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Provisional Peer-Reviewed Toxicity Values for

2-Ethoxyethanol
(CASRN 110-80-5)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

AUTHORS, CONTRIBUTORS, AND REVIEWERS

CHEMICAL MANAGER

Scott C. Wesselkamper, PhD
National Center for Environmental Assessment, Cincinnati, OH

DRAFT DOCUMENT PREPARED BY

ICF International
9300 Lee Highway
Fairfax, VA 22031

PRIMARY INTERNAL REVIEWERS

Ambuja Bale, PhD, DABT
National Center for Environmental Assessment, Washington, DC

Q. Jay Zhao, PhD, MPH, DABT
National Center for Environmental Assessment, Cincinnati, OH

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Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421-3136

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
BMCL	benchmark concentration lower bound 95% confidence interval
BMD	benchmark dose
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
MW	molecular weight
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
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
SD	standard deviation
SE	standard error
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-ETHOXYETHANOL (CASRN 110-80-5)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by a standing panel of National Center for Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

The PPRTV review process provides needed toxicity values in a quick turnaround timeframe while maintaining scientific quality. PPRTV assessments are updated approximately on a 5-year cycle for new data or methodologies that might impact the toxicity values or characterization of potential for adverse human health effects and are revised as appropriate. It is important to utilize the PPRTV database (http://hhpprtv.ornl.gov) to obtain the current information available. When a final Integrated Risk Information System (IRIS) assessment is made publicly available on the Internet (www.epa.gov/iris), the respective PPRTVs are removed from the database.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. Environmental Protection Agency (EPA) programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

QUESTIONS REGARDING PPRTVS

Questions regarding the contents and appropriate use of this PPRTV assessment should 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).

INTRODUCTION

2-Ethoxyethanol (2-EE), also commonly referred to as ethylene glycol monoethyl ether or Cellosolve®, is a glycol ethyl ether that is used in many industrial processes as a solvent and chemical intermediary in the production of ethylene glycol monoethyl ether acetate (NTP, 1993). 2-EE is a colorless liquid with a mild odor that is readily evaporated (NIOSH, 2003). The empirical formula for 2-EE is $C_4H_{10}O_2$ (see Figure 1), and Table 1 provides physicochemical properties for 2-EE.

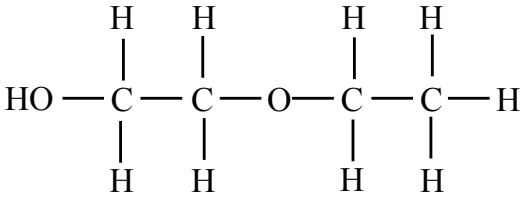

Figure 1. 2-EE Structure (NTP, 1993)

Table 1. Physicochemical Properties Table (2-EE CASRN 110-80-5)[a]

Property (unit)	Value
Boiling point (ºC)	135
Melting point (ºC)	−70
Density (g/cm3)	Not available
Vapor pressure (kPa at 20ºC)	0.5
pH (unitless)	Not available
Solubility in water (g/100 mL at 25ºC)	Miscible
Relative vapor density (air = 1)	3.1
Molecular weight (g/mol)	90.12
Flash point (°C)	44
Octanol/water partition coefficient (log Kow unitless)	−0.540

[a]NIOSH (2003).

No Reference Dose (RfD) or cancer assessment for 2-EE is included in the IRIS database, but a Reference Concentration (RfC) of 0.2 mg/m^3 (UF_C = 300, modifying factor = 1) is provided (U.S. EPA, 2011) based on decreased testis weight, degeneration of the seminiferous tubules, and decreased hemoglobin in New Zealand White rabbits (Barbee et al., 1984b). The Drinking Water Standards and Health Advisories List (U.S. EPA, 2006) does not list reference values. A chronic RfD of 0.4 mg/kg-day (UF_C = 1000), a subchronic RfD of 0.5 mg/kg-day (UF_C = 100), and a subchronic reference concentration of 2 mg/m^3 (UF_C = 30) are reported in the

HEAST (U.S. EPA, 2003). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1994) includes a Health and Environmental Effects Profile (HEEP) for 2-EE esters, which gives an acceptable daily intake of 21.1 mg/day for inhalation exposure (U.S. EPA, 1985). The toxicity of 2-EE has not been reviewed by the ATSDR (2011) or the World Health Organization (WHO, 2011). CalEPA (2008) has derived an acute reference exposure level (REL) value of 0.1 ppm (0.370 mg/m^3) for a 6-hour time-weighted average (TWA) exposure to 2-EE based on reproductive /developmental effects. CalEPA (2008) has also derived a chronic REL of 0.02 ppm (0.070 mg/m^3) based on effects in the reproductive and hematopoietic systems. Additionally, 2-EE is listed under California Proposition 65 as a reproductive hazard. The American Conference of Governmental Industrial Hygienists (ACGIH, 2010) has set an 8-hour TWA occupational exposure limit of 5 ppm along with a skin notation; ACGIH has also set a biological exposure index of 100 mg of 2-ethoxyacetic acid (2-EAA) per gram of creatinine in urine. The National Institute for Occupational Safety and Health (NIOSH, 2005) has established a 10-hour TWA of 0.5 ppm (1.8 mg/m^3) along with a skin designation and an Immediately Dangerous to Life or Health level of 500 ppm. The Occupational Safety and Health Administration (OSHA, 2010) has established a permissible exposure limit 8-hour TWA of 200 ppm (740 mg/m^3) along with a skin designation (i.e., dermal contact should be avoided).

The HEAST (U.S. EPA, 2003) does not report a cancer weight-of-evidence classification. The International Agency for Research on Cancer (IARC, 2011) has not reviewed the carcinogenic potential of 2-EE. 2-EE is not included in the *12th Report on Carcinogens* (NTP, 2011). CalEPA (2008) has not prepared a quantitative estimate of carcinogenic potential for 2-EE.

Literature searches were conducted on sources published from 1900 through March 2011 for studies relevant to the derivation of provisional toxicity values for 2-EE, CAS Number 110-80-5. Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications (NSCEP) and National Environmental Publications Internet Site (NEPIS) database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI, and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following databases outside of HERO were searched for toxicity values: ACGIH, ATSDR, CalEPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 2 provides an overview of the relevant database for 2-EE and includes all potentially relevant repeated short-term-, subchronic-, and chronic-duration studies. NOAELs, LOAELs, and BMDL/BMCLs are provided in HED/HEC units for comparison except that oral noncancer values are not converted to HEDs and are identified in parentheses as adjusted rather than HED/HECs. Principal studies are identified. Entries for the principal studies are bolded.

Table 2. Summary of Potentially Relevant Data for 2-EE (CASRN 110-80-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[b]	LOAEL[a]	Reference (Comments)	Notes[c]
Human								
			1. Oral (mg/kg-d)[a]					
None								
			2. Inhalation (mg/m^3)[a]					
Subchronic	None							
Chronic	None							
Developmental	None							
Reproductive	Unreported number, Chinese male factory employees, occupational exposure (2-Ethoxyethanol [2-EE] and 2-methoxyethanol [2-ME]),	Unknown	Urinary metabolites higher with high exposure; sperm counts and, progressive motility lower; red blood cell count, hemoglobin level, packed cell volume, and white blood cell count lower	Not determinable	Not determinable	Not determinable	Wang et al. (2003)	
	73/0 painters (40 unexposed controls), occupational exposure (2-EE and 2-ME), cross-sectional study	Mean TWA of 9.9 mg/m^3 2-EE; mean TWA of 2.6 mg/m^3 2-ME	Increased semen pH and prevalance of oligospermia; increased odds ratio for lower sperm count per ejaculate	Not determinable	Not determinable	Not determinable	Welch et al. (1988)	PR
	73/0 painters (40 unexposed controls), occupational exposure, cross-sectional study	TWA of 9.9 mg/m^3 2-EE; TWA of 2.6 mg/m^3 2-ME	No fertility effects	Not determinable	Not determinable	Not determinable	Welch et al. (1991)	PR

Table 2. Summary of Potentially Relevant Data for 2-EE (CASRN 110-80-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[b]	LOAEL[a]	Reference (Comments)	Notes[c]
Reproductive	37/0 manufacturing plant employees (39 controls), occupational exposure (roughly 50% ethanol and 50% 2-EE), cross-sectional	33 mg/m^3 (average)	Sperm count per ejaculate decreased; immature forms of sperm increased; proportion of double-headed sperm decreased	Not determinable	Not determinable	Not determinable	Ratcliffe et al. (1986)	
	1019/0 infertility or subfertility cases (475 fertile male controls), case-control study	Unknown	Testis volume, sperm motility, vitality, concentration, morphology and integrity of cell membrane decreased; follicle stimulating hormone (FSH) increased; association between reproductive problems and ethoxyacetic acid (EAA) in urine; association between EAA-positive patients, azoospermia, and oligospermia	Not determinable	Not applicable	Not determinable	Veulemans et al. (1993)	PR
	0/32 manufacturing plant employees (20 controls), occupational exposure, case-control study	24 (average)	EAA in urine increased	Not determinable	Not applicable	Not determinable	Wang et al. (2004)	PR
	0/1712 semiconductor manufacturing plant employees and 0/1295 wives of employees, occupational exposure, retrospective cohort	Unknown (mixtures of glycol ethers)	Spontaneous abortion and subfertility increased in employees	Not determinable	Not applicable	Not determinable	Correa et al. (1996)	PR
Carcinogenic	None							

Table 2. Summary of Potentially Relevant Data for 2-EE (CASRN 110-80-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[b]	LOAEL[a]	Reference (Comments)	Notes[c]
Other studies	7/0 lithographers, occupational exposure, case study	Unknown	Stromal injury with deposition of a granular periodic acid-Schiff positive intracellular material and absolute myeloid hypoplasia in the bone marrow; marrow iron stores, eosinophils, plasma cells, and mast cells increased	Not determinable	Not applicable	Not determinable	Cullen et al. (1983)	PR
	94/0 ship painters (55 controls); cross-sectional study (exposure to 2-EE and 2-ME)	TWA of 9.9 mg/m^3 2-EE; TWA of 2.6 mg/m^3 2-ME (as presented in Sparer et al. (1988)	Lowest quartile for hemoglobin values; increased rate of anemia; 5 painters abnormally low levels of polymorphonuclear leukocytes (significantly different than controls, which showed no abnormal levels)	Not determinable	Not applicable	Not determinable	Welch and Cullen (1988)	PR
	94/0 ship painters (55 controls); cross-sectional study (exposure to 2-EE and 2-ME)	TWA of 9.9 mg/m^3 2-EE; TWA of 2.6 mg/m^3 2-ME (as presented in Sparer et al. (1988)	Pyruvate kinase decreased	Not determinable	Not applicable	Not determinable	Cullen et al. (1992)	PR

Table 2. Summary of Potentially Relevant Data for 2-EE (CASRN 110-80-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[b]	LOAEL[a]	Reference (Comments)	Notes[c]
Animal								
			1. Oral (mg/kg-day)[a]					
Subchronic	**10/10, F344/N rat, diet, 7 d/wk, 13 wk**	**Males: 0, 109, 205, 400, 792, 2240 (adjusted)** **Females: 0, 122, 247, 466, 804, 2061 (adjusted)**	**Both sexes: mortality ≥2061 mg/kg-d** **Males: decreased testis weights and size at 792 mg/kg-d; testicular degeneration ≥400 mg/kg-d; decreased thymus weights ≥205 mg/kg-d; prostate atrophy ≥205 mg/kg-d** **Females: decreased thymus weights at 804 mg/kg-d, increased estrous cycle length ≥247 mg/kg-d (significant at 804 mg/kg-d)** **NOAEL/LOAEL: thymus weights and prostate atrophy**	**109**	**67 (prostate atrophy)**	**205[d]**	**NTP (1993a)**	**PS, PR**
	30/0, F344/N rat, drinking water, 7 d/wk, 60 d (followed by recovery of 0, 30, or 56 d)	0, 407, 792, 2390 (adjusted)	Mortality at 2390 mg/kg-d; body weights, body-weight gains decreased in all groups; testicular weight decreased, and testicular degeneration with no recovery ≥792 mg/kg-d NOAEL/LOAEL: testicular degeneration	407[d]	398 (testicular degeneration)	792[d]	NTP (1993b)	PR

Table 2. Summary of Potentially Relevant Data for 2-EE (CASRN 110-80-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[b]	LOAEL[a]	Reference (Comments)	Notes[c]
Subchronic	10/10, B6C3F$_1$ mouse, drinking water, 13 wk	Males: 0, 587, 971, 2003, 5123, 7284 (adjusted) Females: 0, 722, 1304, 2725, 7255, 11,172 (adjusted)	Males: decreased absolute (≥5123 mg/kg-d) and relative (at 7284 mg/kg-d) testis weights with loss of the germinal epithelium in the seminiferous tubules ≥2003 mg/kg-d; splenic hematopoiesis, lesions of the small testes, and epididymides at 7284 mg/kg-d Females: hypertrophy of the X-zone of the adrenals[e] ≥1304 mg/kg-d; estrous cycle length increased ≥1304 mg/kg-d; splenic hematopoiesis ≥2725 mg/kg-d NOAEL/LOAEL: splenic hematopoiesis in females	1304	1777 (splenic hematopoiesis)	2725[d]	NTP (1993c)	PR
Chronic	50/50, F344 rat, gavage, 103 wk (followed by 1 wk observation period)	0, 357, 714, 1429 (adjusted)	Both sexes: mortality ≥714 mg/kg-d; body weights decreased ≥357 mg/kg-d; stomach ulcerations Males: testis size decreased at 1429 mg/kg-d; enlarged adrenal glands ≥357 mg/kg-d LOAEL: body weight decrease	None	Not run	357[d]	Melnick, (1984a)	PR
	50/50, B6C3F$_1$ mouse, gavage, 103 wk (followed by 1 wk observation period)	0, 357, 714, 1429 (adjusted)	Mortality at 1429 mg/kg-d Males: adrenal glands enlarged and stomach ulceration at 1429 mg/kg-d	Not determinable	Not run	Not determinable	Melnick, (1984b)	PR

Table 2. Summary of Potentially Relevant Data for 2-EE (CASRN 110-80-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[b]	LOAEL[a]	Reference (Comments)	Notes[c]
Reproductive and Developmental[f]	**5/0, Sprague-Dawley rat, gavage, 6 d/wk, 4 wk**	**0, 86, 171, 343, 686 (adjusted)**	**Adult males: marked depletion of all spermatid types at 686 mg/kg-d; dose-related decrease in relative epididymis weight at all doses; relative testis weight decreased at ≥343 mg/kg-d; body weight decreased at ≥171 mg/kg-d; testicular pathology with exfoliation of the germ cells into the tubular lumen at 171 mg/kg-d;** **NOAEL/LOAEL: testicular pathology**	**86[d]**	**Not run for testicular pathology**	**171[d]**	**Yoon et al. (2003)**	**PS, PR**
	10/0 (5 pubertal and 5 adult), Sprague-Dawley rat, gavage, 6 d/wk, 4 wk	0, 43, 86, 171, 343 (adjusted)	Adult males: abnormal spermatogenesis; altered composition of testicular germ cell populations, decreased relative testis weight, decreased relative epididymal weight, and body weight at 343 mg/kg-d Pubertal males: relative testes weight and relative epididymal weight increased in all dose groups; body weights decreased at 343 mg/kg-d NOAEL/LOAEL: testicular pathology in males	171[d]	No fit	343[d]	Yoon et al. (2001)	PR

Table 2. Summary of Potentially Relevant Data for 2-EE (CASRN 110-80-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[b]	LOAEL[a]	Reference (Comments)	Notes[c]
Reproductive and Developmental[f]	9/0, Sprague-Dawley rat, gavage, 6 d/wk, 4 wk	0, 129 (adjusted)	Adult males: body weight, relative adrenal gland, relative testis, and relative epididymis weights decreased; severe degeneration of seminiferous tubules, germ cell necrosis, interstitial Leydig cell hyperplasia and hypertrophy; white blood cell, platelet count, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin concentration, plasma protein content, plasma creatinine concentration, and alkaline phosphatase decreased at 129 mg/kg-d NOAEL/LOAEL: testicular effects and hematopoietic effects in males	None	Not run	129[d]	Yu et al. (1999)	PR
Carcinogenic	None							
2. Inhalation (mg/m^3)[a]								
Subchronic	15/15, Sprague-Dawley rat, inhalation, 6 h/d, 5 d/wk, 13 wk	0, 17, 68, 265	Males: no effects Females: no effects	265[d]	Not run	Not determinable	Barbee et al. (1984a)	PR

Table 2. Summary of Potentially Relevant Data for 2-EE (CASRN 110-80-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[b]	LOAEL[a]	Reference (Comments)	Notes[c]
Subchronic	**10/10, New Zealand white rabbit, inhalation, 6 h/d, 5 d/wk, 13 wk**	**0, 17, 68, 265**	**Males: body weights, and testes weights decreased; hemoglobin, hematocrit, and erythrocyte counts decreased; focal degeneration of seminiferous tubules with loss of epithelium at 265 mg/kg-d** **Females: body weights decreased (not significant), hemoglobin, hematocrit, and erythrocyte counts decreased at 265 mg/kg-d** **NOAEL/LOAEL: testicular effects in males and hematopoietic effects in both sexes**	**68**	**Not run**	**265[d]**	**Barbee et al. (1984b)**	**PS, IRIS, PR**
Chronic	None							
Developmental[f]	0/24, Wistar rat, inhalation, 6 h/d, GDs 6−15	0, 9, 47, 230	Maternal: hemoglobin, hematocrit, and mean cell volume in red blood cells decreased at 230 mg/m^3 Maternal NOAEL/LOAEL: hematopoietic effects Fetal: reduced mean fetal body weight, minor external, visceral, and skeletal defects at 230 mg/m^3 Fetal NOAEL/LOAEL: skeletal defects	Maternal: 47[d] Fetal: 47[d]	Not run	Maternal: 230[d] Fetal: 230[d]	Doe (1984a)	PR
	0/14−15, Sprague-Dawley rat, inhalation, 7 h/d, GDs 7−13 or 14−20	0, 108	Fetal: neuromotor performance decreased; elevations in brain chemistry at 108 mg/m^3 Fetal LOAEL: neurotoxicity	Fetal: none	Not run	Fetal: 108[d]	Nelson et al. (1981)	PR

Table 2. Summary of Potentially Relevant Data for 2-EE (CASRN 110-80-5)

Category	Number of Male/Female, Strain Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[b]	LOAEL[a]	Reference (Comments)	Notes[c]
Developmental[f]	**0/24, Dutch rabbit, inhalation, 6 h/d, GDs 6−18**	**0, 9, 46, 161**	**Maternal: no effects** **Fetal: minor visceral and skeletal defects at 161 mg/m^3** **Fetal NOAEL/LOAEL: skeletal defects**	**Maternal: 161[d]** **Fetal: 46**	**4.23**	**Maternal: not determinable** **Fetal: 161[d]**	**Doe (1984b)**	**PS, PR**
Carcinogenic	None							

[a]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to human equivalent dose (HED in mg/kg-day) or human equivalent concentration (HEC in mg/m^3) units. All of the exposure values of long-term exposure (4 weeks and longer) are converted from a discontinuous to a continuous (weekly) exposure. Values for inhalation (cancer and noncancer) and oral (cancer only) are further converted to an HEC/HED. Values from animal developmental inhalation studies are adjusted to a 24-hour continuous exposure, and then converted to human equivalent concentration (HEC in mg/m^3) units. Following EPA guidance for Category 3 gases (U.S. EPA, 2009), concentrations were converted to adjust for continuous exposure by using the following equation:

$Conc_{ADJ}$ = concentrations in mg/L× 1000 L/m^3 × (hours exposed per 24 day) × (days dosed/ week) ÷ total days). Concentrations were calculated for an extrarespiratory effect for a Category 3 gas. Because the blood:gas (air) partition coefficient lambda for humans is unknown, a default value of 1.0 is used for this ratio. $Conc_{HECEXRESP}$ = $Conc_{ADJ}$× blood:gas (air) partition coefficient of 1.

[b]For studies reporting a BMDL, the critical effect used as the POD is listed first.

[c]Notes: IRIS = utilized by IRIS, date of last update; PR = peer reviewed; PS = principal study.

[d]NOAEL or LOAEL values are determined from the data by the PPRTV authors.

[e]Hypertrophy can be considered an adaptive cell change and is not typically a viable toxicity endpoint.

[f]Additional reproductive and developmental studies are summarized in Table 3 (oral studies) and Table 4 (inhalation studies).

HUMAN STUDIES

Oral Exposures

The effects of oral exposure of humans to 2-EE have not been evaluated in any subchronic-duration, chronic-duration, developmental, reproductive, or carcinogenic studies.

Inhalation Exposures

The effects of inhalation exposure of humans to 2-EE have been evaluated in six primarily occupational studies evaluating various indicators of reproductive function (Welch et al., 1988, 1991; Veulemans et al., 1993; Correa et al., 1996; Ratcliffe et al., 1986; Wang et al., 2003, 2004). There are no clinical studies evaluating the effects of inhalation exposure of humans to 2-EE exposure in short-term-, developmental, subchronic-, or chronic-duration settings. Three other studies evaluated the hematological effects of inhalation exposure of humans to 2-EE (Cullen et al., 1983; Welch and Cullen, 1988; Cullen et al., 1992).

Reproductive Studies

The metabolism of 2-EE, discussed more thoroughly in the following sections of this document, is similar in both humans and animals. 2-EE metabolism is typical of the biotransformation of ethers, with the main pathway being oxidation to the corresponding acid, 2-ethoxyacetic acid or EAA, as it shall be referred to in this document. Many sources attribute the adverse effects of glycol ethers to these acid metabolites and measure those metabolites as an indicator of exposure (Groesenken et al., 1988; Cheever et al., 1984; Medinsky et al., 1990; Wang et al., 2003; Welch et al., 1988).

A number of cross-sectional, case-control, and retrospective cohort studies have examined the association between occupational exposure to 2-EE and potential indicators of male reproductive effects. In a poster session abstract, Wang et al. (2003) described their investigation of the effects of exposure to combined glycol ethers (2-EE and 2-methoxyethanol [2-ME]) among male workers (number not reported) in two Chinese factories manufacturing photopolymer sensitization plates. Workers in areas of the plant that had low or no exposure to glycol ethers served as the "unexposed" comparison group. No exposure metrics were reported. Urine and semen samples were collected from participants. Urine testing measuring for the EAA metabolite showed much higher levels in exposed workers. Analysis of semen samples revealed that the exposed group had significantly lowered sperm count, progressive motility, and percentage of sperm with normal morphology when compared to the unexposed group. Exposed workers also had lowered red blood cell count, hemoglobin level, packed cell volume, and white blood cell count compared to those of the unexposed workers. Blood hormone levels (testosterone, luteinizing hormone, follicle stimulating hormone [FSH], prolactin, and estradiol) were not different between the exposed and unexposed groups.

In a peer-reviewed cross-sectional study of male shipyard workers, Welch et al. (1988) investigated the association between exposure to ethylene glycol ethers and reproductive effects. Sparer et al. (1988) previously concluded that these workers were exposed principally to 2-EE and 2-ME; Welch et al. (1988) also considered other potential exposures that may affect reproduction, including lead and epichlorohydrin.

Welch et al. (1988) sampled for semen characteristics from a group that consisted of 73 male painters and 40 controls. Participants were given a questionnaire and physical examination. Investigators evaluated participants for testicular size, presence of varicocele

(abnormal dilation of the veins of the spermatic cord), and secondary sex characteristics. Urine was collected at the time of interview and again at the sample-collection appointment and was measured for the metabolites of 2-EE and 2-ME. Blood samples were obtained, and hormone levels were measured. Semen samples (collected by participants at home) were analyzed for sperm viability, velocity, motility, count, volume and pH, morphology, and morphometrics.

An analysis of Welch et al. (1988) by Sparer et al. (1988) showed workers were exposed to both inhaled 2-EE and 2-ME. For 2-EE, the TWA of inhalation exposure ranged from 0−80.5 mg/m^3 with a mean of 9.9 mg/m^3 and a median of 4.4 mg/m^3; for 2-ME, the TWA ranged from 0−17.7 mg/m^3 with a mean of 2.6 mg/m^3 and a median of 1.6 mg/m^3. Semen analysis indicated that exposed men had significantly ($p < 0.05$) higher mean semen pH than unexposed men (7.94 ± 0.15 compared to 7.88 ± 0.16). After smokers and nonsmokers were analyzed separately, the exposed group had a significantly higher rate of oligospermia ($p = 0.05$), with an odds ratio of 1.86 and confidence interval (CI) of 0.6−5.6 for this effect. Although the biological significance of these findings is unknown, they indicate that unexposed controls may be more likely than exposed painters to have reported a fertility problem. However, the study authors posit that self-selection bias may have occurred in this study because both control and exposed nonparticipating men were more likely to have experienced fertility problems compared to participants. This bias may have underestimated the reproductive effects of exposure to shipyard paints.

In a cross-sectional study, Ratcliffe et al. (1986) evaluated semen quality in 37 male employees working with a binding slurry (roughly 50% ethanol and 50% 2-EE) used in the preparation of ceramic shells for the manufacture of metal parts, with 2-EE being the only glycol ether used. Workers were mixers of the binder slurry, hand-dippers and/or grabber operators dipping the molds in the slurry, processors who handled the ceramic shells, supervisors, or process engineers. All of the workers inhaled 2-EE circulated by fans and the air recirculation system. The control group consisted of 39 men who worked in other areas of the plant; men who had previously worked in the department with 2-EE were excluded. Participants were interviewed and given a physical exam; semen samples were taken at the participants' homes. Urine voids and spot samples were collected from subsamples of participants. Exposure was estimated using bulk air and breathing zone samples collected in two different months. The mean full-shift breathing zone exposure of 2-EE was 9 ppm ±5.6 ppm (33 ± 21 mg/m^3) with a range of 0−23.8 ppm (0−88 mg/m^3). None of the blood samples contained detectable levels of 2-EE, whereas urine samples indicated some absorption of 2-EE. Although statistical testing was not done on the urine data, EAA concentrations in workers showed higher levels in the hand-dipper (most exposed) when compared to the supervisor (least exposed). No detectable EAA was found in urine from controls. Sperm count per ejaculate was significantly ($p = 0.047$) lower in exposed workers compared to unexposed. The exposed group had a significantly ($p = 0.001$) higher proportion of immature forms of sperm and a significantly lowered proportion of double-headed sperm. When workers in the higher-exposure jobs (e.g., hand-dipper) were compared to the other workers, no significant differences in semen characteristics were found. The study authors state, however, that the mean sperm concentrations of both groups were significantly lower than that for other occupational populations. Also, the number of workers was small, which may preclude the detection of an effect.

In a peer-reviewed retrospective case-control study, Veulemans et al. (1993) evaluated the relationship between exposure to ethylene glycol ethers and spermatogenic disorders in men. The study authors recruited 1019 cases and 475 controls from the population of first-time patients at an outpatient clinic for reproductive disorders between October 1985 and July 1990. Cases had been diagnosed as infertile or subfertile; controls had been diagnosed as fertile. Occupational history and information about potential exposure to spermatotoxic agents was obtained using a questionnaire. Urine samples were collected and analyzed for methoxyacetic acid (MAA) and EAA.

The reproductive disorder cases had significantly lower values for testis volume and sperm motility, sperm vitality (% sperm living), sperm concentration, sperm morphology (% normal), and integrity of the cell membrane (% normal), and a significantly ($p < 0.0001$) higher concentration of FSH when compared to controls. EAA was detected in 45 patients (39 cases and 6 controls) at levels ranging between 1.3 and 71.0 mg/L; the odds ratio was statistically significant (3.11; $p = 0.004$). When the study authors divided the study group according to sperm concentration corrected for motility and morphology, there was a highly statistically significant (chi-square value of 0.0087) association between EAA-positive patients and subcategories of complete azoospermia and severe oligospermia. Though few patients' urine contained MAA, the total number of positive EAA or MAA patients (40 cases versus 8 controls) retained a significant ($p = 0.013$) odds ratio of 2.39 for the detection of 2-EE metabolites in the urine. After stratification for possible confounding by exposure to lead, cadmium, insecticides, weed killers, asphalt and bitumen, carbon disulfide, and welding fumes, the association between urinary EAA and case status remained significant (chi-square probability of 0.011).

The study authors concluded that a highly significant association between impaired fertility and EAA in urine as well as EAA and exposure to products containing solvents such as paints was indicated. Additionally, although urinary EAA levels have been shown to be a reflection of exposure, urinary metabolites are not always an accurate measure of past exposures, and it is difficult in retrospective studies to determine whether the exposure preceded the effects.

In a peer-reviewed case-control study, Wang et al. (2004) investigated the effects of exposure to 2-EE in female workers at factories that made photopolymer sensitization plates and used 2-EE as paint thinner. The study included 32 female workers exposed to 2-EE and a control group of 20 female workers at the same factories that were not exposed. Controls were matched to exposed women based on age range, mean age, and alcohol and cigarette use. All of the subjects underwent one-on-one interviews with physicians to gather demographic data, work and childbirth histories, and any health complaints. The study authors collected blood samples for analysis of blood cells, hormone levels, and plasma aminotransferases. Spot urine samples were also collected at the end of the 8-hour work day and analyzed for EAA. Four workers in the control group and 23 in the exposure group were given organic gas sampling badges to wear on their chest pocket. These devices measured exposure to ambient 2-EE and other solvents over 6−8 hours of work.

The average period of employment for both the exposed and the control groups was just over 2 years. Monitoring badges detected low concentrations of 2-EE (0.56 ppm or 2 mg/m^3) in the control group and nearly 12 times higher concentrations (6.44 ppm or 24 mg/m^3) in the exposed workers. 2-EE was almost the only organic gas detected; two other chemicals were

found at very low concentrations. Protective gloves or masks were not used during the survey period; thus, exposure may have occurred through inhalation and also through the handling of materials containing 2-EE. However, results of urinary analyses revealed that levels of EAA were 40 times higher in the exposed group when compared to controls, although the range was high, suggesting exposure to high 2-EE concentrations. None of the control urine samples showed detectable levels of unmetabolized 2-EE, although 10 samples from the exposed group contained 2-EE (geometric mean, 1.96-mg/g creatinine). There were no significant changes in hematology or blood levels of prolactin. A number of patients in both groups reported irregular menstruation, but no significant differences were noted in the exposed group versus the controls.

The study authors noted that the lack of findings regarding abnormal menstruation may have been caused by the small study size. In addition, this study could not examine effects on pregnancy or childbirth because most women in the study had already given birth prior to ever being exposed to 2-EE. The frequency of the other effects, including swelling of the legs (dropsy), was not different between the control and exposed groups.

Correa et al. (1996) conducted a peer-reviewed retrospective cohort study investigating the association between exposure to ethylene glycol ethers at two semiconductor manufacturing plants (designated I and II) and potential reproductive effects including spontaneous abortion and subfertility in female workers and the wives of male workers. Ethylene glycol ethers are used in these plants primarily as components of a photoresist film, which is used to coat silicon wafers. Exposure occurred through inhalation and skin contact during cleaning and restocking of photoresist containers and in the event of spills. Study participants had to have worked full time for at least 6 months at the plant between 1980 and 1990. Interviews were completed by trained interviewers using a standardized computer-assisted questionnaire. The specific ethylene glycol ether compounds are identified in name only as diethylene glycol dimethyl ether or ethylene glycol monoethyl ether acetate, the latter being metabolized to 2-EE. The study did not report exposure levels. Rather, exposure was ranked as potentially low, intermediate, or high based on the amount of work done with the photoresist mixture; the unexposed population included workers in the clean room using no ethylene glycol ethers. Subfertility was defined as pregnancy that took 1 year or more of unprotected intercourse to conceive.

A total of 1712 female employees and 1295 wives of male employees participated, with 1150 pregnancies among unexposed workers, 561 pregnancies among female semiconductor manufacturers, and 589 pregnancies among wives of male semiconductor manufacturers. Female employees experienced increased rates of spontaneous abortion with calendar year and experienced slightly higher rates in Manufacturing Plant I that increased with the potential of exposure to ethylene glycol ethers (chi-square value of 5.03, $p = 0.02$). The relative risk (RR) of spontaneous abortion increased in female employees with age and potential exposure level. In the high-exposure group, the adjusted RR was 2.9 (95% CI, 1.2−7.0) among female employees. However, wives of male employees did not experience an increased risk.

Subfertility was significantly increased among female employees in an exposure-dependent manner (chi-square value of 5.45, $p = 0.02$). The adjusted odds ratios for subfertility in female employees increased as exposure potential increased; the high-exposure group had nearly a 5-fold higher risk (4.6, 95% CI: 1.6−13.3, with 22 pregnancies) than the unexposed group (1.0 with 260 pregnancies). The study authors considered and ruled out

potential selection bias and recall bias. However, this study is limited by a lack of any specific exposure measurements. In addition, because the study was retrospective in nature, it is unclear whether exposure preceded disease.

Other Studies

Cullen et al. (1983) performed a peer-reviewed study of lithographers occupationally exposed to glycol ethers, including 2-EE, and other solvents used in multicolor offset printing. The investigation began with a case report of a 39-year-old male worker who developed profound pancytopenia (reduced number of red and white blood cells and platelets) and later died; autopsy revealed hemorrhage of many organs and bone marrow that was highly depleted of cellular elements. In the subsequent workplace evaluation, a staff industrial hygienist completed a walk-through, worker interviews, and observation of work. Seven (male) out of 10 employees agreed to participate in the study—5 pressmen and helpers, 1 foreman, and 1 ink mixer. A physician administered a questionnaire and an examination to each participant. Blood was collected, and bone marrow aspiration and biopsies were taken.

Workplace observations revealed consistent background inhalation exposure potential to various substances, although exposure was only measured for one chemical (i.e., dipropylene glycol monomethyl ether). Gloves were frequently not used, prolonged skin contact occurred at times, and respirators were not used. Workers reported skin irritation, but no prolonged illness or systemic disease was found. There were a variety of effects seen in the bone marrow aspirates and biopsies. Three printers (which had the highest assumed exposures) had multifocal areas of stromal injury with deposition of a granular periodic acid-Schiff (PAS) positive intracellular material and absolute myeloid hypoplasia in the bone marrow; two also had ring sideroblasts (possibly indicative of more specific myelodysplastic syndromes). Two cases had significant increases in marrow iron stores, and other workers had significant increases in eosinophils, plasma cells, and mast cells. Another case showed signs of increased intramedullary cell turnover.

The study authors stated that the results strongly supported an association between occupational exposures during multicolor offset printing and bone marrow injury. However, the study was limited by a small population, the lack of preexisting data on marrow injury in the general population with which to compare the data, and the lack of a matched control group with which to compare marrow specimens.

In a peer-reviewed cross-sectional investigation of the shipyard workers discussed in Sparer et al. (1988), investigators evaluated the potential for hematologic (Welch and Cullen, 1988) and male reproductive/fertility effects (Welch et al., 1988) from exposure to 2-EE and 2-ME. For the hematologic portion of the study, the study population included 94 male painters and 55 unexposed controls in jobs with no work aboard the ships (clerks and draftsmen who had not worked with ships in the past 10 years). The study authors gave participants a questionnaire and physical examination and collected blood samples. The cohort for reproduction/fertility portion of the study comprised 73 exposed and 40 unexposed workers. In addition to glycol ethers, the study authors evaluated the potential of lead and benzene, which are known to affect hematological parameters. The maximum detected level of lead in the air was 11 mg/m^3, although the study authors maintained that blood lead levels were not at an amount normally

associated with hematological effects among adults. In 1978, benzene levels in the workplace air were low (ranging from 0.08−0.53 mg/m^3). The current survey of paints and solvents indicated that they did not contain benzene.

Results of a hematology analysis indicated that hemoglobin levels did not differ significantly between the groups. However, when these data were rank-ordered and analyzed, those in the lowest quartile were primarily painters ($p = 0.02$, two-tailed). Painters were also more likely to be categorized as anemic based on hemoglobin level ($p = 0.028$), and the rate of anemia was significantly ($p = 0.04$) different in painters compared to that in controls. Mean values of polymorphonuclear leukocytes of painters were not significantly different than those of controls. However, when the study authors further defined "normal" and "abnormal" levels (with 1800 cells per microliter as the minimum considered "normal"), 0 controls and 5 painters had low levels, which yielded a significant difference between groups. All five of these painters had normal levels when hired.

The study authors stated that the significant differences between painters and controls in distributions of hemoglobin and polymorphonuclear leukocytes may have been affected by race and self-selection bias, but after considering these factors in depth, they concluded that the hematological effects were likely attributable to ethylene glycol ethers.

The study authors also indicated that biologically important differences exist between exposed and unexposed workers in sperm parameters (Welch et al., 1988). Semen samples of 73 exposed (painters) and 40 controls were analyzed for pH, volume, turbidity, liquidity, viability, sperm density and count per ejaculate, motility, morphology, and morphometry. The proportion of exposed men with a sperm count less than or equal to 20 million/cc was 13%, with only 5% expected based on other population surveys. Also, the proportion of painters with azoospermia was 5%, with only 1% expected based on other surveys. Nonsmoking painters were more likely to have oligospermia (defined as a sperm count per ejaculate of ≤100 million), with the odds ratio among the painters increased to 2.8 among the nonsmokers. In a later peer-reviewed study, these authors reported on the reproduction function study of this cohort using a questionnaire (Welch et al., 1991), which showed no effect of exposure on fertility among the 74 married exposed painters when compared to that of 51 married controls even though the groups differed as indicated in sperm count parameters.

A later peer-reviewed cross-sectional study (Cullen et al., 1992) of the same shipyard painters involved a more in-depth investigation of the effects of exposure to 2-EE and 2-ME to the bone marrow and circulating blood cells. This 18-month study took place 2 years after the original investigations. Authors divided previous participants into three groups: Group I consisted of painters with abnormal blood counts (10 painters with hemoglobin <14 g/dL or absolute granulocyte count <1800 cells per microliter), Group II consisted of exposed painters with normal counts (7 men from the prior survey), and Group III was the unexposed participants (8 previously unsurveyed men).

The study authors conducted additional interviews and collected and analyzed blood samples and bone marrow aspirates and biopsies. Liver, renal, and thyroid function tests were performed to determine whether any alternate explanations for depressed blood counts existed; all of the tests were normal in all of the subjects. Group I had a mean myeloid/erythroid ratio significantly lower than that of Group II (2.25 ± 0.70 in Group I versus 2.96 ± 0.65 in Group II).

However, based on an analysis of variance by demographic and exposure characteristics, the only significant predictor of myeloid/erythroid ratio was race. In red cell enzyme and metabolite analyses, there appeared to be a difference in pyruvate kinase levels between groups; men in Group I had significantly ($p = 0.05$) lower levels (7.97 ± 2.57 and 6.97 ± 0.79 for Group I [divided] versus 9.48 ± 1.05 for Group II and 8.89 ± 1.92 for Group III) compared to those of controls. Analysis of sister chromatid exchange in peripheral blood lymphocytes yielded no significant differences in groups. The only finding that indicated anything other than population variation was the depression of red cell pyruvate kinase. This effect was noted by the study authors as being the most consistent defect generally observed in acquired hematologic disorders.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to 2-EE have been evaluated in 3 subchronic-duration (NTP, 1993a,b,c), 2 chronic-duration (Melnick, 1984a,b), 4 developmental (Wier et al., 1987a,b; Hardin et al., 1987; Lamb et al., 1984), and 10 reproductive (Yoon et al., 2003, 2001; Yu et al., 1999; Oudiz and Zenick, 1986a; Horimoto et al., 1996, 2000; Hardin et al., 1987; Lamb et al., 1984; Nagano et al., 1984; Weir et al., 1987) studies; no carcinogenic studies are available. Table 3 presents the reproductive and developmental studies, and a general summary and discussion of the three most sensitive key studies (Yoon et al., 2003, 2001; Yu et al., 1999) are provided in the text below.

Subchronic-duration Studies

The NTP (1993a) study is selected as the principal study for deriving the subchronic p-RfD. In 1993, the National Toxicology Program (NTP) published a report compiling several different types of studies designed to investigate the toxicity of 2-EE. The report includes subchronic-duration oral toxicity studies investigating the effects of 2-EE in F344/N rats and $B6C3F_1$ mice via drinking water for 13 weeks, which will be referred to in this review as NTP (1993a) and NTP (1993c), respectively. Additionally, a stop-exposure study was conducted in male F344/N rats, which will be referred to as NTP (1993b). The rat subchronic-duration portion of the study (NTP, 1993a) is summarized first. The rat stop-exposure study (NTP, 1993b) and the mouse subchronic-duration study (NTP, 1993c) are summarized subsequently. Furthermore, several mutagenicity, genotoxicity, cytogenicity, in vitro reproductive, metabolism/toxicokinetic, mode-of-action/mechanistic, and immunotoxicity studies included in the NTP report are presented in Table 5 (e.g., NTP, 1993d,e,f,g,h).

In a peer-reviewed good laboratory practice (GLP)-compliant subchronic-duration study, 2-EE (purity 99%) was administered in deionized drinking water to ten 5- to 6-week-old F344/N rats (Taconic Farms) per sex per dose for 13 weeks (NTP, 1993a). Animals were treated with doses of 0, 1250, 2500, 5000, 10,000, or 20,000 ppm in drinking water, which was available ad libitum. Due to deaths (5/10 males and 7/10 females) in the 20,000-ppm dose group, exposure was discontinued at 9 weeks. The study authors reported average daily doses of 0, 109, 205, 400, 792, or 2240 mg/kg-day for males and 0, 122, 247, 466, 804, or 2061 mg/kg-day for females.

NTP (1993a) observed animals twice daily with weekly clinical observations; body weights were taken for each animal prior to the study period and then weekly. Hematology (hematocrit, hemoglobin, erythrocytes, mean cell volume, mean cell hemoglobin, mean cell

hemoglobin concentration, platelets, reticulocytes, leukocyte count, differential nucleated erythrocytes, methemoglobin, and total bone marrow cellularity) and clinical chemistry (urea nitrogen, creatinine, total protein, albumin, alkaline phosphatase, alanine aminotransferase, creatine kinase, and bile acids) analysis was conducted on all of the study animals at Weeks 1, 3, and 13. Urinalysis (volume, specific gravity, and pH) was conducted at Week 13. All of the animals were necropsied at the conclusion of the study. Organ weights were taken for heart, right kidney, liver, lung, thymus, and right testis, which were also examined for gross lesions and fixed in 10% formalin for microscopic examination. The study authors conducted histopathological examinations (adrenal glands, femur and marrow, brain, esophagus, eyes, gross lesions, heart, large and small intestines, lymph node, mammary gland, kidneys, larynx, liver, lungs, nasal cavity, ovaries, pancreas, parathyroid glands, pituitary gland, pharynx, preputial or clitoral glands, prostate gland, salivary gland, seminal vesicles, skin, spinal cord, sciatic nerve, spleen, stomach, testes, thigh muscle, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, and vagina) on all of the control animals, the high-dose groups, as well as animals in the "higher dose groups" (including early deaths). Examinations in the lowest dose group were done on the bone marrow, the epididymis, liver, ovaries, preputial or clitoral glands, prostate gland, seminal vesicle, spleen, stomach, testes, thymus, uterus, and vagina. Males in the 0-, 205-, 400-, and 792-mg/kg-day dose groups were examined for reproductive tissue weights and spermatozoal effects. Females in the 0-, 247-, 466-, and 804-mg/kg-day dose groups were examined for estrous cycle length and other cycle effects.

Statistical analysis was performed by NTP (1993a) using the parametric multiple comparisons of Williams or Dunnett to compare organ and body weights of treated and control animals. Clinical chemistry and hematology results were compared using the nonparametric multiple comparisons of Shirley or Dunn. The results of the Jonckheere test of the data were used to choose between the Dunn/Dunnett test ($p \geq 0.10$) and the Shirley/Williams test ($p < 0.10$). Vaginal cytology results were transformed using the arcsine transformation and then examined by multivariate analysis of variance.

NTP (1993a) reported decreased survivorship (50% in males and 30% in females) at Week 9 in the animals exposed in the highest dose group (2240 and 2061 mg/kg-day for males and females, respectively), at which time, further treatment was discontinued (see Table B.1). No other mortality was observed among treated or control animals. Clinical observations in exposed rats included emaciation, diarrhea, abnormal posture, pallor, tachypnea, hypoactivity, and comatose state, although the study authors did not specifically attribute these observations to specific doses. Decreased final mean body weights as well as decreased body-weight gains were also observed with increasing dose in males (e.g., 80% body weight and 66% body-weight gain in the 792-mg/kg-day dose group relative to controls) and females (e.g., 87% body weight and 61% body-weight gain in the 804-mg/kg-day dose group relative to controls) (see Table B.1). Absolute and relative thymus weights were significantly decreased in males in the 205-mg/kg-day (i.e., 71 and 76%, respectively, relative to controls) and greater dose groups and females in the 804-mg/kg-day dose group (i.e., 32 and 41%, respectively, relative to controls) (see Table B.2). Absolute and relative testis weights were significantly decreased in males of the 792-mg/kg-day dose group (i.e., 44 and 59%, respectively, as compared to those of controls) (see Table B.2). Testicular lesions were noted in the 792- and 2240-mg/kg-day dose groups. These lesions were characterized by marked degeneration of the germinal epithelium in the seminiferous tubules. Testicular degeneration in males in the 400-mg/kg-day dose group and greater, as well as prostate atrophy in males in the 205-mg/kg-day dose group and greater, was

observed (see Table B.3). Both testicular and prostate effects were noted to increase in severity with dose, with the prostate atrophy appearing at a dose lower than the testicular effects, indicating the prostate effects to be more sensitive. Other important chemical-related lesions in males (see Table B.3) and females (see Table B.4) were noted in the spleen (hematopoiesis and hemosiderin pigmentation), in the bone marrow, and in the liver (hemosiderin pigmentation in Kupffer cells).

Hematological analysis conducted by NTP (1993a) at Week 1 showed mild anemia in the male rats in the 792-mg/kg-day dose group (decreased red blood cell, hemoglobin, and hematocrit concentrations), which progressed into moderate-to-marked anemia in the Weeks 3 and 13 analyses in both male rats (in the 400- and 792-mg/kg-day dose groups) and female rats (in the 466- and 804-mg/kg-day dose groups), as characterized by decreased red blood cell and hemoglobin concentrations. Mild and moderate leukopenia, which reached significance in the males in the 792-mg/kg-day dose group and females in the 804-mg/kg-day dose group, was observed in the males and females at Weeks 1 and 3, respectively, which transitioned to marked leukocytosis at Week 13. Clinical chemistry results showed decreases in total protein, albumin, and alkaline phosphatase levels. Bile acid concentrations as well as alkaline phosphatase activity markedly increased during Week 3 in males in the 792- and 2240-mg/kg-day dose groups and females in the 466-, 804-, and 2062-mg/kg-day dose groups, which also showed increased alanine aminotransferase activity. Creatinine kinase activity increased in the females in the 247-, 466-, 804-, and 2062-mg/kg-day dose groups in Week 3. None of these effects persisted to Week 13. Urinalysis showed significant decreases in the volume and pH of the males in the 792-mg/kg-day dose group at Week 13; however, no significant changes were observed in the urine of females. The study authors also noted a decrease in average water consumption in exposed animals, although this finding was not considered to be dose related.

NTP (1993a) performed sperm analysis (see Table B.5) showing sperm concentration decreasing in male rats in the 205-, 400-, and 792-mg/kg-day dose groups (the 109-mg/kg-day dose group was not examined) with spermatozoa measurements and motility decreasing to 1% of controls in the 792-mg/kg-day dose group. Spermatozoa concentration was dramatically decreased in the 792-mg/kg-day dose group to about 4% of the control value with the lower dose groups also showing clear significant decreases relative to controls of 86% in the 205-mg/kg-day dose group and 88% in the 400-mg/kg-day dose group. Females in the 804-mg/kg-day dose group were found to have a significantly increased estrous cycle length. This change in estrous cycle length was accompanied by an increased portion of the cycle in the diestrus stage.

On the basis of decreased thymus weights in male rats, NTP (1993a) identified a NOAEL of 109 mg/kg-day. On the basis of histopathologic and hematopoietic effects in the female rats, the study authors identified a NOAEL of 466 mg/kg-day. A $LOAEL_{ADJ}$ of 205 mg/kg-day and a corresponding $NOAEL_{ADJ}$ of 109 mg/kg-day based on thymus weight changes and prostate atrophy in males are identified. It is notable that more specific testicular effects, specifically the spermatozoa concentration, were not examined at the 109-mg/kg-day dose.

NTP (1993b) conducted a peer-reviewed GLP-compliant, stop-exposure study; 2-EE (purity 99%) was administered in deionized drinking water to thirty 6-week-old male F344/N rats (Taconic Farms) per dose for 60 days. Animals were treated with doses of 0, 5000, 10,000, or 20,000 ppm in drinking water, which was available ad libitum. The study authors reported average daily doses of 0, 407, 792, or 2390 mg/kg-day. Ten animals were sacrificed

immediately following the 60-day treatment period, following a 30-day recovery period, or following a 56-day recovery period. Significant mortality was observed in the 2390-mg/kg-day dose group, which led the study authors to add the 5 surviving animals in the 2240-mg/kg-day dose group from the NTP (1993a) subchronic-duration rat study to the 10 surviving animals left in the 2390-mg/kg-day dose group following the 60-day exposure period.

NTP (1993b) examined animals and collected data using the same methods as the NTP (1993a) subchronic-duration rat study with the following exceptions: histologic analysis was only performed on the testes, caput, and cauda of the left epididymis; no clinical pathologic analysis (hematology, urinalysis, and clinical chemistry) was conducted; and no additional reproductive endpoints were measured in any of the dose groups. The statistical analysis was performed using the same methods as those used in the NTP (1993a) subchronic-duration rat study.

NTP (1993b) reported dramatically decreased survivorship with 25 of the 30 animals that were dead or sacrificed before the end of the 60-day exposure period in the animals exposed in the 2390-mg/kg-day dose group (see Table B.6). Additionally, a single unscheduled death following the treatment period occurred in each of the 792- and 2390-mg/kg-day dose groups. Clinical observations in exposed rats included abnormal posture, diarrhea, emaciation, and polyuria, although the study authors did not attribute these observations to specific doses. Mean water consumption in the exposed groups was decreased as compared to that of the controls, although the effect was not statistically significant. Decreased mean body weights as well as decreased body-weight gains were also observed with increasing dose in all of the exposed groups (e.g., 91% at body weight, and 84% body-weight gain, in the 792-mg/kg-day dose group males at study termination, relative to controls). Animals in the 792- and 2390-mg/kg-day dose groups showed increased weight gain as compared to those of the control during the recovery period, although final body weights were still less than those of the control (e.g., 91% in the 792-mg/kg-day dose group relative to controls; data not shown). Chemical-related reduction in testis weight (absolute and relative) was observed in the 792- and 2390-mg/kg-day dose groups following the exposure period, 30 days recovery, and 56 days recovery (see Table B.7). Additionally, absolute testis weight was significantly decreased ($p < 0.01$) in the 407-mg/kg-day dose group following 56 days recovery. Moderate-to-marked testicular degeneration was observed in rats exposed in the 792- and 2390-mg/kg-day dose groups following the exposure period with no evidence of improvement after the 30- and 56-day recovery periods (see Table B.8). Although no degeneration was noted in the 407-mg/kg-day dose group following the exposure period, minimal degeneration was noted after the recovery periods. Sperm analysis was not conducted in this study. No other treatment-related effects were noted by the study authors. A LOAEL of 792 mg/kg-day based on evidence of testicular degeneration, which is seen at increasing incidence and severity at the higher dose, is identified, with a corresponding NOAEL of 407 mg/kg-day.

NTP (1993c) conducted a peer-reviewed GLP-compliant study, where 2-EE (purity 99%) was administered in deionized drinking water to ten 5- to 6-week-old $B6C3F_1$ mice (Taconic Farms) per sex per dose for 13 weeks. Animals were treated with doses of 0, 2500, 5000, 10,000, 20,000, or 40,000 ppm in drinking water, which was available ad libitum. Unlike the NTP (1993a) subchronic-duration rat study, no mortalities occurred in mice over the exposure period. The study authors reported average daily doses of 0, 587, 971, 2003, 5123, or 7284 mg/kg-day for males and 0, 722, 1304, 2725, 7255, or 11,172 mg/kg-day for females.

Animals were examined, and data were collected using the same methods as the NTP (1993a) subchronic-duration rat study with the following exceptions: the gallbladder was subjected to histopathologic analysis; no clinical pathologic analysis (hematology, urinalysis, and clinical chemistry) was performed; and male and female reproductive endpoints (reproductive tissue weights, spermatozoa, and estrous cycle effects) were measured in the 0-, 5000-, 10,000-, and 20,000-ppm dose groups. The statistical analysis was performed using the same methods as the NTP (1993a) subchronic-duration rat study.

NTP (1993c) reported survivorship to be unaffected by treatment with 2-EE, although body-weight gains in male and female mice in the 20,000- (59 and 57% of control, respectively) and 40,000-ppm (52 and 46% of control, respectively) dose groups were decreased (see Table B.9). The only clinical observation in exposed mice was emaciation in males and females of the 20,000- and 40,000-ppm dose groups. Water consumption was variable and not attributed to exposure. Absolute testis weights were significantly decreased in males in the 5123-mg/kg-day dose group (82% as compared to that of controls), and absolute and relative testis weights were significantly decreased in the 7284-mg/kg-day dose group (16 and 19%, respectively, as compared to those of controls) (see Table B.10). Chemical-related lesions of the small testes and epididymides were found in the 7284-mg/kg-day dose group. Histopathologic analysis identified changes in the spleen and testes of male mice and the spleen and adrenal glands of female mice (see Tables B.11 and B.12). Degeneration of the testes in the 7284-mg/kg-day dose group was characterized by marked loss of the germinal epithelium in the seminiferous tubules, similar to the effects observed in male rats (NTP, 1993a). Splenic hematopoiesis was minimally to mildly increased in females (at doses ≥2725 mg/kg) and males (7284-mg/kg-day dose group). Hypertrophy of the X-zone of the adrenals was noted at doses ≥1304 mg/kg-day. No effects were seen in the bone marrow.

Sperm analysis showed that, in contrast to rat sperm, mouse sperm concentration was not affected even in the highest group, although motility was somewhat decreased in the high-dose (5123 mg/kg-day) group (see Table B.13). Females in all dose groups were found to have increased estrous cycle length as compared to that of female controls (see Table B.13).

On the basis of testicular degeneration and increased hematopoiesis in the spleen in male mice, the study authors identified a NOAEL of 5123 mg/kg-day. On the basis of on adrenal gland hypertrophy and increased hematopoiesis in the spleen in the female mice, the study authors identified a NOAEL of 1304 mg/kg-day. While hypertrophy in the adrenal gland was observed at low-dose levels, hypertrophy can be considered an adaptive cell change and is not a viable toxicity endpoint. A $LOAEL_{ADJ}$ of 2725 mg/kg-day and a corresponding $NOAEL_{ADJ}$ of 1304 mg/kg-day are identified based on effects on the increased hematopoiesis in the spleen in the female mice.

Chronic-duration Studies

Melnick (1984a) published the results of a peer-reviewed 2-year oral toxicity study in F344/N rats. 2-EE (>99% pure) was administered by gavage in deionized water at 0, 0.5, 1.0, or 2.0 g/kg of body weight in 5-mL/kg body-weight volume to 50 rats per sex per dose. Animals were treated 5 times per week for 103 weeks, followed by a 1-week observation period. The corresponding adjusted daily doses are 0, 357, 714, and 1429 mg/kg-day. Rats were acquired from Charles River Laboratories at approximately 7 weeks of age. Animals were allowed food and water ad libitum. Results demonstrated no measureable loss of the test compound when

stored for 2 weeks at 25°C. Clinical observations of toxic effects were made twice daily. Body weights were collected weekly for the first 13 weeks and then monthly until the end of the study period. Necropsies were conducted on any moribund animals and on all of the surviving animals at the end of the study period. Microscopic analysis was conducted on all of the animals in the following tissues following necropsy: gross lesions, tissue masses, abnormal lymph nodes, mandibular or mesenteric lymph nodes, salivary gland, thyroid, parathyroids, small intestine, colon, liver, prostate/testes or ovaries/uterus, lungs, mainstream bronchi, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, kidneys, adrenal glands, urinary bladder, pituitary gland, mammary glands, sternebrae, and femur or vertebrae (with marrow). Despite reporting statistical significance of the data, the study authors did not report the statistical methods used to analyze the data. No information regarding compliance with GLP was presented.

Melnick (1984a) reported decreased survival in the males and females in the 1429 mg/kg-day dose group, which caused this exposure level to be terminated at 17−18 weeks. Stomach ulcers were found in many of the high-dose animals and thought to be a cause of death. Additionally, males in this group showed reduced testicular size with no report of prostate effects in those examined. Survival of the males in the 714-mg/kg-day dose group was significantly reduced ($p < 0.05$) over the course of the 103-week exposure duration as compared to that of the control, whereas no negative effect on survival was noted in the 357-mg/kg-day dose group. No negative effects on survival in females of the 357- or 714-mg/kg-day dose groups were observed (see Table B.14). Dose-related decreases in body weight were also observed in the treated male and female animals (see Table B.14). The study author reported that this trend became clear at Week 15 and continued over the duration of the study. By Week 104 of the study, male rats in the 357-mg/kg-day dose group weighed 81% of control, and those in the 714-mg/kg-day dose group weighed 72% of control; female rats in the 357-mg/kg-day dose group weighed 76% of control, and those in the 714-mg/kg-day dose group weighed 71% of control. Gross lesion incidence in some organs was altered compared to that of controls; however, the quantitative data were not provided, and the qualitative data combine the effects in the 357- and 714-mg/kg-day dose groups. The study author noted an increased incidence of enlarged adrenal glands in treated males (357- and 714-mg/kg-day dose groups). This effect was not observed in females. Treatment reportedly decreased the incidence of enlarged spleens and lesions of the pituitary in treated males and females (357- and 714-mg/kg-day dose groups). Subcutaneous tissue masses of the mammary glands were decreased in incidence in exposed females (357- and 714-mg/kg-day dose groups), and incidences of enlarged testes (as opposed to the reduced size noted in the 2-g/kg dose group) were found to decrease in exposed males (357- and 714-mg/kg-day dose groups). The study author reported that the results of the histopathology were not available at the time of publication. The mortality observed in males at 714 mg/kg-day is a frank effect. The observed significant reduction in body weights in the exposed males and females supports identification of a $LOAEL_{ADJ}$ of 357 mg/kg-day. A NOAEL cannot be identified because effects were seen at the lowest dose administered in the study.

Melnick (1984b) published the results of a peer-reviewed 2-year oral toxicity study in $B6C3F_1$ mice. 2-EE (>99% pure) was administered by gavage in deionized water at 0, 0.5, 1.0, or 2.0 g/kg of body weight to 50 mice per sex per dose. Animals were treated 5 times per week, for 103 weeks, followed by a 1-week observation period. The corresponding adjusted daily doses are 0, 357, 714, and 1429 mg/kg-day. Mice were acquired from Charles River Laboratories at approximately 8 weeks of age. Animals were allowed food and water ad libitum.

Clinical observations of toxic effects were made twice daily. Body weights were collected weekly for the first 13 weeks and then monthly until the end of the study period. Necropsies were conducted on any moribund animals and on all of the animals surviving until the end of the study period. Following necropsy, microscopic analysis was conducted in the following tissues from all animals: gross lesions, tissue masses, abnormal lymph nodes, mandibular or mesenteric lymph nodes, salivary gland, thyroid, parathyroids, small intestine, colon, liver, prostate/testes or ovaries/uterus, lungs, mainstream bronchi, heart, esophagus, stomach, brain, thymus, trachea, pancreas, spleen, kidneys, adrenal glands, urinary bladder, gallbladder, pituitary gland, mammary glands, sternebrae, and femur or vertebrae (with marrow). Despite reporting statistical significance, the methods used for the data analysis were not presented in the study. No information regarding compliance with GLP was presented.

The investigator reported decreased survival in the males and females in the 1429-mg/kg-day dose group, which caused this exposure level to be terminated at 17−18 weeks. Stomach ulcers were found in many of the high-dose male animals and thought to be a cause of death, although no consistent incidence of gross lesions could be identified in the females. Additionally, males in this group showed reduced testicular size. Survival of the mice in the 357- and 714-mg/kg-day dose groups was not negatively affected (see Table B.15). No dose-related changes in body weight were observed in the treated male and female animals. Testis size was reduced in male mice compared to that of controls; however, the quantitative data were not provided, and the qualitative data combine the effects in the 357- and 714-mg/kg-day dose groups. The author reported that the results of the histopathology were not available at the time of publication.

The mortality observed in the 1429-mg/kg-day dose group is a frank effect. Although the reported changes in incidence of gross lesions were discussed, adequate quantitative and dose-related data were not provided to support identification of a LOAEL or NOAEL. Also, it is noted that subsequent studies (e.g., NTP, 1993a,b,c) clearly show that rats are more sensitive than mice to the effects of 2-EE. This study is presented in this review as a supporting study and will not be used to support derivation of a chronic p-RfD.

Reproductive and Developmental Studies

The study by Yoon et al. (2003) is selected as the principal study for deriving the chronic p-RfD. In a peer-reviewed reproductive study, Yoon et al. (2003) administered doses of 0- (saline only), 100-, 200-, 400-, or 800-mg/kg 2-EE (purity unreported) by gavage to 5 groups of 5 male Sprague-Dawley rats, 6 times per week, for 4 weeks. The adjusted daily doses are 0, 86, 171, 343, and 686 mg/kg-day. Rats were obtained from the Korea Food and Drug Administration at 8 weeks of age. Animals were allowed to acclimate for 1 week before study initiation. Throughout the study, rats were housed in plastic cages and given pellet food (brand unspecified) and water ad libitum. The GLP compliance of this study was not provided.

Yoon et al. (2003) sacrificed rats after blood samples were obtained by heart puncture. Testes and epididymides were weighed; testes were stored in citrate buffer at −80°C. Both organs were examined under a light microscope after being fixed in 10%-sodium phosphate-buffered formalin solution, embedded in paraffin, and stained with hematoxylin and eosin. Thawed testes were minced and shaken for 30 minutes at room temperature. The resulting cell suspension was filtered to discard tissue debris. Cells were counted using a hemocytometer and light microscope. Testicular cells were then analyzed for DNA content by

flow cytometry. Fluorescence was directly proportionate to the amount of stain absorbed, which in turn, represented the DNA content of each cell. For this review, graphs are analyzed using Coulter Multiparameter Data Acquisition and Display Software. Statistical analyses of data were completed using one-way analysis of variance (ANOVA) with a significance level of $p \leq 0.05$. Authors used the Scheffe test for significant differences between groups.

Yoon et al. (2003) reported that doses of 171 mg/kg-day and higher resulted in a dose-dependent decrease in body weight, suggesting systemic toxicity (see Table B.16). However, at 86 mg/kg-day, weight was increased compared to that of controls. At 343 and 686 mg/kg-day, the weights of the testes were reduced significantly ($p < 0.01$) to 64 and 49% compared to controls, respectively (see Table B.17). Authors reported a dose-related decrease ($p < 0.05$) in epididymis weight in all of the exposed groups. The numbers of testicular cells were reduced ($p < 0.01$) to 44% at 343 mg/kg-day and to 20.0% at 686 mg/kg-day compared to control (see Table B.18). Histopathological examination indicated that spermatogenesis was affected in a dose-dependent manner. Considerable testicular pathology was noted at ≥171 mg/kg-day. The group dosed at 171 mg/kg-day experienced exfoliation of the germ cells into the tubular lumen, whereas the 343-mg/kg-day group showed some large cells with the disappearance of spermatids and moderate testicular generation. A marked depletion of all of the spermatid types was noted in the 686-mg/kg-day group, as well as a decrease in irregularly shaped seminiferous tubules. This group also had increased spermatozoa aggregates in the epididymal tubules and a marked reduction in the density of spermatozoa in the lumen. Flow cytometry revealed that rats exposed to 2-EE experienced differences in proportions of testicular cell types compared to those of controls. Mature haploid cells were reduced ($p < 0.01$) to 50% at 343 mg/kg-day and 4% at 686 mg/kg-day compared to control. Immature haploids were reduced ($p < 0.01$) to 27% at 343 mg/kg-day and 11% at 686 mg/kg-day compared to control. The relative proportions of diploid cells and tetraploid cells were significantly increased ($p < 0.01$) in the 343-mg/kg-day group.

The altered ratios of sperm cell types, reported by Yoon et al. (2003), indicate that 2-EE interferes with spermatogenesis in rats. The depletion in haploid cells was somewhat novel, and authors noted this finding may be due to lethal effects to early cell types (spermatogonia). It is not clear whether the effects on diploid cells and tetraploid cells are an artifact of the cell counting method or an indicator of cytotoxicity. Overall, authors concluded that the study supports the reproductive cytotoxicity of 2-EE in male rats. A LOAEL of 171 mg/kg-day is identified based on testicular pathology (exfoliation of the germ cells in the testicular lumen), with a corresponding NOAEL of 86 mg/kg-day.

In a peer-reviewed study, Yoon et al. (2001) investigated the effects of 2-EE on spermatogenesis in male Sprague-Dawley rats. Twenty-five pubertal rats and 25 adult rats (5 per dose group) were dosed with 0-, 50-, 100-, 200-, or 400-mg/kg 2-EE (purity unreported) by gavage, 6 days per week, for 4 weeks. The adjusted daily doses are 0, 43, 86, 171, and 343 mg/kg-day. Rats were obtained from the Korea Food and Drug Administration. All of the animals were acclimated for 1 week; at study initiation, pubertal rats were 5 weeks of age, and adult rats were 9 weeks of age. Rats were provided with food and water ad libitum. Once a week, investigators weighed and examined rats for behavioral effects. After blood samples were drawn by heart puncture, rats were sacrificed, and the testes and epididymides were collected and weighed. Testes were stored at −80°C in citrate buffer until analysis. The study report did not indicate whether this study is GLP compliant.

Yoon et al. (2001) thawed, minced, and mixed testes for 30 minutes using a magnetic stirrer before analysis. The resulting cell suspension was filtered to remove debris, and cells were stained with propidium iodide. Samples were kept in an ice bath until examined. Flow cytometry was used to analyze the DNA content of the testicular cells. DNA content corresponded to the amount of stain absorbed, as depicted by the degree of fluorescence. For this review, histograms of fluorescence are analyzed using Coulter Multiparameter Data Acquisition and Display Software for the relative proportions of haploid, diploid, and tetraploid cells. Statistical analysis consisted of ANOVA at a significance level of $p \leq 0.05$; the Scheffe test was used for multiple comparisons of significant differences in groups.

Yoon et al. (2001) described significantly increased ($p < 0.05$) relative testis and epididymis weights in pubertal rats treated with ≥43 mg/kg-day compared to those of controls (see Table B.19). Body weights were also reportedly decreased although the data were not presented. Results of cellular analysis by flow cytometry indicated that spermatogenesis in pubertal rats was not significantly affected by 2-EE. No other effects were noted in the exposed pubertal groups.

Yoon et al. (2001) reported that adults administered 343-mg/kg-day 2-EE experienced significantly decreased ($p < 0.01$) relative testis and epididymis weights compared to those of the controls, unlike the trend observed in the pubertal rats (see Table B.19). Body weights were also reportedly decreased, although the data were not presented. Cellular analysis by flow cytometry in adult rats treated with 343 mg/kg-day showed a significant decrease ($p < 0.05$) in the proportions of mature (77%) and immature (52%) haploid cells as well as a significant increase ($p < 0.01$) in the proportions of diploid and tetraploid cells (see Table B.20).

Yoon et al. (2001) concluded that adults appeared to be more sensitive to the effects of 2-EE administration than pubertal rats; however, both showed systemic toxicity at decreased body weights. Although the results did not provide clear evidence of effects of 2-EE on spermatogenesis in pubertal rats, 343-mg/kg-day 2-EE produced clear testicular toxicity in adults. A LOAEL of 343 mg/kg-day is identified based on pathology of the testes of adults, with a NOAEL of 171 mg/kg-day.

Yu et al. (1999) conducted a published peer-reviewed study in which groups of nine male Sprague-Dawley rats were administered 2-EE (purity not reported) by gavage in olive oil. At 9 weeks of age, male, specific pathogen-free Sprague-Dawley rats weighing 315 ± 7 g were separated into experimental groups that received either olive oil vehicle as a control or 2-EE. An initial dose-finding experiment was conducted with 2-EE only administered via gavage at doses of 0, 100, 150, 250, or 500 mg/kg, 6 times per week, for 4 weeks. The corresponding adjusted daily doses are 0, 86, 129, 214, and 429 mg/kg-day. The dose-finding experiment sought to cause measurable testicular atrophy with 2-EE while minimizing changes in weekly body-weight gain, blood biochemistry, hematology, and organ pathology caused by 2-EE. The specific results of this dose-finding experiment were not reported although the study authors chose to administer 150-mg/kg 2-EE in the main experiment also for 4 weeks, 6 days per week for which further specifics and results are available (see below). The corresponding adjusted daily dose is 129 mg/kg-day.

Arteriolar blood was collected and analyzed for aminotransferase, alanine aminotransferase, alkaline phosphatase, glucose, urea nitrogen, total protein, total cholesterol, creatinine, total bilirubin, red and white blood cell counts, and standard blood measures. Adrenal glands, testes, epididymides, heart, lungs, kidneys, spleen, liver, and brain were removed, weighed, fixed in 10% buffered formalin solution, embedded in paraffin, and stained with hematoxylin and eosin for histological analysis. Statistical analyses of body weight, organ weights, blood chemistry, and hematology data were conducted using multiple variance analysis and Duncan multiple range tests. Compliance with GLP guidelines was not reported.

Among animals exposed to the 129-mg/kg-day dose regimen, Yu et al. (1999) reported significantly decreased, right adrenal weight (80%, relative to control), left adrenal weight (72%, relative to control), right and left testes weights (57%, relative to control), right epididymis weight (77%, relative to control) and left epididymis weight (76%, relative to control) all at a relative body weight decreased by only 5% in exposed animals (see Table B.21). Histology of the testes showed "grossly damaged seminiferous tubules," with only 20% of the tubules showing a normal appearance. Additionally, the germ cells in the tubules were necrotic, and the Leydig cells showed signs of hyperplasia and hypertrophy. Hematology showed significant reductions ($p < 0.01$) in platelets (74%, relative to control) and white blood cells (71%, relative to control) (see Table B.22). Hematocrit (92%, relative to control), hemoglobin (93%, relative to control), and mean corpuscular hemoglobin (92%, relative to control) were decreased, which are indicators of bone marrow suppression. Blood biochemistry revealed a reduction ($p < 0.01$) in plasma protein (89%, relative to control) and plasma creatinine concentration (82%, relative to control), which can indicate decreased protein turnover as well as liver and kidney damage. Alkaline phosphatase was decreased (59%, relative to control), but no change was noted in total cholesterol.

A LOAEL based on testicular toxicity as well as changes in hematology of 129 mg/kg-day is identified. Because the effects were observed at the only dose administered, a NOAEL cannot be identified.

Table 3. Summary of Oral Reproductive and Developmental Studies for 2-EE (CASRN 110-80-5)

Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration, Methods	Dosimetry (mg/kg-d)[a], Purity of 2-EE	Critical Effects	NOAEL	LOAEL	Reference
Rat					
Reproductive, 5/0, Sprague-Dawley rat, gavage, 6 d/wk, 4 wk; blood samples, testes and epididymides collected and weighed at study termination	0, 86, 171, 343, 686 (purity not reported)	Body weight decreased ≥171 mg/kg-d; testis weight decreased ≥343 mg/kg-d; testicular pathology at ≥171 mg/kg-d; marked depletion of all of the spermatid types at 686 mg/kg-d. NOAEL/LOAEL: testicular pathology (exfoliation of the germ cells in the testicular lumen)	86	171	Yoon et al. (2003)
Reproductive, 10/0, Sprague-Dawley rat, gavage, 6 d/wk, 4 wk; dose group: 5 pubertal rats and 5 adult rats; body weights and clinical examination weekly; testes and epididymides collected and weighed at study termination	0, 43, 86, 171, 343 (purity not reported)	Adult males: altered composition of testicular germ cell populations; relative testes, relative epididymal, and body weights decreased at 343 mg/kg-d Pubertal males: no effect on testicular growth and germ cell populations; increased relative testes, relative epididymal, and decreased body weights ≥43 mg/kg-d NOAEL/LOAEL: testicular effects (adults)	171	343	Yoon et al. (2001)
Reproductive, 9/0, Sprague-Dawley rat, gavage, 6 d/wk, 4 wk; exposure dose from a range-finding test (0, 86, 129, 214, and 429 mg/kg-d); organ weights, blood chemistry, hematology, and clinical pathology measured study termination	0, 129 (purity not reported)	Adult males: body weight, relative adrenal gland, relative testis, and relative epididymis weights decreased; severe degeneration of seminiferous tubules; germ cell necrosis, interstitial Leydig cell hyperplasia, and decreased white blood cells, platelet count, hematocrit, hemoglobin concentration, mean corpuscular hemoglobin, plasma protein content, plasma creatinine concentration, and alkaline phosphatase at 129 mg/kg-d LOAEL: hematopoietic and testicular effects	None	129	Yu et al. (1999)

Table 3. Summary of Oral Reproductive and Developmental Studies for 2-EE (CASRN 110-80-5)

Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration, Methods	Dosimetry (mg/kg-d)[a], Purity of 2-EE	Critical Effects	NOAEL	LOAEL	Reference
Reproductive, 9−10/0, Sprague-Dawley rat, gavage, 2, 5, or 7 weeks; sacrificed at (2, 5, or 7 wk; sperm motility and sperm counts assessed; body weights, testis and epididymis weights recorded; male rats in 7-wk group bred with untreated females after Week 5; pregnant females sacrificed on GD 14; numbers of implantation sites, resorptions, and live fetuses recorded	0, 250, 500 (purity not reported)	Adult males: decreased body-weight gains, decreased sperm motility, decreased sperm counts, decreased testis and epididymis weights ≥250 mg/kg-day; no effects at 2 wk except slightly decreased testis weight at 500 mg/kg-d; decreased pregnancy index, numbers of implantations and live fetuses from males at 500 mg/kg-d LOAEL: sperm motility	None	250	Horimoto et al. (1996)
Reproductive, 19−20/0, Sprague-Dawley rat, gavage, 35 d; males bred with untreated females; males continued treatment through breeding period (49−52 d); subset sacrificed and necropsied Day 36−39; subset sacrificed and necropsied Day 50−53; pregnant females sacrificed GD 14; body weights, testes and epididymides weights recorded; sperm motility and count analyzed	0, 250, 500 (purity not reported)	Adult males: decreased sperm count, sperm motility, body weights, body-weight gain and epididymis weight (absolute and relative) ≥250 mg/kg-d; decreased testis weight (absolute and relative) ≥500 mg/kg-d; no motile sperm, almost no sperm, decreases in the pregnancy index (30% of control), number of implantation sites (24% of control), and live births (27% of control) in those treated for 7 wk NOAEL/LOAEL: severe male reproductive effects	250	500	Horimoto et al. (2000)
Reproductive, 10/0, Long-Evans hooded rat, gavage, 5 d/wk, 6 wk; males bred with untreated females; semen collected weekly; sperm motility and count analyzed	0, 669 (purity 98%)	Adult males: sperm count decreased (30−40%) and abnormal sperm morphology by Weeks 5 and 6; sperm motility decreased by Week 6; three males azoospermic; no difference in swimming speeds of motile sperm; decreases in testis, epididymis, and caudae epididymis weights LOAEL: sperm and testicular effects	None	669	Oudiz and Zenick (1986a)

Table 3. Summary of Oral Reproductive and Developmental Studies for 2-EE (CASRN 110-80-5)

Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration, Methods	Dosimetry (mg/kg-d)[a], Purity of 2-EE	Critical Effects	NOAEL	LOAEL	Reference
Reproductive, 9/0, Long-Evans hooded rat, tracheal intubation, 5 d/wk, 6 wk; body weights, testes and epididymides weights recorded; hematology evaluation; semen collected weekly; sperm motility and count analyzed	0, 669 (purity 99.9%)	Adult males: body-weight gain reduced, brain and spleen weights increased; hemoglobin and hematocrit decreased; reduced sperm parameters: sperm count and percent normal morphology at Week 5; sperm count, percentage normal morphology, and sperm motility at Week 6; testicular lesions at Week 1 LOAEL: hematopoietic, sperm and testicular effects	None	669	Zenick et al. (1984)
Mouse					
Reproductive and developmental, 0/50, CD-1 mouse, gavage, GDs 6−13; body weights taken GDs 6 and 17; nonbirthing dams sacrificed at GD 22; litter size, birth weight, neonatal growth and survival to Postnatal Day 3	0, 3605 (purity not reported)	Maternal: mortality: 10% Fetal: mortality: 100% 3605 mg/kg-d: frank effect	Not applicable	Not applicable	Hardin et al. (1987)
Reproductive and developmental, 20/20, CD-1 mouse, drinking water, 24 wk; body weights recorded weekly; reproductive endpoints during and at study termination; crossover mating study: (1) mid- and high-dose males bred control females, (2) mid- and high-dose females bred control males, and (3) control males bred control females; sacrificed and necropsied Week 24; litter size, birth weight, neonatal growth, and survival	Males: 0, 1230, 2461, 4921 Females: 0, 1261, 2522, 5044 (purity not reported)	Fetal: embryo mortality (100%) ≥4921 mg/kg-d; number of litters, number of live pups per litter, proportion of pups born alive, and live pup weight decreased ≥2461 mg/kg-d Fetal: crossover mating study: live pups per litter decreased, and increased dead pups per litter in 2522 mg/kg-d females mated with control males; no litters from 5044 mg/kg-d females mated with control males; decreased fertile matings and numbers of live pups per litter from 2461 mg/kg-d males paired with control females NOAEL/LOAEL: fertility and reproductive performance	Fetal: 1230	Fetal: 2461	Lamb et al. (1984)

Table 3. Summary of Oral Reproductive and Developmental Studies for 2-EE (CASRN 110-80-5)

Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration, Methods	Dosimetry (mg/kg-d)[a], Purity of 2-EE	Critical Effects	NOAEL	LOAEL	Reference
Reproductive, 8/0 (4 in control) JCL ICR mouse, gavage, 5 d/wk, 5 wk; necropsied at study termination; body weights, testes and epididymides weights recorded; sperm motility and count analyzed	0, 357, 714, 1429, 2857 (purity not reported)	Adult males: testicular weights, atrophy of seminiferous epithelium, spermatozoa, spermatids, and spermatocytes decreased ≥714 mg/kg-d; decreased testis weight, spermatozoa, and spermatids ≥1429 mg/kg-d NOAEL/LOAEL: sperm and testicular effects	357	714	Nagano et al. (1984)
Developmental, 4−6/0, CD-1 mouse, gavage, GDs 8−14; sacrificed GD 18 and necropsied; maternal body weights and physical examinations on GDs 0, 8, 10, 12, 14, and 18; litter size, fetal weight, fetal growth recorded	0, 1000, 1800, 2600, 3400, 4200 (purity 97%)	Maternal: lethargy, failure to right, uneven gait, abnormal breathing, cold to the touch and/or red vaginal discharge (4200 mg/kg-d only) ≥3400 mg/kg-d; mortality 50% ≥3400 mg/kg-d; decreased maternal body weight (GDs 8−14 and 14−18) ≥1800 mg/kg-d Fetal: mortality 100% at 4200 mg/kg-d; decreased fetal weights ≥1000 mg/kg-d; increased malformed fetuses at 1800 and 2600 mg/kg-d NOAEL/LOAELs maternal weights and increased malformations in the fetus	Maternal: 1000 Fetal: none	Maternal: 1800 Fetal: 1000	Wier et al. (1987a)
Developmental, 0/20−30 treated females mated untreated males, CD-1 mouse, gavage, GDs 8−14; maternal body weights and physical examinations on GDs 0, 8, 10, 12, 14, and 18; litter size, fetal weight, fetal growth recorded	0, 800, 1200 (purity 97%)	Fetal: decreased live-born pups at birth and survival postbirth at 1200 mg/kg-d; malformations of forepaw and kinked tail ≥800 mg/kg-d LOAEL: malformation in pups	None	Fetal: 800	Wier et al. (1987b)

[a]Values for oral reproductive and developmental studies are not converted to human equivalent doses (HEDs). Reproductive studies are presented as duration adjusted doses (from 5−6 d per wk to continuous 7 d/wk). Doses for oral developmental studies are not adjusted beyond continuous daily as dosing is typically every day throughout the developmental period.

Inhalation Exposures

The effects of inhalation exposure of animals to 2-EE have been evaluated in two subchronic-duration (Barbee et al., 1984a,b), no chronic-duration, six developmental (Doe, 1984a,b; Andrew and Hardin, 1984; Nelson et al., 1981, 1982), no reproductive, and no carcinogenic studies. The developmental studies are presented in Table 4, with a general summary and discussion of key studies in the text.

Subchronic-duration Studies

In a peer-reviewed subchronic-duration inhalation study, Barbee et al. (1984a) exposed groups of 15 Sprague-Dawley rats per sex per concentration to 0 (air only), 25, 100, or 400 ppm of 2-EE vapor (in air) (99.59% pure), for 6 hours per day, 5 times per week, for 13 weeks. The analytical means of the 2-EE concentrations were 25, 103, and 403 ppm. The exposure concentration adjustments for continuous exposure and unit conversion are 0, 17, 68, and 265 mg/m^3. Rats were obtained from Charles River Breeding Laboratories and weighed between 149 and 275 g at study initiation. Animals were allowed to acclimate for 15 days before study initiation. Animals had access to food and water ad libitum. Exposure was whole body and conducted in a 10-m^3 stainless steel and glass chamber that provided a complete air change every 3 minutes and a 99% equilibrium time of 15 minutes. Animals were observed twice per day for clinical signs of toxicity. Investigators made physical examinations and weighed animals once per week. The GLP compliance of this study was not provided.

Barbee et al. (1984a) conducted ophthalmic examination before the exposure period and at termination of the overall exposure period. Hematology (red and white blood cell counts, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, platelet counts, total and differential leukocytes, reticulocyte count, and erythrocyte morphology), clinical chemistry (albumin, globulin, albumin/globulin ratio, aspartate transaminase, alanine aminotransferase, alkaline phosphatase, glucose, urea nitrogen, total protein, total cholesterol, creatinine, total bilirubin, direct bilirubin, sodium, potassium, chloride, calcium, and inorganic phosphorus), and urinalysis (specific gravity, pH, protein, glucose, ketones, bilirubin, occult blood, urobilinogen, and microscopic examination for sediment) examinations were performed at the termination of the exposure period. Rats were sacrificed after blood samples were obtained, and full necropsies were performed. Body and organ weights were measured for liver, kidneys, testes including epididymis, brain, spleen, thymus, adrenal glands, and pituitary. The following tissues were collected, preserved, sectioned, and stained from all of the necropsied animals: abdominal aorta, adrenal glands, bone marrow (sternum), brain, eyes with Harderian gland and optic nerve, gonads, heart, intestine, colon, duodenum, ileum, jejunum, kidneys, liver, lungs with trachea, lymph nodes, mammary glands, nasal turbinates, pancreas, pituitary, prostate, salivary gland, sciatic nerve with muscle, seminal vesicles, skin, spinal cord, and vagina. The sections from control and highly exposed animals were examined microscopically. Sperm analysis was not performed. Statistical analyses of data were completed using one-way ANOVA, the Bartlett test, the Dunnett test, the Kruskal-Wallis test, the summed rank test, regression analysis, and the Jonckheere statistic test with a significance level of $p \leq 0.05$.

Barbee et al. (1984a) noted increased lacrimation and nasal discharges in all of the exposed animals in Weeks 2−10, although a concentration-response was not observed. Survivorship was not affected by exposure. No chemical-related effects were noted during the ophthalmoscopic examination. The body weights of treated animals were not significantly

affected by exposure (see Table B.23). Organ weights were largely unaffected by exposure with the exception of decreased pituitary weight ($p < 0.05$) in the high-exposure males (86%, relative to control) and females (92%, relative to control), and spleen weights ($p < 0.01$) in the high-exposure females (85%, relative to control) (see Table B.24). The only changes noted in the hematology were a decreased leukocyte count in high-exposure females, which the study authors noted as being of unknown significance. Differences found in serum biochemistry were not considered biologically significant. No differences were found during urinalysis. Gross pathology and histopathology revealed no treatment-related effects.

Barbee et al. (1984a) concluded that no significant treatment-related effects were noted in rats. The lack of effects noted in rats exposed to subchronic inhalation of 2-EE supports a NOAEL of 265 mg/m^3 although no analysis was performed on the sperm, which is known to be a target tissue by the oral route of 2-EE exposure.

In a peer-reviewed subchronic-duration inhalation study, Barbee et al. (1984b) exposed groups of 10 New Zealand White rabbits per sex per concentration to 0 (air only), 25, 100, or 400 ppm of 2-EE vapor (in air) (99.59% pure) 6 hours per day, 5 times per week, for 13 weeks. The analytical means of the 2-EE concentrations were 25, 103, and 402 ppm. The exposure concentration adjustments for continuous exposure and unit conversion are 0, 17, 68, and 265 mg/m^3. Rabbits were obtained from Dutchland Laboratories (age unreported) and weighed between 2.1 and 3.3 kg at study initiation. Animals were allowed to acclimate for 22 days before study initiation. Animals had access to food and water ad libitum. Exposure was whole body and conducted in a 10-m^3 stainless steel and glass chamber that provided a complete air change every 3 minutes and a 99% equilibrium time of 15 minutes. Animals were observed twice per day for clinical signs of toxicity. Investigators made physical examinations and weighed animals once per week. The GLP compliance of this study was not provided. The study followed the same methods as described for the Barbee et al. (1984a) study in rats with the addition of the examination of the gallbladder from necropsied rabbits.

Barbee et al. (1984b) noted increased lacrimation and nasal discharges in all of the exposed animals in Weeks 2−10 although a concentration-response was not observed. No chemical-related effects were noted during the ophthalmoscopic examination. The body weights of treated animals were slightly depressed, although effects were only significant ($p < 0.05$) in high-exposure males (see Table B.25). Organ weights were largely unaffected by exposure with the exception of decreased adrenal gland weight ($p < 0.05$) in the low-exposure males (72%, relative to control) and decreased testis weights ($p < 0.05$) in the high-exposure males (78%, relative to control) (see Table B.26). Hematology showed decreases in the hemoglobin, hematocrit, and erythrocyte counts in high-exposure males and females, indicating anemia. Although changes were noted in serum biochemistry, they were reportedly not biologically significant. No differences were found during urinalysis. Gross pathology revealed no treatment-related effects. Histopathology of the high-exposure males revealed minimal to slight focal degeneration of the seminiferous tubules of the testes marked by a loss in epithelium (3/10 animals). The study authors stated that spermatogenesis appeared normal as judged by overall organ morphology although no specific sperm analysis was performed.

Barbee et al. (1984b) concluded that subchronic-duration exposure to 2-EE resulted in anemia that stemmed from the destruction of erythrocytes and testicular toxicity, which they used to identify a NOAEL of 68 mg/m^3. The effects seen in the high-exposure rabbits exposed to subchronic-duration inhalation of 2-EE support a LOAEL of 265 mg/m^3 and a corresponding NOAEL of 68 mg/m^3.

Chronic-duration Studies

No studies could be located regarding the effects of chronic-duration inhalation exposure of animals to 2-EE.

Developmental Studies

Doe (1984) presented the results of a peer-reviewed inhalation developmental study of 2-EE in rats and rabbits. This study was sponsored by the Glycol Ethers Program Panel of the Chemical Manufacturers Association and was intended to supplement a previous study conducted for NIOSH. The study authors did not state whether the study followed GLP guidelines. The portion of the study conducted in rats is referenced as Doe (1984a), and the portion conducted in rabbits is referenced as Doe (1984b).

Nulliparous specific pathogen-free female Wistar-derived (Alpk/AP) rats (11−13 weeks of age; initial number not specified) were paired with males (number and strain not specified) until evidence of mating was found by a sperm-positive vaginal smear. The day that a sperm-positive smear was detected was identified as Day 0 of pregnancy. 2-EE (purity >99%) was administered by inhalation to females (24 per group) at concentrations of 0 (control), 10, 50, or 250 ppm on GDs 6−15 for 6 hours per day. The overall atmospheric exposure concentrations were measured as 9.9 ± 0.9, 50.8 ± 2.3, and 249.2 ± 10.4 ppm. The concentrations converted to mg/m^3 and adjusted for continuous exposure (6 hours per 24 hours) are 0, 9, 47, and 230 mg/m^3.

Doe (1984a) exposed whole animals using stainless steel exposure chambers of approximately 3.4 m^3. Each chamber consisted of six cage levels, and there were four cages per level. Air (at a flow rate of 600 L/minute) entered at the front of the chamber and was extracted in the back. Temperature was maintained at 22°C, and relative humidity was maintained at 50%. Animal diets during the experiment were not described.

Females were observed daily for signs of clinical abnormalities. Body weight was recorded on GDs 0, 5, 6−15, 16, and 21. Food consumption was also measured (frequency not specified). On GD 21 of pregnancy, the rats were sacrificed and examined postmortem. Blood samples were collected for hematological assessment, and the spleen, gravid uterus, and thymus were weighed. The uterus was opened, and the number of corpora lutea in each ovary was counted. Numbers of live fetuses, implantations, and early and late uterine deaths were also recorded. Each live fetus was examined for gross abnormalities. Half of the fetuses in each litter were examined for skeletal defects; the other half was examined for visceral defects. Visceral assessments of fetuses included examination of the heart, abdomen, and thorax and determination of sex.

Doe (1984a) reported no treatment-related effects on maternal body weight or food consumption. In addition, no abnormalities related to treatment were observed during clinical or postmortem examinations. In dams exposed at the 230-mg/m^3 level, the study authors noted significant reductions in hemoglobin, hematocrit, and mean cell volume in red blood cells

compared to those of the control. Similar effects were not observed at the 9- and 47-mg/m^3 concentration levels. Preimplantation losses were higher in all of the treatment groups compared to those of the control and were significantly higher in the 9- and 47-mg/m^3 groups because exposure began at GD 6; however, this effect may not be attributable to treatment (see Table B.27). Postimplantation losses were also higher in all of the treatment groups compared to those of the control; however, the differences were not statistically significant for any exposed group. Mean numbers of live fetuses were lower in all of the exposed groups compared to those of the control with the decreases being significantly lower in the 9- and 47-mg/m^3 groups but not in the 230-mg/m^3 group. The study authors reported that mean live fetal weight in the 230-mg/m^3 group was significantly reduced (92%, relative to control); however, mean fetal weights for the other groups were comparable to that of the control (see Table B.27). Occurrences of minor external and visceral defects, as well as skeletal defects, were significantly elevated (18.4 and 97.5%, respectively) in the 230-mg/m^3 group compared to those in the control group (11.7 and 46.3%, respectively) (see Table B.28). The elevated incidence of external and visceral defects was associated with renal pelvic dilation, which occurred in 12.8% of offspring in the 230-mg/m^3 group compared to 6.8% in the control (see Table B.29). However, the study authors noted that this endpoint is not indicative of teratogenicity. The elevated incidences of skeletal defects were associated with increased partial nonossification of parts of the skull, vertebrae, and sternebrae and other skeletal abnormalities (data not reported). The study authors also noted that the number of limb malrotations was significantly increased in the 9-mg/m^3 group compared to that in the control group (see Table B.29), but the study authors believed that this result had no toxicological significance because the effects were limited to the 9-mg/m^3 group. Cardiovascular abnormalities were not observed.

Doe (1984a) concluded that there was evidence of mild maternal toxicity associated with 2-EE due to significantly reduced hemoglobin, hematocrit, and mean cell volume in red blood cells in the 230-mg/m^3 group compared to those in the control group. There was a fetotoxic effect at the 230-mg/m^3 level associated with retarded fetal growth and a significant increase ($p < 0.05$) in minor skeletal defects and skeletal variants. A slight effect at the 47-mg/m^3 level was associated with increased (but not significant) incidences of minor skeletal defects (51% compared to 46% in controls).

The study authors identified 9 mg/m^3 as a clear no-effect level in 2-EE-exposed rats; however, the data do not support concentration-response effects in the 47-mg/m^3 group. A maternal $LOAEL_{ADJ}$ of 230 mg/m^3 with a corresponding $NOAEL_{ADJ}$ of 47 mg/m^3 is identified based on hematopoietic effects. A fetotoxicity $LOAEL_{ADJ}$ of 230 mg/m^3 is identified based on increased skeletal malformations with a corresponding $NOAEL_{ADJ}$ of 47 mg/m^3.

In a peer-reviewed and published developmental study, Nelson et al. (1981) evaluated the possible functional effects in offspring of rats exposed to 2-EE during gestation. The study exposed a total of 29 pregnant Sprague-Dawley rats to 100-ppm 2-EE (98−98.5% purity) in an inhalation chamber, for 7 hours per day, from GDs 7−13 and GDs 14−20. A pilot range-finding study exposed pregnant Sprague-Dawley rats to 200-, 300-, 600-, 900-, or 1200-ppm 2-EE, 7 hours per day, from GDs 7−13 and GDs 14−20. Results of the dose-finding study were complete resorptions of litters at 900 and 1200 ppm and increased mortality of pups as low as a dose of 200 ppm. Thus, the study authors selected 100 ppm as the sole exposure of the main study. Converted to mg/m^3 and adjusted for the daily exposure (7 hours per 24 hours), the concentration is 108 mg/m^3. Fifty-nine pregnant animals were randomly assigned to one of four

groups of exposure (7 hours per day) to 100-ppm 2-EE (369 mg/m^3; 108 mg/m^3 adjusted) (15 animals on GDs 7−13, 14 animals on GDs 14−20, 15 animals each for control exposure on GDs 7−13 and GDs 14−20).

Pups of the mother rats were weighed weekly and observed for abnormalities. Neurochemical analysis was also performed. Behavioral tests were conducted to assess central nervous system functions at several stages of development; rotorod tests, open field tests, activity wheel, avoidance conditioning, and operant conditioning were conducted. Results of behavioral testing of offspring exposed on GDs 7−13 included significantly impaired performance on the rotorod test of neuromuscular ability, prolonged latency of leaving the start area of an open field, and marginal superiority in avoidance conditioning starting on Day 34 of age. Results of animals exposed on GDs 14−20 included significantly increased number of duration of shocks in avoidance conditioning, starting on Day 60 of age, as well as, significantly decreased activity compared to controls in a running wheel.

Results of neurochemical evaluation, of whole-brain samples from newborn pups, found significant decreases in levels of norepinephrine in offspring from both exposure periods. Significant elevations in acetylcholine, norepinephrine, and dopamine were found in the cerebrums from 21-day-old offspring from GDs 7−13. The cerebellums were found to have a more significant increase in acetylcholine, whereas the brainstem had an increase in norepinephrine. The midbrain had excesses of acetylcholine, norepinephrine, and protein. The 21-day-old offspring from the GDs 14−20 exposure group revealed significant elevation in acetylcholine, dopamine, and 5-hydroxytryptamine in the cerebrum relative to the concurrently air-exposed offspring.

The study authors concluded that prenatal exposure to 108 mg/m^3 results in behavioral and neurochemical alternations in offspring. The study authors did not report a NOAEL or LOAEL. A fetal $LOAEL_{ADJ}$ of 108 mg/m^3 is established based on developmental neurotoxicity. Because effects were noted at the only concentration utilized in this study, a NOAEL cannot be identified.

The study by Doe (1984b) is selected as the principal study for deriving the subchronic p-RfC. Doe (1984b) conducted an inhalation developmental study in virgin female Dutch rabbits (5−7 months of age; initial number not specified). Females were housed with males of the same strain until evidence of mating was found by a vaginal smear containing motile sperm. After mating, females received chorionic gonadotropin injections to encourage ovulation. The day of mating was identified as GD 0. 2-EE (>99% pure) was administered by inhalation to females (24 per group) at concentrations of 0 (control), 10, 50, or 175 ppm on GDs 6−18 for 6 hours per day. The overall atmospheric exposure concentrations were measured as 10.1 ± 0.03, 51 ± 4, and 175 ± 3 ppm. The concentrations converted to mg/m^3 and adjusted for continuous exposure (6 hours per 24 hours) are 0, 9, 46, and 161 mg/m^3. Animals were exposed and evaluated in the same manner as the reproductive and developmental study by Doe (1984a), with the exception that animals were exposed from GDs 6−18. Body weights were recorded on GDs 0, 5−19, 24, and 28, and rabbits were sacrificed on GD 29 and examined postmortem.

Doe (1984b) reported no treatment-related effects on maternal body weight or food consumption. No abnormalities related to treatment were observed during clinical or postmortem examinations. In addition, there appeared to be no treatment-related effects on litter data for rabbits (see Table B.30). Occurrences of skeletal defects were significantly elevated in the 161-mg/m^3 group compared to those in the control group (see Table B.31). The elevated incidences of skeletal defects were associated with retarded ossification of the skeleton, increased incidence of extra ribs, and other vertebrae abnormalities. Two major visceral effects occurred in the 161-mg/m^3 group—one fetus had a heart defect and another fetus had an umbilical hernia (see Table B.32).

The study authors concluded that there was marginal evidence of fetotoxicity at the 161-mg/m^3 level due to the occurrence of major defects in two fetuses. There was no clear evidence of maternal or fetal toxicity. The study authors identified 46-mg/m^3 2-EE as the clear no-effect level for fetotoxicity in rabbits. A fetal $LOAEL_{ADJ}$ of 161 mg/m^3 with a corresponding $NOAEL_{ADJ}$ of 46 mg/m^3 is identified based on increased incidence of skeletal defects in offspring of exposed does. No effects were seen in the does following exposure, precluding identification of a maternal $LOAEL_{ADJ}$, although a $NOAEL_{ADJ}$ of 161 mg/m^3 is identified.

Table 4. Summary of Inhalation Developmental Studies for 2-EE (CASRN 110-80-5)

Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration, Methods	Dosimetry (mg/m^3)[a], Purity of 2-EE	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Rat					
Developmental, 0/24, Wistar rat, inhalation, GDs 6−15(6 h/d), dams weighed during study period; dams sacrificed on GD 21; blood collected for hematological assessment; uterus examined; fetuses examined for skeletal defects and visceral defects	0, 9, 47, 230, (>99% purity)	Maternal: Decrease of hemoglobin, hematocrit, and mean cell volume in red blood cells in the 230 mg/m^3 exposure group Maternal NOAEL/LOAEL: hematopoietic effects Fetal: minor external, visceral, and skeletal defects ≥230 mg/m^3 Fetal NOAEL/LOAEL: skeletal defects	Maternal: 47 Fetal: 47	Maternal: 230 Fetal: 230	Doe (1984a)
Developmental, 0/30, Wistar rat, inhalation, separate pregestational exposure for 3 wk followed by exposure GDs 1−19 (7 h/d); food consumption and body weight of dams measured during exposure; dams sacrificed GD 21; uterine contents, maternal viscera, and fetal heads were examined	0 (air-air), 61 (low-air), 103 (air-low), 164 (low-low), 262 (high-air), 392 (air-high), 653 (high-high) (>99% purity)[c]	Maternal: relative spleen weights increased at 61, 392, and 653 mg/m^3; gestational body weight decreased, mean relative lung and kidney weights increased, uterine involution (15/16), and corpora lutea regression of ovaries (9/16) ≥392 mg/m^3 Maternal NOAEL/LOAEL: gestational body weights and histopathology in the uterus and ovaries Fetal: intrauterine growth retardation, body weight, decreased length, and reduced skeletal ossification (with minor skeletal abnormalities) ≥103 mg/m^3, resorptions increased, 100% embryolethality ≥392 mg/m^3 Fetal NOAEL/LOAEL: growth retardation and reduced skeletal ossification	Maternal: 262 Fetal: 61	Maternal: 392 Fetal: 103	Andrew and Hardin (1984a)

Table 4. Summary of Inhalation Developmental Studies for 2-EE (CASRN 110-80-5)

Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration, Methods	Dosimetry (mg/m^3)[a], Purity of 2-EE	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Developmental, 0/14−15, Sprague-Dawley rat, inhalation, GDs 7−13 or 14−20 (7 h/d); behavioral testing to assess central nervous system function (rotorod test, open field test, activity wheel, avoidance conditioning, and operant conditioning)	0, 108 (98.5% purity)	Fetal: Rotorod performance, open field activity, and avoidance conditioning decreased; overall decreased neuromotor performance; elevations in brain chemistry in both exposures at 108 mg/m^3 Fetal LOAEL: developmental neurotoxicity	Fetal: None	Fetal: 108	Nelson et al. (1981)
Developmental, 0/16, Sprague-Dawley rat, inhalation, GDs 7−13 (7 h/d); pups weighed on Days 7, 14, 21, 28, and 35 postpartum; behavioral testing to assess central nervous system function (rotorod test, open field test, activity wheel, avoidance conditioning, and operant conditioning)	0, 215 (98−98.5% purity)	Maternal: maternal weight gain decreased; prolonged pregnancy duration Maternal LOAEL: body weight and gestational duration Fetal: rotorod performance, open field activity, and avoidance conditioning decreased; overall decreased neuromotor performance Fetal LOAEL: behavioral effects	Maternal: None Fetal: None	Maternal: 215 Fetal: 215	Nelson et al. (1982)
Rabbit					
Developmental, 0/24, Dutch rabbit, inhalation, GDs 6−18 (6 h/d); dams weighed during study period and sacrificed GD 29; blood collected for hematological assessment; uterus examined; fetuses examined for skeletal defects and visceral defects	0, 9, 46, 161 (>99% purity)	Maternal: no effects Fetal: minor visceral, and skeletal defects at 161 mg/m^3 Fetal NOAEL/LOAEL: skeletal defects	Maternal: 161 Fetal: 46	Maternal: None Fetal:161	Doe (1984b)

Table 4. Summary of Inhalation Developmental Studies for 2-EE (CASRN 110-80-5)

Study Type, Number of Male/Female, Strain Species, Route of Administration, Study Duration, Methods	Dosimetry (mg/m^3)[a], Purity of 2-EE	Critical Effects	NOAEL[b]	LOAEL[b]	Reference
Developmental, 0/29, New Zealand rabbit, inhalation, GDs 1−18 (7 h/d); food consumption and dams weighed during study period and sacrificed GD 30; uterine contents, maternal viscera, and fetal heads examined	0, 172, 663 (>99% purity)	Maternal: relative liver weights increased ≥172 mg/m^3; mortality (17%), body weight decreased, food consumption and relative kidney weights increased, uterus, ovary and corpora lutea effects at 663 mg/m^3 Maternal NOAEL: 663 mg/m^3 frank effect Fetal: embryolethality, resorptions, major malformations (ventral wall defects and fusion of aorta with pulmonary artery), minor anomalies (renal changes), and skeletal defects at ≥172 mg/mg^3 Fetal LOAEL: fetal malformations and embryolethality	Maternal: 172 Fetal: None	Maternal: None Fetal:172	Andrew and Hardin (1984b)

[a]Conversion Factors: MW = 90.12. Assuming 25ºC and 1 atmosphere, Exposure mg/m^3 = Exposure ppm × MW ÷ 24.45 = 3.69. For developmental effects, this concentration is adjusted for duration to a continuous exposure concentration; therefore, $NOAEL/LOAEL_{ADJ}$ = NOAEL/LOAEL and $Exposure_{ADJ}$ = Exposure hours per day exposed ÷ 24. The $NOAEL_{HEC}$ was calculated for a gas: extrarespiratory effect, assuming periodicity was attained. Because blood:gas (air) lambda values are unknown for the experimental animal species (a) and humans (h), a default value of 1.0 was used for this ratio. $NOAEL_{HEC} = NOAEL_{ADJ}$ × [blood:gas (air) lambda(a) ÷ lambda(h)].

[b]Not reported by the study author(s) but determined from data for this review.

[c]Three exposure levels for pregestational exposure (air [0 mg/m^3], low [115.18 mg/m^3], and high [498.36 mg/m^3]) and three exposure levels for gestational exposure (air [0 mg/m^3], low [217.16 mg/m^3], and high [824.56 mg/m^3]) were time-weighted averaged by duration to form the seven exposure groups shown in the table where average body weight is the body weight provided by the study authors.

Table 5. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Mutagenicity	*Salmonella typhimurium* TA97a, TA98, TA100, and TA102 in buffer or S9 metabolic fraction; 2-EE concentrations up to 20 µg per plate; incubation time unreported, histidine-independent mutant colonies counted	No significant increase in mutant colonies was found with and without S9.	Negative for mutagenic activity	Hoflack et al. (1995)
Mutagenicity	*Salmonella typhimurium* TA1535, TA1537, TA98, and TA100 in buffer or S9 metabolic fraction; 2-EE concentrations up to 104 µg per plate; incubation time unreported; histidine-independent mutant colonies counted	No significant increase in mutant colonies was found with and without S9.	Negative for mutagenic activity	Ong et al. (1980) as cited in U.S. EPA (1981)
Mutagenicity	Streptomycin-dependent *Escherichia coli* Sd-4-73; 2-EE concentrations of 0.01−0.024 mL per plate; incubation time unreported; revertant mutant colonies counted	No significant increase in mutant colonies was found.	Negative for mutagenic activity	Szybalski (1958) as cited in U.S. EPA (1981)
Mutagenicity	*Salmonella typhimurium* (TA98, TA100, TA1535, TA1537, and TA97) in buffer or S9 metabolic fraction; 2-EE concentrations up to 10,000 µg per plate; incubated 2 d; histidine-independent mutant colonies counted	No significant increase in mutant colonies was found with and without S9.	Negative for mutagenic activity	NTP (1993d)
Mutagenicity	Mouse lymphoma L5178Y cells in media alone or media with S9 metabolic fraction; 2-EE concentrations up to 5 µL/mL; incubated 4 h; trifluorothymidine-resistant cells counted	No significant increase in mutant colonies was found without S9; however, slight increases were found with S9.	Negative without S9; weakly positive with S9	NTP (1993e)
Mutagenicity	Adult male flies (*Drosophila melanogaster*); 2-EE by diet for 2 days or single injection; live numbers of offspring counted after mating for two generations	No effects were noted in the germ cells of exposed adult male flies in any exposure group.	Negative for induction of sex-linked recessive mutations	NTP (1993g)

Table 5. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Cytogenicity	Chinese hamster ovary cells in media alone or media with S9 metabolic fraction; 2-EE concentrations up to 9510 µL/mL; incubated 10.5 or 25.5 h; sister chromatid exchanges per cell and chromosomal aberrations counted	Sister chromatid exchanges were found at 3170 and 9510 µg/mL with and without S9. Chromosomal aberrations were found in cells treated without S9. No cell cycle delay was noted.	Positive for sister chromatid exchanges at high doses; positive for chromosomal aberrations without S9	NTP (1993f)
Cytogenicity	Chinese hamster ovary cells in media alone or media with S9 metabolic fraction; 2-EE concentrations up to 30 mg/mL; incubated 10.5 or 25.5 h; sister chromatid exchanges per cell and chromosomal aberrations counted	Cytotoxicity was found at 30 mg/mL (30% survival). Metabolic cooperation was impaired at 10 mg/mL. A 50% recovery in cellular communication was noted after dosing.	Blocked cellular communication before becoming cytotoxic	Loch-Caruso et al. (1984)
Genotoxicity and Cytogenicity	Chinese hamster lung V79 cells evaluated for chromosomal aberrations, micronuclei in polychromatic erythrocytes, alteration of mitotic division apparatus and aneuploidy, and inhibition of intracellular communication between cells; other in vitro tests: chromosomal aberrations in human lymphocytes; micronuclei in polychromatic erythrocytes in mouse bone marrow; and morphological transformation of Syrian hamster embryo cells.	Results were negative for chromosomal aberrations; positive for enhancing chromosomal aberrations in combination with a known clastogen, induction of morphological transformation of Syrian hamster embryo cells, aneuploidy and spindle malformations, equivocal for inducing sister chromatid exchanges, micronuclei induction in vitro and negative in vivo. Positive for inhibiting intercellular communication.	Weakly positive for genotoxicity and epigenetic effects	Elias et al. (1996)
In vitro reproductive	Postimplantation rat embryos (GD 10.5) in serum; 2-EE concentrations of 1.0−15.0 µL/mL; incubated 40 h; toxicity observed	Exposed embryos showed retarded dose-dependent growth and development beginning at 7.3 µL/mL.	Positive for embryotoxicity; NOAEL of 14.2 µL/mL	Brown-Woodman et al. (1994, 1995)

Table 5. Other Studies

Test	Materials and Methods	Results	Conclusions	References
In vitro reproductive	Postimplantation rat embryos (GD 9.5) in serum; 2-EE concentrations of 6.25−100 mM; incubated 2 d; toxicity observed	Exposed embryos showed significant reduction in protein explanted/embryo ratio (12.5 mM), a high frequency of unrotated embryos (50 mM) and completely inhibited embryo development (100 mM).	Positive for embryotoxicity; NOAEL of 6.25 mM	Giavani et al. (1993)
In vitro reproductive	Primary pachytene spermatocytes in buffer; concentrations of 10-mM 2-EE or 1 and 10-mM EAA; incubation unreported; O_2 consumption and ATP production monitored	2-EE exposed embryos showed no effects. EAA interfered with the energetic metabolism of pachytene spermatocytes.	2-EE: negative EAA: altered spermatocyte metabolism	Oudiz and Zenick (1986b)
In vivo short-term reproductive	10 male Wistar rat, gavage; 0-, 100-, 200-, and 400-mg/kg-d 2-EE for 14 consecutive days	Sperm and testicular effects ≥100 mg/kg-d; hematological effects ≥200 mg/kg-d.	Positive for gonadotoxicity and hematotoxicity	Adedara and Farombi (2010)
Metabolism/ toxicokinetic	6 male Sprague-Dawley rat, gavage; 230 mg/kg of ethanol or ethoxy ^{14}C-labeled 2-EE; urinary excretion of metabolites, and composition of testes measured	Elimination of ^{14}C was primarily through urinary excretion within 96 h of dosing (76−80%). Main pathway of metabolism was oxidation, with following conjugation with glycine. The major metabolite EAA and *N*-ethoxy-acetyl glycine composed 73−76% of the 2-EE dose. Half-time was 9.9 h and 12.5 h, depending on the label. EAA found in testes.	Major metabolites: EAA and *N*-ethoxy-acetyl glycine EAA found in testes	Cheever et al. (1984)

Table 5. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	5 male humans; inhalation; 200 min; male Wistar rat (unreported number) gavage; 2-EE concentrations of 10 mg/m^3, 20 mg/m^3, or 40 mg/m^3; urinary excretion of EAA and inhaled and exhaled 2-EE measured	The half-life of EAA elimination was 42.0 ± 4.7 h in humans compared to 7.20 ± 1.54 h in rats. Total recovery was dose-dependent in rats and amounted to between 13.4% and 36.8%, while in humans, the recovery was estimated between 30 and 35% regardless of concentration. Based on higher recovery in humans than rats at low doses, the metabolic conversion to EAA appears more important in humans than in the rat.	Blood concentrations could be three times higher in man than rat.	Groeseneken et al. (1988)
Metabolism/ toxicokinetic	Human blood; head space vial of physiological saline or olive oil; 2 µl of 2-EE; gas chromatographic analysis	Study authors reported the partition coefficients of 2-EE to be: water/air—23069; blood/air—22093; water/blood—1.044; oil/air—962; oil/water—0.042; and oil/blood—0.044.	Evidence of high levels of respiratory uptake, uniform tissue distribution	Johanson and Dynésius (1988)
Metabolism/ toxicokinetic	physiologically based pharmacokinetic model for pregnant rats effects to a pregnant woman; vapor exposures of 50 and 100 ppm (6 h per d); 5 d (GDs 11−15) and controlled exposure in a pregnant woman published by Groeseneken et al. (1988); exposure of 2-EE (8 h per d); 5 d per week; 270 d; calculated for a 58-kg pregnant woman to match internal venous blood concentration	The human inhaled concentration equivalent to rat NOAEL for EGEEA/2-EE (50 ppm) was predicted to be 25 ppm by using the maternal blood average daily area under the curve (AUC) and 40 ppm by using the maximum concentration achieved in maternal blood (C_{max}). Similarly, the rat LOEL for EGEEA (100 ppm) was determined to be 55 ppm using the maternal blood average daily AUC and 80 ppm using the maternal blood C_{max}.	AUC metric: NOAEL of 8.3 ppm (designated for 8 h only) and 18.3 ppm (adjusted for continuous exposure)	Gargas et al. (2000)

Table 5. Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ toxicokinetic	8- to 10-wk-old adult male Sprague-Dawley rat; unreported administration method; 113-mM 2-EE or up to 9.85 mM EAA; metabolites of mitochondria from livers and testes identified	2-EE did not affect mitochondrial respiration. EAA caused decreased state-3 succinate oxidation and a respiratory control ratio above 3.85 mM.	2-EE: negative EAA: positive for mitochondrial toxicity	Beattie and Brabec (1986)
Metabolism/ toxicokinetic	Primary mixed Sertoli and germ testis cells in media; concentrations 50 mM 2-EE, 2 mM MAA or 10 mM EAA; incubated 72 h; toxicity observed and metabolites identified	2-EE produced no sign of cellular toxicity. MAA and EAA caused degeneration of the pachytene and dividing spermatocytes.	2-EE: negative MAA and EAA: positive for testicular toxicity	Gray et al. (1985)
Mode of action/ mechanistic	36 male/0 female rats (strain unreported); gavage; 2-EE at 250, 500 or 1000 mg/kg-d or 2-ME at 50, 100, 250 or 500 mg/kg-d; 11 d; testicular histology examined	2-EE and 2-ME primarily damaged spermatocytes undergoing maturation and division. 2-ME was estimated to be 5-fold more potent than 2-EE.	2-EE and 2-ME: positive for spermatocyte toxicity	Foster et al. (1984)
Immunotoxicity	0 male/6−8 female Hybrid B6C3F_1 (C57Bl/6 female × C3H) and CD2F1 (BALB/c female × DBA/2 inbred) mice; gavage; 2-EE at 600, 1200, or 2400-mg/kg; Days −12, −8, −5, −1; mouse lymphoid leukemia L1210 tumors (3×10^6, 1×10^5, 3×10^3, or 1×10^2) implanted Day 0	Allogenic mice treated with 2-EE and tumor cells showed increased survivorship as compared to control.	Positive for antileukemic activity	Houchens et al. (1984)
Immunotoxicity	20 male/0 female Fisher rats; drinking water; 2-EE at 2500 or 5000-ppm (231−255 or 438−459 mg/kg-d); 9 wk; F344 rat leukemia cells (2.5×10^7) implanted Day 0	The 5000-ppm dose reduced effects of leukemia by approximately 50%, with 100% survival.	Positive for antileukemic activity	NTP (1993h)

OTHER DATA (SHORT-TERM TESTS, MECHANISTIC STUDIES, OTHER EXAMINATIONS)

Data evaluating the genotoxicity, cytogenicity, and embryotoxicity are included in Table 5 (Hoflack et al., 1995; Ong et al., 1980, as cited in U.S. EPA, 1981; Szybalski, 1958, as cited in U.S. EPA, 1981; NTP, 1993d,e,f,g; Loch-Caruso et al., 1984; Elias et al., 1996; Brown-Woodman et al., 1994, 1995; Giavani et al., 1993; Oudiz and Zenick, 1986b). Table 5 also summarizes studies investigating the kinetics and metabolism of 2-EE in human, animal, and in vitro systems (Cheever et al., 1984; Gargas et al., 2000; Groeseneken et al., 1988; Johanson and Dynésius, 1988; Beattie and Brabec, 1986; Gray et al., 1985) and the immunotoxic effects of 2-EE (Houchens et al., 1984; NTP, 1993h). A general discussion of the data is presented in this section; the key studies pertaining to the physiologically based pharmacokinetic (PBPK) model (Gargas et al., 2000) are presented in greater detail in the text.

Tests Evaluating Mutagenicity, Cytogenicity, and Embryotoxicity

Mutagenicity, cytogenicity, and embryotoxicity tests have been conducted for 2-EE in several strains of *Salmonella typhimurium* (TA97a, TA98, TA100, TA102, TA1535, and TA1537), *Escherichia coli*, mouse lymphoma L5178Y cells, *Drosophila melanogaster*, and in vitro reproductive studies (Hoflack et al., 1995; Ong et al., 1980, as cited in U.S. EPA, 1981; Szybalski, 1958, as cited in U.S. EPA, 1981; NTP, 1993d,e,f,g; Loch-Caruso et al., 1984; Elias et al., 1996; Brown-Woodman et al., 1994, 1995; Giavani et al., 1993; Oudiz and Zenick, 1986b). The results from tests evaluating genotoxicity and/or mutagenicity have been largely negative in prokaryotic systems exposed to 2-EE (Hoflack et al., 1995; Ong et al., 1980, as cited in U.S. EPA, 1981; Szybalski, 1958, as cited in U.S. EPA, 1981; NTP, 1993d). However, there have been some positive genotoxic and cytotoxic results in some eukaryotic mammalian cell cultures (NTP, 1993e,f; Loch-Caruso et al., 1984; Elias et al., 1996). In vitro reproductive studies showed embryotoxicity and developmental impairment following 2-EE exposure (Brown-Woodman et al., 1994; Giavani et al., 1993); effects on spermatocytes were not observed following 2-EE exposure (Oudiz and Zenick, 1986b).

Genotoxicity and cytotoxicity tests have been conducted for 2-EE in Chinese hamster ovary (CHO) cells and *Salmonella typhimurium* with and without S9 activation, *Escherichia coli*, Chinese hamster lung V79 cells, human lymphocytes, human and mouse erythrocytes, mouse bone marrow, Syrian hamster embryo cells, rat embryos, and primary pachytene rat spermatocytes (Hoflack et al., 1995; Ong et al., 1980, as cited in U.S. EPA, 1981; Szybalski, 1958, as cited in U.S. EPA, 1981; NTP, 1993f; Loch-Caruso et al., 1984; Elias et al., 1996; Brown-Woodman et al., 1994, 1995; Giavani et al., 1993; Oudiz and Zenick, 1986b). Sister chromatid exchanges occurred in CHO cells at 2-EE concentrations of 3170 and 9510 µg/mL both with and without S9 activation (NTP, 1993f). Chromosomal aberrations also occurred in CHO cells, but this effect was only significant without S9 activation (NTP, 1993f). Although positive results were seen in Chinese hamster ovary cells (NTP, 1993f; Loch-Caruso et al., 1984), only weakly positive results were obtained from studies of Chinese hamster lung V79 cells (Elias et al., 1996).

Cytotoxic effects ranging from inhibition of cellular communication (measured in vitro by an assay that depends on the transfer of metabolites via gap junctions, i.e., metabolic cooperation) at concentrations of 10 mg/mL to approximately 70% cytotoxicity at 30 mg/mL were also observed in CHO cells (Loch-Caruso et al., 1984). Cells were able to recover from effects on cellular communication in vitro at 2-EE concentrations of up to 20 mg/mL

(Loch-Caruso et al., 1984). Specifically, Loch-Caruso et al. (1984) outlined the potentially harmful effects of decreased gap junction communication. Briefly, gap junction communication, although not yet clearly defined, is thought to have an important function in morphogenesis. The study authors hypothesized that these effects may be related to significantly lengthened gestation observed in rats and mice exposed to 2-EE. Increased length of parturition or labor and other effects to organs such as the heart and the intestines and the male reproductive system that are dependent on gap junction communication may also arise through this putative mechanism of action.

Cultured rat embryos have also been examined for embryotoxicity in vitro. Concentrations, as low as 12.5 mM, caused effects on embryo protein content (Giavani et al., 1993). Effects on growth and development occurred in a dose-dependent manner (Brown-Woodman et al., 1994, 1995; Giavani et al., 1993). There were no cytotoxic effects of 2-EE exposure on primary pachytene rat spermatocytes in vitro, but there were significant effects from exposure to EAA, a metabolite of 2-EE, which the study authors noted could contribute to some of the toxic effects on spermatocytes observed in vivo (Oudiz and Zenick, 1986a,b).

Other Toxicity Studies

The metabolism of administered 2-EE (Cheever et al., 1984), a PBPK model (Gargas et al., 2000), and the effects of oral 2-EE exposure in leukemia inhibition studies in mice and rats have been evaluated (Houchens et al., 1984; NTP, 1993h).

In a peer-reviewed short-term-duration reproductive study, Adedara and Farombi (2010) administered doses of 0- (saline only), 100-, 200-, and 400-mg/kg-day 2-EE (purity unreported) by gavage to four groups of 10 adult male Wistar rats for 14 consecutive days. Rats were obtained from the University of Ibadan, Ibadan, Nigeria. Throughout the study, rats were housed in plastic suspended cages and given pellet food (brand unspecified) and water ad libitum. The GLP compliance of this study was not reported.

Twenty-four hours after the last treatment, rats were sacrificed, body weights were recorded, and blood was collected for hematological analysis. Testes, epididymides, seminal vesicles, and prostate glands were removed and weighed. Testes and epididymis samples were fixed, sectioned, and stained with hematoxylin and eosin for microscopy. For biochemical assays, glutathione (GSH), vitamin C, malondialdehyde (MDA), and lactate dehydrogenase (LDH) levels as well as superoxide dismutase (SOD), catalase (CAT), and glutathione-S-transferase activities were measured in the testes and epididymal spermatozoa. Epididymal spermatozoa number and motility were assessed, and daily spermatozoa production and testicular spermatozoa number were quantified. Spermatozoa were also assayed for morphological abnormalities and percentage viability. Statistical analyses were performed using one-way ANOVA followed by Student's *t*-test.

Adedara and Farombi (2010) reported no significant changes in the weights of the testes, epididymides, seminal vesicles, and prostate glands of rats exposed to 2-EE. Body-weight gain was significantly ($p < 0.05$) decreased in the 200- and 400-mg/kg-day groups compared to controls. At doses ≥100 mg/kg-day, significant ($p < 0.05$) decreases were observed in epididymal and testicular spermatozoa number, daily spermatozoa production, and spermatozoa motility, and total spermatozoa abnormalities were significantly ($p < 0.05$) increased. No effect was observed on the spermatozoa live-dead ratio at all doses tested. There was a significant

($p < 0.05$) decrease in testicular GSH levels and SOD and CAT activities, as well as a significant ($p < 0.05$) increase in MDA levels and GST and LDH activities in the 200- and 400-mg/kg-day groups compared to controls. Vitamin C content in the testes was unchanged. In the spermatozoa, there was a significant ($p < 0.05$) decrease in SOD and LDH activities as well as GSH and vitamin C levels at doses ≥100 mg/kg-day, and CAT and GST activities in the 200- and 400-mg/kg-day groups. MDA was significantly ($p < 0.05$) increased in spermatozoa at doses ≥100 mg/kg-day. Histopathological analysis of the testes revealed treatment-related lesions (e.g., congestion and hemorrhage at the interstitium of the seminiferous tubules, erosion of the germinal epithelium, and necrosis of germinal cells with reduced number of sertoli cells) at all doses tested, but the epididymides were only mildly affected at 400 mg/kg-day. Hematological analysis showed that white blood cells, platelets, neutrophils, and mean corpuscular hemoglobin concentration were significantly ($p < 0.05$) lower, whereas lymphocytes were increased in the 200- and 400-mg/kg-day groups compared to controls.

Adedara and Farombi (2010) reported a LOAEL of 100 mg/kg-day based on sperm and testicular effects. A NOAEL cannot be identified.

Metabolism/Toxicokinetic Studies

Studies have investigated the kinetics and metabolism of 2-EE in human, animal, and in vitro systems (Cheever et al., 1984; Gargas et al., 2000; Groeseneken et al., 1988; Johanson and Dynésius, 1988; Beattie and Brabec, 1986; Gray et al., 1985).

The metabolism of 2-EE is understood to involve initial oxidation by alcohol dehydrogenase followed by aldehyde dehydrogenase-forming acid metabolites, principally ethoxy acetic acid or EAA, which are measurable in the urine (Groeseneken et al., 1988; Cheever et al., 1984). Studies by Beattie and Brabec (1986) and Gray et al. (1985) have implicated major metabolites of 2-EE in mitochondrial and cellular toxicity, respectively; the particular toxic mechanism of action remains unclear. Johanson and Dynésius (1988), to better understand the absorption and distribution potential of 2-EE, experimentally calculated several partition coefficients, which led the study authors to the conclusion that 2-EE could be highly absorbed through inhalation and that it would distribute in a relatively uniform manner in the tissues once absorbed. Importantly, Groeseneken et al. (1988) compared the metabolism and kinetics of exposed humans with those of rats. This study provided important quantitative data, utilized by Gargas et al. (2000) to develop a PBPK model of inhaled 2-EE for purposes of cross-species extrapolation of fetal effects from pregnant rats to the pregnant woman. Further description of the Gargas et al. (2000) study is presented below.

Gargas et al. (2000) developed a PBPK model for inhaled 2-EE for purposes of cross-species extrapolation of fetal effects from pregnant rats to the pregnant woman. The basis underlying this extrapolation is to estimate external human exposures equivalent with the rat based on internal measures of dose. PBPK models utilize the anatomical and physiological structure and functions of the organism (animal or human) by representing blood flows, pulmonary ventilation rate, and organ volumes as compartments. The model also utilizes metabolic values to estimate the kinetics of a toxic species, including concentrations of a given chemical species at specific organs or sites. General and chemical-specific toxicokinetic values used in the construction of the models were generated from measurements made by other researchers as documented in Gargas et al. (2000). These parameters included estimates of in vivo metabolic transformation rates of 2-EE to EAA and EAA to ethylene glycol for both

humans and rats from comparative in vitro studies with species-specific hepatocytes (Green et al., 1996). Fetal and placental tissues were grouped with the richly perfused compartment, as well as rat fetal and maternal blood concentrations for 2-EE and 2-EAA. They were assumed to be valid correlates of one another, based on observations with related glycol ethers (2-ME and its principal metabolite, 2-MAA; Welsch et al., 1995), showing that rat maternal blood and rat fetal tissue concentrations are nearly identical or proportional.

The rat model constructed by Gargas et al. (2000) was validated by comparing model outputs with measurements made by Gargas et al. (2000) in whole-body vapor inhalation exposures of pregnant Sprague-Dawley rats at 50 and 100 ppm, 6 hours per day, to the acetate of 2-EE[1] for 5 consecutive days (GDs 11−15). These comparisons included EAA concentrations in maternal venous blood and fetal tissue during and following GD 15 for pregnant rats exposed to 100- or 5-ppm 2-EE/EGEEA on GDs 11−15. The human model thus constructed was validated with the human experiments of Groeseneken et al. (1988) by predicting values for (1) the rate of urinary excretion of EAA from human volunteers exposed to 20-mg/m^3 2-EE, (2) as in (1) with 28-mg/m^3 2-EE, (3) exhaled breath concentrations of 2-EE from these human volunteers exposed to 20-mg/m^3 2-EE, and (4) as in (3) with 28-mg/m^3 2-EE. In all of these experiments with both rats and humans volunteers, the modeled outputs were shown to be very near and comparable to the observed values.

On the precept that a common internal dose would produce similar effects in similar tissues, the validated rat model was used to estimate the internal venous blood concentration in the pregnant rats at a steady state under the exposure conditions of 50- and 100-ppm 2-EE for 6 hours per day. The validated human model was then used to find the external exposure concentrations of 2-EE of 8 hours in duration, 5 days per week, for 270 days to a 58-kg pregnant woman that would match this internal venous blood concentration. These concentrations, which may be termed human equivalent concentrations, were obtained for two different measures of tissue dose, i.e., maternal venous blood: the area under the curve (AUC) and the maximum concentration achieved in maternal blood (C_{max}). The human inhaled concentration equivalent to the rat exposure of 50-ppm 2-EE from the study of Doe (1984) was estimated to be somewhat lower at 25 ppm using the maternal blood average daily AUC and 40 ppm using the C_{max}. The human inhaled concentration equivalent to the rat exposure of 100-ppm 2-EE was likewise estimated at 55 ppm using the maternal blood average daily AUC and 80 ppm using the maternal blood C_{max}.

It is these concentrations, preferably the lower AUC values, which could be utilized in deriving the HEC values for the rat study of Doe (1984a). There are, however, a number of limitations present, both with the data used and with the model itself. The PBPK model simulation was applied to the human data of Groesneken et al. (1988). These data are considered marginal and could be better judged if more human data (which are available from Groesneken et al., 1987a,b) were used in the development of the model. Also, these human data were obtained from male workers with the weight adjustment to the pregnant female made only on body-weight allometry. As mentioned above, a number of sensitive physiochemical/physiological parameters in the human model were calculated based on related

[1]The acetate is rapidly and nearly completely hydrolyzed to the subject ether, 2-EE, and thence to the putative active metabolite 2-EAA in both rats (Gargas et al., 2000; Stott and McKenna, 1985) and in humans (Groeseneken et al., 1987a,b).

compounds, e.g., the acetate of 2-EE and 2-ME, or based on in vitro measurements. Also, the data sets used in developing the model are rather small (the human sample size was five). Lastly, the model simulation was for a work shift (8 hours) not for a continuous exposure as is required for derivation of p-RfC values, thus necessitating further manipulation of the modeled estimates. In consideration of the multiple aforementioned limitations, these estimated values cannot be used in developing the HEC values from the rat study of Doe (1984a).

Foster et al. (1984) undertook an investigation into the mechanism of toxicity of glycol ethers, including both 2-ME and 2-EE, to the testicular tissues of rats. Groups of male rats (n = 36) were treated orally for 11 days with either 2-ME at 50, 100, 250, or 500 mg/kg-day or with 2-EE at 250, 500, or 1000 mg/kg-day. Groups of controls were given an equivalent volume of water. At sequential times in the treatment regime (6 and 24 hours after a single dose and 1, 2, 4, 7, and 11 days after repeated daily doses), groups of animals were sacrificed, and testicular histology was examined. The major site of damage following treatment with either of these glycol ethers was shown to be the primary spermatocytes undergoing maturation and division. Further, 2-ME was estimated to be about 5-fold more potent than 2-EE. Equimolar doses of either MAA (4 days at 500 mg/kg) or EAA (11 days at 500 mg/kg)—the primary metabolites of these glycol ethers—also induced injury similar to the corresponding parent. Administration of MAA but not 2-ME to in vitro germ cell cultures resulted in adverse effects analogous to those seen in vivo. These investigators also showed that inhibitors of aldehyde dehydrogenase administered before a toxic dose of 2-ME (500 mg/kg) afforded complete protection against testicular toxicity. These results indicate that it is likely the acetic acid metabolite (or possibly the corresponding aldehyde) and not the parent glycol ether that is responsible for the testicular damage observed. Aldehydes are also known to have profound effects on cells undergoing division. However, the precise biochemical lesion affecting the spermatocytes undergoing division was not elucidated.

Immunotoxicity Studies

Houchens et al. (1984) published the results of a cell-mediated immunity assay in a peer-reviewed study in which syngeneic mice and mice allogenic for the leukemia cell tumor were pretreated with 2-EE and then injected with leukemia cells. All syngeneic mice died from the effects of the leukemia treatment 8−9 days following the injection. Similarly, allogenic mice pretreated with water instead of 2-EE died an average of 8 days following leukemia cell treatment. However, the allogenic mice pretreated with 2-EE survived for more than 43 days, on average, with survival between 80−100% per treatment group. The study authors concluded that the increased survivorship of the 2-EE pretreated mice may indicate a preventative effect of 2-EE or a stimulation of the immune system.

A published peer-reviewed study by NTP (1993h) investigated the activity of 2-EE in a cellular leukemia transplant model in rats to examine the effect of treatment on cancer progression. 2-EE given orally (2.5 mg/mL in drinking water) to transplant recipients showed a roughly 50% reduction in clinical, morphological, and histopathological signs of leukemia. While the mechanism of action was not explored in this study, the effects of 2-EE occurred at levels below which toxicity was observed.

DERIVATION OF PROVISIONAL VALUES

Table 6 presents a summary of noncancer reference values. Table 7 presents a summary of cancer values.

Table 6. Summary of Noncancer Reference Values for 2-EE (CASRN 110-80-5)

Toxicity Type (units)	Species/Sex	Critical Effect	Reference Value	POD Method	POD	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/M	Prostate atrophy	1×10^{-1}	$BMDL_{10\%}$	33.7	300	NTP (1993a)
Chronic p-RfD (mg/kg-d)	Rat/M	Pathological effects in the testes	9×10^{-2}	NOAEL	86	1000	Yoon et al. (2003)
Subchronic p-RfC (mg/m^3)	Rabbit pups (both sexes)	Increased percentage of rabbit offspring showing major skeletal defects	4×10^{-2}	$BMCL_{5\%HEC}$	4.23	100	Doe (1984b)
RfC (IRIS)[a] (mg/m^3)	Rabbit/M	Decreased testis weight, degeneration of the seminiferous tubules, and decreased hemoglobin	2×10^{-1}	NOAEL	68	300	Barbee et al. (1984b)

[a]All the reference values obtained from IRIS are indicated with the latest revision date (05/01/1991).

Table 7. Summary of Cancer Reference Values for 2-EE (CASRN 110-80-5)

Toxicity Type	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF	None			
p-IUR	None			

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

The study by NTP (1993a) is selected as the principal study for deriving the subchronic p-RfD. This 90-day study is peer reviewed and performed according to GLP principles, and the results were reviewed by the NTP Pathology Working Group (PWG). The study meets the standard of study design and performance, with numbers of animals (10 per sex per dose), examination of potential toxicity endpoints, and presentation of information. Details are provided in the "Review of Potentially Relevant Data" section. The observed atrophy of the prostate in the NTP (1993a) study represents the most appropriate and most sensitive effect for developing a subchronic p-RfD among the acceptable studies. The study authors do not discuss the methods for evaluating the severity of prostate atrophy, nor did they use this endpoint in the derivation of the NOAEL. However, this endpoint is considered toxicologically relevant and is the most sensitive endpoint of the available acceptable subchronic-duration studies for 2-EE.

Oral administration of 2-EE is shown within the literature database to elicit varied effects. There was a significant decrease in thymus weight observed in a subchronic-duration study in male rats by NTP (1993a), and hematopoietic effects were also observed in male rats (Yu et al., 1999). However, reproductive effects, particularly in the testes, were noted in a number of studies. A significant decrease in testis weight coupled with testicular degeneration was observed in a stop-exposure study in male rats by NTP (1993b). Testicular pathology was observed in a reproductive study in adult male rats by Yoon et al. (2001), and testicular effects were observed in a reproductive study in male rats by Yu et al. (1999). Prostate atrophy is similar in that it is also a toxicologically relevant reproductive effect, and compared to testicular effects, it is a more sensitive endpoint. The dose at which prostate atrophy was identified is within 2-fold of the doses at which testicular effects occurred. This adds further confidence in the selection of prostate atrophy as the critical effect.

A duration adjustment is not required because the study authors adjusted for continuous exposure. A POD of 67 mg/kg-day is identified using BMD analysis. This POD is protective against other effects from subchronic-duration exposure, including those seen in the testes and epididymides of rats and mice at slightly higher dose levels (Yoon et al., 2001; Yu et al., 1999; Horimoto et al., 1996, 2000; Zenick et al., 1984; NTP, 1993a,b; Nagano, 1984). It is also noted that prostate effects were not reported in the available chronic-duration rat study performed by Melnick (1984a).

All EPA Benchmark Dose Software (BMDS version 2.1.2) dichotomous models were fit to the data for incidence of prostate atrophy. The high-dose data (2240 mg/kg-day) were excluded due to the observed 50% (5/10) chemical-related mortality in that dose group. A benchmark response (BMR) of 10% extra risk was used to estimate the BMD, as recommended by EPA (2000). Table 8 presents BMD input data for the incidence of prostate atrophy in male rats after 13 weeks of oral exposure via drinking water. Appendix C presents the BMD model output.

Table 8. Prostate Atrophy in the Male F344/N Rat Following a 13-Week Oral Exposure to 2-EE Used for BMD Analysis[a]

Dose[b] (mg/kg-d)	Number of Rats	Incidence
0	10	0
109	10	0
205	10	6
400	10	7
792	10	10

[a]NTP (1993a).
[b]The highest dose group data (2240 mg/kg-day) were excluded due to the observed frank effect of 50% mortality.

Table 9 summarizes the BMD modeling results for the male prostate atrophy. The Log-Probit model fit the data best compared to other models. The Logistic and Probit models are eliminated because they failed the *p*-value criteria (i.e., *p*-score less than 0.1). The Log-Probit model, as well as the Multistage, Gamma, Log-Logistic, and Weibull models, have a goodness-of-fit *p*-value greater than 0.1. Among the models that pass the *p*-value criteria, the Multistage model provides the lowest AIC value of 33.368. Therefore, the $BMDL_{10\%}$ of 33.7 mg/kg-day from the Multistage model is used as a POD for the derivation of the subchronic p-RfD (see Table 9).

Table 9. Model Predictions for Prostate Atrophy in the Male F344/N Rat Following a 13-Week Oral Exposure to 2-EE[a]

Model	Goodness of Fit *p*-value	AIC[b] for Fitted Model	$BMD_{10\%}$ (mg/kg-d)	$BMDL_{10\%}$ (mg/kg-d)	Conclusions
Gamma	0.23	34.868	108.3	45.1	
Logistic	0.09	37.578	113.1	72.7	*p*-score 4 < 0.1
Log-Logistic	0.24	34.783	113.3	61.2	
Log-Probit	0.27	34.303	118.3	66.9	
Multistage	**0.31**	**33.368**	**102.2**	**33.7**	**Lowest AIC**
Probit	0.097	37.037	110.7	70.8	*p*-score 4 < 0.1
Weibull	0.22	35.301	93.6	38.4	

[a]NTP (1993a).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the BMD

The subchronic p-RfD is based on the $BMDL_{10\%}$ of 33.7 mg/kg-day for prostate atrophy calculated from male rats exposed to 2-EE for 13 weeks (NTP, 1993a) and is derived as follows:

$$\textbf{Subchronic p-RfD} = \textbf{BMDL}_{\textbf{10\%}} \div \textbf{UF}_{\textbf{C}}$$
$$= 33.7 \text{ mg/kg-day} \div 300$$
$$= \mathbf{1 \times 10^{-1} \text{ mg/kg-day}}$$

The composite uncertainty factor (UF_C) for the subchronic p-RfD for 2-EE is 300 as explained in Table 10.

Table 10. Uncertainty Factors for Subchronic p-RfD of 2-EE

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to the reproductive effects of 2-EE.
UF_D	3	A UF_D of 3 is selected because the database includes four acceptable developmental studies in mice (Wier et al., 1987a,b; Hardin et al., 1987; Lamb et al., 1984), but there are no acceptable two-generation reproductive studies. Although thymus effects are observed in rats in a subchronic-duration study, these effects are not observed following chronic-duration exposure, and there is no other indication of immunotoxicity. Thus, there is likely no potential for immunotoxicity, and the absence of a comprehensive immunotoxicity study is not a concern.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a NOAEL.
UF_S	1	A UF_S of 1 is applied because a subchronic-duration study was utilized.
UF_C ≤3000	300	

The confidence of the subchronic p-RfD for 2-EE is medium as explained in Table 11.

Table 11. Confidence Descriptors for Subchronic p-RfD for 2-EE		
Confidence Categories	**Designation[a]**	**Discussion**
Confidence in study	H	Confidence in the key study is high. NTP (1993a) examined appropriate reproductive toxicity endpoints and used 10 males per dose group. The study was peer reviewed and is GLP compliant. The study examined multiple effects, and a thorough description of general methods and data was provided. However, the methodology for characterization of the critical effect used—prostate atrophy—is not entirely clear.
Confidence in database	M	The database includes subchronic-duration toxicity studies in two species (rat and mouse), chronic-duration toxicity studies in two species (rat and mouse), developmental toxicity studies in one species (mouse), and no two-generation reproductive studies.
Confidence in subchronic p-RfD[b]	M	The overall confidence in the subchronic p-RfD is medium.

[a]L = low, M = medium, H = high.
[b]The overall confidence cannot be greater than the lowest entry in the table.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

The study by Yoon et al. (2003) is selected as the principal study for deriving the chronic p-RfD. This 28-day study is a published peer-reviewed study and meets the standard of study design and performance, with numbers of animals (five per dose), examination of potential toxicity endpoints, and presentation of information. Details are provided in the "Review of Potentially Relevant Data" section. The critical effect is the pathology findings in the testes, which consisted of exfoliation of the germ cells in the testicular lumen in male Sprague-Dawley rats administered 2-EE by gavage for 4 weeks. While the subchronic duration of this study is only 28 days, the study is supported by other observations that the testes appear to be a target of 2-EE. This study also notes that testicular toxicity due to this compound was dose dependent with indices of testicular pathology (i.e., decreased testis weight, exfoliation of germ cells in the tubular lumen, etc.). These study results are supported by an earlier study by Yoon et al. (2001) that also reported similar testicular pathology in treated adult male rats. Available data from other oral studies support the testes (and related prostate tissues) as a target organ (with particular effects noted in sperm) for toxicity in rats and mice (Yu et al., 1999; Horimoto et al., 1996, 2000; Zenick et al., 1984; NTP, 1993a,b; Nagano, 1984) as well as humans (Wang et al., 2003; Ratcliffe et al., 1986; Veulemans et al., 1993, Welch et al., 1988).

Although prostate atrophy was more sensitive compared to testicular effects following subchronic-duration exposure to 2-EE (NTP, 1993a), prostate effects were examined but not observed following chronic-duration exposure at higher doses and in multiple animal species, thus precluding its choice as a critical effect for deriving a chronic p-RfD. Therefore, the next most sensitive endpoint that was also observed following chronic-duration exposure was pathological effects in the testes observed in the 28-day Yoon et al. (2003) study. Although the principal study for the chronic p-RfD was shorter in duration than that for the subchronic p-RfD (13 weeks vs. 28 days), the duration of exposure is not a concern with respect to the testicular effects observed in the study by Yoon et al. (2003) because testicular toxicity is the most appropriate sensitive endpoint that occurs in the potentially most sensitive rat strain (i.e., Sprague-Dawley). This is evidenced by the higher LOAEL (400 mg/kg-day) for testicular

effects observed after a 13-week exposure in F344/N rats (NTP, 1993a) versus a LOAEL of 171 mg/kg-day following a 28-day exposure in Sprague-Dawley rats (Yoon et al., 2003). Additionally, induction of testicular damage by chronic-duration exposure to 2-EE is also evident in studies where chronic-duration exposure (at comparatively higher doses than the subchronic-duration studies) induced testicular atrophy in both F344/N rats and $B6C3F_1$ mice (Melnick, 1984a,b). Therefore, the critical effect is pathology of the testes observed in rats by Yoon et al. (2003).

The testicular pathology data from Yoon et al. (2003) are not amenable to BMD modeling. Thus, the $NOAEL_{ADJ}$ of 86 mg/kg-day based on pathological effects in the testes of male Sprague-Dawley rats exposed to 2-EE for 4 weeks (Yoon et al., 2003) is chosen as the POD to derive the chronic p-RfD. The chronic p-RfD for 2-EE, based on the $NOAEL_{ADJ}$, is derived as follows:

$$\textbf{Chronic p-RfD} = \textbf{NOAEL}_{\textbf{ADJ}} \div \textbf{UF}_{\textbf{C}}$$
$$= 86 \text{ mg/kg-day} \div 1000$$
$$= \mathbf{9 \times 10^{-2} \text{ mg/kg-day}}$$

The UF_C for the chronic p-RfD for 2-EE is 1000, as explained in Table 12.

Table 12. Uncertainty Factors for Chronic p-RfD of 2-EE

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to the reproductive effects of 2-EE.
UF_D	3	A UF_D of 3 is applied because the database includes four acceptable developmental studies in mice (Wier et al., 1987a,b; Hardin et al., 1987; Lamb et al., 1984), but there are no acceptable two-generation reproduction studies.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a NOAEL.
UF_S	3	A partial UF_S of 3 is applied for using data from a subchronic-duration study to assess potential effects from chronic-duration exposure. Although there are data on testicular effects from chronic-duration studies (Melnick, 1984a,b) in F344/N rats at comparatively higher doses, these studies were not performed in the potentially most sensitive rat strain (i.e., Sprague-Dawley), therefore a partial UF_S is warranted.
$UF_C \leq 3000$	1000	

The confidence of the chronic p-RfD for 2-EE is medium as explained in Table 13.

Table 13. Confidence Descriptors for Chronic p-RfD for 2-EE

Confidence Categories	Designation[a]	Discussion
Confidence in study	M	Confidence in the key study is medium. Yoon et al. (2003) examined appropriate reproductive toxicity endpoints, although only five male rats per dose group were used. The study was peer reviewed. GLP compliance is unknown. The study examined multiple effects, and a thorough description of methods and data was provided. The data used as the critical effect are well supported within the database. The key endpoint of pathology in the testes is seen in multiple independent studies and in two species (rat and mouse).
Confidence in database	M	The database includes subchronic-duration toxicity studies in two species (rat and mouse), chronic-duration toxicity studies in two species (rat and mouse), developmental toxicity studies in one species (mouse), and no two-generation reproductive studies.
Confidence in subchronic p-RfD[b]	M	The overall confidence in chronic p-RfD is medium.

[a]L = low, M = medium, H = high.
[b]The overall confidence cannot be greater than lowest entry in the table.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

Derivation of Subchronic Provisional RfC (Subchronic p-RfC)

IRIS (U.S. EPA, 2011) has provided an RfC based on the critical effect of decreased testis weight, seminiferous tubule degeneration, and decreased hemoglobin following subchronic-duration exposure of New Zealand White rabbits to 2-EE reported by Barbee et al. (1984b). However, when compared on the basis of duration-adjusted concentrations, major and minor fetal skeletal defects in the offspring of Dutch rabbits described by Doe (1984b) in a developmental inhalation study are a more sensitive endpoint than testicular effects in adult New Zealand White rabbits reported by Barbee et al. (1984b) following subchronic-duration exposure (LOAELs of 161 vs. 265 mg/m^3, respectively, with associated NOAELs of 46 and 68 mg/m^3, respectively). The occurrence of fetal skeletal defects is also supported in a developmental inhalation study in Wistar rats where a dose-dependent increase in minor skeletal defects was observed with a LOAEL of 230 mg/m^3 and an associated NOAEL of 47 mg/m^3 (Doe, 1984a). Maternal effects from the Doe (1984a) studies also support a LOAEL of 230 mg/m^3 with an associated NOAEL of 47 mg/m^3, based on reported hematology effects in the exposed does; however, the quantitative data were not presented in the study report.

The characteristics of 2-EE indicate that it is a Category 3 gas, and thus exhibits systemic toxicity (U.S. EPA, 2009). Because Category 3 gases cause extrarespiratory effects, the concentrations in the study were converted to adjusted doses (to account for continuous exposure) and then to HEC concentrations utilizing a default blood:gas (air) partition coefficient of 1 because the actual value is unknown.

The following dosimetric adjustments are made for unit conversion and inhalation exposure in adjusting for continuous exposure for extrarespiratory effects. The example calculation given below is for the inhalation exposure on GDs 6−18 in the Doe (1984a) study:

$$\begin{aligned} \mathbf{NOAEL_{ADJ}} &= \mathbf{NOAEL \times (molecular\ weight \div 24.45) \times (hours\ per\ day \div 24) \times (days\ exposed \div total\ days)} \\ &= 50\ ppm \times (90.12\ g/mol \div 24.45) \times (6\ hours/day \div 24) \times (13\ days\ exposed \div 13\ total\ days) \\ &= 50\ ppm \times 3.68 \times 0.25 \times 1 \\ &= \mathbf{46\ mg/m^3} \end{aligned}$$

$$\begin{aligned} \mathbf{NOAEL_{HEC}} &= \mathbf{NOAEL_{ADJ} \times blood{:}gas\ partition\ coefficient} \\ &= 46\ mg/m^3 \times 1 \\ &= \mathbf{46\ mg/m^3} \end{aligned}$$

where blood:gas partition coefficient = 1

BMD modeling could not be applied to the less sensitive testis weight or seminiferous tubule generation endpoints from Barbee et al. (1984b); an abnormally large standard deviation was reported for one of the testis weight values, and no quantitative data on seminiferous tubule generation were provided in the study.

Although the *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991) suggest that analysis of endpoints of developmental toxicity preferably be performed on a per-litter basis, the major fetal skeletal defect data from Doe (1984b) are presented on a per-pup basis. However, this does not necessarily preclude these data from BMD modeling. Using the number of pups showing any major skeletal defects and the percentage of pups showing major skeletal defects, the sample size of each exposure group was calculated for use in BMD modeling. The EPA Benchmark Dose Software (BMDS version 2.1.2) dichotomous variable models were fit to the major fetal skeletal defect data, and a BMR of a 5% extra risk was used based on current EPA practice for developmental toxicity endpoints. Table 14 presents BMD input data for the incidence of major fetal skeletal defects in offspring of Dutch rabbits exposed to 2-EE via inhalation on GDs 6–18. The BMD model output is presented in Appendix C.

Table 14. Major Skeletal Defects in Offspring of Dutch Rabbits Exposed to 2-EE via Inhalation on GDs 6–18 Used for BMD Analysis[a]

Dose (mg/m^3, HEC)	Number of Offspring	Response[b]
0	136	51.5
9	140	60.0
46	96	64.6
161	134	79.1

[a]Doe (1984b).
[b]Percentage of offspring showing any major defects.

Table 15 summarizes the BMD modeling results for major fetal skeletal defects. The Log-Logistic model fit the data best compared to other models. All models have a goodness of fit *p*-value greater than 0.1, and the Log-Logistic model provides the lowest AIC value of 643.971.

Table 15. Model Predictions for Major Skeletal Defects in Offspring of Dutch Rabbits Exposed to 2-EE via Inhalation on GDs 6–18[a]

Model	Goodness of Fit *p*-value	AIC for Fitted Model	$BMC_{5\%HEC}$ (mg/m^3)	$BMCL_{5\%HEC}$ (mg/m^3)	Conclusions
Gamma	0.54	644.252	10.43	7.42	
Logistic	0.48	644.471	12.75	9.66	
Log-Logistic	**0.62**	**643.971**	**6.99**	**4.23**	**Lowest AIC and lowest BMCL in a range of 4.23–20.27**
Log-Probit	0.30	645.407	28.24	20.27	
Multistage	0.53	646.942	10.91	7.69	
Probit	0.47	644.516	13.20	10.14	
Weibull	0.54	644.252	10.43	7.42	
Quantal-Linear	0.54	644.252	10.43	7.42	

[a]Doe (1984b).

AIC = Akaike's Information Criteria; BMC = benchmark concentration; BMCL = lower confidence limit (95%) on the BMC

The $BMC_{5\%HEC}$ and $BMCL_{5\%HEC}$ associated with the best-fitting model (Log-Logistic) were 6.99 and 4.23 mg/m^3, respectively (see Table 15). Therefore, the $BMCL_{5\%HEC}$ of 4.23 mg/m^3 calculated from the major fetal skeletal defect data observed in rabbits in Doe (1984b) was chosen as the POD, and a subchronic p-RfC is derived as follows:

$$\textbf{Subchronic p-RfC} = \textbf{BMCL}_{\textbf{5\%HEC}} \div \textbf{UF}_{\textbf{C}}$$
$$= 4.23\ mg/m^3 \div 100$$
$$= \mathbf{4 \times 10^{-2}\ mg/m^3}$$

The UF_C for the subchronic p-RfC for 2-EE is 100 as explained in Table 16.

Table 16. Uncertainty Factors for Subchronic p-RfC of 2-EE

UF	Value	Justification
UF_A	3	A UF_A of 3 is applied for animal-to-human extrapolation to account for the toxicodynamic portion of the UF_A because the toxicokinetic portion ($10^{0.5}$) has been addressed in the dosimetric conversions.
UF_D	3	A UF_D of 3 is applied, because the database includes six acceptable developmental studies in rats and rabbits (Doe, 1984a,b; Andrew and Hardin, 1984a,b; Nelson et al., 1981, 1982), but there are no acceptable two-generation reproduction studies.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a $BMCL_{5\%HEC}$.
UF_S	1	A UF_S of 1 is applied because a subchronic-duration study was utilized.
$UF_C \leq 3000$	100	

The confidence of the subchronic p-RfC 2-EE is medium as explained in Table 17.

Table 17. Confidence Descriptors for Subchronic p-RfC for 2-EE

Confidence Categories	Designation[a]	Discussion
Confidence in study	M	Confidence in the key study is medium. Doe (1984b) examined appropriate developmental toxicity endpoints. The study was peer reviewed, although GLP compliance is unknown. The key endpoint of fetal skeletal defects is seen in two species (rabbit and rat).
Confidence in database	M	The database includes subchronic-duration toxicity studies in two species (rat and rabbit), no chronic-duration toxicity studies, developmental toxicity studies in two species (rat and rabbit), and no two-generation reproductive studies.
Confidence in subchronic p-RfC[b]	M	The overall confidence in the p-RfC is medium.

[a]L = low, M = medium, H = high.
[b]The overall confidence cannot be greater than the lowest entry in the table.

Derivation of Chronic Provisional RfC (Chronic p-RfC)

IRIS (U.S. EPA, 2011) has provided an RfC. No additional studies that may be relevant have been discovered. A chronic p-RfC is not developed.

CANCER WEIGHT-OF-EVIDENCE (WOE) DESCRIPTOR

Table 18 identifies the cancer weight-of-evidence (WOE) descriptor for 2-EE. Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), 2-EE is classified as *"Inadequate Information to Assess Carcinogenic Potential."*

Table 18. Cancer WOE Descriptor for 2-EE			
Possible WOE Descriptor	**Designation**	**Route of Entry**	**Comments**
"Carcinogenic to Humans"	N/A	N/A	No human cancer studies are available.
"Likely to Be Carcinogenic to Humans"	N/A	N/A	No strong animal cancer data are available.
"Suggestive Evidence of Carcinogenic Potential"	N/A	N/A	There is not enough evidence from human and animal studies to be suggestive of carcinogenicity.
"Inadequate Information to Assess Carcinogenic Potential"	**Selected**	**Inhalation and oral**	**Adequate information is not available to assess carcinogenic potential.**
"Not Likely to Be Carcinogenic to Humans"	N/A	N/A	No strong evidence of noncarcinogenicity in humans is available.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

No human or animal studies examining the carcinogenicity of 2-EE following oral exposure have been identified. Therefore, derivation of a provisional oral slope factor is precluded.

Derivation of Provisional Inhalation Unit Risk (p-IUR)

No human or animal studies examining the carcinogenicity of 2-EE following inhalation exposure have been identified. Therefore, derivation of a provisional inhalation unit risk is precluded.

APPENDIX A. PROVISIONAL SCREENING VALUES

No screening values are presented.

APPENDIX B. DATA TABLES

Table B.1. Survival, Weight Loss, and Water Consumption in F344/N Rats Following Oral Administration of 2-EE via Drinking Water for 13 Weeks[a]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[b]					
	0 ppm	1250 ppm (109)	2500 ppm (205)	5000 ppm (400)	10,000 ppm (792)	20,000 ppm (2240)[e]
Male rats						
Sample size	10	10	10	10	10	10
Survival	10	10	10	10	10	5
Initial body weight (g)[c]	142	142 (100)	146 (103)	144 (101)	142 (100)	143 (101)
Final body weight (g)[d]	333	331 (99)	325 (98)	315 (95)	268 (80)	204 (61)
Body weight change (g)[c]	191	189 (99)	179 (94)	171 (90)	127 (66)	61 (32)
Water consumption (g/d)[c]	21.2	20.7 (98)	19.4 (92)	18.3 (86)	16.6 (78)	18.4 (87)
Parameter	**Exposure Group (Average Daily Dose, mg/kg-d)[b]**					
	0 ppm	**1250 ppm (122)**	**2500 ppm (247)**	**5000 ppm (466)**	**10,000 ppm (804)**	**20,000 ppm (2061)[e]**
Female rats						
Sample size	10	10	10	10	10	10
Survival	10	10	10	10	10	3
Initial body weight (g)[c]	123	123(100)	124 (101)	127 (103)	126 (102)	126 (102)
Final body weight (g)[d]	197	194 (98)	190(96)	186 (94)	171 (87)	185 (94)
Body weight change (g)[c]	74	71 (96)	66 (89)	59 (80)	45 (61)	59 (80)
Water consumption (g/d)[c]	17.9	16.3 (91)	16.2 (91)	14.8(83)	12.4(69)	14.6 (82)

[a]NTP (1993a).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1÷ body weight) × (days dosed ÷ total days).
[c]Mean, (% change relative to controls calculated for this review).
[d]Mean, (% change relative to controls calculated by the study authors).
[e]Exposure terminated at Week 9 due to decreased survivorship.

Table B.2. Selected Organ Weights and Organ-weight Ratios in F344/N Rats Following Oral Administration of 2-EE via Drinking Water for 13 Weeks[a]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[b]					
	0 ppm	1250 ppm (109)	2500 ppm (205)	5000 ppm (400)	10,000 ppm (792)	20,000 ppm (2240)[f]
Male rats						
Sample size	10	10	10	10	10	5
Necropsy body weight (g)[c]	315	309 (98)	296 (94)[e]	295 (94)[d]	236 (75)[e]	–
Absolute right testis weight (g)[c]	1.394	1.431(103)	1.443 (104)	1.342 (96)	0.618 (44)[e]	–
Relative right testis weight (mg/g body weight)[c]	4.43	4.64 (105)	4.89 (110)	4.56 (103)	2.62 (59)[d]	–
Absolute thymus weight (g)[c]	0.299	0.270 (90)	0.213 (71)[e]	0.258 (86)[e]	0.154 (52)[e]	–
Relative thymus weight (mg/g body weight)[c]	0.95	0.87 (92)	0.72 (76)[e]	0.87 (92)[d]	0.65 (68)[e]	–
Parameter	**Exposure Group (Average Daily Dose, mg/kg-d)[b]**					
	0 ppm	**1250 ppm (122)**	**2500 ppm (247)**	**5000 ppm (466)**	**10,000 ppm (804)**	**20,000 ppm (2061)[f]**
Female rats						
Sample size	10	10	10	10	10	3
Necropsy body weight (g)[c]	185	183 (99)	177 (96)	173 (94)[e]	149 (81)[e]	–
Absolute thymus weight (g)[c]	0.214	0.210 (98)	0.221 (103)	0.186 (87)	0.069 (32)[e]	–
Relative thymus weight (mg/g body weight)[c]	1.16	1.15 (99)	1.25 (108)	1.07 (92)	0.47 (41)[e]	–

[a]NTP (1993a).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Mean, (% change relative to controls calculated for this review).
[d]Significantly different ($p < 0.05$) from the control group by Dunn's or Shirley's test.
[e]Significantly different ($p < 0.01$) from the control group by Dunn's or Shirley's test.
[f]Exposure terminated at week 9 due to decreased survivorship.

Table B.3. Incidence and Severity of Selected Histopathologic Lesions in Male 344/N Rats Following Oral Administration of 2-EE via Drinking Water for 13 Weeks[a]

Parameter		Exposure Group (Average Daily Dose, mg/kg-d)[b]					
		0 ppm	1250 ppm (109)	2500 ppm (205)	5000 ppm (400)	10,000 ppm (792)	20,000 ppm (2240)[d]
Sample size		10	10	10	10	10	5
Liver	Degeneration[c]	0	0	0	0	0	5 (2.4)
	Pigmentation[c]	0	0	0	0	10 (1.0)	5 (1.0)
	Hematopoiesis[c]	0	0	0	0	9 (1.7)	0
Bone marrow	Cellular depletion[c]	0	0	0	0	0	5 (3.6)
	Hyperplasia[c]	0	0	0	0	10 (2.7)	0
Spleen	Hematopoiesis[c]	0	0	0	10 (2.0)	10 (3.2)	0
	Pigmentation[c]	0	0	0	0	0	5 (2.6)
	Atrophy[c]	0	0	0	0	0	4 (2.3)
Thymus	Atrophy[c]	0	0[f]	0[e]	0	4 (2.0)	2 (4.0)[e]
Testes	Degeneration[c]	0	0	0	10 (1.1)	10 (3.5)	5 (4.0)
Prostate	Atrophy[c]	0	0	6 (1.3)	7 (1.4)	10 (2.0)	5 (3.4)

[a]NTP (1993a).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Incidence, (average severity of the number of animals with lesions: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked).
[d]Exposure terminated at Week 9 due to decreased survivorship.
[e]Sample size was 3 for this measurement.
[f]Sample size was 2 for this measurement.

Table B.4. Incidence and Severity of Selected Histopathologic Lesions in Female F344/N Rats Following Oral Administration of 2-EE via Drinking Water for 13 Weeks[a]

Parameter		Exposure Group (Average Daily Dose, mg/kg-d)[b]					
		0 ppm	1250 ppm (122)	2500 ppm (247)	5000 ppm (466)	10,000 ppm (804)	20,000 ppm (2061)[d]
Sample size		10	10	10	10	10	7
Liver	Degeneration[c]	0	0	0	0	0	6 (1.8)
	Pigmentation[c]	0	0	0	0	10 (1.0)	7 (1.0)
	Hematopoiesis[c]	0	0	0	0	9.0 (2.0)	0
Bone marrow	Cellular depletion[c]	0	0	0	0	0	7 (3.3)
	Hyperplasia[c]	0	0	0	0	10 (3.0)	0
Spleen	Hematopoiesis[c]	0	0	0	0	10 (2.5)	0
	Pigmentation[c]	0	0	0	0	0	7 (2.7)
	Atrophy[c]	0	0	0	0	0	6 (2.2)
Thymus	Atrophy[c]	0	-[e]	-[e]	0	10 (1.3)	6 (4.0)[c]
Uterus	Atrophy[c]	0	0	0	0	9 (2.7)	7 (3.7)

[a]NTP (1993a).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Incidence, (average severity of the number of animals with lesions: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked).
[d]Sample size was 7 for this measurement.
[e]Not applicable; tissue not examined for animals in this dose group.

Table B.5. Summary of Reproductive Tissue and Estrous Cycle Analysis in F344/N Rats Following Oral Administration of 2-EE via Drinking Water for 13 Weeks[a]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[b]					
	0 ppm	1250 ppm (109)	2500 ppm (205)	5000 ppm (400)	10,000 ppm (792)	20,000 ppm (2240)[h]
Male rats						
Spermatid heads (10^7/g testis)[c]	8.980 ± 0.352	–	9.630 ± 0.273 (107)	9.410 ± 0.376 (105)	1.610 ± 0.399[e] (18)	–
Spermatid heads (10^7/testis)[c]	13.12 ± 0.58	–	14.63 ± 0.50 (112)	13.27 ± 0.60 (101)	1.17 ± 0.31[e] (9)	–
Spermatid count (mean/10^{-4} mL suspension)[c]	65.58 ± 2.90	–	73.15 ± 2.49 (112)	66.35 ± 3.00 (101)	5.83 ± 1.57[e] (9)	–
Spermatozoal motility (%)[c]	96.55 ± 1.02	–	97.88 ± 0.67 (101)	97.07 ± 0.93 (101)	0.56 ± 0.44[e] (1)	–
Spermatozoal concentration[c] (10^6/g caudal epididymal tissue)[c]	763.9 ± 23.1	–	658.3 ± 14.8[e] (86)	669.0 ± 25.2[e] (88)	27.2 ± 5.2[e] (4)	–
Parameter	**Exposure Group (Average Daily Dose, mg/kg-d)**					
	0 ppm	**1250 ppm (122)**	**2500 ppm (247)**	**5000 ppm (466)**	**10,000 ppm (804)**	**20,000 ppm (2061)[d]**
Female rats						
Estrous cycle length (days)[c]	5.40 ± 0.15	–	5.83 ± 0.40 (108)	5.83 ± 0.26[g] (108)	6.50 ± 0.43[d,f] (120)	–
Estrous stages: Diestrus (% of cycle)	36.7	–	37.3	42.5	55.0	–
Estrous stages: Proestrus (% of cycle)	15.0	–	11.0	15.8	10.0	–
Estrous stages: Estrus (% of cycle)	39.2	–	44.1	30.0	25.8	–
Estrous stages: Metestrus (% of cycle)	9.2	–	7.6	11.7	9.2	–

[a]NTP (1993a).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Mean ± standard error (% change relative to controls calculated for this review).
[d]Significantly different ($p < 0.05$) from the control group by Shirley's test.
[e]Significantly different ($p < 0.01$) from the control group by Shirley's test.
[f]Estrous cycle longer than 12 days or unclear in 4/10 animals.
[g]Estrous cycle longer than 12 days or unclear in 1/10 animals.
[h]Not applicable; tissue not examined for animals in this dose group.

Table B.6. Survival, Weight Loss, and Water Consumption in Male F344/N Rats Following Oral Administration of 2-EE via Drinking Water for 60 Days[a]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[b]			
	0 ppm	5000 ppm (407)	10,000 ppm (792)	20,000 ppm (2390)[c]
Male rats				
Sample size	30	30	30	35
Survival[d]	10	10	9	5
Initial body weight (g)[e]	164	164 (100)	165 (101)	161 (98)
Day 60 body weight (g)[f]	302	284 (94)	255 (84)	157 (52)
Final body weight (g)[f]	388	361 (93)	353 (91)	277 (71)
Body weight change (g)[e]	224	197 (88)	188 (84)	116 (52)
Water consumption (g/d)[e]	21.2	19.3 (91)	17.5 (83)	19.9 (94)

[a]NTP (1993b).

[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).

[c]Twenty rats in this group died at or before Day 60; one rat died after Day 60. Because of the excessive mortality of rats administered 20,000-ppm 2-ethoxyethanol in the stop-exposure and rat subchronic-duration NTP (1993a) study, the five surviving rats from the subchronic-duration study were moved to the 20,000-ppm stop-exposure group at Day 60.

[d]Number surviving at the end of the recovery period; number surviving does not include animals sacrificed after 60 days of treatment or 30 days of recovery.

[e]Mean, (% change relative to controls calculated for this review).

[f]Mean, (% change relative to controls calculated by the study authors).

Table B.7. Selected Organ Weights and Organ-weight Ratios in Male F344/N Rats Following Oral Administration of 2-EE via Drinking Water for 60 Days[a]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[b]			
	0 ppm	5000 ppm (407)	10,000 ppm (792)	20,000 ppm (2390)[c]
60-d treatment period				
Sample size	10	10	10	4
Necropsy body weight (g)[d]	306 ± 7	285 ± 6[e] (93)	259 ± 5[f] (85)	138 ± 21[f] (45)
Absolute right testis weight (g)[d]	1.368 ± 0.019	1.400 ± 0.016 (102)	0.609 ± 0.044[f] (45)	0.361 ± 0.096[f] (26)
Relative right testis weight (mg/g body weight)[d]	4.48 ± 0.09	4.93 ± 0.10 (110)	2.37 ± 0.19[f] (53)	2.51 ± 0.27[e] (56)
Absolute epididymis weight (g)[d]	0.441 ± 0.012	0.420 ± 0.014 (95)	0.228 ± 0.012[f] (52)	0.114 ± 0.018[f] (26)
Relative epididymis weight (mg/g body weight)[d]	1.44 ± 0.03	1.48 ± 0.06 (103)	0.88 ± 0.04[f] (61)	0.83 ± 0.06[f] (58)
30-d recovery period				
Sample size	10	10	10	5
Necropsy body weight (g)[d]	339 ± 8	339 ± 6 (100)	303 ± 3[f] (89)	237 ± 37[f] (70)
Absolute right testis weight (g)[d]	1.460 ± 0.030	1.415 ± 0.021 (97)	0.652 ± 0.029[f] (45)	0.395 ± 0.038[f] (27)
Relative right testis weight (mg/g body weight)[d]	4.32 ± 0.05	4.19 ± 0.10 (97)	2.15 ±0.10[f] (50)	1.72 ± 0.10[f] (40)
Absolute epididymis weight (g)[d]	0.507 ± 0.018	0.497 ± 0.017 (98)	0.311 ± 0.015[f] (61)	0.204 ± 0.014[f] (40)
Relative epididymis weight (mg/g body weight)[d]	1.49 ± 0.04	1.47 ± 0.05 (99)	1.03 ± 0.05[f] (69)	0.91 ± 0.11[f] (61)
56-d recovery period				
Sample size	10	10	9	5
Necropsy body weight (g)[d]	384 ± 6	362 ± 8[e] (94)	352 ± 6[f] (92)	272 ± 29[f] (71)
Absolute right testis weight (g)[d]	1.486 ± 0.022	1.362 ± 0.026[f] (92)	0.678 ± 0.044[f] (46)	0.444 ± 0.023[f] (30)
Relative right testis weight (mg/g body weight)[d]	3.88 ± 0.07	3.77 ± 0.06 (97)	1.92 ± 0.12[f] (49)	1.72 ± 0.23[f] (44)
Absolute epididymis weight (g)[d]	0.533 ± 0.015	0.544 ± 0.021 (102)	0.319 ± 0.019[f,g] (60)	0.255 ± 0.024[f] (48)
Relative epididymis weight (mg/g body weight)[d]	1.39 ± 0.04	1.51 ±0.06 (109)	0.91 ± 0.05[f,g] (65)	0.95 ± 0.04[f] (68)

[a]NTP (1993b).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Twenty rats in this group died at or before Day 60; one rat died after Day 60. Because of the excessive mortality of rats administered 20,000-ppm 2-EE in the stop-exposure and rat subchronic-duration NTP (1993a) study, the five surviving rats from the subchronic-duration study were moved to the 20,000-ppm stop-exposure group at Day 60.
[d]Mean ± S.E. (% change relative to controls conducted for this review).
[e]Significantly different ($p < 0.05$) from the control group by the Dunn's or Shirley's test.
[f]Significantly different ($p < 0.01$) from the control group by the Dunn's or Shirley's test.
[g]Sample size was 10 for this measurement.

Table B.8. Incidence and Severity of Testicular Degeneration in Male F344/N Rats Following Oral Administration of 2-EE via Drinking Water for 60 Days[a]

Time	Exposure Group (Average Daily Dose, mg/kg-d)[b]			
	0 ppm	5000 ppm (407)	10,000 ppm (792)	20,000 ppm (2390)[c]
60-d treatment period[d]	0/10	0/10	10/10 (2.9)	24/24 (4.0)
30-d recovery period[d]	0/10	6/10 (1.0)	11/11 (2.7)	5/5 (4.0)
56-d recovery period[d]	0/10	7/10 (1.0)	9/9 (2.7)	5/5 (4.0)

[a]NTP (1993b).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Twenty rats in this group died at or before Day 60; one rat died after Day 60. Because of the excessive mortality of rats administered 20,000-ppm 2-ethoxyethanol in both the stop-exposure and rat subchronic-duration NTP (1993a) study, the five surviving rats from the subchronic-duration study were moved to the 20,000-ppm stop-exposure group at Day 60.
[d]Incidence, (average severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked).

Table B.9. Survival, Weight Loss, and Water Consumption in $B6C3F_1$ Mice Following Oral Administration of 2-EE via Drinking Water for 13 Weeks[a]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[b]					
	0 ppm	2500 ppm (587)	5000 ppm (971)	10,000 ppm (2003)	20,000 ppm (5123)	40,000 ppm (7284)
Male mice						
Sample size	10	10	10	10	10	10
Survival	10	10	10	10	10	10
Initial body weight (g)[c]	22.7	23.7 (104)	23.5 (104)	22.8 (100)	23.4 (103)	23.9 (105)
Final body weight (g)[d]	39.2	41.7 (106)	43.1 (110)	41.0 (105)	33.2 (85)	32.5 (83)
Body weight change (g)[c]	16.5	18.0 (109)	19.6 (119)	18.2 (110)	9.8 (59)	8.6 (52)
Water consumption (g/d)[c]	6.7	7.6 (113)	6.5 (97)	6.3 (94)	7.8 (116)	5.2 (78)
Parameter	**Exposure Group (Average Daily Dose, mg/kg-d)[b]**					
	0 ppm	**2500 ppm (722)**	**5000 ppm (1304)**	**10,000 ppm (2725)**	**20,000 ppm (7255)**	**40,000 ppm (11,172)**
Female mice						
Sample size	10	10	10	10	10	10
Survival	10	10	10	10	10	10
Initial body weight (g)[c]	19.3	19.0(98)	18.9 (98)	19.1 (99)	19.1 (99)	19.0 (98)
Final body weight (g)[d]	32.0	34.0 (106)	34.1(107)	30.2 (94)	26.4 (83)	24.9 (78)
Body weight change (g)[c]	12.7	15.0 (118)	15.2 (120)	11.1 (87)	7.3 (57)	5.9 (46)
Water consumption (g/d)[c]	8.7	7.5 (86)	6.9 (79)	6.9 (79)	8.7(100)	6.1 (70)

[a]NTP (1993c).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Mean, (% change relative to controls conducted for this review).
[d]Mean, (% change relative to controls as calculated by the study authors).

Table B.10. Selected Organ Weights and Organ-weight Ratios in Male B6C3F$_1$ Mice Following Oral Administration of 2-EE via Drinking Water for 13 Weeks[a]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[b]					
	0 ppm	2500 ppm (587)	5000 ppm (971)	10,000 ppm (2003)	20,000 ppm (5123)	40,000 ppm (7284)
Male mice						
Sample size	10	10	10	10	10	10
Necropsy body weight (g)[c]	38.9	40.9 (105)	43.0 (111)	40.5 (104)	33.6 (86)[d]	31.9 (82)[e]
Absolute right testis weight (g)[c]	0.119	0.124 (104)	0.123 (103)	0.119 (100)	0.097 (82)[e]	0.019 (16)[e]
Relative right testis weight (mg/g body weight)[c]	3.08	3.05 (99)	2.86 (93)	2.95 (96)	2.88 (94)	0.59 (19)[e]

[a]NTP (1993c).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Mean, (% change relative to controls calculated for this review).
[d]Significantly different (p <0.05) from the control group by Dunn's or Shirley's test.
[e]Significantly different (p <0.01) from the control group by Dunn's or Shirley's test.

Table B.11. Incidence and Severity of Selected Histopathologic Lesions in Male B6C3F$_1$ Mice Following Oral Administration of 2-EE via Drinking Water for 13 Weeks[a]

Parameter		Exposure Group (Average Daily Dose, mg/kg-d)[b]					
		0 ppm	2500 ppm (587)	5000 ppm (971)	10,000 ppm (2003)	20,000 ppm (5123)	40,000 ppm (7284)
Sample size		10	10	10	10	10	10
Spleen	Hematopoiesis[c]	0	–[d]	0	0	0	10 (1.6)
Testes	Degeneration[c]	0	–[d]	0	0	0	10 (4.0)

[a]NTP (1993c).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Incidence, (Average severity of the number of animals with lesions: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked).
[d]Not applicable; tissue not examined for animals in this dose group.

Table B.12. Incidence and Severity of Selected Histopathologic Lesions in Female B6C3F$_1$ Mice Following Oral Administration of 2-EE via Drinking Water for 13 Weeks[a]

Parameter		Exposure Group (Average Daily Dose, mg/kg-d)[b]					
		0 ppm	2500 ppm (722)	5000 ppm (1304)	10,000 ppm (2725)	20,000 ppm (7255)	40,000 ppm (11,172)
Sample size		10	10	10	10	10	10
Spleen	Hematopoiesis[c]	0	–[d]	0	1 (1.0)	9 (1.3)	10 (1.8)
Adrenal gland	X-zone, hypertrophy[c]	0	–[d]	1 (2.0)	8 (1.8)	10 (2.8)	9 (2.4)

[a]NTP (1993c).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Incidence, (Average severity of the number of animals with lesions: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked).
[d]Not applicable; tissue not examined for animals in this dose group.

Table B.13. Summary of Reproductive Tissue and Estrous Cycle Analysis in B6C3F$_1$ Mice Following Oral Administration of 2-EE via Drinking Water for 13 Weeks[a]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[b]					
	0 ppm	2500 ppm (587)	5000 ppm (971)	10,000 ppm (2003)	20,000 ppm (5123)	40,000 ppm (7284)
Male mice						
Spermatid heads (10^7/g testis)[c]	19.160 ± 0.745	–[g]	19.340 ± 0.767 (101)	19.970 ± 0.961 (104)	18.710 ± 1.018 (98)	–[g]
Spermatid heads (10^7/testis)[c]	2.26 ± 0.10	–[g]	2.27 ± 0.15 (100)	2.39 ± 0.10 (106)	1.72 ± 0.12[d] (76)	–[g]
Spermatid count (mean/10^{-4} mL suspension)[c]	70.68 ± 3.16	–[g]	70.85 ± 4.74 (100)	74.68 ± 3.18 (106)	53.68 ± 3.88[d] (76)	–[g]
Spermatozoal motility (%)[c]	98.65 ± 0.24	–[g]	98.40 ± 0.30 (100)	97.92 ± 0.25 (99)	97.35 ± 0.45[d] (99)	–[g]
Spermatozoal concentration (10^6/g caudal epididymal tissue)[c]	1126.7 ± 55.7	–[g]	1036.2 ± 94.5 (92)	1133.2 ± 63.4 (101)	1139.7 ± 91.0 (101)	–[g]
Parameter	**Exposure Group (Average Daily Dose, mg/kg-d)[b]**					
	0 ppm	**2500 ppm (722)**	**5000 ppm (1304)**	**10,000 ppm (2725)**	**20,000 ppm (7255)**	**40,000 ppm (11,172)**
Female mice						
Estrous cycle length (days)[c]	4.30 ± 0.11	–[g]	4.85 ± 0.15[d] (113)	5.25 ± 0.23[e] (122)	5.50 ± 0.47[e,f] (128)	–[g]
Estrous stages: Diestrus (% of cycle)	31.7	–[g]	27.5	32.5	40.8	–[g]
Estrous stages: Proestrus (% of cycle)	23.3	–[g]	20.8	18.3	19.2	–[g]
Estrous stages: Estrus (% of cycle)	29.2	–[g]	41.7	37.5	33.3	–[g]
Estrous stages: Metestrus (% of cycle)	15.8	–[g]	10.0	11.7	6.7	–[g]

[a]NTP (1993c).
[b]Doses are converted from ppm intake using the following equation: $Dose_{ADJ}$ = dose × food consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Mean ± standard error (% relative to controls).
[d]Significantly different ($p < 0.05$) from the control group by Shirley's test.
[e]Significantly different ($p < 0.01$) from the control group by Shirley's test.
[f]Estrous cycle longer than 12 days or unclear in 1/10 animals.
[g]Not applicable; tissue not examined for animals in this dose group.

Table B.14. Survivorship and Mean Body Weights in F344/N Rats Exposed to 2-EE by Gavage for 103 Weeks[a]

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-d)[b]			
	0 g/kg (0)	0.5 g/kg (357)	1.0 g/kg (714)	2.0 g/kg (1429)
Male rats				
Sample size	50	50	50	50
Survivorship	30	38	18[d]	–[f]
Mean body weights (g)[c]	421	339 (81)	304 (72)	–[f]
Parameter	**Exposure Group (Adjusted Daily Dose, mg/kg-d)[b]**			
	0 g/kg (0)	**0.5 g/kg (357)**	**1.0 g/kg (714)**	**2.0 g/kg (1429)**
Female rats				
Sample size	50	50	50	50
Survivorship	26	46[e]	25	–[f]
Mean body weights (g)[c]	315	239 (76)	225 (71)	–[f]

[a]Melnick (1984a).
[b]Doses are converted from % of food to ppm by multiplying by 10,000 (1% = 10,000 ppm), and then ppm intake in food is adjusted using the following equation: $Dose_{ADJ}$ = Dose × food consumption per day × (1 ÷ body weight) × (days dosed ÷ total days).
[c]Mean body weight at 104 weeks, (% change relative to controls as calculated by the study author).
[d]Significantly decreased relative to control ($p < 0.05$) as reported by the study author.
[e]Significantly increased relative to control ($p < 0.01$) as reported by the study author .
[f]Not applicable; tissue not examined for animals in this dose group.

Table B.15. Survivorship and Mean Body Weights in B6C3F$_1$ Mice Exposed to 2-EE by Gavage for 103 Weeks[a]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[b]			
	0 g/kg (0)	0.5 g/kg (357)	1.0 g/kg (714)	2.0 g/kg (1429)
Male mice				
Sample size	50	50	50	50
Survivorship	36	28	33	-
Mean body weights (g)[c]	44	40 (91)	41 (93)	-
Parameter	**Exposure Group (Adjusted Daily Dose, mg/kg-d)[b]**			
	0 g/kg (0)	**0.5 g/kg (357)**	**1.0 g/kg (714)**	**2.0 g/kg (1429)**
Female mice				
Sample size	50	50	50	50
Survivorship	36	38	32	–[d]
Mean body weights (g)[c]	40	38 (95)	39 (98)	–[d]

[a]Melnick (1984b).
[b]Doses are converted from g/kg body weight to mg/kg-day by converting grams to milligrams and multiplying by days dosed ÷ total days using the following equation: $Dose_{ADJ}$ = dose × (1000 mg ÷ 1g) × (days dosed ÷ total days).
[c]Mean body weight at 104 weeks, (% change relative to controls as calculated by the study authors).
[d]Not applicable; tissue not examined for animals in this dose group.

Table B.16. Body-Weight Gain in Male Sprague-Dawley Rats Exposed to 2-EE for 4 Weeks[a,b]

Body-weight Gain (g)	Exposure Group (Average Daily Dose, mg/kg-day)[c]				
	0 mg/kg (0)	100 mg/kg (86)	200 mg/kg (171)	400 mg/kg (343)	800 mg/kg (686)
Sample size	5	5	5	5	5
Week 1	15.7	22.5	11.8	3.91	0.00
Week 2	33.9	41.4	20.9	24.8	0.094
Week 3	61.4	84.4	50.2	33.4	20.8
Week 4	72.08	80.8	66.5	47.2	25.7

[a]Yoon et al. (2003).
[b]Data extracted from study graph(s) using Coulter Multiparameter Data Acquisition and Display Software.
[c]Doses are converted from g/kg body weight to mg/kg-day by converting grams to milligrams and multiplying by days dosed ÷ total days using the following equation: $Dose_{ADJ}$ = dose × (1000 mg ÷ 1g) × (days dosed ÷ total days).

Table B.17. Mean Testis and Epididymis Weights in Male Sprague-Dawley Rats Exposed to 2-EE for 4 Weeks[a,b]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[c]				
	0 mg/kg (0)	100 mg/kg (86)	200 mg/kg (171)	400 mg/kg (343)	800 mg/kg (686)
Sample size	5	5	5	5	5
Testis (mg/100 g bw)[d]	337.5 ± 14.52	320.21 ± 22.18 (95)	318.3 ± 13.76 (94)	217.6 ± 32.89[f] (64)	165.19 ± 26.77[f] (49)
Epididymis (mg/100 g bw)[d]	123.6 ± 8.072	113.9 ± 9.463[e] (92)	107.03 ± 10.58[f] (87)	95.41 ± 9.463[f] (77)	82.94 ± 6.401[f] (67)

[a]Yoon et al. (2003).
[b]Data extracted from study graph(s) using Coulter Multiparameter Data Acquisition and Display Software.
[c]Doses are converted from g/kg body weight to mg/kg-day by converting grams to milligrams and multiplying by days dosed ÷ total days using the following equation: $Dose_{ADJ}$ = dose × (1000 mg ÷ 1/g) × (days dosed ÷ total days).
[d]Mean ± SD (% relative to controls).
[e]Statistically significantly different from control ($p < 0.05$) as reported by the study authors.
[f]Statistically significantly different from control ($p < 0.01$) as reported by the study authors.

Table B.18. Testicular Cell Numbers in Male Sprague-Dawley Rats Exposed to 2-EE for 4 Weeks[a,b]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[c]				
	0 mg/kg (0)	100 mg/kg (86)	200 mg/kg (171)	400 mg/kg (343)	800 mg/kg (686)
Total[d]	20.54 ± 4.057	21.72 ± 3.934 (106)	20.81 ± 3.872 (101)	9.084 ± 2.828[e] (44)	4.057 ± 1.600[e] (20)
Mature haploid[d]	43.75 ± 4.321	43.62 ± 3.086 (100)	39.78 ± 3.086 (91)	21.74 ± 6.800[e] (50)	1.854 ± 1.852[e] (4)
Immature haploid[d]	25.27 ± 3.717	26.511 ± 3.725 (105)	25.90 ± 1.856 (102)	6.700 ± 3.260[e] (27)	2.791 ± 0.939[e] (11)
Diploid[d]	17.77 ± 3.615	18.45 ± 1.205 (104)	19.12 ± 3.615 (108)	42.70 ± 6.024[e] (240)	71.08 ± 9.639[e] (400)
S-phase[d]	1.460 ± 1.171	0.9757 ± 0.329 (67)	0.9675 ± 0.1463 (66)	1.728 ± 0.4390 (118)	1.208 ± 0.5852 (83)
Tetraploid[d]	4.157 ± 2.174	5.073 ± 1.957 (122)	5.557 ± 1.522 (134)	14.30 ± 2.610[d] (344)	9.346 ± 6.087 (225)

[a]Yoon et al. (2003).
[b]Data extracted from study graph(s) using Coulter Multiparameter Data Acquisition and Display Software.
[c]Doses are converted from g/kg body weight to mg/kg-day by converting grams to milligrams and multiplying by days dosed ÷ total days using the following equation: $Dose_{ADJ}$ = Dose × (1000 mg ÷ 1g) × (days dosed ÷ total days).
[d]Number (1×10^6) ± SD.
[e]Statistically significantly different from control ($p < 0.01$) as reported by the study authors.

Table B.19. Relative Testis and Epididymis Weight in Male Sprague-Dawley Rats Dosed by Gavage with 2-EE for 4 Weeks[a,b]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[c]				
	0 mg/kg (0)	50 mg/kg (43)	100 mg/kg (86)	200 mg/kg (171)	400 mg/kg (343)
Pubertal					
Epididymis[d]	101 ± 10	116 ± 4[e]	111 ± 5[e]	124 ± 6[f]	113 ± 6[e]
Testis[d]	406 ± 21	452 ± 8[e]	426 ± 19[e]	445 ± 27[e]	440 ± 12[e]
Adult					
Epididymis[d]	125 ± 7	129 ± 19	111 ± 21	113 ± 11	111 ± 13[f]
Testis[d]	352 ± 35	380 ± 56	354 ± 47	349 ± 19	233 ± 57[f]

[a]Yoon et al. (2001).
[b]Data extracted from study graph(s) using Coulter Multiparameter Data Acquisition and Display Software.
[c]Doses are converted from g/kg body weight to mg/kg-day by converting grams to milligrams and multiplying by days dosed per total days using the following equation: $Dose_{ADJ}$ = dose × (1000 mg ÷ 1 g) × (days dosed ÷ total days).
[d]Weight (mg/100g bw) ± SD.
[e]Statistically significantly different from control ($p < 0.05$) as reported by the study authors.
[f]Statistically significantly different from control ($p < 0.01$) as reported by the study authors.

Table B.20. Relative Alteration of Testicular Cell Populations in Male Sprague-Dawley Rats Dosed by Gavage with 2-EE for 4 Weeks[a,b]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[c]				
	0 mg/kg (0)	50 mg/kg (43)	100 mg/kg (86)	200 mg/kg (171)	400 mg/kg (343)
Mature haploid					
Pubertal[d]	35 ± 6	40 ± 5	39 ± 2	40 ± 1	40 ± 6
Adult[d]	39 ± 2	40 ± 4	39 ± 2	35 ± 5	30 ± 6[e]
Immature haploid					
Pubertal[d]	28 ± 4	27 ± 4	28 ± 3	29 ± 5	27 ± 4
Adult[d]	29 ± 3	27 ± 4	26 ± 1	27 ± 6	15 ± 8[e]
Diploid					
Pubertal[d]	25 ± 6	21 ± 4	22 ± 3	21 ± 4	22 ± 4
Adult[d]	23 ± 3	24 ± 2	24 ± 2	26 ± 5	38 ± 5[f]
S-phase					
Pubertal[d]	1.4 ± 0.33	1.4 ± 0.40	1.2 ± 0.35	1.3 ± 0.42	1.3 ± 0.18
Adult[d]	1.4 ± 0.33	1.3 ± 0.19	1.3 ± 0.12	1.4 ± 0.20	2 ± 0.70
Tetraploid					
Pubertal[d]	6.3 ± 2.5	6.6 ± 2.2	5.8 ± 1.4	7.3 ± 1.7	7 ± 3.1
Adult[d]	3.7 ± 0.6	3.9 ± 1.1	4.3 ± 0.84	5.9 ± 2.2	9.4 ± 2.0[f]

[a]Yoon et al. (2001).
[b]Data extracted from study graph(s) using Coulter Multiparameter Data Acquisition and Display Software.
[c]Doses are converted from g/kg body weight to mg/kg-day by converting grams to milligrams and multiplying by days dosed per total days using the following equation: $Dose_{ADJ}$ = dose × (1000 mg ÷ 1 g) × (days dosed ÷ total days).
[d]Percentage of population (%) ± SD.
[e]Statistically significantly different from control ($p < 0.05$) as reported by the study authors.
[f]Statistically significantly different from control ($p < 0.01$) as reported by the study authors.

Table B.21. Body and Organ Weights in Male Sprague-Dawley Rats Dosed by Gavage with 2-EE for 4 Weeks[a]

Parameter	Exposure Group (Average Daily Dose, mg/kg-d)[b]	
	0 mg/kg (0)	150 mg/kg (129)
Body weight (g)[c]	385 ± 17	364 ± 17[d] (95)
Adrenal gland (R) (mg/ 100 g body weight)[c]	9.8 ± 2.4	7.8 ± 2.8 (80)
Adrenal gland (L) (mg/ 100 g body weight)[c]	12.1 ± 4.9	8.7 ± 2.1[e] (72)
Testis (R) (mg/ 100 g body weight)[c]	425 ± 28	244 ± 32[e] (57)
Testis (L) (mg/ 100 g body weight)[c]	433 ± 25	247 ± 38[e] (57)
Epididymis (R) (mg/ 100 g body weight)[c]	149 ± 22	114 ± 15[e] (77)
Epididymis (L) (mg/ 100 g body weight)[c]	148 ± 23	112 ± 15[e] (76)

[a]Yu et al. (1999).
[b]Doses are converted from mg/kg body weight to mg/kg-day by multiplying by days dosed ÷ total days using the following equation: $Dose_{ADJ}$ = dose × (days dosed ÷ total days).
[c]Mean ± SD (% change relative to controls calculated for this review)
[d]Statistically significantly different from control ($p < 0.05$) as reported by the study authors.
[e]Statistically significantly different from control ($p < 0.01$) as reported by the study authors.

Table B.22. Hematology and Blood Chemistry in Male Sprague-Dawley Rats Dosed by Gavage with 2-EE for 4 Weeks[a]

Grade	Exposure Group (Average Daily Dose, mg/kg-d)[b]	
	0 mg/kg (0)	150 mg/kg (129)
White blood cells (10^3/mm^3)[c]	5.8 ± 0.1	4.1 ± 0.8[d] (71)
Red blood cells (106/mm^3)[c]	8.1 ± 0.4	8.3 ± 0.6 (102)
Hematocrit (%)[c]	49.8 ± 2.8	45.6 ± 3.6[d] (92)
Hemoglobin (g/dL)[c]	15.2 ± 0.4	14.2 ± 0.8[d] (93)
Mean corpuscular volume (μ^3)[c]	54.2 ± 2.0	55.2 ± 3.5 (102)
Mean corpuscular hemoglobin (pg)[c]	18.9 ± 0.9	17.3 ± 0.5[d] (92)
Mean corpuscular hemoglobin concentration (%)[c]	30.7 ± 1.1	31.3 ± 1.7 (102)
Platelet counts ($10^3/\mu^3$)[c]	1010 ± 72	750 ± 144[d] (74)
Total protein (mg/dL)[c]	6.6 ± 0.3	5.9 ± 0.2[d] (89)
Blood urea nitrogen (mg/dL)[c]	17.7 ± 1.5	18.9 ± 2.8 (107)
Creatinine (mg/dL)[c]	0.61 ± 0.0	0.50 ± 0.0[d] (82)
Total bilirubin (mg/dL)[c]	0.20 ± 0.0	0.19 ± 0.1 (95)
Glucose (mg/dL)[c]	127 ± 25	125 ± 12 (98)
Total cholesterol (mmol/L)[c]	59 ± 8	54 ± 7 (92)
Aspartate aminotransferase (units/L)[c]	137 ± 25	125 ± 23 (91)
Alanine aminotransferase (units/L)[c]	39 ± 3	34 ± 6 (87)
Alkaline phosphatase (units/L)[c]	377 ± 82	223 ± 76[d] (59)

[a]Yu et al. (1999).
[b]Doses are converted from mg/kg body weight to mg/kg-day by multiplying by days dosed ÷ total days using the following equation: $Dose_{ADJ}$ = dose × (days dosed ÷ total days).
[c]Mean ± SD (% change relative to controls calculated for this review).
[d]Statistically significantly different from control ($p < 0.01$) as reported by the study authors.

Table B.23. Body-Weight Gain in Sprague-Dawley Rats Exposed to 2-EE Vapor for 13 Weeks[a]

Body-weight Gain (g)	Exposure Group (Human Equivalent Concentration, mg/m^3)[b]			
	0 ppm (0)	**25 ppm (17)**	**100 ppm (68)**	**400 ppm (265)**
Male rats				
Sample size	**15**	**15**	**15**	**15**
Week 0[c]	249 ± 11	254 ± 9 (102)	250 ± 10 (100)	251 ± 14 (101)
Week 3[c]	358 ± 21	356 ± 19 (99)	367 ± 22 (103)	365 ± 24 (102)
Week 6[c]	404 ± 36	403 ± 27 (100)	423 ± 36 (105)	415 ± 27 (103)
Week 9[c]	460 ± 46	453 ± 30 (98)	468 ± 44 (102)	448 ± 41 (97)
Week 13[c]	475 ± 53[d]	472 ± 38 (99)	481 ± 38 (101)	471 ± 56 (99)
Terminal[c]	442 ± 49[d]	446 ± 34 (101)	454 ± 39 (103)	439 ± 48 (99)
Female rats				
Sample size	**15**	**15**	**15**	**15**
Week 0[c]	171 ± 8	169 ± 6 (99)	169 ± 7 (99)	170 ± 8 (99)
Week 3[c]	218 ± 11	208 ± 22 (95)	210 ± 13 (96)	215 ± 8 (99)
Week 6[c]	247 ± 15	253 ± 30 (102)	246 ± 20 (100)	249 ± 15 (101)
Week 9[c]	264 ± 18	262 ± 18 (99)	259 ± 21 (98)	270 ± 28 (102)
Week 13[c]	278 ± 23	275 ± 23[d] (99)	267 ± 26 (96)	282 ± 38[d] (101)
Terminal[c]	252 ± 23	253 ± 21[d] (100)	244 ± 23 (97)	250 ± 20[d] (99)

[a]Barbee et al. (1984a).
[b]Doses are converted using conversion factors: MW = 90.12 and assuming 25ºC and 1 atmosphere. HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × (days per week exposed ÷ 7) × blood:gas (air) partition coefficient.
[c]Mean ± SD (% change relative to controls calculated for this review).
[d]Sample size is 14 rats.

Table B.24. Mean Pituitary and Spleen Weights in Sprague-Dawley Rats Exposed to 2-EE Vapor for 13 Weeks[a]

Parameter	Exposure Group (Human Equivalent Concentration, mg/m^3)[b]			
	0 ppm (0)	**25 ppm (17)**	**100 ppm (68)**	**400 ppm (265)**
Male rats				
Sample size	**15**	**15**	**15**	**15**
Pituitary (g)[c]	10.9 ± 1.4[d]	10.7 ± 1.3 (98)	10.2 ± 1.0 (94)	9.4 ± 1.0[d,e] (86)
Spleen (g)[c]	0.67 ± 0.13[d]	0.68 ± 0.09 (101)	0.71 ± 0.11 (106)	0.62 ± 0.08 (93)
Female rats				
Sample size	**15**	**15**	**15**	**15**
Pituitary (g)[c]	15.6 ± 2.6	14.5 ± 1.9 (93)	13.7 ± 1.7 (88)	14.4 ± 2.8[e] (92)
Spleen (g)[c]	0.52 ± 0.07	0.46 ± 0.05 (88)	0.46 ± 0.07[e] (88)	0.44 ± 0.06[d,f] (85)

[a]Barbee et al. (1984a).
[b]Doses are converted using conversion factors: MW = 90.12 and assuming 25ºC and 1 atmosphere. HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × (days per week exposed ÷ 7) × blood gas partition coefficient.
[c]Mean ± SD (% relative to controls).
[d]Sample size is 14 rats.
[e]Statistically significantly different from control ($p < 0.05$) as reported by the study authors.
[f]Statistically significantly different from control ($p < 0.01$) as reported by the study authors.

Table B.25. Body-weight Gain in New Zealand White Rabbits Exposed to 2-EE Vapor for 13 Weeks[a]

Body-weight Gain (g)	Exposure Group (Human Equivalent Concentration, mg/m^3)[b]			
	0 ppm (0)	**25 ppm (17)**	**100 ppm (68)**	**400 ppm (265)**
Male rabbits				
Sample size	**10**	**10**	**10**	**10**
Week 0[c]	2900 ± 100	2900 ± 100 (100)	2900 ± 100 (100)	2900 ± 200 (100)
Week 3[c]	3200 ± 200	3100 ± 200 (97)	3100 ± 200 (97)	3000 ± 100[e] (94)
Week 6[c]	3500 ± 200	3400 ± 100 (97)	3300 ± 100 (94)	3200 ± 200[f] (91)
Week 9[c]	3600 ± 200	3500 ± 100 (97)	3500 ± 100 (97)	3300 ± 200[f] (92)
Week 13[c]	3900 ± 300	3700 ± 200[e] (95)	3800 ± 200 (97)	3500 ± 200[f] (90)
Terminal[c]	3700 ± 300	3500 ± 200[e] (95)	3600 ± 200 (97)	3400 ± 200[f] (92)
Female rabbits				
Sample size	**10**	**10**	**10**	**10**
Week 0[c]	2400 ± 200	2400 ± 100 (100)	2400 ± 200 (100)	2400 ± 200 (100)
Week 3[c]	2800 ± 200	2700 ± 200 (96)	2900 ± 200 (104)	2700 ± 200 (96)
Week 6[c]	3300 ± 200	3000 ± 300 (91)	3200 ± 200 (97)	2900 ± 400[d,e] (88)
Week 9[c]	3400 ± 300	3200 ± 300[d] (94)	3400 ± 300 (100)	3100 ± 300 (91)
Week 13[c]	3800 ± 300	3400 ± 400[d,e] (89)	3700 ± 400 (97)	3400 ± 300[d] (89)
Terminal[c]	3700 ± 300[d]	3300 ± 400[d,e] (89)	3500 ± 300 (95)	3300 ± 300[d] (89)

[a]Barbee et al. (1984b).
[b]Doses are converted using conversion factors: MW = 90.12 and assuming 25ºC and 1 atmosphere. HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × (days per week exposed ÷ 7) × blood:gas (air) partition coefficient.
[c]Mean ± SD (% relative to controls).
[d]Sample size is 9 rabbits.
[e]Statistically significantly different from control ($p < 0.05$) as reported by the study authors.
[f]Statistically significantly different from control ($p < 0.01$) as reported by the study authors.

Table B.26. Mean Adrenal Gland, Testis, and Brain Weights in New Zealand White Rabbits Exposed to 2-EE Vapor for 13 Weeks[a]

Parameter	Exposure Group (Human Equivalent Concentration, mg/m^3)[b]			
	0 ppm (0)	25 ppm (17)	100 ppm (68)	400 ppm (265)
Male rabbits				
Sample size	**10**	**10**	**10**	**10**
Adrenal (g)[c]	424 ± 86[d]	304 ±78[e] (72)	364 ± 86 (86)	351 ± 89 (115)
Testis (g)[c]	8.19 ± 0.82	8.73 ± 119[g] (107)	8.73 ± 0.69 (107)	6.36 ± 0.99[f] (78)
Brain (g)[c]	9.50 ± 0.57	9.50 ± 0.42 (100)	9.71 ± 0.35 (102)	9.29 ± 0.39 (98)
Female rabbits				
Sample size	**10**	**10**	**10**	**10**
Adrenal (g)[c]	324 ± 79	318 ± 30[d] (98)	289 ± 54 (89)	282 ± 60 (89)
Brain (g)[c]	9.20 ± 0.55	9.42 ± 0.37 (102)	9.20 ± 0.48 (100)	9.33 ± 0.39 (99)

[a]Barbee et al. (1984b).
[b]Doses are converted using conversion factors: MW = 90.12 and assuming 25ºC and 1 atmosphere. HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × (days per week exposed ÷ 7) × blood:gas (air) partition coefficient.
[c]Mean ± SD (% relative to controls).
[d]Sample size is 9 rabbits.
[e]Statistically significantly different from control ($p < 0.05$) as reported by the study authors.
[f]Statistically significantly different from control ($p < 0.01$) as reported by the study authors.
[g]The SD value reported here reflects the number reported in the study. There is no way to confirm this value.

Table B.27. Litter Data for Female Wistar Rats Exposed to 2-EE via Inhalation on GDs 6−15[a]

Parameter	(Human Equivalent Concentration, mg/m^3)[b]			
	0 ppm (0)	10 ppm (9)	50 ppm (47)	250 ppm (230)
No. pregnant	23/24	24/24	23/24	21/24
Preimplantation loss (%)	2.4	9.7[d]	14.3[c]	6.2
Postimplantation loss (%)	5.5	7.6	8.9	12.6
Mean no. of live fetuses	12.2	10.6[c]	10.8[c]	11.1
Mean live fetus weight (g)	5.1	5.2	5.1	4.7[d]

[a]Doe (1984a).
[b]Developmental studies are adjusted for hourly but not for weekly exposure. Conversion Factors: MW = 90.12. Assuming 25ºC and 1 atmosphere; HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × blood:gas (air) partition coefficient.
[c]Significantly different from control ($p < 0.05$); statistical test methods were not specified.
[d]The significance of this result cannot be confirmed. Study text indicated that this result was statistically significant; however, Table 4 in the study did not. This represents a discrepancy in the article.

Table B.28. Fetal, Visceral, External, and Skeletal Defects in Offspring of Wistar Rats Exposed to 2-EE via Inhalation on GDs 6−15[a]

Parameter	(Human Equivalent Concentration, mg/m^3)[b]			
	0 ppm (0)	10 ppm (9)	50 ppm (47)	250 ppm (230)
External and Visceral Defects				
No. (%) showing any minor defects	33 (11.7)	41 (16.1)	29 (11.6)	43 (18.4)[c]
No. (%) showing any major defects	0	0	0	1 (0.4)
Skeletal Defects				
No. (%) showing any minor defects	68 (46.3)	52 (39.7)	66 (51.2)	119 (97.5)[c]
No. (%) showing any major defects	0	0	0	0

[a]Doe (1984a).
[b]Developmental studies are adjusted for hourly but not for weekly exposure. Conversion Factors: MW = 90.12. Assuming 25ºC and 1 atmosphere; HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × blood:gas (air) partition coefficient.
[c]Significantly different from control ($p < 0.05$); statistical test methods were not specified.

Table B.29. Incidence of Specific Visceral and External Defects in Offspring of Wistar Rats Exposed to 2-EE via Inhalation on GDs 6−15[a]

Parameter	Exposure Group (Human Equivalent Concentration, mg/m^3)[b]			
	0 ppm (0)	10 ppm (9)	50 ppm (47)	250 ppm (230)
Minor Defects				
No. (%) renal pelvic dilation	19 (6.8)	25 (9.8)	22 (9.8)	30 (12.8)[c]
No. (%) hydroureter	13 (4.6)	26 (2.4)	4 (1.6)	10 (4.3)
No. (%) bladder distended	0	1 (0.4)	0	0
No. (%) dermal hemorrhage	2 (0.7)	2 (0.8)	0	0
No. (%) limb malrotation	0	9 (3.5)[c]	2 (0.8)	3 (1.3)
No. (%) lateral ventricles of brain dilated	0	0	1 (0.8)	0
Major Defects				
Right ureter/kidney fused to left kidney, right kidney vestigial	0	0	0	1 (0.4)

[a]Doe (1984a).
[b]Developmental studies are adjusted for hourly but not for weekly exposure. Conversion Factors: MW = 90.12. Assuming 25ºC and 1 atmosphere; HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × blood:gas (air) partition coefficient.
[c]Significantly different from control ($p < 0.05$); statistical test methods were not specified.

Table B.30. Litter Data for Female Dutch Rabbits Exposed to 2-EE via Inhalation on GDs 6−18[a]

Parameter	Exposure Group (Human Equivalent Concentration, mg/m^3)[b]			
	0 ppm (0)	10 ppm (9)	50 ppm (46)	175 ppm (161)
No. pregnant	21/24	21/24	16/24	22/24
Preimplantation loss (%)	19.5	17.6	22.1	25.7
Postimplantation loss (%)	5.7	8.4	8.8	5.7
Mean no. of live fetuses	6.5	6.6	6.0	6.1
Mean live fetus weight (g)	34.1	34.2	35.7	36.1

[a]Doe (1984b).
[b]Developmental studies are adjusted for hourly but not for weekly exposure. Conversion Factors: MW = 90.12. Assuming 25ºC and 1 atmosphere; HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × blood:gas (air) partition coefficient.

Table B.31. Fetal, Visceral, External, and Skeletal Defects in Offspring of Dutch Rabbits Exposed to 2-EE via Inhalation on GDs 6−18[a]

Parameter	Exposure Group (Human Equivalent Concentration, mg/m^3)[b]			
	0 ppm (0)	10 ppm (9)	50 ppm (46)	175 ppm (161)
External and Visceral Defects				
No. (%) showing any minor defects	6 (4.4)	8 (5.8)	4 (4.2)	2 (1.5)
No. (%) showing any major defects	1 (0.7)	1 (0.7)	0	2 (1.5)
Skeletal Defects				
No. (%) showing any minor defects	44 (32.4)	72 (52.2)	35 (36.5)	87 (64.5)[c]
No. (%) showing any major defects	70 (51.5)	84 (60.0)	62 (64.6)	106 (79.1)[c]

[a]Doe (1984b).
[b]Developmental studies are adjusted for hourly but not for weekly exposure. Conversion Factors: MW = 90.12. Assuming 25ºC and 1 atmosphere; HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × blood:gas (air) partition coefficient.
[c]Significantly different from control ($p < 0.05$); statistical test methods were not specified.

Table B.32. Incidence of Specific Visceral in Offspring of Dutch Rabbits Exposed to 2-EE via Inhalation on GDs 6−18[a]

Parameter	Exposure Group (Human Equivalent Concentration, mg/m^3)[b]			
	0 ppm (0)	10 ppm (9)	50 ppm (46)	175 ppm (161)
Right subclavian artery, absent, aorta and heart reduced in size (%)	0	0	0	1 (0.7)
Extreme pelvic dilatation of both kidneys	1 (0.7)	0	0	0
Umbilical hernia	0	0	0	1 (0.7)

[a]Doe (1984b).
[b]Developmental studies are Adjusted for hourly but not for weekly exposure. Conversion Factors: MW = 90.12. Assuming 25ºC and 1 atmosphere; HEC_{EXRESP} = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × blood:gas (air) partition coefficient.

APPENDIX C. BMD MODELING OUTPUTS FOR 2-EE

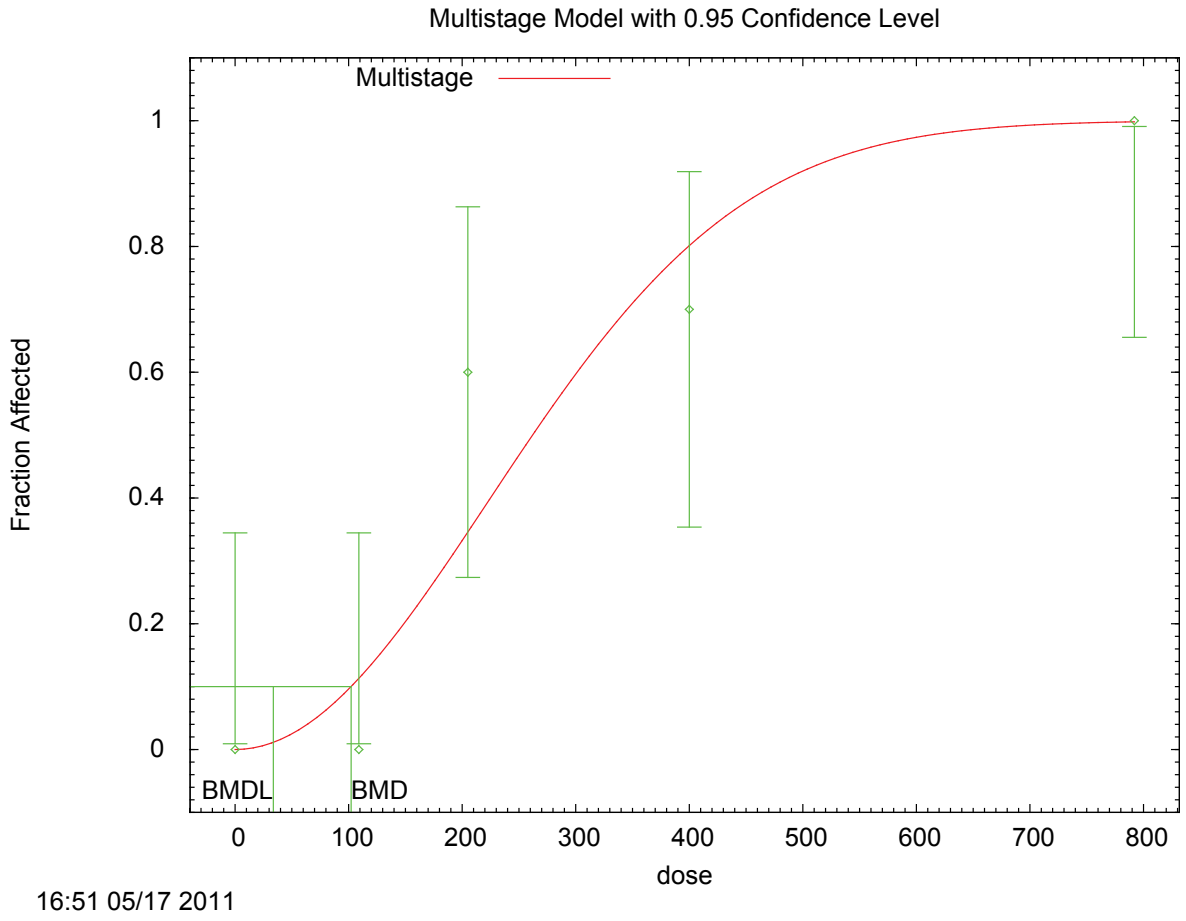

Figure C.1. Multistage BMD Model for Male Prostate Atrophy Data (NTP [1993a])

Text Output for Multistage BMD Model for Male Prostate Atrophy Data (NTP [1993a])

```
 ======================================================================
         Multistage Model. (Version: 3.2;  Date: 05/26/2010)
         Input Data File: C:/USEPA/BMDS21/Data/mst_2EE_prostate_atrophy_Mst-BMR10-
Restrict.(d)
         Gnuplot Plotting File:  C:/USEPA/BMDS21/Data/mst_2EE_prostate_atrophy_Mst-
BMR10-Restrict.plt
                                              Tue May 17 16:51:03 2011
 ======================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]
```

```
   The parameter betas are restricted to be positive


   Dependent variable = Incidence
   Independent variable = Dose

 Total number of observations = 5
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =  1.6426e+014


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(2)

   Beta(2)            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0           *               *                  *
        Beta(1)                0           *               *                  *
        Beta(2)     1.00902e-005           *               *                  *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -12.8388         5
   Fitted model        -15.6842         1       5.6909      4          0.2235
  Reduced model        -34.4972         1      43.3169      4         <.0001

           AIC:         33.3684


                                Goodness  of  Fit
                                                        Scaled
```

Dose	Est._Prob.	Expected	Observed	Size	Residual
0.0000	0.0000	0.000	0.000	10	0.000
109.0000	0.1130	1.130	0.000	10	-1.129
205.0000	0.3456	3.456	6.000	10	1.692
400.0000	0.8010	8.010	7.000	10	-0.800
792.0000	0.9982	9.982	10.000	10	0.134

Chi^2 = 4.79 d.f. = 4 P-value = 0.3092

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 102.185

BMDL = 33.6961

BMDU = 130.329

Taken together, (33.6961, 130.329) is a 90 % two-sided confidence interval for the BMD

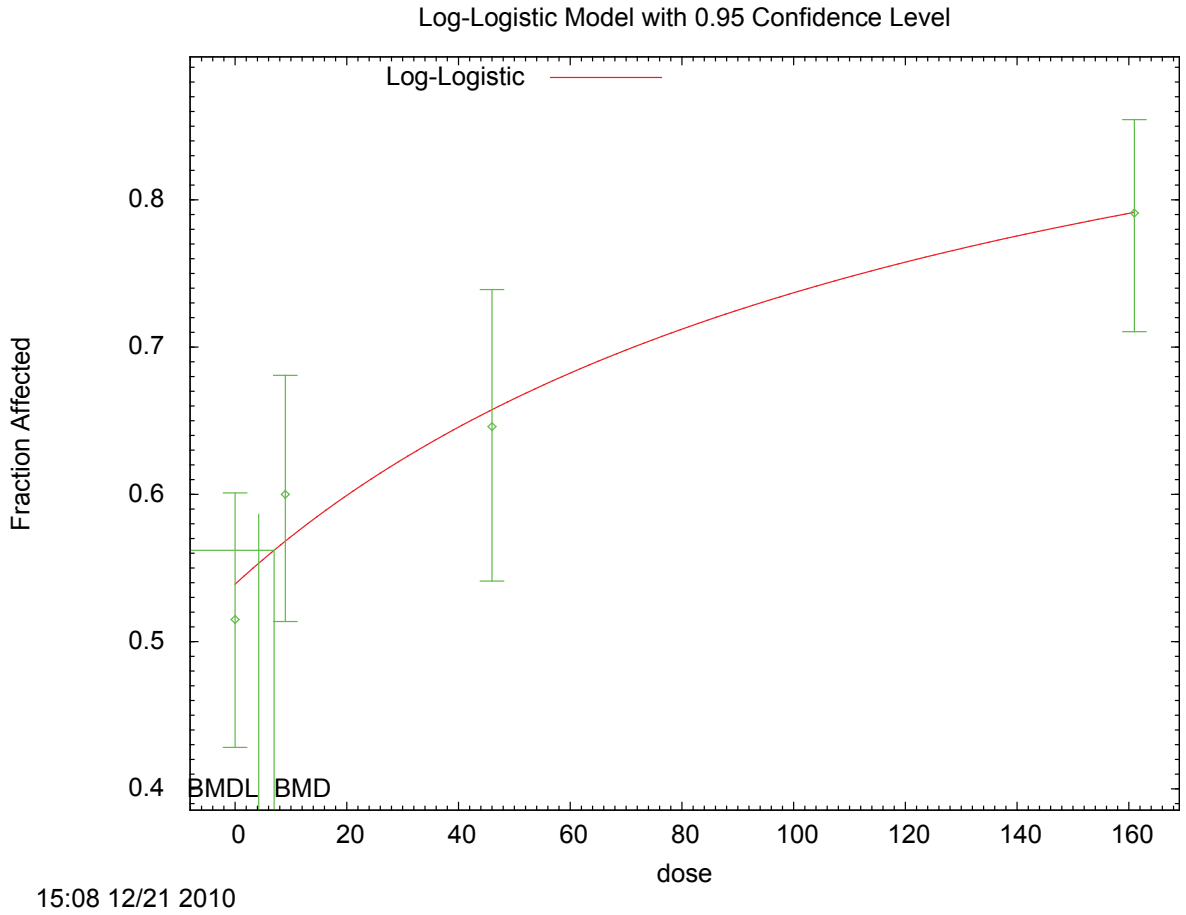

Figure C.2. Log-Logistic BMD Model for Major Fetal Skeletal Defect Data (Doe [1984b])

Text Output for Log-Logistic BMD Model for Major Fetal Skeletal Defect Data (Doe [1984b])

```
================================================================
        Logistic Model. (Version: 2.13; Date: 10/28/2009)
        Input Data File: C:/USEPA/BMDS21/Data/lnl_2EE_skeletal_Lnl-BMR05-Restrict.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS21/Data/lnl_2EE_skeletal_Lnl-BMR05-
Restrict.plt
                                              Tue Dec 21 15:08:43 2010
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-slope*Log(dose))]


   Dependent variable = Percent_Positive
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 4
   Total number of records with missing values = 0
```

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

User has chosen the log transformed model

Default Initial Parameter Values
background = 0.515
intercept = -4.71869
slope = 1

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.59
intercept	-0.59	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.538916	*	*	*
intercept	-4.88826	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-319.51	4			
Fitted model	-319.985	2	0.950696	2	0.6217
Reduced model	-331.646	1	24.2718	3	<.0001

AIC: 643.971

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.5389	73.293	70.040	136	-0.559
9.0000	0.5682	79.548	84.000	140	0.760
46.0000	0.6576	63.129	62.016	96	-0.239
161.0000	0.7917	106.081	105.994	134	-0.019

Chi^2 = 0.95 d.f. = 2 P-value = 0.6226

```
   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        6.98538

            BMDL =        4.23203
```

APPENDIX D. REFERENCES

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