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Provisional Peer-Reviewed Toxicity Values for Ethylbenzene (CASRN 100-41-4)

Derivation of a Subchronic Oral Provisional-RfD and a Subchronic Inhalation Provisional-RfC

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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

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR ETHYLBENZENE (CASRN 100-41-4) DERIVATION OF A SUBCHRONIC ORAL PROVISIONAL-RFD AND A SUBCHRONIC INHALATION PROVISIONAL-RFC

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, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in 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

Ethylbenzene has a chronic RfD, a chronic RfC, and a cancer descriptor of "*Not classifiable as to human carcinogenicity*" on IRIS (U.S. EPA, 1991a). Thus, only subchronic toxicity values are presented in this toxicity assessment. There is an Agency for Toxic Substances and Disease Registry (ATSDR, 1999) assessment of ethylbenzene. ATSDR has since posted an updated version of Toxicological Profile for Ethylbenzene (ATSDR, 2007) in September 2007, but it is only a draft for public comment—not the official citable final report. Therefore, any potential changes or updates in toxicity values (critical effects, principal study, etc) are not described here in the PPRTV document. In order to determine whether newer data might be available to support subchronic noncancer toxicity values for ethylbenzene, a targeted literature search was conducted to identify human or in vivo animal studies of appropriate duration and quality to serve this purpose. Literature searches were limited to studies published between 1999 and August 2007 in the following databases: MEDLINE, TOXLINE, BIOSIS, TSCATS, DART/ETIC, GENETOX, HSDB, and Current Contents. The searches included terms to identify human exposure studies (epidemiologic, occupational) and animal studies for noncancer endpoints and less-than-chronic durations. The searches included health effects and toxicity information available from the U.S. EPA (IRIS), ATSDR, and other relevant federal, state or international governmental or quasi-governmental agencies, including, but not limited to, ACGIH, NIOSH, OSHA, NTP, IARC, WHO, and CalEPA. In addition, electronic databases, including CURRENT CONTENTS, MEDLINE, TOXLINE, BIOSIS/TOXCENTER, TSCATS/TSCATS2, CCRIS, DART/ETIC, GENETOX, HSDB, and RTECS, were searched. Studies having the potential ability to inform the derivation of subchronic noncancer toxicity values were retrieved and a critical study was selected.

The derivation of subchronic toxicity values for ethylbenzene is discussed below. A brief rationale is provided for the selection of the critical study and endpoint, a summary of the critical study is presented, and the subchronic toxicity value derivations are described. Further information on the toxicology and toxicokinetics of ethylbenzene can be found in the

ATSDR (1999) Toxicological Profile for Ethylbenzene or on IRIS (www.epa.gov/iris). The health effects associated with ethylbenzene exposure are currently being reassessed by the IRIS Program (see IRIS Track at http://cfpub.epa.gov/ncea/iristrac/index.cfm).

REVIEW OF PERTINENT DATA AND DERIVATION OF PROVISIONAL SUBCHRONIC TOXICITY VALUES FOR ETHYLBENZENE

Subchronic p-RfD

The chronic RfD for ethylbenzene on IRIS (0.1 mg/kg-day) was verified in May 1985 based on liver and kidney toxicity in a "subchronic-to-chronic" rat study (Wolf et al., 1956). It includes a UF of 10 for subchronic-to-chronic extrapolation. There is no intermediate duration oral MRL for ethylbenzene; ATSDR (1999) considered the Wolf et al. (1956) study to be of inadequate quality for the purpose of MRL derivation.

Only one oral study potentially useful for subchronic p-RfD derivation was identified in the update literature searches: a 13-week rat gavage study (Mellert et al., 2007). This study was conducted in compliance with GLP and OECD guidelines, used both male and female rats, evaluated a wide variety of endpoints, and reported both data and results of statistical analysis on all relevant findings. In contrast, the study by Wolf et al. (1956) utilized for the IRIS RfD used only female rats, evaluated only a subset of endpoints, and reported results qualitatively. Mellert et al. (2007) identifies LOAEL and NOAEL values (250 and 75 mg/kg-day, respectively), which are very near the values reported by Wolf et al. (1956) (291 and 97 mg/kg-day), and they identified the same target organs (liver and kidney). The Mellert et al. (2007) study is considered a more suitable study for determining a POD than Wolf et al. (1956) and, therefore, is used to derive the subchronic p-RfD for ethylbenzene.

Mellert et al. (2007) treated groups of Wistar rats (10/sex/dose) with ethylbenzene (99.7% pure) by gavage at doses of 0, 75, 250, or 750 mg/kg-day 7 days/week[1] for 13 weeks. Animals were examined daily for mortality and signs of toxicity, while a detailed clinical examination was performed weekly. Weekly measurements were made of food and water intake and body weights. Urine was collected for analysis (color, turbidity, volume, specific gravity, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood, microscopic examination of sediment) and blood samples for hematology and clinical chemistry (details of each not given) were collected at study termination. Ophthalmology, functional observational battery (FOB) for neurobehavioral effects and motor activity were evaluated during the final week of treatment. All animals were necropsied, and major organs (adrenal glands, brain, epididymes, heart, kidneys, liver, ovaries, spleen, testes, thymus, thyroid, and uterus) were weighed. Microscopic examination of a comprehensive list of tissues (>45 tissues) was performed in the control and high-dose animals, while the liver, kidney, and pancreas were examined in all groups. Male kidneys were also examined using Mallory-Heidenhain staining for hyaline droplets.

Clinical signs in treated animals included postdosing salivation (all mid- and high-dose animals, as well as one low-dose male) and discolored urine observed in the bedding (but not on urinalysis) in high-dose animals of both sexes (Mellert et al., 2007). Body weights were

[1]Daily gavage administration confirmed by personal communication, Dr. Bennard van Ravenzwaay.

significantly lower than controls in high-dose males beginning in Week 5; terminal body weights in this group were 14% lower than controls ($p < 0.01$). Water consumption was significantly increased in mid- and high-dose males and in high-dose females ($p < 0.01$), and food consumption was increased in high-dose males ($p < 0.05$). The FOB revealed a significant decrease in landing foot-splay in high-dose males, which the authors attributed to decreased body weight. Motor activity was significantly increased in high-dose females ($p < 0.01$), but the pattern of changes was considered inconsistent with treatment-related effects; the authors reported that treatment-related effects are usually observed at the beginning or end of measurement, whereas effects in the high-dose females were observed intermittently. Hematology analysis indicated a statistically significant ($p < 0.01$) increase in mean corpuscular volume in high-dose males (5% higher than controls) and mid- and high-dose females (2–4%), as well as a significant reduction ($p < 0.01$) in platelet count in high-dose females (15%); there were no other hematology changes. Absolute and relative thymus weights were decreased in mid- and high-dose females, but no pathology was observed upon microscopic examination of this organ.

Table 1 shows relevant changes in clinical chemistry, urinalysis, organ weights, and histopathology. Evidence for liver toxicity at the mid- and high-doses included clinical chemistry effects (e.g., increases in ALT, GGT, and bilirubin), dose-related increases in absolute and relative liver weight, and dose-related increases in the incidence of centrilobular hepatocyte hypertrophy. Kidney effects in mid- and high-dose animals included clinical chemistry changes in both sexes (e.g., increased serum urea, potassium, and calcium in males; increased serum potassium in females), urinalysis findings (increased incidences of transitional epithelial cells and granular and epithelial cell casts in males), dose-related increases in relative (both sexes) and absolute (males only) kidney weights, and increased severity of hyaline droplet nephropathy in male rats. The only treatment-related finding at the low dose was increased relative—but not absolute—liver weight in male rats (4% higher than controls, $p < 0.01$).

The study authors identified the low dose (75 mg/kg-day) as a NOAEL and the mid-dose (250 mg/kg-day) as a LOAEL for centrilobular hepatocyte hypertrophy with clinical chemistry changes indicative of liver and kidney effects. Findings at 250 mg/kg-day that support the identification of a LOAEL based on liver toxicity include histopathology (centrilobular hepatocyte hypertrophy) and increased absolute and relative liver weight, in conjunction with changes in several clinical chemistry measures (increased ALT, GGT, bilirubin, and cholesterol in males; increased cholesterol and decreased prothrombin time in females).

Mellert et al. (2007) observed liver and kidney effects at lower doses than other endpoints. Evidence of mild kidney impairment in males exposed to the LOAEL included urinalysis changes (transitional epithelial cells and granular and epithelial cell casts in urine), clinical chemistry findings (increased potassium and calcium), increased relative kidney weights, and an increase in the severity of hyaline droplet nephropathy. Hyaline droplet nephropathy is related to the accumulation of α_{2u}-globulin, an effect that is specific to the male rat and not relevant to humans (U.S. EPA, 1991b). Evidence for the role of α_{2u}-globulin includes the increased incidence and severity of hyaline droplet formation and granular cell casts in the urine. The only kidney effect observed in female rats exposed to the LOAEL was a slight—but statistically significant ($p < 0.01$)—increase in relative kidney weight (7% above controls). In females at the high-dose, there were slight increases in sodium, potassium, and magnesium concentrations along with increased relative kidney weight (13%) that indicate a potential effect

Table 1. Significant Effects on Liver and Kidney in Rats Treated with Ethylbenzene via Gavage for 13 Weeks[a]

	Control	75 mg/kg-day	250 mg/kg-day	750 mg/kg-day
Males				
Clinical Chemistry				
ALT (µkat/L)	0.62 ± 0.12[b]	0.70 ± 0.12	0.89 ± 0.26[c]	1.11 ± 0.23[c]
GGT (nkat/L)	2 ± 3	6 ± 6	10 ± 6[c]	10 ± 6[c]
Total bilirubin (µmol/L)	2.20 ± 0.35	2.41 ± 0.53	3.05 ± 0.80[c]	3.40 ± 0.83[c]
Albumin (g/L)	36.8 ± 1.0	37.0 ± 0.6	37.6 ± 0.6	38.1 ± 1.0[d]
Cholesterol (mmol/L)	1.76 ± 0.26	1.68 ± 0.11	2.23 ± 0.24[c]	2.21 ± 0.26[c]
Creatinine (mmol/L)	54.7 ± 3.8	53.6 ± 3.2	52.1 ± 4.2	49.2 ± 3.2[c]
Serum urea (mmol/L	4.13 ± 0.46	4.01 ± 0.44	4.07 ± 0.59	4.87 ± 0.78[d]
Potassium (mmol/L)	4.59 ± 0.26	4.71 ± 0.27	4.89 ± 0.32[d]	4.98 ± 0.36[d]
Calcium (mmol/L)	2.75 ± 0.04	2.78 ± 0.07	2.84 ± 0.05[c]	2.82 ± 0.10[d]
Magnesium (mmol/L)	0.92 ± 0.04	0.94 ± 0.03	0.94 ± 0.06	1.00 ± 0.04[c]
Urinalysis[e]				
Transitional epithelial cells, grade ≥2	1/10	4/10	7/10[c]	8/10[c]
Granular and epithelial cell casts	3/10	7/10	9/10[c]	8/10[d]
Body Weight on Day 91 (% change from control)	372 g	-0.9%	-2.7%	-13.8% [c]
Organ Weights				
Absolute liver weight (g)	8.02 ± 0.55	8.26 ± 0.81	10.25 ± 0.98[c]	9.88 ± 0.98[c]
Liver/body weight (%)	2.26 ± 0.08	2.36 ± 0.08[c]	3.01 ± 0.14[c]	3.31 ± 0.13[c]
Absolute kidney weight (g)	2.08 ± 0.13	2.14 ± 0.15	2.37 ± 0.15[c]	2.37 ± 0.31[c]
Kidney/body weight (%)	0.59 ± 0.04	0.61 ± 0.03	0.70 ± 0.05[c]	0.79 ± 0.06[c]
Histopathology[e]				
Centrilobular hepatocyte hypertrophy	1/10	1/10	6/10[d]	8/10[c]
Hyaline droplet nephropathy (severity)	8/10 (1.5)	9/10 (1.7)	10/10 (3.1)	10/10 (3.2)
Females				
Clinical Chemistry				
ALT (µkat/L)	0.58 ± 0.18	0.55 ± 0.08	0.60 ± 0.12	0.73 ± 0.19[d]
Total bilirubin (µmol/L)	2.75 ± 0.39	2.56 ± 0.42	2.94 ± 0.37	3.65 ± 0.64[c]
Total protein (g/L)	71.8 ± 2.6	71.3 ± 3.0	72.4 ± 2.6	75.6 ± 3.1[d]
Albumin (g/L)	40.2 ± 1.5	39.9 ± 1.5	40.9 ± 1.3	41.8 ± 1.5[d]
Globulins (g/L)	31.6 ± 1.3	31.4 ± 1.8	31.6 ± 2.0	33.8 ± 1.9[c]
Cholesterol (mmol/L)	1.35 ± 0.27	1.41 ± 0.31	1.81 ± 0.31[c]	2.16 ± 0.20[c]
Prothrombin time (s)	28.4 ± 0.8	28.3 ± 2.2	27.0 ± 1.1[c]	26.3 ± 1.8[d]
Sodium (mmol/L)	141.4 ± 1.4	140.6 ± 1.3	141.5 ± 0.8	139.1 ± 1.0[c]
Potassium (mmol/L)	4.28 ± 0.21	4.38 ± 0.29	4.28 ± 0.27	4.62 ± 0.21[c]
Magnesium (mmol/L)	0.97 ± 0.06	0.99 ± 0.06	0.97 ± 0.04	1.04 ± 0.03[c]
Body Weight on Day 91(% change from control)	222 g	+3.1%	-1.6%	-1.4%
Organ Weights				
Absolute liver weight (g)	5.40 ± 0.30	5.72 ± 0.53	6.11 ± 0.36[c]	7.15 ± 0.50[c]
Liver/body weight (%)	2.63 ± 0.13	2.70 ± 0.16	3.03 ± 0.12[c]	3.52 ± 0.18[c]
Kidney/body weight (%)	0.67 ± 0.03	0.68 ± 0.04	0.72 ± 0.03[c]	0.76 ± 0.03[c]
Histopathology[e]				
Centrilobular hepatocyte hypertrophy (incidence)	0/10	0/10	5/10[d]	10/10[c]

[a]Mellert et al., 2007
[b]Mean ± standard deviation
[c]$p < 0.01$
[d]$p < 0.05$
[e]Incidence of effect; statistical analysis conducted for this review using Fisher's exact test

on the kidney unrelated to hyaline droplet nephropathy. However, given that liver effects were observed in the same dose range as the kidney effects and the possible role of α_{2u}-globulin accumulation in the kidney effects observed in male rats at the LOAEL and the limited effects observed in female rats even at the high-dose of ethylbenzene, kidney effects were not considered as the basis for the subchronic p-RfD.

Several measures of liver toxicity were significantly affected ($p < 0.01$) at the LOAEL: incidences of centrilobular hepatocyte hypertrophy (males and females); absolute and relative liver and kidney weights (males and females); and serum alanine aminotransferase [ALT] (males), gamma glutamyl transferase [GGT] (males), bilirubin (males); and cholesterol (males and females). Examination of these clinical chemistry findings and organ weight changes suggests that male rats may be slightly more sensitive to the liver effects of ethylbenzene than females, as there were more significant findings at the LOAEL in males than in females. Furthermore, a 4-fold increase in GGT was observed in male rats exposed at the LOAEL, while no change in GGT was observed in female rats at this dose. GGT is a sensitive indicator of liver toxicity (U.S. EPA, 2002). Based on these observations, the data on liver changes in male rats were considered for BMD modeling.

Endpoints to which benchmark dose modeling was applied include the following: GGT, bilirubin, cholesterol, absolute and relative liver weight, and incidence of centrilobular hepatocyte hypertrophy in male rats (see Table 1 for data). Biologically relevant benchmark response (BMR) values for the continuous endpoints (serum chemistry changes and liver weight) were not located; thus, the default BMR of 1 standard deviation (SD) from the control mean (U.S. EPA, 2000) was used for these endpoints. The BMR used for modeling incidence of centrilobular hepatocyte hypertrophy was the default value of 10% increase over the control incidence. Serum ALT in males was not modeled because the observed increases, while statistically significant ($p < 0.01$), were less than 2-fold increase, and were of unknown biological significance. Because body weights were significantly reduced (14%; $p < 0.01$) in the high-dose males, this dose group was not used in modeling of absolute and relative liver weight, as the liver weights were confounded by body weight changes. However, even without the high-dose group, efforts to apply benchmark dose modeling to the data on relative liver weight were not successful (the model failed to converge and no results were produced). In addition, modeling of cholesterol changes did not result in any model fit (see Appendix A).

Details of the benchmark dose modeling and results, as well as graphs of the best-fitting model for each endpoint, are provided in Appendix A. Benchmark dose modeling of serum GGT in male rats resulted in model fit using the linear model with modeled variance. The BMD_{1SD} (benchmark dose associated with 1SD from the control mean response) and $BMDL_{1SD}$ (lower confidence limit on this benchmark dose) calculated from these data were 96 and 53 mg/kg-day, respectively. Model fit was achieved using the linear model with modeled variance for the data on total serum bilirubin. The BMD_{1SD} and $BMDL_{1SD}$ calculated from these data were 105 and 62 mg/kg-day, respectively. Modeling of absolute liver weight gave reasonable fit using the linear model with homogenous variance. The BMD_{1SD} and $BMDL_{1SD}$ predicted by the linear model were 84 and 63 mg/kg-day, respectively. BMD modeling of centrilobular hepatocyte hypertrophy in male rats resulted in model fit for several quantal models. The log-probit model provided the best fit and the BMD_{10} and $BMDL_{10}$ predicted by the

log-probit model for the data on centrilobular hepatocyte hypertrophy in male rats were 79 and 48 mg/kg-day (respectively). Table 2 shows the BMDs and BMDLs calculated from each of the liver toxicity endpoints.

Table 2. Comparison of BMDs and BMDLs Predicted by Modeling of Liver Effect Endpoints in Male Rats

Endpoint Modeled	Best-fitting Model	BMD (mg/kg-day)	BMDL (mg/kg-day)
Serum GGT	Linear (modeled variance)	95.95	53.06
Total Serum Bilirubin	Linear (modeled variance)	105.43	62.04
Absolute Liver Weight	Linear (constant variance)	83.80	63.30
Incidence of Centrilobular Hepatocyte Hypertrophy	Log-probit	78.95	48.26

The lowest BMDL (48 mg/kg-day), derived from modeling centrilobular hepatocyte hypertrophy in male rats, was used as the point of departure (POD) for subchronic p-RfD derivation. Using this BMDL as the POD is expected to provide protection against potential kidney effects, since there was no evidence of kidney effects at the NOAEL. The **subchronic p-RfD** for ethylbenzene is derived as follows:

$$\begin{aligned} \textbf{Subchronic p-RfD} &= BMDL_{10} \div UF \\ &= 48 \text{ mg/kg-day} \div 1{,}000 \\ &= \textbf{0.05 mg/kg-day or } \mathbf{5 \times 10^{-2}} \textbf{ mg/kg-day} \end{aligned}$$

A composite Uncertainty Factor (UF) of 1,000 was applied to the $BMDL_{10}$ to calculate the subchronic p-RfD for ethylbenzene. The composite UF included a factor of 10 for interspecies extrapolation, a factor of 10 for human variability, and a factor of 10 for database uncertainties, as follows:

- A full UF_A of 10 was applied for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to the liver and/or kidney toxicity of ethylbenzene.
- A full UF_A of 10 was applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
- A full UF_D of 10 was applied to account for database uncertainty. There are only two subchronic studies of oral exposure to ethylbenzene (i.e., Mellert et al., 2007; Wolf et al., 1956) and no oral studies of developmental or reproductive toxicity. Further, studies of inhaled ethylbenzene have identified ototoxicity as the most sensitive endpoint (see subchronic p-RfC derivation below). A short-term (2-week) study of the ototoxicity of orally administered ethylbenzene (Gagnaire and Langlais, 2005) reported histopathological evidence of ototoxicity at the only dose tested, 8.47 mmol/kg-day (900 mg/kg-day), which indicates that this endpoint may be relevant to oral exposure but cannot be evaluated with current information.

Confidence in the principal study (Mellert et al., 2007) is high because the study tested 10 rats per sex at 4 dose levels (including controls), and a broad array of endpoints was evaluated. Confidence in the database is low reflecting the limited oral toxicity data (only two subchronic studies), the lack of multigeneration reproductive and developmental toxicity studies, and the lack of information on potential ototoxicity from oral exposure. Reflecting high confidence in the principal study and low confidence in the database, confidence in the provisional subchronic p-RfD is medium.

Comparison of the subchronic p-RfD (0.05 mg/kg-day) with the chronic RfD for ethylbenzene (0.1 mg/kg-day) on IRIS indicates that the subchronic p-RfD is lower than (one-half of) the existing chronic RfD. The chronic RfD for ethylbenzene that is currently posted on IRIS was derived in 1985 using U.S. EPA guidance and methods that have since been updated and revised. The subchronic p-RfD for ethylbenzene is derived using a new study and current U.S. EPA guidance and methods, resulting in a lower subchronic value. The chronic RfD for ethylbenzene on IRIS is currently being reassessed (see IRIS Track at www.epa.gov/iris); when the reassessment is complete, the chronic RfD will also reflect the new data and the use of current EPA guidance and methods.

Subchronic p-RfC

The chronic RfC for ethylbenzene (1 mg/m^3) on IRIS was verified in December 1990 based on developmental toxicity studies in rats and rabbits exposed during gestation or for 3 weeks prior to gestation and during gestation (Andrew et al., 1981; Hardin et al., 1981), and it is supported by subchronic and chronic studies in several species (NTP, 1989, 1990; Cragg et al., 1989; Elovaara et al., 1985; Clark, 1983; Wolf et al., 1956). No UF for exposure duration was used. The intermediate duration inhalation MRL (1 ppm, or 4.3 mg/m^3) was derived in 1999 and was also based on Andrew et al. (1981).

A number of inhalation studies in animals were identified in the update literature searches: a multigeneration reproductive toxicity study in rats (Faber et al., 2006, 2007), three developmental toxicity studies in rats (Saillenfait et al., 2003, 2006, 2007), and four studies of the ototoxic effects of ethylbenzene in rats (Cappaert et al., 1999, 2000, 2001, 2002; Gagnaire et al., 2007). Of the ototoxicity studies, only Gagnaire et al. (2007) employed a subchronic exposure duration (13 weeks). The other ototoxicity studies (Cappaert et al., 1999, 2000, 2002) were of short-term duration (5 days) and, thus, were not considered pertinent to the derivation of a subchronic RfC.

Table 3 shows a comparison of the recent studies identified through the literature search and the developmental toxicity study that was used as the basis of both the chronic RfC and the intermediate duration inhalation MRL for ethylbenzene. As the table shows, the recent reproductive and developmental toxicity studies (Saillenfait et al., 2003, 2006, 2007; Faber et al., 2006, 2007) support the LOAEL (1,000 ppm or 4,340 mg/m^3) identified in the study by Andrew et al. (1981). In contrast, the ototoxic effects were observed at a lower concentration than developmental toxicity; Gagnaire et al. (2007) identified a LOAEL of 200 ppm (868 mg/m^3) for persistent ototoxic effects. Ototoxicity studies of shorter duration (Cappaert et al., 1999, 2000, 2001, 2002) identify LOAELs in the range of 300–400 ppm, providing support for the sensitivity of this endpoint when compared with developmental toxicity. Thus, the study by Gagnaire et al. (2007) was selected as the critical study for derivation of the subchronic p-RfC.

Table 3. Comparison of Recent (1999–2007) Inhalation Studies with Critical Study Used for Chronic RfC and Intermediate MRL Derivation for Ethylbenzene

Species	Sex	Exposure Concentration (ppm)	Exposure	NOAEL (ppm)	LOAEL (ppm)	Responses	Comments	Reference
Rat Subchronic Ototoxicity Study	M	0, 200, 400, 600, and 800	6 hr/d, 6 d/wk for 13 wks	NA	200	Minimal LOAEL; loss of 3rd row outer hair cells from organ of Corti	Increased audiometric thresholds were observed at ≥400 ppm	Gagnaire et al., 2007
Rats Developmental Toxicity Study	F	0, 100, 500, 1,000, and 2,000	6 hr/d during GD 6–20.	500 (maternal and fetal)	1,000 (maternal and fetal)	Reduced weight gain (maternal) Reduced fetal body weight (fetal)		Saillenfait et al., 2003
Rats Developmental Toxicity Study	F	0, 250, and 1,000	6 hr/d during GD 6–20.	250 (maternal and fetal)	1,000 (maternal and fetal)	Reduced weight gain (maternal) Reduced fetal body weight (fetal)	Data collected as part of a study on interaction with methyl ethyl ketone	Saillenfait et al., 2006
Rats Developmental Toxicity Study	F	0, 250, and 1,000	6 hr/d during GD 6–20.	250 (maternal and fetal)	1,000 (maternal and fetal)	Reduced weight gain (maternal) Reduced fetal body weight (fetal)	Data collected as part of a study on interaction with butyl acetate	Saillenfait et al., 2007
Rats 2-generation Reproductive Toxicity	M/F	0, 25, 100, and 500	6 hr/d for at least 70 days premating, through mating and gestation for 2 generations	500	NA		Reduced estrous cycle length observed in F_0, but there was no effect on fertility or time to mating and F_1 females were not affected at this concentration	Faber et al., 2006, 2007
Rat and rabbit Developmental Toxicity	M/F	0, 100, and 1,000	7 hr/d, 5 d/wk for 3 weeks premating, through mating pregnancy daily through GD 19.	100 (maternal and fetal)	1,000 (maternal and fetal)	Increased incidence supernumerary ribs in rats; slightly reduced litter size in rabbits	This study was used for the IRIS chronic RfC and ATSDR intermediate MRL. A weight of evidence approach was used by IRIS to identify the LOAEL based on a cluster of mild effects in rats and rabbits	Andrew et al., 1981; Hardin et al., 1981

Gagnaire et al. (2007) exposed groups of 14 male Sprague-Dawley rats to ethylbenzene (99% pure) vapors (whole body exposure) at concentrations of 0, 200, 400, 600, or 800 ppm 6 hours/day, 6 days/week for 13 weeks followed by 8 untreated weeks. The rats were about 14 weeks of age at the time testing commenced. Mortality was monitored and body weights were recorded weekly. Auditory thresholds at different sound frequencies (2, 4, 8, and 16 kHz) were measured by brainstem auditory-evoked responses (using surgically implanted electrodes and a computerized recording device) assessed at the end of the 4th, 8th, and 13th weeks of exposure and at the end of the 8-week recovery period. After the 8 untreated weeks, 8 rats/exposure-concentration were sacrificed for microscopic examination of the organ of Corti. The microscopic examination was used to quantify loss of outer hair cells in the organ of Corti; these results were presented as histocochleograms (graphs of cell loss of inner hair cells and the three rows of outer hair cells).

A single rat in the 800-ppm group died of unknown causes and a second was sacrificed after developing a large tumor on the neck (Gagnaire et al., 2007). A third rat lost its head plug and could not undergo audiometric threshold testing. Ethylbenzene treatment did not affect body-weight gain in any group. Audiometric thresholds at all four frequencies were statistically significantly ($p < 0.05$) increased over controls in groups exposed to 400 ppm and higher beginning in the 4th week of exposure. The magnitudes of the threshold shifts, depending on frequency, exhibited some dose-dependency, ranging from 23 to 27 decibels (dB) in the 400-ppm group and from 44 to 49 dB in the 600- and 800-ppm groups. The threshold increases observed at 4 weeks did not change with additional exposure and persisted through the 8-week untreated period, with no evidence of recovery. There was no change in audiometric threshold in the rats exposed to 200-ppm ethylbenzene. Microscopic examination of the organs of Corti revealed significant dose-related and, in some cases, marked losses of both inner and outer hair cells. There was no evidence of biological significant hair cell loss in the controls. At the highest concentrations (600 and 800 ppm), there was nearly complete loss of all three rows of outer hair cells, as well as less marked inner hair cell loss (14% and 32% in the 600 and 800 ppm groups, respectively). At 400 ppm, there was limited loss of the inner hair cells, but still marked loss of outer hair cells, especially in the third row. At 200 ppm, significant outer hair cell loss (up to 30% in the mid-frequency region) was observed in the third row in 4/8 rats examined.

This study identified a minimal LOAEL of 200 ppm for histopathological evidence of ototoxicity without functional changes in audiometric threshold; no NOAEL can be determined from these data. Because a NOAEL was not identified, Gagnaire et al. (2007) calculated theoretical lowest adverse effect levels[2] (TLAELs) based on the upper confidence limits of the average hair cell losses observed in the controls. TLAELs were calculated to be 114, 120, and 130 ppm for the 95, 99, and 99.9% upper confidence limits.

The LOAEL identified from the data reported by Gagnaire et al. (2007) was associated with histopathological evidence of ototoxicity (loss of outer hair cells). The data for this endpoint were reported graphically and were not amenable to BMD modeling. Thus, the

[2]To calculate the TLAELs, the concentration-response relationship (mean cell loss in the third row of the OHC versus exposure concentration) was fitted using a logistic regression model. The regression analysis was used to estimate the concentrations associated with the upper confidence limits (95%, 99%, and 99.9%) on the control mean response; these concentrations were termed the TLAELs. The study authors did not report the parameters of the regression model.

LOAEL of 200 ppm (868 mg/m^3) was used as the POD for derivation of the subchronic p-RfC. No adjustment for continuous exposure was made because available data indicate that inhaled ethylbenzene is rapidly absorbed, metabolized, and excreted through the urine (ATSDR, 1999). As a result, effects of inhaled exposure are considered to be more correlated with concentration than with duration of exposure. Studies of 5-day exposures (Cappaert et al., 1999, 2000, 2001, 2002) identify ototoxicity LOAELs only slightly higher (300–400 ppm) than the subchronic study (200 ppm; Gagnaire et al., 2007), providing support for a minimal effect of exposure duration on otoxicity.

The LOAEL was converted to a human equivalent concentration ($LOAEL_{HEC}$) based on the guidance provided in U.S. EPA (1994). It is not clear from the available information whether exposure to the inner ear of the rats occurred primarily via direct contact or via absorption in the lungs and transport via the bloodstream. However, because ototoxicity is an extrarespiratory effect, ethylbenzene was treated as a Category 3 gas, and the ratio of blood:gas partition coefficients was used to make the dosimetric adjustment, as shown below:

$$LOAEL_{HEC} = LOAEL \times [(H_{b/g})_A/(H_{b/g})_A]$$

Where: $(H_{b/g})_A$ = blood/gas partition coefficient in rats
$(H_{b/g})_H$ = blood/gas partition coefficient in humans

Abraham et al. (2005) reported human and rat blood:gas partition coefficients of 28 and 30, respectively, for ethylbenzene. Because $(H_{b/g})_A > (H_{b/g})_H$, a default value of 1 was used for the animal-to-human blood:gas ratio in accordance with U.S. EPA (1994) guidance. Thus, the $LOAEL_{HEC}$ is equal to 868 mg/m^3, calculated as follows:

$$\begin{aligned} LOAEL_{HEC} &= LOAEL \times [(H_{b/g})_A/(H_{b/g})_A] \\ &= 868 \text{ mg/m}^3 \times 1 \\ &= 868 \text{ mg/m}^3 \end{aligned}$$

This value would be the same if no dosimetric adjustment was made under the assumption that exposure to the inner ear occurred via direct contact. The **subchronic p-RfC** for ethylbenzene is derived as follows:

$$\begin{aligned} \textbf{Subchronic p-RfC} &= LOAEL \div UF \\ &= 868 \text{ mg/m}^3 \div 100 \\ &= \textbf{9 or } \mathbf{9 \times 10^0 \text{ mg/m}^3} \end{aligned}$$

The composite UF of 100 includes the following:

- A partial UF_A of 3 ($10^{0.5}$) was applied for interspecies extrapolation to account for potential toxicodynamic differences between rats and humans when a dosimetric adjustment is used.
- A full UF_H of 10 was used to account for intraspecies differences for potentially susceptible individuals in the absence of information on the variability of response in humans.

- A partial UF_L of 3 ($10^{0.5}$) was applied for use of a minimal LOAEL. The effects observed at the LOAEL consisted of histopathological evidence of limited outer hair cell loss in the third row only and in four/eight rats, without functional changes in auditory threshold. Further, Gagnaire et al. (2007) estimated TLAELs in the range of 114–130 ppm based on the statistical upper confidence limits on outer hair cell loss in the control group. These values are in the range of one-half the LOAEL, providing further support for a partial UF for LOAEL-to-NOAEL extrapolation.
- A UF_D of 1 was applied for database uncertainty. No database UF was required, because the toxicological database for inhaled ethylbenzene includes high-quality subchronic bioassays, as well as developmental toxicity and multi-generation reproduction studies and a number of studies of ototoxicity.

Confidence in the principal study is medium. Gagnaire et al. (2007) is an adequate subchronic oral toxicity study using a sufficient number of animals, an appropriate range of exposure levels and measuring sensitive endpoints, but the study used only one gender (males) and did not identify a NOAEL. Confidence in the database is high. The animal database contains high quality studies on a variety of endpoints and in multiple species. Further, there are some limited data suggesting that the critical effect (ototoxicity) is relevant to humans; hearing loss has been reported in solvent abusers and in workers exposed to both solvents and sound, which may interact synergistically (Gagnaire et al., 2007). Confidence in the subchronic p-RfC is, therefore, medium.

Table 4 summarizes the subchronic noncancer assessments for ethylbenzene.

Table 4. Summary of Subchronic Noncancer Reference Values for Ethylbenzene

	POD Type	POD	UF	Reference Value	Critical Effect	Species/ Sex	Principal Study
p-sRfD	$BMDL_{10}$	48 mg/kg-day	1,000	5×10^{-2} mg/kg-day	Centrilobular hepatocyte hypertrophy	Rat/M	Mellert et al., 2007
p-sRfC	$LOAEL_{HEC}$	868 mg/m^3	100	9×10^{0} mg/m^3	Ototoxicity	Rat/M	Gagnaire et al., 2007

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APPENDIX A. DETAILS OF BENCHMARK DOSE MODELING FOR SUBCHRONIC ORAL RfD

Modeling Procedure Continuous Data Modeling

The model fitting procedure for continuous data is as follows. When a biologically-defined BMR is not available, the default BMR of 1 standard deviation from the control mean response is used (U.S. EPA, 2000). 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. If the linear model adequately fits the means ($p \geq 0.1$), then it is selected as the model for BMD derivation. If the linear model does not adequately fit the means, then the more complex 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 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 applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model provides an adequate fit ($p \geq 0.1$) to the variance data, then the fit of the linear model to the means is evaluated. If the linear model does not provide adequate fit to the means while the nonhomogenous variance model is applied, then 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.

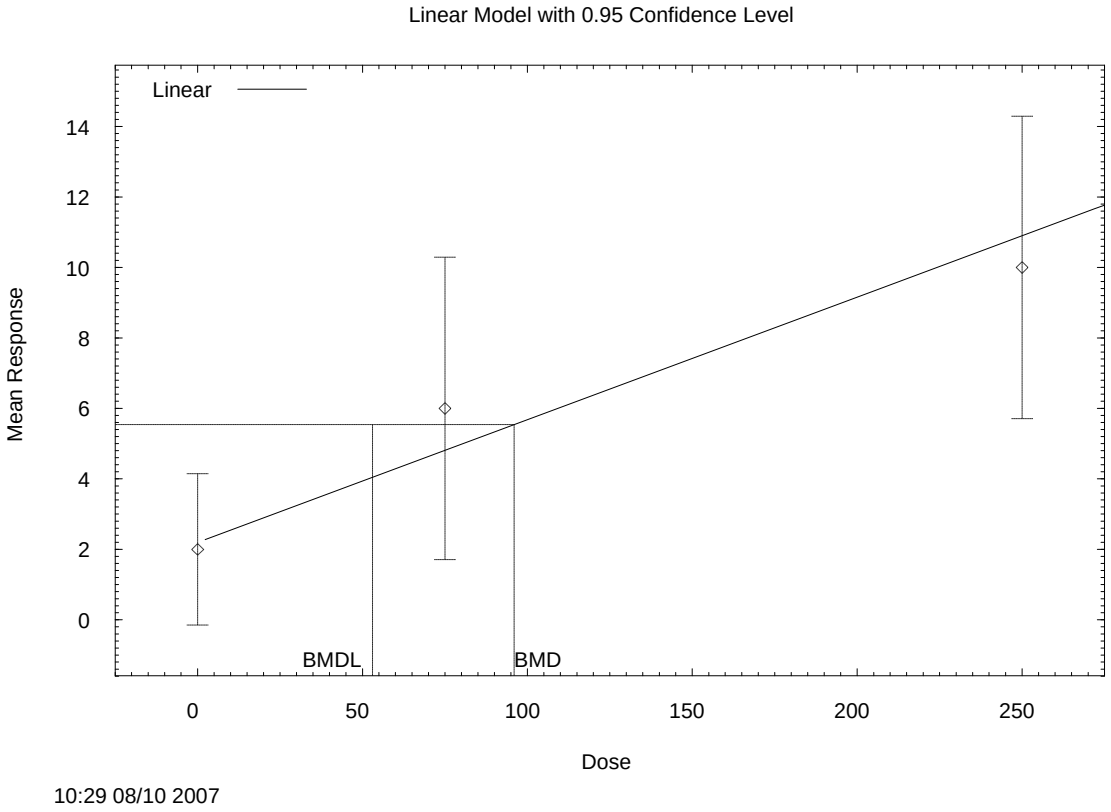
Modeling of Data on Serum GGT in Male Rats

Following the above procedure, continuous-variable models in the EPA BMDS (version 1.3.2) were fit to the data shown in Table 2 for increased serum GGT in male rats (Mellert et al., 2007) using a default BMR of 1 standard deviation from the control mean. Using these data, the constant variance model provided adequate fit to the variance data. However, the linear model with constant variance did not provide an adequate fit to the means, as shown in Table A-1. Further, none of the remaining models provided adequate fit to the means (there were not enough dose groups to apply the Hill model). In order to achieve model fit, the high-dose group was dropped from the analysis. With the reduced data set, the homogenous variance model did not fit the variance data adequately. With the modeled variance, the linear model provided adequate fit to the means (Figure A-1). The BMDs and the 95% lower confidence limits (BMDLs) associated with a change of 1 standard deviation (SD) from the control were calculated using the linear model with modeled variance.

Table A-1. Model Predictions for Serum GGT in Male Rats Exposed Orally to Ethylbenzene for 13 Weeks[a]

Model	Variance *p*-Value[b]	Means *p*-Value[b]	BMD_{1SD} (mg/kg-day)	$BMDL_{1SD}$ (mg/kg-day)
All dose groups				
Linear (constant variance)[c]	0.1352	0.03645	651.15	404.85
Polynomial (constant variance)[c, d]	0.1352	0.01006	651.15	404.85
Power (constant variance)[e]	0.1352	0.01006	651.15	404.85
Hill (constant variance)[e]	NA[f]	NA	NA	NA
Without high-dose group				
Linear (constant variance)[c]	0.07308	0.4167	164.06	108.12
Linear (modeled variance)[c]	0.4848	0.1416	95.95	53.06

[a]Mellert et al., 2007
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Coefficients restricted to be positive
[d]2-degree polynomial selected
[e]Power restricted to ≥1
[f]NA = not applicable (insufficient dose groups available to apply this model)



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

Figure A-1. Fit of Linear Model (Modeled Variance) to Data on Serum GGT in Male Rats (Mellert et al., 2007)

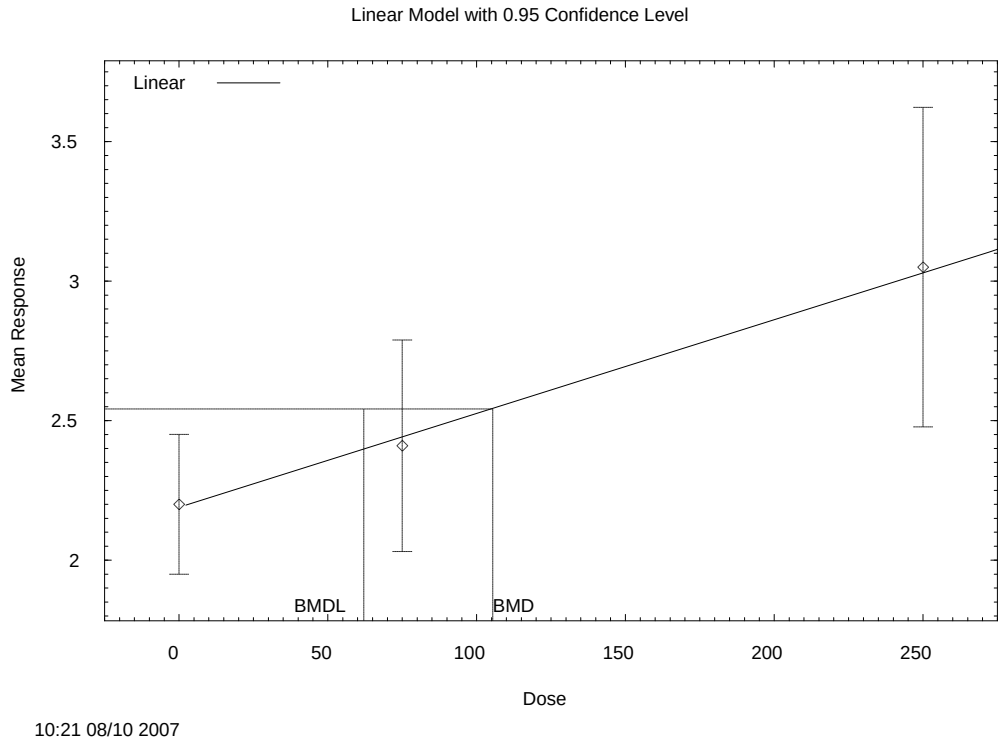
Modeling of Data on Total Serum Bilirubin in Male Rats

Following the above procedure, continuous-variable models in the EPA BMDS (version 1.3.2) were fit to the data shown in Table 2 for increased total serum bilirubin in male rats (Mellert et al., 2007) using a default BMR of 1 standard deviation from the control mean. Using these data, the constant variance model did not provide adequate fit to the variance data. When the variance was modeled using the power model in the BMDS, the linear model did not provide an adequate fit to the means, as shown in Table A-2. Further, none of the remaining models provided adequate fit to the means (there were not enough dose groups to apply the Hill model). In order to achieve model fit, the high-dose group was dropped from the analysis. With the reduced data set, the homogenous variance model did not fit the variance data adequately. With the modeled variance, the linear model provided adequate fit to the means (Figure A-2). The BMDs and the 95% lower confidence limits (BMDLs) associated with a change of 1 standard deviation (SD) from the control were calculated using the linear model with modeled variance.

Table A-2. Model Predictions for Total Serum Bilirubin in Male Rats Exposed Orally Ethylbenzene for 13 Weeks[a]

Model	Variance *p*-Value[b]	Means *p*-Value[b]	BMD_{1SD} (mg/kg-day)	$BMDL_{1SD}$ (mg/kg-day)
All dose groups				
Linear (constant variance)[c]	0.03826	0.1883	427.04	300.05
Linear (modeled variance)[c]	0.7171	0.04065	252.03	141.00
Polynomial (modeled variance)[c, d]	0.7171	0.01138	252.03	141.00
Power (constant variance)[e]	0.717	0.01138	252.03	141.00
Hill (constant variance)[e]	NA [f]	NA	NA	NA
Without high-dose group				
Linear (constant variance)[c]	0.0393	0.8397	162.49	107.38
Linear (modeled variance)[c]	0.5809	0.9404	105.43	62.04

[a]Mellert et al., 2007
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Coefficients restricted to be positive
[d]2-degree polynomial selected
[e]Power restricted to ≥1
[f]NA = not applicable (insufficient dose groups available to fit this model)



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

Figure A-2. Fit of Linear Model (Modeled Variance) to Data on Total Serum Bilirubin in Male Rats (Mellert et al., 2007)

Modeling of Data on Serum Cholesterol in Male Rats

Following the above procedure, continuous-variable models in the EPA BMDS (version 1.3.2) were fit to the data shown in Table 2 for increased serum cholesterol in male rats (Mellert et al., 2007) using a default BMR of 1 standard deviation from the control mean. Using these data, the constant variance model did not provide adequate fit to the variance data. Further, the variance model included in the BMDS did not provide an adequate fit to the variance, as shown in Table A-3. In an attempt to achieve model fit, the high-dose group was dropped from the analysis. However, the results were the same as with the full dataset; neither the homogenous nor modeled variance options resulted in adequate fit to the variance data. Thus, this data set was not considered suitable for BMD analysis.

Table A-3. Model Predictions for Serum Cholesterol in Male Rats Exposed Orally to Ethylbenzene for 13 Weeks[a]

Model	Variance *p*-Value[b]	Means *p*-Value[b]	BMD_{1SD} (mg/kg-day)	$BMDL_{1SD}$ (mg/kg-day)
All dose groups				
Linear (constant variance)[c]	0.05241	0.0001022	413.53	292.77
Linear (modeled variance)[c]	0.05377	<0.001	372.14	210.33
Without high-dose group				
Linear (constant variance)[c]	0.02868	0.009652	107.86	78.30
Linear (modeled variance)[c]	0.01563	0.005013	109.90	77.92

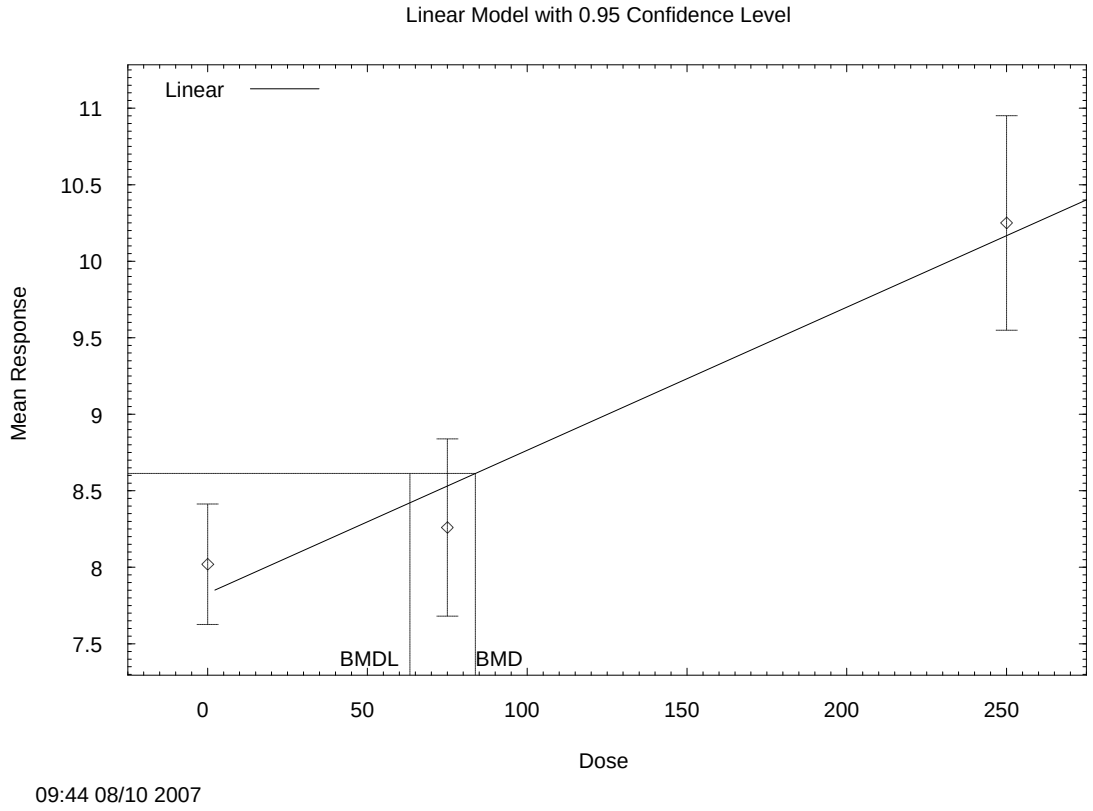
[a]Mellert et al., 2007
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Coefficients restricted to be positive

Modeling of Data on Absolute Liver Weight in Male Rats

Following the above procedure, continuous-variable models in the EPA BMDS (version 1.3.2) were fit to the data shown in Table 2 for increased absolute liver weight in male rats (Mellert et al., 2007) using a default BMR of 1 standard deviation from the control mean. As noted in the text, the high-dose group was excluded from the analysis a priori due to the confounding effect of reduced body weight on liver-weight changes. Using this reduced data set, the linear model with constant variance model provided adequate fit to both the variance and means data (Table A-4 and Figure A-3). The BMDs and the 95% lower confidence limits (BMDLs) associated with a change of 1 standard deviation (SD) from the control were calculated using the linear model with constant variance.

Table A-4. Model Predictions for Absolute Liver Weight in Male Rats Exposed Orally to Ethylbenzene for 13 Weeks[a]				
Model	**Variance *p*-Value[b]**	**Means *p*-Value[b]**	**BMD_{1SD} (mg/kg-day)**	**$BMDL_{1SD}$ (mg/kg-day)**
Without high-dose group				
Linear (constant variance)[c]	0.2048	0.1617	83.80	63.30

[a]Mellert et al., 2007
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria
[c]Coefficients restricted to be positive



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

Figure A-3. Fit of Linear Model (Constant Variance) to Data on Absolute Liver Weight in Male Rats (Mellert et al., 2007)

Modeling Procedure for Dichotomous Data

The benchmark dose (BMD) modeling for dichotomous data was conducted with the EPA's BMD software (BMDS version 2.1). For all the dichotomous data, the original data were modeled with all the dichotomous models (i.e., Gamma, Multistage, Logistic, Log-logistic, Probit, Log-Probit, Weibull, and Quantal linear models) available within the software with a default benchmark response (BMR) of 10% extra risk. An adequate fit was judged based on the goodness of fit *p*-value ($p > 0.1$), scaled residual at the range of benchmark response (BMR), and visual inspection of the model fit. Among all the models provided adequate data fit, the lowest BMDL will be selected if the BMDLs estimated from different models if the range is considered sufficiently large; otherwise, the BMDL from the model with the lowest Akaike's Information Criterion (AIC) would be considered appropriate for the data set.

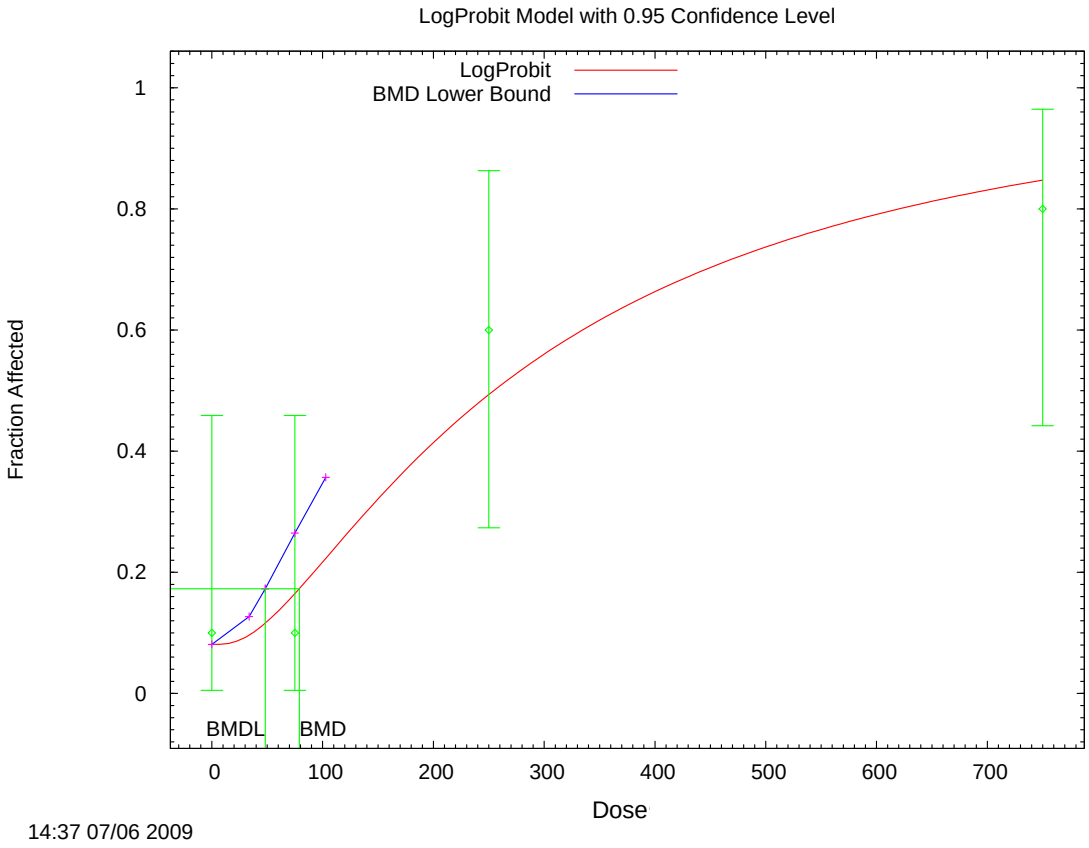
Modeling of Data on Centrilobular Hepatocyte Hypertrophy in Male Rats

Table 2 shows the dose-response data for incidence of centrilobular hepatocyte hypertrophy in male rats (Mellert et al., 2007). These data were modeled according to the procedure outlined above. As assessed by the χ^2 goodness-of-fit test, all models in the software provided adequate fits to the data for the incidence of centrilobular hepatocyte hypertrophy in male rats (χ^2 $p \geq 0.1$) (Table A-5). The Log-probit model provided the best fit, as assessed by AIC. The fit of the log-probit model to the data is shown in Figure A-4.

Table A-5. Model Predictions for Incidence of Centrilobular Hepatocyte Hypertrophy in the Male Rats Exposed Orally to Ethylbenzene for 13 Weeks[a]

Model	Degrees of Freedom	χ^2	χ^2 Goodness of Fit *p*-Value	AIC	BMC_{10} (mg/m^3)	$BMCL_{10}$ (mg/m^3)
Log Logistic	1	1.04	0.3068	43.55	73.08	18.24
Gamma	1	1.60	0.2059	44.15	61.59	29.13
Multistage (degree of polynomial = 1)[b]	2	1.62	0.4457	42.24	46.8785	28.92
Multistage (degree of polynomial = 2)[b]	2	1.62	0.4457	42.24	46.8785	28.92
Multistage (degree of polynomial = 3)[b]	2	1.62	0.4457	42.24	46.8785	28.92
Weibull	1	1.62	0.2026	44.21	54.54	29.00
Quantal Linear	2	1.62	0.4457	42.24	46.88	28.92
Log Probit	2	0.98	0.6119	41.48	78.95	48.26
Probit	2	3.44	0.1792	43.91	112.02	76.32
Logistic	2	3.46	0.1772	43.95	114.34	73.53

[a] Mellert et al., 2007
[b]Degree of polynomial initially set to (*n*-1) where *n* = number of dose groups including control. Betas restricted to ≥0.



BMDs and BMDLs indicated are associated with an extra risk of 10% and are in units of mg/kg-day.

Figure A-4. Fit of Log-Probit Model to Incidence of Centrilobular Hepatocyte Hypertrophy in the Male Rat (Mellert et al., 2007)

```
=====================================================================
        Probit Model. (Version: 3.1;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21Beta\Temp\4tmp110E.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21Beta\Temp\4tmp110E.plt
                                              Mon Jul 06 14:44:29 2009
=====================================================================

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

  The form of the probability function is:

  P[response] = Background
              + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

  where CumNorm(.) is the cumulative normal distribution function


  Dependent variable = Incidence
  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 (and Specified) Parameter Values
background = 0.1
intercept = -6.15205
slope = 1.07357

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	-0.37
intercept	-0.37	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
background	0.0810598	0.07279	-0.061606	0.223726
intercept	-5.65033	0.317859	-6.27332	-5.02734
slope	1	NA		

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

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-18.2358	4			
Fitted model	-18.741	2	1.01037	2	0.6034
Reduced model	-26.9205	1	17.3693	3	0.0005933

AIC: 41.482

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0811	0.811	1.000	10	0.219

```
   75.0000     0.1650         1.650     1.000          10       -0.553
  250.0000     0.4934         4.934     6.000          10        0.674
  750.0000     0.8474         8.474     8.000          10       -0.417

 Chi^2 = 0.98      d.f. = 2        P-value = 0.6119


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       78.9472

            BMDL =       48.2564
```