in macaques. Groups of 6−14 pregnant macaques (*Macaca fascicularis*) were given gavage doses of 0-, 12-, 24-, or 36-mg/kg-day 2-ME (99.9% pure) in 15 ml water during organogenesis on GDs 20−45. A second control group of 3 animals was given ethanol, equivalent to the highest dose of 2-ME. On GD 100 or upon abortion if prior to GD 100, maternal body weights were measured, and the fetus from each mother (there were no multiple pregnancies) was collected by Caesarian section. Table 23 summarizes selected data. Anorexia and maternal body-weight loss occurred in a dose-related manner in all treated groups. Loss of appetite was so severe in the two high-dose groups that the researchers sometimes gave the animals nutrition by gavage. The macaques regained their appetite after the end of treatment, and body weights were generally similar to controls by the time of Caesarian section. The data for hematological parameters (red blood cell [RBC], hemoglobin, and hematocrit) measured on GD 45 showed no statistically significant effects of treatment. Chemical-related embryo lethality claimed 0/6, 0/3 (ethanol controls), 3/13 (23%), 3/10 (30%), and 8/8 (100%) embryos in

macaques treated with 0-, 12-, 24-, or 36-mg/kg-day 2-ME, respectively. One additional embryonic death in each of the low- and mid-dose groups was not considered treatment related by the researchers (one was attributed to a severe fight between the mother and a cage mate and the other to a spontaneous abortion). Although no spontaneous abortions were observed in controls in this study, the authors estimated that spontaneous embryonic death occurs in about 10−20% of pregnancies in *M. fascicularis*. In the study authors' experience, spontaneous abortion of *M. fascicularis* results in excessive vaginal bleeding, rapid reduction in uterine size, and expulsion of uterine contents. However, the dead embryos observed in this study were held within the uterus, with minimal autolysis, until hysterotomy at GD 100. This led the study authors to conclude that the observed embryo lethality was chemical related (except for the two cases noted above, which were not counted in the incidence data). From the 36-mg/kg-day dose, one of the dead fetuses had bilateral forelimb malformations (missing digit). Among the fetuses that were not aborted, fetal body and organ weights were not statistically different from controls in the 12- and 24-mg/kg-day groups (no data for the 36-mg/kg-day group, as all fetuses in that group were aborted). The lowest dose tested, 12 mg/kg-day, is a 25-day LOAEL for both maternal effects (anorexia and reduced body weights) and embryo lethality in macaques in this study.

Table 23. Selected Maternal and Developmental Effects of Gavage Doses of 2-Methoxyethanol During Organogenesis on GDs 20−45 in Female Macaques

Parameter	Dose (mg/kg-day)				
	0 (Control)[c]	0 (Ethanol Control)	12	24	36
Mean maternal body weight (kg)					
GD 20	3.45 ± 0.17	3.53 ± 0.16	3.79 ± 0.15	3.48 ± 0.12	4.25 ± 0.33
GD 45	3.47 ± 0.18	3.63 ± 0.11	3.72 ± 0.11	3.28 ± 0.15	3.88 ± 0.35
GD 100[a]	4.02 ± 0.28	4.23 ± 0.22	4.40 ± 0.14	4.27 ± 0.26	NA[d]
Maternal Anorexia[b]	0/6	0/3	3/13	6/10	8/8
Chemical-related Embryo Lethality	0/6	0/3	3/13[e]	3/10[e]	8/8
Bilateral forelimb malformations (missing digit)	0/6	0/3	0/13	0/10	1/8

[a]Final maternal body weight measured at GD 100 or at spontaneous abortion of fetus.
[b]Authors reported anorexia to increase in severity with dose: "slight" at 12 mg/kg-day and "severe" at 36 mg/kg-day.
[c]Includes data from three macaques from a previous study by these authors in the same laboratory.
[d]All pregnancies aborted; no maternal-weight data reported.
[e]One additional embryonic death in each of the low- and mid-dose groups was attributed by the researchers to a severe fight between the mother and a cage mate or to a spontaneous abortion.

Source: Scott et al. (1989).

Nelson et al. (1989) performed two developmental experiments in which pregnant Sprague-Dawley rats were fed liquid diets containing 2-ME (purity not reported) on GDs 7−18. In the first experiment, groups of 10 rats were fed diets containing 0, 0.006, 0.012, 0.025, 0.05, 0.1, 0.25, or 0.5% 2-ME. The investigators estimated corresponding doses of 0, 16, 31, 73, 140,

198, 290, or 620 mg/kg-day. Animals were observed daily for clinical signs. The dams were weighed and sacrificed for examination of the uterine contents on GD 20. Maternal effects included statistically significantly reduced mean body-weight gain ($p < 0.05$), ranging from 20% less than controls in the 140-mg/kg-day group to frank weight loss from preexposure levels in the 620-mg/kg-day group. Overt signs of toxicity (diarrhea, respiratory difficulties, alopecia, and malaise) were seen in the 620-mg/kg-day group. Statistically significantly increased embryo lethality occurred in rats fed 73 mg/kg-day. No data were reported for higher dose groups because all fetuses were resorbed. In the 73-mg/kg-day group, 44% of litters were resorbed, while 100% of litters were resorbed in ≥140-mg/kg-day group. No litter resorption was seen in rats fed <73 mg/kg-day. In groups that littered successfully (see Table 24), fetal body weights were statistically significantly reduced in a dose-related manner. A LOAEL of 16 mg/kg-day was identified for reduced fetal body weights, with no developmental NOAEL. The maternal NOAEL and LOAEL were 73 and 140 mg/kg-day, respectively, for decreased weight gain.

Table 24. Developmental Effects in Rats Fed Liquid Diets Containing 2-Methoxyethanol on GDs 7−18

	Dose (mg/kg-day)				
Effect	**Control**	**16**	**31**	**73**	**≥140[a]**
Male fetal body weights (kg)	3.3	2.9[b]	2.8[b]	2.3[b]	NT
Female fetal body weights (kg)	3.3	2.8[b]	2.7[b]	2.5[b]	NT
Embryo lethality (%)[c]	11	7	14	92	NT

[a]Rats in the ≥140-mg/kg-day groups did not litter.
[b]Significantly different from controls ($p < 0.05$).
[c]No statistical analysis of this endpoint was performed by the researchers.

NT = not tested.

Source: Nelson et al. (1989).

In the second experiment, groups of 12 rats were fed diets containing 0, 0.006, 0.012, or 0.014% 2-ME on GDs 7−18 for evaluation of behavioral parameters in the 42- to 63-day-old offspring (Nelson et al., 1989). The investigators estimated corresponding doses of 0, 17, 33, or 40 mg/kg-day. Dams were observed for mortality, clinical signs, and weight gain. The behavioral parameters observed in the offspring were figure-8 activity (test of general activity), the Cincinnati maze (a problem-solving test), the startle response (reflex test), and conditioned lick suppression (operant conditioning). There were no effects on maternal body-weight gain or lactation. A statistically significant and dose-related increase in gestation length was noted in all treated groups (see Table 25). Statistically significantly increased mortality of offspring during the pre- and postweaning periods was observed in rats fed the 33- and 40-mg/kg-day diets (see Table 25). Too few offspring from rats fed the 40-mg/kg-day diet survived for behavioral testing. The only behavioral effect attributed to treatment was statistically significantly increased error ($p < 0.05$) in the Cincinnati maze test in the offspring of rats fed 33 mg/kg-day. In this experiment, 17 mg/kg-day, the lowest dose tested, was a LOAEL for increased gestation length; no NOAEL was identified.

Table 25. Gestational and Postnatal Effects in Rats Fed Liquid Diets Containing 2-Methoxyethanol on GDs 7−18

Effect	Dose (mg/kg-day)			
	Control	17	33	40
Length of gestation (days)	21.9	22.4[a]	22.8[a]	22.9[a]
% Pups dead at				
Birth	2	1	7	14
PNDs 1−25	9	8	51[a]	83[a]
PNDs 26−63	0	0	3	NT
Birth weight (g)	5.6	6.2	6.1	5.8

[a]Significantly different from controls ($p < 0.05$).

NT = not tested.

Source: Nelson et al. (1989).

Toraason and coworkers (Toraason and Breitenstein, 1988; Toraason et al., 1986, 1985) investigated the effects on the fetal heart after gavage treatment of pregnant Sprague-Dawley rats with 2-ME (purity not reported) on GDs 7−19. In the first study, groups of 8−11 rats were treated with 0-, 25-, 50-, or 100-mg/kg-day 2-ME on GDs 7−13 (Toraason et al., 1985). Maternal body weights were recorded daily. Fetal electrocardiograms (EKGs) were recorded on GD 20, after which, the fetuses were examined for external and visceral abnormalities. Treatment with 100 mg/kg-day resulted in early resorption of all fetuses; therefore, fetal effects could not be studied in this group. Apparent dose-related reductions in live litter size and maternal body weights were observed in rats treated with 25 and 50 mg/kg-day, as well as an increase in fetal resorptions at 50 mg/kg-day, but the changes were not statistically significant. There were also slight, apparently dose-related reductions in mean fetal body weights in both groups of treated rats, but these changes, too, were not statistically significant. A statistically significant and dose-related increase in the percent of fetuses with prolonged QRS intervals (probably reflecting intraventricular conduction delay) was seen in both treated groups ($p < 0.05$). Several fetuses from the 50-mg/kg-day group exhibited one or more cardiovascular malformations. Double aortic arch was exhibited in 1/74 fetuses in the 25-mg/kg-day group. The occurrence of double aortic arch in the 25-mg/kg-day group cannot be unequivocally attributed to 2-ME exposure, as this particular anomaly was not seen in any of the 38 fetuses (from six different litters) treated with 50 mg/kg-day. The investigators noted no correlation between altered EKG and the presence of cardiovascular anomalies in individual animals. In this study, 25 mg/kg-day is considered a developmental LOAEL for slight changes in the fetal EKG and a maternal LOAEL for decreased body weights. No developmental or maternal NOAEL was identified.

In the second study, Toraason et al. (1986) explored the developmental effect of 2-ME on the heart in rats. Doses of 0- or 25-mg/kg-day 2-ME (purity not reported) were given on GDs 7−13 or 13−19 to groups of 13 pregnant Sprague-Dawley rats. Ornithine decarboxylase (ODC) activity in the heart was used as an indicator of cardiac development. ODC activity

following isoproterenol administration was used as an indicator of cardiac autonomic nervous system response in the offspring. ODC measurements, with or without previous treatment with isoproterenol, were made on PNDs 3, 9, 16, and 22. Treatment had no effect on maternal body weights, litter weights, number of viable offspring, or the heart or body weights of the offspring through PND 22. Prolonged gestation was observed in both treated groups but was statistically significant ($p < 0.05$) only in those treated on GDs 7−13. Statistically significantly reduced ODC activity on PND 3 occurred in rats treated on GDs 7−13. Isoproterenol pretreatment had no effect on ODC activity. The 25-mg/kg-day dose is a developmental LOAEL, associated with biochemical evidence of retarded cardiac development; no developmental NOAEL was identified. The maternal NOAEL is 25 mg/kg-day but is based on limited observations.

In a study designed to determine whether the effects on EKG observed in the 20-day fetuses persisted into the postnatal period, Toraason and Breitenstein (1988) treated groups of 25−30 rats with 0, 50, or 75 mg/kg-day on GDs 7−13. EKG recordings were made when the pups were 3 and 6 weeks old. A statistically significant, dose-related decrease in maternal body weight gain was observed in both treated groups, which was attributed to statistically significantly reduced litter size (increased resorptions). Statistically significantly prolonged gestation and a statistically significant ($p < 0.05$) and dose-related decrease in postnatal survival were also observed in both treated groups. No offspring from dams treated with 75 mg/kg-day survived more than 3 days. Statistically significantly increased QRS or T-wave intervals were observed in male and female 50-mg/kg-day offspring at both periods of measurement. Statistically significantly decreased body weights and increased relative heart weights were observed when the 50-mg/kg-day offspring were sacrificed at 8 weeks of age. The low dose of 50 mg/kg-day was a maternal and developmental LOAEL associated with prolonged gestation, increased fetal resorptions, high pup lethality, decreased postnatal weights, increased relative heart weights, and EKG changes in pups; no NOAEL was identified.

Sleet and Ross (1997) exposed groups of 4−10 pregnant CRL:CD rats on GD 13 to a single gavage dose of 0, 50, 100, or 250 mg/kg-day of 2-ME (purity not reported). Dams and their litters were sacrificed on either Day 15 or 20, and body weights were recorded for dams and fetuses. Gross observations were made for embryonic/fetal abnormalities. No maternal lethality occurred during the study; maternal body weights, corrected for gravid uterine weights, were not different between control and treated groups. On Day 15, embryonic body weights in all exposed groups were statistically significantly decreased relative to controls, and the incidence of limb-bud dysmorphogenesis was statistically significantly ($p < 0.05$) elevated in a dose-related manner, occurring in 36, 65, and 98% of embryos (75−100% of litters from treated dams) in the 50-, 100-, and 250-mg/kg-day groups, respectively. Embryonic limb-bud paddle area was decreased following exposure to ≥100 mg/kg-day, while interdigital distance (distance between developing paw digits) was statistically significantly increased at both 50 and 100 mg/kg-day (measurements could not be made for the 250-mg/kg-day group due to the distorted state of the condensing and noncondensing regions of the limb bud). Intralitter differences were not reported for these endpoints. Exposure to 100 mg/kg-day resulted in decreased fetal body weights, while exposure to 250 mg/kg-day resulted in decreased fetal body weights and an increase in the incidence of malformations of the digits. This study did not identify a developmental NOAEL; a LOAEL of 50 mg/kg-day was identified for decreased embryonic weights and limb-bud malformations. The high dose of 250 mg/kg-day was a NOAEL for maternal effects.

Nagano et al. (1984, 1981) examined maternal and fetal effects of gavage doses of 2-ME given to JCL-ICR mice from GDs 7−14. Groups of 21−24 mice were given 0, 31.25, 62.5, 125, 250, 500, or 1000 mg/kg-day of 2-ME (purity not reported) in distilled water. Dams were observed daily for clinical signs, and body weights were measured every 2−4 days. Dams were sacrificed on GD 18. Blood was taken from dams for leukocyte counting, while observations were made for live, dead, and resorbed fetuses. Gross and microscopic observations were made of skeletal abnormalities. Maternal effects included statistically significantly ($p < 0.05$) reduced body-weight gain in mice receiving ≥250 mg/kg-day (~38−45% lower terminal body weights than controls) and leukocytopenia (46% fewer leukocytes than controls) in the 1000-mg/kg-day group. There were no live pups born in the 1000-mg/kg-day group, 0.3% of pups were born alive in the 500-mg/kg-day group, and 47% of pups were born alive in the 250-mg/kg-day group. Groups receiving ≤125 mg/kg-day had ≥89% of pups born alive. Exencephaly was statistically significantly ($p < 0.05$) increased (19% of fetuses, compared to 0.03% in controls) in the 250-mg/kg-day group. Other fetal effects included statistically significant dose-related increases in incidences of skeletal anomalies in all treated groups (see Table 26). The ribs, vertebrae, and fingers were affected. Skeletal anomalies appeared to be the most sensitive indicator of developmental toxicity in the mice. The lowest dose tested—31.25 mg/kg-day—was a developmental LOAEL for skeletal anomalies in mice; the maternal NOAEL and LOAEL were 125 and 250 mg/kg-day, respectively, for reduced maternal weight gain.

Table 26. Skeletal Anomalies in Pups Born to Mice Given Gavage Doses of 2-Methoxyethanol on GDs 7−14					
	Dose (mg/kg-day)				
Anomaly	**Control**	**31.25**	**62.5**	**125**	**250**
Number of fetuses examined	173	174	229	178	77
Cervical ribs	1	7	23	55[a]	44[a]
Lumbar ribs	63	76	131	170[a]	64[a]
Bifurcated/split cervical vertebrae	46	74[b]	108[a]	101[a]	68[a]
Supernumerary lumbar vertebrae	0	1	11	63[a]	39[a]
Asymmetrical sternebrae	14	27	61[a]	50[a]	29
Fused ribs	0	0	1	48[b]	71[a]
Fused cervical vertebrae	0	0	1	8	32[a]
Agenesis of cervical vertebrae	0	0	0	2	13[a]
Fused thoracic vertebrae	0	0	0	28[a]	63[a]
Fused lumbar vertebrae	0	0	0	41[a]	60[a]
Fused postlumbar vertebrae	0	0	0	5	55[a]
Spina bifida occulta	1	2	19[b]	25[a]	17[a]
Abnormal fingers (oligodactyly)	0	0	0	0	17[a]

[a]Significantly different from controls ($p < 0.01$).
[b]Significantly different from controls ($p < 0.05$).

Sources: Nagano et al. (1984, 1981).

Greene et al. (1987) treated groups of three to four pregnant CD-1 mice by gavage with 0, 100, 175, 250, 350, 400, 450, or 500 mg/kg-day of 2-ME (analytical grade, purity not reported) in distilled water on GD 11. The purpose of the study was to identify the threshold of cytotoxicity in the limb buds and anomalies of the digits in the offspring. Maternal effects were not reported. Histological evidence of cell death in the limb buds was apparent in fetuses treated with ≥100 mg/kg-day. A statistically significant and dose-related increase in malformations of the forepaw occurred at doses ≥175 mg/kg-day. The lowest dose tested—100 mg/kg-day—was identified as a fetal LOAEL for limb-bud cell death. Maternal effects cannot be determined from this study.

Horton et al. (1985) examined maternal and fetal toxicity in mice given 2-ME on various days of gestation. Groups of 10 pregnant CD-1 mice were given gavage doses of 0-, 100-, 175-, 250-, 300-, 350-, 400-, 450-, or 500-mg/kg-day 2-ME (purity not reported) in distilled water. Some mice were given a single dose on GDs 9, 10, 11, 12, or 13; others were treated on 2 or 3 consecutive days at various times during GDs 7−11. Dams were observed for clinical signs and changes in body-weight gain. Offspring were observed for embryo lethality and gross abnormalities. Treatment caused no apparent maternal toxicity, although some (unspecified) groups had reduced body-weight gain, which the authors attributed to reduced litter size due to embryo lethality (Horton et al., 1985). The incidence of skeletal anomalies, particularly of the digits, increased statistically significantly and in a dose-related manner in mice treated with ≥175 mg/kg-day on GD 11. Embryo lethality was statistically significantly increased in mice receiving 250 mg/kg-day on at least two GDs. Fetal body weights were statistically significantly ($p < 0.05$) reduced in mice given a single dose of ≥250 mg/kg-day on GD 11. The developmental NOAEL and LOAEL for skeletal abnormalities were 100 and 175 mg/kg-day, respectively; the data were not sufficiently reported to identify the NOAEL and LOAEL for maternal toxicity.

Hardin and Eisenmann (1987) treated groups of 14 or 16 pregnant CD-1 mice on GD 11 with 0- or 304-mg/kg-day gavage doses of 2-ME (>99% pure) in distilled water. Dams were sacrificed on GD 18 and examined for maternal body weights and gravid uterus weights. Live and dead fetuses were counted. Live fetuses were weighed and observed for paw malformations. Maternal and fetal body weights and number of live fetuses were not affected by treatment. Statistically significant ($p < 0.001$) increases in the incidence of paw defects occurred in treated mice (14/16 litters and 88.5% of the fetuses, compared to 2/14 litters and 13% of fetuses in the control group). The hind limbs were affected more frequently than the forelimbs. Syndactyly (fused digits) was the predominant anomaly. In this study, 304 mg/kg-day was a developmental LOAEL for paw defects; no NOAEL or maternal LOAEL was identified.

One study was located that investigated the developmental toxicity of 2-ME acetate. As part of a large preliminary assessment of chemicals for developmental toxicity, groups of 50 pregnant CD-1 mice were given gavage doses of 0- or 1225-mg/kg-day 2-ME acetate (purity and dosing vehicle not specified) for 8 days on GDs 6−13 (Hardin et al., 1987). The treatment concentration was determined during prior dose-finding studies to be the LD_{10} (details not reported). Dams were observed for mortality, and body weights were measured prior to treatment and again on GD 18. Live and dead pups were counted, and litters were weighed on PNDs 1 and 3. Uteri were examined for the presence of implantation sites in dams that failed to litter. Maternal body weights were also measured on PND 3. There was no maternal mortality,

and no changes in body weights were reported. No pups were born alive. No results were reported for presence or absence of implantation sites. A developmental LOAEL of 1225 mg/kg-day in mice was identified for 2-ME acetate in this study.

Inhalation Exposure

Unless otherwise indicated, all inhalation exposures discussed below were whole-body exposures conducted using 2-ME in exposure chambers rather than nose-only exposures. No inhalation studies were available for animals exposed to 2-ME acetate.

Subchronic Studies

Miller et al. (1983a) exposed rats and rabbits to airborne 2-ME. Groups of Sprague-Dawley rats (10/sex/group) and New Zealand white rabbits (5/sex/group) were exposed to concentrations of 0-, 30-, 100-, or 300-ppm (0-, 93-, 310-, or 930-mg/m^3) 2-ME, 6 hours/day, 5 days/week, for 13 weeks. Observations for clinical signs were made daily, while body weights were recorded weekly. Blood samples were collected at 4 and 12 weeks. Hematological tests included measurements of hemoglobin, PCV, MCV, MCH, MCHC, and RBC, total and differential leukocyte (white blood cell [WBC], and platelet counts. Evaluated clinical chemistry parameters included blood urea nitrogen (BUN), AST, alkaline phosphatase, glucose, total protein, albumin, globulins, and total bilirubin concentrations. Urinalysis was performed on rats (only) at 12 weeks and included measurements of bilirubin, ketones, glucose, protein, pH, urobilinogen, and specific gravity. At necropsy, weights of livers, kidneys, brains, spleens, thymuses, and testes were measured. Including the nasal turbinates, 39 tissue types were collected and subjected to histopathological evaluation.

No mortality occurred in the rats. Miller et al. (1983a) reported statistically significant body weight decreases among 300-ppm males (13% below controls) and 100- and 300-ppm females (9−18% below controls). Both sexes of 300-ppm groups exhibited pancytopenia, thymic atrophy (incidence not reported), and decreases in thymus (42−66% lower than controls) and liver (15−17% lower than controls) weights and serum concentrations of total protein, albumin, and globulin. In the 300-ppm males, decreased testicular weights (59% lower than controls), small flaccid testes, and severe degeneration of the seminiferous tubules (incidence not reported) were observed. No gross or histological changes were seen in the 30- or 100-ppm males or females. A NOAEL of 100 ppm (310 mg/m^3) and a LOAEL of 300 ppm (930 mg/m^3) were identified for rats on the IRIS database (U.S. EPA, 2010a) based on testicular degeneration.

In rabbits, two of five males exposed to 300 ppm and two of five females in each of the 100- and 300-ppm groups died or were sacrificed moribund (Miller et al., 1983a). The researchers considered the deaths to be of uncertain relationship to 2-ME exposure (one was due to inner ear infection, one to pneumonia, one to acute hemorrhagic enteritis, one to inanition, and two to undetermined causes). Males and females in the 300-ppm group exhibited statistically significant ($p < 0.05$) body-weight reduction (9−13% below controls), pancytopenia, and relative thymus-weight reduction (35−59%) and atrophy (incidence not reported). All males in the 300-ppm group had small flaccid testes by gross observation (see Table 27), and mean testicular weights were statistically significantly and markedly reduced in this group (73% lower than controls). Some males in the 100- and 30-ppm groups also showed slight-to-moderate decreases in gross testes size, although mean testicular weights in these groups did not differ from controls. Histopathological examination of males revealed testicular degeneration that increased in

incidence and severity with exposure concentration. Severe diffuse seminiferous tubule degeneration occurred in all males at 300 ppm, while more moderate degeneration was observed in the three affected males at 100 ppm, and relatively minor changes (thinner than normal germinal epithelium with few spermatozoa) were observed in the one male affected at 30 ppm (see Table 27). No gross or histological effects on reproductive organs were observed in treated females. IRIS (U.S. EPA, 2010a) identified a NOAEL of 30 ppm (93 mg/m^3) and a LOAEL of 100 ppm (310 mg/m^3) for rabbits in this study, based on testicular effects in males.

Table 27. Gross and Histological Testicular Changes of Male New Zealand White Rabbits Exposed to Airborne 2-Methoxyethanol, 6 Hours/Day, 5 Days/Week, for 13 Weeks

Testicular Parameter	Control[a]	30 ppm (93 mg/m^3)	100 ppm (310 mg/m^3)	300 ppm (930 mg/m^3)
Gross reduction in testes size	0/5	2/5	4/5[b]	5/5[b]
Seminiferous tubule degeneration	0/5	1/5	3/5	3/3[b]

[a]Control incidence implied, but not explicitly reported by researchers.
[b]Significantly different ($p < 0.05$) from controls by Fisher's exact test, performed for this review.

Source: Miller et al. (1983a).

In a follow-up study, Miller et al. (1982, as summarized on the IRIS database [U.S. EPA, 2010a]) further investigated the toxicity of 2-ME exposure in male rabbits. Groups of 10 male New Zealand white rabbits were exposed to airborne concentrations of 0-, 3-, 10-, or 30-ppm (0-, 9-, 31-, or 93-mg/m^3) 2-ME, 6 hours/day, 5 days/week, for 13 weeks. Observations were made for clinical signs, and body weights were recorded. At necropsy, testicular weights were recorded, and major organs (including the testes) were examined for gross and histological abnormalities. No statistically significant differences were observed between the control and any treated groups for any of the endpoints examined. The high exposure concentration of 30 ppm (93 mg/m^3) was identified as a NOAEL on IRIS (U.S. EPA, 2010a). The results of this study support identification of 30 ppm as a NOAEL in the previous study by Miller et al. (1983a).

Reproductive Studies

Rao et al. (1983) examined fertility effects in rabbits exposed to 2-ME for 13 weeks. Groups of 20−30 male and female Sprague-Dawley rats were exposed to airborne concentrations of 0-, 30-, 100-, or 300-ppm (0-, 93-, 310-, or 930-mg/m^3) 2-ME, 6 hours/day, 5 days/week, for 13 weeks. The exposed rats were then bred with nonexposed partners. Clinical signs were observed daily and body weights collected weekly from all rats during the exposure period, after which, male weights were collected weekly and female weights collected at littering. Observed litter parameters included gestation duration; litter size; pup viability on PNDs 1, 4, 7, 14, and 21; pup weights; and gross abnormalities. Mated males and females were sacrificed at 23 and 15 weeks, respectively, following cessation of exposure. Organ weights were collected for liver, kidney, brain, spleen, thymus, and testes. Histological examinations were performed on spleen, thymus, bone marrow, lymph nodes, testes, epididymides, ovaries, and uteri. A subset of unexposed females were mated with exposed males and sacrificed 12 days after last cohabitation.

2-Methoxyethanol
2-Methoxyethanol Acetate

Dominant lethality was assessed by observing the male fertility index, rate of preimplantation loss, and resorption rate. A second subset of exposed males (0 or 300 ppm) and unexposed females were bred at 13 and 19 weeks after cessation of exposure to assess recovery of fertility.

Terminal body weights were statistically significantly reduced (8−13% decrease from controls) in both sexes of the 300-ppm groups. None of the treated female groups exhibited reproductive effects. Fertility was reduced in the 300-ppm males, as only 4/20 unexposed females were impregnated by the exposed males from this group, compared to 29/30 pregnancies from matings with control males. In the four impregnated dams, complete fetal resorption was observed for all conceptuses. After the 13-week postexposure recovery period, 55% of 300-ppm males were fertile. High-dose males also exhibited decreased testicular size and atrophy of the seminiferous tubules. No effects on fertility or testicular weights were observed in the 30- or 100-ppm males. A NOAEL of 100 ppm (310 mg/m^3) and an associated LOAEL of 300 ppm (930 mg/m^3) were identified on the IRIS database (U.S. EPA, 2010a) for reduced fertility and testicular atrophy in male rats, which is consistent with the results of the Miller et al. (1983a) rat study.

Developmental Studies

Nelson et al. (1984a) examined the teratogenic effects of 2-ME in pregnant rats. Groups of 8−34 pregnant Sprague-Dawley rats were exposed to airborne concentrations of 0-, 50-, 100-, or 200-ppm (0-, 155-, 310-, or 620-mg/m^3) 2-ME, for 7 hours/day, on GDs 7−15. On GD 20, dams were weighed and sacrificed. There were no overt signs of toxicity in the dams. Observed developmental endpoints included number of resorption sites, live fetuses, fetal body weights, and gross and microscopic skeletal and soft tissue abnormalities. All of the litters in the 200-ppm group were resorbed. In the 100-ppm group, 50% of all fetuses were resorbed, with resorptions occurring in all litters. A statistically significant, 3-fold increase in fetal resorptions was observed in the 50-ppm group. This group also exhibited fetal body weights 20% lower than controls. Statistically significant increases in fetuses with cardiac malformations were observed in the 100-ppm group, while increased skeletal malformations were observed in the 50- and 100-ppm groups (see Table 28). This study identified a LOAEL of 50 ppm (155 mg/m^3) for fetal resorptions and skeletal malformations in rats. A NOAEL was not identified.

Table 28. Fetal Body Weights and Incidences of Malformations in Pups Born to Rats Exposed to Airborne 2-Methoxyethanol, for 7 Hours/Day on GDs 7−15

Malformation	Control	50 ppm (155 mg/m^3)	100 ppm (310 mg/m^3)
Fetal body weight—males (g)	3.46	2.84[a]	2.29[a]
Fetal body weight—females (g)	3.64	2.91[a]	2.49[a]
Cardiac IV septal defect	0/270	4/103	20/65[a]
Wavy ribs	0/137	28/53[a]	16/31[a]

[a]Significantly different from controls ($p < 0.05$).

Source: Nelson et al. (1984a).

Hanley et al. (1984) conducted a study of developmental toxicity from 2-ME in rats, mice, and rabbits. Groups of 24−32 pregnant rats, mice, and rabbits were exposed to 99.96% pure 2-ME for 6 hours/day on GDs 6−15 (mice and rats) or 6−18 (rabbits). Fischer 344 rats and New Zealand white rabbits were exposed to airborne concentrations of 0, 3, 10, or 50 ppm (0, 9.3, 31, or 155 mg/m^3), while CF-1 mice were exposed to airborne concentrations of 0, 10, or 50 ppm (0, 31, or 155 mg/m^3). Daily observations were made for clinical signs, while body weights and food and water consumption were measured at 3-day intervals. On GDs 18, 21, or 29 (for mice, rats, and rabbits, respectively), dams were sacrificed, and maternal body, liver, spleen, and thymus weights were recorded. Blood samples were collected for analysis of RBC and WBC counts, hemoglobin, PCV, MCV, MCH, and MCHC. Counts were made of corpora lutea (rats and rabbits); live, dead, and resorbed fetuses; and implantation sites. Fetuses were weighed, measured, and examined for gross and microscopic abnormalities.

In rats, no statistically significant effects on maternal body or organ weights were observed (Hanley et al., 1984). Statistically significant decreases in hemoglobin and PCV (3−6 and 4−6%, respectively) were observed in all treated adult groups, and decreased RBC counts (5%) occurred in the 50-ppm group. Statistically significantly increased incidences of fetuses with lumbar spurs and delayed ossification of the vertebral centra were also observed in the 50-ppm group (see Table 29). Thus, a developmental NOAEL and LOAEL of 10 and 50 ppm (31 and 155 mg/m^3), respectively, were identified for minor skeletal abnormalities in rats. The maternal NOAEL was 50 ppm (155 mg/m^3).

Table 29. Incidences of Select Skeletal Variations in Fetuses of Rats Exposed to Airborne 2-Methoxyethanol for 6 Hours/Day on GDs 6−15

Malformation	Control	3 ppm (9 mg/m^3)	10 ppm (31 mg/m^3)	50 ppm (155 mg/m^3)
Lumbar spurs	18/287 (21/29)[a,b]	13/283 (10/28)	20/293 (13/28)	57/307[c] (26/30)
Delayed ossification of vertebral centra	4/287 (4/29)	3/283 (3/28)	6/293 (5/28)	19/307[c] (13/30)

[a]Parentheses indicate litter incidences.
[b]The number of affected litters cannot exceed the number of affected fetuses, so the litter incidence reported by the researchers (and duplicated here) for lumbar spurs in controls is likely in error.
[c]Significantly different from control ($p < 0.05$).

Source: Hanley et al. (1984).

In mice, Hanley et al. (1984) reported a statistically significant decrease in maternal body-weight gain (18%) from GDs 12 to 15 in the 50-ppm group. Statistically significantly increased fetal incidences of extra lumbar ribs and unilateral testicular hypoplasia also were observed in the 50-ppm group (see Table 30). Thus, a maternal and developmental NOAEL and LOAEL of 10 and 50 ppm (31 and 155 mg/m^3), respectively, were identified for transient decreases in maternal body weight gain and fetal skeletal and soft tissue abnormalities in mice.

Table 30. Incidences of Select Alterations in Fetuses of Mice Exposed to Airborne 2-Methoxyethanol for 6 Hours/Day on GDs 6−15

Malformation/lesion	Control	10 ppm (31 mg/m^3)	50 ppm (155 mg/m^3)
Extra lumbar ribs	48/317 (14/26)[a]	49/260 (14/23)	82/251[b] (21/24)
Unilateral testicular hypoplasia	2/165 (2/26)	3/136 (3/23)	8/132[b] (6/24)

[a]Parentheses indicate litter incidences.
[b]Significantly different from controls ($p < 0.05$).

Source: Hanley et al. (1984).

In rabbits, a decrease in body-weight gain (42% compared with controls) occurred in the 50-ppm group during exposure (Hanley et al., 1984). A statistically significantly higher fetal resorption rate (500% increase) and lower fetal mean body weights (9%) were both observed in the 50-ppm group when compared with controls. Of all 50-ppm fetuses, 63% exhibited at least one gross malformation, with abnormalities in the extremities (shortened or missing digits) appearing most often (see Table 31). Table 31 notes other observed malformations occurring in the 50-ppm group. A maternal and developmental NOAEL and LOAEL of 10 and 50 ppm (31 and 155 mg/m^3), respectively, were identified for decreases in maternal body-weight gain and numerous skeletal and soft tissue abnormalities in fetal rabbits.

Doe et al. (1983) studied the developmental effects of 2-ME (99% pure) in rats that were allowed to give birth. Groups of 20 pregnant Wistar rats were exposed to airborne concentrations of 0-, 100-, or 300-ppm (0-, 310-, or 930-mg/m^3) 2-ME, for 6 hours/day, on GDs 6−17. Dams were allowed to birth, and pups were observed for 3 days. Maternal body weights were measured throughout the study. Numbers of live and dead pups and pup body weights were recorded on Postpartum Days 1 and 3. Dams not birthing by GD 24 were sacrificed and evaluated for pregnancy status. Body-weight gain in the 300-ppm group was statistically significantly less (weights not reported) than controls throughout the study (Doe et al., 1983). None of the 300-ppm dams produced litters. Only 9/20 100-ppm dams littered, with gestation extending 1.6 days longer than controls. The mean number of live pups/litter (eight fewer/litter), total live pup ratio (22% lower), and pups surviving to Postpartum Day 3 (29% lower) were statistically significantly reduced in the 100-ppm group. This study identified a LOAEL of 100 ppm (310 mg/m^3) for decreased live-pup birth and pup survival in rats. No NOAEL was identified.

Table 31. Incidences of Select Malformations in Fetuses of New Zealand White Rabbits Exposed to Airborne 2-Methoxyethanol for 6 Hours/Day on GDs 6−18

Malformation	Control	3 ppm (9 mg/m^3)	10 ppm (31 mg/m^3)	50 ppm (155 mg/m^3)
Limb defects	0/173 (0/23)[a]	1/172 (1/23)	1/187 (1/24)	55/145[b] (16/22)
Digit defects	0/173 (0/23)	0/172 (0/23)	0/187 (0/24)	17/145[b] (8/22)
Ventral wall defects	0/173 (0/23)	0/172 (0/23)	0/187 (0/24)	11/145[b] (4/22)
Cardiovascular defects	0/95 (0/23)	0/93 (0/23)	0/101 (0/24)	34/80[b] (15/22)
Coarctation of the aortic arch	0/95 (0/23)	0/93 (0/23)	0/101 (0/24)	13/80[b] (6/22)
Aplastic/hypoplastic spleen	0/95 (0/23)	0/93 (0/23)	0/101 (0/24)	26/80[b] (13/22)
Renal defects	0/95 (0/23)	2/93 (2/23)	1/101 (1/24)	29/80[b] (14/22)
Missing bones	0/173 (0/23)	0/172 (0/23)	0/187 (0/24)	11/145[b] (5/22)

[a]Parentheses indicate incidence/litter.
[b]Significantly different from controls ($p < 0.05$).

Source: Hanley et al. (1984).

Nelson et al. (1984b) examined the reproductive and neurodevelopmental effects of 2-ME in a cross-breeding study in rats. There were 15 male Sprague-Dawley rats exposed to airborne concentrations of 25-ppm (78-mg/m^3) 2-ME, (>98% pure) 7 hours/day, 7 days/week, for 6 weeks. No male controls were used. These rats were then mated with unexposed females. In a separate experiment, groups of 15 pregnant Sprague-Dawley rats were exposed to airborne concentrations of 0- or 25-ppm (0- or 78-mg/m^3) 2-ME, for 7 hours/day ,on GDs 7−13 or 14−20. Parental body weights were recorded. Food and water consumption were recorded in the pregnant female-only experiment. At birth, litters were evaluated for litter number and allowed to nurse with the biological mothers. From each litter, four male and four female pups were randomly selected for further observation. Pup body weights were taken on Postpartum Days 7, 14, 21, 28, and 35. On Postpartum Days 10−90, one male and female from each litter were selected for behavioral evaluation via six tests for neuromuscular function, activity, and learning. At birth and Postpartum Day 21, representative pups ($n \geq 10$) were sacrificed, and the brains were removed and analyzed for concentrations of protein, acetylcholine, dopamine, norepinephrine, and 5-hydroxytryptamine.

No treatment-related effects were observed for parental body weights or water or food consumption (Nelson et al., 1984b). Litter sizes and pup body-weight gain through Postpartum Day 90 were not affected by treatment. Of the behavioral parameters measured, only avoidance

behavior in pups from dams exposed on GD 7−13 was statistically significantly affected. Avoidance was tested by placing pups in shuttle boxes in which an electrical shock could be delivered via the metal grid floor on one side of the box. A warning tone was sounded, followed by a 5-second delay and administration of a shock. Pups from the treated dams experienced statistically significantly ($p < 0.01$ using Wilcoxson test) fewer number and shorter duration of electrical shocks compared to controls. No treatment-related behavioral changes were observed in pups sired by treated males. In contrast, brain tissue analysis revealed statistically significant differences of all monitored neurochemicals in various segments of the brain in both paternally and maternally exposed 21-day pups. Increases or decreases in neurochemicals were not consistent for various brain segments. The toxicological significance of these changes or their association (if any) with treatment-related changes in avoidance-conditioning is unclear. This study identified a LOAEL of 25 ppm (78 mg/m^3) for neurobehavioral changes in rats exposed in utero.

OTHER STUDIES

Data from occupational studies indicate that the dermal route is the major route of absorption in the workplace (Sparer et al., 1988; Piacitelli et al., 1990; Chang et al., 2004; Kezic et al., 1997). In addition, physicochemical properties of 2-ME and 2-ME acetate, especially their solubility in both water and lipids, indicate the likelihood of important dermal absorption of the liquid. Dugard (1984) found the dermal penetration rate of 2-ME in vitro in human abdominal skin to be 2.82 mg/cm^2/hour. Dermal contact with either the liquid or its concentrated aqueous solutions raised the biological concentrations of MAA statistically significantly above the concentrations reached during inhalation-only exposure at 5 ppm (Johanson, 1988). Dermal absorption of dilute aqueous solutions of glycol ethers and their acetates, including 2-ME and 2-ME acetate, were reported to be higher than for neat compounds (Johanson, 1988). Studies of volunteers exposed to vapors and liquid 2-ME showed extensive dermal absorption of both the vapor and the liquid, with uptake of the vapor estimated to be 55% of the total dose. Dermal uptake of undiluted 2-ME placed in a 27-cm^2 glass chamber on the volar forearm for 60 minutes exceeded the uptake by an 8-hour inhalation exposure to 5 ppm (Kezic et al., 1997) by 100 times. Thus, dermal absorption appears to be a principal route of exposure to the liquid, as well as the vapor.

Starek et al (2008) injected groups of 5, 12-week old Wistar rats, initially weighing about 300 g each, with 2-ME in saline solution at daily doses of 0, 1.25, 2.5, or 5 millimoles (mM)/kg for 29 days. These correspond to doses of 0, 95, 190, and 380 mg/kg-day. While control rats gained weight, all treated rats lost weight; mean body weights correlated negatively with dose ($p < 0.0001$). All 2-ME doses resulted in decreased RBC, PCV, and HGB, and increased reticulocyte counts. These data indicate a 29-day LOAEL of 95 mg/kg-day for hematological effects in rats injected with 2-ME.

Bagchi and Waxman (2008) reviewed the impact of 2-ME and its active biological oxidation product, MAA, on testicular gene expression. MAA primarily affects tissues with rapidly dividing cells and high rates of energy metabolism, including testes, thymus and the fetus. Testicular toxicity is one of the most prominent consequences of 2-ME and MAA exposure, which results from apoptosis of primary spermatocytes and is associated with changes in the expression of various genes and signaling pathways. Of particular importance are the genes that code for oxidative stress response factors, protein kinases, and nuclear hormone

receptors. Nuclear receptors and protein kinases regulate multiple cellular processes and are critical for signaling events required for spermatogenesis. De-regulation of their activity by 2-ME or MAA leads to inappropriate signaling in testicular cells. Oxidative stress in spermatocytes exposed to MAA triggers mitochondrial release of cytochrome C, activation of caspases, and ultimately, apoptosis.

Tonkin et al. (2009) gavaged groups of five ~10-week old male Wistar rats with 0-, 50-, or 150-mg/kg/day 2-ME (99.9% pure) in sterilized water for 3 days and collected testes for histopathological (left testicle) and gene-expression analysis (right testicle). Histopathological changes in the testes, consisting of degeneration and necrosis of spermatocytes and reductions in spermatocyte numbers, were observed only in high-dose (150 mg/kg-day) animals. Microarray analysis of testicular samples from these animals revealed a large number of differentially expressed genes from animals exposed to 50- or 150-mg/kg 2-ME (>900 each at >1.5-fold changed). Expression Analysis Systematic Explorer (EASE) analysis of these genes demonstrated enrichments in gene protein transport, endocytosis, protein kinase activity, cell cycle, and meiosis. Quantitative polymerase chain reaction confirmed increased expression of the actin-binding protein cortactin and the transcription factor Wilm's tumor 1 (Wt1) following 2-ME exposure. Increased localization of cortactin in abnormal spermatocytes was also observed by immunohistochemistry, consistent with a possible role for this protein in the mechanism of toxicity.

Acute and Short-term Toxicity

Doe et al. (1983) studied the effects of 2-ME in male rats. Groups of 10 male Wistar rats were exposed to airborne concentrations of 0-, 100-, or 300-ppm (0-, 310-, or 930-mg/m^3) 2-ME (~99% pure), 6 hours/day, for 10 consecutive days. Daily clinical observations were made. On Day 10, rats were sacrificed and subjected to postmortem evaluation; blood was collected for hematological analysis (hemoglobin, hematocrit, MCH, and total RBC and WBC counts), and testes and thymus were removed for histological evaluation. Body-weight gain in the 300-ppm males was statistically significantly reduced (body weights not reported). Statistically significant decreases were observed for all hematological parameters, testicular size, and thymus weights, while spermatocytic degeneration was statistically significantly increased (no quantitative data reported) in the 300-ppm group. No hematological, gross, or histological differences were observed in controls or 100-ppm rats. This study identified a NOAEL and LOAEL of 100 and 300 ppm (310 and 930 mg/m^3), respectively, for hematological, testicular, and spermatocytic effects.

Hong et al. (1988) gave male and female B6C3F1 mice (group size not reported) daily gavage doses of 0-, 50-, 100-, or 250-mg/kg-day 2-ME (99.6% purity) for 4 consecutive days. The mice were evaluated for hematology (hemoglobin, hematocrit, MCV, and erythrocyte and lymphocyte counts) and bone marrow cellularity at 1, 5, and 14 days after the last treatment. Seven mice/sex/group were sacrificed the day after the last treatment for body- and organ-weight measurements and histopathological examination of lung, heart, liver, kidneys, adrenal glands, spleen, thymus, stomach, bone marrow, urinary bladder, small and large intestines, and uterus or testes. No treatment-related effects were seen for body weights or relative liver, spleen, kidney, or thymus weights. Relative testes weights were statistically significantly reduced in the 250-mg/kg-day group (24% less than controls). Mild-to-moderate degeneration of seminiferous tubules was observed in the 250-mg/kg-day males. Erythrocyte counts and hematocrit were

statistically significantly reduced in ≥100-mg/kg-day females, while lymphocyte counts were statistically significantly reduced in ≥50-mg/kg-day males (see Table 32). Bone marrow cellularity was statistically significantly reduced in ≥100-mg/kg-day males on Days 1 and 14 and in females treated with ≥50 mg/kg-day on Day 1, ≥100 mg/kg-day on Day 5, and 250 mg/kg-day on Day 14 (comparative values not reported). A LOAEL of 50 mg/kg-day was identified for hematological effects in mice; no NOAEL was identified.

Table 32. Selected Hematological Effects in Mice Given Gavage Doses of 2-Methoxyethanol for 4 Days				
Hematological Parameter	**Control**	**50 mg/kg-day**	**100 mg/kg-day**	**250 mg/kg-day**
Males				
Erythrocytes	7.9 ± 0.1^a	7.9 ± 0.1	8.0 ± 0.1	7.9 ± 0.1
Hematocrit	37.5 ± 0.5	37.2 ± 0.4	36.8 ± 0.4	36.8 ± 0.7
Lymphocytes	4.9 ± 0.3	3.1 ± 0.1^b	2.6 ± 0.2^b	1.9 ± 0.2^b
Females				
Erythrocytes	8.3 ± 0.1	8.1 ± 0.1	7.7 ± 0.2^b	7.7 ± 0.1^b
Hematocrit	38.3 ± 0.8	37.4 ± 0.5	35.3 ± 0.8^b	35.2 ± 0.2^b
Lymphocytes	4.0 ± 0.2	3.8 ± 0.2	4.8 ± 0.7	3.9 ± 0.3

[a]Mean ± standard error for five mice/group.
[b]Significantly different from controls ($p < 0.01$).

Source: Hong et al. (1988).

Immunotoxicity was identified in several acute studies. Williams et al. (1995) gave male Fischer 344 rats (six/group) four daily gavage doses of 0, 25, 50, 100, or 200 mg/kg-day of 2-ME (purity not reported). Animals were immunized with sheep erythrocytes (SRBCs) or trinitrophenyl-lipopolysaccharide (TNP-LPS) on Day 1 or 2 and assessed for a primary antibody response to SRBCs or TNP-LPS using a direct plaque-forming cell (PFC) assay. The lymphoproliferative response was assessed by in vitro mitogenic response of splenic lymphocytes to a variety of T- and B-cell mitogens. Rats were sacrificed 24 hours after the final dose. Body, spleen, and thymus weights were measured. Body weights were not affected by treatment. Rats in the 200-mg/kg-day group showed statistically significantly ($p < 0.05$) decreased spleen weights, and rats given ≥50 mg/kg-day exhibited statistically significantly ($p < 0.05$) decreased thymic weights. A statistically significantly ($p < 0.05$) increased lymphoproliferative response to in vitro mitogen stimulation occurred in the 200-mg/kg-day group. Doses of ≥50 mg/kg-day resulted in a statistically significantly ($p < 0.05$) decreased PFC response of spleen cells in rats immunized with SRBCs on Day 1 and TNP-LPS on Day 2 of dosing. No body or organ weights, or immune responses were reported. This study identified a NOAEL of 25 mg/kg-day and a LOAEL of 50 mg/kg-day for decreased thymus weights and decreased primary antibody responses in rats.

Smialowicz et al. (1991) gave male Fischer 344 rats (six to eight/sex/group) daily gavage doses of 0-, 50-, 100-, or 200-mg/kg-day 2-ME (purity not reported) for 10 days. Rats were

immunized with SRBC or TNP-LPS on Day 1 or 2 and assessed for a primary antibody response to SRBC or TNP-LPS using a direct PFC assay. Rats were sacrificed 48 hours after the last treatment. Body, spleen, testes, and thymus weights were recorded. Multiple T- and B-cell mitogens were used to assess the in vitro lymphoproliferative response of splenic lymphocytes. Splenocytes were collected and assessed for in vitro interleukin-2 (IL-2) and natural killer cell activity. Lymph node cells were collected and assessed for in vitro cytotoxic T-lymphocyte production. Treatment did not affect body or spleen weights or natural killer cell activity. Statistically significantly ($p < 0.05$) decreased testes weights were observed in the 200-mg/kg-day group. A statistically significant ($p < 0.05$) dose-related decrease in thymus weights was seen in the ≥50-mg/kg-day groups. Histological examination of the testes revealed degeneration of the seminiferous tubules in the ≥100-mg/kg-day groups. Rats in the ≥50-mg/kg-day groups showed a statistically significantly ($p < 0.05$) decreased splenic lymphoproliferative response to mitogen stimulation, as well as a decreased PFC response to TNP-LPS. IL-2 production was statistically significantly ($p < 0.05$) reduced in all treated groups. In a separate test in which groups of six male and female rats were given 10 daily gavage doses of 0-, 25-, 50-, 100-, or 200-mg/kg-day 2-ME, males were more sensitive to the decreased PFC response to TNP-LPS than females, with statistically significant ($p < 0.05$) reduction in PFC response observed in the ≥50-mg/kg-day males and ≥100-mg/kg-day females. Males given 200 mg/kg-day exhibited a decreased ability to expel worms following infection with *Trichinella spiralis* larvae. No quantitative values were reported. This study identified a LOAEL of 50 mg/kg-day for decreases in thymus and testes weights and ex vivo immunologic responses; no NOAEL was identified.

Smialowicz et al. (1992a) also gave male Fischer 344 rats (six/group) daily gavage doses of 0-, 50-, 100-, 200-, or 400-mg/kg-day 2-ME (purity not reported) in distilled water for 2 consecutive days following a single immunization with TNP-LPS; 3 days later, the primary antibody response to TNP-LPS was determined with a PFC assay. In all treated groups, 2-ME caused a statistically significant ($p < 0.05$) decrease in PFC response to TNP-LPS (32% lower than controls). This effect was blocked by coadministration of 4-methylpyrazole, an alcohol dehydrogenase inhibitor. The study authors suggested that 4-methylpyrazole protection against reduced PFC response indicates that oxidative metabolism of 2-ME is required for immunotoxicity. This study identified a LOAEL of 50 mg/kg-day for decreased primary antibody response in male rats; no NOAEL was identified.

Smialowicz et al. (1992b) gave female Fischer 344 rats and C57BL/6J mice (six/group) daily gavage doses of 0-, 50-, 100-, 200-, or 400-mg/kg-day 2-ME (purity not reported) in distilled water for 10 consecutive days. The animals were weighed and sacrificed 48 hours after the last dose, and the spleen and thymus were removed and weighed. The lymphoproliferative response of splenic lymphocytes was determined following in vitro stimulation with multiple B- and T-cell mitogens. In rats, 2-ME treatment did not affect body or spleen weights. Thymus weights were decreased in a dose-related manner, with statistically significant differences from controls occurring at ≥100 mg/kg-day. A decreased lymphoproliferative response in rat splenic lymphocytes was seen in all dose groups. In rats injected with TNP-LPS on Day 9 of 2-ME exposure, statistically significantly ($p < 0.05$) decreased PFC responses were seen in the ≥100-mg/kg-day groups. In contrast, exposure of mice to 2-ME did not affect body, spleen, or thymus weights or lymphoproliferative responses. Likewise, no effect on PFC response was seen in mice exposed to TNP-LPS on Day 9 of exposure to 2-ME. No quantitative values were

reported. These studies identified a LOAEL of 50 mg/kg-day for decreased lymphoproliferative response in female rats; a NOAEL for rats was not identified. A NOAEL of 400 mg/kg-day, the highest dose tested, was identified for changes in body weights, organ weights, and lymphoproliferative response in female mice.

Toxicokinetics
2-Methoxyethanol
At air concentrations <5 ppm, metabolism is a first-order process, and the relationship between uptake and biological concentrations of metabolites is linear (Groeseneken et al., 1989). 2-ME is rapidly and extensively absorbed in the lungs, with 76% of an inhaled dose translocated across pulmonary tissues in humans, resulting in a measured uptake rate of 97 µg/min (Groeseneken et al., 1989). No data were given regarding gastrointestinal uptake of 2-ME. Partition coefficients for 2-ME at body temperature are as follows (Johanson and Dynesius, 1988):

blood-gas 32,800
water-gas 35,900
oil-gas 529

Initially, it is widely distributed across body tissues with the exception of adipose tissues, in which a very low tissue:blood partition coefficient of 0.02 has been measured. Radiolabel studies have shown 2-ME to initially distribute rapidly throughout the body's water and soft tissues and subsequently localize in the liver, kidney, bone marrow, and epididymis. In pregnant mice, 2-ME rapidly crosses the placenta into the conceptus. 2-ME is predominantly oxidized to MAA via methoxyacetaldehyde. In human volunteers, 85% of a 4-hour inhalation exposure to 16-mg/m^3 2-ME was metabolized to MAA. MAA appeared in the urine shortly after the start of exposure to 2-ME or 2-ME acetate; the concentration rose during the exposure. The concentration usually peaked 4−8 hours after the end of exposure and remained measurable for at least 5 days after a single exposure (Groeseneken et al., 1989). The decline of MAA concentration was reported to be monophasic with an apparent elimination half-life of 77.1 hours, measured in human volunteers at rest and "calculated from the slope of the linear part of the log-linear excretion rate-time curve" (Groeseneken et al., 1989). Volunteers were still excreting MAA at one-third of the peak rate 5 days after a 4-hour experimental exposure. In a workplace study, Shih et al., 2001 reported that MAA rose daily in urine specimens taken at the end of the shift throughout the week. Seven days after no exposure, MAA in the urine was still elevated, indicating accumulation of MAA over the workweek. In workplace settings, the half-life has been close to that established in laboratory studies, about 77 hours.

Alcohol dehydrogenase (in mice and rats) and aldehyde dehydrogenase (in mice, rats, hamsters, rabbits, guinea pigs, and humans) in the testes are capable of 2-ME oxidation to 2-MAA. This metabolite produces testicular toxicity in male rats (Foster et al., 1987) and embryo toxicity in female rats at doses equivalent to those of 2-ME and 2-ME acetate that produce the same effects (Rawlings et al., 1985). Welsch (2005) also concluded that 2-MAA is the putative toxicant for reproductive and developmental effects in animals. Ethanol reduces the metabolism of ethylene glycol monoethyl ether (EGEE) and ethylene glycol monoethyl ether acetate (EGEEA) in rats and probably affects the metabolism of 2-ME and 2-ME acetate in humans, due to competitive inhibition of alcohol dehydrogenase (Johanson, 1988). 2-ME also

has been shown to be demethylated in humans to ethylene glycol. It may also be conjugated to a glucuronide or sulfate. Elimination occurs via urinary excretion of the metabolites. There are 10 urinary metabolites that have been identified, including ethylene glycol, glycolic acid, glycine, methoxyethanol-β-D-glucuronide, methoxyethylsulfate, MAA, methoxyacetyl-β-D-glucuronide, methoxy-*N*-acetylglycine, methoxycitrate, and methoxybutenoic acid.

Shih et al. (1999) monitored 27 workers exposed to 2-ME in a semiconductor copper laminate circuit board plant and 30 nonexposed controls for 2-ME exposure for the entire workweek, using personal sampling. Eighteen of the 27 exposed workers, whose exposures were confined to airborne exposure as well as vapor only dermal exposures to 2-ME, showed a good correlation between both end of the workweek MAA and the mean weekly exposure to 2-ME. The authors found a weekly mean concentration of 4.46 ppm (SD = 2.56) and a mean Friday afternoon MAA of 46.5-mg/g creatinine (SD = 33.5). Even after 7 days away from work, the mean before-shift urinary MAA concentration was elevated, demonstrating that the half-life of MAA is close to that established in laboratory studies, about 77 hours. Of the 30 "control" workers monitored, exposure measurements for three workers near the heat press operation ranged from 0.2−0.3 ppm. These three workers were the only ones who had detectable concentrations of MAA in their urine in the range of 0.4−0.5 mg/L.

Shih et al. (2000, 2003) conducted two studies on the effects of 2-ME exposure on hematological and spermatotoxicity. In the first study, Shih et al. (2000) monitored 53 workers from two semiconductor copper laminate circuit board assembly plants for 2-ME exposure on Friday using personal samplers. These workers also submitted an end-of-shift urine specimen for MAA analysis. A group of 121 lamination workers with indirect exposure to 2-ME were selected as controls. The GM air concentration of 2-ME in the first plant was 3.98 ppm (GSD = 2.88, *n* = 55 personal samples), with a range of 0.65 to 30.1 ppm, while the GM 2-ME concentration in the second plant was 4.27 ppm (GSD = 2.19, *n* = 11 personal samples) with a range of 1.7 to 20.0 ppm. The GM urinary MAA concentrations were 19.95-mg/g creatinine (GSD = 2.19, *n* = 30) in the first plant and 20.89-mg/g creatinine (GSD = 2.19, *n* = 15) in the second plant. The air concentrations reported for the control group were between nondetectable and 0.28 ppm, *n* = 9. The GM urinary concentration of MAA in the control facility was 1.26-mg/g creatinine (GSD = 1.62, *n* = 32) with a range from nondetectable to 4.22-mg/g creatinine.

In the second study, Shih et al. (2003) studied the same group of 29 impregnation workers in a copper laminated circuit board plant. An initial survey was conducted in February 1997 with follow-up studies conducted in April and in August of the same year. The aim of the study was to examine the air concentrations of 2-ME, urinary MAA concentrations, and hematological effects following an aggressive workplace improvement program. There were three groups: an exposed group (*n* = 29), a low-exposure group of heat-press workers (*n* = 32), and a nonexposed control group (*n* = 58) of administrative workers. Full-shift 8-hour TWA personal air samples were collected on Fridays for exposed groups and randomly among the administrative workers. End-of-shift urine specimens were collected from exposed workers and randomly among the administrative workers. Nine of the 32 heat-press operators were randomly selected for personal air sampling. Ten randomly selected area air samples were collected from the 58 administrative workers. After improved engineering controls were instituted, the GM concentrations of 2-ME declined in the 29 workers from the initial survey of 9.62 ppm

(GSD = 4.75; range 0.75−320) to 2.34 ppm (GSD = 1.76, range 0.2−10) in April to 0.34 ppm (GSD = 2.69; range, 0.1−3.5) in August, confirming the impact of engineering controls to reduce exposure. During the same corresponding intervals, urinary end-of-shift MAA in the same 29 workers dropped from an initial GM baseline of 50.7-mg/g creatinine (GSD = 1.67; range, 24.3−139) to a GM of 19.7-mg/g creatinine (GSD = 2.09; range, 4.6−54.9) in April and a mean of 6.77-mg/g creatinine (GSD = 4.19; range, 0.95−25.2) in August. The comparison group of nine randomly selected heat-press workers had an initial baseline GM exposure of 0.08 ppm (GSD = 5.09; range not detected (ND) to 0.80, *n* = 9 randomly selected workers). No 2-ME was detected in the air to which administrative controls were exposed. The heat-press workers' mean MAA was 0.53-mg/g creatinine (GSD = 3.40; range not detected to 4.22, *n* = 32). These results from the heat press operators show a GM of 0.53-mg/g creatinine MAA at a GM 2-ME air concentration of 0.08 ppm.

In another study by this group, Chang et al. (2004) examined the protective effectiveness of gloves in 74 exposed workers in the same semiconductor copper laminated circuit board plant as before, along with 80 nonexposed controls. As in other plants, there were two groups of workers, "regular operations" with lower exposures and "special operations" with higher exposures, as well as more contact with liquid 2-ME. One 8-hour TWA 2-ME sample was collected on the last day of the workweek. The exposures were quite constant throughout the workweek. The GM exposure in the regular operations group was 2.14 ppm (GSD = 2.01; range 0.57−9.28, *n* = 49) and 8.13 ppm (GSD = 1.62; range 3.18−15.64, *n* = 25) in special operations, with the lowest detected exposure of 0.57 ppm. Urine specimens collected at the end of the last shift on Friday showed GMs of 5.44-mg/g creatinine (GSD = 3.59; range 0.52−40.7, *n* = 49) for the regular operations group and 72.6-mg/g creatinine (GSD = 2.04; range 16.39−178.0, *n* = 25) for the special operations group. The authors did not show correlations between 2-ME and MAA in urine. The amount of MAA in the urine of "regular operations" workers was much less than reported in previous papers by the same group, perhaps an indication of less exposure through the dermal route.

2-Methoxyethanol Acetate
2-ME acetate is more soluble in oil and less soluble in water than 2-ME. 2-ME acetate in blood is immediately hydrolyzed to 2-ME (Johanson and Dynesius, 1988). No data are available for describing the absorption, distribution, metabolism, and elimination of 2-ME acetate in vivo in humans or animals. However, in vitro studies in animal tissues suggest hydrolysis of the acetate to 2-ME. In mice, nasal mucosal epithelium homogenates exhibited equimolar hydrolysis of 2-ME acetate to 2-ME and acetic acid via carboxylesterase, with apparent Vmax and Km values of 0.9 mM/min and 13.7 mM, respectively (Stott and McKenna, 1985). No differences were observed between sexes, but carboxylesterase activity in mice was 13% lower than dogs, 19% higher than rats, and 147% higher than rabbits. Further, the specific activity of carboxylesterase for 2-ME acetate hydrolysis in homogenized mouse nasal mucosa (667 nmoles/mg protein/min) was similar to that of liver (677 nmoles/mg protein/min) and higher than kidney, lung, or blood (266, 177, and 84 nmoles/mg protein/min, respectively). Bogdanffy et al. (1987) showed that carboxylesterase activity was 3−6 times higher in olfactory epithelium compared to respiratory epithelium. In rat plasma, 2-ME acetate is quickly hydrolyzed by plasma esterases to 2-ME, with an in vitro half-life of ~12 minutes (Hoffman, 1984, as cited in ECETOC, 1984).

Groeseneken et al. (1989) determined that inhaled 2-ME acetate is exhaled as 2-ME after hydrolysis. ACGIH (2006b) concluded that the systemic effects of inhalation exposure to 2-ME acetate occurred at lower concentrations than were likely to cause respiratory tract effects.

In vivo animal studies of glycol ethers support the notion that hydrolysis of 2-ME acetate to 2-ME is rapid and extensive. Oral exposure of mice to propylene glycol monomethyl ether (Miller et al., 1983b) or its acetate (Miller et al., 1984) resulted in almost identical profiles of metabolite production and urinary elimination, suggesting that the acetate is rapidly and extensively hydrolyzed. For 2-ME acetate, the occurrence of reduced testes weights and increased testicular atrophy and tubule degeneration at ≥500 ppm in mice was also seen at approximately equimolar exposures of ≥250-ppm 2-ME (Nagano et al., 1979). This suggests equivalent internal dosimetry of 2-ME and its metabolites in animals exposed to the acetate, and that the generation of acetic acid from the oral exposures tested does not contribute substantially to 2-ME acetate toxicity.

PBPK Models
Several studies described the development and application of PBPK models for estimation of internal 2-ME dosimetry across species. Welsch et al. (1995) developed single and multiple-compartment physiological models in the mouse (for oral or i.v.-administered 2-ME) capable of predicting maternal plasma, embryo, and extraembryonic fluid concentrations of 2-ME and its putative reproductive toxicant, 2-MAA. The multicompartment model was scaled to rats (using rat-specific values for physiological and metabolic characteristics) and humans (via linkage to a previously published human model for pregnancy [O'Flaherty et al., 1992]). The human model was not exercised against actual 2-ME or 2-MAA data from humans. Hays et al. (2000) developed a PBPK model to simulate oral and i.v. exposures of 2-ME in the rat. This model is capable of predicting blood and tissue concentrations of both 2-ME and 2-MAA. Gargas et al. (2000) further developed the Hays et al. (2000) rat model to include inhalation exposures and dynamic physiological changes associated with pregnancy. Predictions of maternal blood 2-ME and fetal 2-ME and 2-MAA concentrations were similar to observations. This model was allometrically scaled to humans and exercised against human urinary 2-MAA data but not in pregnant humans. The available PBPK models were designed to predict internal dosimetry as it related to maternal 2-ME exposures and organogenesis but do not have the capability for predicting male reproductive endpoints, such as testicular concentrations of 2-ME or 2-MAA.

Genotoxicity
Available data on the genotoxicity of 2-ME have been mixed. Tests of mutagenic activity in the Ames test using *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535, TA1537, and TA1538 with or without an S-9 activation system have been negative (Hoflack et al., 1995; Guzzie et al., 1986; McGregor et al., 1983; Ong, 1980). 2-ME was negative in the cell culture test for unscheduled DNA synthesis (McGregor et al., 1983; Guzzie et al., 1986) and point mutations in mouse lymphoma L5178Y cells or Chinese hamster ovary cells (Ma et al., 1993; McGregor, 1984). Weakly positive results (Chapin et al., 1985b; McGregor et al., 1983) and negative results (Rao et al., 1983) in the dominant lethal test in rats, strong positive results in mouse sperm abnormalities (Chapin et al., 1985a,b; McGregor et al., 1983), and inconsistent results in the sex-linked recessive lethal test in *Drosophila* (McGregor et al., 1983) were reported for 2-ME. Sister chromatid exchange was observed in

mouse bone marrow cells following i.p. injection (Arashidani et al., 1998) but not following gavage (Au et al., 1993) or in vitro exposure of human peripheral blood (Arashidani et al., 1998) or Chinese hamster ovary cells (Chiewchanwit and Au, 1994).

DERIVATION OF SUBCHRONIC AND CHRONIC
p-RfDS FOR 2-METHOXYETHANOL

Available human oral studies consist of case reports of acute accidental ingestion. As such, they are not suitable for use as key studies in the derivation of a p-RfD.

Available studies in animals have reported adverse effects of 2-ME on a number of endpoints following oral exposure (see Table 33). Studies in adult animals have identified changes in body-weight gain, decreased organ weights (thymus, spleen, and testes), anemia, and diminished immune function as sensitive endpoints (NTP, 1993; Smialowicz et al., 1992a,b, 1991; Hong et al., 1988; Nagano et al., 1984; Mellon Institute, 1962). Oral exposure to 2-ME has also been shown to have adverse effects on reproductive function and reproductive organs, including testicular and seminiferous tubule degeneration, morphologically altered sperm and decreased sperm number, and ovarian histopathology and estrous cycle alterations (Berndtson and Foote, 1997; Foote et al., 1995; NTP, 1993; Smialowicz et al., 1991; NTP, 1990, 1989, 1988a,b; Hong et al., 1988; Chapin et al., 1985a,b; Nagano et al., 1984; Dodo et al., 2009). 2-ME also induced adverse effects on the developing organism including fetal death, decreased fetal/pup body weights, increased gestation length, and developmental malformations, including cardiovascular malformations and alterations of the digits (Sleet and Ross, 1997; Nelson et al., 1989; Scott et al., 1989; Toraason and Breitenstein, 1988; Greene et al., 1987; Hardin and Eisenmann, 1987; Toraason et al., 1986, 1985; Horton et al., 1985; Nagano et al., 1981). Reproductive or developmental effects have been reported in rats, mice, hamsters, guinea pigs, rabbits, and macaques. Tonkin et al. (2009) provided some elucidation of the MOA for testicular toxicity using gene-expression analysis.

Table 33. Summary of Oral Toxicity Studies of 2-Methoxyethanol and 2-Methoxyethanol Acetate in Animals				
Reference	**Description**	**NOAEL (mg/kg-day)**	**LOAEL (mg/kg-day)**	**Observed Responses**
2-Methoxyethanol acetate				
Nagano et al. (1979)	A gavage study in male JCL-ICR mice exposed for 5 weeks, 5 days/week Daily doses: 0, 62.5, 125, 250, 500, 1000, or 2000 mg/kg-day	250 (179, adjusted for continuous exposure)	500 (357, adjusted for continuous exposure)	Reduced testes weights and spermatocyte counts (Data shown in Table 1)
2-Methoxyethanol				
Short-term systemic studies				
Nagano et al. (1979)	A gavage study in male JCL-ICR mice exposed for 5 weeks, 5 days/week Daily doses: 0, 62.5, 125, 250, 500, 1000, or 2000 mg/kg-day	125 (89, adjusted for continuous exposure)	250 (179, adjusted for continuous exposure)	Reduced testes weights and spermatocyte counts (Data shown in Table 1)
Hong et al. (1988)	A gavage study in male and female B6C3F1 mice exposed for 4 days Daily doses: 0, 50, 100, or 250 mg/kg-day	ND	50	Reduced lymphocytes in males (Data shown in Table 32)
Immunotoxicity studies				
Williams et al. (1995)	A gavage study in male F344 rats exposed for 4 days Daily doses: 0, 25, 50, 100, or 200 mg/kg-day	25	50	Decreased thymus weight and primary antibody response
Smialowicz et al. (1991)	A gavage study in male F344 rats exposed for 10 days Daily doses: 0, 50, 100, or 200 mg/kg-day	ND	50	Decreased thymus and testes weights and primary antibody response

Table 33. Summary of Oral Toxicity Studies of 2-Methoxyethanol and 2-Methoxyethanol Acetate in Animals				
Reference	**Description**	**NOAEL (mg/kg-day)**	**LOAEL (mg/kg-day)**	**Observed Responses**
Smialowicz et al. (1992a)	A gavage study in male F344 rats exposed for 2 days Daily doses: 0, 50, 100, 200, or 400 mg/kg-day	ND	50	Decreased primary antibody response
Smialowicz et al. (1992b)	A gavage study in female F344 rats and female C57BL/6J mice exposed for 10 days Daily doses: 0, 50, 100, 200, or 400 mg/kg-day	Rats: ND Mice: 400	Rats: 50 Mice: ND	Rats: Decreased lymphoproliferative response Mice: No treatment-related changes in body or organ weights or lymphoproliferative response
Systemic studies				
NTP (1993)	A drinking water study in F344/N rats exposed for 13 weeks Drinking water concentrations: 0, 750, 1500, 3000, 4500, or 6000 ppm Estimated doses (mg/kg-day)[a]: Males: 0, 71, 165, 324, 715, or 806 Females: 0, 70, 135, 297, 546, or 785	ND	Male: 71 (750 ppm in drinking water) Female: 70 (750 ppm in drinking water)	Male: Testicular lesions and reduced semen quality; decreased thymus weight Female: Decreased thymus weight (Data shown in Tables 6−8)

Table 33. Summary of Oral Toxicity Studies of 2-Methoxyethanol and 2-Methoxyethanol Acetate in Animals				
Reference	**Description**	**NOAEL (mg/kg-day)**	**LOAEL (mg/kg-day)**	**Observed Responses**
NTP (1993)	A drinking water study in B6C3F1 mice exposed for 13 weeks Drinking water concentrations: 0, 2000, 4000, 6000, 8000, or 10,000 ppm Estimated doses (mg/kg-day)[a]: Males: 0, 295, 529, 765, 992, or 1367 Females: 0, 492, 902, 1194, 1489, or 1839	ND	Male: 295 (2000 ppm in drinking water) Female: 492 (2000 ppm in drinking water)	Male: Decreased spermatozoal concentration Female: Adrenal hypertrophy and splenic hematopoiesis (Data shown in Tables 9 and 10)
Mellon Institute (1962)	A dietary exposure study in DW albino rats exposed for 3 months Dietary concentrations: 0, 0.01, 0.05, 0.25% Estimated doses (mg/kg-day)[a]: Males: 0, 7, 40, or 178 Females: 0, 8, 43, or 201	7 (0.01% diet)	40 (0.05% diet)	Decreased body weight gain
Reproductive studies				
Foster et al. (1984, 1983)	A gavage study in male Sprague-Dawley rats exposed for 11 days Daily doses: 0, 50, 100, or 200 mg/kg-day	50	100	Spermatocyte degeneration
Chapin et al. (1985a)	A gavage study in male F344 rats exposed for 5 days Daily doses: 0, 50, 100, or 200 mg/kg-day	ND	50	Reduced sperm counts and increased incidences of abnormal sperm morphology

2-Methoxyethanol
2-Methoxyethanol Acetate

Table 33. Summary of Oral Toxicity Studies of 2-Methoxyethanol and 2-Methoxyethanol Acetate in Animals				
Reference	Description	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Observed Responses
Chapin et al. (1985b)	A gavage study in male F344 rats exposed for 5 days Daily doses: 0, 50, 100, or 200 mg/kg-day	ND	50	Increased severity of testicular histopathology
NTP (1990)	A two-generation drinking water study in Sprague-Dawley rats cohabitating for 6 weeks Drinking water concentrations: 0, 0.01, 0.03, or 0.1% Estimated doses (mg/kg-day)[a]: F0 Males: 0, 9, 24, or 76 F1 Males: 0, 9, or 27 F0 Females: 0, 13, 36, or 132 F1 Females: 0, 15, or 41	ND	9 (F1 males) (0.01% dw)	Increased testicular degeneration, decreased sperm density (Data shown in Table 16)
NTP (1988a,b; 1989)	A two-generation drinking water study in C57BL/6, CD-1, and C3H mice cohabitating for 6 weeks Drinking water concentrations: 0, 0.03, 0.1, or 0.3% Estimated doses (mg/kg-day)[a]: F0 Males: 0, 53−64, 170−219, or 505−636 F0 Females: 0, 54−63, 174−235, or 543−645	CD-1: ND C57BL/6: ND C3H: 64 (0.03% dw)	CD-1: 60 (0.03% dw) C57BL/6: 53 (0.03% dw) C3H: 219 (0.1% dw)	CD-1: Reduced number of live pups/litter in F1 males C57BL/6: Reduced survival of F0 and F1 male offspring, reduced sperm quality C3H: Testicular lesions, reduced sperm quality, fetal toxicity, impaired fertility (Data shown in Tables 17−19)

		NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	
Reference	**Description**			**Observed Responses**
Berndtson and Foote et al. (1997)	A drinking water study in male Dutch rabbits, 5 days/week, for 12 weeks Doses: 0, 12.5, 25, 37.5, or 50 mg/kg-day	25 (18, adjusted for continuous exposure)	37.5 (27, adjusted for continuous exposure)	Reduced spermatids
Foote et al. (1995)	A drinking water study in male Dutch rabbits, 5 days/week, for 12 weeks Doses: 0, 12.5, 25, 37.5, or 50 mg/kg-day	12.5 (9, adjusted for continuous exposure)	25 (18, adjusted for continuous exposure)	Reduced sperm motility and semen quality (Data shown in Table 22)
Developmental studies				
Nelson et al. (1989)	A liquid diet study in pregnant Sprague-Dawley rats exposed on GDs 7−18 Liquid dietary concentrations: 0, 0.006, 0.012, 0.025, 0.05, 0.1, 0.25, or 0.5% Estimated doses (mg/kg-day)[a]: 0, 16, 31, 73, 140, 198, 290, or 620 mg/kg-day	Maternal: 73 (0.025% diet) Fetal: ND	Maternal: 140 (0.05% diet) Fetal: 16 (0.006% diet)	Maternal: Reduced body weight gain Fetal: Reduced body weight (Data shown in Table 24)
Nelson et al. (1989)	A liquid diet study in pregnant Sprague-Dawley rats exposed on GDs 7−18 Liquid dietary concentrations: 0, 0.006, 0.012, or 0.14% Estimated doses (mg/kg-day)[a]: 0, 17, 33, or 40 mg/kg-day	Maternal: ND Fetal: ND	Maternal: 17 (0.006% diet) Fetal: 17 (0.006% diet)	Maternal: Increased length of gestation Fetal: Increased length of gestation; no effect on neurobehavioral battery (Data shown in Table 25)
Sleet and Ross (1997)	A gavage study in pregnant CRL:CD rats exposed on GD 13 Daily doses: 0, 50, 100, or 250 mg/kg-day	Maternal: 250 Fetal: ND	Maternal: ND Fetal: 50	Maternal: No effects identified Fetal: Limb-bud malformations; decreased embryonic weights

Table 33. Summary of Oral Toxicity Studies of 2-Methoxyethanol and 2-Methoxyethanol Acetate in Animals

Table 33. Summary of Oral Toxicity Studies of 2-Methoxyethanol and 2-Methoxyethanol Acetate in Animals				
Reference	**Description**	**NOAEL (mg/kg-day)**	**LOAEL (mg/kg-day)**	**Observed Responses**
Toraason et al. (1985)	A gavage study in pregnant Sprague-Dawley rats exposed on GDs 7−13 Daily doses: 0, 25, 50, or 100 mg/kg-day	Maternal: ND Fetal: ND	Maternal: 25 Fetal: 25	Maternal: Reduced body weight Fetal: Slight changes in EKG
Toraason et al. (1986)	A gavage study in pregnant Sprague-Dawley rats exposed on GDs 7−13 or 13−19 Daily doses: 0 or 25 mg/kg-day	Maternal: 25 Fetal: ND	Maternal: ND Fetal: 25	Maternal: No effects identified Fetal: Retarded cardiac development
Toraason and Breitenstein (1988)	A gavage study in pregnant Sprague-Dawley rats exposed on GDs 7−13 Daily doses: 0, 50, or 75 mg/kg-day	Maternal: ND Developmental: ND	Maternal: 50 Developmental: 50	Maternal: Reduced body weight and prolonged gestation Developmental: Fetal resorptions, pup lethality, decreased postnatal weights, increased relative heart weights, and EKG changes
Nagano et al. (1981)	A gavage study in pregnant JCL-ICR mice exposed on GDs 7−14 Daily doses: 0, 31.25, 62.5, 125, 250, 500, or 1000 mg/kg-day	Maternal: 125 Fetal: ND	Maternal: 250 Fetal: 31.25	Maternal: Reduced body weight Fetal: Dose-related increase in skeletal anomalies (Data shown in Table 26)
Greene et al. (1987)	A gavage study in pregnant CD-1 mice exposed on GD 11 Daily doses: 0, 100, 175, 250, 350, 400, 450, or 500 mg/kg-day	Maternal: ND Fetal: ND	Maternal: ND Fetal: 100	Maternal: Not reported Fetal: Histological evidence of limb-bud cell death
Horton et al. (1985)	A gavage study in pregnant CD-1 mice exposed on GD 9, 10, 11, 12, or 13 Daily doses: 0, 100, 175, 250, 350, 400, 450, or 500 mg/kg-day	Maternal: ND Fetal: 100	Maternal: ND Fetal: 175	Maternal: Reduced body weight gain in unspecified groups Fetal: Dose-related increase in skeletal (particularly digital) malformations

2-Methoxyethanol
2-Methoxyethanol Acetate

Table 33. Summary of Oral Toxicity Studies of 2-Methoxyethanol and 2-Methoxyethanol Acetate in Animals				
Reference	**Description**	**NOAEL (mg/kg-day)**	**LOAEL (mg/kg-day)**	**Observed Responses**
Hardin and Eisenmann (1987)	A gavage study in pregnant CD-1 mice exposed on GD 11 Daily doses: 0 or 304 mg/kg-day	Maternal: ND Fetal: ND	Maternal: ND Fetal: 304	Maternal: Not reported Fetal: Paw development defects, predominantly syndactyly
Scott et al. (1989)	A gavage study in pregnant *M. fascicularis* (macaques) exposed on GDs 20−45 Daily doses: 0, 12, 24, or 36 mg/kg-day	Maternal: ND Fetal: ND	Maternal: 12 Fetal: 12	Maternal: Dose-related anorexia and maternal body weight loss Fetal: Dose-related embryo lethality

[a]Calculated by study authors based on measurements of body weight and average daily drinking water or food consumption.

ND = not determined.

Based on the above studies, the most sensitive endpoints appear to be reproductive, including alterations in testicular histology in parents and embryo lethality. A number of studies (Berndtson and Foote, 1997; NTP, 1993; Smialowicz et al., 1991; NTP, 1990, 1989, 1988a,b; Hong et al., 1988; Chapin et al., 1985a,b) have reported testicular degeneration following oral exposure to 2-ME in rats, mice, and rabbits. The study by NTP (1990) identified the lowest LOAEL in available animal studies (9.07 mg/kg-day for testicular degeneration and decreased sperm density in F1 male rats in a two-generation reproduction study) but did not identify a NOAEL. This LOAEL is lower than the majority of the NOAELs identified by other studies, suggesting that the juvenile male is more sensitive than the adult to reproductive effects. The next lowest LOAEL in the database was 12 mg/kg-day for embryo lethality in macaques exposed during gestation, also without an accompanying NOAEL (Scott et al., 1989). The data from NTP (1990) and Scott et al. (1989), as well as data from other studies identifying LOAELs of ≤25 mg/kg-day (Foote et al., 1995; Nelson et al., 1989; Toraason et al., 1986) and NTP (1993), were evaluated as candidates for benchmark dose (BMD) modeling to estimate a point of departure (POD) for RfD derivation. Three of the these data sets (Foote et al., 1995; Nelson et al., 1989; Toraason et al., 1986) are not amenable to BMD modeling, either because the effects were not dose-related, or data for variability about the mean were not reported for continuous endpoints. These studies are not considered further for p-RfD derivation because the LOAELs identified in each study (16−25 mg/kg-day) were higher than the LOAELs of 9.07 mg/kg-day identified by NTP (1990) and 12 mg/kg-day from Scott et al. (1989).

BMD modeling (BMDS version 2.1) was conducted using the dichotomous data of NTP (1993, 1990) for seminiferous tubule degeneration in rats, and Scott et al. (1989) for embryo lethality in macaques, as well as the continuous data of NTP (1990) for reduced sperm density in F1 rats. Appendix A contains details of the modeling. The 95% lower confidence limit on the BMD (BMDL) estimates obtained are as follows:

- 0.75 mg/kg-day for seminiferous tubule degeneration in F1 male rats (NTP, 1993)
- 1.64 mg/kg-day for embryo lethality in macaques (Scott et al., 1989)
- 14.3 mg/kg-day for reduced sperm density in F1 rats (NTP, 1990)

BMD modeling of the testicular degeneration data from NTP (1990) was not successful because the models would not run.

The NTP (1993) data for seminiferous tubule degeneration in F1 male rats provided the lowest BMDL. However, these results were considered unreliable due to weaknesses in the data set (see Appendix A), which provided insufficient data to inform the dose-response curve near the calculated BMDL. These weaknesses included no NOAEL, a LOAEL 95 times higher than the $BMDL_{10}$, and a 70% response rate at the LOAEL.

The $BMDL_{1SD}$ of 14.3 mg/kg-day from the NTP (1990) sperm density data also is considered unreliable because it is higher than the LOAEL of 9.07 mg/kg-day identified for this data set. In addition, the sperm density in controls predicted by the BMD model is lower than observed, while the prediction for sperm density at the LOAEL dose is considerably higher than observed. These discrepencies appear to result from the large variability in control sperm density data and the shallow slope of the fitted dose-response curve.

The $BMDL_5$ of 1.64 mg/kg-day based on embryo lethality in macaques is lower than the LOAEL of 12 mg/kg-day in this study and is lower than either the NOAELs or LOAELs identified in any of the studies, or the BMDL for effects on sperm density from NTP (1990). In addition, BMD curve for these data fits the data well. Scott et al. (1989) was selected as the critical study for the derivation of a provisional subchronic and chronic RfDs, because it provides the lowest reliable POD and because it is based on a response in macaques, a species more closely related to humans than rodents.

Although PBPK models for 2-ME have been developed (see above), they are not suitable for extrapolating internal dosimetry for testicular effects in rats because they do not predict internal doses relevant to male reproductive endpoints, such as testicular concentrations of 2-ME or 2-MAA, and they have not been evaluated for their ability to predict internal dosimetry in macaques.

Data from occupational studies indicate that the dermal route is the major route of absorption in the workplace (Sparer et al., 1988; Piacitelli et al., 1990; Chang et al., 2004; Kezic et al., 1997). In addition, physicochemical properties of 2-ME and 2-ME acetate indicate the likelihood of substantial dermal absorption of the liquid (Dugard, 1984; Johanson, 1988). Studies done in volunteers exposed to vapors and liquid 2-ME showed extensive dermal absorption of both the vapor and the liquid. (Kezic et al., 1997). Because data indicate that dermal absorption occurs readily, the potential for dermal exposure must be considered when applying the p-RfDs and p-RfCs for 2-ME and its acetate.

The **subchronic p-RfD** is calculated as follows:

$$\textbf{Subchronic p-RfD} \quad = \quad BMDL_5 \div UF$$
$$= \quad \text{1.64 mg/kg-day/100}$$
$$= \quad \textbf{0.0164 or 2} \times \textbf{10}^{-2} \textbf{ mg/kg-day}$$

The composite UF of 100 is composed of the following:

- A default UF_A of 10 is applied for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between macaques and humans.

- A default UF_H of 10 for intraspecies differences is applied to account for potentially susceptible individuals in the absence of information on the variability of response in humans.

- An UF_L is not needed because a $BMDL_5$ is used as the POD for derivation of the p-RfD.

- An UF_S is not needed because the POD is from a 26-day developmental toxicity study with exposure during organogenesis and because subchronic studies resulted in effects only at higher doses.

- An UF_D is not needed because because the database for this chemical includes numerous supporting developmental, reproductive, immunotoxicity, and subchronic toxicity studies in rats, mice, and rabbits.

Confidence in the critical study is high. The study evaluated appropriate endpoints of reproductive toxicity in adequately sized groups of female macaques exposed to three appropriately chosen gavage doses. Confidence in the database also is high; high-quality subchronic studies are available, while the reproductive and developmental toxicity of 2-ME has been tested in numerous studies. Therefore, confidence in the subchronic p-RfD is high.

The **chronic p-RfD** is calculated as follows:

Chronic p-RfD	=	$BMDL_{10} \div UF$
	=	1.64 mg/kg-day/300
	=	**0.00547 or 5×10^{-3} mg/kg-day**

The composite UF of 300 is composed of the following:

- A default UF_A of 10 is applied for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between macaques and humans.

- A default UF_H of 10 for intraspecies differences is applied to account for potentially susceptible individuals in the absence of information on the variability of response in humans.

- An UF_L is not needed because a $BMDL_{10}$ is used as the POD for derivation of the p-RfD.

- An UF_S of 3 ($10^{0.5}$) is applied for use of a 26-day developmental toxicity study with exposure during organogenesis as the source of the POD, for derivation of the chronic p-RfD. Although subchronic studies resulted in effects only at higher doses, there were no toxicity data from studies longer than 13 weeks in rodents to assure that lifetime exposure might result in effects at lower doses.

- An UF_D is not needed for database insufficiencies. The database contains adequate developmental toxicity and multigeneration reproduction studies, as well as subchronic systemic toxicity studies; although no chronic oral exposure studies for 2-ME are available.

Confidence in the critical study is high. The study evaluated the appropriate endpoints of reproductive toxicity in adequately sized groups of female macaques exposed to three appropriately chosen gavage doses. Confidence in the database is medium; although the reproductive and developmental toxicity of 2-ME have been appropriately tested in numerous studies, an adequate chronic study was not located. Therefore, confidence in the chronic p-RfD is medium.

DERIVATION OF SUBCHRONIC AND CHRONIC
p-RfDs FOR 2-METHOXYETHANOL ACETATE

Nagano et al. (1979) showed that, when daily doses were converted from mg/kg-day to mmol/kg-day, the reduction in relative testes weights were identical for mice treated with 2-ME acetate or its hydrolysis product, 2-ME. Further, similar testicular histopathology was observed in mice administered daily doses of 2-ME that were half that of 2-ME acetate, which corresponds with the 0.64 ratio of molecular weights. This suggests that the internal dosimetry of the putative toxicant for the acetate or glycol ether is equivalent, and equivalent external exposures may be estimated by adjusting doses by molar ratios of the two compounds. Thus, the subchronic and chronic p-RfDs for 2-ME acetate are derived by multiplying the subchronic and chronic RfDs for 2-ME by the 2-ME acetate-to-2-ME molecular weight ratio of $118.13 \div 76.09 = 1.55$.

Data from occupational studies indicate that the dermal route is the major route of absorption in the workplace (Sparer et al., 1988; Piacitelli et al., 1990; Chang et al., 2004; Kezic et al., 1997). In addition, physicochemical properties of 2-ME and 2-ME acetate indicate the likelihood of substantial dermal absorption of the liquid (Dugard, 1984; Johanson, 1988). Because data indicate that dermal absorption occurs readily, the potential for dermal exposure must be considered when applying the p-RfDs and p-RfCs for 2-ME and its acetate.

The **subchronic p-RfD** is calculated as follows:

Subchronic p-RfD	=	2-ME subchronic p-RfD × 1.55
	=	0.0165 mg/kg-day × 1.55
	=	**0.025 or 3×10^{-2} mg/kg-day**

The **chronic p-RfD** is calculated as follows:

Chronic p-RfD	=	2-ME chronic p-RfD × 1.55
	=	0.00547 mg/kg-day × 1.55
	=	**0.00848 or 8×10^{-3} mg/kg-day**

Because the p-RfDs for 2-ME acetate were derived explicitly from those of 2-ME, the uncertainties associated with the PODs for 2-ME and confidence in the critical studies and database for 2-ME also apply to 2-ME acetate.

DERIVATION OF A SUBCHRONIC p-RfC FOR 2-METHOXYETHANOL

IRIS (U.S. EPA,2010a) includes a chronic RfC of 2×10^{-2} mg/m^3 for 2-ME based on a NOAEL of 30 ppm (93 mg/m^3) for testicular effects in male rabbits exposed for 13 weeks (Miller et al., 1983a). A duration-adjusted HEC of 17 mg/m^3 was derived using a dosimetric adjustment and was modified by an UF of 1000, which included an UF_S of 10 for use of a subchronic NOAEL, an UF_H of 10 for sensitive human populations, and a combined factor of 10 for both interspecies extrapolation (UF_A) and database deficiencies (UF_D).

Several occupational studies of 2-ME inhalation exposure have reported statistically significant changes in hematological parameters and subjective neurological symptoms among workers (Cohen, 1984; Cook et al., 1982; Greenburg et al., 1938; Parsons and Parsons, 1938; Donley, 1936; Shih 2003). With the exception of Shih (2003), these data are limited by design (cross-sectional sampling vs. cohort), observational bias, low numbers of subjects, and confounding exposures.

Animal studies have identified multiple toxicological effects from subchronic inhalation exposures to 2-ME (see Table 34). These include reduction in body weights, changes in hematological and immunological parameters, and changes in organ weights (Hanley et al., 1984; Doe et al., 1983; Miller et al., 1983a; Rao et al., 1983). However, like oral exposures, the most consistently observed effects in multiple species are reproductive and developmental. Induction of fetal soft tissue and skeletal abnormalities has been observed in rats (Hanley et al., 1984; Nelson et al., 1984a; Doe et al., 1983) and mice and rabbits (Hanley et al., 1984). Adverse responses of male reproductive tissues have been exhibited in both short-term (Doe et al., 1983) and subchronic (Miller et al., 1983a; Rao et al., 1983) studies in rats and rabbits, including decreased testicle size, testicular atrophy, degeneration of the seminiferous tubules, spermatocyte degeneration, and reduced ability to successfully sire pups.

The NOAELs and LOAELs for the studies given in Table 34 have been adjusted for continuous exposure and converted to an HEC ($NOAEL_{HEC}$ or $LOAEL_{HEC}$) as follows, using the NOAEL from a 6 hours/day, 5 days/week exposure, as an example:

$$NOAEL_{ADJ} = NOAEL \times (6 \text{ hours} \div 24 \text{ hours}) \times (5 \text{ days} \div 7 \text{ days})$$
$$NOAEL_{HEC} = NOAEL_{ADJ} \times [(H_{b/g}) \div (H_{b/g})_H]$$

where $NOAEL_{HEC}$ is calculated as the dosimetric adjustment from the $NOAEL_{ADJ}$ in animals to a NOAEL in humans based on the treatment of 2-ME as a Category 3 gas exhibiting extrarespiratory effects. This is accomplished by multiplying the $NOAEL_{ADJ}$ by the ratio of animal and human blood:gas (air) partition coefficients ($H_{b/gA}:H_{b/gH}$) (U.S. EPA, 1994b). For $NOAEL_{HEC}$s based on rat data, rat and human blood:air partition coefficients of 31,300 and 32,836, respectively (Hays et al., 2000 and Johanson and Dynesius, 1988, respectively), yielded an animal:human blood:gas(air) partition coefficient ratio of 0.95. No blood:gas(air) partition coefficients were located for other species. Therefore, a default value of 1 was used for the ratio of animal to human blood:gas(air) partition coefficients for species other than the rat. For studies reporting exposures that were uninterrupted by weekends (7 days/week exposures), duration adjustment was made only for hours of exposure per day. Current EPA (2002) policy, which differs from the policy in place at the time of the IRIS RfC derivation, is to duration-adjust exposures in developmental toxicity studies, as is done for other types of studies. As a result, the HECs reported here for developmental toxicity studies differ from those reported on IRIS for the same studies.

2-Methoxyethanol
2-Methoxyethanol Acetate

		NOAEL (mg/m^3)	LOAEL (mg/m^3)	
Reference	**Description**			**Observed Responses**
Systemic				

Table 34. Summary of Inhalation (Whole-Body) Toxicity Studies of 2-Methoxyethanol in Animals

Reference	Description	NOAEL (mg/m^3)	LOAEL (mg/m^3)	Observed Responses
		Systemic and reproductive studies		
Doe et al. (1983)	An inhalation study in male Wistar rats exposed 6 hours/day for 10 consecutive days Inhalation concentrations: 0, 100, or 300 ppm	310 (100 ppm) HEC[a]: 74	930 (300 ppm) HEC: 222	Decreases in hematological parameters, testicular atrophy, and spermatocytic degeneration
Rao et al. (1983)	An inhalation study in F344/N rats exposed for 6 hours/day, 5 days/week for 13 weeks Inhalation concentrations: 0, 50, 100, or 300 ppm	310 (100 ppm) HEC: 53	930 (300 ppm) HEC: 158	Reduced parental body weight; reduced fertility, decreased testicular size, and atrophy of the seminiferous tubules in males
Miller et al. (1983a)	An inhalation study in Sprague-Dawley rats exposed for 6 hours/day, 5 days/week for 13 weeks Inhalation concentrations: 0, 30, 100, or 300 ppm	310 (100 ppm) HEC: 53	930 (300 ppm) HEC: 158	Testicular degeneration and decreased testicular weight in males; also pancytopenia, lymphoid atrophy, and reduced body weight
Miller et al. (1983a)	An inhalation study in New Zealand white rabbits exposed 6 hours/day, 5 days/week for 13 weeks Inhalation concentrations: 0, 30, 100, or 300 ppm	93 (30 ppm) HEC: 17	310 (100 ppm) HEC: 56	Testicular degeneration and reduced testes size (Data shown in Table 27)

		NOAEL (mg/m³)	LOAEL (mg/m³)	
Reference	**Description**			**Observed Responses**
Miller et al. (1982)	An inhalation study in New Zealand white rabbits exposed 6 hours/day, 5 days/week for 13 weeks Inhalation concentrations: 0, 3, 10, or 30 ppm	93 (30 ppm) HEC: 17	ND ND	No treatment-related effects were identified
		Developmental studies		
Hanley et al. (1984)	An inhalation study in pregnant F344/N rats exposed for 6 hours/day on GDs 6−15 Inhalation concentrations: 0, 3, 10, or 50 ppm	31 (10 ppm) HEC: 7	155 (50 ppm) HEC: 37	Minor skeletal abnormalities in fetuses; no maternal effects (Data shown in Table 29)
Hanley et al. (1984)	An inhalation study in pregnant CF-1 mice exposed for 6 hours/day on GDs 6−15 Inhalation concentrations: 0, 10, or 50 ppm	31 (10 ppm) HEC: 8	155 (50 ppm) HEC: 39	Fetal skeletal and soft tissue abnormalities; transient decreases in maternal body weight gain (Data shown in Table 30)
Hanley et al. (1984)	An inhalation study in pregnant New Zealand white rabbits exposed for 6 hours/day on GDs 6−18 Inhalation concentrations: 0, 3, 10, or 50 ppm	31 (10 ppm) HEC: 8	155 (50 ppm) HEC: 39	Numerous fetal skeletal and soft tissue abnormalities; transient decreases maternal body weight gain (Data shown in Table 31)
Nelson et al. (1984a)	An inhalation study in pregnant Sprague-Dawley rats exposed for 7 hours/day on GDs 7−15 Inhalation concentrations: 0, 50, 100, or 200 ppm	ND	155 (50 ppm) HEC: 43	Fetal resorptions, decreased fetal body weight, and skeletal malformations (Data shown in Table 28)

Table 34. Summary of Inhalation (Whole-Body) Toxicity Studies of 2-Methoxyethanol in Animals

		NOAEL (mg/m^3)	LOAEL (mg/m^3)	
Table 34. Summary of Inhalation (Whole-Body) Toxicity Studies of 2-Methoxyethanol in Animals				
Reference	**Description**	**NOAEL (mg/m^3)**	**LOAEL (mg/m^3)**	**Observed Responses**
Nelson et al. (1984b)	An inhalation study in which (1) male Sprague-Dawley rats were exposed 7 hours/day, 7 days/week, for 6 weeks and then mated to unexposed females; or (2) pregnant females were exposed for 7 hours/day on GDs 7−13 or 14−20 Inhalation concentrations: 0 (females only) or 25 ppm	ND	78 (25 ppm) HEC: 21	Altered avoidance behavior in pups (maternal exposure on GDs 7−13 only); neurochemical changes of uncertain toxicological significance in 21-day-old pups (maternal or paternal exposure)
Doe et al. (1983)	An inhalation study in pregnant Wistar rats exposed for 6 hours/day on GDs 6−17 Inhalation concentrations: 0, 100, or 300 ppm	ND	310 (100 ppm) HEC: 53	Decreased live-pup birth and pup survival

[a]HEC = N(L)OAEL$_{ADJ}$ × ((H$_{b/g}$)$_A$/(H$_{b/g}$)$_H$); where N(L)OAEL$_{ADJ}$ = N(L)OAEL × (6 hours/24 hours) × (5 days/7 days), and (H$_{b/g}$)$_A$/(H$_{b/g}$)$_H$, the ratio of animal to human blood:air partition coefficients, is 0.95 for rats based on measured data and 1 for all other species by default.

ND = not determined.

2-Methoxyethanol
2-Methoxyethanol Acetate

LOAEL$_{HEC}$s were 158−222 mg/m^3 for testicular and fertility effects in male rats
(Doe et al., 1983; Miller et al., 1983a; Rao et al., 1983). Rabbits were more sensitive than rats,
with testicular effects occurring with a LOAEL$_{HEC}$ of 56 mg/m^3 and NOAEL$_{HEC}$ of 17 mg/m^3
(Miller et al., 1983a, 1982). Developmental effects were seen at LOAEL$_{HEC}$s ranging from 21 to
53 mg/m^3 in rats, mice, and rabbits, with corresponding NOAEL$_{HEC}$s of 7−8 mg/m^3
(Hanley et al., 1984; Nelson et al., 1984a,b; Doe et al., 1983). The data sets for testicular effects
in rabbits from Miller et al. (1983a) and developmental effects in rats, mice, and rabbits from
Hanley et al. (1984) were considered further for dose-response modeling because these studies
identified the most sensitive effects and included ranges of exposure concentrations, giving both
NOAELs and LOAELs.

BMC modeling was performed using the most sensitive endpoints from these studies
(gross reduction in testes size and seminiferous tubule degeneration in rabbits [Miller et al.,
1983a]; delayed ossification of the vertebral centra in rat pups; extra lumbar ribs and unilateral
testicular hypoplasia in mouse pups; and limb and kidney defects in rabbit kits [Hanley et al.,
1984]). Appendix B provides details of the modeling. Table 35 shows the resulting benchmark
concentration (BMC) and 95% lower confidence limit on the BMCs and BMCLs as HECs.
BMCL$_{10HEC}$s for these endpoints ranged from 0.73 to 9.9 mg/m^3, well below all LOAELs in the
database. The low BMCL$_{10HEC}$ of 0.73 mg/m^3 derived from the data for gross reduction in testes
size in male rabbits (Miller et al., 1983a) was selected as the POD for derivation of the
subchronic p-RfC.

Table 35. BMCs Calculated for the Most Sensitive Endpoints from Inhalation (Whole-Body) Studies of 2-Methoxyethanol		
Endpoint	**BMC$_{10HEC}$ (mg/m^3)**	**BMCL$_{10HEC}$ (mg/m^3)**
Gross reduction in testes size in rabbits (Miller et al., 1983a)	6.8	0.73
Seminiferous tubule degeneration in rabbits (Miller et al., 1983a)	6.1	3.1
Delayed ossification of vertebral centra in rats (Hanley et al., 1984)	13.3	9.6
Extra lumbar ribs in mice (Hanley et al., 1984)	4.3	2.9
Unilateral testicular hypoplasia in mice (Hanley et al., 1984)	18.6	7.5
Limb defects in rabbits (Hanley et al., 1984)	13.7	9.9
Renal defects in rabbits (Hanley et al., 1984)	4.8	3.3

The **subchronic p-RfC** is calculated as follows:

$$\textbf{Subchronic p-RfC} = BMCL_{10HEC} \div UF$$
$$= 0.73 \text{ mg/m}^3 \div 100$$
$$= \textbf{0.0073 or } 7 \times 10^{-3} \textbf{ mg/m}^3$$

The composite UF of 100 is composed of the following:

- An UF_H of 10 for intraspecies differences is applied to account for potentially susceptible individuals in the absence of information on the variability of response in humans.

- An UF_A of 3 ($10^{0.5}$) is applied for interspecies extrapolation to account for potential pharmacodynamic differences not quantified by the dosimetric adjustment between rabbits and humans.

- An UF_L is not needed because a $BMCL_{10HEC}$ is used as the POD for derivation of the p-RfC.

- An UF_S is not needed because a subchronic toxicity study is used as the source of the POD for derivation of the subchronic p-RfC.

- An UF_D of 3 ($10^{0.5}$) is applied for database insufficiencies (lack of a multigeneration reproductive toxicity study by inhalation exposure and minimal evaluation of respiratory effects).

Confidence in the critical study is medium. Miller et al. (1983a) is a well-designed toxicity study in which data for multiple endpoints of toxicity, including the results of histological examination of reproductive tissues, were evaluated for rats and rabbits. However, group sizes in the rabbit study were small, and reporting of pathology findings was incomplete. Confidence in the database is medium. Several high-quality subchronic systemic toxicity and developmental toxicity studies in multiple animal species are available; however, the database is lacking a multigeneration reproduction study by inhalation exposure. Neurotoxicity endpoints were not reported in the subchronic studies, and respiratory tract toxicity was only indirectly reported (lung histology). Given medium confidence in the critical study and in the database, confidence in the subchronic p-RfC is medium.

Data from occupational studies indicate that the dermal route is the major route of absorption in the workplace (Sparer et al., 1988; Piacitelli et al., 1990; Chang et al., 2004; Kezic et al., 1997). In addition, physicochemical properties of 2-ME and 2-ME acetate indicate the likelihood of substantial dermal absorption of the liquid (Dugard, 1984; Johanson, 1988). Because data indicate that dermal absorption occurs readily, the potential for dermal exposure must be considered when applying the p-RfDs and p-RfCs for 2-ME and its acetate.

The subchronic p-RfC derived here for 2-ME is lower than the chronic RfC available for this chemical on IRIS (U.S. EPA, 2010a). The subchronic p-RfC and chronic RfC are based on the same endpoint (testicular effects in male rabbits studied by Miller et al., 1983a) but were

derived independently. BMC modeling and duration adjustment in the HEC calculation performed for the subchronic p-RfC (but not the older chronic RfC) lowered the POD from 17 mg/m^3 to 0.73 mg/m^3. The lack of an UF to extrapolate across durations for the subchronic p-RfC partially offsets this change so that (after rounding) the subchronic p-RfC is nearly a factor of 3 lower than the chronic RfC on IRIS (U.S. EPA, 2010a).

DERIVATION OF SUBCHRONIC AND CHRONIC p-RfCs
FOR 2-METHOXYETHANOL ACETATE

No human or animal data for 2-ME acetate inhalation exposure were available for derivation of a p-RfC. Chronic and subchronic p-RfDs for 2-ME acetate were derived by analogy to 2-ME; however, the database to support a derivation of a p-RfC by analogy to 2-ME is much more limited. Unlike oral exposures to 2-ME or its acetate, it is unclear if inhalation of the acetate would result in substantial upper respiratory tract effects (i.e., because of hydrolytic production of acetic acid) that were not observed in the inhaled 2-ME database. However, because the potential acetic acid solution is unlikely to result in a pH sufficiently low to cause substantial upper respiratory tract irritation (ACGIH, 2006b), p-RfCs are derived from the Miller (1983a) subchronic data in rats used to derive the subchronic p-RfC for 2-ME acetate, based on differences in molecular weight.

The low BMCL$_{10HEC}$ of 0.73 mg/m^3 is derived from the data for gross reduction in testes size in male rabbits (Miller et al., 1983a). This BMCL$_{10HEC}$ for 2-ME is multiplied by the molecular-weight difference between the alcohol and acetate of 1.55, to calculate the POD of 1.13 mg/m^3 for derivation of the subchronic p-RfC for 2-ME acetate.

The **subchronic p-RfC** is calculated as follows:

$$\textbf{Subchronic p-RfC} \quad = \quad \text{POD} \div \text{UF}$$
$$= \quad 1.13 \text{ mg/m}^3 \div 100$$
$$= \quad \textbf{0.0113 or } 1 \times 10^{-2} \textbf{ mg/m}^3$$

As for the subchronic derivation for 2-ME, the composite UF of 100 is composed of the following:

- An UF$_H$ of 10 for intraspecies differences is applied to account for potentially susceptible individuals in the absence of information on the variability of response in humans.

- An UF$_A$ of 3 ($10^{0.5}$) is applied for interspecies extrapolation to account for potential pharmacodynamic differences not quantified by the dosimetric adjustment between rabbits and humans.

- An UF$_L$ is not needed because a BMCL$_{10HEC}$ is used as the POD for derivation of the p-RfC.

- An UF$_S$ is not needed because a subchronic toxicity study is the source of the POD for derivation of the subchronic p-RfC.

- An UF$_D$ of 3 ($10^{0.5}$) is applied for database insufficiencies (lack of a multigeneration reproductive toxicity study by inhalation exposure and minimal evaluation of respiratory effects).

Confidence in the critical study is medium. Miller et al. (1983a) is a well-designed toxicity study in which data for multiple endpoints of toxicity, including the results of histological examination of reproductive tissues, were evaluated for rats and rabbits. However, group sizes in the rabbit study were small, reporting of pathology findings was incomplete, and the data were for the alcohol rather than the acetate. Confidence in the database is medium. Several high-quality subchronic systemic toxicity and developmental toxicity studies in multiple animal species are available for the alcohol; however, the database is lacking a multigeneration reproduction study by inhalation exposure. Neurotoxicity endpoints were not reported in the subchronic studies, and respiratory tract toxicity was only indirectly reported (lung histology). Given medium confidence in the critical study and in the database, confidence in the subchronic p-RfC is medium.

Data from occupational studies indicate that the dermal route is the major route of absorption in the workplace (Sparer et al., 1988; Piacitelli et al., 1990; Chang et al., 2004; Kezic et al., 1997). In addition, physicochemical properties of 2-ME and 2-ME acetate indicate the likelihood of substantial dermal absorption of the liquid (Dugard, 1984; Johanson, 1988). Because data indicate that dermal absorption occurs readily, the potential for dermal exposure must be considered when applying the p-RfDs and p-RfCs for 2-ME and its acetate.

The chronic p-RfC for 2-ME acetate was calculated similarly from the subchronic POD, applying an additional UF$_S$ of 10 for extrapolation of the subchronic data to chronic exposure, as follows:

The **chronic p-RfC** is calculated as follows:

$$
\begin{array}{lll}
\textbf{Chronic p-RfC} & = & \text{POD} \div \text{UF} \\
 & = & 1.13 \text{ mg/m}^3 \div 1000 \\
 & = & \textbf{0.00113 or } \mathbf{1 \times 10^{-3} \text{ mg/m}^3}
\end{array}
$$

This chronic p-RfC for 2-ME acetate is a factor of 30 lower than the value of 0.03 mg/m^3 that would be calculated from the IRIS RfC of 0.02 mg/m^3 for the alcohol, because of the much lower POD calculated by BMC modeling.

The discussion of confidence in the chronic p-RfC is identical to that for the subchronic p-RfC, with the same conclusion of medium confidence in the chronic p-RfC for 2-ME acetate.

Data from occupational studies indicate that the dermal route is the major route of absorption in the workplace (Sparer et al., 1988; Piacitelli et al., 1990; Chang et al., 2004; Kezic et al., 1997). In addition, physicochemical properties of 2-ME and 2-ME acetate indicate the likelihood of substantial dermal absorption of the liquid (Dugard, 1984; Johanson, 1988).

Because data indicate that dermal absorption occurs readily, the potential for dermal exposure must be considered when applying the p-RfDs and p-RfCs for 2-ME and its acetate.

PROVISIONAL CARCINOGENICITY ASSESSMENT
FOR 2-METHOXYETHANOL AND 2-METHOXYETHANOL ACETATE

WEIGHT-OF-EVIDENCE CLASSIFICATION

The human database for 2-ME exposure is limited to case reports of acute exposures. The animal data do not include any chronic-exposure oral or inhalation bioassays; none of the available studies are longer than ~3 months in duration. None of these data suggest the development of precancerous lesions. The mutagenicity data for 2-ME (Guzzie et al., 1986; McGregor et al., 1983; Ong, 1980) do not suggest substantial mutagenic activity leading to carcinogenicity. No human or animal data are available on the carcinogenicity of 2-ME acetate. Because 2-ME acetate is rapidly and extensively hydrolyzed to 2-ME, the carcinogenic potential of the acetate is likely to be very similar, if not identical, to that of the glycol ether. Overall, under the EPA (2005) *Guidelines for Carcinogen Risk Assessment*, these data are considered to provide "*Inadequate Information to Assess Carcinogenic Potential*" for 2-ME or its acetate.

QUANTITATIVE ESTIMATES OF CARCINOGENIC RISK

Due to the lack of adequate human or animal data, no quantitative estimate for carcinogenic risk from exposure to 2-ME or its acetate is derived.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2006a) 2-Methoxyethanol. Documentation of the threshold limit values for chemical substances. 7th Edition (update). Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

ACGIH (American Conference of Governmental Industrial Hygienists). (2006b) 2-Methoxyethanol Acetate. Documentation of the threshold limit values for chemical substances. 7th Edition (update). Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

ACGIH (American Conference of Governmental Industrial Hygienists). (2010) Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists.

Ariashidani, K; Kawamoto, T; Kodama, Y. (1998) Induction of sister chromatid exchange by ethylene glycol monomethyl ether and its metabolite. Ind Health 36:127−131.

ATSDR (Agency for Toxicological Substances and Disease Registry). (2010) Internet HazDat toxicological profile query. U.S. Department of Health and Human Services, Public Health Service. Atlanta, GA. Available online at http://www.atsdr.cdc.gov (accessed May 2010).

Au, WW; Morris, DL; Legator, MS. (1993) Evaluation of the clastogenic effects of 2-methoxyethanol in mice. Mut Res 300:273−279.

Berndtson, WE; Foote, RH. (1997) Disruption of spermatogenesis in rabbits consuming ethylene glycol monomethyl ether. Reprod Toxicol 11(1):29−36.

Bagchi G. and D.J. Waxman. 2008. Toxicity of ethylene glycol monomethyl ether: impact on testicular gene expression. International Journal of Andrology. 31:269−274.

Bogdanffy, MS; Randall, HW; Morgan, KT. (1987) Biochemical quantitation and histochemical localization of carboxylesterase in the nasal passages of the Fischer 344 rat and B6C3F1 mice. Toxicol Appl Pharmacol 88:183−194.

CalEPA (California Environmental Protection Agency). (2000a) Chronic toxicity summary: Ethylene glycol monomethyl ether. OEHHA Appendix D.3: Chronic RELs and toxicity summaries, p. 253−259. Office of Environmental Health Hazard Assessment. Available online at http://www.oehha.ca.gov/air/hot_spots/2008/AppendixD3_final.pdf#page=253 (accessed May 2010).

CalEPA (California Environmental Protection Agency). (2000b) Chronic toxicity summary: Ethylene glycol monomethyl ether acetate. OEHHA Appendix D.3: Chronic RELs and toxicity summaries, p. 260−263. Office of Environmental Health Hazard Assessment. Available online at http://www.oehha.ca.gov/air/hot_spots/2008/AppendixD3_final.pdf#page=260 (accessed May 2010).

Chang HY; Lin CC; Shih TS; et al. 2004. Evaluation of the protective effectiveness of gloves from occupational exposure to 2-methoxyethanol using the biomarkers of 2-methoxyacetic acid levels in the urine and plasma. Occup Environ Med 61:697−702.

Chapin, RE; Dutton, SL; Ross, MD; et al. (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, RE; Dutton, SL; Ross, MD; et al. (1985b) The recovery of the testes over 8 weeks after short-term dosing with ethylene glycol monomethyl ether: histology, cell-specific enzymes and discrete testis fluid protein. Fundam Appl Toxicol 5:515−525.

Chiewchanwit, T; Au, W. (1994) Cytogenic effects of 2-methoxyethanol and its metabolite, methoxyacetaldehyde, in mammalian cells in vitro. Mut Res 320:125−132.

Cohen, R. (1984) Reversible subacute ethylene glycol monomethyl ether toxicity associated with microfilm production: a case report. Am J Ind Med 6(6):441−446.

Cook, RR; Bodner, KM; Kolesar, RC; et al. (1982) A cross-sectional study of ethylene glycol monomethyl ether process employees. Arch Environ Health 37(6):346−351.

Dodo T., Y. Taketa, M. Sugiyama, A. Inomata, J. Sonoda, Y. Okuda, H. Mineshima, S. Hosokawa, T. Aoki. 2009. Collaborative work on evaluation of ovarian toxicity. 11) Two- or four-week repeated-dose studies and fertility study of ethylene glycol monomethyl ether in female rats. J Toxicol Sci. 2009;34 Suppl 1:121−128.

Doe, JE; Samuels, DM; Tinston, DJ; et al. (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(1):43−47.

Donley, DF. (1936) Toxic encephalopathy and volatile solvents in industry. Report of a case. J Ind Hyg Toxicol 18(8):571−577.

Dugard P. 1984. Absorption of some glycol ethers through human skin in vitro. Environ Health Perspect 57:193−197.

ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals). (1984) Technical report no. 17. The toxicology of glycol ether and its relevance to man: an updating of ECETOC Technical Report No. 4. Brussels, Belgium: European Chemical Industry Ecology and Toxicology Centre.

Foote, RH; Farrell, PB; Schlafer, DH; et al. (1995) Ethylene glycol monomethyl ether effects on health and reproduction in male rabbits. Reprod Toxicol 9(6):527−539.

Foster, PMD; Creasy, DM; Foster, JR; et al. (1983) Testicular toxicity of ethylene glycol monomethyl and monomethyl ethers in the rat. Toxicol Appl Pharmacol 69:385−399.

Foster, PMD; Creasy, DM; Foster, JR; et al. (1984) Testicular toxicity produced by ethylene glycol monomethyl and monomethyl ethers in the rat. Environ Health Persp 57:207−217.

Foster PMD; Lloyd SC; Blackburn DM. 1987. Comparison of the in vivo and in vitro testicular effects produced by methoxy-, ethoxy- and n-butoxy acetic acids in the rat. Toxicology 43:17−30.

Gargas, ML; Tyler, TR; Sweeney, LM; et al. (2000) A toxicokinetic study of inhaled ethylene glycol monomethyl ether (2-ME) and validation of a physiologically based pharmacokinetic model for the pregnant rat and human. Toxicol Appl Pharmacol 165:53−62.

Greenburg, L; Mayers, MR; Goldwater, JL; et al. (1938) Health hazards in the manufacture of "fused collars". I. Exposure to ethylene glycol monomethyl ether. J Ind Hyg Toxicol 20(2):134−147.

Greene, JA; Sleet, RB; Morgan, KT; et al. (1987) Cytotoxic effects of ethylene glycol monomethyl ether in the forelimb bud of the mouse embryo. Teratology 36(1):23−34.

Groeseneken, D; Veulemans, H; Masschelein, RM; et al. (1989) Experimental human exposure to ethylene glycol monomethyl ether. Int Arch Occup Environ Health 61:243−247.

Guzzie, PJ; Slesinski, RS; Hengler, WC; et al. (1986) Assessment of 2-ethoxyethanol for genotoxicity using a battery of in vitro and in vivo test systems. Environ Mutagen 8(Suppl 6):33.

Hanley, TR; Yano, BL; Nitschke, KD; et al. (1984) Comparison of the teratogenic potential of inhaled ethylene glycol monomethyl ether in rats, mice and rabbits. Toxicol Appl Pharmacol 75:409−422.

Hardin, BD; Eisenmann, CJ. (1987) Relative potency of four ethylene glycol ethers for induction of paw malformations in the CD-1 mouse. Teratology 35:321−328.

Hardin BD, Schuler RL, Burg JR, et al. (1987) Evaluation of 60 chemicals in a preliminary evelopmental toxicity test. Teratog Carcinog Mutagen 7:29−48.

Hays, SM; Elswick, BA; Blumenthal, GM; et al. (2000) Development of a physiologically based pharmacokinetic model of 2-methoxyethanol and 2-methoxyacetic acid in pregnant rats. Toxicol Appl Pharmacol 163:67−74.

Hoffman, HD. (1984) Bericht uberdie Prufung der Stabilitat von Glykoletheracetaten in Rattenplasma. BASF, internal report. (as cited in ECETOC, 1984)

Hoflack, JC; Lambolez, L; Elias, Z; et al. (1995) Mutagenicity of ethylene glycol ethers and of their metabolites in Salmonella typhimurium his⁻. Mut Res 341:281−287.

Hong, HL; Canipe, J; Jameson, CJ; et al. (1988) Comparative effects of ethylene glycol and ethylene glycol monomethyl ether exposure on hematopoiesis and histopathology in B6C3F1 mice. J Environ Pathol Toxicol Oncol 8(7):27−38.

Horton, VL; Sleet, RB; John-Greene, JA; et al. (1985) Developmental phase-specific and dose-related teratogenic effects of ethylene glycol monomethyl ether in CD-1 mice. Toxicol Appl Pharmacol 80(1):108−118.

HSDB (Hazardous Substances Data Bank). 2007. National Library of Medicine. Available online at: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~uXADP7:1 (accessed May 2010).

IARC (International Agency for Research on Cancer). (2010) Search IARC monographs. Available online at http://monographs.iarc.fr/ (accessed May 2010).

Johanson G. 1988. Aspects of biological monitoring of exposure to glycol ethers. Toxicol Lett 43:5−21.

Johanson, G; Dynesius, B. (1988) Liquid/air partition coefficients of six commonly used glycol ethers. Br J Ind Med 45:561−564.

Johanson, G. (2000) Toxicity review of ethylene glycol monomethyl ether and its acetate ester. Crit Rev Toxicol 30:307−345.

Kezic S; Mahieu K; Monster AC; deWolff FA. 1997. Dermal absorption of vaporous and liquid 2-methoxyethanol and 2-ethoxyethanol in volunteers. Occup Environ Med 54:38−43.

Larese F; Fiorito A; DeZotti R. (1992). The possible haematological effects of glycol monomethyl ether in a frame factory. Br J Ind Med 49:131−133.

Ma, H; An, J; Hsie, AW; et al. (1993) Mutagenicity and cytotoxicity of 2-methoxyethanol and it metabolites in Chinese hamster cells (the CHO/HPRT and AS52/GPT assays). Mut Res 298:219−225.

McGregor, DB. (1984) Genotoxicity of glycol ethers. Environ Health Perspect 57:97−104.

McGregor, DB; Willins, JM; Holmstrom, M; et al. (1983) Genetic effects of 2-methoxy ethanol and bis-(2-methoxy ethyl) ether. Toxicol Appl Pharmacol 70(2):303−316.

McManus K; Moseley C; Lowry L; et al. 1989. Health hazard evaluation: Electric boat division, General Dynamics Corporation, Grotton, CT. DHHS HETA No 84-474-1946. National Institute for Occupational Safety and Health, Cincinnati, OH.

Mellon Institute. (1962) Initial submission: gross results of 3-months of inclusion of methyl cellosolve in the diet of rats with cover letter. U.S. EPA/OPTS Public Files, Fiche #OTS0571090.

Miller, RR; Calhoun, LL; Yano, BL. (1982) Ethylene glycol monomethyl ether: 13-week vapor inhalation study in male rabbits. Report prepared for Chemical Manufacturers Association, March 25, 1982.

Miller, RR; Ayres, JA; Young, JT; et al. (1983a) Ethylene glycol monomethyl ether. I. Subchronic vapor inhalation study with rats and rabbits. Fundam Appl Toxicol 3(1):49−54.

Miller, RR; Hermann, A; Landvardt, PW; et al. (1983b) Comparative metabolism and distribution of ethylene glycol monomethyl ether and propylene glycol monomethyl ether in male rats. Toxicol Appl Pharmacol 67:229−237.

Miller, RR; Hermann, EA; Young, JT; et al. (1984) Propylene glycol monomethyl ether acetate (PGMEA) metabolism, disposition, and short-term vapor inhalation toxicity studies. Toxicol Appl Pharmacol 75:521−530.

Nagano, K; Nakayama, E; Koyano, M; et al. (1979) Mouse testicular atrophy induced by ethylene glycol monoalkyl ethers. Submitted to the U.S. Environmental Protection Agency under TSCA section FYI. FYI-OTS-0381-0105. OTS0000105 (pdf pages 76−85) (English translation of Jap J Ind Health 21:29−35).

Nagano, K; Nakayama, E; Oobayashi, H; et al. (1981) Embryotoxic effects of ethylene glycol monomethyl ether in mice. Toxicology 20:335−343.

Nagano, K; Nakayama, E; Oobayashi, H; et al. (1984) Experimental studies on toxicity of ethylene glycol alkyl ethers in Japan. Environ Health Perspect 57:75−84.

Nelson, BK; Setzer, JV; Brightwell, WS; et al. (1984a) Comparative inhalation teratogenicity of four glycol ether solvents and an amino derivative in rats. Environ Health Perspect 57:261−271.

Nelson, BK; Brightwell, WS; Burg, JR; et al. (1984b) Behavioral and neurochemical alterations in the offspring of rats after maternal or paternal inhalation exposure to the industrial solvent 2-methoxyethanol. Pharmacol Biochem Behav 20(2):269−279.

Nelson, BK; Vorhees, CV; Scott, WJ, Jr; et al. (1989) Effects of 2-methoxyethanol on fetal development, postnatal behavior, and embryonic intracellular pH of rats. Neurotoxicol Teratol 11:273−284.

NIOSH (National Institute for Occupational Safety and Health). (1991) Criteria for a Recommended Standard: Occupational Exposure to Ethylene Glycol Monomethyl Ether, Ethylene Glycol Monoethyl Ether, and Their Acetates. NIOSH Publication No. 91-119. Available online at http://cdc.gov/niosh/91-119.html (accessed September 2010).

NIOSH (National Institute for Occupational Safety and Health). (2005) NIOSH pocket guide to chemical hazards. Available online at http://www.cdc.gov/niosh/npg/npgd0401.html (accessed May 2010).

Nitter-Hauge, S. (1970) Poisoning with ethylene glycol monomethyl ether: report of two cases. Acta Med Scand 188:277−280.

NTP (National Toxicology Program). (1988a) Ethylene glycol monomethyl ether reproduction and fertility assessment in C57BL/6 mice when administered in drinking water. Environmental Health Research and Testing, Inc., Lexington, KY under Contract No. NO1-ES-65142. NTP, Research Triangle Park, NC. NTIS PB88-192240.l

NTP (National Toxicology Program). (1988b) Ethylene glycol monomethyl ether reproduction and fertility assessment in CD-1 mice when administered in drinking water. Environmental Health Research and Testing, Inc., Lexington, KY under Contract No. NO1-ES-65142. NTP, Research Triangle Park, NC. NTIS PB88-211446.

NTP (National Toxicology Program). (1989) Ethylene glycol monomethyl ether reproduction and fertility assessment in C3H mice when administered in drinking water. Environmental Health Research and Testing, Inc., Lexington, KY under Contract No. NO1-ES-65142. NTP, Research Triangle Park, NC. NTIS PB89-152565.

NTP (National Toxicology Program). (1990) Reproductive toxicity of ethylene glycol monomethyl ether (CAS No. 109-86-4) in Sprague-Dawley rats, litter two. Environmental Health Research and Testing, Inc., Lexington, KY under Contract No. NO1-ES-65142. NTP, Research Triangle Park, NC. NTIS PB90-252313.

NTP (National Toxicology Program). (1993) NTP technical report on toxicity studies of ethylene glycol ethers 2-methoxyethanol, 2-ethoxyethanol, 2-butoxyethanol administered in drinking water to F344/n rats and B6C3F1 mice. National Toxicology Program. Research Triangle Park, NC; Toxicity report no. 26. Available online at http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox026.pdf (accessed May 2010)

NTP (National Toxicology Program). (2005) 11th Report on carcinogens. Available online at http://ntp.niehs.nih.gov/index.cfm?objectid=32BA9724-F1F6-975E-7FCE50709CB4C932 (accessed May 2010).

NTP (National Toxicology Program). (2010) Management status report. Available online at http://ntp.niehs.nih.gov/index.cfm?objectid=78CC7E4C-F1F6-975E-72940974DE301C3F (Accessed May 2010).

O'Flaherty, EJ; Scott, WJ, Jr; Schreiner, C; et al. (1992) A physiologically-based kinetic model of rat and mouse gestation: disposition of a weak acid. Toxicol Appl Pharmacol 112:245−256.

Ong, T. (1980) Mutagenicity testing of 2-ethoxy-madhouse ethanol. Internal NIOSH Communication Sept. 29, 1980. (Cited in U.S. EPA, 1986)

OSHA (Occupational Safety and Health Administration). (2010) Regulations for air contaminants (Standards 29 CFR 1910.1000). Available online at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=999 2 (accessed May 2010).

Parsons, CE; Parsons, MEM. (1938) Toxic encephalopathy and "granulopenic anemia" due to volatile solvents in industry: report of two cases. J Ind Hyg Toxicol 20(2):124−133.

Piacitelli GM; Votaw DM; Krishman ER (1990) An exposure assessment of industries using ethylene glycol ethers. Appl Occup Environ Hyg 5:107−114.

Rao, KS; Cobel-Geard, SR; Young, JT; et al. (1983) Ethylene glycol monomethyl ether. II. Reproductive and dominant lethal studies in rats. Fundam Appl Toxicol 3:80−85.

Rawlings SJ; Shuker DEG; Randall JL; et al. (1985). The teratogenic potential of alkoxy acids in post-implantation rat embryo cultures: structure activity relationships. Toxicol Lett 28:49−58.

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2-Methoxyethanol Acetate

Scott, WJ; Fradkin, R; Wittfoht, W; et al. (1989) Teratologic potential of 2-methoxyethanol and transplacental distribution of its metabolite, 2-methoxyacetic acid, in non-human primates. Teratology 39:363−373.

Shih TS; Liou SH; Chen CY; Chou JS. (1999). Correlation between urinary 2-methoxy acetic acid and exposure of 2-methoxyethanol. Occup Environ Med 56:674−678.

Shih TS; Hsieh AT; Liao GD; et al. 2000. Hematological and spermatotoxic effects of ethylene glycol monomethyl ether in copper clad laminate factories. Occup Environ Med 57:348−352.

Shih TS; Liou SH; Chen CY; Smith TJ. 2001. Urinary 2-methoxy acetic acid accumulation in response to 2-methoxy ethanol exposure. Arch Environ Health 56:20−25.

Shih TS; Hsieh AT; Chen YH; et al. (2003). Follow-up study of hematological effects in workers exposed to 2-methoxyethanol. Occup Environ Med 60:130−135.

Sleet, RB; Ross, WP. (1997) Serine-enhanced restoration of 2-methoxyethanol-induced dysmorphogenesis in the rat embryo and near-term fetus. Toxicol Appl Pharmacol 145(2):415−424.

Smialowicz, RJ; Riddle, MM; Luebke, RW; et al. (1991) Immunotoxicity of 2-methoxyethanol following oral administration in Fischer 344 rats. Toxicol Appl Pharmacol 109(3):494−506.

Smialowicz, RJ; Williams, WC; Riddle, MM; et al. (1992a) Comparative immunosuppression of various glycol ethers orally administered to Fischer 344 rats. Fundam Appl Toxicol 18(4):621−627.

Smialowicz, RJ; Riddle, MM; Williams, WC; et al. (1992b) Differences between rats and mice in the immunosuppressive activity of 2-methoxyethanol and 2-methoxyacetic acid. Toxicology 74(1):57−67.

Sparer J; Welch LS; McManus K; et al. (1988). Effects of exposure to ethylene glycol ethers on shipyard painters: I. Evaluation of exposure. Am J Ind Med 14:497−507.

Starek, A; Szymczak, W; Zapor, L. (2008). Hematological effects of four ethylene glycol monoalkyl ethers in short-term repeated exposure in rats. Arch Toxicol 82:125−136.

Stott, WT; McKenna, MJ. (1985) Hydrolysis of several glycol ether acetates and acrylate esters by nasal mucosal carboxylesterase in vitro. Fundam Appl Toxicol 5(2):399−404.

Tonkin E.G., M. Coopera, L.O. Lollini, P.A. Day-Lollini, J. Allard, K.L. Kolaja, S.J. Platz, S.M. Chanda. 2009. Testicular gene expression profiling following 2-methoxyethanol and 2-ethoxyethanol exposure in male rats reveals abnormal expression of the actin binding protein cortactin in degenerating spermatocytes. Toxicology Letters 190:193−201.

Toraason, M; Breitenstein, M. (1988) Prenatal ethylene glycol monomethyl ether (EGME) exposure produces electrocardiographic changes in the rat. Toxicol Appl Pharmacol 95:321−327.

Toraason, M; Stringer, B; Stober, P; et al. (1985) Electrocardiographic study of rat fetuses exposed to ethylene glycol monomethyl ether (EGME). Teratology 32:33−39.

Toraason, M; Stringer, B; Smith, R. (1986) Ornithine decarboxylase activity in the neonatal rat heart following prenatal exposure to ethylene glycol monomethyl ether. Drug Chem Toxicol 9(1):1−14.

U.S. EPA (Environmental Protection Agency). (1984) Health effects assessment for glycol ethers. Prepared by the Office of Research and Development, Cincinnati, OH, for the Office of Office of Emergency and Remedial Response, Washington, DC EPA/540/1-86-052.

U.S. EPA (Environmental Protection Agency). (1986) Health and environmental effects profile for 2-methoxyethanol. Prepared by the Environmental Criteria and Assessment Office, Cincinnati, OH, for the Office of Solid Waste and Emergency Response.

U.S. EPA (Environmental Protection Agency). (1987) Health and environmental effects profile for 2-methoxyethanol acetate. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH, for the Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA (Environmental Protection Agency). (1991) Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC.

U.S. EPA (Environmental Protection Agency). (1994a) Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC.

U.S. EPA (Environmental Protection Agency). (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Office of Research and Development, Washington, DC EPA/600/8-90/066F. Available online at http://www.epa.gov/osa/raf/publications/pdfs/RFCMETHODOLOGY.PDF (accessed May 2010)

U.S. EPA (Environmental Protection Agency). (2000) Benchmark dose technical guidance document (draft). Risk Assessment Forum, Washington, DC. EPA/630/R-00/001. Available online at http://www.epa.gov/nceawww1/pdfs/bmds/BMD-External_10_13_2000.pdf (accessed May 2010)

U.S. EPA (Environmental Protection Agency). (2002) A review of the reference dose and reference concentration processes. Risk Assessment Forum, Washington, DC. EPA/630/P-02/002F. Available online at http://www.epa.gov/ncea/iris/RFD_FINAL1.pdf (accessed May 2010)

U.S. EPA (Environmental Protection Agency). (2005) Guidelines for carcinogen risk assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001F. Federal Register 70(66):17765−17817. Available online at http://www.epa.gov/raf/publications/pdfs/CANCER_GUIDELINES_FINAL_3-25-05.PDF (accessed May 2010).

U.S. EPA (Environmental Protection Agency). (2009) Drinking water standards and health advisories. Office of Water, Washington, DC. Available online at http://www.epa.gov/waterscience/criteria/drinking/dwstandards2009.pdf (accessed April 2010).

U.S. EPA (Environmental Protection Agency). (2010a) Integrated Risk Information System (IRIS) summary for 2-Methoxyethanol. Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Available online at http://www.epa.gov/IRIS/subst/0525.htm (accessed May, 2010).

U.S. EPA (Environmental Protection Agency). (2010b) Health Effects Assessment Summary Tables (HEAST). FY-1997 Update. Prepared by the Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC. EPA/540/R-97/036. NTIS PB 97-921199.

Welch LS; Cullen MR. 1988. Effects of exposure to ethylene glycol ethers on shipyard painters: III. Hematological effects. Am J Ind Med 14:527−536.

Welch LS; Schrader SM; Turner TW; et al. 1988. Effects of exposure to ethylene glycol ethers on shipyard painters: II. Male reproduction. Am J Ind Med 14:509−526.

Welsch, F. (2005) The mechanism of ethylene glycol ether reproductive and developmental toxicity and evidence for adverse effects in humans. Toxicol Lett 156:13−28.

Welsch, F; Blumenthal, GM; Conolly, RB. (1995) Physiologically based pharmacokinetic models applicable to organogenesis: extrapolation between species and potential use in prenatal toxicity risk assessments. Toxicol Lett 82:539−547.

WHO (World Health Organization). (1990) Environmental health criteria 115. 2-Methoxyethanol, 2-ethoxyethanol, and their acetates. Available online at http://www.inchem.org/documents/ehc/ehc/ehc115.htm (accessed May 2010).

Williams, WC; Riddle, MM; Copeland, CB; et al. (1995) Immunological effects of 2-methoxyethanol administered dermally or orally to Fischer 344 rats. Toxicology 98:215−223.

Young, EG; Woolner, LB. (1946) A case of fatal poisoning from 2-methoxy-ethanol. J Ind Hyg Toxicol 28:267−268.

Zavon, MR. (1963) Methyl cellosolve intoxication. Proceed Ind Hyg Assoc J 24:36−41.

APPENDIX A. DETAILS OF BENCHMARK DOSE MODELING FOR SUBCHRONIC AND CHRONIC p-RfDs

MODEL-FITTING PROCEDURE FOR QUANTAL NONCANCER DATA
        The model-fitting procedure for dichotomous noncancer data is as follows. All available dichotomous models in the EPA BMDS (version 2.1) are fit to the incidence data using the extra risk option. The multistage model is run for all polynomial degrees up to $n - 1$ (where n is the number of dose groups including control). Adequate model fit is judged by three criteria: goodness-of-fit p-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark response rate (BMR). Among all of the models providing adequate fit to the data, the lowest BMDL is selected as the POD when the difference between the BMDLs estimated from these models is >3-fold (unless it appears to be an outlier); otherwise, the BMDL from the model with the lowest Akaike Information Criterion (AIC) is chosen. In accordance with EPA (2000) guidance, BMDs and BMDLs associated with an extra risk of 10% BMR generally are calculated for all models; a 5% BMR is used for developmental data.

Model Fitting for Incidence of Seminiferous Tubule Degeneration in Rats (NTP, 1993)
        Following the above procedure, the quantal models in the EPA BMDS (version 2.1) were fit to the incidence data for seminiferous tubule degeneration in rats from the NTP (1993) study shown in Table 7. Table A-1 shows the modeling results. Only the log logistic model produced a nonzero p-value ($p = 0.22$). However, modeling of these data was problematic, based on limitations in the data set for BMD modeling, including the lack of data points in the region of the BMR, very high response rate at the lowest dose (70 vs. 0% in controls), fractional response in only one dose group, and a plateau at the maximum response rate at higher doses and actual decrease in response rate to 90% at the second highest dose. The models other than the log logistic failed due to the drop in response at the second highest dose. When the models were run again after dropping the two high dose groups, most models achieved perfect fit. This model over-fit reflects the lack of information in the available data (fractional response at only one data point).

                                                    2-Methoxyethanol
                                                    2-Methoxyethanol Acetate

Table A-1. Model Predictions for the Incidence of Seminal Tubule Degeneration in Male Rats						
Model	**Degrees of Freedom**	**χ^2**	**χ^2 Goodness of Fit p-Value[a]**	**AIC**	**BMD_{10} (mg/kg-day)**	**$BMDL_{10}$ (mg/kg-day)**
All doses						
Gamma[b]	5	178.22	0.00	35.08	-	-
Logistic	4	99.57	0.00	46.69	-	-
Log logistic[c]	**4**	**5.75**	**0.22**	**28.13**	**3.92**	**0.75**
Log probit[c]	5	34.26	0.00	28.59	-	-
Multistage (degree = 1)[d]	5	178.22	0.00	35.08	-	-
Multistage (degree = 2)[d]	5	178.22	0.00	35.08	-	-
Multistage (degree = 3)[d]	5	178.22	0.00	35.08	-	-
Multistage (degree = 4)[d]	5	178.22	0.00	35.08	-	-
Multistage (degree = 5)[d]	5	178.22	0.00	35.08	-	-
Probit	4	29.56	0.00	50.07	-	-
Weibull[b]	5	178.22	0.00	35.08	-	-
Quantal-linear	5	178.22	0.00	35.08	-	-
Two highest doses dropped						
Gamma[b]	3	0	1.00	14.22	45.61	3.42
Logistic	2	0	1.00	16.22	60.22	14.81
Log logistic[c]	3	0	1.00	14.22	59.95	4.83
Log probit[c]	2	0	1.00	16.22	53.06	6.20
Multistage (degree = 1)[d]	3	0.61	0.89	15.17	2.50	1.51
Multistage (degree = 2)[d]	**3**	**0.61**	**0.89**	**15.17**	**0.49**	**0.30**
Multistage (degree = 3)[d]	2	0	1.00	16.22	31.52	3.42
Probit	2	0	1.00	16.22	50.56	13.44
Weibull[b]	2	0	1.00	16.22	32.14	3.42
Quantal-linear	3	0.61	0.89	15.17	5.13	3.10

[a]p-Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.
[d]Betas restricted to ≥0.

Source: NTP (1993).

2-Methoxyethanol
2-Methoxyethanol Acetate

Model Fitting for Incidence of Testicular Degeneration in Rats (NTP, 1993)

The quantal models in the EPA BMDS (version 2.1) were fit to the incidence data for testicular degeneration in rats from the NTP (1990) study shown in Table 16. However, it was not possible to model these data, as they caused unrecoverable software failures in the BMDS system. This was probably due to the higher incidence in the mid-dose group than the high-dose group. However, due to the availability of data for only three dose groups, it was not possible to drop the high-dose group.

Model Fitting for Incidence of Embryo Lethality in Macaques (Scott et al., 1989)

Scott et al. (1989) found a dose-related increase in incidence of embryo lethality following gavage exposures during GDs 20−45. The data of Scott et al. (1989), showing increasing response (0/6, 3/13, 3/10, or 8/8) over four evenly-spaced doses (0, 12, 24, or 36 mg/kg-day), were subjected to BMD modeling, as described above, using a 5% BMR for developmental data. Table A-2 shows the modeling results. Adequate fit ($p > 0.01$) is achieved with several models; fit for the 1-degree polynomial model is marginal. Of these, the 2-degree polynomial model had the lowest AIC, indicating best fit to the data, and one of the lowest $BMDL_5$s. Figure A-1 shows a graph of the 2-degree polynomial model superimposed on the data. Although model fit at the 2 higher-dose groups is marginal (scaled residuals of 1.3−1.6), fit at the control and low-dose groups, which bracket the BMR of 5%, is reasonably good (scaled residuals of 0−0.48). The $BMDL_5$ estimated from the 2-degree multistage model for these data is 1.64 mg/kg-day.

Table A-2. BMD_5 Modeling Results for Incidence of Embryo Lethality in Pregnant Macaques Given Gavage Doses of 2-Methoxyethanol on GDs 20−45

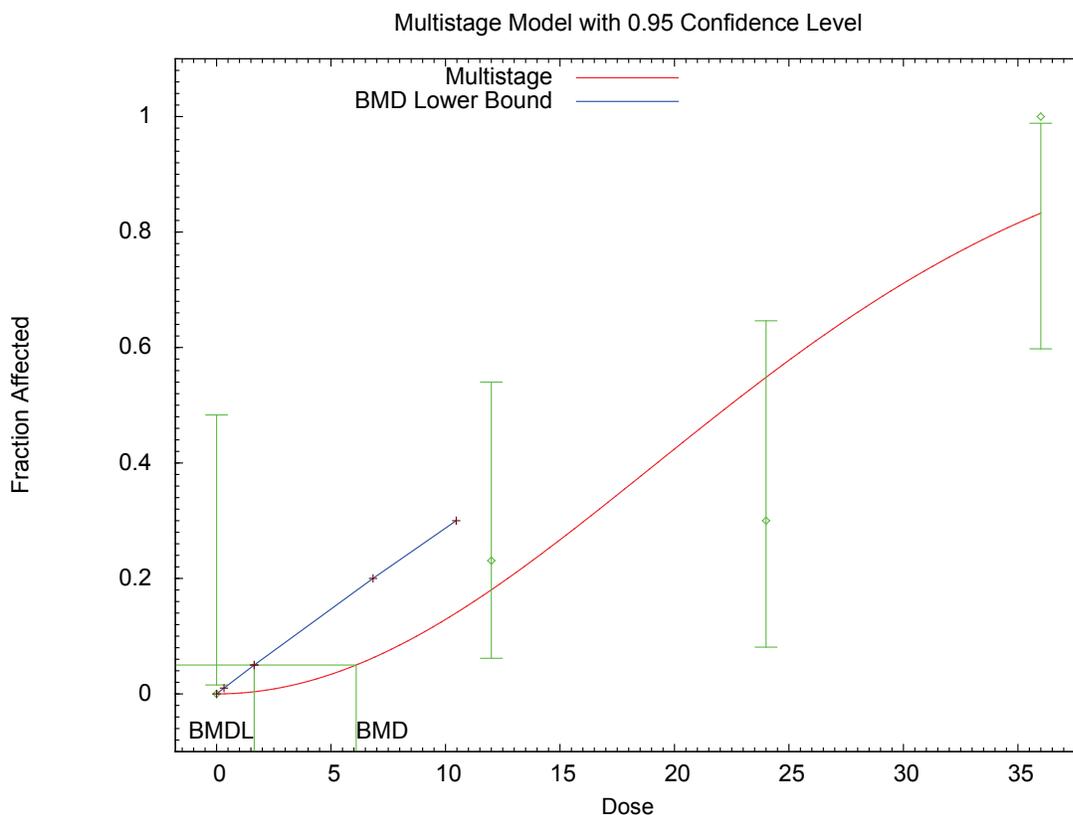| Model	Degrees of Freedom	χ^2	χ^2 Goodness of Fit p-Value[a]	AIC	BMD_5 (mg/kg-day)	$BMDL_5$ (mg/kg-day)
Gamma[b]	2	4.89	0.09	36.45	-	-
Logistic	2	4.11	0.13	35.37	7.60	3.91
Log logistic[c]	2	5.31	0.07	37.13	-	-
Log probit[c]	2	5.38	0.07	37.13	-	-
Multistage (degree = 1)[d]	3	6.30	0.10	37.09	2.73	1.09
Multistage (degree = 2)[d]	**3**	**4.32**	**0.23**	**33.92**	**8.15**	**1.64**
Multistage (degree = 3)[d]	2	3.64	0.16	34.85	5.01	1.70
Probit	2	4.03	0.13	35.19	7.17	3.59
Weibull[b]	1	1.64	0.20	34.79	21.1	10.0

[a]p-Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.
[d]Betas restricted to ≥0.

Sources: Scott et al. (1989).

Multistage Model with 0.95 Confidence Level

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BMD and BMDL indicated are associated with an extra risk of 5% and are in units of mg/kg-day.

Source: Scott et al. (1989).

Figure A-1. Fit of 2-Degree Multistage Model to Data on Incidence of Embryo Lethality in Macaques

MODEL-FITTING PROCEDURE FOR CONTINUOUS DATA

The model-fitting procedure for continuous data using the EPA benchmark dose software (BMDS) is as follows. The simplest model (linear) is first applied to the data while assuming constant variance. If the data are consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of the linear model to the means is evaluated, and the polynomial, power, and Hill models are fit to the data while assuming constant variance. Adequate model fit is judged by three criteria: goodness-of-fit p-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest BMDL is selected as the POD when the difference between the BMDLs estimated from these models is >3-fold (unless it appears to be an outlier); otherwise, the BMDL from the model with the lowest AIC is chosen. If the test for constant variance is negative, the linear model is run again while applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model provides an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear

model to the means is evaluated, and the polynomial, power, and Hill models are fit to the data and evaluated while the variance model is applied. Model fit and POD selection proceed as described earlier. If the test for constant variance is negative and the nonhomogenous variance model does not provide an adequate fit to the variance data, then the data set is considered unsuitable for modeling.

Model Fitting for Sperm Density in Rats (NTP, 1990)

All available continuous-variable models in the EPA BMDS (version 2.1) were fit to the data of NTP (1990) for sperm density in rats treated with 2-ME (see Table 16). Data were not available for the percent reduction in sperm density in rats or humans resulting in diminished reproductive capacity. Therefore, a default BMR of one SD from the control means was used. For the F1 sperm density data of NTP (1990), the calculated BMD_{1SD} and the $BMDL_{1SD}$ are estimates of the doses associated with a negative change of one SD from the control mean. Table A-3 shows the modeling results. The assumption of constant variance did not hold, but the nonhomogenous variance model provided adequate fit to the variance data. The linear model adequately fit the means ($p \geq 0.1$). The higher order models, except the Hill model for which there were insufficient data points to fit the model, defaulted back to the linear model. Thus, the linear model was selected for BMD derivation. The resulting BMD_{1SD} and $BMDL_{1SD}$ are 26.0 and 14.3 mg/kg-day, respectively. Figure A-2 shows a graph of the linear model fit.

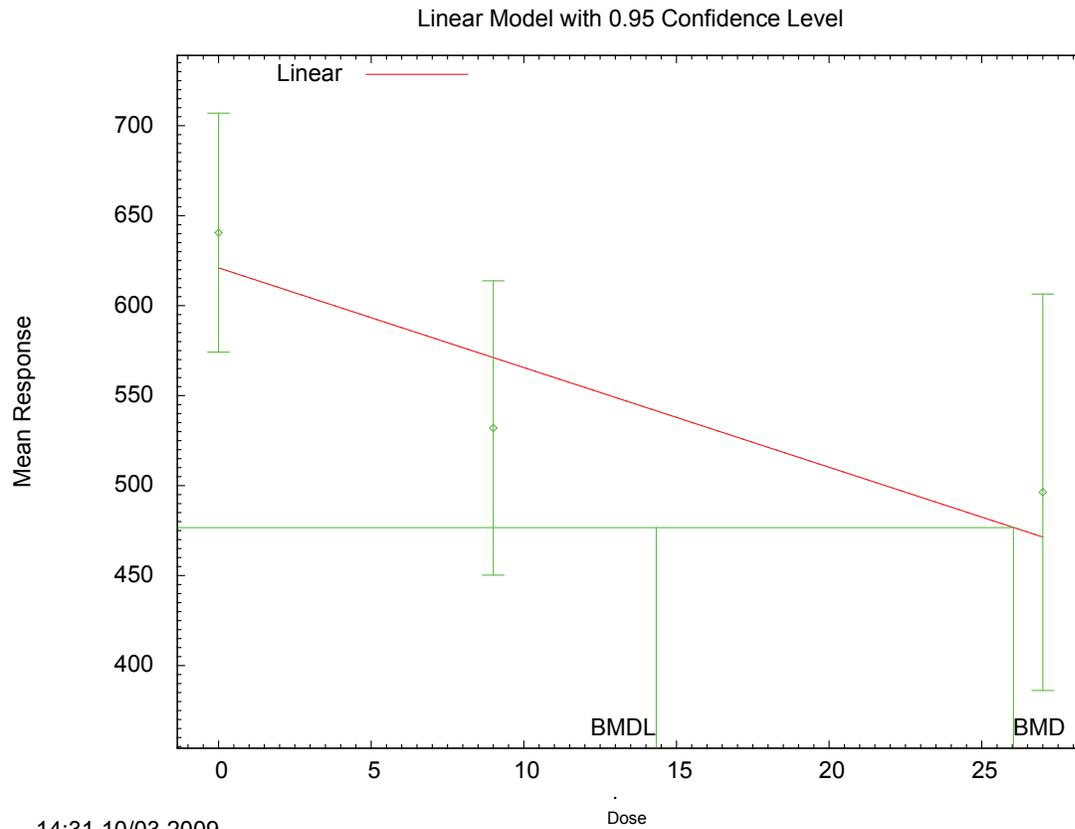| **Table A-3. Model Predictions for Reduced Sperm Density in F1 Rats**					
Model	**Variance *p*-Value[a]**	**Means *p*-Value[a]**	**AIC**	**BMD$_{1SD}$ (mg/kg-day)**	**BMDL$_{1SD}$ (mg/kg-day)**
Constant variance					
Linear[b]	0.0738	0.2396	692.6674	38.1162	21.945
Nonconstant variance					
Hill[c]	NA				
Linear[b]	**0.5608**	**0.2283**	**689.861**	**26.0378**	**14.3388**
Polynomial (2-degree)[b]	0.5608	0.2283	689.861	26.0378	14.3388
Power[c]	0.5608	0.2283	689.861	26.0378	14.3388

[a]*p*-Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Coefficients restricted to be negative.
[c]Power restricted to ≥1.

NA = Too many parameters in model for number of observations.

Source: NTP (1990).

Linear Model with 0.95 Confidence Level

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BMD and BMDL indicated are associated with a change of 1 SD from the control mean and are in units of mg/kg-day.

Source: NTP (1990).

Figure A-2. Fit of Linear Model to Data for Sperm Density in Rats

2-Methoxyethanol
2-Methoxyethanol Acetate

APPENDIX B. DETAILS OF BENCHMARK DOSE MODELING FOR SUBCHRONIC p-RfC

MODEL-FITTING PROCEDURE FOR QUANTAL NONCANCER DATA
  The model-fitting procedure for dichotomous noncancer data is as follows. All available dichotomous models in the EPA BMDS (version 2.1) are fit to the incidence data using the extra risk option. The multistage model is run for all polynomial degrees up to $n-1$ (where n is the number of dose groups including control). Adequate model fit is judged by three criteria: goodness-of-fit p-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined BMR. Among all of the models providing adequate fit to the data, the lowest BMCL is selected as the POD when the difference between the BMCLs estimated from these models is >3-fold (unless it appears to be an outlier); otherwise, the BMCL from the model with the lowest AIC is chosen. In accordance with EPA (2000) guidance, BMCs and BMCLs associated with an extra risk of 10% BMR are calculated for all models.

Model Fitting for Incidence of Gross Reduction in Testes Size in Rabbits (Miller et al., 1983a)
  Following the above procedure, the quantal models in the EPA BMDS (version 2.1) were fit to the incidence data for gross reduction in testes size in rabbits from the Miller et al. (1983a) study shown in Table 27. HECs, calculated as shown in Table 34, were used for modeling, so results are reported as HECs as well. Table B-1 shows the modeling results. All models produced adequate fit ($p > 0.01$). With the exception of the BMCL from the log logistic model, the BMCLs all were within a factor of 3. The best fitting model, with the lowest AIC, was the quantal linear (1-degree multistage), giving BMC_{10HEC} and $BMCL_{10HECs}$ of 3.5 and 1.8 mg/m^3, respectively. However, because the log logistic model also provided excellent fit and the lowest BMCL, its BMC_{10HEC} of 6.76 and $BMCL_{10HEC}$ of 0.73 mg/m^3 were selected for derivation of the p-RfC. Figure B-1 shows the fit of this model to the data.

2-Methoxyethanol
2-Methoxyethanol Acetate

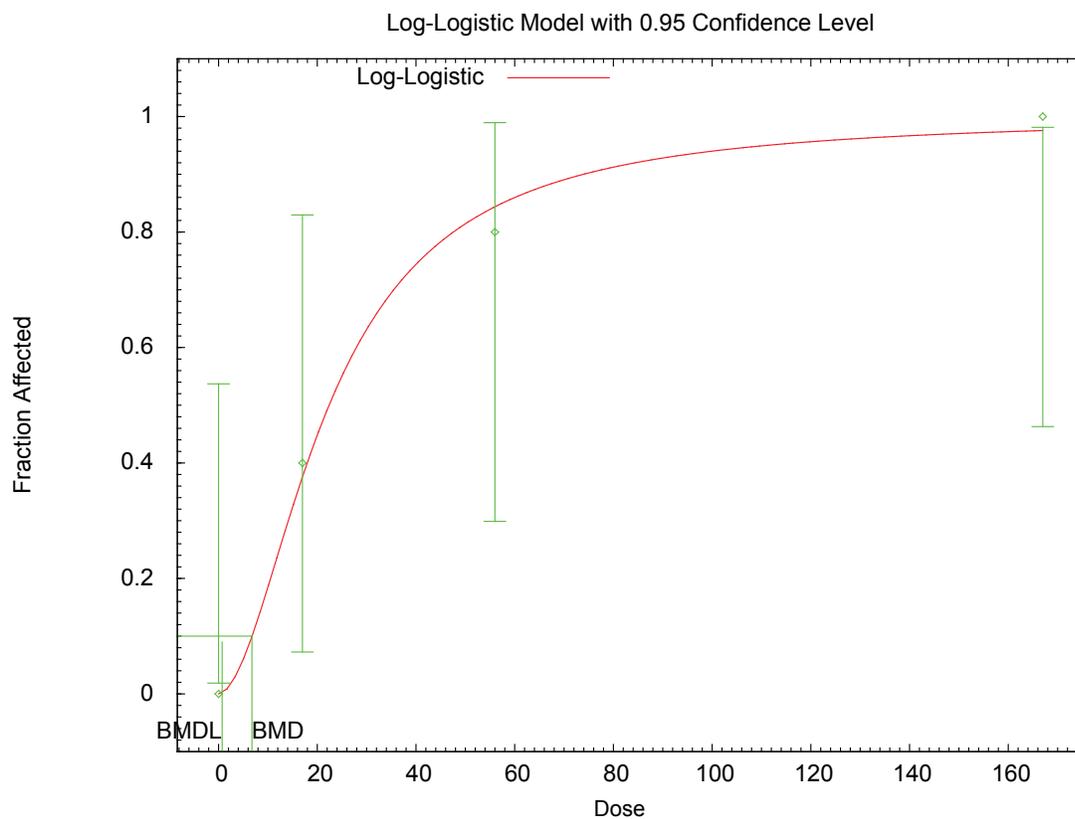| Table B-1. Model Predictions for the Incidence of Gross Reduction in Testes Size in Rabbits						
Model	Degrees of Freedom	χ^2	χ^2 Goodness of Fit *p*-Value[a]	AIC	BMC$_{10HEC}$ (mg/m^3)	BMCL$_{10HEC}$ (mg/m^3)
Gamma[b]	2	0.04	0.9793	15.8006	4.0251	1.8352
Logistic	2	1.11	0.5734	17.282	10.9333	5.32525
Log logistic[c]	**2**	**0.21**	**0.8996**	**16.0589**	**6.75982**	**0.727736**
Log probit[c]	2	0.14	0.9321	15.9437	6.96769	3.03858
Multistage (degree = 1)[d]	3	0.04	0.9979	13.8069	3.49396	1.83389
Multistage (degree = 2)[d]	2	0.03	0.9849	15.7739	3.86908	1.84076
Multistage (degree = 3)[d]	2	0.02	0.9921	15.7527	3.74913	1.84522
Probit	2	1.07	0.5868	17.1681	10.5005	5.53729
Weibull[b]	2	0.04	0.9797	15.7952	4.05573	1.83632

[a]*p*-Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.
[d]Betas restricted to ≥0.

Source: Miller et al.. (1983a).

2-Methoxyethanol
2-Methoxyethanol Acetate

Log-Logistic Model with 0.95 Confidence Level

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BMC and BMCL indicated are associated with an extra risk of 10% and are in units of mg/m^3 as HEC.

Source: Miller et al. (1983a).

Figure B-1. Fit of Log Logistic Model to Incidence Data for Gross Reduction in Testes Size in Rabbits

Model Fitting for Incidence of Seminiferous Tubule Degeneration in Rabbits (Miller et al., 1983a)

Following the above procedure, the quantal models in the EPA BMDS (version 2.1) were fit to the incidence data for seminiferous tubule degeneration in rabbits from the Miller et al. (1983a) study shown in Table 27. HECs, calculated as shown in Table 34, were used for modeling, so results are reported as HECs as well. Table B-2 shows the modeling results. All models produced adequate fit ($p > 0.01$). With the exception of the BMCL from the log logistic model, which appears to be an outlier, the other BMCLs were all within a factor of 3. The best fitting model, with the lowest AIC, was the quantal linear (1-degree multistage), giving BMC_{10HEC} and $BMCL_{10HECS}$ of 6.1 and 3.1 mg/m^3, respectively. Figure B-2 shows the fit of this model to the data.

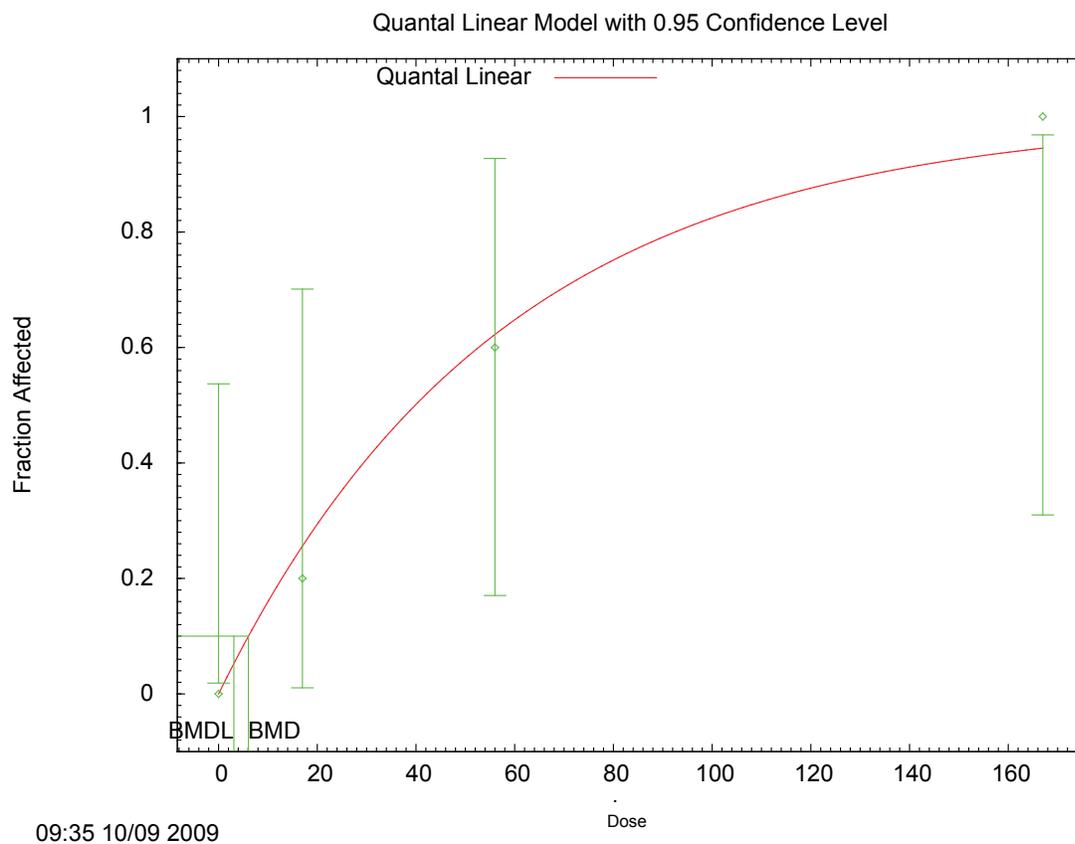|	Table B-2. Model Predictions for the Incidence of Seminiferous Tubule Degeneration in Rabbits					
Model	**Degrees of Freedom**	**χ^2**	**χ^2 Goodness of Fit p-Value[a]**	**AIC**	**BMC_{10HEC} (mg/m^3)**	**$BMCL_{10HEC}$ (mg/m^3)**
Gamma[b]	2	0.11	0.9468	15.8979	11.1269	3.20073
Logistic	2	0.5	0.7796	16.4504	19.1263	9.39131
Log logistic[c]	2	0.32	0.8524	16.2222	12.447	1.89119
Log probit[c]	2	0.27	0.872	16.1352	12.8247	5.57338
Multistage (degree = 1)[d]	**3**	**0.27**	**0.9661**	**14.1695**	**6.05074**	**3.09883**
Multistage (degree = 2)[d]	2	0.03	0.9853	15.7776	9.52467	3.24906
Multistage (degree = 3)[d]	2	0	0.9992	15.7367	8.35829	3.266
Probit	2	0.43	0.8059	16.341	17.6572	9.04958
Weibull[b]	2	0.08	0.962	15.8418	10.9506	3.22303
Quantal-linear	**3**	**0.27**	**0.9661**	**14.1695**	**6.0507**	**3.09883**

[a]p-Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.
[d]Betas restricted to ≥0.

Source: Miller et al. (1983a).

BMC and BMCL indicated are associated with an extra risk of 10% and are in units of mg/m^3 as HEC.

Source: Miller et al. (1983a).

Figure B-2. Fit of Quantal-Linear Model to Incidence Data for Seminiferous Tubule Degeneration in Rabbits

Model Fitting for Incidence of Delayed Ossification of Vertebral Centra in Rats (Hanley et al., 1984)

Following the above procedure, the quantal models in the EPA BMDS (version 2.1) were fit to the incidence data for delayed ossification of vertebral centra in rats from the Hanley et al. (1984) study shown in Table 29. Because the litter is the preferred unit of statistical analysis for developmental studies, the data modeled were those for incidence of litters affected. HECs, calculated as shown in Table 34, were used for modeling, so results are reported as HECs as well. Exposures for this gestational exposure study were duration-adjusted as part of the HEC calculation, as currently recommended by EPA (2002). Table B-3 shows the modeling results. All models produced adequate fit ($p > 0.01$). The BMCLs were all within a factor of 3. The best fitting model, with the lowest AIC, was the probit, giving BMC_{10HEC} and $BMCL_{10HEC}$s of 13.3 and 9.6 mg/m^3, respectively. Figure B-3 shows the fit of this model to the data.

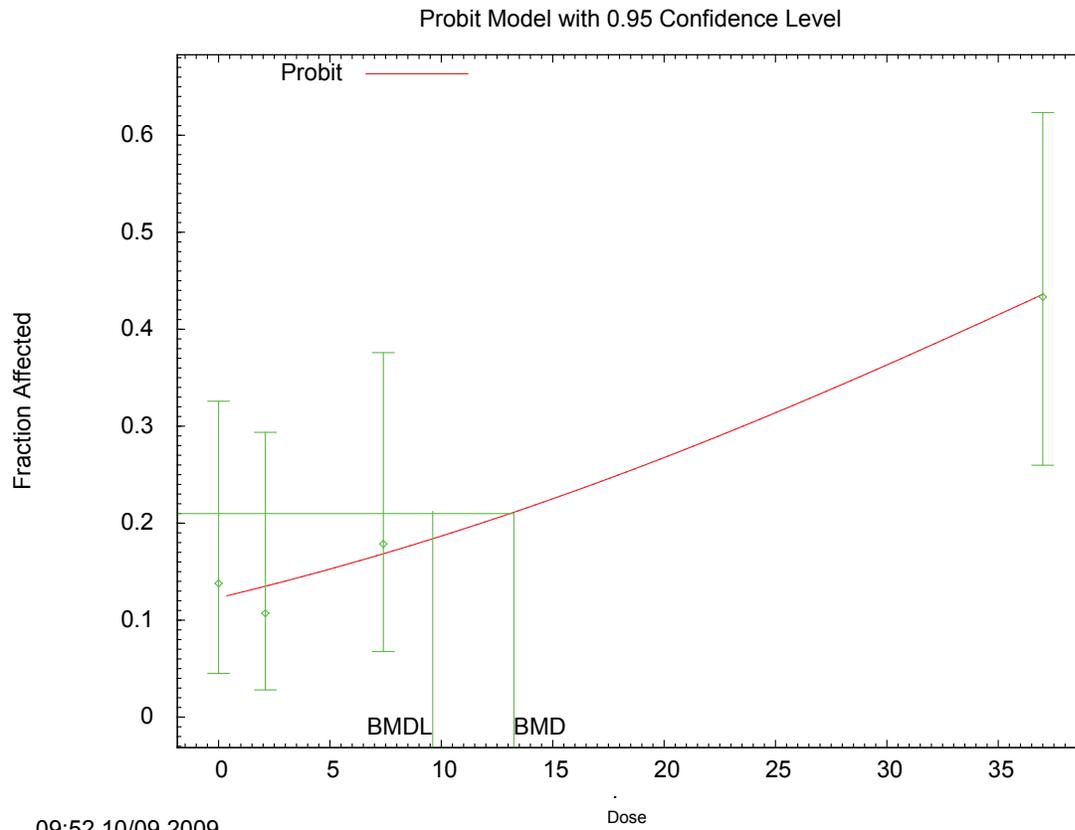| Table B-3. Model Predictions for the Litter Incidence of Delayed Ossification of Vertebral Centra in Rats						
Model	**Degrees of Freedom**	χ^2	χ^2 **Goodness of Fit** p**-Value**[a]	**AIC**	BMC_{10HEC} **(mg/m**^{3}**)**	$BMCL_{10HEC}$ **(mg/m**^{3}**)**
Gamma[b]	1	0.22	0.6364	115.897	12.4033	5.37379
Logistic	2	0.27	0.8736	113.946	14.0009	10.241
Log logistic[c]	1	0.22	0.6404	115.891	12.0016	4.18566
Log probit[c]	2	0.32	0.8538	113.987	14.9242	9.44885
Multistage (degree = 1)[d]	2	0.38	0.8266	114.05	8.98591	5.30379
Multistage (degree = 2)[d]	1	0.26	0.608	115.938	13.0639	5.35445
Multistage (degree = 3)[d]	1	0.26	0.608	115.938	13.0639	5.35445
Probit	**2**	**0.27**	**0.8751**	**113.942**	**13.2573**	**9.61316**
Weibull[b]	1	0.23	0.6309	115.904	12.5187	5.3702
Quantal-linear	2	0.38	0.8266	114.05	8.98591	5.30379

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.
[d]Betas restricted to ≥0.

Source: Hanley et al. (1984).

BMC and BMCL indicated are associated with an extra risk of 10% and are in units of mg/m^3 as HEC.

Source: Hanley et al. (1984).

Figure B-3. Fit of Probit Model to Data on Litter Incidence of Delayed Ossification of Vertebral Centra in Rats

Model Fitting for Incidence of Extra Lumbar Ribs in Mice (Hanley et al., 1984)

Following the above procedure, the quantal models in the EPA BMDS (version 2.1) were fit to the incidence data for extra lumbar ribs in mice from the Hanley et al. (1984) study shown in Table 30. Because the litter is the preferred unit of statistical analysis for developmental studies, the data modeled were those for incidence of litters affected. HECs, calculated as shown in Table 34, were used for modeling, so results are reported as HECs as well. Exposures for this gestational exposure study were duration-adjusted as part of the HEC calculation, as currently recommended by EPA (2002). Table B-4 shows the modeling results. There were insufficient degrees of freedom available to fit several of the models. Among the models that produced adequate fit ($p > 0.01$), the BMCLs were all within a factor of 3. The best fitting model, with the lowest AIC, was the probit, giving BMC_{10HEC} and $BMCL_{10HEC}$s of 4.3 and 2.9 mg/m^3, respectively. Figure B-4 shows the fit of this model to the data.

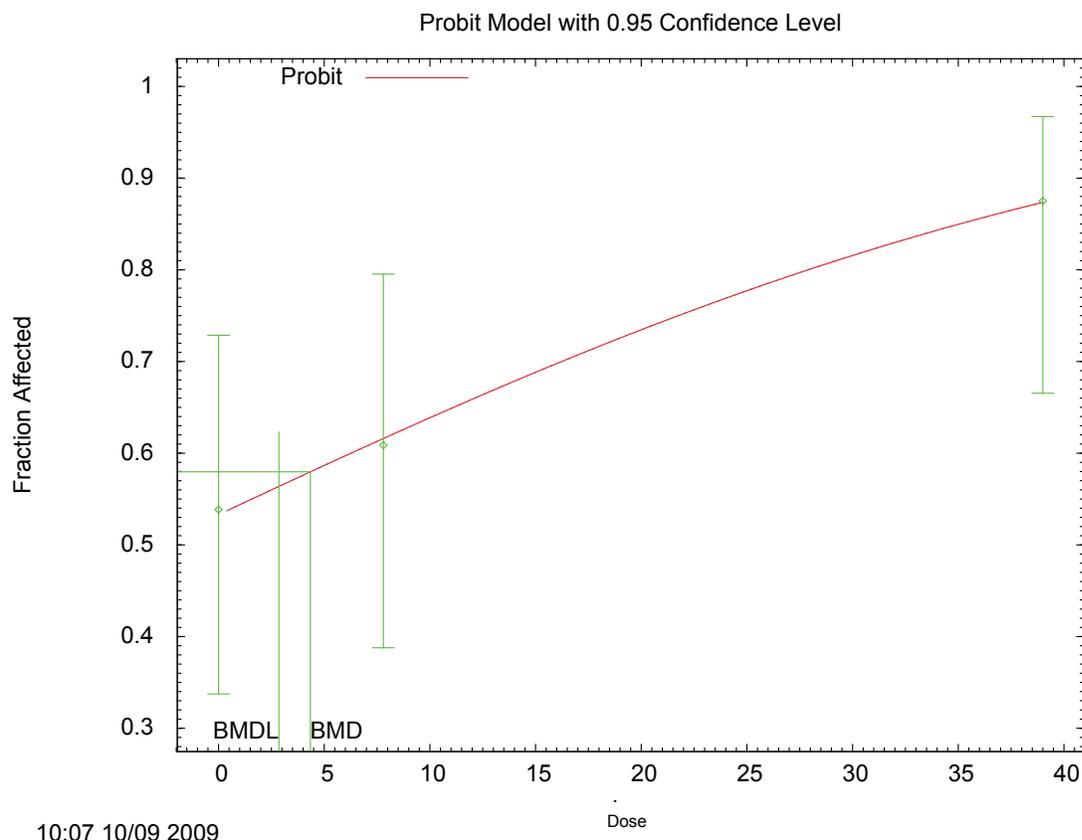|	Table B-4. Model Predictions for the Litter Incidence of Extra Lumbar Ribs in Mice					
Model	**Degrees of Freedom**	χ^2	χ^2 **Goodness of Fit *p*-Value[a]**	**AIC**	**BMC_{10HEC} (mg/m^3)**	**$BMCL_{10HEC}$ (mg/m^3)**
Gamma[b]	0	0	NA	90.7637	5.60675	1.77342
Logistic	1	0.02	0.8968	88.7805	4.10839	2.55048
Log logistic[c]	0	0	NA	90.7637	5.86561	0.842663
Log probit[c]	0	0	NA	90.7637	6.07437	2.989
Multistage (degree = 1)[d]	1	0.08	0.7716	88.8476	3.19226	1.75927
Multistage (degree = 2)[d]	0	0	NA	90.7637	5.22928	1.77342
Probit	**1**	**0.01**	**0.9257**	**88.7724**	**4.34628**	**2.85615**
Weibull[b]	0	0	NA	90.7637	5.49992	1.77342
Quantal-linear	1	0.08	0.7716	88.8476	3.19226	1.75927

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.
[d]Betas restricted to ≥0.

NA = not applicable.

Source: Hanley et al. (1984).

2-Methoxyethanol
2-Methoxyethanol Acetate

Probit Model with 0.95 Confidence Level

BMC and BMCL indicated are associated with an extra risk of 10% and are in units of mg/m^3 as HEC.

Source: Hanley et al. (1984).

**Figure B-4. Fit of Probit Model to Data on Litter Incidence of
Extra Lumbar Ribs in Mice**

Model Fitting for Unilateral Testicular Hypoplasia in Mice (Hanley et al., 1984)

Following the above procedure, the quantal models in the EPA BMDS (version 2.1) were fit to the incidence data for unilateral testicular hypoplasia in mice from the Hanley et al. (1984) study shown in Table 30. Because the litter is the preferred unit of statistical analysis for developmental studies, the data modeled were those for incidence of litters affected. HECs, calculated as shown in Table 34, were used for modeling, so results are reported as HECs as well. Exposures for this gestational exposure study were duration-adjusted as part of the HEC calculation, as currently recommended by EPA (2002). Table B-5 shows the modeling results. All models produced adequate fit ($p > 0.01$). The BMCLs were all within a factor of 3. The best fitting model, with the lowest AIC, was the log logistic, giving BMC_{10HEC} and $BMCL_{10HEC}$s of 18.6 and 7.5 mg/m^3, respectively. Figure B-5 shows the fit of this model to the data.

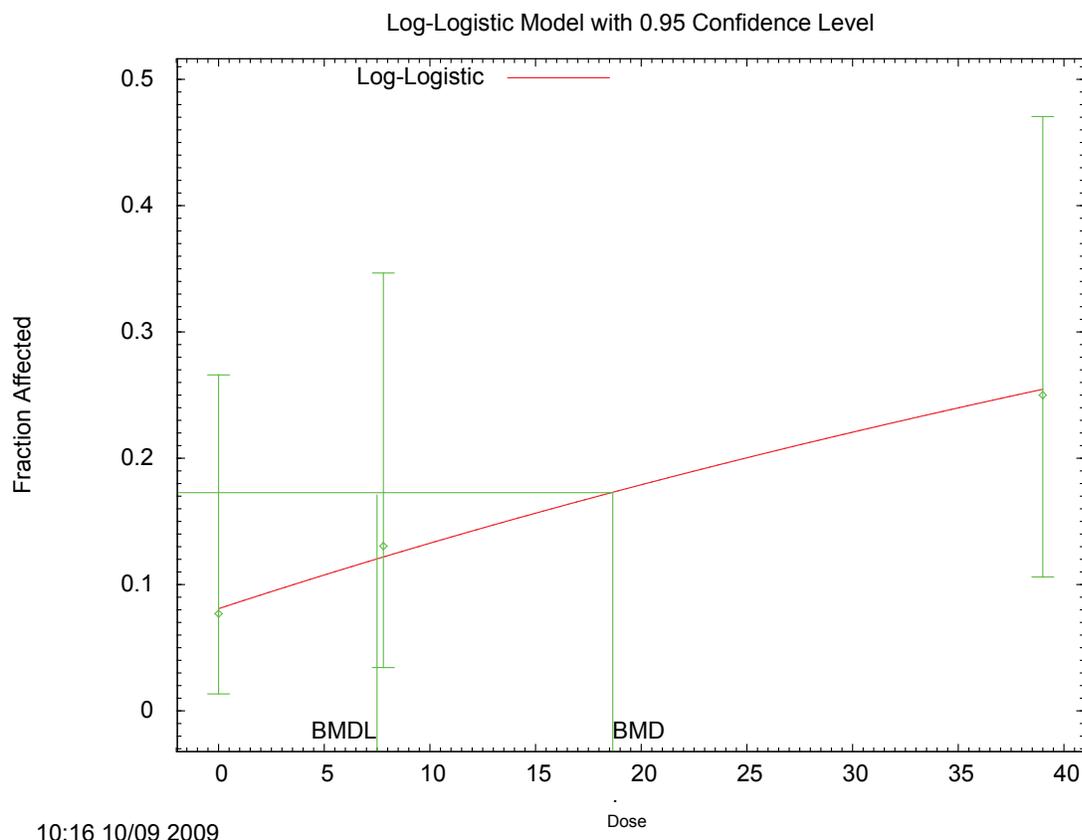| Table B-5. Model Predictions for the Litter Incidence of Unilateral Testicular Hypoplasia in Mice						
Model	**Degrees of Freedom**	**χ^2**	**χ^2 Goodness of Fit p-Value[a]**	**AIC**	**BMC_{10HEC} (mg/m^3)**	**$BMCL_{10HEC}$ (mg/m^3)**
Gamma[b]	1	0.04	0.8457	62.9431	19.7721	8.91306
Logistic	1	0.13	0.717	63.0362	25.4106	15.9418
Log logistic[c]	**1**	**0.02**	**0.8766**	**62.9296**	**18.6536**	**7.49865**
Log probit[c]	1	0.32	0.5712	63.2242	27.954	15.2951
Multistage (degree = 1)[d]	1	0.04	0.8457	62.9431	19.772	8.91306
Multistage (degree = 2)[d]	1	0.04	0.8457	62.9431	19.772	8.91306
Probit	1	0.12	0.7318	63.0222	24.6053	14.9646
Weibull[b]	1	0.04	0.8457	62.9431	19.7721	8.91306
Quantal-linear	1	0.04	0.8457	62.9431	19.772	8.91306

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.
[d]Betas restricted to ≥0.

Source: Hanley et al. (1984).

BMC and BMCL indicated are associated with an extra risk of 10% and are in units of mg/m^3 as HEC.

Source: Hanley et al. (1984).

Figure B-5. Fit of Log Logistic Model to Data on Litter Incidence of Unilateral Testicular Hypoplasia in Mice

Model Fitting for Limb Defects in Rabbits (Hanley et al., 1984)

Following the above procedure, the quantal models in the EPA BMDS (version 2.1) were fit to the incidence data for limb defects in rabbits from the Hanley et al. (1984) study shown in Table 31. Because the litter is the preferred unit of statistical analysis for developmental studies, the data modeled were those for incidence of litters affected. HECs, calculated as shown in Table 34, were used for modeling, so results are reported as HECs as well. Exposures for this gestational exposure study were duration adjusted as part of the HEC calculation, as currently recommended by EPA (2002). Table B-6 shows the modeling results. All models produced adequate fit ($p > 0.01$). With the exception of the BMCL from the quantal linear/1-degree multistage model, which was considered to be an outlier due to poor fit to the mid-dose group (the point closest to the BMR; scaled residual $= -1.7$), the BMCLs were all within a factor of 3. The best fitting model, with the lowest AIC, was the Probit, giving a BMC_{10HEC} and a $BMCL_{10HECS}$ of 13.7 and 9.9 mg/m^3, respectively. Figure B-6 shows the fit of this model to the data.

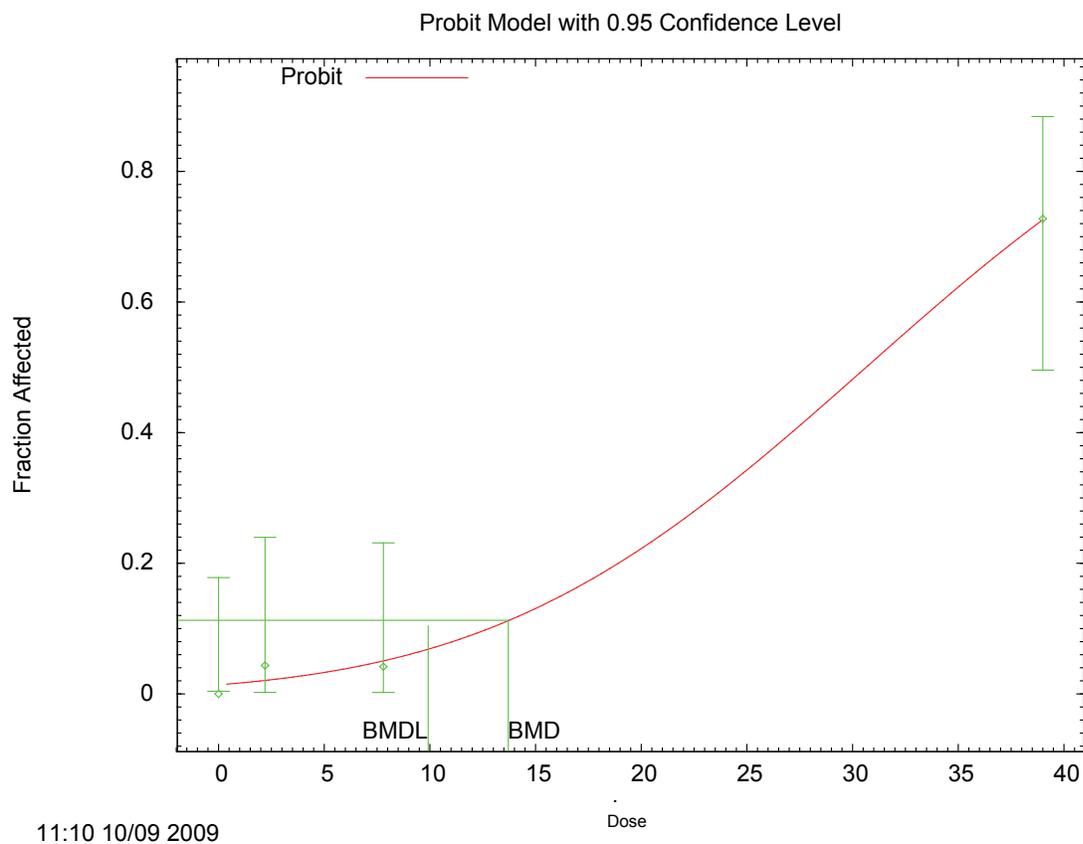| Table B-6. Model Predictions for the Litter Incidence of Limb Defects in Rabbits						
Model	**Degrees of Freedom**	**χ^2**	**χ^2 Goodness of Fit p-Value[a]**	**AIC**	**BMC_{10HEC} (mg/m^3)**	**$BMCL_{10HEC}$ (mg/m^3)**
Gamma[b]	1	1.03	0.3093	49.717	13.2462	4.67047
Logistic	2	0.87	0.6475	47.4831	15.6353	11.085
Log logistic[c]	1	1.03	0.309	49.7061	13.1984	5.14219
Log probit[c]	1	1.02	0.3117	49.7303	12.429	6.14406
Multistage (degree = 1)[d]	3	4.11	0.2498	49.5567	4.26928	2.92108
Multistage (degree = 2)[d]	1	1.47	0.225	49.7311	9.48507	4.38246
Multistage (degree = 3)[d]	1	1.12	0.2896	49.2537	10.5605	4.58557
Probit	**2**	**0.96**	**0.6203**	**47.4635**	**13.7093**	**9.91939**
Weibull[b]	1	1.05	0.3055	49.6823	13.7322	4.62413
Quantal-linear	3	4.11	0.2498	49.5567	4.26928	2.92108

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.
[d]Betas restricted to ≥0.

Source: Hanley et al. (1984).

Probit Model with 0.95 Confidence Level

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BMC and BMCL indicated are associated with an extra risk of 10% and are in units of mg/m^3 as HEC.

Source: Hanley et al. (1984).

Figure B-6. Fit of Probit Model to Litter Data on Limb Defects in Rabbits

2-Methoxyethanol
2-Methoxyethanol Acetate

Model Fitting for Renal Defects in Rabbits (Hanley et al., 1984)

Following the above procedure, the quantal models in the EPA BMDS (version 2.1) were fit to the incidence data for limb defects in rabbits from the Hanley et al. (1984) study shown in Table 31. Because the litter is the preferred unit of statistical analysis for developmental studies, the data modeled were those for incidence of litters affected. HECs, calculated as shown in Table 34, were used for modeling, so results are reported as HECs as well. Exposures for this gestational exposure study were duration adjusted as part of the HEC calculation, as currently recommended by EPA (2002). Table B-7 shows the modeling results. All models produced adequate fit ($p > 0.01$) except the 2-degree multistage, although none fit especially well. BMCLs from the adequate models were all within a factor of 3 (approximately). The best fitting model, with the lowest AIC, was the quantal linear/1-degree multistage, giving a BMC_{10HEC} and a $BMCL_{10HEC}$s of 4.8 and 3.3 mg/m^3, respectively. Figure B-7 shows the fit of this model to the data.

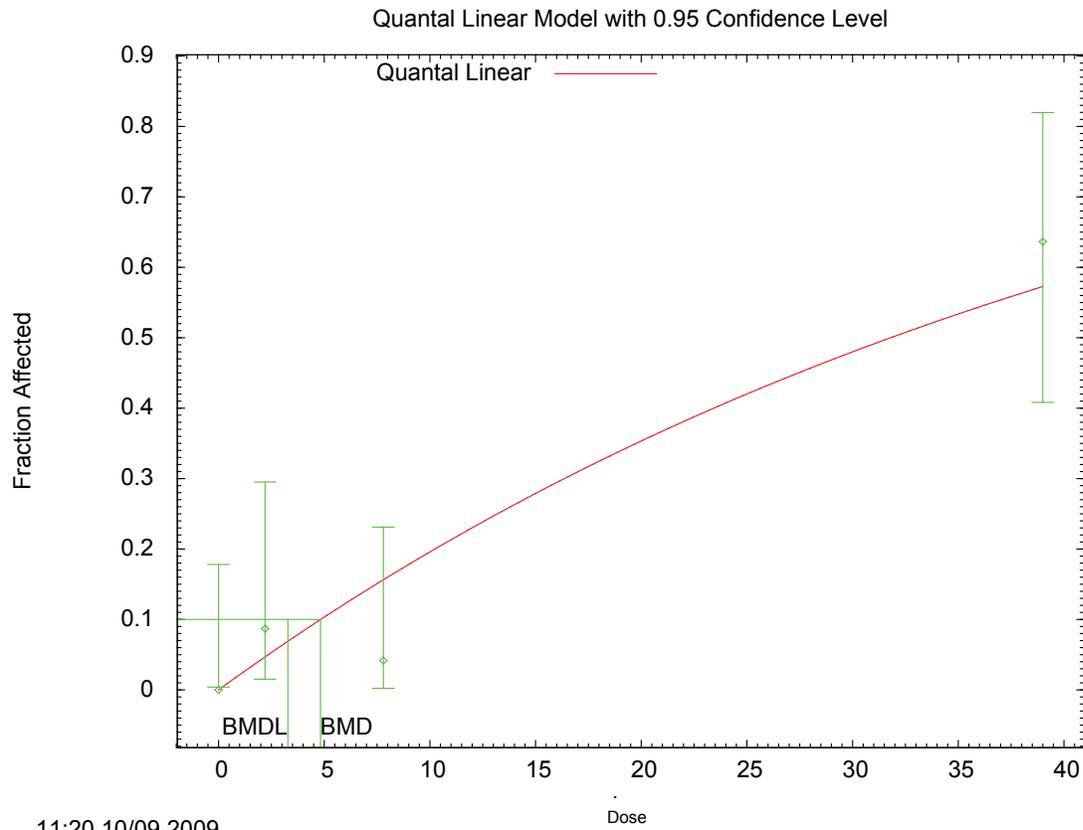| Table B-7. Model Predictions for the Litter Incidence of Renal Defects in Rabbits						
Model	**Degrees of Freedom**	χ^2	**χ^2 Goodness of Fit p-Value[a]**	**AIC**	**BMC_{10HEC} (mg/m^3)**	**$BMCL_{10HEC}$ (mg/m^3)**
Gamma[b]	1	2.12	0.1453	59.61	25.4759	3.67068
Logistic	2	2.55	0.2797	57.5421	15.0818	10.9677
Log logistic[c]	1	2.12	0.1453	59.61	29.2418	3.75507
Log probit[c]	1	2.12	0.1453	59.61	24.003	6.94654
Multistage (degree = 1)[d]	**3**	**3.58**	**0.3101**	**57.0039**	**4.82348**	**3.28055**
Multistage (degree = 2)[d]	1	2.92	0.0874	59.8557	11.5469	3.58575
Multistage (degree = 3)[d]	1	2.58	0.1081	59.4755	13.8241	3.72181
Probit	2	2.7	0.2592	57.5999	13.3542	9.86922
Weibull[b]	1	2.12	0.1453	59.61	30.6671	3.67068
Quantal-linear	**3**	**3.58**	**0.3101**	**57.0039**	**4.82348**	**3.28055**

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]Power restricted to ≥1.
[c]Slope restricted to ≥1.
[d]Betas restricted to ≥0.

Source: Hanley et al. (1984).

BMC and BMCL indicated are associated with an extra risk of 10% and are in units of mg/m^3 as HEC.

Source: Hanley et al. (1984).

Figure B-7. Fit of Quantal-Linear Model to Litter Data on Renal Defects in Rabbits