Provisional Peer-Reviewed Toxicity Values for

Diethylene Glycol Monobutyl Ether
(DGBE, CASRN 112-34-5)

Superfund Health Risk Technical Support Center
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COMMONLY USED ABBREVIATIONS

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
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

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DIETHYLENE GLYCOL MONOBUTYL ETHER (DGBE, CASRN 112-34-5)**

Background

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

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

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

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

Disclaimers

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

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths

and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

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

INTRODUCTION

Diethylene glycol monobutyl ether (DGBE) is a colorless liquid. Its synonyms include 2-(2-butoxyethoxy)-ethanol, butoxydiglycol, butadigol, and butyl carbitol. The formula for DGBE is $C_8H_{18}O_3$ (Figure 1) with a molecular weight of 162.23, boiling temperature of 230.4 C°, and melting point of -68.1C°.
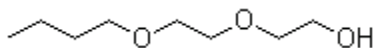

Figure 1. Diethylene Glycol Monobutyl Ether Structure

There is no RfD assessment for DGBE on IRIS (U.S. EPA, 2008) or in the HEAST (U.S. EPA, 1997) or Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). Subchronic and chronic RfDs of 0.04 and 0.004 mg/kg-day, respectively, were derived for DGBE in a draft Health and Environmental Effects Document (HEED) on glycol ethers (SRC, 1992). The RfDs in the draft HEED were based on a subchronic LOAEL of 36 mg/kg-day for hematological effects in rats (Hobson et al., 1987) and an UF of 1,000 (subchronic RfD) or 10,000 (chronic RfD). An earlier Health Effects Assessment (HEA) on glycol ethers (U.S. EPA, 1984) did not derive acceptable subchronic or chronic oral intake values for DGBE. This HEA is the only document relevant to DGBE in the Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991, 1994a).

There is no RfC assessment for DGBE on IRIS (U.S. EPA, 2008) or in the HEAST (U.S. EPA, 1997). No occupational exposure limits have been recommended or promulgated by the American Conference of Governmental Industrial Hygienists (ACGIH, 2001, 2007), the National Institute of Occupational Safety and Health (NIOSH, 2005), or the Occupational Safety and Health Administration (OSHA, 2008).

DGBE is not listed in the HEAST cancer table (U.S. EPA, 1997), and no carcinogenicity assessment is available on IRIS (U.S. EPA, 2008) or indicated in the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). The carcinogenicity of DGBE has not been assessed by the International Agency for Research on Cancer (IARC, 2008) or the National Toxicology Program (NTP, 2005, 2008).

There are no Toxicological Profiles available from ATSDR (2008) or World Health Organization Environmental Health Criteria documents (WHO, 2008) for DGBE. CalEPA (2002, 2005a,b) has not derived oral or inhalation recommended exposure limits (RELs) or a cancer potency factor for DGBE.

Literature searches were conducted from the 1960s through August 2009 for studies relevant to the derivation of provisional toxicity values for DGBE. Databases searched included: MEDLINE (including cancer subset), TOXLINE (Special), BIOSIS, TSCATS 1/TSCATS 2, CCRIS, DART/ETIC, GENETOX, HSDB, RTECS, and Current Contents (February−August 2009).

REVIEW OF PERTINENT DATA

Human Studies
No pertinent data were located regarding health effects of DGBE in humans following oral or inhalation exposure.

Animal Studies
Animal studies are summarized in Table 1.

Oral Exposure
Short-term Studies—As a preliminary study to a 13-week oral subchronic toxicity investigation discussed above, Johnson et al. (2005) administered DGBE (99.2% pure) in drinking water to Fischer 344 (F344) rats (five/sex/group) at doses of 0, 1,000, 1,500, or 2,000 mg/kg-day for 2 weeks. Animals were evaluated for clinical signs, body weight, food consumption, water consumption, urinalysis variables, serum chemistry, and hematological variables, and were given a complete gross necropsy at termination. There were no effects on mortality and no clinical signs other than urine soiling (all doses). Body weight, food consumption, and water consumption were reduced at all doses (but not statistically significant), with effects greatest in high-dose males (14% lower body weight than controls by study termination). Red blood cell count (RBC), hemoglobin (Hgb), and hematocrit (Hct) were decreased (not statistically significant) relative to controls at all doses, with the largest decreases observed in the 2,000 mg/kg-day dose group (all variables: 10−13% decrease for males, 5−7% decrease for females). The data showed no statistically significant treatment-related adverse effects on serum chemistry, urinalysis, or organ weights. The only effect observed at gross necropsy was urine staining of the perineal fur (all doses).

Table 1. Summary Table for Diethylene Glycol Monobutyl Ether						
Species and Study Type	**Exposure**	**Critical Effects**	**NOAEL**	**LOAEL**	**Comments**	**Reference**
Oral Studies						
Rat Short-term	0, 51, 94, 210, 650, 970, or 1,830 mg/kg-day in drinking water for 30 days.	Reduced water intake at ≥94 mg/kg-day and histopathological changes in the liver (congestion and slight cloudy swelling) and kidney tubules (cloudy swelling and increased secretion) at ≥650 mg/kg-day.	Cannot be determined	Cannot be determined	Endpoints included mortality, water intake, body weight and histology of the liver, kidneys, spleen, and testis. Small groups of rats (five/sex/dose). This is a range-finding study that is limited in scope and inadequately reported.	Smyth, 1940. Smyth and Carpenter, 1948.
Rat Subchronic	891, 1,782, or 3,564 mg/kg-day by gavage on 5 days/week, for 6 weeks. (average daily dose: 636, 1,273, or 2,546 mg/kg-day)	Hyperkeratosis of the stomach at ≥891 mg/kg-day and hematologic effects (reduced erythrocyte count, hemoglobin concentration, and MCHC and increased MCV) at ≥1,782 mg/kg-day.	891 mg/kg-day (average daily dose of 636 mg/kg-day)	1,782 mg/kg-day (average daily dose of 1,273 mg/kg-day) Hematological effects	Endpoints included clinical signs, food consumption, body weight, hematology, clinical chemistry, organ weights, gross pathology, and histopathology. Human relevance of gastric hyperkeratosis is questionable due to bolus exposure and likely direct irritant properties of DGBE.	Kodak, 1984.

		Table 1. Summary Table for Diethylene Glycol Monobutyl Ether					
Species and Study Type	**Exposure**	**Critical Effects**	**NOAEL**	**LOAEL**	**Comments**	**Reference**
Rat Subchronic	0, 70, 330, or 1,630 mg/kg-day (males) or 0, 50, 250, or 1,270 mg/kg-day (females) by gavage on 5 days/week, for 13 weeks. (Average daily dose: 0, 50, 236, or 1,164 mg/kg-day [males]; 0, 36, 179, or 907 mg/kg-day [females].)	Decreased total WBC and lymphocyte counts and MCHC in females at ≥50 mg/kg-day. Mortality in both sexes at ≥250/330 mg/kg-day (some of the deaths were due to gavage error).	N/A	50 mg/kg-day (average daily dose of 36 mg/kg-day) Lymphopenia	Endpoints included clinical signs, food consumption, body weight, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, and histopathology. The histological examinations did not include the stomach.	Hobson et al, 1987.
Rat Subchronic	0, 50, 250, or 1,000 mg/kg-day in drinking water for 13 weeks.	Decreased RBC count, hemoglobin, and hematocrit at ≥250 mg/kg-day. Other effects only occurred at 1,000 mg/kg-day and mainly involved the liver; these included increases in organ weight and hepatic cytochrome P450s and UGT levels, decreases in serum total protein, cholesterol, and serum AST, and hepatocyte hypertrophy and individual hepatocyte degeneration.	50 mg/kg-day	250mg/kg-day Reduced RBC count and Hgb in both sexes.	Endpoints included clinical signs, food and water consumption, body weight, hematology, clinical chemistry, urinalysis, functional observational battery, sperm analysis, liver metabolic enzymes, organ weights, gross pathology, and histopathology. Study possibly useful for subchronic and chronic p-RfD derivation.	Johnson et al, 2005.

Table 1. Summary Table for Diethylene Glycol Monobutyl Ether						
Species and Study Type	**Exposure**	**Critical Effects**	**NOAEL**	**LOAEL**	**Comments**	**Reference**
Rat Reproductive	0, 250, 500, or 1,000 mg/kg-day by gavage in water. Untreated males were bred to treated females and vice versa. The males were treated for 60 days prior to mating, and the females were treated from 14 days prior to mating to GD 13 or until weaning of the offspring.	Slight reduction in pup weight during the last week of lactation in the offspring of the females dosed with 1,000 mg/kg-day. No maternal toxicity or reproductive effects were observed.	500 mg/kg-day	1,000 mg/kg-day Decreased pup weight during the last week of lactation.	Endpoints included body weight in both sexes; numbers of corpora lutea, implants, resorptions, viable embryos and live or dead pups; and clinical condition, body weight, and external development in pups.	Nolen et al, 1985.
Rat Reproductive and Developmental	0, 25, 115, or 633 mg/kg-day in diet on GD 0−20. Rats were killed on GD 20 for uterine and fetal examinations or allowed to deliver pups that were reared until 10 weeks of age.	Reduced maternal body weight gain during pregnancy at ≥25 mg/kg-day (the effect was variable and not dose-related). No prenatal or postnatal developmental toxicity.	633 mg/kg-day		Maternal endpoints included clinical signs, food consumption, and body weight. Developmental endpoints included numbers of corpora lutea, implantations, litters, and live fetuses per litter, pre and postimplantation losses, fetal and placental weights, sex ratio, external and oral cavity anomalies, skeletal and internal anomalies, gestation length, numbers of live newborns, and pup body weight and survival.	Ema et al., 1988.
Mouse Developmental	0 or 500 mg/kg-day gavage in water on GD 7−14.	No maternal or developmental toxicity.	500 mg/kg-day		Developmental toxicity screening assay. Study endpoints included maternal body weight, fetal survival, pup perinatal and postnatal survival, and pup body weight. Teratogenicity not evaluated.	Schuler et al., 1984.

Table 1. Summary Table for Diethylene Glycol Monobutyl Ether						
Species and Study Type	**Exposure**	**Critical Effects**	**NOAEL**	**LOAEL**	**Comments**	**Reference**
Inhalation Study						
Rat Subchronic	0, 2, 6, or 18 ppm (0, 13, 40, or 119 mg/m^3) for 6 hours/day, 5 days/week, for up to 22 exposures in 5 weeks. (average daily concentration: 0, 2.3, 7.1, or 21.3 mg/m^3)	Liver histopathology (slight hepatocyte vacuolization consistent with fatty change) in females at ≥6 ppm and gross paleness of the liver in 3/10 females at 18 ppm.	2 ppm (average concentration of 13 mg/m^3)	6 ppm (average concentration of 40 mg/m^3)	Endpoints included clinical signs, body weight, hematology, clinical chemistry, urinalysis, organ weights, gross pathology, and histopathology. Histological exams were comprehensive at 0 and 18 ppm but limited to the liver at 2 and 6 ppm. Study used to derive subchronic and chronic RfCs in previous PPRTV document.	Gushow et al., 1984.

In an earlier drinking water study, groups of Sherman rats (5/sex/group) were exposed to DGBE (reported as butyl carbitol) in drinking water at reported doses of 0, 51, 94, 210, 650, 970, or 1,830 mg/kg-day for 30 days (Smyth, 1940; Smyth and Carpenter, 1948). The study was summarized only briefly. Endpoints included mortality, water intake, body weight, and histology of the liver, kidney, spleen, and testis. No effects were observed at 51 mg/kg-day. Deaths did not occur at any dose. Water intake was reduced at ≥94 mg/kg-day, but growth was not statistically significantly affected at any dose level. Histopathological changes occurred at ≥650 mg/kg-day that included cloudy swelling and increased secretion in the kidney tubules and congestion and slight cloudy swelling of the liver. The limited scope and inadequate reporting of this study precludes identification of a NOAEL or LOAEL.

Subchronic Studies—Groups of 10 male albino rats (Charles River COBS, CD, BR) were administered 0, 891, 1,782, or 3,564 mg/kg-day doses of undiluted DGBE (>99.5% pure) by gavage on 5 days/week, for 6 weeks (Kodak, 1984). The group of control rats was treated with distilled water equal in volume to that of the highest-dose group. Endpoints included clinical condition, food consumption and body weight, measured hematology values (Hgb, Hct, RBC, and total and differential white blood cell counts [WBC]) and calculated red blood cell indices (mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], and mean corpuscular hemoglobin concentration [MCHC]), several serum chemistry indicators of liver damage and kidney function (alanine aminotransferase [ALT or SGPT], aspartate aminotransferase [AST or SGOT], alkaline phosphatase [ALP], lactate dehydrogenase [LDH], blood urea nitrogen [BUN], creatinine, and glucose), organ weights (liver, kidneys, heart, testes, brain, and spleen), and comprehensive gross and histopathology (~30 tissues). Deaths attributed to gavage error occurred in the high- (4/10), mid- (2/10), and low- (1/10) dose groups. An additional two rats in the high-dose group were killed in moribund condition. Effects observed in the high-dose group included clinical signs of toxicity (dyspnea, prostration, and unkempt hair coat, as well as bloody urine and blood around the nose and mouth in one animal), reduced body-weight gain and food consumption, and histopathological changes in the spleen (congestion, red pulp hypocellularity, and hemosiderin-like pigmentation). Statistically significant effects occurring in the mid- and high-dose groups included hematologic indications of RBC damage (clear dose-related reductions in RBC [14% and 28%], Hgb [11% and 15%], and MCHC [9% and 18%] and increases in MCV [14% and 45%] and MCH [18% at high dose only], with no effect on total or differential WBC), decreased serum glucose concentration, increased absolute and relative liver and spleen weights, and histopathological changes in the kidneys (hyaline droplet degeneration, proteinaceous casts, and hemosiderin in the proximal tubules). The investigators noted that the renal proteinaceous casts and hemosiderin appeared to be compound-related but may have been secondary to the hematological effects, and that the significance of the hyaline droplet degeneration is uncertain because it was also seen in all 10 control rats. The only clear treatment-related effect at the low dose was hyperkeratosis of the stomach (10/10), which was also observed in the mid- and high-dose groups (10/10 and 8/10 incidences, respectively) compared to 0/10 in the controls. The human relevance of the gastric hyperkeratosis is questionable due to the bolus (undiluted gavage) type of exposures and likely direct irritant properties of DGBE, as indicated by aspiration-related respiratory tract lesions after gavage exposure in the Hobson et al. (1987) study summarized below. Based on the hematological effects, the NOAEL and LOAEL of 891 and 1,782 mg/kg-day were identified, and the corresponding average daily doses were 636 and 1,273 mg/kg-day.

A 13-week study was conducted, in which groups of 16 male and 16 female F344 rats were treated with DGBE (99% pure) by gavage in water (0.2% of body weight) 5 days/week (Hobson et al., 1987). Reported dose levels were 0, 70, 330, and 1,630 mg/kg-day in the males and 0, 50, 250, and 1,270 mg/kg-day in the females. An interim sacrifice was conducted at 6 weeks, after which all groups except the high dose consisted of 10 rats/sex; the high-dose group had 4 rats/sex due to early mortality, as discussed below. Endpoints included clinical condition, food consumption and body weight, hematology (RBC and platelet counts, Hgb, Hct, MCV, MCHC, and total and differential WBC), serum chemistry (11 indices, including BUN), urine chemistry (14 indices, including blood in urine), organ weights, and comprehensive gross and histopathology (~30 tissues, but not including stomach). A dose-related increase in mortality occurred in mid- and high-dose rats of both sexes. Deaths occurred in mid-dose males starting in Week 8, mid-dose females starting in Week 4, and high-dose rats of both sexes starting in Week 1. Surviving to termination were 10, 10, 4, and 2 males and 10, 9, 8, and 1 females in the control, low-, mid-, and high-dose groups, respectively. The small numbers of survivors in the high-dose group precluded statistical analysis of endpoints evaluated at termination for this group.

Other effects in the mid- and high-dose rats of both sexes included inflammatory lesions in the respiratory tract (Hobson et al., 1987). Respiratory tract lesions that were commonly observed in the females at ≥250 mg/kg-day and males at ≥330 mg/kg-day included acute rhinitis, laryngitis, tracheitis, pulmonary congestion, and edema. Mild squamous metaplasia of the nasal epithelium was found in two mid-dose and five high-dose males and one high-dose female. The study authors suggested that these effects, as well as sporadic observations of foreign body pneumonia and acute pleuritis, indicated that gavage-related aspiration of DGBE may have occurred, contributing to mortality in some of the rats. Study authors reported that over the entire study, respiratory tract lesions in three males and six females in the mid-dose groups and four males and five females in the high-dose groups were consistent with gavage accident. Although a number of the deaths were clearly attributable to gavage error, the dose-related distribution of the mortality suggests that some deaths were related to systemic toxicity. Histological findings in the other mid- and high-dose rats (i.e., unclear pathogenesis of respiratory lesions) and experimental observations by the dosing technicians also did not completely support a definitive relationship between gavage procedure and mortality. The lack of histological examinations of the stomach precludes possibly corroborating the gastric hyperkeratosis observed in the Kodak (1984) 6-week study. Gastric hyperkeratosis is not necessarily expected to have been induced in the Hobson et al. (1987) study due to testing of lower doses diluted in water compared to higher undiluted doses in the Kodak (1984) study discussed above.

Hematological effects after 13 weeks of DGBE exposure (Hobson et al., 1987) included dose-related decreases in total WBC and lymphocyte counts in surviving females at 50 (by 32.1% in WBC and 34.9% in lymphocyte counts) and 250 mg/kg-day (by 42.6% in WBC and 47.9% in lymphocyte counts) (see Table 2). No decrease in these parameters was seen in the lone surviving female at 1,270 mg/kg-day or in males at any dose at 13 weeks. RBC and Hgb were not significantly reduced at any dose in either sex at 13 weeks. However, the researchers reported that at 6 weeks (i.e., before all the animals died), RBC and Hgb were significantly decreased (data not reported) in high-dose males and females, and there were dose-dependent hemoglobinuria in males and females exposed to DGBE. In addition, lymphocyte counts were significantly decreased (data not reported) in high-dose DGBE males; nevertheless, neutrophil

counts were significantly increased in mid- and high-dose males at the interim sacrifice. At 13 weeks, nonhematological effects of DGBE that were statistically significant ($p < 0.05$) included increased absolute liver weight in males at ≥70 mg/kg-day, increased relative liver weight in males at 330 mg/kg-day, increased BUN and serum alkaline phosphatase values in males at ≥330 mg/kg-day, and increased hyaline droplet formation in the renal tubular epithelium in females at 1,270 mg/kg-day. The hyaline droplets in females were considered by the researchers to be indicative of hemoglobinuria. Renal hyaline droplets were commonly observed in males of all dose and control groups and thereby regarded as being within normal physiologic limits. The available data indicate that hematological changes are the most sensitive effects of DGBE and that the 50 mg/kg-day-dose (average daily dose of 36 mg/kg-day) is most appropriately classified as a LOAEL based on the induction of lymphopenia in only females, and no lymphopenia in the high-dose female rat. MCHC was also reduced in females at ≥50 mg/kg-day, but is not clearly reflective of an adverse effect in the absence of significant decreases in RBC count, Hgb, or Hct.

Table 2. Data Sets for Leukopenia/Lymphopenia in Female Rats Exposed to DGBE by Gavage for 13 Weeks[a]

Endpoint (Mean ± SD)	Duration-Adjusted Dose[b] (mg/kg-day)		
	0	**35.7**	**178.6**
Number Examined	7	9	7
WBC count ($\times 10^3/mm^3$)	4.46 ± 0.73	3.03 ± 0.59[c] (67.9%)[d]	2.56 ± 0.31[c] (57.4%)
Lymphocyte count ($\times 10^3/mm^3$)	3.55 ± 0.6	2.31 ± 0.42[c] (65.1%)	1.85 ± 0.31[c] (52.1%)

[a]Hobson et al. (1987)
[b]Duration-Adjusted Dose = Dose × 5/7 days
[c]Statistically significantly different from controls ($p < 0.05$)
[d]Percentage relative to the control

Note: the results from the high-dose group are not presented because this dose group was dropped from further analysis due to high mortality.

Johnson et al. (2005) administered DGBE (99.2% pure) in drinking water daily to groups (10 rats/sex) of F344 rats at doses of 0, 50, 250, or 1,000 mg/kg-day for 13 weeks. Animals were observed twice daily for general appearance and were assessed weekly for detailed clinical evaluation, body weight, and food and water consumption. Ophthalmological examinations and a behavioral functional observational battery (FOB) were administered before initiation of exposure and during the last week of the study. Clinical pathology, including hematology, serum chemistry, and urinalysis, was assessed prior to sacrifice and necropsy. Evaluations of sperm and liver metabolic enzymes were also made prior to necropsy. A comprehensive necropsy, including organ-weight determinations, was conducted for all rats. Comprehensive histopathological evaluations were made for control and high-dose rats, with subsequent evaluations of selected organs from the low- and mid-dose groups as indicated based on findings at the high dose.

All rats survived to study termination, and other than some urine soiling in the high-dose group, there were no treatment-related clinical signs (Johnson et al., 2005). Decreases in water and food consumption and concomitant reductions in body weight were noted in both sexes at the high dose throughout the study, with the greatest reductions noted in males; body weight at the end of the study was reduced by 10% and 6% in males and females, respectively, in comparison with controls. Water consumption among high-dose rats was 7−8% less than controls throughout the study, and weekly food consumption among high-dose rats was 5−11% less than control values. No treatment-related effects on body weight or food or water consumption were noted in the mid- and low-dose groups. No treatment-related effects on urinalysis, ophthalmology or the FOB (sensory evaluation, rectal temperature, grip performance, motor activity) were observed for any treatment group (data not shown). There were small, but statistically significant, dose-related decreases in RBC hematological variables relative to controls in mid- (RBC, Hgb) and high-dose (RBC, Hgb, Hct) rats of both sexes. The decreases ranged from 2−4% at 250 mg/kg-day and 5−9% at 1,000 mg/kg-day. RBC counts in both sexes and Hgb in males were outside of the historical control range for the similar study in the same strain rats (see Table 3). There were no other treatment-related effects on hematological variables, including total and differential WBC (data not shown).

Table 3. Data Sets for Anemia in Male and Female Rats Exposed to DGBE in Drinking Water for 13 Weeks[a]				
	Dose (mg/kg-day)			
Endpoint	**0**	**50**	**250**	**1000**
Males				
Number Examined	10	10	10	10
RBC Count ($\times 10^6$/μL)	9.27 ± 0.35	9.13 ± 0.22 (98.5%)[c]	8.94 ± 0.34[b] (96.4%)	8.53 ± 0.31[b] (92.0%)
Hgb (g/dL)	15.8 ± 0.5	15.7 ± 0.4 (99.3%)	15.3 ± 0.4[b] (96.8%)	14.8 ± 0.4[b] (93.6%)
Females				
Number Examined	10	10	10	10
RBC Count ($\times 10^6$/μL)	8.26 ± 0.22	8.06 ± 0.31 (97.6%)	8.07 ± 0.24[b] (97.7%)	7.54 ± 0.17[b] (91.3%)
Hgb (g/dL)	15.6 ± 0.3	15.2 ± 0.4 (97.4%)	15.2 ± 0.4[b] (97.4%)	14.8 ± 0.3[b] (94.9%)

[a]Johnson et al. (2005)
[b]Statistically significantly different from controls ($p < 0.05$)
[c]Percentage relative to the control

Note: historical control ranges: RBC in males 9.11−9.22 (10^6/μL), RBC in females 7.81−8.20 (10^6/μL), Hgb in males 15.0−15.6 (g/dL), and Hgb in females 14.3−14.9 (g/dL).

Clinical chemistry findings were generally unremarkable, although there were slight decreases in serum protein, cholesterol, and AST in high-dose males and females (Johnson et al., 2005). Liver metabolic enzymes were slightly increased relative to controls only for high-dose animals as follows: EROD[1] (24% both sexes); PROD[2] (38% males, 24%, females); and UGT[3] (17% males, 16% females). Organ weight results included increased absolute and relative liver and kidney weights in the high-dose groups of both sexes, despite decreased body weight in these groups. Absolute and/or relative spleen weights were increased in all male and female dose groups, but the changes from controls were small (absolute weight changes ≤6% in males and ≤3% in females) and did not increase with dose. The only treatment-related histopathological changes were noted in the livers of high-dose females. These changes were considered by the study authors to be treatment-related and consisted of hepatocellular hypertrophy ("very slight" in 6/10 high-dose females) and foci of necrotic cells in the centrolobular region. No treatment-related histopathologic changes were noted in any other tissues, including kidneys, spleen, or male livers (incidence data not shown). No treatment-related effects on sperm count, sperm morphology or motility, or male reproductive tissues were observed (data not shown). The NOAEL for this study is 50 mg/kg-day, and the LOAEL is 250 mg/kg-day on the basis of significantly reduced RBC count and Hgb in both sexes.

Chronic Studies—No pertinent data were located regarding health effects of DGBE in animals following chronic oral exposure.

Reproductive/Developmental Studies—Groups of 25 male and 25 female Charles River CD rats were treated with 0, 250, 500, or 1,000 mg/kg-day doses of DGBE (95% pure) by gavage in distilled water in a fertility study (Nolen et al., 1985). Untreated males were bred to treated females and vice versa. The males were treated for 60 days prior to mating, and the females were treated from 14 days prior to mating to gestation day (GD) 13 or until weaning of the offspring. Half of the females in each group were killed on GD 13 for examination of uterine contents. The remaining females delivered their young, and the offspring were followed to weaning. Endpoints included weekly body weight in both sexes; numbers of corpora lutea, implants, resorptions, viable embryos, and live or dead pups; and clinical condition, body weight, and external development in pups. The only effect attributable to treatment was a slight (~8%) reduction in pup weight during the last week of lactation among the offspring of the females dosed with 1,000 mg/kg-day relative to the control group. The NOAEL and LOAEL were 500 and 1,000 mg/kg-day, respectively, for mild toxicity to the neonate, while no maternal or reproductive effects were found even at 1,000 mg/kg-day.

These findings are supported by another study in rats. Groups of 19−21 pregnant Wistar rats were fed DGBE (purity not reported) in the diet at reported intake levels of 0, 25, 115, or 633 mg/kg-day from GD 0−20 (Ema et al., 1988). Some 14 or 15 rats in each group were killed on Day 20 for uterine and fetal examinations, and the remaining 5 or 6 rats/group were allowed to deliver spontaneously. From each litter, eight pups (four rats/sex) were reared until 10 weeks

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[1]Ethoxyresorufin-O-dealkylase.
[2]Pentoxyresorufin-O-dealkylase.
[3]UDP-glucuronosyltransferase.

of age. Maternal endpoints included daily evaluations for clinical signs of toxicity and effects on food consumption and body weight. Prenatal developmental endpoints included numbers of litters and corpora lutea, implantations and live fetuses per litter, pre and postimplantation losses, fetal and placental weights, sex ratio, external and oral cavity anomalies, skeletal anomalies (approximately half the fetuses in each litter), and internal anomalies (remaining half of the fetuses). Postnatal endpoints included gestation length, numbers of live newborns, body weight, and survival rate. The only effect noticed was reduced maternal body-weight gain during pregnancy at ≥25 mg/kg-day (12−18% lower than controls, $p < 0.05$). The maternal body-weight gain is not only affected by changes in the maternal weight, but also changes in litter size and fetal body weight. Thus, the absolute changes in maternal weight during the gestation in any of these dose groups were relatively small (<10%); therefore, this endpoint is not considered to be an adverse response to DGBE. The lack of any prenatal or postnatal developmental effects indicates that 633 mg/kg-day is a NOAEL for developmental as well as maternal toxicity.

Similar results were found in mice. In a screening assay, groups of 50 pregnant CD-1 mice were treated with 0 or 500 mg/kg doses of DGBE (>99% pure) by gavage in aqueous solution on GD 7−14 (Schuler et al., 1984). Study endpoints included pup survival in utero (percent of live litters/pregnant survivors), pup perinatal and postnatal survival (numbers of live and dead pups per litter and pup survival to age 2.5 days), and pup body weights (at birth and age 2.5 days). Maternal indices included body weight on GD 7, GD 18, and Day 3 postpartum. No treatment-related maternal, litter, or pup effects were observed, indicating that 500 mg/kg-day was a NOAEL for maternal and developmental toxicity.

Inhalation Exposure
Chronic Studies—No pertinent data were located regarding health effects of DGBE in animals following chronic inhalation exposure.

Subchronic Studies—In a subchronic inhalation study, groups of 15 male and 15 female F344 rats were exposed to 0, 2, 6, or 18 ppm of DGBE (~98.6% pure) (0, 13, 40, or 119 mg/m^3) for 6 hours/day, 5 days/week, for up to 22 exposures in 5 weeks (Gushow et al., 1984). The 119 mg/m^3 concentration was the highest sustainable vapor concentration of DGBE due to its low vapor pressure. RBC fragility was tested in five rats/sex/group following the 15th exposure. Endpoints evaluated in the remaining 10 rats/sex/group included clinical condition and body weight throughout the study; hematology (RBC, total and differential WBC, platelet, Hgb, Hct, MCV, MCH, and MCHC), clinical chemistry (BUN, ALT, ALP, glucose, albumin, total protein, and total bilirubin), and urine chemistry (pH, glucose, ketones, bilirubin, urobilinogen, occult blood, protein, and specific gravity) after 16−22 exposures; and organ weights, gross pathology, and histology after 22 exposures. The histological examinations were comprehensive (including nasal turbinates and lungs) in the control and 119-mg/m^3 groups but limited to the liver in the 13 and 40 mg/m^3 groups because this was the only tissue identified as a possible target at 119 mg/m^3. Statistically significant ($p < 0.05$) decreases in serum glucose levels were observed in females at ≥13 mg/m^3 and males at ≥40 mg/m^3, but the reductions were minimal (9−16% lower than controls). Other effects included slightly increased WBC in males (within the normal range of variation and not considered to be toxicologically significant by the researchers) and hepatic changes in both sexes at ≥40 mg/m^3. The hepatic effects included inconsistent changes in relative liver weight (decreased in males at ≥40 mg/m^3 and increased in

females at 119 mg/m^3) with no effects on absolute liver weight, histological changes (slight hepatocyte vacuolization consistent with fatty change) in females at ≥40 mg/m^3, and gross paleness of the liver in 3/10 females at 119 mg/m^3. Hepatocytes in the control and 13-mg/m^3 females were vacuolated to a somewhat lesser degree (i.e., very slight), indicating that the effect was only minimally increased at ≥40 mg/m^3. No other biologically significant effects were found in clinical chemistry, hematology, and urine chemistry. The low concentration of DGBE (i.e., 13 mg/m^3) is identified as a NOAEL based on significant incidences of hepatocyte vacuolization consistent with fatty change that occurred in female rats at ≥40 mg/m^3 and is supported by increased relative liver weight at 119 mg/m^3.

Other Studies

There are several short-term and subchronic toxicity studies (Proctor and Gamble, 1982; Bio/dynamics, Inc., 1989; Auletta et al., 1993), reproductive toxicity studies (Bio/dynamics, Inc., 1989; Auletta et al., 1993), and a subchronic neurotoxicity study (Bio-Research Labs, 1989) with dermal exposure to DGBE. The only changes reported by Proctor and Gamble (1982) (hematological parameters or hematuria) and those (occult blood in the urine) reported by Bio/dynamics, Inc. (1989) and Auletta et al. (1993) are consistent with the effects that were seen following oral exposure to DGBE.

Genotoxicity

The genotoxicity of DGBE has been tested in several assay systems. In in vitro assays, DGBE was negative for reverse mutation in *Salmonella typhimurium* with or without metabolic activation (Thompson et al., 1984; Zeiger et al., 1992), unscheduled DNA synthesis in primary rat hepatocytes (Thompson et al., 1984), forward mutation (HGPRT locus) in Chinese hamster ovary (CHO) cells (Gollapudi et al., 1993) and sister chromatid exchanges (SCEs) in CHO cells (Thompson et al., 1984), and weakly positive for forward mutations in mouse lymphoma L5178Y cells (Thompson et al., 1984). In vivo testing of DGBE was negative for sex-linked recessive mutations in *Drosophila melanogaster* (Thompson et al., 1984) and induction of micronuclei in bone marrow cells of mice (Gollapudi et al., 1993).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfD VALUES FOR DIETHYLENE GLYCOL MONOBUTYL ETHER (DGBE)

Information on systemic effects of repeated oral exposure to DGBE is available from several studies in rats as summarized in Table 1. A range-finding study was conducted in which groups of five Sherman rats/sex that were exposed to DGBE in drinking water at doses ranging from 51−1,830 mg/kg-day for 30 days had no effects at 51 mg/kg-day, reduced water intake at ≥94 mg/kg-day, and histopathological changes in the kidneys and liver at ≥650 mg/kg-day (Smyth, 1940; Smyth and Carpenter, 1948). However, a NOAEL or LOAEL cannot be identified from this study due to the limited number of endpoints investigated and inadequate reporting. A more recent range-finding study (Johnson et al., 2005) in which groups of five F344 rats/sex were exposed to DGBE in drinking water at doses of 1,000−2,000 mg/kg-day for 2 weeks noted decreases in body weight, food consumption, water consumption, RBC, Hgb, and Hct; however, these changes were not statistically significant in comparison with controls.

In a 6-week gavage study, DGBE was administered to groups of 10 male rats (Charles River COBS, CD, BR) at doses of 891, 1,782, or 3,564 mg/kg-day on 5 days/week, for 6 weeks (Kodak, 1984). This study identified a hematological NOAEL and LOAEL of 891 and 1,782 mg/kg-day (average daily doses of 636 and 1,273 mg/kg-day), respectively, based on reduced RBC counts, Hgb, and MCHC, with related effects, including bloody urine, occurring at 3,564 mg/kg-day. In a 13-week study, DGBE was administered to groups of 16 male/16 female F344 rats at doses of 0, 50/70, 250/330, or 1,270/1,630 mg/kg-day by gavage on 5 days/week, for 13 weeks (Hobson et al., 1987). This study identified a LOAEL of 50 mg/kg-day (36 mg/kg-day average daily dose) based on leukopenia and lymphopenia in female rats (Hobson et al., 1987). In a subsequent comprehensive drinking water study, groups of 10 F344 rats/sex were exposed to DGBE in drinking water at doses of 0, 50, 250, and 1,000 mg/kg-day for 13 weeks (Johnson et al., 2005). This study identified a NOAEL of 50 mg/kg-day based on the critical effects of decreased RBC and Hgb noted at doses ≥250 mg/kg-day in both sexes.

The database for DGBE consistently indicates that hematological effects are the most sensitive response after exposure to DGBE; however, inconsistent findings of leukopenia and lymphopenia were reported. The most sensitive response was reported from Hobson et al. (1987), noting leukopenia and lymphopenia in female rats at doses ≥50 mg/kg-day. However, confidence in the findings from this study (Hobson et al., 1987) is limited by gavage accidents and associated deaths and respiratory tract pathology in the higher-dose groups (≥250 mg/kg-day), inconsistent changes in neutrophil counts (increased) in male rats in the mid- and high-dose groups (≥330 mg/kg-day) at 6 weeks, lack of leukopenia and lymphopenia in male rats at the end of experiment, and no similar responses in the lone surviving female in the highest-dose group. In addition, neither leukopenia nor lymphopenia was reported in the same strain of rats in a 13-week drinking water study (Johnson et al., 2005) at dose levels as high as 1,000 mg/kg-day, and no similar effects were found in male rats in the Kodak (1984) 6-week gavage study (females were not tested) at doses at high as 3,564 mg/kg-day. In contrast to the leukopenia and lymphopenia responses, consistent findings of decreased RBC counts and Hgb levelswere reported in male rats treated with DGBE ≥1,782 mg/kg-day for 6-weeks (Kodak, 1984), in male rats at a dose level of 1,630 mg/kg-day and female rats at a dose level of 1,270 mg/kg-day at 6-week interim sacrifice (Hobson et al., 1987), and in both sexes at a dose level of ≥250 mg/kg-day at the end of 13 weeks of exposure (Johnson et al., 2005). Therefore, decreased RBC counts and Hgb levels after 13-weeks of treatment (Johnson et al., 2005) are considered the critical effects for the DGBE-induced hematological response.

There are no indications that reproductive or developmental toxicity are effects of concern for DGBE, as multiple oral studies in rats and mice found no effects at doses of 500 and 633 mg/kg-day and only a mild, transitory effect on neonate weight at 1,000 mg/kg-day (Ema et al., 1988; Nolen et al., 1985; Schuler et al., 1984). Dermal studies also showed no effects on reproduction or development (Bio/dynamics, Inc., 1989; Nolen et al., 1985).

There are no indications that neurotoxicities are effects of concern for DGBE, as an oral study in rats found no behavioral effects at doses of 50, 250, or 1,000 mg/kg-day (Johnson et al., 2005). A dermal study in rats also showed no effects on a number of neurotoxicity endpoints at doses corresponding to 200, 600, or 2,000 mg/kg (Bio-Research Labs, 1989).

Based on the available studies, a potential point of departure (POD) for the derivation of p-RfD values is the NOAEL of 50 mg/kg-day for anemia (reduced RBC counts, Hgb levels) in rats (Johnson et al., 2005). The relevant data sets for these endpoints are shown in Table 3 (Johnson et al., 2005). No duration adjustment for the doses for the Johnson et al. (2005) study is needed because exposure was continuous for the duration of the study. Attempts to apply BMD modeling to the data sets (RBC and Hgb) were successful for RBC counts in male (BMDL of 81 mg/kg-day) and female rats (BMDL of 280 mg/kg-day), and for Hgb levels in males (BMDL of 328 mg/kg-day) but not in females (Appendix A). Because the changes in Hgb levels in females were comparable to those in males (see Table 3), a POD for this endpoint is expected to be at the range close to 328 mg/kg-day based on male data. Thus, the lowest BMDL of 81 mg/kg-day (based on changes in RBC in male rats) among these four endpoints is the most sensitive POD and is used for the derivation of both subchronic and chronic p-RfDs.

Subchronic p-RfD

A **subchronic p-RfD** is derived by applying an UF of 300 to the BMDL of 81 mg/kg-day as follows:

$$\textbf{Subchronic p-RfD} \quad = \quad \text{BMDL} \div \text{UF}$$
$$= \quad 81 \text{ mg/kg-day} \div 300$$
$$= \quad \textbf{0.3 mg/kg-day or } 3 \times 10^{-1} \textbf{ mg/kg-day}$$

The composite UF is composed of the following factors:
- An UF of 10 is applied for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans.
- An UF of 10 for intraspecies differences is used to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
- An UF for LOAEL to NOAEL extrapolation is not needed because the POD is a BMDL.
- An UF of 3 ($10^{0.5}$) is applied to account for deficiencies in the database. The database includes three subchronic studies in rats, two developmental studies in rats and mice, and a one-generation reproductive study in rats; however, the database lacks a multigeneration reproduction study and a subchronic study in a second species.

Confidence in the principal study (Johnson et al., 2005) is medium. The principal study was well designed, and the critical effect is consistent with that found from other short-term studies (Kodak, 1984, Hobson et al., 1987). However, a 13-week study (Hobson et al., 1987) exposed F344 rats to DGBE by gavage for the same duration but did not identify a similar response at the end of treatment at doses of $\geq 1,270$ mg/kg-day. Instead, this study (Hobson et al., 1987) identified a LOAEL of 50 mg/kg-day for decreased WBC and lymphocyte counts, which were not shown by Johnson et al. (2005). Confidence in the database is medium due to some inconsistent findings from subchronic studies (Johnson et al., 2005; Hobson et al., 1987), a lack of a multigeneration reproduction study, and a subchronic study conducted in a second species. Overall, confidence in the subchronic p-RfD is medium.

Chronic p-RfD

A **chronic p-RfD** is similarly derived by applying an UF of 3,000 to the BMDL of 81 mg/kg-day as follows:

$$\textbf{Chronic p-RfD} \quad = \quad \text{BMDL} \div \text{UF}$$
$$= \quad 81 \text{ mg/kg-day} \div 3{,}000$$
$$= \quad \textbf{0.03 mg/kg-day or } 3 \times 10^{-2} \textbf{ mg/kg-day}$$

The composite UF is composed of the following factors:

- An UF of 10 is applied for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans.
- An UF of 10 for intraspecies differences is used to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
- An UF for LOAEL to NOAEL extrapolation is not needed because the POD is a BMDL.
- An UF of 10 was applied for using a study with a subchronic duration of exposure to approximate chronic exposure.
- An UF of 3 ($10^{0.5}$) is applied to account for deficiencies in the database. The database includes critical three subchronic studies in rats, two developmental studies in rats and mice, and a one-generation reproductive study in rats; however, the database lacks a multigeneration reproduction study and a subchronic study in a second species.

Confidence in the principal study (Hobson et al., 1987) is medium, as discussed above for the subchronic p-RfD. Confidence in the database is low due to some inconsistent findings from subchronic studies (Johnson et al., 2005; Hobson et al., 1987), a lack of chronic oral toxicity studies, and a lack of a multigeneration reproduction study. Overall, confidence in the chronic p-RfD is low.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR DIETHYLENE GLYCOL MONOBUTYL ETHER (DGBE)

Information on the inhalation toxicity of DGBE is essentially limited to the results of a comprehensive 5-week study in which rats were exposed to 0, 13, 40, or 119 mg/m^3 for 6 hours/day, 5 days/week (Gushow et al., 1984). Evaluation of clinical condition, body weight, hematology (including RBC fragility), blood and urine chemistry, organ weights, gross pathology, and histopathology (including nasal turbinates and lungs) showed slight hepatocyte vacuolization consistent with fatty change in female rats at concentrations of ≥40 mg/m^3 and increased relative liver weight and gross paleness of the liver at 119 mg/m^3. Although there were inconsistent changes (decreases) in relative liver weight in male rats at ≥40 mg/m^3 with no effects on absolute liver weight, the hepatic changes in female rats were considered biologically significant. The potential for hepatic effects is supported by findings of increased liver weight and congestion/cloudy swelling in subchronic oral studies of DGBE (Johnson et al., 2005; Hobson et al., 1987; Kodak, 1984; Smyth, 1940; Smyth and Carpenter, 1948). Exposure to concentrations higher than 119 mg/m^3 could not be tested because this level was the maximum sustainable vapor concentration of DGBE.

Subchronic and chronic p-RfCs were derived for DGBE using the data for hepatocelluar vacuolization in female rats (Gushow et al., 1984) based on U.S. EPA (1994b) RfC methodology. BMD modeling was conducted for the incidence data on hepatocyte vacuolization (Table 4), and detailed results are presented in Appendix A. Because the exposure was not continuous in the principal study (Gushow et al., 1984), duration-adjusted concentrations and human equivalent concentration (HEC) (see Table 4) were calculated before BMD modeling. An example of the duration adjustment calculation is presented below:

$$\text{NOAEL}_{ADJ} = \text{NOAEL mg/m}^3 \times 6/24 \text{ hr} \times 5/7 \text{ d}$$
$$= 13 \text{ mg/m}^3 \times 6/24 \text{ hr} \times 5/7 \text{ d}$$
$$= 2.3 \text{ mg/m}^3$$

For purposes of deriving a p-RfC based on extrarespiratory effects, DGBE was treated as a Category 3 gas. The HEC was calculated assuming periodicity was attained using the following equation:

$$\text{NOAEL}_{HEC} = \text{NOAEL}_{ADJ} \times (H_{b/g})_A/(H_{b/g})_H$$

where $(H_{b/g})_A/(H_{b/g})_H$ is the ratio of blood:gas (air) partition coefficients of the chemical in the test animals and humans. Since a blood:gas partition coefficient is not available for DGBE in humans or rats, a unity value is assumed for the ratio (U.S. EPA, 1994b). The BMD modeling resulted in an estimated BMC_{10ADJ} of 1.7 mg/m^3 and $BMCL_{10ADJ}$ of 0.32 mg/m^3, and the $BMCL_{10ADJ}$ is equivalent to $BMCL_{10HEC}$.

Table 4. Data Sets for Hepatic Changes in Female Rats Exposed to DGBE Through Inhalation for 5 Weeks[a]				
	Concentration (mg/m^3)			
Endpoint	**0**	**13**	**40**	**119**
Females				
Duration-adjusted concentration (mg/m^3)[b]	0	2.3	7.1	21.3
Human equivalent concentration (mg/m^3)[c]	0	2.3	7.1	21.3
# Examined	3	4	10	10
Slight vacuolization consistent with fatty change	3	4	9[d]	10[d]

[a]Gushow et al. (1984)
[b]The duration adjustment was calculated as follows:
Conc$_{ADJ}$ = 119 mg/m^3 × 6/24 hr × 5/7 d = 21.3 mg/m^3
[c]HEC = Conc$_{ADJ}$ × $(H_{b/g})_A/(H_{b/g})_H$
[d]Statistically significantly different from controls ($p < 0.05$)

Subchronic p-RfC

A **subchronic p-RfC** is derived by applying an UF of 300 to the $BMCL_{10HEC}$ of 0.32 mg/m^3 as follows:

$$
\begin{aligned}
\textbf{Subchronic p-RfC} \quad &= \quad BMCL_{10HEC} \div UF \\
&= \quad 0.32 \text{ mg/m}^3 \div 300 \\
&= \quad \textbf{0.001 or } \mathbf{1 \times 10^{-3}} \textbf{ mg/m}^3
\end{aligned}
$$

The composite UF of 300 is composed of the following factors:
- An UF of 3 ($10^{0.5}$) is applied for interspecies extrapolation to account for potential pharmacodynamic differences between rats and humans. Converting the rat data to human equivalent concentrations by the dosimetric equations, accounts for pharmacokinetic differences between rats and humans; thus, an UF of 10 for interspecies extrapolation was not used.
- An UF of 10 for intraspecies differences is used to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
- An UF of 10 is applied to account for deficiencies in the database. The database includes only one 5-week study; it lacks developmental toxicity studies and a multigeneration reproduction study.
- An UF_L is not applied, as a BMCL was used as the POD.
- An UF_S is not applied because the principal study is a subchronic study.

The principal study was well conducted with respect to scope of examinations, numbers of animals, and exposure levels but is given low-to-medium confidence because the duration was short (5 weeks). Confidence in the database is low due to the lack of a 90-day inhalation study, supporting data in a second species, a two-generation reproduction study, and developmental toxicity studies. With regard to the latter, however, it is noteworthy that studies by oral and dermal exposure did not identify reproductive and developmental endpoints as sensitive for this chemical. Low confidence in the subchronic p-RfC follows.

Chronic p-RfC

A **chronic p-RfC** is derived by applying an UF of 3,000 to the $BMCL_{10HEC}$ of 0.32 mg/m^3 as follows:

$$
\begin{aligned}
\textbf{Chronic p-RfC} \quad &= \quad BMCL_{10HEC} \div UF \\
&= \quad 0.32 \text{ mg/m}^3 \div 3,000 \\
&= \quad \textbf{0.0001 mg/m}^3 \textbf{ or } \mathbf{1 \times 10^{-4}} \textbf{ mg/m}^3
\end{aligned}
$$

The composite UF of 3,000 is composed of the following factors:
- An UF of 3 ($10^{0.5}$) is applied for interspecies extrapolation to account for potential pharmacodynamic differences between rats and humans. Converting the rat data to human equivalent concentrations by the dosimetric equations accounts for pharmacokinetic differences between rats and humans; thus, an UF of 10 for interspecies extrapolation was not used.
- An UF of 10 for intraspecies differences is used to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
- An UF of 10 is applied for using a study with a subchronic duration of exposure to approximate chronic exposure.

- An UF$_L$ was not applied, as a BMCL was used as the POD.
- An UF of 10 is applied to account for deficiencies in the database. The database includes only one 5-week study; it lacks chronic toxicity studies, developmental toxicity studies, and a multigeneration reproduction study.

Confidence in the chronic RfC is low, for the same reasons as described above for the subchronic RfC, with the additional shortcoming that chronic studies are also lacking from the database.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR DIETHYLENE GLYCOL MONOBUTYL ETHER (DGBE)

Weight-of-Evidence Descriptor

There is no adequate information on the carcinogenicity of DGBE due to a lack of oral, inhalation, or dermal studies in animals longer than 13 weeks in duration (Johnson et al., 2005; Bio/dynamics, Inc., 1989; Gushow et al., 1984; Hobson et al., 1987; Kodak, 1984). Genotoxicity testing of DGBE in vitro with bacteria and mammalian cells and in vivo with Drosophila and mice have yielded negative results in all but one study (a weakly positive mutagenic response in mouse lymphoma cells in vitro) (Thompson et al., 1984; Zeiger et al., 1992; Gollapudi et al., 1993). Under current U.S. EPA (2005) cancer guidelines, "*there is inadequate information to assess the carcinogenic potential*" of DGBE.

Quantitative Estimates of Carcinogenic Risk

Derivation of quantitative estimates of cancer risk for DGBE is precluded by the lack of carcinogenicity data.

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APPENDIX A. DETAILS OF BENCHMARK DOSE MODELING
FOR SUBCHRONIC/CHRONIC p-RfD

Model Fitting Procedure for Continuous Data

The model fitting procedure for continuous data is as follows. The simplest model (linear) is first applied to the data while assuming constant variance. If the data are consistent with the assumption of constant variance ($p \geq 0.1$), then the fit of the linear model to the means is evaluated, and the polynomial, power, and Hill models are fit to the data while assuming constant variance. Among the models providing adequate fit to the means ($p \geq 0.1$), the one with the lowest Akaike Information Criterion (AIC) for the fitted model is selected for BMD derivation. If the test for constant variance is negative, the linear model is run again while modeling the variance as a power function of the mean to account for nonhomogenous variance. If the nonhomogenous variance model provides an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear model to the means is evaluated, and the polynomial, power, and Hill models are fit to the data and evaluated while the variance model is applied. Among those providing adequate fit to the means ($p \geq 0.1$), the one with the lowest AIC for the fitted model is selected for BMD derivation. If the test for constant variance is negative and the nonhomogenous variance model does not provide an adequate fit to the variance data, then the data set is considered unsuitable for modeling. If after these attempts, no model provides an adequate fit to the data, the highest dose is dropped, if appropriate, and the entire procedure is repeated. If no fit is obtained after dropping the highest dose, the next highest dose is dropped, if appropriate, and the procedure is repeated. Dose-dropping continues until (1) adequate fit is obtained; (2) there are only controls and two dose groups remaining; or (3) it is inappropriate to continue dropping doses due to a lack of statistical significance or biologically important differences between controls and the remaining treatment groups. If no fit is obtained following application of this procedure, then the data set is not considered to be amenable to BMD modeling.

Model Fitting Results for Decreased RBCs in Male Rats (Johnson et al., 2005)

Following the above procedure, continuous-variable models in the EPA Benchmark Dose Software (BMDS) (version 2.1) were fit to the data shown in Table 3 (main text) for decreased RBCs in male rats. In the absence of a biologically relevant response level, the benchmark response (BMR) was chosen to be 1 standard deviation (SD) from the control mean, as recommended by U.S. EPA (2000). As shown in Table A-1, all models provide adequate fit to the data. The Hill model (Figure A-1) estimated a significantly lower BMDL than other models; therefore, the BMDL for this endpoint is 81 mg/kg-day.

Table A-1. Model Predictions for Decreased RBCs in Male Rats[a]					
Data Set/Model	**Variance *p*-Value[b]**	**Means *p*-Value[b]**	**AIC**	**BMD$_{1SD}$ (mg/kg-day)**	**BMDL$_{1SD}$ (mg/kg-day)**
Linear (constant variance)[c]	0.4943	0.454	-50.5176	440.732	328.449
2-Degree Polynomial (constant variance)[c]	0.4943	0.454	-50.5176	440.732	328.449
3-Degree Polynomial (constant variance)[c]	0.4943	0.454	-50.5176	440.732	328.449
Power (constant variance)[d]	0.4943	0.454	-50.5176	440.732	328.449
Hill (constant variance)[d]	**0.4943**	**0.6312**	**-49.8664**	**222.31**	**81.408**

[a]Johnson et al. (2005)
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Coefficients restricted to be *negative*
[d]Power restricted to ≥1

Hill Model with 0.95 Confidence Level
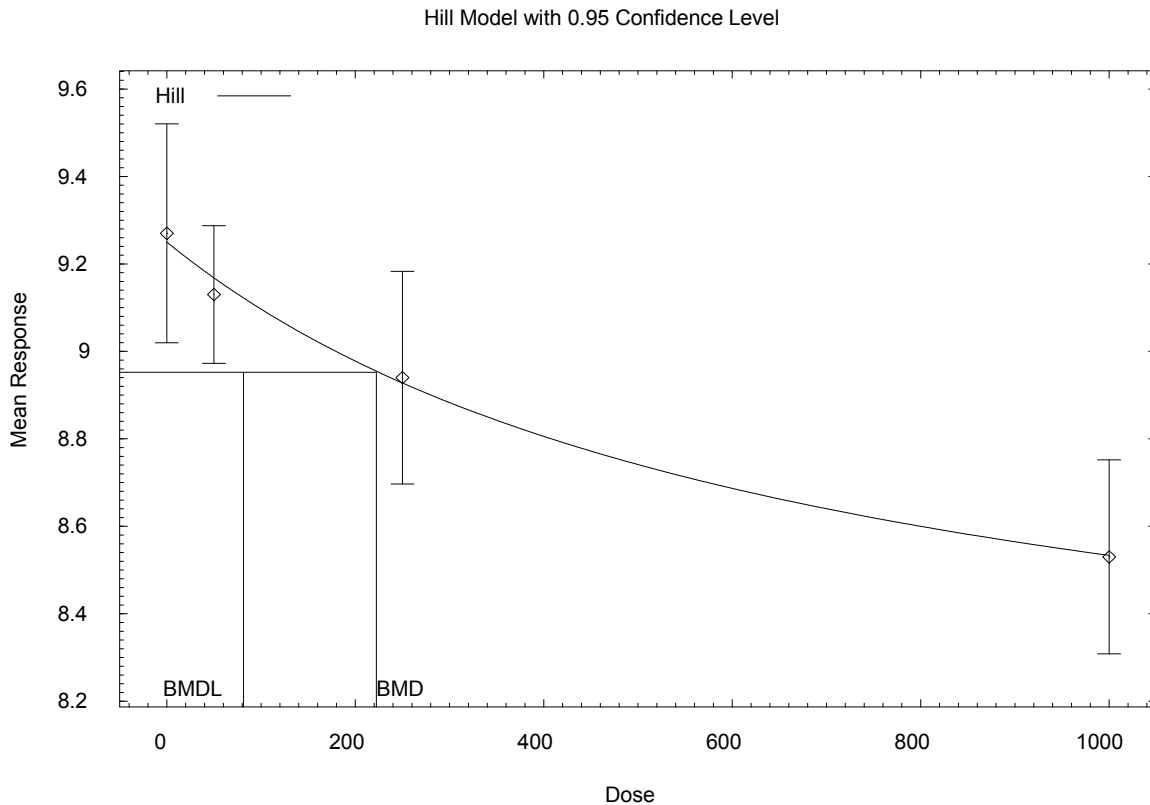
16:27 07/10 2008

**Figure A-1. Fit of Hill Model to Data on Decreased RBCs
in Male Rats (Johnson et al., 2005)**

```
====================================================================
      Hill Model. (Version: 2.14;  Date: 06/26/2008)
      Input Data File: C:\USEPA\BMDS21\Data\hilRBCmaleHill.(d)
      Gnuplot Plotting File: C:\USEPA\BMDS21\Data\hilRBCmaleHill.plt
                                     Fri Aug 28 16:22:41 2009
====================================================================

BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = intercept + v*dose^n/(k^n + dose^n)


  Dependent variable = Mean
  Independent variable = Dose
  rho is set to 0
  Power parameter restricted to be greater than 1
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
```

26

Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

                    Default Initial Parameter Values
                            alpha =   0.09565
                              rho =   0   Specified
                        intercept =   9.27
                                v =   -0.74
                                n =   0.812681
                                k =   323.171

            Asymptotic Correlation Matrix of Parameter Estimates

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

                    alpha   intercept   v   k

       alpha   1   1.8e-009   2.2e-008   -9.9e-009

   intercept   1.8e-009   1   0.33   -0.57

           v   2.2e-008   0.33   1   -0.94

           k   -9.9e-009   -0.57   -0.94   1

                            Parameter Estimates

                                              95.0% Wald Confidence
Interval
        Variable   Estimate   Std. Err.   Lower Conf. Limit   Upper Conf.
Limit
          alpha   0.0865823   0.0193604   0.0486366
0.124528
      intercept   9.24677   0.080516   9.08896
9.40457
              v   -1.20681   0.578497   -2.34065   -
0.0729792
              n   1   NA
              k   689.459   784.134   -847.415
2226.33

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.

      Table of Data and Estimated Values of Interest

  Dose   N   Obs Mean   Est Mean   Obs Std Dev   Est Std Dev   Scaled Res.
  ------   --- --------   --------   -----------   -----------   ----------

      0   10   9.27   9.25   0.35   0.294   0.25
     50   10   9.13   9.17   0.22   0.294   -0.378
    250   10   8.94   8.93   0.34   0.294   0.155
   1000   10   8.53   8.53   0.31   0.294   -0.0263

27

```
Model Descriptions for likelihoods calculated


 Model A1:     Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

 Model A2:     Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:     Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

 Model  R:     Yi = Mu + e(i)
          Var{e(i)} = Sigma^2


                  Likelihoods of Interest

          Model      Log(likelihood)   # Param's      AIC
           A1          29.048402          5        -48.096804
           A2          30.246635          8        -44.493271
           A3          29.048402          5        -48.096804
         fitted        28.933200          4        -49.866400
            R          16.206159          2        -28.412317


                  Explanation of Tests

 Test 1: Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
 Test 2: Are Variances Homogeneous? (A1 vs A2)
 Test 3: Are variances adequately modeled? (A2 vs. A3)
 Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

                  Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df       p-value

   Test 1            28.081            6           <.0001
   Test 2            2.39647           3           0.4943
   Test 3            2.39647           3           0.4943
   Test 4            0.230404          1           0.6312
```

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data

```
         Benchmark Dose Computation

Specified effect =              1

Risk Type        =      Estimated standard deviations from the control mean

Confidence level =          0.95

         BMD =          222.31

        BMDL =        81.4084
```

Model Fitting Results for Decreased RBCs in Female Rats (Johnson et al., 2005)
Following the above procedure, continuous-variable models in the EPA BMDS (version 2.1) were fit to the data shown in Table 2 (main text) for decreased RBCs in female rats. In the absence of a biologically relevant response level, the BMR was chosen to be 1 SD from the control mean, as recommended by U.S. EPA (2000). As shown in Table A-2, the linear and power models (linear model shown in Figure A-2) provide identical fit to the data and yielded AIC values that were significantly lower than the other models fitted. Thus, the estimated BMDL for this endpoint is 280 mg/kg-day.

Table A-2. Model Predictions for Decreased RBCs in Female Rats[a]					
Data Set/Model	Variance *p*-Value[b]	Means *p*-Value[b]	AIC	BMD$_{1SD}$ (mg/kg-day)	BMDL$_{1SD}$ (mg/kg-day)
Linear (constant variance)[c]	**0.3052**	**0.2239**	**-69.286**	**364.222**	**279.607**
2-Degree Polynomial (constant variance)[c]	0.3052	0.0862	-67.3357	408.969	280.868
3-Degree Polynomial (constant variance)[c]	0.3052	0.08802	-67.3694	429.854	281.283
Power (constant variance)[d]	0.3052	0.2239	-69.286	364.876	280.266
Hill (constant variance)[d]	0.3052	0.08352	-67.2847	364.222	279.604

[a]Johnson et al. (2005)
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Coefficients restricted to be *negative*
[d]Power restricted to ≥1

Linear Model with 0.95 Confidence Level
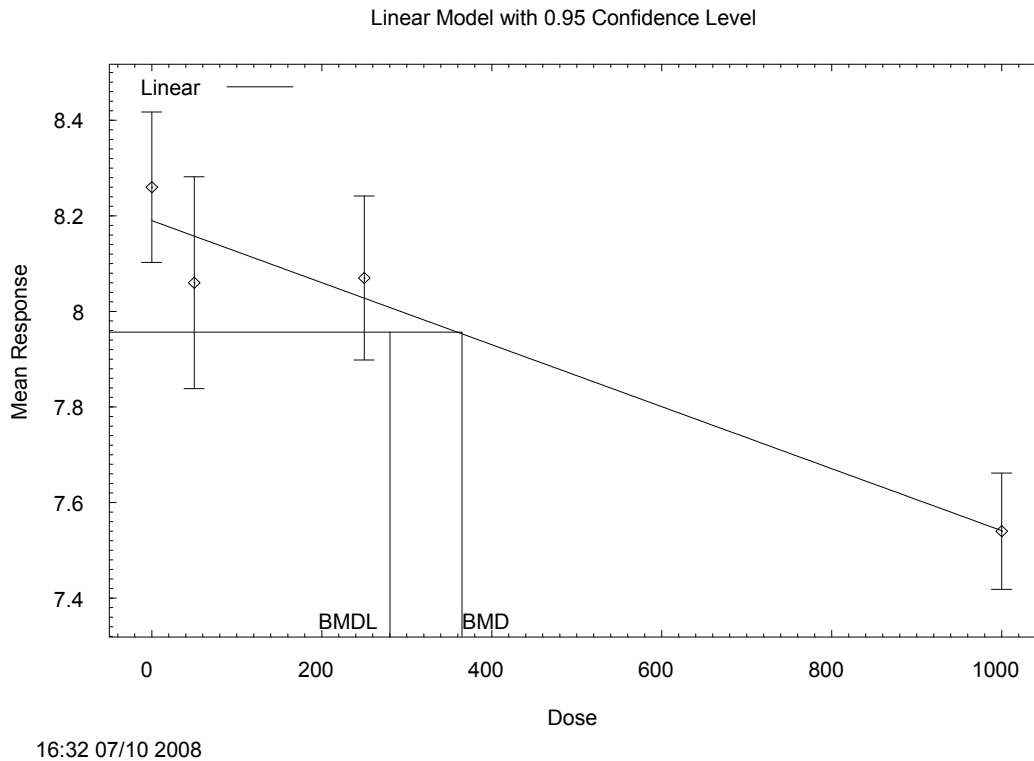

16:32 07/10 2008

**Figure A-2. Fit of Linear Model to Data on Decreased RBCs in Female Rats
(Johnson et al., 2005)**

BMDs and BMDLs indicated are associated with a change of 1 SD from the control and are in units mg/kg-day

```
=====================================================================
        Hill Model. (Version: 2.14;  Date: 06/26/2008)
        Input Data File: C:\USEPA\BMDS21\Data\hilRBCmaleHill.(d)
        Gnuplot Plotting File: C:\USEPA\BMDS21\Data\hilRBCmaleHill.plt
                                        Fri Aug 28 16:57:54 2009
=====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the response function is:

  Y[dose] = intercept + v*dose^n/(k^n + dose^n)


  Dependent variable = Mean
  Independent variable = Dose
  rho is set to 0
  Power parameter restricted to be greater than 1
  A constant variance model is fit

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
```

31

Parameter Convergence has been set to: 1e-008


```
                  Default Initial Parameter Values
                          alpha =      0.05775
                            rho =            0   Specified
                      intercept =         8.26
                              v =        -0.72
                              n =     0.970124
                              k =      490.566
```


                Asymptotic Correlation Matrix of Parameter Estimates

          (*** The model parameter(s) -rho   -n
              have been estimated at a boundary point, or have been specified by
the user,

              and do not appear in the correlation matrix)

```
                 alpha    intercept           v            k

    alpha            1      0.00028      0.0044      -0.0044

intercept      0.00028            1       0.061      -0.065

        v       0.0044        0.061           1           -1

        k      -0.0044       -0.065          -1            1
```


                              Parameter Estimates

                                                     95.0% Wald Confidence
Interval
        Variable   Estimate   Std. Err.   Lower Conf. Limit   Upper Conf.
Limit
          alpha   0.056016   0.0125257   0.0314661
0.0805658
      intercept   8.19343   0.048292   8.09878
8.28808
              v   -240.216   6001.98   -12003.9
11523.5
              n   1   NA
              k   369305   9.25312e+006   -1.77665e+007
1.85051e+007

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.

        Table of Data and Estimated Values of Interest

```
 Dose      N    Obs Mean     Est Mean    Obs Std Dev  Est Std Dev   Scaled Res.
------    ---   --------     --------    -----------  -----------   ----------

    0     10      8.26         8.19         0.22         0.237         0.889
   50     10      8.06         8.16         0.31         0.237        -1.35
  250     10      8.07         8.03         0.24         0.237         0.522
 1000     10      7.54         7.54         0.17         0.237        -0.0632
```

Model Descriptions for likelihoods calculated

Model A1:   Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2

Model A2:   Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

Model A3:   Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
    Model A3 uses any fixed variance parameters that
    were specified by the user

Model R: Yi = Mu + e(i)
          Var{e(i)} = Sigma^2

                    Likelihoods of Interest

           Model   Log(likelihood)   # Param's   AIC
            A1   39.139849   5   -68.279698
            A2   40.951049   8   -65.902099
            A3   39.139849   5   -68.279698
          fitted   37.642370   4   -67.284740
            R   21.815106   2   -39.630212

                    Explanation of Tests

 Test 1: Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2: Are Variances Homogeneous? (A1 vs A2)
 Test 3: Are variances adequately modeled? (A2 vs. A3)
 Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

                    Tests of Interest

   Test   -2*log(Likelihood Ratio)   Test df   p-value

   Test 1   38.2719   6   <.0001
   Test 2   3.6224   3   0.3052
   Test 3   3.6224   3   0.3052
   Test 4   2.99496   1   0.08352

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is less than .1. You may want to try a different
model

        Benchmark Dose Computation

34

```
Specified effect =              1

Risk Type        =      Estimated standard deviations from the control mean

Confidence level =         0.95

            BMD =        364.222

           BMDL =        279.607
```

Model Fitting Results for Decreased Hemoglobin Concentration in Male Rats (Johnson et al., 2005)

Following the above procedure, continuous-variable models in the EPA BMDS (version 1.4.1c) were fit to the data shown in Table 3 (main text) for decreased hemoglobin concentration in male rats. In the absence of a biologically relevant response level, the BMR was chosen to be 1 SD from the control mean, as recommended by U.S. EPA (2000). As shown in Table A-3, the linear, polynomial, and power models provide identical linear fit to the data. Fit to the Hill Model cannot be tested due to insufficient degrees of freedom. Fit to the linear model is illustrated in Figure A-3. For this endpoint, the estimated BMDL is 328 mg/kg-day.

Table A-3. Model Predictions for Decreased Hemoglobin in Male Rats[a]					
Data Set/Model	Variance *p*-Value[b]	Means *p*-Value[b]	AIC	BMD$_{1SD}$ (mg/kg-day)	BMDL$_{1SD}$ (mg/kg-day)
Linear (constant variance)[c]	**0.8494**	**0.2992**	**-23.84147**	**439.943**	**328.015**
2-Degree Polynomial (constant variance)[c]	0.8494	0.2992	-23.84147	439.943	328.015
3-Degree Polynomial (constant variance)[c]	0.8494	0.2992	-23.84147	439.943	328.015
Power (constant variance)[d]	0.8494	0.2992	-23.84147	439.943	328.015
Hill (constant variance)[d]	Fit cannot be tested for this model due to inadequate degrees of freedom				

[a]Johnson et al. (2005)
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Coefficients restricted to be *negative*
[d]Power restricted to ≥1

Linear Model with 0.95 Confidence Level
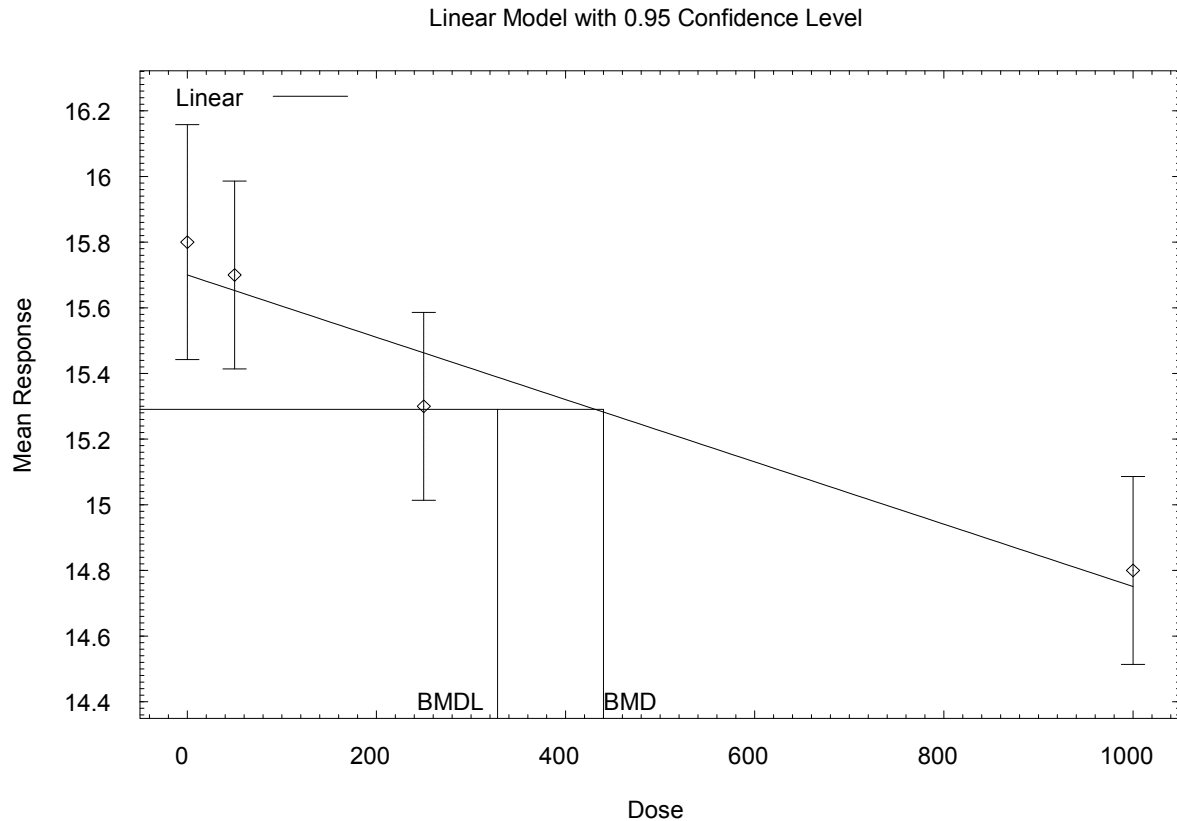
16:41 07/10 2008

**Figure A-3. Fit of Linear Model to Data on Decreased
Hemoglobin Concentration in Male Rats (Johnson et al., 2005)**

BMDs and BMDLs indicated are associated with a change of 1 SD from the control and are in units mg/kg-day

```
=====================================================================
        Polynomial Model. (Version: 2.13;  Date: 04/08/2008)
        Input Data File: C:\USEPA\BMDS21\Data\linRBCmaleLinear.(d)
        Gnuplot Plotting File: C:\USEPA\BMDS21\Data\linRBCmaleLinear.plt
                                        Fri Aug 28 17:11:28 2009
=====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
   Independent variable = Dose
   rho is set to 0
   The polynomial coefficients are restricted to be negative
   A constant variance model is fit
```

```
   Total number of dose groups = 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




                 Default Initial Parameter Values
                        alpha =        0.1825
                          rho =             0   Specified
                       beta_0 =       15.7086
                       beta_1 = -0.000949416


            Asymptotic Correlation Matrix of Parameter Estimates

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

                    alpha        beta_0        beta_1

      alpha             1     -1.3e-009      1.7e-009

     beta_0     -1.3e-009             1         -0.63

     beta_1      1.7e-009         -0.63             1




                         Parameter Estimates

                                                95.0% Wald Confidence
Interval
        Variable         Estimate        Std. Err.     Lower Conf. Limit    Upper Conf.
Limit
          alpha         0.174464        0.0390113          0.0980032
0.250925
         beta_0          15.7086        0.0850278            15.5419
15.8752
         beta_1     -0.000949416     0.000164784          -0.00127239            -
0.000626445



      Table of Data and Estimated Values of Interest

   Dose        N     Obs Mean     Est Mean    Obs Std Dev  Est Std Dev   Scaled Res.
   ------     ---    --------     --------    -----------  -----------   ----------

      0       10       15.8         15.7          0.5          0.418        0.692
     50       10       15.7         15.7          0.4          0.418        0.295
    250       10       15.3         15.5          0.4          0.418        -1.3
   1000       10       14.8         14.8          0.4          0.418        0.309



  Model Descriptions for likelihoods calculated


  Model A1:      Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
```

```
 Model A2:      Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma(i)^2

 Model A3:      Yij = Mu(i) + e(ij)
          Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:       Yi = Mu + e(i)
          Var{e(i)} = Sigma^2
```

```
                    Likelihoods of Interest

            Model       Log(likelihood)   # Param's      AIC
             A1            16.127312           5      -22.254625
             A2            16.527404           8      -17.054808
             A3            16.127312           5      -22.254625
           fitted         14.920736           3      -23.841473
             R             2.835616           2       -1.671231
```

```
                    Explanation of Tests

 Test 1: Do responses and/or variances differ among Dose levels?
         (A2 vs. R)
 Test 2: Are Variances Homogeneous? (A1 vs A2)
 Test 3: Are variances adequately modeled? (A2 vs. A3)
 Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note: When rho=0 the results of Test 3 and Test 2 will be the same.)
```

```
                    Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1           27.3836              6        0.0001227
   Test 2          0.800183              3          0.8494
   Test 3          0.800183              3          0.8494
   Test 4           2.41315              2          0.2992
```

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

The p-value for Test 3 is greater than .1. The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1. The model chosen seems
to adequately describe the data


```
              Benchmark Dose Computation
```

Specified effect =   1

Risk Type   =   Estimated standard deviations from the control mean

Confidence level =   0.95

```
BMD =        439.943

BMDL =       328.015
```

Model Fitting Results for Decreased Hemoglobin Concentration in Female Rats (Johnson et al., 2005)

Following the above procedure, continuous-variable models in the EPA BMDS (version 1.4.1c) were fit to the data shown in Table 3 (main text) for decreased hemoglobin concentration in female rats. In the absence of a biologically relevant response level, the BMR was chosen to be 1 SD from the control mean, as recommended by U.S. EPA (2000). As shown in Table A-4, none of the models provides an adequate fit to the data, even with the highest dose dropped. Dropping the highest dose reduces the data set to controls and two dose groups; therefore, it is not possible to drop further dose groups to attempt to identify a fit. Therefore, this data set is not amenable to BMD modeling.

Table A-4. Model Predictions for Decreased Hemoglobin in Female Rats[a]					
Data Set/Model	**Variance *p*-Value**[b]	**Means *p*-Value**[b]	**AIC**	**BMD$_{1SD}$ (mg/kg-day)**	**BMDL$_{1SD}$ (mg/kg-day)**
All Doses					
Linear (constant variance)[c]	0.652	0.05345	-35.534	579.683	408.287
2-Degree Polynomial (constant variance)[c]	0.652	0.05345	-35.534	579.683	408.287
3-Degree Polynomial (constant variance)[c]	0.652	0.05345	-35.534	579.683	408.287
Power (constant variance)[d]	0.652	0.05345	-35.534	579.683	408.287
Hill (constant variance)[d]	0.652	0.03974	-35.163	127.36	11.921
Highest Dose Dropped					
Linear (constant variance)[c]	0.5991	0.03223	-22.281	331.251	170.263
2-Degree Polynomial (constant variance)[c]	0.5991	0.03223	-22.281	331.251	170.263
Power (constant variance)[d]	0.5991	0.03223	-22.281	331.251	170.263
Hill (constant variance)[d]	# observations < # parameters: cannot run this model				

[a]Johnson et al. (2005)
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Coefficients restricted to be *negative*
[d]Power restricted to ≥1

Modeling Procedure for Hepatocyte Vacuolization in Females (Gushow et al., 1984)

The BMD modeling for dichotomous data was conducted with the EPA's BMD software (BMDS version 2.1). For all the dichotomous data, the incidence data on hepatocyte vacuolization with duration-adjusted concentrations (see Table 4 of main text) were modeled with eight dichotomous models (i.e., Gamma, Multistage, Logistic, Log-logistic, Probit, Log-probit, Weibull, and Quantal-linear models) with a BMR of 10% extra risk. An adequate model fit was judged based on the goodness-of-fit p-value ($p > 0.1$), scaled residual at the range of BMR, and visual inspection of the model fit. As shown in Table A-5 and Figures A-4 and A-5, all the models provided adequate goodness-of-fit p-values (≥ 0.1), and estimated BMCLs from these models are sufficiently close (ranging from 0.32 to 0.60 mg/m^3). Therefore, the BMCL of 0.32 mg/m^3 estimated from the Multistage model (Figure A-5), with the lowest AIC from these five models, was considered to be an appropriate estimate for this endpoint.

Table A-5. Model Predictions for Increased Hepatocyte Vacuolization in Female Rats[a]					
Data Set/Model	**Goodness-of-Fit p-Value[b]**	**AIC**	**Scaled Residual**	**BMC$_{10}$ (mg/m^3)**	**BMCL$_{10}$ (mg/m^3)**
Gamma[c]	0.9917	38.18	0.001	2.0	0.32
Logistic	0.8180	36.58	0.367	0.9	0.52
Log-logistic[d]	0.8523	38.24	0.053	2.1	0.50
Multistage[e]	**0.9889**	**36.20**	**-0.119**	**1.7**	**0.32**
Probit	0.8330	36.54	0.338	0.8	0.55
Log-probit[d]	0.9398	38.19	0.017	2.1	0.60
Weibull[c]	0.9999	38.18	0.000	1.9	0.32
Quantal-linear	0.5132	37.60	0.360	0.48	0.27

[a]Gushow et al. (1984)
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Power restricted to ≥ 1
[d]Slope restricted to ≥ 1
[e]Betas restricted to ≥ 0
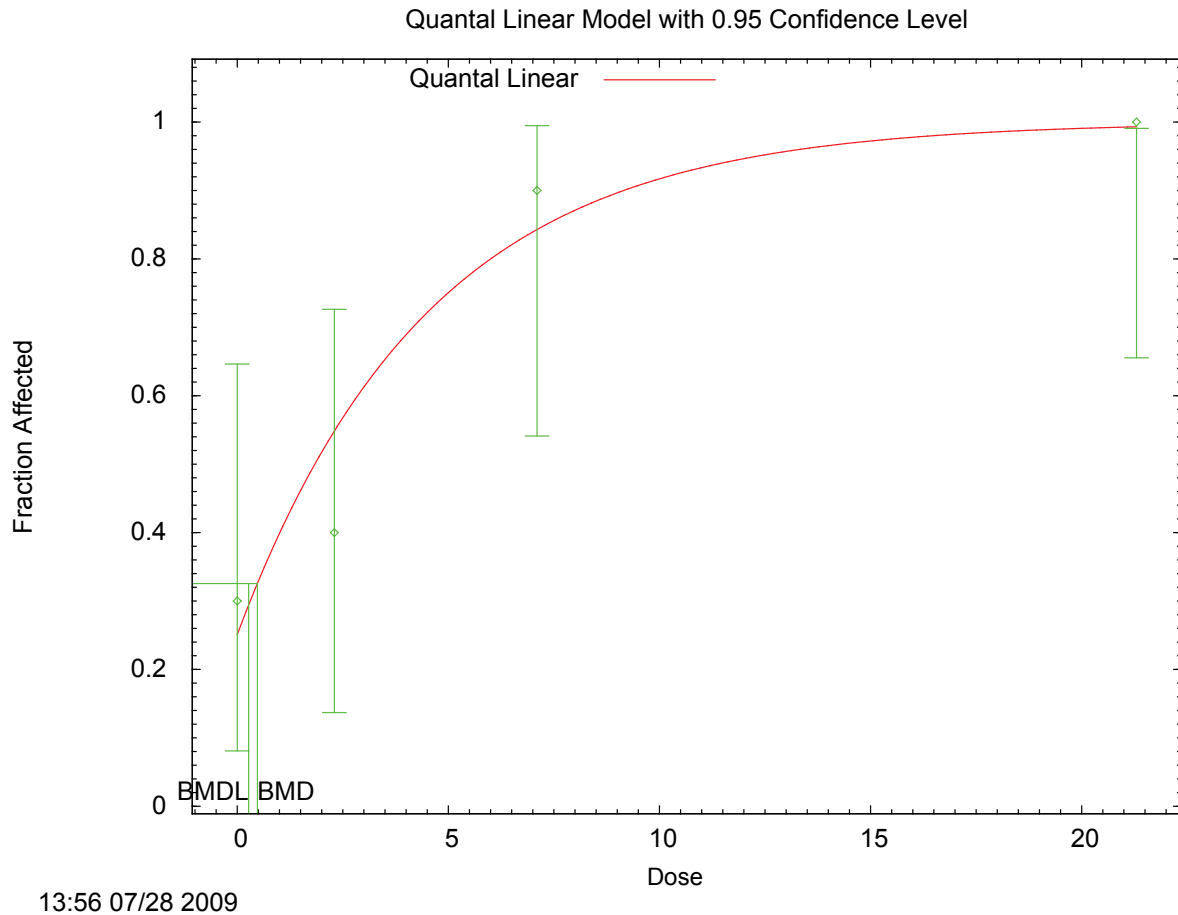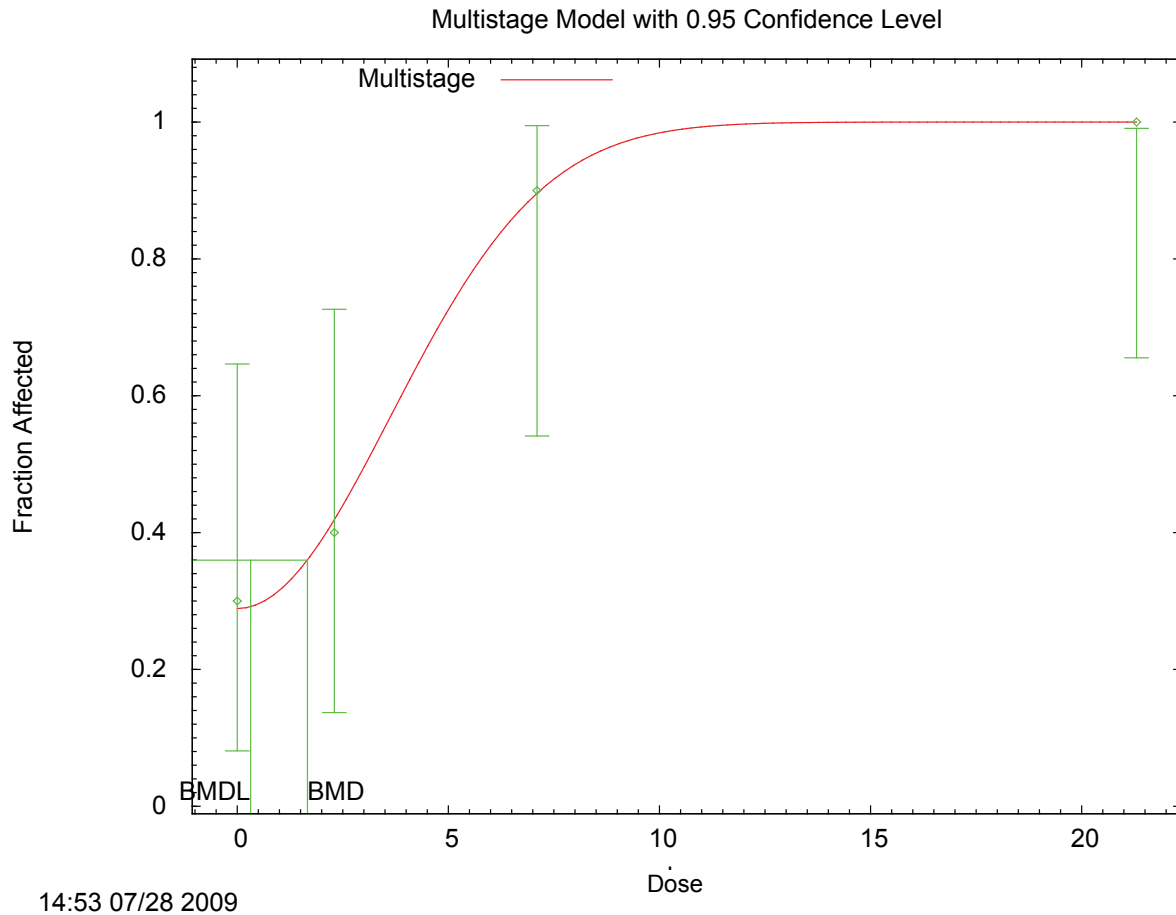
Quantal Linear Model with 0.95 Confidence Level

**Figure A-4. Fit of Quantal-Linear Model to Data
on Increased Hepatocyte Vacuolization in Female Rats (Gushow et al., 1984)**

BMCs and BMCLs indicated are associated with a change of 10% extra risk from the control, and are in units mg/m^3

Multistage Model with 0.95 Confidence Level

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**Figure A-5. Fit of Multistage Model to Data
on Increased Hepatocyte Vacuolization in Female Rats (Gushow et al., 1984)**

BMCs and BMCLs indicated are associated with a change of 10% extra risk from the control, and are in units mg/m^3

```
====================================================================
      Multistage Model. (Version: 3.0;  Date: 05/16/2008)
      Input Data File: C:\USEPA\BMDS21\Data\mstHepaticmulti.(d)
      Gnuplot Plotting File: C:\USEPA\BMDS21\Data\mstHepaticmulti.plt
                                    Fri Aug 28 17:38:06 2009
====================================================================

 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 = Response
   Independent variable = Dose
```

```
Total number of observations = 4
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) = 2.27362e+017


            Asymptotic Correlation Matrix of Parameter Estimates

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

                 Background      Beta(2)

Background              1        -0.41

   Beta(2)          -0.41              1



                            Parameter Estimates

                                              95.0% Wald Confidence
Interval
         Variable        Estimate        Std. Err.    Lower Conf. Limit    Upper Conf.
Limit
      Background        0.288586            *                *                   *
         Beta(1)               0            *                *                   *
         Beta(2)       0.0381152            *                *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model       Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -16.0896        4
    Fitted model        -16.1007        2      0.0222818      2        0.9889
   Reduced model        -25.8979        1       19.6166       3       0.0002038

           AIC:      36.2015



                            Goodness  of  Fit
                                                          Scaled
       Dose     Est._Prob.    Expected    Observed    Size    Residual
     -------------------------------------------------------------------
      0.0000      0.2886       2.886       3.000       10       0.080
      2.3000      0.4185       4.185       4.000       10      -0.119
```

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```
   7.1000     0.8958        8.958     9.000          10        0.043
  21.3000     1.0000       10.000    10.000          10        0.000

 Chi^2 = 0.02      d.f. = 2        P-value = 0.9889


    Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =       Extra risk

Confidence level =           0.95

         BMD =         1.66261

        BMDL =         0.318188
```