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# **1,3-Butadiene**

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TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

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## Chapter 1 Summary Tables and Figure

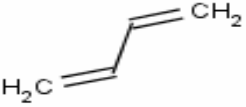
Table 1 provides a summary of health- and welfare-based values based on an acute and chronic evaluation of 1,3-butadiene (BD). Table 2 provides summary information on BD's physical/chemical data.

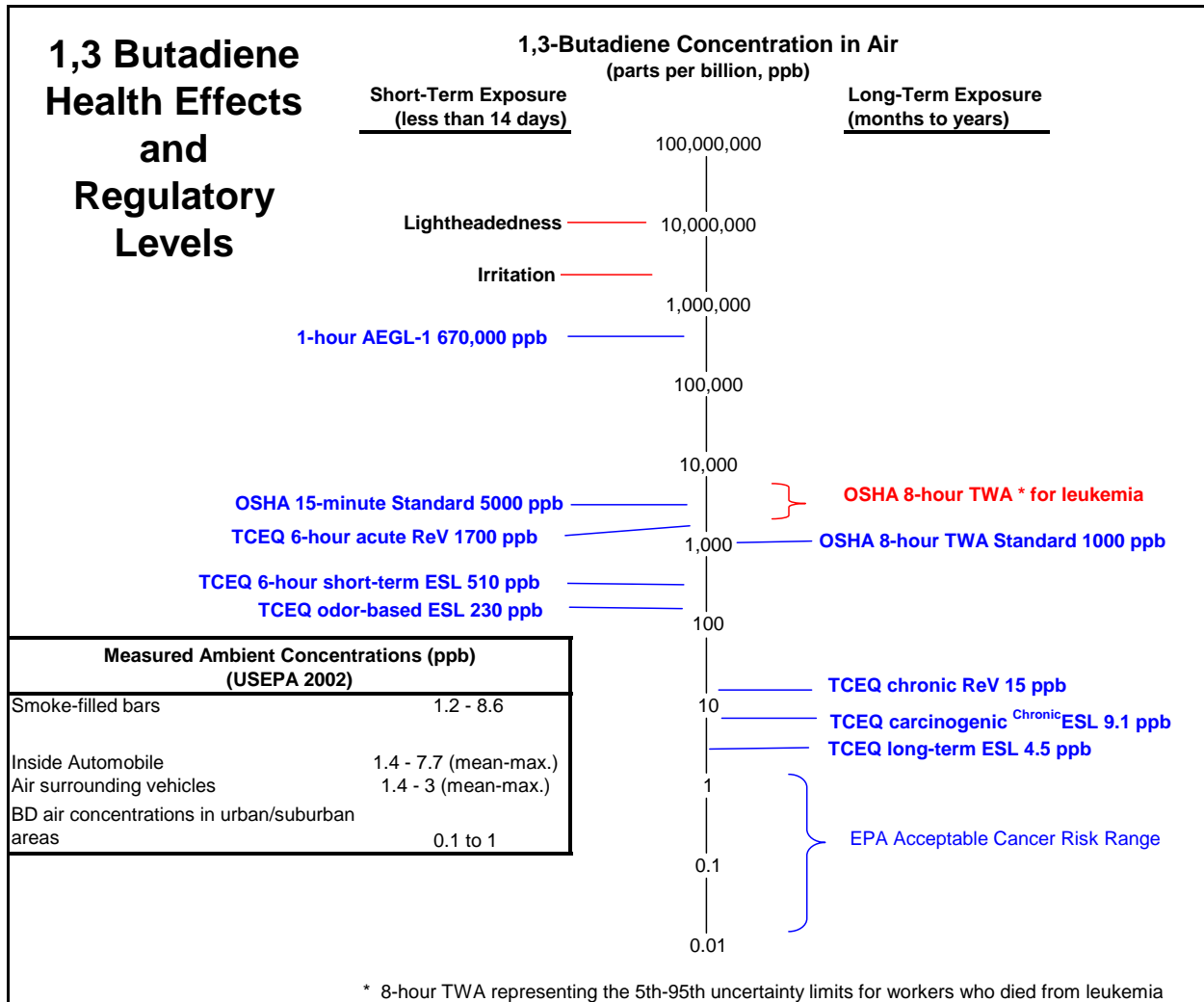
<b>Table 1. Health- and Welfare-Based Values</b>		
<b>Short-Term Values</b>	<b>Concentration</b>	<b>Notes</b>
<sup>acute</sup> ESL [6 h] (HQ = 0.3)	1,100 µg/m <sup>3</sup> (510 ppb)	<b>Critical Effect:</b> Developmental toxicity; reduction in extragestational weight gain and in fetal body weight in CD-1 mice
Acute ReV [6 h] (HQ = 1.0)	3,700 µg/m <sup>3</sup> (1,700 ppb) <sup>a</sup>	
<sup>acute</sup> ESL <sub>odor</sub>	510 µg/m <sup>3</sup> (230 ppb) <sup>a</sup> <b>Short-Term ESL for Air Permit Reviews</b>	50% detection threshold, mild aromatic odor
<sup>acute</sup> ESL <sub>veg</sub>	---	Concentrations producing vegetative effects were significantly above other ESLs
<b>Long-Term Values</b>	<b>Concentration</b>	<b>Notes</b>
<sup>chronic</sup> ESL <sub>nonlinear(nc)</sub> (HQ = 0.3)	9.9 µg/m <sup>3</sup> (4.5 ppb) <b>Long-Term ESL for Air Permit Reviews</b>	<b>Critical Effect:</b> Reproductive toxicity: ovarian atrophy in B6C3F1 mice
Chronic ReV (HQ = 1.0)	33 µg/m <sup>3</sup> (15 ppb) <sup>a</sup>	
<sup>chronic</sup> ESL <sub>linear(c)</sub>	20 µg/m <sup>3</sup> (9.1 ppb) <sup>a, b</sup>	<b>Cancer Endpoint:</b> Leukemia in occupational exposure study of styrene-butadiene synthetic rubber production workers
<sup>chronic</sup> ESL <sub>veg</sub>	---	No data found

<sup>a</sup> Values that may be used for evaluation of air monitoring data

<sup>b</sup> Based on unit risk factor (URF) = 5.0E-07 per µg/m<sup>3</sup> (1.1E-06 per ppb) and a risk level of 1 in 100,000 excess cancer risk

Abbreviations used: **HQ**, hazard quotient; **ppb**, part per billion; **mg/m<sup>3</sup>**, milligrams per cubic meter; **µg/m<sup>3</sup>**, micrograms per cubic meter; **h**, hour; **ESL**, Effects Screening Levels; **ReV**, Reference Value; <sup>acute</sup>**ESL**, acute health-based ESL; <sup>acute</sup>**ESL<sub>odor</sub>**, acute odor-based ESL; <sup>acute</sup>**ESL<sub>veg</sub>**, acute vegetation-based ESL; <sup>chronic</sup>**ESL<sub>linear(c)</sub>**, chronic health-based ESL for linear dose-response cancer effect; <sup>chronic</sup>**ESL<sub>nonlinear(nc)</sub>**, chronic health-based ESL for nonlinear dose-response noncancer effects; and <sup>chronic</sup>**ESL<sub>veg</sub>**, chronic vegetation-based ESL

<b>Table 2. Chemical and Physical Data</b>		
<b>Parameter</b>	<b>Value</b>	<b>Reference</b>
Molecular Formula	C <sub>4</sub> H <sub>6</sub> or H <sub>2</sub> C:CHHC:CH <sub>2</sub>	Lewis 1993
Chemical Structure		ChemIDplus Lite
Molecular Weight	54.1	TRRP 2006
Physical State	gas/organic	TRRP 2006
Color	colorless	Lewis 1993
Odor	mild aromatic odor	ACGIH 2001
CAS Registry Number	106-99-0	TRRP 2006
Synonyms	vinylethylene; erythrene; bivinyl; divinyl; biethylene; pyrrolylene; a,g- butadiene	Lewis 1993 NTP 1993
Solubility in water	735 mg/L	TRRP 2006
Log K <sub>ow</sub>	2.03	TRRP 2006
Vapor Pressure	2,100 mm Hg at 20 °C	TRRP 2006
Vapor Density (air = 1)	1.87	Lewis 1992
Density (water = 1)	0.6211 (liquid at 20 °C)	Lewis 1993
Melting Point	-113° C	Lewis 1992
Boiling Point	-4.41° C	Lewis 1993
Conversion Factors	1 µg/m <sup>3</sup> = 0.45 ppb @ 25°C 1 ppb = 2.21 µg/m <sup>3</sup>	NTP 1993



**Figure 1. BD Health Effects and Regulatory Levels.** This figure compares BD’s acute toxicity values (acute ReV, odor-based ESL, and health-based, short-term ESL) and chronic toxicity values (chronic ReV and long-term ESL) found in Table 1 to USEPA’s acceptable cancer risk range (USEPA 2002), OSHA’s occupational values, and the AEGL-1 value (AEGL 2005). USEPA’s (2002) acceptable cancer risk range is based on an older epidemiology study that has recently been updated to include additional information with validated, more accurate BD exposure estimates.

Abbreviations used: **BD**, 1,3-butadiene; **TCEQ**, Texas Commission on Environmental Quality; **TWA**, Time-Weighted Average; **ESL**, Effects Screening Level; **ReV**, Reference Value; **OSHA**, Occupational Safety and Health Administration; **USEPA**, United State Environmental Protection Agency; and **AEGL-1**, Level 1-Acute Exposure Guideline Levels.

## Chapter 2 Major Sources or Uses

BD is used as an intermediate in the production of polymers, elastomers, and other chemicals. Its major uses are in the manufacture of styrene-butadiene rubber (SBR) (synthetic rubber) and thermoplastic resins. Elastomers of BD are used in the manufacture of tires, footwear, sponges, hoses and piping, luggage, packaging, and a variety of other molded products. In addition, BD is used as an intermediate to produce a variety of industrial chemicals, including the fungicides captan and captfol. The primary way that BD is released into the environment is via emissions from gasoline- and diesel-powered vehicles and equipment. Lesser releases occur from the combustion of other fossil fuels and biomass. Minor releases occur in production processes, tobacco smoke, gasoline vapors, and vapors from the burning of plastics as well as rubber (Miller 1978; USEPA 2002). United States Environmental Protection Agency's (USEPA) (2001) National-Scale Air Toxics Assessment of emissions from the 1996 National Toxics Inventory indicates that statewide BD emissions from mobile sources (onroad and nonroad) accounted for approximately 54% of the National Toxics Inventory BD emissions in Texas, with major facility sources and area/other sources (e.g., smaller facilities) comprising the remainder of 46%.

## Chapter 3 Acute Evaluation

### 3.1 Health-Based Acute ReV and <sup>acute</sup>ESL

#### 3.1.1 Physical/Chemical Properties and Key Studies

##### 3.1.1.1 Physical/Chemical Properties

BD is a highly volatile, colorless gas with a mildly aromatic odor. The main chemical and physical properties of BD are summarized in Table 2. It is soluble in ethanol, diethyl ether, and organic solvents, and only slightly soluble in water.

##### 3.1.1.2 Key Studies

This section is based on USEPA (2002) and AEGL (2005). Both of these sources state "The acute toxicity of BD is of low order." (USEPA 2002; AEGL 2005). A review of the scientific literature since 2002 indicates that a subchronic inhalation study in rats conducted by the American Chemistry Council (ACC 2003) is a new animal study that was not considered by USEPA (2002), and the findings of Spencer *et al.* (2001) and Chi *et al.* (2002) on the possible reproductive/developmental mode of action of BD were not considered. Therefore, these studies are discussed in Sections 3.1.1.2.2 and 3.1.2.2, respectively. Animal data show BD is a potential reproductive/developmental hazard to humans. Since the reproductive/developmental effects of BD in rats and mice are among the effects observed at the lowest exposure levels following acute inhalation exposure, the following sections focus on these health effects. Chapter 5 of *Health Assessment of 1,3-Butadiene* (USEPA 2002) provides a detailed discussion on potential reproductive/developmental effects in humans and animals, and AEGL (2005) discusses other types of acute toxicity data.

##### 3.1.1.2.1 Human Studies

Albertini *et al.* (2007) conducted a molecular epidemiological study of BD-exposed Czech workers to compare female to male responses. The focus of the study was to collect data on urine concentrations of

BD metabolites and blood concentrations of BD-metabolite hemoglobin adducts. However, questionnaire responses for female-specific adverse health questions in control and exposed females were also obtained. There were 26 female control workers and 23 female BD-exposed workers. The years of employment were  $17.6 \pm 9.3$  years for control and  $19.4 \pm 9.9$  years for exposed females (mean  $\pm$  S.D.). Multiple external exposure measurements were obtained (10 full 8-hour (h) shift measures by personal monitoring per worker) over a 4-month period before biological samples were collected. Mean 8-h time-weighted average (TWA) exposure levels were 0.008 milligram per cubic meter ( $\text{mg}/\text{m}^3$ ) (0.0035 parts per million (ppm)) for controls and  $0.397 \text{ mg}/\text{m}^3$  (0.180 ppm) for exposed. Individual single 8-h TWA values were as high as  $9.793 \text{ mg}/\text{m}^3$  (4.45 ppm). Analysis of questionnaire responses for female-specific adverse health questions showed no significant differences between controls and exposed for miscarriages, still births, ectopic pregnancies, molar pregnancies, low birth weight (<2,500 g) babies, or pre-term births, based on information collected on all pregnancies. The ability of the study to detect differences in the evaluated endpoints may be limited because there were few subjects evaluated.

The health effects observed in humans occur at high concentrations and include the following: odor perception (ACGIH 2001; Ruth 1986; and Nagata 2003); slight smarting of the eyes and difficulty in focusing on instrument scales (Carpenter *et al.* 1944); and tingling sensation and dryness of the nose and throat (Larionov *et al.* 1934) (Table 3). A poorly reported study conducted by Ripp (1967) in human volunteers reported effects of olfactory perception at  $4.0 \text{ mg}/\text{m}^3$  (1.8 ppm) and sensitivity of the eye to light at  $3.9 \text{ mg}/\text{m}^3$  (1.7 ppm). There were no effects on the occurrence of an electrocortical conditioned reflex at  $3 \text{ mg}/\text{m}^3$  (1.4 ppm). Khalil *et al.* (2007) reported that BD produced increased neurological risks in a random cohort of 310 patients who had been exposed to accidental leakage and release of BD due to an explosion. The environmental contamination persisted for a few hours to several days in the atmosphere of the areas surrounding the plant. Exposure concentrations of BD or information on other chemicals that may have been released during the explosion were not provided.

Study	Concentration (Exposure Duration)	Subjective Symptoms	Differences Observed
Carpenter <i>et al.</i> 1944 2 males 1-hour (h) lunch break Nominal Concentrations	2,000 ppm <sup>1</sup> (7 h)	Slight smarting of the eyes; difficulty in focusing on instrument scales	Results of tapping test and steadiness test – no differences
	4,000 ppm (6 h)	Slight smarting of the eyes; difficulty in focusing on instrument scales	
	8,000 ppm (8 h)	No subjective complaints <sup>2</sup>	
Larionov <i>et al.</i> (1934) No details on number of subjects and gender	1% (10,000 ppm) 5 minute (min)	Tingling sensation and dryness of the nose and throat.	Slight increase in pulse rate. No effects on blood pressure or respiration

<sup>1</sup> Difficulty in focusing on instrument scales was the basis of the AEGL-1 value. The 1-h AEGL-1 value of 670 ppm = 2,000 ppm divided by an intraspecies uncertainty factor of 3.

<sup>2</sup> No subjective complaints because of slight anxiety of subjects concerning the possibility of an explosion.

### 3.1.1.2.2 Animal Studies

#### 3.1.1.2.2.1 Reproductive/Developmental Toxicity in Rats

In 1982, Hackett *et al.* (International Institute of Synthetic Rubber Producers (IISRP) 1982) conducted a reproductive/developmental study that included exposure of pregnant rats at 0, 200, 1,000, and 8,000 ppm 6 hours/day (h/day) on gestation day (GD) 6-15 and then sacrifice on GD 20. The most sensitive endpoints were a significant decrease in maternal body weight gain on GD 6-9 and extragestational weight gain (lowest observed adverse effect level (LOAEL) of 1,000 ppm and no observed adverse effect level (NOAEL) of 200 ppm for both endpoints). Minor skeletal defects were found to be significantly elevated at the lowest concentration, and the percentage of fetuses with major skeletal defects was significantly elevated at 1,000 ppm and above. The incidence of marked-to-severe wavy ribs and the total number of abnormal ossifications and irregular ossification of the ribs were elevated at 8,000 ppm.

In 1987, Hackett *et al.* (1987a) repeated the IISRP (1982) study at slightly lower concentrations to confirm the 1982 findings in rats and to compare the effects of similar BD exposures in mice (Hackett *et al.* 1987b). The results of the Hackett *et al.* (1987b) study in mice are discussed in the next section. Pregnant rats (Hackett *et al.* 1987a) were exposed for 10-days via inhalation to 0, 40, 200, and 1,000 ppm on GD 6-15 for 6 h/day (Hackett *et al.* 1987a). For rats, the most sensitive short-term endpoints were decreases in maternal body weight gain on GD 6-11 and decreases in extragestational weight gain (NOAEL of 200 ppm and LOAEL of 1,000 ppm for both endpoints). Effects from BD exposure for fetal measures were not observed (i.e., no developmental toxicity was observed).

In 2003, a subchronic reproductive/developmental study in rats sponsored by the American Chemistry Council was conducted by WIL Research Laboratories, Inc (ACC 2003). Since this study was not available for USEPA's BD assessment (USEPA 2002), the major findings of the study are discussed below. The study was conducted using the following guidelines:

- USEPA TSCA Good Laboratory Practice Standards;
- The protocol met or exceeded applicable regulations of the Organisation for Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals, Guideline 421, Reproduction/Development Toxicity Screening Test (July 27, 1995) and Office of Prevention, Pesticides & Toxic Substances (USEPA) 870.3550 (July 2000) requirements.

This study was conducted to provide information on the potential adverse effects of BD on male and female reproduction within the scope of a screening study. Assessments of gonadal function, mating behavior, conception, gestation, parturition, lactation of the F<sub>0</sub> generation, and the development of F<sub>1</sub> offspring from conception through weaning and post-weaning exposure were included. Three groups of F<sub>0</sub> animals, each consisting of 12 male and 12 female CrI:CD®(Sprague-Dawley) IGS BR rats, were exposed to 300, 1,500, and 6,000 ppm BD via whole-body inhalation exposure 6 h/day for 14 days prior to the breeding period and continuing throughout the gestation and lactation periods. A control group was exposed to clean, filtered air on a comparable regimen. For F<sub>0</sub> dams, the daily inhalation exposures were suspended on GD 21 through lactation day 4, to avoid any confounding effects of exposure on nesting or nursing behavior. Exposures were resumed for these dams on lactation day 5. The F<sub>1</sub> generation pups were potentially exposed to BD *in utero* and through nursing during lactation until weaning. Beginning on postnatal day (PND) 21, one male and one female from each litter were exposed for seven consecutive days to the same concentration of the BD concentration as its dam. Beginning on PND 28, one previously

unexposed male and one previously unexposed female per litter were exposed for seven consecutive days to the same BD concentration as its dam.

Under the conditions of the current study, there were no adverse BD-related effects on any parameter measured in either the F<sub>0</sub> or F<sub>1</sub> animals at the exposure level of 300 ppm. Adverse BD-related effects were noted at 1,500 and 6,000 ppm and consisted of persistent reductions in body weight parameters in F<sub>0</sub> and F<sub>1</sub> males and females and transient reductions in food consumption (week 0-1) for F<sub>0</sub> males and females.

Adverse BD-related effects noted exclusively at 6,000 ppm consisted of clinical observations indicative of chromodacryorrhea, chromorhinorrhea, and salivation in F<sub>0</sub> males and females as well as infrequent occurrences of dried red material in the perioral and perinasal regions of four exposed F<sub>1</sub> pups (three males and one female).

Based on the results of this study, an exposure level of 300 ppm was considered to be the NOAEL in rats for F<sub>0</sub> parental systemic toxicity and for systemic toxicity for F<sub>1</sub> animals following post-weaning 6-h daily exposures (PND 21-27 or PND 28-34). The NOAEL for effects on gonadal function, mating behavior, conception, gestation, parturition, lactation of the F<sub>0</sub> generation, and the development of F<sub>1</sub> offspring from conception through weaning was considered to be 6,000 ppm.

The findings of this subchronic reproductive/developmental study showed effects of reduction in body weight parameters as the most sensitive endpoint in male and female rats with a NOAEL of 300 ppm. Developmental effects were not observed. This study is included in the acute toxicity section because it is a well-conducted, high-quality study with a NOAEL of 300 ppm, which is slightly higher than the NOAEL of 200 ppm determined in previous rat studies (IISRP 1982; Hackett *et al.* 1987a).

### ***3.1.1.2.2 Reproductive/Developmental Toxicity in Mice***

Hackett *et al.* (1987b) exposed pregnant mice for 10 days via inhalation at 0, 40, 200, and 1,000 ppm (analytical concentrations of 0, 39.9, 200, and 1,000 ppm) on GD 6-15 for 6 h/day. Maternal toxicity manifested as reduced body weight gain (GD 11-16) and extragestational weight gain was observed at 200 and 1,000 ppm. Total body weight at GD 18 was decreased at 1,000 ppm. Therefore, the NOAEL for maternal toxicity was 40 ppm. Hackett *et al.* (1987b) reported the most sensitive short-term developmental endpoint was decreased fetal body weight in male mice at 40 ppm. BD caused reduced fetal body weight and increased frequency of skeletal variations at 200 and 1,000 ppm which are concentrations corresponding to maternal toxicity expressed as reduced body weight. Major malformations in the mouse fetus were not detected although the potential for altered development was indicated by a dose-related increase in supernumerary ribs and reduced ossifications, particularly of the sternbrae.

Hackett *et al.* (1987b) reported that statistical differences were observed at the lowest exposure concentration of 40 ppm for male fetal body weight. Therefore, a NOAEL was not identified for this effect. However, Hackett *et al.* (1987b) conducted analyses of variance (ANOVA) on the average pup weight followed-up by Student's t-tests comparing the average pup weight for different treatment groups. Their pairwise comparisons using Student's t-test did not adjust significance levels for the number of multiple tests. In addition, their analyses did not adjust for well-known important covariate effects such as litter size. Christian (1996) noted that the apparent significant decrease in male fetal body weight in the 40 ppm group was the result of the statistical analysis used, which was considered to be inappropriate.

Data reported by Hackett *et al.* (1987b) were reanalyzed by Green (2003). The Green (2003) reanalysis was based on analysis of covariance (ANCOVA) on the average pup weight adjusted for covariates and used the Dunnett-Hsu test to compare the mean weights for each of the exposed groups to the mean weight for the control group. Application of the statistical analysis indicates that the 40 ppm exposure concentration is a NOAEL in this study. Other previously analyzed endpoints were also analyzed by more appropriate methodology (Green 2003). In each instance, the NOAEL was at least as high as previously reported. For a few endpoints, a higher NOAEL was found. The overall NOAEL for this study is 40 ppm, based on the fetal body weights.

In order to assess the Green (2003) reanalysis, Sielken *et al.* (Appendix 1) conducted a review of the Hackett *et al.* (1987b) study and the Green (2003) reanalysis, concentrating on male fetal body weight. The Sielken *et al.* review (Appendix 1) indicates that Green's (2003) conclusions are reasonable and based on standard statistical analyses practices that were overlooked by Hackett *et al.* (1987b). Green used the Dunnett-Hsu test to compare the mean weights for each of the exposed groups to the mean weight for the control group after both were adjusted for the effects of the covariates. The Dunnett-Hsu test was specifically designed for this situation. In addition to reviewing the statistical methodology used in the Hackett *et al.* (1987b) and Green (2003) studies, Sielken *et al.* (Appendix 1) re-analyzed the fetal body weight data to confirm the numerical results obtained by Green (2003). Sielken *et al.* (Appendix 1) also performed a sensitivity analysis with respect to the effects of covariates and determined the outcome of the more powerful statistical analyses where the individual pup weights were analyzed and the dams were treated as random effects. These analyses support the finding that the NOAEL based on either male or female fetal body weight for this study is 40 ppm (Sielken *et al.* (Appendix 1)).

Table 4 is similar to Table 5-6 in USEPA (2002) but only contains parameters that were significantly different from controls. There were no statistical differences in number of pregnant dams, litters with live fetuses, implantations per dam, resorptions per litter, dead fetuses per litter, fetuses per number of litters examined, or sex ratio (% males) between treated mice and control mice (data not shown). The highlighted cells in Table 4 have been corrected based on the Hackett *et al.* (1987b) study reanalyses by Green (2003) and Sielken *et al.* (Appendix 1). The appropriate NOAEL for early resorptions is 1,000 ppm (not 200 ppm as reported by Hackett *et al.* (1987b)), and the LOAEL for decreases in male fetal body weight is 200 ppm (not 40 ppm). Decreases in male fetal body weight occur at the same concentrations as decreases in maternal weight gain (Table 6).

Table 5 is similar to Table 5-7 in USEPA (2002) but only contains parameters that were significantly different from controls. There were no results contrary to those of the Hackett *et al.* (1987b) after the reanalysis by Green (2003). The only fetal effects noted were significant increases in minor skeletal abnormalities at 200 and/or 1,000 ppm indicative of growth retardation (i.e., increases in supernumerary ribs and reduced ossification in the sternbrae). These effects occurred at the same concentrations as decreases in maternal weight gain (Table 6).

Parameters	Concentration (ppm)			
	0	40	200	1,000
Early resorptions	1.00 ± 0.23	0.58 ± 0.21	0.43 ± 0.13 <sup>c, g</sup>	0.75 ± 0.16
Fetal body weight (gram (gm)) (Mean per litter)	1.34 ± 0.03 <sup>b</sup>	1.28 ± 0.01	1.13 ± 0.02 <sup>c</sup>	1.04 ± 0.03 <sup>c</sup>
Females	1.30 ± 0.03 <sup>b</sup>	1.25 ± 0.01	1.10 ± 0.02 <sup>c</sup>	1.06 ± 0.02 <sup>c, f</sup>
Males	1.38 ± 0.03 <sup>b</sup>	1.31 ± 0.02 <sup>c, d</sup>	1.13 ± 0.02 <sup>c</sup>	1.06 ± 0.02 <sup>c</sup>
Placental weight (mg) (Mean per litter)	86.8 ± 2.99 <sup>b</sup>	85.4 ± 2.29	78.6 ± 3.24 <sup>c</sup>	72.6 ± 1.88 <sup>c</sup>
Females	83.1 ± 3.03 <sup>b</sup>	80.9 ± 2.46	74.7 ± 3.52	70.1 ± 2.33 <sup>c</sup>
Males	89.3 ± 3.03 <sup>b, e</sup>	89.5 ± 2.27	80.1 ± 2.35 <sup>c</sup>	74.5 ± 1.81 <sup>c</sup>

<sup>a</sup> All values mean ± standard error from USEPA (2002)

<sup>b</sup>  $p \leq 0.05$ , significant linear trend

<sup>c</sup>  $p \leq 0.05$ , pairwise comparison with corresponding control parameter based on Hackett *et al.* (1987b)

<sup>d</sup>  $p > 0.05$  based on Green (2003) and Sielken *et al.* reanalyses(Appendix 1)

<sup>e</sup>  $89.3 \pm 3.05$  (Hackett *et al.* 1987b)

<sup>f</sup>  $1.02 \pm 0.02$  (Hackett *et al.* 1987b)

<sup>g</sup>  $p \geq 0.05$  based on Green (2003)

Source: USEPA (2002)

Parameters	Concentration (ppm)			
	0	40	200	1,000
Variations: Abnormal sternebrae <sup>a, b</sup>	0.6 ± 0.9	0.4 ± 0.7	0.4 ± 0.8	0.8 ± 1.3 <sup>c</sup>
Variations: Supernumerary ribs <sup>a, b</sup>	1.7 ± 2.3	1.6 ± 2.1	6.0 ± 3.6 <sup>c</sup>	9.9 ± 3.0 <sup>c</sup>
Reduced ossification (all sites combined) <sup>a</sup>	1.7 ± 1.7	1.2 ± 1.5	2.7 ± 2.7	3.9 ± 2.6 <sup>c</sup>

<sup>a</sup> Mean percentage per litter (mean ± SD)

<sup>b</sup>  $p \leq 0.05$ , significant linear trend, orthogonal contrast test

<sup>c</sup>  $p \leq 0.05$ , Tukey's test

<sup>d</sup>  $p \leq 0.05$ , Fisher exact test (fetal incidence)

Source: USEPA (2002) and Hackett *et al.* (1987b)

Parameters	Concentration (ppm)			
	0	40	200	1,000
Whole-body weight (gm)				
Day 0	28.4 ± 0.25	28.3 ± 0.32	28.3 ± 0.32	28.4 ± 0.32
Day 18	54.9 ± 1.21 <sup>b</sup>	55.4 ± 1.09	52.5 ± 1.01	50.8 ± 0.86 <sup>c, f</sup>
Body weight gain (gm)				
Days 0-6	2.7 ± 0.3	3.0 ± 0.3	2.5 ± 0.2	2.3 ± 0.2
Days 6-11	5.5 ± 0.4	5.8 ± 0.3	5.6 ± 0.3	4.8 ± 0.3
Days 11-16	13.3 ± 0.6 <sup>b</sup>	12.7 ± 0.4	11.4 ± 0.5 <sup>c</sup>	10.6 ± 0.4 <sup>c</sup>
Days 16-18	5.5 ± 0.3 <sup>b</sup>	5.7 ± 0.3	4.7 ± 0.4	4.8 ± 0.3
Gravid uterine weight (gm)	19.3 ± 1.00 <sup>b</sup>	20.3 ± 0.80	18.0 ± 0.87	16.8 ± 0.67 <sup>c, g</sup>
Extragestational weight (gm) <sup>d</sup>	35.5 ± 0.48 <sup>b</sup>	35.1 ± 0.44	34.5 ± 0.46	34.1 ± 0.36 <sup>c</sup>
Extragestational weight gain (gm) <sup>e</sup>	7.60 ± 0.48 <sup>b</sup>	6.99 ± 0.38	6.20 ± 0.38 <sup>c</sup>	5.91 ± 0.28 <sup>c</sup>

<sup>a</sup> All values mean ± standard error from USEPA (2002)

<sup>b</sup>  $p \leq 0.05$ , significant linear trend

<sup>c</sup>  $p \leq 0.05$ , pairwise comparison with corresponding control parameter

<sup>d</sup> Body weight on GD 18 minus gravid uterine weight

<sup>e</sup> Extragestational weight minus body weight on GD 0

<sup>f</sup>  $50.8 \pm 0.87$  (Hackett *et al.* 1987b)

<sup>g</sup>  $16.7 \pm 0.67$  (Hackett *et al.* 1987b)

Source: USEPA (2002)

Table 6 is similar to Table 5-5 in USEPA (2002) but only lists data on maternal weight loss measures which are the main parameters that were significantly different from controls. There were no results contrary to those of Hackett *et al.* (1987b) based on the reanalysis of Green (2003). Table 6 indicates that there was a statistical reduction in extragestational weight gain (i.e., maternal weight minus gravid uterine weight) and weight gain (GD 11-16) at 200 ppm. A statistical decrease in gravid uterine weight occurred at 1,000 ppm. These results suggest that BD produces maternal toxicity but little or no intrauterine effects at 200 ppm. For mice and rats, body weight changes and changes in body weight gain in pregnant dams with no change in gravid uterine weight usually indicate maternal toxicity as discussed by Pohl *et al.* (1998):

“Changes in maternal body weight corrected for gravid uterine weight at sacrifice may indicate whether the effect is primarily maternal or fetal. For example, there may be a significant reduction in weight gain and in gravid uterine weight throughout gestation but no change in corrected maternal weight gain, which would generally indicate an intrauterine effect. Conversely, a change in corrected weight gain and no change in gravid uterine weight generally suggest maternal toxicity and little or no intrauterine effect.”

Although reduction in maternal body weight gain was an effect that was consistently observed in studies in rats (at higher concentrations) and mice (IISRP 1982; Hackett *et al.* 1987a, 1987b; and ACC 2003), there is experimental evidence that BD exposure causes a reduction in serum progesterone which may

result in fetal/placental effects (Section 3.1.2.2 *MOA for Reproductive/ Developmental Effects*). Therefore, the data from the following developmental and maternal toxicity endpoints observed in mice (Hackett *et al.* (1987b) was evaluated using benchmark dose modeling to determine a point of departure (POD) because they had a positive dose-response relationship:

- Developmental endpoints: decreased placental weight and fetal body weight, abnormal sternbrae, reduced ossification for all sites and increased incidence of supernumerary ribs
- Maternal toxicity: decreases in extragestational weight gain, body weight gain (GD 11-16), whole-body weight (day 18), gravid uterine weight, and extragestational weight

### 3.1.2 Mode-of-Action (MOA) Analysis

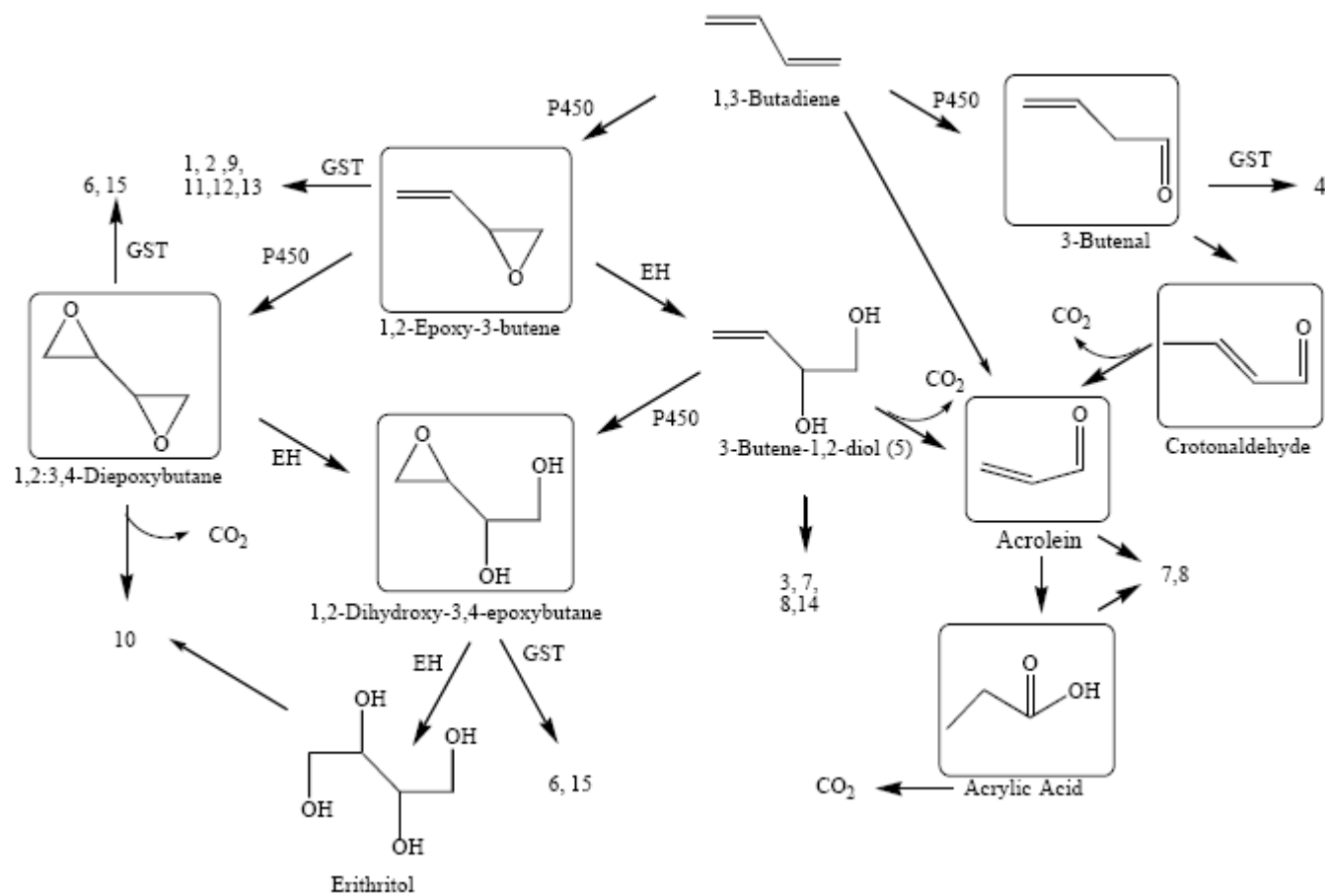
It is generally agreed that BD produces toxicity when it is metabolized to its reactive metabolites after animals are exposed to BD. However, there is a difference in the metabolism amongst species. The basis of the species differences between rats and mice may be related to the greater production of toxic intermediates and a lower capacity for detoxification of these intermediates (USEPA 2002).

#### 3.1.2.1 Metabolism

The following chemical terminology, similar to the terminology in USEPA (2002), is used in the DSD. Figure 2 is Figure 3.1 from USEPA (2002):

- 1,2-Epoxy-3-butene (EB). EB is also used for epoxybutene, 1,3-butadiene monoepoxide, 1,3-butadiene monoxide, 1,2-epoxybutene-3, vinyl oxirane, and 3,4-epoxy-1-butene;
- 1,2:3,4-Diepoxybutane (DEB). DEB is also used for diepoxybutane, butadiene diepoxide, and butadiene bisoxide;
- 3-Butene-1,2-diol (butene-diol). Butene-diol is also used for 1,2-dihydroxybut-3-ene; and
- 1,2-Dihydroxy-3,4-epoxybutane (EBD). EBD is also used for epoxybutanediol, 3,4-epoxybutanediol, 3,4-epoxybutane-1,2-diol, and 3,4-epoxy-1,2-butanediol.

The general metabolic scheme of BD, which has been reviewed by Himmelstein *et al.* (1997), is shown in Figure 2. BD is first metabolized to 1,2-epoxy-3-butene (EB), a process that is primarily associated with cytochrome P450 (CYP) 2E1, but can also be accomplished by additional isoforms including CYP 2A6 and 4B1. This electrophilic metabolite can be detoxified by conjugation with glutathione and subsequent excretion in the urine as urinary metabolites 1-hydroxy-2-(N-acetylcysteinyl)-3-butene and 2-hydroxy-1-(N-acetylcysteinyl)-3-butene (collectively known as M2 metabolite). It can also undergo hydrolysis by epoxide hydrolase (EH) to form 3-butene-1,2-diol (butene-diol). Butene-diol can also be conjugated with glutathione and subsequently excreted in the urine as urinary 1,2-dihydroxy-4-(N-acetylcysteinyl)-butane (M1 metabolite). It can be further oxidized by cytochrome P450 to the 1,2-dihydroxy-3,4-epoxybutane (EBD). An alternative pathway for the metabolism of EB is oxidation to the 1,2:3,4-diepoxybutane (DEB) which can be further hydrolyzed to EBD or conjugated by glutathione. This series of epoxidation and detoxication steps generates three electrophilic metabolites: EB, DEB, and EBD.



**Figure 2. Schematic of BD Metabolism** (Figure 3-1 from USEPA (2002))

P450 stands for cytochrome P450, EH stands for epoxide hydrolase, GST stands for glutathione transferase, and GSH stands for glutathione. The reactive metabolites are shown inside boxes. The urinary metabolites are numbered and listed in Table 3-1 of USEPA (2002).

Cochrane and Skopek (1994) have shown that DEB is 100 times more mutagenic than EB and 200 times more mutagenic than EBD in human lymphocytes. Kligerman and Yu (2007) used an *in vitro* system of lymphocytes treated with EB or DEB and measured sister chromatid exchange and chromosome aberrations. DEB-induced damage for both sister chromatid exchange and chromosome aberrations was persistent in G<sub>0</sub> cells and DEB was much more genotoxic than EB. EB did not induce sister chromatid exchange in lymphocytes unless actively cycling cells were treated. The extent to which DEB is produced and reaches target tissues will play a role in the toxicity. The ability of EB to reach actively dividing or repair deficient cells will also contribute somewhat to toxicity (Kligerman and Yu 2007). Mice form more DEB than rats or humans whereas EBD is more readily formed in humans than in rats (Slikker *et al.* 2004; Swenberg *et al.* 2007).

Human genetic polymorphisms are likely to affect individual susceptibility to BD and its metabolites. Metabolic activation rates in humans exhibit a high degree of variability and appear to span the range of activation rates between mice and rats when evaluated with *in vitro* systems measuring enzyme kinetics (greater than ten-fold). Other *in vitro* studies and *in vivo* molecular epidemiological studies indicate the range of increased sensitivity due to human genetic polymorphisms is approximately two- to four-fold (Albertini *et al.* 2001, 2003; Begemann *et al.* 2001; Fustinoni *et al.* 2002; Hayes *et al.* 1996, 2000, 2001; Smith *et al.* 2001; and Zhao *et al.* 2000, 2001). Several genes appear to be important in the BD metabolic pathway. Inherent susceptibilities have been shown for both EB and DEB (Weincke and Kelsey 1993), which may be due to glutathione S-transferase theta (GSTT1) status. Also, glutathione S-transferase GSTM1 appears to be an important detoxifying factor for EB, so that GSTM1 null individuals would be expected to have greater effects following formation of EB. Unfortunately, no data have been published on the effects of GST polymorphisms of EBD. Genetic polymorphisms have also been identified for EH and CYP 2E1 that would be expected to affect susceptibility to BD and its metabolites. The role of these proteins in the toxicokinetics of numerous chemicals is reasonably well known. Three *in vitro* studies (Csanády *et al.* 1992; Seaton *et al.* 1995; and Duescher and Elfarra 1994) using rodent and human tissue samples have demonstrated that CYP 2E1 plays a role in the oxidation of both BD and EB.

Polymorphisms that reduce EH activity may increase susceptibility to BD-induced effects. Likewise, rapid CYP 2E1 metabolizers may potentially be at greater risk. As previously mentioned, mice are much more sensitive to BD's reproductive/developmental effects than rats. The basis of the species differences between rats and mice may be related to the greater production of toxic intermediates, specifically DEB, and a lower capacity for detoxification of these intermediates in mice (USEPA 2002). Conjugation with GSH is an important detoxification route. Himmelstein *et al.* (1997) points out that GSH depletion occurs at longer exposure duration or at higher concentrations leading to higher body burdens of EB and DEB (Himmelstein *et al.* 1997).

### **3.1.2.2 MOA for Reproductive/Developmental Effects**

The most sensitive reproductive effect observed in 2-year chronic exposure studies was ovarian atrophy in female mice (NTP 1993). Ovarian atrophy is discussed in greater detail in Chapter 4. The specific mechanism of action for the reproductive/developmental effects produced by BD is unknown, although the MOA may involve DEB-induced ovarian atrophy and a decrease in serum progesterone levels (Spencer *et al.* 2001; Chi *et al.* 2002). Both Spencer *et al.* (2001) and Chi *et al.* (2002) hypothesize that DEB inhibits ovarian function, leading to a decrease in progesterone. Both estrogen and progesterone acting together, followed by progesterone postimplantation levels, are required for endocrine support for mammalian gestation. DEB does not appear to alter relative levels of estrogen receptor  $\alpha$  mRNA

expression (Spencer *et al.* 2001). Spencer *et al.* (2001) demonstrated that four daily intraperitoneal (i.p.) injections of DEB caused a dose-dependent decrease in endometrial weight, protein, and DNA, with decreases in serum progesterone in pseudo-pregnant Sprague-Dawley rats. Inducible nitric oxide synthase, pituitary adenylate cyclase-activating polypeptide (PACAP) mRNA expression, and matrix metalloproteinase-9 (MMP-9) activity were also decreased. These enzymes are important in implantation of the blastocyst and tissue remodeling. These changes lead to an inhibitory effect on uterine decidual growth/differentiation. Similar results were obtained when pregnant Sprague-Dawley rats were treated with four daily i.p. doses of DEB (Chi *et al.* 2002). Serum progesterone levels were significantly decreased as well as placental PACAP mRNA expression and MMP-9 activity (Chi *et al.* 2002). Chi *et al.* (2002) concluded:

In summary, the reproductive toxicity of diepoxybutane in pregnant rats apparently involved coordinated inhibition of placental molecular mechanisms (PACAP and MMP-9), uterine developmental processes (implantation and fetal metabolism) and progesterone secretion.

Based on the above information and consistent with USEPA (2002), the reproductive/developmental effects in mice are considered to have a threshold (i.e., a nonlinear MOA) and to be concentration and duration dependent.

### 3.1.3 Dose Metric

For the reproductive/developmental key study (Hackett *et al.* 1987b), data on the exposure concentration of the parent chemical are available. Since the MOA of the toxic response is not fully elucidated and data on other more specific dose metrics are not available (e.g. blood concentration of parent chemical, area under blood concentration curve of parent chemical, or putative metabolite concentrations in blood or target tissue), the exposure concentration of the parent chemical was used as the default dose metric.

### 3.1.4 Points of Departure (PODs) for Key Studies

The LOAEL for maternal toxicity in rats (1500 ppm) reported from a subchronic study conducted by the American Chemistry Council (ACC 2003) is more than seven times the LOAEL for developmental effects and maternal toxicity observed in mice (200 ppm). In addition, the slope of the rat dose-response curve is not steep, so the data from maternal toxicity in rats will not be considered. Data from mice for the following developmental and maternal toxicity endpoints (Section 3.1.1.2.2.2 *Reproductive/Developmental Toxicity in Mice*), which are all continuous data, were modeled with Benchmark Dose Modeling (BMDS) Software (Version 1.4.1c) using continuous models:

- Developmental endpoints: decreased placental weight, fetal body weight, abnormal sternebrae, reduced ossification for all sites, and increased incidence of supernumerary ribs
- Maternal toxicity: decreases in extragestational weight gain, body weight gain (GD 11-16), whole-body weight (day 18), gravid uterine weight, and extragestational weight

Since the selected endpoints are from a single study (Hackett *et al.* 1987b) and the same dosimetric adjustments and uncertainty factors will be applied to each endpoint, the endpoint with the lowest POD determined with BMD modeling may be the critical effect, if the endpoint is considered adverse, biologically plausible, and consistent with the proposed MOA.

### **3.1.4.1 Critical Effect Size**

If there is an accepted level of change in the endpoint that is considered to be biologically significant, then that amount of change is chosen for evaluation (USEPA 2000). For dichotomous data, this level is typically expressed as a certain increase in the incidence of adverse outcomes and is referred to as the benchmark response (BMR). In order to distinguish continuous data from dichotomous data, Dekkers *et al.* (2001) recommended the term “critical effect size” (CES) be used instead of the term “BMR,” since for continuous data, the effect measure is expressed on a continuous scale. A CES defines the demarcation between non-adverse and adverse changes in toxicological effect parameters for continuous data (Dekkers *et al.* 2001). For example, a CES of 10% or CES<sub>10</sub> for continuous data (i.e., a 10% change in the mean of a treated group compared to the control mean) is not the same as a BMR of 10% or BMR<sub>10</sub> (i.e., 10% of total animals responding for dichotomous data).

#### **3.1.4.1.1 Critical Effect Size for Developmental Endpoints – Linear Model**

Changes in fetal and placental weight were analyzed using the average fetal or placental weight for each litter. For a decrease in fetal body weight, a CES was defined in terms of a prespecified level of response, corresponding to a 5% relative decrease in the mean when compared to controls (CES<sub>05</sub>) (Kavlock *et al.* 1995; Allen *et al.* 1996). It was also assumed that a CES<sub>05</sub> for placental weight was the demarcation between non-adverse and adverse changes, although empirical data are not available for this endpoint. For abnormal sternebrae, reduced ossification for all sites, and increased incidence of supernumerary ribs (usually associated with maternal stress/weight loss), a 5% relative decrease in the mean when compared to controls (CES<sub>05</sub>) was used based on the findings by Allen *et al.* (1994) that indicated the CES<sub>05</sub> for malformed fetuses was similar to study NOAELs. The CES results for one standard deviation (SD) (CES<sub>1 SD</sub>) were calculated and are presented in Table 7 for comparison purposes as suggested by USEPA (2000).

#### **3.1.4.1.2 Critical Effect Size for Maternal Endpoints – Linear Model**

A 10% reduction in body weight or organ weight relative to the mean body weight in the control animals (CES<sub>10</sub>) is typically considered an adverse effect (USEPA 2000; Dekkers *et al.* 2001). It was assumed that a CES<sub>10</sub> for decreased maternal extragestational weight gain, decreased maternal body weight gain (GD 11-16), whole-body weight (day 18), gravid uterine weight, and extragestational weight was adverse. The CES<sub>1 SD</sub> was calculated and is presented in Table 7 for comparison purposes, as suggested by USEPA (2000).

#### **3.1.4.1.3 Unrestricted Power Model and CES<sub>1 SD</sub>**

As shown in Table 7, the differences between BMC<sub>05</sub> and BMCL<sub>05</sub> values for fetal/placental endpoints or BMC<sub>10</sub> and BMCL<sub>10</sub> values for maternal endpoints using the unrestricted power model ranged from approximately 20- to 100,000-fold (Table 7) which may be due to the unrealistically high slope in the low dose region at the level of the CES<sub>05</sub> or CES<sub>10</sub>. Therefore, the CES<sub>1 SD</sub> was a more relevant choice for the unrestricted power model because it avoids the steep-slope region (Appendix 2 *Benchmark Modeling Results Using the Power Model (11/19/07 Email from Bruce Allen)* and corresponds to USEPA guidance (2000). A CES of 1 SD from control mean corresponds to an approximately 10% excess risk for individuals below the 2<sup>nd</sup> percentile or above the 95<sup>th</sup> percentile of the control distribution for normally distributed effects (USEPA 2000). The BMC<sub>05</sub> and BMCL<sub>05</sub> values or BMC<sub>10</sub> and BMCL<sub>10</sub> values are presented in Table 7 and in Appendix 2, but are not discussed in the following sections.

### 3.1.4.2 Benchmark Concentration Modeling

Appendix 2 contains the dose-response data (i.e., dose, mean, SD, number of litters, percent control response, and coefficient of variation) (Tables 2A and 2B) and summary tables of modeling results from BMDS Software (Version 1.4.1c) (Tables 2C, 2D, 2E) for all ten endpoints. Table 7 and Figures 3 and 4 contain a summary of modeling results for the endpoints that could be adequately modeled. Modeling results using the unrestricted polynomial model (i.e., 2<sup>nd</sup> degree polynomial) produced a nonmonotonic dose-response curve, which is not considered biologically plausible, so unrestricted polynomial model results were not considered. The Hill model was not used because it is not the best choice for estimating the dose-response in the lower end of the data. The Hill model inherently gives too much weight to the higher doses, compromising the fit to the lower doses. Use of the Hill model with only four concentrations resulted in overparameterization of the data (i.e., model estimates of the dose-response curve artificially passed through every data point). The only models that adequately modeled the experimental data with 95% confidence (i.e., goodness of fit p-value and scaled residual values did not imply rejection at the 5% significance level and the model was not over-parameterized) and visual inspection of the dose-response curve indicated an adequate fit were the linear model (i.e., 1<sup>st</sup> degree polynomial model) and the unrestricted power model (Table 7 and Appendix 2, Tables 2C and 2D). Results from the restricted power model were identical to the linear model. A discussion of BMC modeling results from the linear model and the unrestricted power model is presented below.

Continuous data were modeled using continuous models in USEPA's BMDS software (version 1.4.1c). The TS did not attempt to change continuous data into dichotomous data and model the resulting dose-response curve with dichotomous models. USEPA (2000) noted that when continuous data were changed into dichotomous data, it potentially resulted in loss of information about the magnitude of response. Other investigators have noted the following when modeling continuous data as dichotomized data:

- Kavlock *et al.* (1995) found evidence that the confidence limits on the maximum likelihood estimates were larger when "quantalizing" continuous fetal body weight data;
- Gaylor (1996) found considerable precision was lost upon explicitly dichotomizing the data, even for moderate sample sizes; and
- West and Kodel (1999) noted the implicit approach (i.e., continuous data) gave substantially better results than modeling explicitly dichotomized data for sample sizes in the range of 10-20 animals per dose group, which is the number of pregnant dams in the Hackett *et al.* study (1987b).

#### 3.1.4.2.1 Data Not Amenable to Modeling

According to guidance in USEPA (2000), if the data for an endpoint are not amenable to modeling, the POD will be the statistically-derived study NOAEL. The following endpoints could not be modeled with confidence in either the linear model (all exposure concentrations), linear model (highest concentration excluded), or the unrestricted power model, because the modeling was not acceptable with respect to either test one (i.e., no significant difference (p value > 0.05) between responses and/or variances among the dose levels, so modeling the data with a dose/response curve may not be appropriate) or test four (i.e., the goodness of fit p value was less than 0.1) (Appendix 2, Tables 2C and 2D). That is, for the following endpoints, none of the three models passed test one or none of the three models passed test four (Appendix 2, Tables 2C and 2D). The coefficient of variations were very large for increased incidence of supernumerary ribs, abnormal sternbrae, and reduced ossification for all sites (Appendix 2, Table 2B). The study NOAEL will be used as the POD for the following toxicity endpoints:

- increased incidence of supernumerary ribs (test four); NOAEL = 40 ppm;

- abnormal sternebrae (test one); NOAEL = 200 ppm;
- reduced ossification for all sites (test four); NOAEL = 200 ppm;
- gravid uterine weight (test one); NOAEL = 200 ppm; and
- extragestational weight (test one); NOAEL = 200 ppm.

#### 3.1.4.2.2 Decreased Placental Weight

Decreased placental weight could be adequately modeled with confidence including all four exposure concentrations with the linear model and the unrestricted power model (Table 7 and Figure 3):

- Linear model:
  - $BMC_{05} = 344$  ppm,  $BMCL_{05} = 256$  ppm
  - $BMC_{1SD} = 1,063$  ppm,  $BMCL_{1SD} = 734$  ppm
- Unrestricted power model:
  - $BMC_{1SD} = 874$  ppm,  $BMCL_{1SD} = 233$  ppm.

Both a nonhomogeneous and homogeneous variance were used to model the data. The scaled residuals for a nonhomogeneous variance were slightly smaller in the low-dose region of the dose response curve, so the results from a nonhomogeneous variance are reported. The Akaike's Information Criterion (AIC) for the linear model was smaller than the AIC for the unrestricted power model, indicating the most appropriate POD for decreased placental weight is the  $BMCL_{05}$  of 256 ppm based on the linear model.

#### 3.1.4.2.3 Decreased Fetal Body Weight

Fetal body weight could be adequately modeled with confidence with the linear model when the highest concentration of 1,000 ppm was eliminated (Table 7 and Figure 3). Both a nonhomogeneous and homogeneous variance were used to model the data. The scaled residuals for a nonhomogeneous variance were slightly smaller in the low-dose region of the dose response curve, so the results from a nonhomogeneous variance are reported. Decreased fetal body weight had a  $BMC_{05}$  of 65.8 ppm and  $BMCL_{05}$  of 54.7 ppm and a  $BMC_{1SD}$  of 94.8 ppm and  $BMCL_{1SD}$  of 71.8 ppm. The POD for decreased fetal body weight is the  $BMCL_{05}$  of 54.7 ppm

#### 3.1.4.2.4 Decreased Maternal Extragestational Weight Gain

Decreased extragestational weight gain\* could be adequately modeled with confidence including all concentrations with the unrestricted power model (Table 7 and Figure 3):  $BMC_{1SD} = 723$  ppm and  $BMCL_{1SD} = 51.3$  ppm. The POD for decreased extragestational weight gain is the  $BMCL_{1SD}$  of 51.3 ppm.

#### 3.1.4.2.5 Decreased Maternal Body Weight Gain (GD11-16)

When the highest exposure concentration of 1,000 ppm was eliminated, decreased maternal body weight gain (GD11-16) could be adequately modeled with confidence with the linear model. Decreased maternal body weight gain (GD11-16) could be adequately modeled with confidence including all exposure concentrations with the unrestricted power model (Table 7 and Figure 4):

---

\* Extragestational weight is maternal body weight on GD 18 minus gravid uterine weight. Extragestational weight gain is extragestational weight minus body weight on GD 0

- Linear model without the highest dose:
  - $BMC_{10} = 145$  ppm,  $BMCL_{10} = 94.3$  ppm
  - $BMC_{1SD} = 238$  ppm;  $BMCL_{1SD} = 148$  ppm
- Unrestricted power model:
  - $BMC_{1SD} = 392$  ppm;  $BMCL_{1SD} = 63.5$  ppm

The AIC for the linear model with three doses cannot be compared to the AIC for the unrestricted power model with four doses because the number of doses differ, so the TS chose the  $BMCL_{1SD}$  of 63.5 ppm from the unrestricted power model because it was the lowest POD, included all concentrations, and captured the nonlinear characteristics of the dose-response relationship. The POD for decreased maternal body weight gain (GD11-16) is the  $BMCL_{1SD}$  of 63.5 ppm.

#### **3.1.4.2.6 Decreased Maternal Whole Body Weight**

Decreased maternal whole body weight could be adequately modeled with confidence including all concentrations with the linear model and the unrestricted power model (Table 7 and Figure 4):

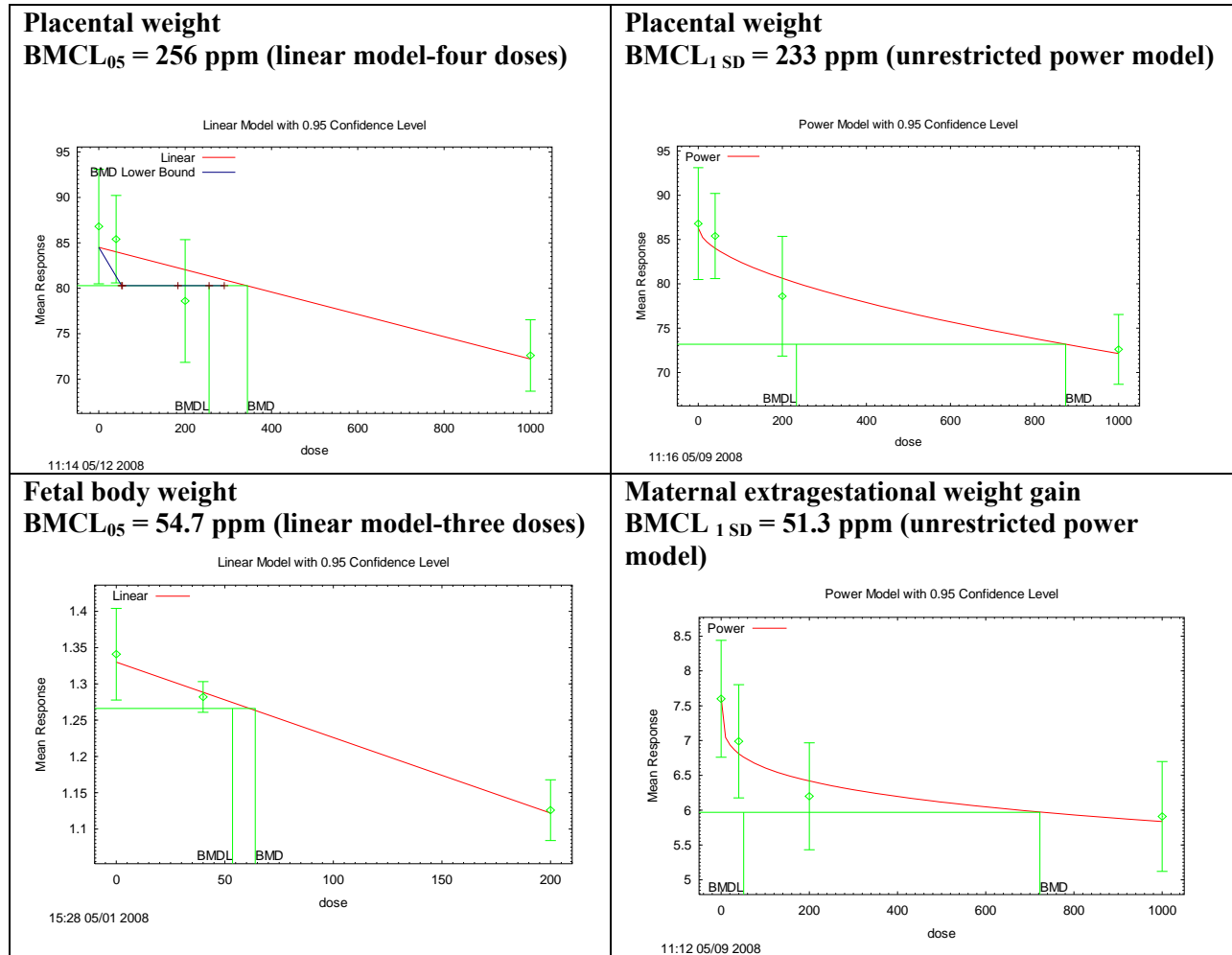
- Linear model:
  - $BMC_{10} = 1,344$  ppm,  $BMCL_{10} = 896$  ppm;
  - $BMC_{1SD} = 1,121$  ppm,  $BMCL_{1SD} = 732$  ppm
- Unrestricted power model:
  - $BMC_{1SD} = 962$  ppm and  $BMCL_{1SD} = 304$  ppm

The AIC for the linear model was equal to the AIC for the unrestricted power model, so the TS chose the lowest  $BMCL_{1SD}$  of 304 ppm from the unrestricted power model. The POD for decreased maternal body weight gain (GD11-16) is the  $BMCL_{1SD}$  of 304 ppm.

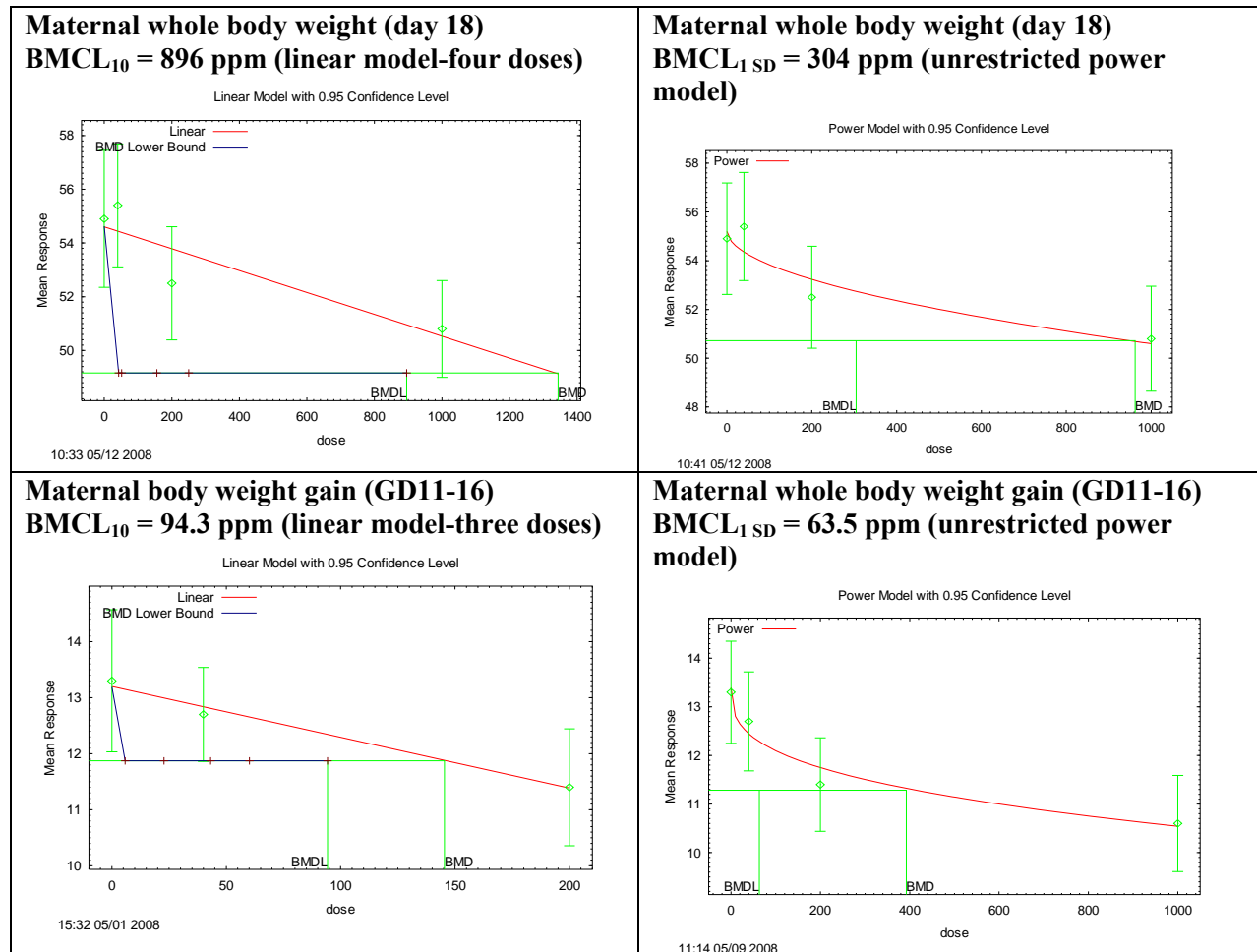
<b>Table 7. BMC Modeling Results for Maternal/Developmental Toxicity</b>								
<b>Endpoint</b>	<b>BMD Model</b>	<b>BMC (ppm)</b>	<b>BMCL (ppm)</b>	<b>BMC (ppm)</b>	<b>BMCL (ppm)</b>	<b>p-value for fit</b>	<b>AIC</b>	<b>Scaled Residual *</b>
	<b>Critical Effect Size</b>	<b>0.05</b>		<b>1 SD</b>				
<b>Placental weight</b>	Linear **	344	256	1063	734	0.767	466	<   2
	Power ** (unrestricted)	123	4.17	874	233	0.984	468	<   2
<b>Fetal body weight</b>	Linear ** without highest dose	65.8	54.7	94.8	71.8	0.350	-212	<   2
	<b>Critical Effect Size</b>	<b>0.10</b>		<b>1 SD</b>				
<b>Maternal whole body weight</b>	Linear	1344	896	1121	732	0.257	321	<   2
	Power (unrestricted)	1403	599	962	304	0.194	321	<   2
<b>Maternal body weight gain (GD11-16)</b>	Linear without highest dose	145	94.3	238	148	0.734	153	<   2
	Power (unrestricted)	108	5.96	392	63.5	0.339	200	<   2
<b>Maternal extra-gestational weight gain</b>	Power (unrestricted)	31.4	0.0000345	723	51.3	0.424	164	<   2

\* All scaled residuals at each concentration were less than an absolute value of 2 (< | 2 |) (Appendix 2, Table 2E)

\*\* Both a nonhomogeneous and homogeneous variance were used to model the data. The scaled residuals for a nonhomogeneous variance were slightly smaller in the low-dose region of the dose response curve, so the results from a nonhomogeneous variance are reported.



**Figure 3. BMC Dose-Response Curves for Placental Weight, Fetal Body Weight, and Maternal Extragestational Weight Gain**



**Figure 4. BMC Dose-Response Curves – Maternal Body Weight and Weight Gain**

### 3.1.4.2.7 Summary of Modeling Results

A summary of BMCL<sub>05</sub> values for developmental effects and BMCL<sub>10</sub> values for maternal effects from the linear model, and a summary of BMCL<sub>1SD</sub> values from the unrestricted power model, is shown in Table 8 with study NOAELs for comparison. If data from an endpoint cannot be modeled, USEPA (2000) suggests the study NOAEL for that endpoint be used as the POD.

Reduction in maternal extragestational weight gain with a BMCL<sub>1SD</sub> of 51.3 ppm and reduction in fetal body weight with a BMCL<sub>05</sub> of 54.7 ppm will be the PODs and endpoints selected by the TS to be critical effects. These effects are adverse, relevant PODs to the proposed MOA (i.e., decreased serum progesterone levels) and produced the lowest PODs. Both of these values are comparable to, although slightly higher than, the study NOAEL of 40 ppm.

	<b>BMCL<sub>1 SD</sub> Unrestricted power</b>	<b>BMCL<sub>05</sub> or BMCL<sub>10</sub> Linear Model</b>	<b>NOAEL</b>
placental weight	233 ppm	<b>BMCL<sub>05</sub> = 256 ppm<sup>1, 2</sup></b>	40
fetal body weight	--- <sup>4</sup>	<b>BMCL<sub>05</sub> = 54.7 ppm<sup>1</sup></b>	40
extragestational weight gain	<b>51.3 ppm<sup>1</sup></b>	--- <sup>4</sup>	40
body weight gain (GD 11-16)	<b>63.5 ppm<sup>1, 3</sup></b>	BMCL <sub>10</sub> = 94.3 ppm	40
whole-body weight (day 18)	<b>304 ppm<sup>1, 3</sup></b>	BMCL <sub>10</sub> = 896 ppm	200
increased incidence of supernumerary ribs	--- <sup>4</sup>	--- <sup>4</sup>	40 <sup>4</sup>
abnormal sternebrae	--- <sup>4</sup>	--- <sup>4</sup>	200 <sup>4</sup>
reduced ossification for all sites	--- <sup>4</sup>	--- <sup>4</sup>	200 <sup>4</sup>
gravid uterine weight	--- <sup>4</sup>	--- <sup>4</sup>	200 <sup>4</sup>
extragestational weight	--- <sup>4</sup>	--- <sup>4</sup>	200 <sup>4</sup>

<sup>1</sup> POD for selected endpoint shown in bold and highlighted cells

<sup>2</sup> lowest AIC value

<sup>3</sup> lowest BMCL value chosen as POD for selected endpoint

<sup>4</sup> toxicity endpoint could not be modeled with confidence (test 4) or trend test failed (test 1)

Increased incidence of supernumerary ribs was a toxicity endpoint that could not be adequately modeled. Hackett *et al.* (1987b) indicated this endpoint is associated with reduced fetal body weight and with maternal toxicity as evidenced by a reduction in maternal weight gain during gestation, which were adequately modeled. The critical effects chosen by the TS are decreased extragestational weight gain with a POD of 51.3 ppm and reduced fetal body weight with a POD of 54.7 ppm, which would also be protective of potential teratogenicity as suggested by increased incidence of supernumerary ribs in mice.

### **3.1.4.2.8 BMC Modeling Results from USEPA (2002)**

USEPA used several different approaches to model fetal body weight dose-response data and reported BMC modeling results adjusted to reflect a 24-h exposure duration (Table 10-13, USEPA 2002). Refer to USEPA (2002) for a complete discussion of the advantages and disadvantages of each model (log-logistic, three-dose continuous power, and hybrid) and cutoff values used by USEPA. The values in Table 10-13 (USEPA 2002) were converted from a 6 h/day exposure to continuous exposure (6/24). In contrast, Table 9 shows the results from Table 10-13 (USEPA 2002), except data are shown for a 6-h/day exposure (i.e., the original duration of the Hackett *et al.* (1987b) study). USEPA's results from the restricted three-dose continuous power model (BMC<sub>05</sub> = 65.1 ppm and BMCL<sub>05</sub> = 53.5) (Table 9) are almost identical to results derived by the TS using the three-dose linear model (BMC<sub>05</sub> = 65.8 and BMCL<sub>05</sub> = 54.7 ppm) (Table 7) (i.e., modeling results from the restricted continuous power model are the same as the continuous linear model).

<b>Model</b>	<b>Response</b>	<b>Cutoff</b>	<b>BMC (ppm)</b>	<b>BMCL (ppm)</b>
Log-logistic (four dose groups)	Individual fetal body weight	BMR = 5 <sup>th</sup> percentile	27.6	11.6
		BMR = 10 <sup>th</sup> percentile	40	18.8
Continuous power (three dose groups)	Fetal body weight/litter	CES = 5% relative reduction	65.1	53.5
		CES = 25 <sup>th</sup> percentile	5.12	36.7
		CES = 0.5 SD absolute reduction	52.4	42.6
Hybrid model (4 dose groups)	Fetal body weight/litter	P <sub>0</sub> = 0.05	28.3	13.3

\* Adapted from Table 10-13 (USEPA 2002), except the data are for an exposure duration of 6 h, not 24 h

USEPA (2002) used a 5<sup>th</sup> percentile BMR and BMCL<sub>05</sub> of 11.6 ppm as their POD because the log-logistic model:

- fit all four exposure levels adequately;
- accounted for intralitter correlation or litter size; and
- was a more health-protective choice to use for the POD.

For reasons previously discussed in Section 3.1.4.2 *Benchmark Concentration Modeling*, the TS did not consider BMC modeling results from the log-logistic model (which involves converting continuous data to dichotomous data). The advantages and disadvantages of using the hybrid approach to model reduction in fetal body weight after exposure of pregnant dams to BD are discussed by USEPA (2002). For all modeling results, the BMC and BMCL values based on a CES<sub>1 SD</sub> are provided (i.e. results equivalent to the hybrid approach\*).

### 3.1.5 Dosimetric Adjustments

The USEPA closely examined the physiologically-based toxicokinetic (PBTK) models for BD to determine if additional modeling could reduce uncertainties in the interspecies scaling between mice and humans for ovarian atrophy and other endpoints (USEPA 2002, Chapter 9). USEPA stated that despite advances in the models over the past decade, the current models are inadequate for this purpose. For example, the PBTK models do not yet accurately describe the distribution of the major metabolites in various compartments, do not yet include the reportedly important epoxydiol metabolites, and have not been adequately validated. A PBTK model not included in USEPA (2002) was developed by Smith *et al.* (2001), who investigated genetic and dietary factors affecting human metabolism of BD. Human volunteers were exposed to 2 ppm BD for a 20-min exposure with a 40-min washout period. Smith *et al.* (2001) fitted a three-compartment PBTK model to investigate BD uptake and estimate model parameters.

\* A CES of 1 SD from control mean corresponds to an approximately 10% excess risk for individuals below the 2<sup>nd</sup> percentile or above the 95<sup>th</sup> percentile of the control distribution for normally distributed effects (USEPA 2000).

Recently, Filser *et al.* (2007) measured and evaluated the BD-dependent blood burden of the following metabolites in rats and mice: EB, DEB, EBD and butene-diol (refer to Figure 2). Brochot *et al.* (2007) conducted a global sensitivity analysis for a proposed PBTK model. However, relevant parameters and a validated PBTK model for extrapolation from animals to humans are still lacking. Therefore, default duration exposure and dosimetric adjustments from animal-to human exposure were used.

### **3.1.5.1 Critical Effect and Default Exposure Duration Adjustments**

Both decreased maternal extragestational weight gain and reduced fetal body weight occurred at similar concentrations and are considered developmental endpoints since they are highly correlated. Since the POD is derived from a developmental endpoint, the exposure duration will not be adjusted to 1 h according to ESL Guidelines (TCEQ 2006) due to potential sensitive windows of exposure. The BMCL<sub>1SD</sub> of 51.3 ppm based on the Hackett *et al.* (1987b) study for reduction in extragestational weight gain is used as the POD since it is slightly lower than the BMCL<sub>05</sub> of 54.7 ppm for decreased fetal body weight and is adverse, biologically plausible, and consistent with the proposed MOA.

### **3.1.5.2 Default Dosimetry Adjustments from Animal-to-Human Exposure**

BD is only slightly soluble in water and is moderately soluble in blood (USEPA 2002). It is readily absorbed from the air into the blood through the lungs. The health effects it produces at lower concentrations are mainly remote effects, so dosimetric adjustments were performed as a Category 3 gas which is consistent with USEPA (2002) and based on guidance in USEPA (1994). For Category 3 gases, the default dosimetric adjustment from animal-to-human exposure is conducted using the following equation:

$$POD_{HEC} = POD_{ADJ} \times [(H_{b/g})_A / (H_{b/g})_H]$$

where:

$H_{b/g}$	=	ratio of the blood:gas partition coefficient
A	=	animal
H	=	human

For BD, the blood:gas partition coefficients for mice range from 1.2 to 3.0 with a mean of 1.67 (Appendix 3 of USEPA 2005a) and for humans  $1.22 \pm 0.30$  (mean  $\pm$  SD) (Brochot *et al.* 2007). When  $(H_{b/g})_A / (H_{b/g})_H > 1$ , a default value of 1 is used for  $(H_{b/g})_A / (H_{b/g})_H$ , the regional gas dose ratio (RGDR) (USEPA 1994).

Reduction in extragestational weight gain

$$POD_{HEC} = POD_{6h} \times RGDR = 51.3 \text{ ppm} \times 1 = 51.3 \text{ ppm}$$

### **3.1.6 Adjustments of the POD<sub>HEC</sub>**

The MOA by which BD produces maternal/developmental toxicity is assumed to be nonlinear (Section 3.1.2.2), so a POD was determined and uncertainty factors (UFs) were applied to derive a ReV. The following UFs were applied to the 6-h POD<sub>HEC</sub> of 51.3 ppm: 10 for intraspecies variability (UF<sub>H</sub>), 3 for extrapolation from animals to humans (UF<sub>A</sub>), and 1 for database uncertainty (UF<sub>D</sub>), a total UF = 30:

Reduction in extragestational weight gain  
acute ReV =  $POD_{HEC} / (UF_H \times UF_A \times UF_D)$   
= 51.3 ppm / (10 x 3 x 1)  
= 1.71 ppm  
= 1,710 ppb

A full  $UF_H$  of 10 was used to account for intraspecies variability. There is experimental evidence that indicates BD-sensitive human subpopulations may exist due to metabolic genetic polymorphisms (USEPA 2002), although recent studies indicate that variability due to genetic polymorphisms is less than 10 based on metabolism of BD in humans with different genotypes. While the results examining metabolic differences between humans with different genotypes in some cases are inconsistent, overall, the differences between genotypes have been small (i.e., generally a factor of two to four) (Albertini *et al.* 2001, 2003; Begemann *et al.* 2001; Fustinoni *et al.* 2002; Hayes *et al.* 1996, 2000, 2001; Smith *et al.* 2001; and Zhao *et al.* 2000, 2001).

A  $UF_A$  of 3 was used for extrapolation from animals to humans\* because default dosimetric adjustments from animal-to-human exposure were conducted, which account for toxicokinetic differences but not toxicodynamic differences. This approach is likely conservative, since existing studies indicate that mice are relatively sensitive laboratory animals in regards to the reproductive effects of BD (e.g., greater production of toxic intermediates and a lower capacity for detoxification of these intermediates (USEPA 2002)).

A database  $UF_D$  of 1 was used because the overall acute toxicological database for BD meets the requirements for a high confidence database for an acute ReV (TCEQ 2006):

- acute inhalation studies in humans;
- two inhalation bioassays in different species investigating a wide range of endpoints; and
- two prenatal developmental toxicity studies in different species (USEPA 2002; AEGl 2005).

Both the quality of the studies and the confidence in the acute database is high.

### 3.1.7 Health-Based Acute ReV and <sup>acute</sup>ESL

The 6-h acute ReV value of 1,710 ppb was rounded to two significant figures at the end of all calculations resulting in an acute ReV of 1,700 ppb (3,700  $\mu\text{g}/\text{m}^3$ ). The rounded acute ReV was then used to calculate the 6-h <sup>acute</sup>ESL. At the target hazard quotient of 0.3, the 6-h <sup>acute</sup>ESL is 510 ppb (1,100  $\mu\text{g}/\text{m}^3$ ) (Table 10). This acute ReV and <sup>acute</sup>ESL are considered to be conservative since pregnant mice exposed to BD and their offspring develop maternal/developmental toxicity much easier than similarly- exposed rats, available scientific information suggests mice are more sensitive than humans, and BD-induced reproductive/ developmental effects have never been observed in humans.

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\* For the chronic assessment, as discussed in Section 4.1.5.2 *Toxicokinetic Adjustments from Animal-to-Human Exposure*, the total  $UF_A$  for ovarian atrophy was reduced to 1 based on strong MOA evidence that DEB (not EB or BD) causes ovarian atrophy (Doerr *et al.* 1995) and toxicokinetic data that DEB levels in mice are much higher than in humans (Section 4.1.5.2). Doerr *et al.* (1995) investigated ovarian atrophy in both mice and rats after exposure to BD, EB, and DEB. However, for the acute assessment, there is not strong evidence that DEB alone is responsible for reproductive/developmental effects because Spencer *et al.* (2001) and Chi *et al.* (2002) only evaluated the effects of DEB in rats. Therefore, a full toxicodynamic  $UF_A$  of 3 was used.

<b>Table 10. Derivation of the Acute ReV and <sup>acute</sup>ESL</b>	
Study	Hackett <i>et al.</i> 1987b
Study population	CD-1 mice (18-21 pregnant mice per dose group)
Study quality	High
Exposure Methods	0, 40, 200, and 1,000 ppm on gestation days (GD) 6-15 for 6 h/day
Critical Effects	Reduction in extragestational weight gain and fetal body weight; developmental toxicity
POD	51.3 ppm (BMCL <sub>1 SD</sub> )
Exposure Duration	6 h
Extrapolation to 1 h	No adjustment because the critical effect was a maternal/developmental endpoint
POD (6 h)	51.3 ppm
6-h POD <sub>HEC</sub>	51.3 ppm (gas with systemic effects, based on default RGDR = 1.0)
Total uncertainty factors (UFs)	30
<i>Interspecies UF</i>	3
<i>Intraspecies UF</i>	10
<i>LOAEL UF</i>	Not applicable
<i>Incomplete Database UF</i>	1
<i>Database Quality</i>	High
<b>acute ReV [6 hr] (HQ = 1)</b>	<b>3,700 µg/m<sup>3</sup> (1,700 ppb)</b>
<b><sup>acute</sup>ESL [6 h] (HQ = 0.3)</b>	<b>1,100 µg/m<sup>3</sup> (510 ppb)</b>

### 3.1.8 Comparison of <sup>acute</sup>ESL to Generic ESL

When a subacute study is used to derive a 1-h <sup>acute</sup>ESL, Section 3.2.3 of the ESL guidelines (TCEQ 2006) suggests a generic ESL (<sup>acute</sup>ESL<sub>generic</sub>) be derived using approaches in Section 3.6 for comparison to the 1-h <sup>acute</sup>ESL to ensure the derived value is not overly conservative. The Threshold of Concern (TOC) approach utilizes the lowest reported inhaled concentration which produced death in 50% of the study specimens after exposure (LC<sub>50</sub>). Shugaev (1969) reported the 2-h LC<sub>50</sub> of BD in mice was 122,000 ppm and the 4-h LC<sub>50</sub> in rats was 128,000 ppm which would classify BD as a TOC Category 5 gas, and the corresponding <sup>acute</sup>ESL<sub>generic</sub> would be 1,000 µg/m<sup>3</sup> for a 1-h exposure duration (Table 3-3 of the ESL guidelines (TCEQ 2006)). The 6-h <sup>acute</sup>ESL of 1,100 µg/m<sup>3</sup> based on the subacute study cannot be directly compared to the 1-h <sup>acute</sup>ESL<sub>generic</sub> because the exposure durations are different. However, the 6-h <sup>acute</sup>ESL is slightly higher than the 1-h <sup>acute</sup>ESL<sub>generic</sub> of 1,000 µg/m<sup>3</sup> for a Category 5 gas. This provides confidence that the derived value is not overly conservative.

## ***3.2. Welfare-Based Acute ESLs***

### **3.2.1 Odor Perception**

ACGIH (2001) reports BD has a mildly aromatic odor with recognition occurring at 1 to 1.6 ppm. Ruth (1986) states the 50% odor detection threshold is  $352 \mu\text{g}/\text{m}^3$  (160 ppb) and the 100% recognition threshold is  $2,860 \mu\text{g}/\text{m}^3$  (1,300 ppb). The 50% odor detection threshold for BD determined by the triangular odor bag method was 230 ppb (Nagata 2003). Both Ruth (1986) and Nagata (2003) are listed as sources of information for odor thresholds in Appendix B of the ESL Guidelines (TCEQ 2006). However, only the Nagata (2003) study meets the criteria for acceptable odor threshold measurement techniques developed by the American Industrial Hygiene Association (TCEQ 2006). Therefore, the  ${}^{\text{acute}}\text{ESL}_{\text{odor}}$  is 230 ppb ( $510 \mu\text{g}/\text{m}^3$ ). Since odor is a concentration-dependent effect, the same 1-h  ${}^{\text{acute}}\text{ESL}_{\text{odor}}$  is assigned to all averaging times.

### **3.2.2 Vegetation Effects**

BD concentrations that produce vegetative effects, such as abscission and inhibition of growth, are orders of magnitude higher than concentrations of ethylene, propylene, and acetylene that produce similar effects (USDHEW 1970). Since concentrations producing vegetative effects (approximately  $> 10,000$  ppm) are significantly above other health- and odor-based concentrations, an  ${}^{\text{acute}}\text{ESL}_{\text{veg}}$  was not developed for BD.

## ***3.3. Short-Term ESL and Values for Air Monitoring Evaluation***

The acute evaluation resulted in the derivation of the following values:

- 6-h  ${}^{\text{acute}}\text{ESL} = 1,100 \mu\text{g}/\text{m}^3$  (510 ppb)
- 6-h acute ReV =  $3,700 \mu\text{g}/\text{m}^3$  (1,700 ppb)
- 1-h  ${}^{\text{acute}}\text{ESL}_{\text{odor}} = 510 \mu\text{g}/\text{m}^3$  (230 ppb)

The short-term ESL for air permit evaluations is the  ${}^{\text{acute}}\text{ESL}_{\text{odor}} = 510 \mu\text{g}/\text{m}^3$  (230 ppb) as it is lower than the health-based 6-h  ${}^{\text{acute}}\text{ESL}$  of  $1,100 \mu\text{g}/\text{m}^3$  (510 ppb) (Table 1). If the predicted 1-h maximum ground level concentration ( $\text{GLC}_{\text{max}}$ ) is less than the health-based 6-h  ${}^{\text{acute}}\text{ESL}$ , then no acute health effects would be expected. If the  $\text{GLC}_{\text{max}}$  exceeds the health-based 6-h  ${}^{\text{acute}}\text{ESL}$ , then it will be necessary to calculate a 6-h  $\text{GLC}_{\text{max}}$  in order to evaluate potential health effects.

For the evaluation of ambient air monitoring data, the  ${}^{\text{acute}}\text{ESL}_{\text{odor}}$  of  $510 \mu\text{g}/\text{m}^3$  (230 ppb) is lower than the acute ReV of  $3,700 \mu\text{g}/\text{m}^3$  (1,700 ppb), although both values may be used for the evaluation of ambient air monitoring data (Table 1). The  ${}^{\text{acute}}\text{ESL}$  (HQ = 0.3) is not used to evaluate ambient air monitoring data. If measured 1-h ambient air monitoring data is less than the 6-h acute ReV, then no acute health effects would be expected. If the health-based 6-h acute ReV is exceeded, and it is possible to calculate a 6-h value (i.e., automatic gas chromatographic data), then a 6-h averaged value will be calculated in order to evaluate potential health effects.

## ***3.4 Comparison of TCEQ's Acute ReV versus USEPA's Acute Reference Concentration***

USEPA (2002) derived a 24-h acute reference concentration (RfC) of  $3.2 \mu\text{g}/\text{m}^3$  (7 ppb) based on decreased fetal body weight. A value of 2.9 ppm for a 24-h  $\text{POD}_{\text{HEC}}$  is reported in Table 10-25 of USEPA (2002) using the log-logistic model (i.e., continuous data was changed into dichotomous data and

modeled with a dichotomous model). USEPA applied UFs of 3 for interspecies variability, 10 for intraspecies variability, 4 for effect level extrapolation factor (to decrease risk to below the benchmark response level; analogous conceptually to the LOAEL-to-NOAEL UF), and 3 for incomplete database because a neurodevelopmental toxicity study had not been completed (total UF = 400) (Table 11).

The TS evaluated ten different toxicity endpoints using BMC modeling. The acute ReV for a 6-h exposure duration is based on decreased extragestational weight gain with a POD being 51.3 ppm, although reduction in fetal body weight had a similar POD of 54.7. A UF of 3 was applied for interspecies extrapolation and 10 for intraspecies variability (total UF = 30). An effect level extrapolation factor (somewhat equivalent to a LOAEL-to-NOAEL UF) was not applied because BMC modeling was used to determine the POD, considered an appropriate NOAEL surrogate. An acute database UF was not applied because the acute database for BD meets the minimum database with high confidence for an acute ReV (TCEQ 2006). Table 11 compares the derivation of the 6-h acute ReV and 6-h<sup>acute</sup>ESL to USEPA's 24-h acute RfC (USEPA 2002).

POD <sub>HEC</sub>	Inter-species	Intra-species	Effect Level Extrapolation Factor	Incomplete Database	Total UF	Acute Reference Value
TCEQ 51.3 [6 h] <sup>1</sup> Decreased extragestational weight gain	3	10	---	---	30	acute ReV [6 h] 1,700 ppb  acute <sup>ESL</sup> [6 h] 510 ppb
USEPA 2.9 ppm [24 h] <sup>2</sup> Decreased fetal body weight	3	10	4	3	400	acute RfC [24 h] 7 ppb

<sup>1</sup> Lowest adverse POD determined from an evaluation of ten toxicity endpoints

<sup>2</sup> The unadjusted 6-h BMCL<sub>05</sub> for decreased fetal body weight was 11.6 ppm using a log-logistic BMC model (continuous data was converted to dichotomous data)

USEPA's RfC is approximately 240 times lower than TCEQ's ReV due to the following reasons:

- The exposure duration for USEPA's RfC is 24 h, whereas the exposure duration for TCEQ's ReV is 6 h, which makes USEPA's RfC approximately four times lower;
- The TCEQ did not use an effect level extrapolation factor of 4 because BMC modeling was used to determine the POD, considered an appropriate NOAEL surrogate, or a UF<sub>D</sub> of 3 which makes USEPA's RfC 12 times lower; and
- The TCEQ used the BMCL<sub>1SD</sub> of 51.3 ppm as a POD for reduction in extragestational weight gain because it was the lowest POD of adverse effects based on BMC analysis of ten toxicity endpoints, whereas USEPA used the BMCL<sub>05</sub> from a log-logistic model (i.e., continuous data for fetal body weight was converted into dichotomous data), which makes USEPA's RfC approximately 4.4 times lower.

Consideration of the above differences accounts for approximately a 210-fold difference (4 x 12 x 4.4). While an exact partitioning of the 240-fold difference may not be possible, there are science-based and logical explanations accounting for most of the differences.

## **Chapter 4 Chronic Evaluation**

### ***4.1 Noncarcinogenic Potential***

#### **4.1.1 Physical/Chemical Properties and Key Studies**

Refer to Section 3.1.1.1 for a discussion of physical/chemical properties.

This section is based on USEPA (2002). Chapter 5 of USEPA (2002) discusses the chronic reproductive/developmental effects of BD. Animal data indicate that BD is a potential reproductive hazard because reproductive effects are observed at the lowest concentrations tested in animals. Chapter 6 of USEPA (2002) discusses other subchronic and chronic health effects observed in animals exposed to BD. Few adverse noncarcinogenic effects have been observed other than reproductive and developmental effects, except for hematological effects in mice exposed to higher concentrations and increases in organ weights in rats (USEPA 2002, Chapter 6). Hematological effects in mice may not be relevant for humans, as demonstrated by Tsai *et al.* (2005). Tsai *et al.* (2005) conducted a hematology surveillance study of petrochemical workers at two Shell facilities and reported there were no significantly increased abnormalities for any hematology parameter among exposed employees (404 exposed employees and 733 comparison employees).

A review of the scientific literature since 2002 did not reveal any other chronic inhalation studies that could be used instead of the 2-year chronic bioassays conducted by the National Toxicology Program (NTP 1993) which are summarized in the following sections but discussed in detail in USEPA (2002).

##### ***4.1.1.1 Human Studies***

Albertini *et al.* (2007) conducted a molecular epidemiological study of BD-exposed Czech workers to compare female to male responses as discussed previously in Section 3.1.1.2.1. Briefly, there were no significant differences reported between control and exposed groups for miscarriages, still births, ectopic pregnancies, molar pregnancies, low birth weight (< 2,500 g) babies, or pre-term births, based on information collected on all pregnancies. The ability of the study to detect differences in the evaluated endpoints may be limited because there were only a few subjects evaluated.

##### ***4.1.1.2 Animal Studies***

The most sensitive reproductive effects observed in 2-year chronic exposure studies were ovarian atrophy in female mice and testicular atrophy in male mice (NTP 1993). Testicular atrophy was primarily a high-exposure effect, so this section focuses on ovarian atrophy. In this bioassay, groups of 70 female B6C3F1 mice were exposed by inhalation 6 h/day, 5 days/week to 0, 6.25, 20, 62.5, or 200 ppm BD for up to 103 weeks, and groups of 90 female mice were exposed to 625 ppm. An interim evaluation of ovarian atrophy was conducted at 9 months on ten mice per group and also at 15 months. Significant concentration-related decreases in survival were seen in female mice exposed to concentrations  $\geq 20$  ppm, primarily due to the development of malignant neoplasms. Statistically significant increases in the incidence of ovarian

atrophy were observed in all exposure groups following lifetime exposures. The LOAEL for ovarian atrophy was observed at the lowest exposure level (6.25 ppm, 6 h/day, 5 days/week, for 2 years). Uterine atrophy was also observed in the highest exposure groups; however, this is likely to be a secondary effect of ovarian atrophy. Rats exposed to 0, 1,000, and 8,000 ppm did not develop adverse reproductive effects, thus providing further evidence that rats are less sensitive to the effects of BD than mice (HLE 1981; Owen *et al.* 1987; Owen and Glaister 1990).

#### 4.1.2 MOA Analysis

Refer to Section 3.1.2 for a discussion of BD metabolism. There is strong evidence that ovarian atrophy is mediated by the diepoxide metabolite, DEB, the most reactive of BD metabolites (Doerr *et al.* 1995, 1996; USEPA 2002). There are marked species differences in effects seen between rats, which do not exhibit BD-induced ovarian atrophy, and mice, which do exhibit BD-induced ovarian atrophy. Doerr *et al.* (1995, 1996) evaluated the ovarian effects of the metabolites of BD in mice and rats and also examined 4-vinylcyclohexene, a structurally similar compound. Doerr *et al.* (1995, 1996) showed that the diepoxide of BD or 4-vinylcyclohexene is required for ovarian toxicity to occur in the rat. EB was ovotoxic to mice but not rats. Thus, the resistance of the rat to ovarian toxicity of BD is likely due to the decreased ability of the rat to produce DEB. Filser *et al.* (2007) was unable to detect DEB in venous blood of male Sprague-Dawley rats (detection limit 0.01  $\mu\text{mol/l}$ ) when they were exposed to 1,200 ppm for 6-8 h, whereas DEB was detected in B6C3F1 mice at 3.2  $\mu\text{mol/l}$  at 1,280 ppm BD. Humans are similar to rats in that they do not readily produce the diepoxide metabolite (refer to Section 4.1.5.2.2 *Estimate for the Toxicokinetic UF<sub>A</sub> Based on Empirical Data*).

Swenberg *et al.* (2007) compared results in Czech Republic occupationally-exposed workers to results in mice and rats for a N,N-(2,3-dihydroxy-1,4-butadiyl) valine (pyr-Val) hemoglobin adduct specific for DEB at similar BD concentrations (Table 12). The pyr-Val adduct was not detected in human females or males, while female mice were 78 times more likely than human females to produce DEB as evaluated with pyr-Val adducts (Table 12). Pyr-Val adducts for human females were based on the limit of quantitation (LOQ) because pyr-Val adducts were not detected (Swenberg *et al.* 2007). At the 2007 and 2008 Society of Toxicology meetings, Georgieva *et al.* (2007; 2008) presented results using a more sensitive analytical method to measure pyr-Val adducts in the Czech Republic workers. Pyr-Val adducts were detected at low concentrations in Czech Republic workers. There was not a clear dose-response relationship between pyr-Val adducts and BD concentrations from the Georgieva *et al.* (2007) study, and the authors hypothesized that the pyr-Val adducts could have been formed from other unknown sources. However, the Georgieva *et al.* (2008) study showed the amount of pyr-Val was significantly higher in the polymerization workers than in the monomer workers and controls.

**Table 12. DEB-Specific pyr-Val Hb Adduct in Mouse, Rat, and Human from Swenberg *et al.* (2007)**

Concentration	1 ppm BD 6 h/day 4 weeks (4.0 ppm-weeks)		1 ppm BD 6 h/day 4 weeks (4.0 ppm-weeks)		Mean 0.18 ppm for 4 months (3.1 ppm-weeks)	Mean 0.37 ppm for 4 months (6.3 ppm-weeks)
Species	Female mice	Male mice	Female rat	Male rat	Female human	Male human
Pyr-VAL Hb adducts  (pmol/g in 50 mg globin)	23.5 ± 3.1  female mice have 78 times more pyr-Val adducts than female humans	30.8 ± 4.6  male mice have 103 times more pyr-Val adducts than male humans	0.7 ± 0.1	0.9 ± 0.03	< 0.3 limit of quantitation (LOQ)	< 0.3 LOQ

### 4.1.3 Dose Metric

For ovarian atrophy, data on the exposure concentration of the parent chemical are available, whereas data on more specific dose metrics, such as the monoepoxide or diepoxide metabolites in blood or target tissue, are not available. As discussed previously in Section 3.1.5, a validated PBTK model for extrapolation from animals to humans is still lacking. Therefore, the exposure concentration of the parent chemical was used as the default dose metric.

### 4.1.4 PODs for Key Studies and Critical Effect

Using benchmark concentration dose modeling and a Weibull time-to-response model, USEPA (2002) calculated a BMC<sub>10</sub> of 1.05 ppm and BMCL<sub>10</sub> of 0.88 ppm based on the 1993 NTP 2-year inhalation bioassay, including interim sacrifice data. In calculating the BMC<sub>10</sub> and BMCL<sub>10</sub>, lesion severity was not taken into account, and the 625 ppm group was excluded because of high early mortality. In addition, ovarian atrophy was modeled to reflect extra risks only until age 50, because BD-induced ovarian atrophy is believed to result from follicular failure, and after menopause, follicles would no longer be available.

The PODs for all prenatal deaths (dominant lethal effect) (BMCL<sub>05</sub> = 10 ppm) and for testicular atrophy (BMCL<sub>10</sub> = 16 ppm) were also determined by USEPA (2002) and were significantly higher than the BMCL<sub>10</sub> of 0.88 ppm. Therefore, ovarian atrophy was selected as the critical effect (USEPA 2002).

Sielken *et al.* (Appendix 3) repeated the BMC modeling performed by USEPA using the same procedures described above and calculated the BMC<sub>05</sub> and BMCL<sub>05</sub> as well as the BMC<sub>10</sub> and BMCL<sub>10</sub> (Appendix 3). The BMCL<sub>05</sub> has generally been considered a conservative NOAEL surrogate (Barnes *et al.* 1995; Fowles *et al.* 1999; Filipsson *et al.* 2003) whereas the BMCL<sub>10</sub> may be analogous to a NOAEL or LOAEL. The BMC<sub>10</sub> and BMCL<sub>10</sub> calculated by Sielken *et al.* (Appendix 3) were 1.15 ppm and 0.881 ppm, respectively, which agreed with the BMC<sub>10</sub> of 1.05 ppm and BMCL<sub>10</sub> of 0.88 ppm calculated by USEPA (2002).

USEPA (2002) analyzed ovarian atrophy data excluding the highest dose group and also including all the data. Traditionally, EPA drops the highest dose group when the model does not fit the data well or when quantal data are fit with a quantal model and there is high mortality in the highest dose group. The ovarian atrophy data, however, were modeled with a time-to-response model (i.e., a model that accounts for the time of death) as opposed to a quantal model which does not account for time of death. Furthermore, the model fit to the data that excluded the highest dose group was not better than the model fit to the data that included the highest dose group as shown by Sielken *et al.* (Appendix 3). However, USEPA (2002) excluded the highest dose group because of early mortality. The  $BMC_{05}$  and  $BMCL_{05}$  were 0.560 ppm and 0.429 ppm, respectively, excluding the highest dose and 0.607 ppm and 0.462 ppm, respectively, including the highest dose. Since a time-to-response model was used, the TS used the  $BMCL_{05}$  modeling result of 0.462 ppm as the POD (uses all the data).

Because the Weibull time-to-response model in these analyses is linear in dose, the  $BMC_{05}$  and  $BMCL_{05}$  values are approximately half the corresponding  $BMC_{10}$  and  $BMCL_{10}$  values. The values of  $BMC_{05}$  and  $BMCL_{05}$  can be used if the dose-response relationship below the lowest experimental dose is believed to be the linear Weibull time-to-response model fit to the data. The assumption of linearity below the lowest experimental dose is usually conservative and, therefore, health protective. However, there is less uncertainty behind the benchmark dose methodology when it is used to identify the POD ( $BMC_{05}$  and  $BMCL_{05}$ ) within the range of the experimental data (the range of the non-zero doses in the experimental data) and to be a dose whose risk can be reasonably reliably estimated without undue sensitivity to the dose-response model selected or the model estimation. Here, the  $BMC_{05}$  and  $BMCL_{05}$  are below the range of the experimental data and, hence, introduce an additional element of uncertainty into the POD. However, the  $BMCL_{05}$  for ovarian atrophy was used as the POD because the TS preferentially uses a benchmark response level of 5% for more severe effects such as ovarian atrophy, and the  $BMCL_{05}$  is considered to be a conservative NOAEL surrogate (TCEQ 2006).

#### **4.1.5 Dosimetric Adjustments**

Based on the summary of information in Section 3.1.5 and the detailed discussion in USEPA (2002, Chapter 9), default duration exposure from animal-to-human exposure were not used. Instead, empirical data were used to estimate BD-specific toxicokinetic adjustments from animal-to-human exposure.

##### ***4.1.5.1 Default Exposure Duration Adjustments***

The  $BMCL_{05} = 0.462$  ppm for ovarian atrophy (Appendix 3) represents exposure concentrations that were already adjusted from discontinuous to continuous exposures.

##### ***4.1.5.2 Toxicokinetic Adjustments from Animal-to-Human Exposure***

The following sections discuss methods for a toxicokinetic adjustment from animal-to-human exposure as opposed to a toxicodynamic adjustment. The standard toxicokinetic UF is 3 and the toxicodynamic UF is 3 for a total  $UF_A = 10$ . If default toxicokinetic dosimetry adjustments from animal-to-human exposure based on procedures in USEPA (1994) are used, a toxicokinetic  $UF_A$  of 1 may be justified as demonstrated in Section 4.1.5.2.2. However, there is empirical evidence to indicate that the toxicokinetic UF is considerably less than 1 because mice metabolize BD to the reactive metabolite DEB much more than humans as discussed in Section 4.1.2 MOA Analysis. Although the experimental data are not sufficient to develop a chemical-specific adjustment factor (CSAF) for BD, it would support a  $UF_A$  substantially less than 1. The toxicokinetic  $UF_A$  that will be used by the TS is 0.3, although it may be

substantially less than 0.3, as discussed below. If a BD-specific toxicokinetic  $UF = 0.3$  is used with the standard toxicodynamic  $UF = 3$ , the total  $UF_A = 1$ .

#### 4.1.5.2.1 Default Dosimetry Adjustments from Animal-to-Human Exposure

As discussed previously in Section 3.1.5.2, dosimetric adjustments were performed as a Category 3 gas which is consistent with USEPA (2002) and based on guidance in USEPA (1994) with a  $RGDR = 1$ :

$$\begin{aligned} \text{POD}_{\text{HEC}} &= \text{POD}_{\text{ADJ}} \times \text{RGDR} \\ &= 0.462 \text{ ppm} \times 1 \\ &= 0.462 \text{ ppm} \end{aligned}$$

The toxicokinetic  $UF_A$  using these default procedures is 1. However, procedures discussed in Section 4.1.5.2.2 were used to justify a toxicokinetic  $UF_A$  less than 1.

#### 4.1.5.2.2 Estimate for the Toxicokinetic $UF_A$ Based on Empirical Data

Humans produce much lower levels of DEB than mice as demonstrated by experimental data on DEB-specific pyr-Val Hb adducts (Section 4.1.2) and urinary metabolites (Sabourin *et al.* 1992 as reviewed by Henderson *et al.* 1996 and Henderson 2001)). DEB is the BD metabolite responsible for ovarian atrophy (Section 4.1.2; USEPA 2002). The toxicokinetic  $UF_A$  may range from less than 0.2 to 0.01 based on data discussed in Sections 4.1.5.2.2.1 to 4.1.5.2.2.3. There is uncertainty in these estimates since data on a more specific dose metric in humans and mice (i.e., area under the curve DEB blood concentration or tissue DEB concentration) are not available. The TS will use a toxicokinetic  $UF_A$  of 0.3 based on the following experimental data:

- comparison of specific pyr-Val Hb adducts in humans and mice (Section 4.1.5.2.2.1);
- comparison of the total butadiene metabolites in blood from monkeys and mice (Section 4.1.5.2.2.2);
- comparison of DEB blood concentrations from rats and mice (Section 4.1.5.2.2.3); and
- comparison of DEB tissue levels from rats and mice (Section 4.1.5.2.2.3).

##### 4.1.5.2.2.1 Human-to-mouse experimental data

Swenberg *et al.* (2007) noted humans form 100-times less pyr-Val adducts than similarly exposed mice, a humans-to-mouse ratio of 0.01. At the present time, procedures for developing a chemical-specific-adjustment factor based on pyr-Val Hb adducts are not available because the rate constant for the association between DEB and Hb adducts is unknown (i.e., the DEB blood concentration area under the curve, the dose metric appropriate for chronic exposure, cannot be estimated, see example from Fennell *et al.* 2005 for acrylamide).

##### 4.1.5.2.2.2 Monkey-to-mouse experimental data

Sabourin *et al.* 1992 (as reviewed by Henderson *et al.* 1996 and Henderson 2001) showed that monkeys and humans had similar urinary excretion of the M1 and MII metabolites of BD. Dahl and Henderson (2000) showed the *in vitro* metabolism of BD by hepatic microsomes from cynomolgus monkeys and

humans is similar. This indicates experimental data in monkeys may be applicable to humans since they metabolize BD similarly. Dahl *et al.* (1990; 1991) demonstrated that the uptake of BD as a result of metabolism was much lower in monkeys than in mice or rats. For equivalent inhalation exposures, the concentrations of total BD metabolites in the blood were 5-50 times lower in the monkey than in the mouse, a monkey-to-mouse ratio of 0.2 to 0.02. These results indicate that epoxide levels in monkey tissue would be lower than mouse tissue since blood epoxide concentrations were lower in the monkey than in rats or mice (Dahl *et al.* 1991).

#### **4.1.5.2.3 Rat-to-mice experimental data**

For the purpose of approximating a bounding estimate of  $UF_A$  between mice and humans, a comparison of rat data to mice data may be informative. Primates and humans metabolize BD more similarly to rats than mice (Henderson *et al.* 1996; Henderson 2001). Swenberg *et al.* (2007) demonstrated humans form at least 3-times less pyr-Val than similarly exposed rats, and Dahl *et al.* (1991) showed total BD metabolites in the blood were 4-14 times lower in monkey than in the rat. Several investigators have measured DEB blood levels in rats and mice (reviewed in Filser *et al.* 2007). There was a difference in DEB blood concentrations between mice and rats of more than one order of magnitude based on data from several laboratories, when exposed to around 65 ppm BD, a rat-to-mice ratio  $< 0.1$ . Thornton-Manning *et al.* (1995) demonstrated that DEB-tissue levels in mice were 40- to 163-fold greater than those in rats (4-h exposure to around 65 ppm), a rat-to-mice ratio of 0.025 to 0.0006.

### **4.1.6 Adjustments of the $POD_{HEC}$**

The MOA by which BD produces ovarian atrophy is metabolism of the parent compound to DEB (Section 4.1.2), which is considered a threshold, nonlinear MOA. Therefore, a POD was determined and UFs applied to derive a ReV. The following UFs were applied to the  $POD_{ADJ}$  of 0.462 ppm: 10 for intraspecies variability ( $UF_H$ ), 1 for extrapolation from animals to humans ( $UF_A$ ), 1 for extrapolation from a LOAEL-to-NOAEL ( $UF_L$ ) and 3 for database uncertainty ( $UF_D$ ), a total  $UF = 30$ :

$$\begin{aligned} \text{Chronic ReV} &= \text{POD}_{ADJ} / (UF_H \times UF_A \times UF_L \times UF_D) \\ &= 0.462 \text{ ppm} / (10 \times 1 \times 1 \times 3) \\ &= 0.0154 \text{ ppm} \end{aligned}$$

- A full  $UF_H$  of 10 was used to account for intraspecies variability. There is experimental evidence to indicate that BD-sensitive human subpopulations may exist due to metabolic genetic polymorphisms (USEPA 2002), although the differences between genotypes have generally been a factor of two to four as previously discussed in Section 3.1.6.1.
- The  $UF_A$  is composed of a toxicokinetic and toxicodynamic component. A toxicokinetic  $UF_A$  of 0.3 was used for extrapolation from animal to human based on empirical data (Section 4.1.5.2.2). A toxicodynamic  $UF_A$  of 3 was used because the key sequence of events and understanding of how DEB interacts in different species to produce ovarian atrophy is not available. The resulting total  $UF_A$  was 1.
- A  $UF_L$  of 1 was used because BMC modeling was performed to determine a POD based on the  $BMCL_{05}$  (TCEQ 2006).
- The toxicological database for BD is extensive. However, a  $UF_D$  of 3 was applied because of the absence of a multigenerational reproductive study, consistent with USEPA (2002).
- Both the quality of the studies and the confidence in the chronic database is high.

#### 4.1.7 Health-Based Chronic ReV and <sup>chronic</sup>ESL<sub>nonlinear(nc)</sub>

The chronic ReV value based on ovarian atrophy was rounded to two significant figures at the end of all calculations resulting in a chronic ReV of 15 ppb (33 µg/m<sup>3</sup>). The rounded chronic ReV was then used to calculate the <sup>chronic</sup>ESL<sub>nonlinear(nc)</sub>. At the target hazard quotient of 0.3, the <sup>chronic</sup>ESL<sub>nonlinear(nc)</sub> is 4.5 ppb (9.9 µg/m<sup>3</sup>) (Table 13).

<b>Table 13. Derivation of the Chronic ReV and <sup>chronic</sup>ESL<sub>nonlinear(nc)</sub></b>	
Study	2-year bioassays (NTP 1993)
Study Population	70 female B6C3F1 mice; 90 female mice
Study Quality	high
Exposure Method	103 week exposures via inhalation at 0, 6.25, 20, 62.5, or 200 ppm; 90 female mice exposed to 625 ppm
Critical Effects	ovarian atrophy in female mice
POD (original animal study)	Not available. BMD modeling was conducted on data already adjusted from discontinuous to continuous exposure
Exposure Duration	6 h/day, 5 days/week, for 2 years
Extrapolation to continuous exposure (POD <sub>ADJ</sub> )	0.462 ppm (BMCL <sub>05</sub> )
POD <sub>HEC</sub>	0.462 ppm Adjustment not applicable; a toxicokinetic UF <sub>A</sub> based on empirical data was used
Total UFs	30
<i>Interspecies UF</i>	1
<i>Intraspecies UF</i>	10
<i>LOAEL UF</i>	1
<i>Subchronic to chronic UF</i>	Not applicable
<i>Incomplete Database UF</i>	3
<i>Database Quality</i>	high
<b>Chronic ReV (HQ = 1)</b>	<b>33 µg/m<sup>3</sup> (15 ppb)</b>
<b><sup>chronic</sup>ESL<sub>nonlinear(nc)</sub> (HQ = 0.3)</b>	<b>9.9 µg/m<sup>3</sup> (4.5 ppb)</b>

#### 4.1.8 Derivation of Chronic ReV versus USEPA’s Chronic RfC

Table 14 provides a comparison of the derivation of the chronic ReV of 33  $\mu\text{g}/\text{m}^3$  (15 ppb) versus the chronic RfC of 2  $\mu\text{g}/\text{m}^3$  (0.9 ppb) (USEPA 2002). USEPA’s RfC is approximately 17 times lower than TCEQ’s ReV due to the following reasons:

- The TCEQ did not use an effect level extrapolation factor of 10 which makes USEPA’s RfC 10 times lower;
- The TCEQ used a  $\text{UF}_A$  of 1 based on data that DEB is the reactive metabolite responsible for ovarian atrophy and empirical data demonstrating DEB is produced in much lower concentrations than humans, whereas USEPA used a  $\text{UF}_A$  of 3, which makes USEPA’s RfC three times lower; and
- The TCEQ used a  $\text{BMCL}_{05}$  of 462 ppb which included the highest dose whereas USEPA used a  $\text{BMCL}_{10}$  of 880 ppb which excluded the highest dose, which makes USEPA’s RfC approximately 2 times higher.

Consideration of the above differences accounts for approximately a 15-fold difference ( $10 \times 3 \times 0.5$ ). While an exact partitioning of the 17-fold difference may not be possible, there are science-based and logical explanations accounting for most of the differences.

Chronic Toxicity Value	$\text{POD}_{\text{HEC}}$	$\text{UF}_H$	$\text{UF}_A$	$\text{UF}_L$ or Effect Level Extrapolation Factor	$\text{UF}_{\text{Sub}}$	$\text{UF}_D$	Total UFs	Chronic Toxicity Value
ReV based on ovarian atrophy (TCEQ)	462 ppb $\text{BMCL}_{05}$ including highest dose	10	1	1 $\text{UF}_L$	1	3	30	15 ppb
RfC based on ovarian atrophy (USEPA)	880 ppb $\text{BMCL}_{10}$ excluding highest dose	10	3	10 Effect Level extrapolation Factor	1	3	1,000	0.88 ppb

## 4.2 Carcinogenic Potential

### 4.2.1 Carcinogenic Weight of Evidence and MOA

USEPA has classified BD as known to be carcinogenic to humans by inhalation (DHHS 2000; USEPA 2002) based on the following findings:

- Increased lymphohematopoietic cancers in workers occupationally exposed via inhalation to BD based on epidemiologic studies (leukemias in polymer workers and non-Hodgkin’s lymphoma in

monomer workers);

- BD causes a variety of tumors in mice and rats by inhalation in various studies;
- Demonstration that BD is metabolized into genotoxic metabolites by experimental animals and humans.

Table 15 provides information on the carcinogenic weight of evidence provided by other organizations. Although the mechanism of action, as opposed to the MOA, by which BD produces tumors is unknown, scientific evidence suggests that carcinogenic effects are mediated by genotoxic metabolites of BD (i.e., EB, DEB, and EBD, Section 3.1.2 and Figure 2). A detailed review of the weight of evidence, carcinogenic hazard assessment, and MOA analysis for lifetime exposure potential is included in USEPA (2002). Preston (2007) recently reviewed the evidence that BD works through a mutagenic MOA and concluded: “For butadiene, the MoA is DNA-reactivity and subsequent mutagenicity and so following the EPA’s cancer guidelines, a linear extrapolation is used from the POD, unless additional data support a non-linear extrapolation.” Therefore, an inhalation unit risk factor (URF) and <sup>chronic</sup>ESL<sub>linear(c)</sub> (i.e., air concentration at 1 in 100,000 excess cancer risk) was developed for BD.

Although a linear extrapolation from a POD will be used to calculate a URF based on the MOA of BD, a free-standing NOAEL for biomarkers of effect (hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutations and chromosome aberrations) at mean BD exposure concentrations of 0.800 ppm has been demonstrated by Albertini *et al.* (2001) in a small initial study of workers in the Czech Republic.

<b>Table 15. Carcinogenic Weight of Evidence</b>	
International Agency for Research on Cancer (IARC 2007)	Group 1, Carcinogenic to humans
National Institute for Occupational Safety and Health 1997	Potential occupational carcinogen
Occupational Safety and Health Administration 1996	“Potential occupational carcinogen” There is strong evidence that workplace exposure to BD poses an increased risk of death from cancers of the lymphohematopoietic system.
ACGIH 2001	A2, Suspected Human Carcinogen
USEPA 2002; DHHS 2000	Carcinogenic to humans by inhalation

#### 4.2.2 Epidemiological Studies and Exposure Estimates

Chapter 7 of USEPA (2002) discusses the epidemiologic studies of carcinogenicity for BD, and Chapter 10 discusses the dose-response assessment of the preferred occupational epidemiological study conducted by researchers at the University of Alabama at Birmingham (UAB) (Delzell *et al.* 1995; 1996). Numerous epidemiology studies were reviewed, but USEPA (2002) concluded the UAB exposure estimates provided the best published set of data to evaluate human cancer risk from BD exposure. USEPA published an inhalation URF of  $3.0 \times 10^{-5}$  per  $\mu\text{g}/\text{m}^3$  or 0.08 per ppm based on leukemia mortality data from the UAB occupational epidemiological study (Delzell *et al.* 1995; 1996).

Delzell *et al.* (1995, 1996) investigated a cohort of synthetic rubber production workers exposed to BD in a retrospective cohort mortality study. The investigators developed a job exposure matrix (JEM) for BD, styrene, and benzene based on industrial hygiene data, which contained estimates of the average daily exposure (in ppm based on the 8-h TWA) and the number of annual peaks (defined as > 100 ppm for BD and >50 ppm for styrene) for each area and job code for each study year. The investigators were then able to estimate cumulative exposures (part-per-million (ppm)-years) and number of peak exposures (peak years) for each individual worker by linking the JEM with the study subjects' work histories.

Recently, the exposure estimates and the UAB epidemiology study of leukemia were updated:

- The UAB butadiene exposure estimates were updated and estimates for styrene and dimethyldithiocarbamate (DMDTC) were calculated (DMDTC is an immune system depressant) (Macaluso *et al.* 2004);
- The UAB epidemiology study and analysis of leukemia data was updated (Sathiakumar *et al.* 2005; Graff *et al.* 2005) (see below for additional details);
- Dr. Delzell and associates finalized a Health Effects Institute (HEI) report that discussed the updated exposure estimates and analysis of leukemia mortality data. Additional analyses requested by the Health Effects Review committee were included in the HEI report (HEI 2006).

The Health Review Committee (HEI 2006) thoroughly reviewed Delzell's findings and concluded "An analysis of butadiene that controlled for the possibly carcinogenic coexposures to styrene and DMDTC produced the most important result of the investigation: the clear and consistent exposure-response relation observed between cumulative exposure to butadiene and mortality from leukemia. . . . and support the presence of a linear increase in the relative rate of leukemia mortality with increasing cumulative exposures to butadiene."

After the HEI report was finalized, an exposure estimate validation study was conducted on the updated UAB butadiene exposure estimates (Sathiakumar *et al.* 2007). At lower concentrations, there was reasonably good agreement between measured versus estimated BD exposures; whereas at higher exposures, the estimates tended to be less than the measured values. On average, estimates were about 10% lower than measurements. Based on the validation study of Sathiakumar *et al.* (2007), the updated exposure estimates of Macaluso *et al.* (2004) have a higher confidence than original exposure estimates. Dose-response modeling was conducted based on the updated studies (Cheng *et al.* 2007; Sielken *et al.* 2007). These new, updated studies were used by the TS to update the USEPA (2002) assessment. A review of the scientific literature indicated there were no other epidemiology studies (e.g., Tsai 2005; Alder *et al.* 2006) that would be appropriate to evaluate human cancer risk from BD exposure.

Subjects included in the updated study were 16,579 men classified as having worked (for at least one year before 1 January 1992) at any of six synthetic rubber plants located in Texas (two plants), Louisiana (two plants), Kentucky (one plant) and Canada (one plant). Of the 16,579 subjects in the updated study, 488 subjects were excluded because they dropped out of follow-up at ages younger than the youngest leukemia decedent (age 33 years) (Cheng *et al.* 2007). Thus, results of leukemia analyses were based on 16,091 subjects and 485,732 person-years of observation. The updated study provided seven more years of follow-up (through 1998), a larger number of decedents, and a total of 81 deaths with leukemia as the primary or contributing cause. The association of BD exposure to lymphoid and myeloid neoplasms was investigated. BD-exposure estimates were also updated, and quantitative estimates of each subject's

exposure to butadiene, styrene and dimethyldithiocarbamate (DMDTC), an immune system depressant (Irons and Pyatt 1998; Irons *et al.* 2001) were determined.

## 4.2.3 Dose-Response Assessment

### 4.2.3.1 Beta coefficient ( $\beta$ ) and Standard Error Based on Observed Data

Cheng *et al.* (2007) investigated the dose-response relationship between BD and leukemia rate ratios using a log-linear exponential Cox regression analysis. Three BD exposure indices were evaluated by Cheng *et al.* (2007): (1) continuous, time-dependent BD exposure indices (ppm-years); (2) the total number of exposures to BD concentrations >100 ppm (number of peak exposures) and (3) average intensity of BD. All three BD exposure indices were positively associated with leukemia. The term “peak” is used by the UAB group to refer to the cumulative number of exposures to >100 ppm BD. These exposures were frequently of short duration (several seconds to several minutes). However, the term “peak” or “peak exposures” is misleading and will not be used in this assessment. Instead, the more descriptive term “number of high-intensity tasks” (i.e., number of HITS) is used. The dose metric used by the TS for the dose-response assessment was cumulative BD ppm-years, a dose metric commonly used for dose-response modeling based on epidemiological studies.

The data needed to conduct a detailed mechanism of action analysis were not available, so the use of a biologically-based model was not possible. Rather, standard epidemiological models such as the log-linear Cox proportional hazards models with age included as an index variable and the linear Poisson regression, a conservative linear default model, were selected. Whereas Cheng *et al.* (2007) used the log-linear Cox regression analysis with continuous, untransformed data and mean-scored deciles (grouped data), Sielken *et al.* (2007) used a linear Poisson regression analysis with mean-scored deciles (grouped data) to investigate the relationship between BD and leukemia rate ratios. Cheng *et al.* (2007) and Sielken *et al.* (2007) calculated betas ( $\beta$ ) (maximum likelihood estimates (MLEs)) and standard errors (SE) from the updated UAB epidemiological study and updated exposure estimates (Table 16).

The Cox regression analysis using continuous cumulative exposure estimates is preferred to the Cox with mean-scored deciles (grouped data) because the former uses the best estimate of cumulative BD ppm-years. Additionally, Cox regression analyses use individual data and adjust for the effects of age in an optimal way (age is used as the index variable and implicitly a covariate) and are preferred over Poisson regression analyses.

Cheng *et al.* (2007) also determined the  $\beta$  and SE for data restricted to the lower 95% of the exposure range of all subjects since spline regression analysis indicated that above an exposure level of 1,123 BD ppm-year, the data were sparse, and the dose-response relationship was erratic (Figure 5a). Figure 5b shows the dose-response relationship below 1,123 BD ppm-years. Spline regression indicated that the ln hazard ratio for leukemia increased in a fairly linear fashion in the exposure range below the 95% of exposure, although the choice of “knots” may affect the appearance of spline curves. The  $\beta$  estimates obtained from restricted data were higher (i.e., more conservative). Evaluating the  $\beta$  and SE for restricted data, which are more conservative, may address concerns that data were sparse (there were only four leukemia decedents), and exposure-response trends were erratic for cumulative BD above 1,123 ppm-years (Cheng *et al.* 2007; Steenland 2005). Sielken *et al.* (2007) examined the results of progressively restricting the data to lower concentrations (i.e., < 1,338, 1,000, 500, 400, 300, 200, and 100 ppm-years).

Interestingly, these analyses showed the absence of a statistically significant low-dose risk versus cumulative BD ppm-years for restricted data less than 300 ppm-years.

Covariates	Model	Source	$\beta$ (MLE) $\pm$ SE p-Value	$\beta$ (95% UCL) <sup>b</sup>
Age	Cox log-linear ppm-years continuous <sup>c</sup>	Cheng <i>et al.</i> (2007)	2.9E-04 $\pm$ 1.0E-04 < 0.01	4.545E-04
	Cox log-linear ppm-years mean-scored deciles <sup>c</sup>	Cheng <i>et al.</i> (2007)	7.5E-04 $\pm$ 2.2E-04 < 0.01	1.112E-03
	Cox log-linear (restricted to lower 95% of exposure range) ppm-years continuous <sup>c</sup>	Cheng and Delzell <sup>d</sup>	1.58E-03 $\pm$ 3.9E-04 < 0.001	2.221E-03
	Poisson linear ppm-years mean-scored deciles <sup>c</sup>	Sielken <i>et al.</i> (2007)	1.68E-03 $\pm$ 8.21E-04 < 0.001	3.031E-03
Age & Other Covariates <sup>f</sup>	Cox log-linear ppm-years continuous <sup>c</sup>	Cheng <i>et al.</i> (2007)	3.0E-04 $\pm$ 1.4E-04 0.04	5.303E-04
	Cox log-linear ppm-years mean-scored deciles <sup>c</sup>	Cheng <i>et al.</i> (2007)	5.8E-04 $\pm$ 2.7E-04 0.03	1.024E-03
	Cox log-linear (restricted to lower 95% of exposure range) ppm-years continuous <sup>c</sup>	Cheng <i>et al.</i> (2007)	1.31E-03 $\pm$ 4.7E-04 < 0.01	2.083E-03

<sup>a</sup> units are in ppm-years and based on occupational exposure concentrations

<sup>b</sup>  $\beta$  (95% UCL) =  $\beta$ (MLE) + (1.645 x SE)

<sup>c</sup> ppm-years is included as a continuous variable (untransformed) in a parametric model of the effect of ppm-years

<sup>d</sup> Personal communication, 1/30/2008 email from Cheng and Delzell. Cheng *et al.* (2007) reported results for Cox log-linear (restricted to lower 95% of exposure range) ppm-years continuous for age & other covariates, but not age only. The 1/30/2008 email provided the values for Cox log-linear (restricted to lower 95% of exposure range) ppm-years continuous for age.

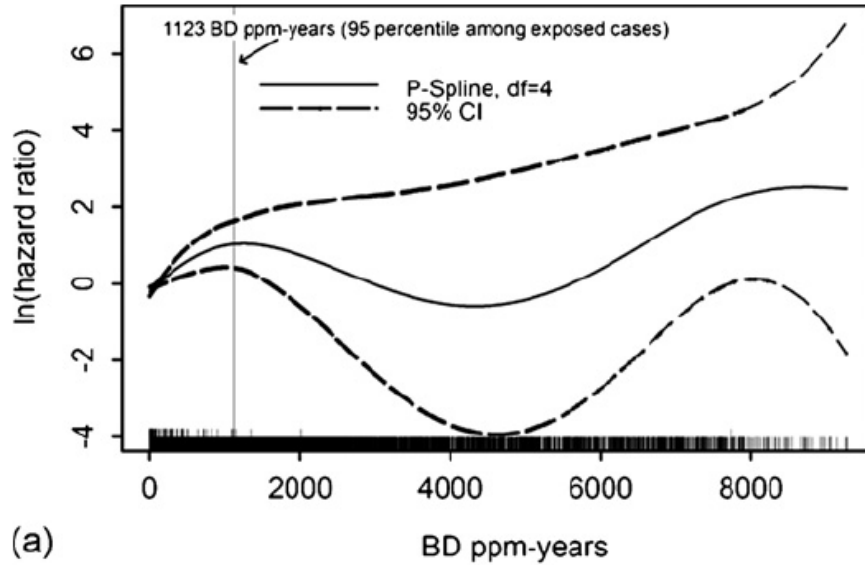
<sup>e</sup> ppm-years is included as a continuous variable with values grouped into mean-scored deciles (untransformed) in a parametric model of the effect of ppm-years

<sup>f</sup> other covariates are year of birth, race, DMDTC, years since hire and plant

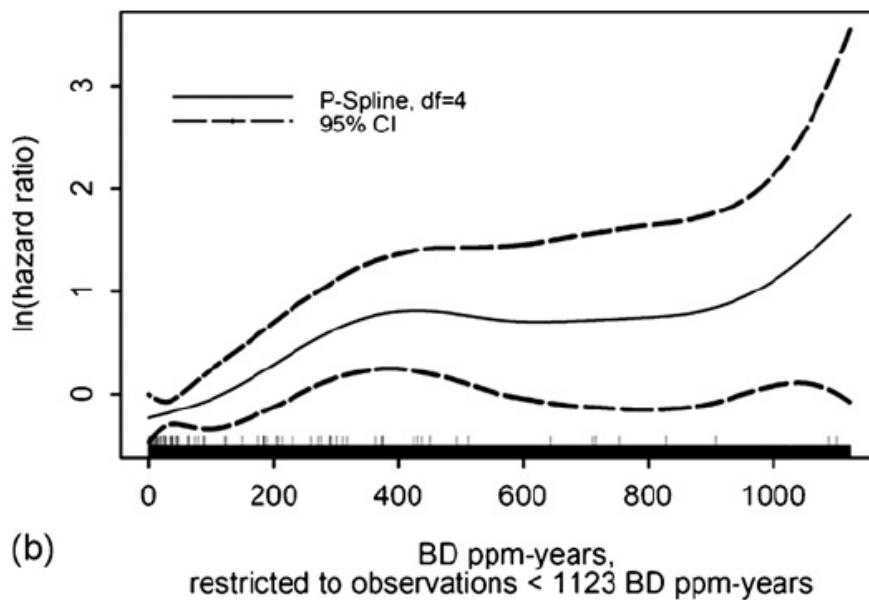
Table 16 shows results from the BD dose-response relationship conducted by Cheng *et al.* (2007) using log-linear Cox regression procedures, continuous data or mean-scored decile data, adjusted either for age as a covariate or adjusted for other covariates (age, year of birth, race, plant, years since hire and DMDTC) for the full range of exposure data and to data restricted to the lower 95% of the exposure range. The linear Poisson mean-scored decile data adjusted for age as a covariate is also included in Table 16. The TS used these values to calculate 95% upper confidence limit (UCL) values, URFs and corresponding air concentrations at 1 in 100,000 excess cancer risk (Table 17). Cheng *et al.* (2007) results support the presence of a relationship between high cumulative exposure and leukemia and high intensity of exposure and leukemia.

Beta estimates were also calculated by Cheng *et al.* (2007) for unlagged and lagged BD exposure but these  $\beta$  estimates were not used by the TS because lagging BD exposure had little impact on the dose-response relationship between leukemia and BD ppm-years. Sielken & Associates have shown that when windows of exposure were evaluated in the model, there was little impact on the dose-response relationship between leukemia and BD ppm-years (personal communication from Sielken & Associates). The association of BD exposure with leukemia, lymphoid neoplasms, and myeloid neoplasms was investigated by both Cheng *et al.* (2007) and Sielken *et al.* (2007). Lymphoid neoplasms were associated with ppm-years and myeloid neoplasms were associated with number of HITs in models that controlled only for age but not after adjusting for multiple covariates (age, year of birth, race, plant, years since hire and DMDTC). These potency estimates were not used by the TS because evidence of an association between BD and all lymphoid neoplasm or all myeloid neoplasms was not persuasive (Cheng *et al.* 2007; Sielken *et al.* 2007).

Sielken *et al.* (2007) used a linear Poisson regression model to examine the dose-response relationships adjusted for age as a categorical covariate (Table 16), age + number of HITs as covariates, and multiple covariates. Sielken *et al.* (2007) found that if the exposure dosimetric is cumulative ppm-years, the performance of the predictor for leukemia rate ratio is statistically significantly improved if the categorical covariates age + number of HITs are included in the Poisson regression model. If covariates other than age + number of HITs are included, the model fit using cumulative ppm-years was not significantly improved except for styrene. However, if styrene was included as a covariate, the slope was negative, so styrene was not included as a covariate. Although Sielken *et al.* (2007) performed this statistical analyses for covariates using Poisson regression models, their findings are also generally applicable for the Cox proportional hazards models.



(a)



(b)

**Figure 5. Exposure-Response in Models using Continuous BD Variables and Restricted Data** (Figure 1 (a, b) from Cheng *et al.* (2007), reproduced with permission). Penalized splines for BD ppm-years and leukemia (a and b). Rugs just above the x-axis of each figure depict the frequency of observations (lower rug) and leukemias (upper rug) at corresponding BD variable values. There were only four leukemia decedents above 1,123BD ppm years.

#### 4.2.3.2 *Dosimetric Adjustments*

Occupational concentrations were converted to environmental concentrations for the general population using the following equation (TCEQ 2006):

$$\text{Concentration}_{\text{HEC}} = \text{Concentration}_{\text{OC}} \times (\text{VE}_{\text{ho}}/\text{VE}_{\text{h}}) \times (\text{days per week}_{\text{oc}}/\text{days per week}_{\text{res}})$$

where:  $\text{VE}_{\text{ho}}$  = occupational ventilation rate for an 8-h day ( $10 \text{ m}^3/\text{day}$ )  
 $\text{VE}_{\text{h}}$  = non-occupational ventilation rate for a 24-h day ( $20 \text{ m}^3/\text{day}$ )  
 $\text{days per week}_{\text{oc}}$  = occupational weekly exposure frequency (default of 5 days per week)  
 $\text{days per week}_{\text{res}}$  = residential weekly exposure frequency (7 days per week)

#### 4.2.3.3 *Extrapolation to Lower Exposures*

##### 4.2.3.3.1 **URFs and Air Concentrations at 1 in 100,000 Excess Cancer Risk**

Table 17 shows estimates of air concentrations at 1 in 100,000 excess cancer risk ( $10^{-5}$ -risk air concentrations) based on  $\beta$ s (column three) and 95% UCLs (column five) using the log-linear Cox regression and linear Poisson regression models. Air concentrations were solved iteratively with life-table analyses using the BEIR IV approach (NRC 1988). Air concentrations based on extra risk were calculated as opposed to added risk. The following mortality and survival rates were used to calculate air concentrations based on a lifetime exposure of 70 years, the default used by TCEQ for exposure analysis (TCEQ 2006):

- US mortality rates for 2000-2003 for all leukemia (Surveillance, Epidemiology, and End Results database (SEER 2006)) (Appendix 4)
- US survival rates for 2000 (Arias 2002) (Appendix 4).

Columns four and six of Table 17 provide URFs calculated using the linear extrapolation default approach (USEPA 2005a; TCEQ 2006). Risk estimates are obtained by first calculating a  $\text{POD}_{\text{HEC}}$  at the low end of the range of observations using appropriate models and then extrapolating to zero by means of a straight line (linear extrapolation default). The air concentration at 0.1% excess risk level (i.e., 1 in 1,000 excess cancer risk) is chosen for determining the POD because it is within the observable response range of leukemia deaths. The URFs in units of  $\text{ppm}^{-1}$  at the  $\text{POD}_{\text{HEC}}$  (when the  $\text{POD}_{\text{HEC}}$  was set to either the effective concentration ( $\text{EC}_{001}$ ) or the 95% UCL lowest effective concentration ( $\text{LEC}_{001}$ )) were calculated as follows:

$$\begin{aligned}\text{URF} &= 0.001/\text{EC}_{001} \\ \text{URF} &= 0.001/\text{LEC}_{001}\end{aligned}$$

Columns four and six of Table 17 also provide  $10^{-5}$ -risk air concentrations based on the corresponding URFs. Air concentrations calculated using the corresponding URFs are more conservative than air concentrations calculated based on the Cox regression model, because this model is a log-linear model. As a health-protective policy decision,  $10^{-5}$ -risk air concentrations calculated with URFs based on the default linear approach were adopted and all subsequent discussions will refer to the URF (MLE) or URF (95%UCL) and their corresponding  $10^{-5}$ -risk air concentration values.

<b>Table 17. URFs and Air Concentrations Corresponding to 1 in 100,000 Extra Leukemia Risk</b>					
Covariates	Model  type of data	$\beta$ (MLE)	EC <sub>001</sub>	$\beta$ (95% UCL)	LEC <sub>001</sub>
		Air Concentration 1 in 100,000 excess cancer risk using model	URF (MLE) <sup>a</sup>  Air Concentration 1 in 100,000 excess cancer risk using URF	Air Concentration 1 in 100,000 excess cancer risk using model	URF (95% UCL) <sup>b</sup>  Air Concentration 1 in 100,000 excess cancer risk using URF
Age	Cox log-linear ppm-years continuous <sup>c</sup> Cheng <i>et al.</i> (2007)	87.36 ppb	1.371E-04/ppm  72.93 ppb	55.74 ppb	2.149E-04/ppm  46.53 ppb
	Cox log-linear ppm-years mean-scored deciles <sup>d</sup> Cheng <i>et al.</i> (2007)	33.78 ppb	3.546E-04/ppm  28.20 ppb	22.78 ppb	5.258E-04/ppm  19.02
	Cox log-linear (restricted to lower 95% of exposure range) ppm-years continuous <sup>c</sup> Cheng and Delzell <sup>f</sup>	16.03 ppb	7.471E-04/ppm  13.39 ppb	11.41 ppb	1.050E-03/ppm  9.523 ppb
	Poisson linear ppm-years mean-scored deciles <sup>d</sup> Sielken <i>et al.</i> (2007)	15.11 ppb	6.614E-04/ppm  15.12 ppb	8.376 ppb	1.193E-03/ppm  8.381 ppb
Age & Other Covariates <sup>e</sup>	Cox log-linear ppm-years continuous <sup>c</sup> Cheng <i>et al.</i> (2007)	84.45 ppb	1.418E-04/ppm  70.50 ppb	47.77 ppb	2.507E-04/ppm  39.88 ppb
	Cox log-linear ppm-years mean-scored deciles <sup>d</sup> Cheng <i>et al.</i> (2007)	43.68 ppb	2.742E-04/ppm  36.47	24.74 ppb	4.842E-04  20.65
	Cox log-linear (restricted to lower 95% of exposure range) ppm-years continuous <sup>c</sup> Cheng <i>et al.</i> (2007)	19.34 ppb	6.194E-04/ppm  16.14 ppb	12.16 ppb	9.849E-04/ppm  10.15 ppb

<sup>a</sup> URF = 0.001/EC<sub>001</sub>

<sup>b</sup> URF = 0.001/LEC<sub>001</sub>

<sup>c</sup> ppm-years is included as a continuous variable (untransformed) in a parametric model of the effect of ppm-years

<sup>d</sup> ppm-years is included as a continuous variable with values grouped into mean-scored deciles (untransformed) in a parametric model of the effect of ppm-years

<sup>e</sup> Other covariates are year of birth, race, DMDTC, years since hire and plant

<sup>f</sup> Personal communication, 1/30/2008 email from Dr. Cheng and Dr. Delzell. Cheng *et al.* (2007) reported results for Cox log-linear continuous (restricted to lower 95% of exposure range) ppm-years for age & other covariates, but not age only or age + # HITs. Dr. Cheng and Dr. Delzell provided the  $\beta$  and SE values for Cox log-linear continuous (restricted to lower 95% of exposure range) ppm-years for age and age + # HITs in the 1/30/2008 email.

#### 4.2.3.3.2 Age as a Covariate

Models that only include age as a non-exposure covariate have the advantage of model parsimony (i.e., the model includes as few variables as necessary to explain the relationship when there is not sufficient biological knowledge to justify the inclusion or exclusion of a variable). When age is included as a covariate (Table 18), the 10<sup>-5</sup>-risk air concentrations using the Poisson linear model were the most conservative: 15.12 ppb (MLE) and 8.381 ppb (95% UCL). However, as stated previously, Cox log-linear analysis using continuous, untransformed data are preferred over the linear Poisson regression analysis with mean-scored deciles (grouped data) because it uses the best estimate of cumulative BD ppm-years, uses individual data, and adjusts for the effects of age in an optimal way. Using the Cox log-linear model and restricted data, the 10<sup>-5</sup>-risk air concentrations of 13.39 ppb (MLE) and 9.523 ppb (95% UCL) were more conservative than Cox log-linear mean-scored deciles (28.20 ppb MLE and 19.02 ppb 95% UCL) and continuous, untransformed data (72.93 ppb MLE and 46.53 ppb 95% UCL).

<b>Model</b>	<b>EC<sub>001</sub></b>	<b>LEC<sub>001</sub></b>
	<b>URF (MLE)<sup>a</sup></b>	<b>URF (95% UCL)<sup>b</sup></b>
<b>type of data</b>	<b>10<sup>-5</sup>-risk air concentration using URF</b>	<b>10<sup>-5</sup>-risk air concentration using URF</b>
Cox log-linear Cheng <i>et al.</i> (2007)	1.371E-04/ppm	2.149E-04/ppm
ppm-years continuous <sup>c</sup>	72.93 ppb	46.53 ppb
Cox log-linear Cheng <i>et al.</i> (2007)	3.546E-04/ppm	5.258E-04/ppm
ppm-years mean-scored deciles <sup>d</sup>	28.20 ppb	19.02
Cox log-linear (restricted to lower 95% of exposure range) Cheng <i>et al.</i> (2007)	7.471E-04/ppm	1.050E-03/ppm
ppm-years continuous <sup>c</sup>	13.39 ppb	9.523 ppb
Poisson linear Sielken <i>et al.</i> (2007)	6.614E-04/ppm	1.193E-03/ppm
ppm-years mean-scored deciles <sup>d</sup>	15.12 ppb	8.381 ppb

<sup>a</sup> URF = 0.001/EC<sub>001</sub>

<sup>b</sup> URF = 0.001/LEC<sub>001</sub>

<sup>c</sup> ppm-years is included as a continuous variable (untransformed) in a parametric model of the effect of ppm-years

<sup>d</sup> ppm-years is included as a continuous variable with values grouped into mean-scored deciles (untransformed) in a parametric model of the effect of ppm-years

The 10<sup>-5</sup>-risk air concentration estimates based on restricted data are preferred because the impact of sparse data and the erratic exposure-response trends above 1,123 ppm-years are reduced. The EC<sub>001</sub> and LEC<sub>001</sub> values from the Cox regression models with continuous restricted data are approximately 5-fold smaller than the values from the Cox regression models with all the data, 2-fold smaller than the values from the Cox regression models with all the data and mean-scored deciles, and within 13% from the values of the Poisson regression model with mean-scored deciles. The use of mean-scored deciles in the

Cox log-linear and Poisson linear models may reduce the impact of misclassification because it reduces the influence of data at the extreme exposure estimates (Cheng *et al.* 2007), although it may increase misclassification at the low end, where exposure estimates are likely to be better. Therefore, continuous, untransformed data are preferred (also refer to Section 4.2.3.1 *Beta coefficient ( $\beta$ ) and Standard Error Based on Observed Data* for additional reasons).

#### **4.2.3.3.3 Other Covariates**

##### ***4.2.3.3.3.1 Models that adjusted for multiple covariates***

Cheng *et al.* (2007) fit models that adjusted for age, year of birth, race, DMDTC, years since hire and plant. Except for the exposure covariate DMDTC, an immune system depressant (Irons and Pyatt 1998; Irons *et al.* 2001), these covariates are typically evaluated in epidemiology dose-response models. Sielken *et al.* (2007) included statistically-based covariates and determined that if covariates other than age + number of HITs are included, the model fit using cumulative ppm-years was not significantly improved except for styrene. However, if styrene was included as a covariate, the slope was negative, so styrene was not included as a covariate. Although Sielken *et al.* (2007) performed this statistical analysis for covariates using the Poisson regression model, his findings are generally applicable for the Cox regression model. Therefore, URF (MLE) and URF (95% UCL) from models that adjusted for age, year of birth, race, DMDTC, years since hire, and plant were not considered as potency factors by the TS, although these values are provided in Tables 16 and 17 for comparison purposes. There were minor differences between  $10^{-5}$ -risk air concentrations in models that adjusted for age only or models that adjusted for multiple covariates (Table 17).

##### ***4.2.3.3.3.2 Models that adjusted for age + number of HITs > 100 ppm***

The cumulative number of HITs > 100 ppm may better explain the increased leukemia mortality observed in the BD worker cohort (Cheng *et al.* 2007; Sielken *et al.* 2007). Sielken *et al.* (2007) demonstrated that when the categorical covariates of age + number of HITs are included in the Poisson regression model, the model's ability to predict the leukemia rate ratio was statistically improved. The UAB group evaluated the number of HITs > 100 ppm because BD ppm-years could not by itself adequately explain worker's leukemia risk. Since the USEPA Science Advisory Board (USEPA 1998) recommended that consideration of peak exposures to BD be evaluated during its review of the draft health risk assessment of BD (USEPA 1998b), the TS did evaluate the effect of including number of HITs. However, BD ppm-years and number of HITs are both exposure variables and may be correlated, so it may not be appropriate to include both of them in the same model. Cheng *et al.* (2007) found these BD exposure variables were weakly correlated for continuous values (Pearson correlation coefficient of 0.30) as opposed to grouped (deciles) values (Pearson correlation coefficient of 0.80). Therefore, the TS evaluated Cox regression models using continuous (untransformed) variables that adjusted for age and the continuous (ungrouped) value of cumulative number of HITs > 100 ppm. The  $10^{-5}$ -risk air concentration based on the URF (MLE) increased approximately 18% and the  $10^{-5}$ -risk air concentration based on the URF (95% UCL) increased approximately 6% (Tables 18 and 19 of subsection 4.2.5.2 *Estimating Risks for the General Population from Occupational Workers*) when number of HITs > 100 ppm was included as a covariate. These models were not the preferred models selected to represent excess leukemia mortality risk, but are useful for evaluating uncertainty of estimating risks to the general population when data are based on occupational workers who are exposed to peak BD exposures > 100 ppm. Section 4.2.5 *Uncertainty Analysis*, subsection 4.2.5.2 *Estimating Risks for the General Population from Occupational Workers* provides a more detailed discussion.

#### 4.2.4 Potency Estimate Selected to Represent Excess Leukemia Mortality Risk

Of the various estimates presented in Table 17, the potency estimate of 1.050E-03 per ppm ( $10^{-5}$ -risk air concentrations of 9.523 ppb) from the Cox regression model using restricted continuous data, age as a covariate, the URF based on the 95% UCL, and a linear default approach is selected to represent the excess leukemia mortality risk from the occupational data. However, refer to Section 4.2.4.1 *Evaluating Susceptibility from Early-Life Exposures* and Section 4.2.4.2 *Relevance of Estimated Risk to the Texas General Population* for additional adjustments to the URF (95% UCL) and  $10^{-5}$ -risk air concentrations. The ranges in the cancer potency estimates from the different models were within a factor of five:

- The cancer potency estimates and  $10^{-5}$ -risk air concentrations using URFs (MLE) in Table 17 range from 7.471E-04 per ppm (13.39 ppb) to 1.371E-04 per ppm (72.93 ppb).
- The cancer potency estimates and  $10^{-5}$ -risk air concentrations using URFs (95% UCL) in Table 17 range from 1.193E-03 per ppm (8.381 ppb) to 2.149E-04 per ppm (46.53 ppb).

The UAB group recommended the estimate of the dose-response relationship that is based on the continuous, untransformed form of BD ppm-years, age included as the index variable, and the full range of exposure data (2.9E-04 ( $\beta$ ), 1.0E-04 (S.E.)). However, due to the high potential for distortion of the dose-response relationship as a result of exposure misclassification at high exposure concentrations, Cheng *et al.* (2007) also recommended that an uncertainty analysis be incorporated into any risk assessment that uses these data. However, since the purpose of this assessment is to calculate a health-protective  $10^{-5}$ -risk air concentration for evaluation of air permits and ambient air monitoring data, the TS decided as a policy decision to use the results based on restricted data because they are more conservative and to address concerns about sparse data and an erratic exposure-response relationship at high exposure concentrations.

The Cox regression analysis using continuous, untransformed data are preferred over the Cox log-linear and linear Poisson regression analysis with mean-scored deciles (grouped data) because it uses the best estimate of cumulative BD ppm-years, uses individual data, and adjusts for the effects of age in an optimal way. A linear default was used to extrapolate to lower concentrations and the URF (95% UCL) was preferred to account for uncertainties as discussed in the uncertainty section. The confidence intervals are indicators of the variability, and to some extent the uncertainty, in the dose-response curve for mortality. The risk to the general population will be lowered since using the URF (95% UCL) adds conservatism to the estimate. There was only a 1.4 fold difference between estimates using the MLE compared to the 95% UCL, which supports the quality of the epidemiological data.

##### 4.2.4.1 Evaluating Susceptibility from Early-Life Exposures

USEPA (2005b) provides default, age-dependent adjustment factors (ADAFs) to account for potential increased susceptibility in children due to early-life exposure when a chemical has been identified as acting through a mutagenic MOA for carcinogenesis and the cancer assessment did not include exposures at an early age (generally before age 16). This is the case for the epidemiological leukemia data utilized in this evaluation. BD is currently identified by USEPA as having a mutagenic MOA. USEPA (2005b) states:

“The following adjustments represent a practical approach that reflects the results of the preceding analysis, which concluded that cancer risks generally are higher from early-life exposure than from similar exposure durations later in life:

- For exposures before 2 years of age (i.e., spanning a 2-year time interval from the first day of birth up until a child's second birthday), a 10-fold adjustment.
- For exposures between 2 and <16 years of age (i.e., spanning a 14-year time interval from a child's second birthday up until their sixteenth birthday), a 3-fold adjustment
- For exposures after turning 16 years of age, no adjustment.”

The ADAF is an adjustment to the slope factor (as opposed to an adjustment to the dose metric). The ADAF is to be applied on an age-specific basis. That is, the ADAFs are applied to each relevant year in a life and the risks for all years summed to get the lifetime risk, as opposed to calculating a lifetime excess risk without ADAFs and then multiplying this calculated value by a constant ADAF.

When the dose metric is cumulative exposure and when using a life-table analysis BEIR IV approach (NRC 1988), an implementation consistent with USEPA guidelines is to calculate the excess risk in each year using the age-specific dose (cumulative dose) for that year and multiply the slope by the age-specific ADAF for that year (age). This is consistent with USEPA's guidelines from the point of view of both excess risk being calculated using age-specific exposures and ADAFs being age-specific modifiers of the slope (potency). That is, the excess risk in year “i” is calculated with the  $\beta$  or 95% UCL multiplied by ADAF(i). Refer to Appendix 5 *Calculating Excess Risk with Age-Dependent Adjustment Factors using a Life-Table Analysis*.

The TS calculated potency factors both with and without ADAFs. When the ADAFs are not applied, the selected potency estimate is 1.050E-03 per ppm (9.523 ppb  $10^{-5}$ -risk air concentration). When the ADAFs are incorporated into the life-table analyses using the BEIR IV approach (NRC 1988), the selected potency estimate is 1.062E-03 per ppm (9.416 ppb  $10^{-5}$ -risk air concentration). There is a minor difference between potency estimates calculated with and without ADAFs, when the URF is rounded to two significant figures at the end of all calculations. Toxicokinetic and toxicodynamic evidence indicates children are not more susceptible to chemical leukemogenesis than adults for acute myeloid leukemia and acute nonlymphocytic leukemia (Johnsrud *et al.* 2003; Levine and Bloomfield 1992; Pyatt *et al.* 2005; Pyatt *et al.* 2007; USEPA 1997), so the application of ADAFs may not be justified. USEPA (1997) provides a detailed discussion of the critical steps that may contribute to BD leukemogenesis.

#### **4.2.4.2 Relevance of Estimated Risks to the Texas General Population**

There is uncertainty about whether potency estimates are representative of the mortality risks that might be associated with environmental BD exposures in Texas because potency estimates were developed based on the leukemia mortality experience of predominantly male workers in the styrene-butadiene rubber industry, total US rates of mortality from leukemia and total US survival rates (Appendix 4). In order to address this uncertainty, Texas-specific mortality rates for 1999-2003 for all leukemia and Texas-specific survival rates for 2003 were kindly provided by the Texas Department of State Health Services, Cancer Epidemiology and Surveillance Branch, Texas Cancer Registry. There were minor differences in calculated air concentrations when Texas versus US all leukemia mortality rates and survival rates were used because the Texas-specific rates are very similar to US rates (Appendix 4). The selected potency estimate is 1.062E-03 per ppm (9.416 ppb  $10^{-5}$ -risk air concentration) using US rates of mortality from leukemia and total US survival rates when ADAFs are incorporated (Section 4.2.4.1) and is 1.097E-03 per ppm (9.112 ppb  $10^{-5}$ -risk air concentration) using Texas-specific mortality rates for 1999-2003 for all

leukemia and Texas-specific survival rates for 2003 when ADAFs are incorporated. There is no difference between potency estimates calculated with either US rates or Texas rates when the URF is rounded to two significant figures at the end of all calculations (i.e., 1.1E-03 per ppm). The  $^{chronic}ESL_{linear(c)}$  or air concentration at 1 in 100,000 excess cancer risk is 9.1 ppb (20  $\mu\text{g}/\text{m}^3$ ).

## 4.2.5 Uncertainty Analysis

### 4.2.5.1 Estimating Risks for other Potentially Sensitive Subpopulations

Leukemia mortality was evaluated based on healthy male workers employed at North American plants that manufactured SBR. Since these workers were healthy, they may underestimate risks to the general population that are comprised of sensitive subpopulations. It is unknown whether workers with genetic polymorphisms as discussed in Section 3.1.2 (i.e., genes that regulate the metabolism of BD to mutagenic intermediates and genes that regulate the detoxification of those metabolites) were represented in the cohort. Populations with certain lifestyle choices may be more sensitive to health effects caused by BD. Children may or may not be more sensitive to mutagenic carcinogens (see Section 4.2.4.1).

Studies in which animals were exposed to high BD concentrations suggest that female animals may be more sensitive than male animals for cancer effects after exposure to BD (USEPA 2002). Initial studies conducted in humans by Albertini *et al.* (2007) indicate that except for lower production of both urine BD metabolites in females, no female-male differences in response to low BD exposures were detected (mean 8-h TWA exposure levels were 0.180 ppm for BD-exposed female workers and 0.370 ppm for BD-exposed male workers as discussed in Section 4.5). A significant finding from Albertini *et al.* (2007) is “females showed lower concentrations of both M1 and M2 metabolites in the urine per unit of BD exposure than did males while exhibiting the same M1/(M1 + M2) ratio, reflecting the same relative utilization of the hydrolytic (producing M1) and the conjugation (producing M2) detoxication pathways as males.” This may indicate that females absorb less BD per unit of exposure than male workers.

The UAB group has analyzed mortality results for 4,863 female workers employed in the SBR industry from 1943 to 2002 (Sathiakumar and Delzell 2007a, b). Preliminary results indicate that standard mortality rates (SMRs) for lung and bladder cancer were elevated in female workers. Both excesses were concentrated among ever-hourly employees and among ever-hourly employees with 20+ years since hire, but neither cancer displayed a pattern of increasing SMRs with increasing duration of employment. For lung cancer, analyses of cumulative exposure indices were conducted. Results for lung cancer indicated a moderately positive association with each agent, without exposure-response. The SMRs for leukemia, non-Hodgkin lymphoma or other forms of lymphohematopoietic cancers, breast cancer, and ovarian cancer were not elevated (Sathiakumar and Delzell 2007b). For lung and bladder cancer, the absence of any trend of increasing SMRs with increasing duration of employment, the lack of any exposure-response trend for cumulative exposure to BD, styrene, or DMDTC and the absence of positive results in studies of male employees indicate that these occupational exposures may not have been responsible for the observed excesses of lung and bladder cancers among women in the industry (Sathiakumar and Delzell 2007b).

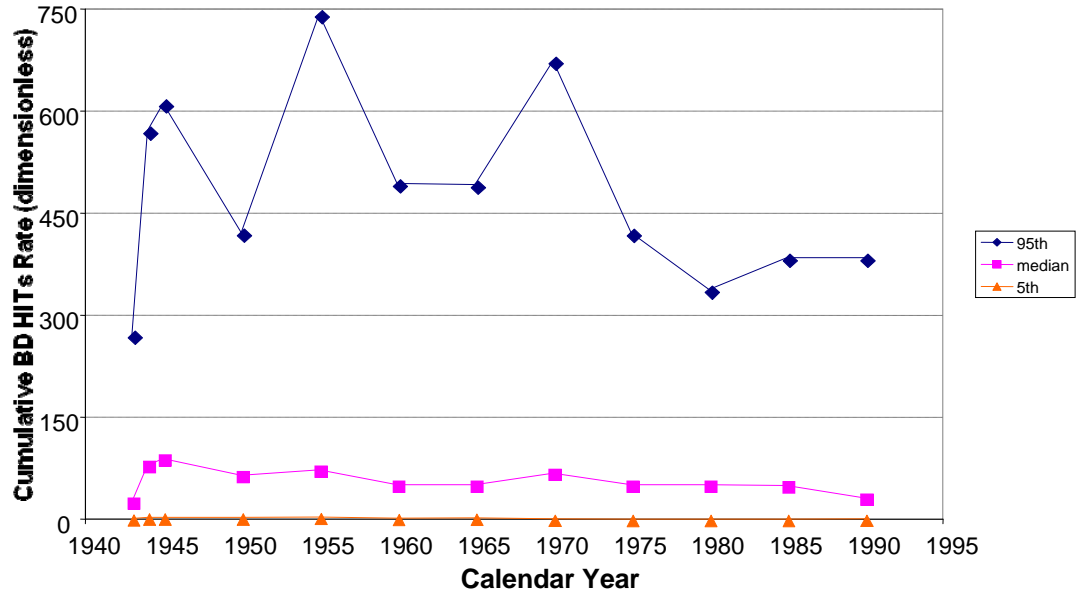
Since the UAB cohort was comprised primarily of males, a linear default was used to extrapolate to lower concentrations, and the URF (95% UCL) was used instead of the URF (MLE) to account for the uncertainty of calculating potency estimates for the general population.

#### **4.2.5.2 Estimating Risks for the General Population from Occupational Workers**

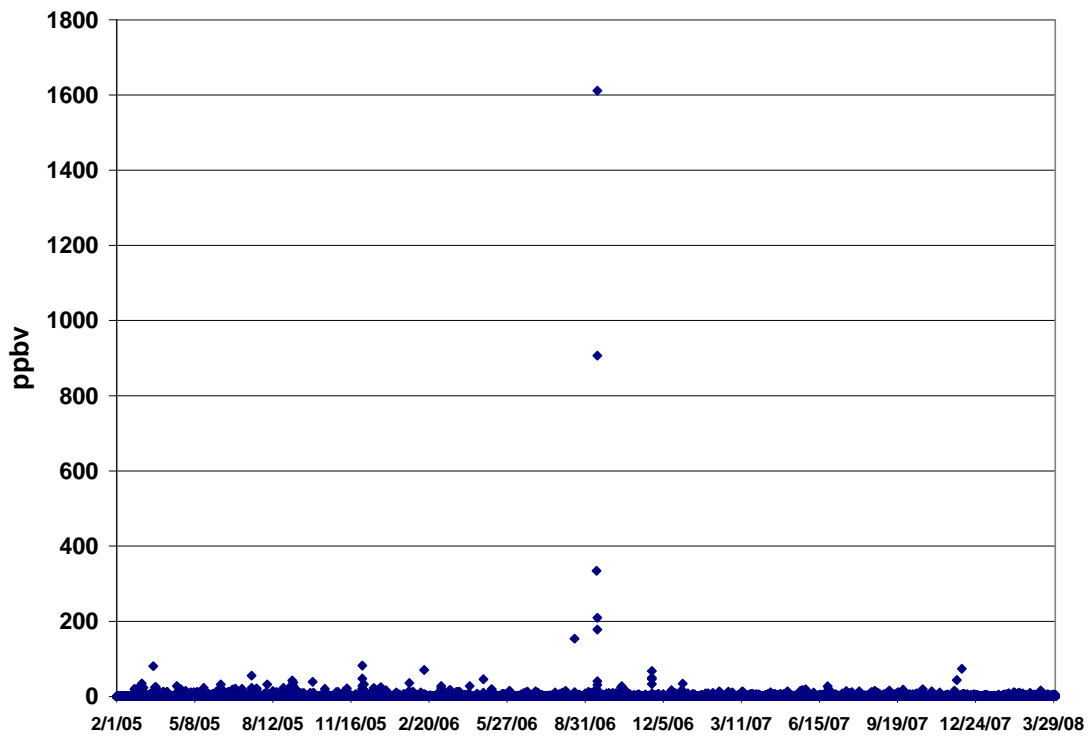
There is uncertainty regarding the extrapolation of risks from occupational workers exposed to high BD concentrations and to BD HITs > 100 ppm to risks for the general population who are exposed to much lower BD concentrations and not exposed to BD HITs > 100 ppm. Epidemiological studies in Texas, at sites downwind of facilities that produce styrene-butadiene rubber that investigated BD exposures and increased mortality from any cause at low concentrations typical for the general population have not found a significant association between mortality from leukemia and exposure to BD, although there are only a few epidemiology studies that have been conducted (reviewed by Grant *et al.* (2007)). Figure 6 shows the 5<sup>th</sup>, 50<sup>th</sup>, and 95<sup>th</sup> percentiles of the distribution of the cumulative number of BD HITs > 100 ppm in the UAB cohort study indicating SBR workers were frequently exposed to BD HITs > 100 ppm. In contrast, air monitoring data in Texas do not indicate the general population is exposed to BD HITs > 100 ppm. For example, Figure 7 provides 40-min BD concentrations (ppbv) at a monitoring site at Milby Park (2005 thru the first quarter of 2008). Milby Park is located predominantly downwind of nearby major industrial sources of BD emissions. There were only four times in a two-year period that the concentration of BD exceeded 200 ppb and the maximum peak BD concentration was 1,600 ppb. Maximum 40-min BD concentration data from 25 other ambient air monitoring sites in Texas indicate peak concentrations have not approached 1,600 ppb; in fact, maximum concentrations are less than 150 ppb. Other exposure studies indicate that the general population is exposed to concentrations of BD much lower than occupational workers (USEPA 2002, Gordon *et al.* 1999; Sapkota and Buckley 2003; Sapkota *et al.* 2005; Grant *et al.* 2007).

The inclusion of age and number of HITs > 100 ppm BD as covariates in the Cox regression modeling may result in cancer potency estimates that are more relevant to BD exposures experienced by the general population. Once age is in the model, inclusion of number of BD HITs results in a significant improvement in the fit (likelihood) (Sielken *et al.* 2007). The general population is not expected to be exposed to BD concentrations greater than 100 ppm, so adjusting for BD HITs > 100 ppm as a covariate produces cancer potency estimates more relevant to BD exposures experienced by the general population.

Slikker *et al.* (2004) provides a discussion of the role of dose-dependent transitions in mechanisms of toxicity for BD as well as several other chemicals. Exposure to BD at high concentrations may result in a change from the hydrolytic pathways that are normally used by humans to form EBD to the formation of the more toxic metabolite, DEB (i.e., metabolic enzymes may be saturated) (Figure 1). In addition, DNA repair mechanisms as well as protective enzymes may become saturated and other protective cellular constituents may be depleted which could result in mechanisms of toxic tissue injury that are not relevant at exposures significantly less than 100 ppm. As mentioned previously, Albertini *et al.* (2001) showed a clear NOAEL for biomarkers of effect (hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutations and chromosome aberrations) at mean BD exposure concentrations of 0.800 ppm in a study of workers in the Czech Republic (see Section 4.5 for additional information) and Sielken *et al.* (2007) analyses showed the absence of a statistically significant low-dose risk versus cumulative BD ppm-years for restricted data less than 300 ppm-years.



**Figure 6. Distribution of BD HITs > 100 ppm among BD-Exposed Workers in a Calendar Year.** The cumulative number of BD HITs rate (dimensionless) versus calendar year is shown for the 5<sup>th</sup>, 50<sup>th</sup>, and 95<sup>th</sup> percentiles of the distribution among BD-exposed workers included in the UAB cohort study.



**Figure 7. Forty-Minute BD Concentrations (ppbv) at Milby Park (2005 – first quarter of 2008).** Milby Park is located predominantly downwind of nearby major industrial sources of BD emissions (Grant *et al.* 2007). Forty-minute auto gas chromatography data.

Table 19 contains  $\beta$ , SE, and 95% UCL values when age & number of HITS > 100 ppm are included as covariates for the different models in Table 18. Table 20 contains URFs and  $10^{-5}$ -risk air concentrations using Texas-specific mortality rates for 1999-2003 for all leukemia and Texas-specific survival rates for 2003 when ADAFs are incorporated based on the  $\beta$  and 95% UCL values in Table 16 (age only) and Table 19 (age & number of HITS).

<b>Table 19. Age &amp; Number of HITS &gt; 100 ppm<sup>a</sup></b>				
<b>Covariates</b>	<b>Model</b>	<b>Source</b>	<b><math>\beta</math> (MLE) <math>\pm</math> SE</b>	<b><math>\beta</math> (95% UCL)<sup>b</sup></b>
<b>Age &amp; Number of HITS &gt; 100 ppm</b>	Cox log-linear  ppm-years continuous <sup>c</sup> # of HITS continuous <sup>e</sup>	Cheng <i>et al.</i> (2007)	2.5E-04 $\pm$ 1.2E-04 <sup>g</sup>	4.474E-04
	Cox log-linear  ppm-years mean-scored deciles <sup>h</sup> # of HITS categorical <sup>f</sup>	Sielken <i>et al.</i> (Appendix 6)	2.8E-04 $\pm$ 2.4E-04	6.748E-04
	Cox regression (restricted to lower 95% of exposure range)  ppm-years continuous <sup>c</sup> # of HITS continuous <sup>e</sup>	Cheng and Delzell <sup>d</sup>	1.34E-03 $\pm$ 4.6E-04	2.097E-03
	Poisson linear  ppm-years mean-scored deciles <sup>h</sup> # of HITS categorical <sup>f</sup>	Sielken <i>et al.</i> (2007)	1.89E-04 $\pm$ 3.6E-04	7.812E-04

<sup>a</sup> units are in ppm-years and based on occupational exposure concentrations

<sup>b</sup>  $\beta$  (95% UCL) =  $\beta$ (MLE) + (1.645 x SE)

<sup>c</sup> ppm-years is included as a continuous variable (untransformed) in a parametric model of the effect of ppm-years

<sup>d</sup> Personal communication, 1/30/2008 email from Dr. Cheng and Dr. Delzell. Cheng *et al.* (2007) reported results for Cox log-linear (restricted to lower 95% of exposure range) ppm-years continuous for age & other covariates, but not age only or age + # HITS. Dr. Cheng and Dr. Delzell provided the  $\beta$  and SE values for Cox log-linear continuous (restricted to lower 95% of exposure range) ppm-years for age and age + # HITS in the 1/30/2008 email.

<sup>e</sup> number of HITS > 100 ppm is included as a continuous variable (untransformed) in a parametric model of the effect of the number of HITS > 100 ppm

<sup>f</sup> number of HITS > 100 ppm is included as a categorical variable (based on quintiles) in a nonparametric model of the effect of the number of HITS > 100 ppm

<sup>g</sup> back calculated from the corresponding p-value in Cheng *et al.* (2007)

<sup>h</sup> ppm-years is included as a continuous variable with values grouped into mean-scored deciles (untransformed) in a parametric model of the effect of ppm-years

<b>Table 20. Age &amp; Number of HITS &gt; 100 ppm; URFs and Air Concentrations Corresponding to 1 in 100,000 Extra Leukemia Risk <sup>a</sup></b>				
<b>Model</b>	<b>EC<sub>001</sub></b>		<b>LEC<sub>001</sub></b>	
	<b>URF (MLE) <sup>b</sup></b>		<b>URF (95% UCL) <sup>c</sup></b>	
	<b>10<sup>-5</sup>-risk air concentration using URF</b>		<b>10<sup>-5</sup>-risk air concentration using URF</b>	
<b>type of data</b>				
Cox log-linear Cheng <i>et al.</i> (2007)	age	1.433E-04/ppm 69.79 ppb	age	2.246E-04/ppm 44.53 ppb
ppm-years continuous <sup>d</sup>	age & HITS <sup>g</sup> <b>1.2-fold higher</b>	1.235E-04/ppm 80.95 ppb	age & HITS <sup>g</sup> <b>1.02-fold higher</b>	2.210E-04/ppm 45.24 ppb
Cox log-linear Cheng <i>et al.</i> (2007) and Sielken <i>et al.</i> (Appendix 6)	age	3.706E-04/ppm 26.98 ppb	age	5.494E-04/ppm 18.20 ppb
ppm-years mean-scored deciles <sup>f</sup>	age & HITS <sup>h</sup> <b>2.7-fold higher</b>	1.383E-04/ppm 72.28 ppb	age & HITS <sup>h</sup> <b>1.6-fold higher</b>	3.334E-04/ppm 29.99 ppb
Cox regression (restricted to lower 95% of exposure range) Cheng and Delzell <sup>e</sup>	age	7.807E-04/ppm 12.81 ppb	age	1.097E-03/ppm 9.112 ppb
ppm-years continuous <sup>d</sup>	age & HITS <sup>g</sup> <b>1.2-fold higher</b>	6.621E-04/ppm 15.10 ppb	age & HITS <sup>g</sup> <b>1.1-fold higher</b>	1.036E-03/ppm 9.651 ppb
Poisson linear Sielken <i>et al.</i> (2007)	age	6.976E-04/ppm 14.33 ppb	age	1.258E-03/ppm 7.946 ppb
ppm-years mean-scored deciles <sup>f</sup>	age & HITS <sup>h</sup> <b>8.9-fold higher</b>	7.846E-05/ppm 127.4ppb	age & HITS <sup>h</sup> <b>3.9-fold higher</b>	3.243E-04/ppm 30.83 ppb

<sup>a</sup> using Texas-specific mortality rates for 1999-2003 for all leukemia and Texas-specific survival rates for 2003 when ADAFs are incorporated

<sup>b</sup> URF = 0.001/EC<sub>001</sub>

<sup>c</sup> URF = 0.001/LEC<sub>001</sub>

<sup>d</sup> ppm-years is included as a continuous variable (untransformed) in a parametric model of the effect of ppm-years

<sup>e</sup> Personal communication, 1/30/2008 email from Dr. Cheng and Dr. Delzell. Cheng *et al.* (2007) reported results for Cox log-linear (restricted to lower 95% of exposure range) ppm-years continuous for age & other covariates, but not age only or age + # HITS. Dr. Cheng and Dr. Delzell provided the β and SE values for Cox log-linear continuous (restricted to lower 95% of exposure range) ppm-years for age and age + # HITS in the 1/30/2008 email.

<sup>f</sup> ppm-years is included as a continuous variable with values grouped into mean-scored deciles (untransformed) in a parametric model of the effect of ppm-years

<sup>g</sup> number of HITS > 100 ppm is included as a continuous variable (untransformed) in a parametric model of the effect of the number of HITS > 100 ppm

<sup>h</sup> number of HITS > 100 ppm is included as a categorical variable (based on quintiles) in a nonparametric model of the effect of the number of HITS > 100 ppm

Using URFs (MLE), the  $10^{-5}$ -risk air concentrations for Cox log-linear, restricted continuous data if age + number of HITS are included as covariates, is 15.10 ppb (Table 20) as compared to 12.81 ppb if age is included as a covariate, approximately 1.2-fold higher. Using URFs (95% UCL), the  $10^{-5}$ -risk air concentrations for Cox log-linear, restricted continuous data if age + number of HITS are included as covariates, is 9.651 ppb (Table 20) as compared to 9.112 ppb if age is included as a covariate approximately 1.1-fold higher. Therefore, estimated risks for the model based on the restricted data that adjusts for age only result in estimates of risks that are 6-20% higher than estimates from the same model that also adjusts for the number of BD HITS > 100 ppm. Similar results were obtained when the full data set was examined (i.e., 2% higher for the URF (95% UCL) to 20% higher for the URF (MLE)).

When categorical data were used (i.e., mean-scored deciles), the differences between the  $10^{-5}$ -risk air concentrations for age only and age + # HITS were much greater: 1.6-fold higher for the URF (95% UCL) to 2.7-fold higher for the URF (MLE) for Cox log-linear mean-scored deciles and 3.9-fold higher for the URF (95% UCL) to 8.9-fold higher for the URF (MLE) for Poisson linear mean-scored deciles. As mentioned previously, Cheng *et al.* (2007) found that BD ppm-years and # of HITS > 100 ppm, both exposure variables, were weakly correlated for continuous (ungrouped) values (Pearson correlation coefficient of 0.30) as opposed to deciles (grouped) values (Pearson correlation coefficient of 0.80).

#### **4.2.5.3 Effect of Occupational Exposure Estimation Error**

One of the limitations of most epidemiological studies is potential exposure estimation error. Health Canada (2000) and USEPA (2002) expressed concerns about the validity of exposure estimates from the Delzell (1995, 1996) study. In the updated exposure estimates, Macaluso *et al.* (2004) used a more in-depth job, task, and exposure classification for the cohort, and exposure estimates were developed using exposure modeling, historical exposure data, and plant equipment analysis. Recently, Sathiakumar *et al.* (2007) assessed the validity of the BD exposure estimates by measuring the differences and correlations between calendar year- and job-specific estimates and measurements of BD concentrations at the Canadian Sarnia plant (a latex operation), one plant included in the UAB cohort. Sathiakumar *et al.* (2007) stated in their abstract, "Exposure misclassification may have been more severe for subjects from the validation study plant than for subjects from other plants in the mortality study." BD measurements from the late 1970s onward were available. Estimated concentrations were lower than measured concentrations before 1984 by approximately two-fold, whereas after 1984, estimated concentrations were higher than measured concentrations by approximately three-fold. On average, estimates were about 10% lower than measurements.

Macaluso *et al.* (2004) characterized each of the exposures in the JEM by a distribution. The analyses in Sielken *et al.* (2007) and Cheng *et al.* (2007) used the average of this distribution to characterize job exposure in the JEM and calculations of cumulative ppm-years. Sielken and Associates (2008) (Appendix 7) conducted a sensitivity analysis in order to investigate the effects of the exposure estimation errors identified by Sathiakumar *et al.* (2007) on the  $\beta$  and SE using the full data set and log-linear Cox regression modeling. Beta and SE from the following alternative data sets were determined:

1. The first alternative data set altered the exposure estimate (JEM) values so that prior to 1984 the exposure estimate JEM values were increased approximately 2-fold (i.e., 1.98-fold), and in 1984 and later years the exposure estimate JEM values were decreased approximately 3-fold (i.e., (1/0.37)-fold).

2. The second alternative data set altered the JEM values so that the exposure estimates prior to 1977 were left unchanged, the exposure estimate JEM values for 1977 through 1983 were increased approximately 2-fold (1.98-fold), and the exposure estimates JEM values for 1984 through 1991 were decreased approximately 3-fold [(1/0.37)-fold]. This alternative is the same as the first alternative except that the exposure estimates prior to 1977 were left unchanged because these years were not specifically addressed in Sathiakumar *et al.* (2007).
3. The third alternative data set altered the JEM values so that the exposure estimates prior to 1977 were left unchanged and the exposure estimate (JEM values) for each specific year of 1977 through 1991 were multiplied by the calendar-year specific value for “measurement / estimate” as shown by Table 1 in Appendix 7. The third alternative is the same as the second alternative except the calendar-year specific findings for 1977 to 1991 in Sathiakumar *et al.* (2007) were used.
4. The fourth alternative data set altered the JEM values so that these estimates are all divided by 0.90 corresponding to estimate (JEM value) = 0.90 x measurement because Sathiakumar *et al.* (2007) noted that, “On average, estimates were about 10% lower than measurements.”

<b>Data Set</b> <b>Description of JEM Values</b>	<b><math>\beta \pm</math> Standard Deviation of Estimate of <math>\beta</math></b>	<b>95% UCL on <math>\beta</math></b>	<b>EC<sub>001</sub></b> <b>10<sup>-5</sup>-risk air concentration using URF</b>	<b>LEC<sub>001</sub></b> <b>10<sup>-5</sup>-risk air concentration using URF</b>
<b>Original</b> Average in Macaluso Distribution	2.911E-04 $\pm$ 1.03E-04	4.60E-04	72.65 ppb	45.94 ppb
<b>1<sup>st</sup> Alternate</b> Sathiakumar Average Calendar-Year Correction before 1984 and Average Calendar-Year Correction after 1983	1.469E-04 $\pm$ 5.21E-05	2.33E-04	143.97 ppb	90.93 ppb
<b>2<sup>nd</sup> Alternate</b> Sathiakumar Average Calendar-Year Correction for 1977 through 1983 and Average Calendar-Year Correction for 1984 through 1991	2.478E-04 $\pm$ 8.66E-05	3.90E-04	85.35 ppb	54.19 ppb
<b>3<sup>rd</sup> Alternate</b> Sathiakumar Calendar-Year Specific Correction for 1977 through 1991	2.468E-04 $\pm$ 8.62E-05	3.89E-04	85.70 ppb	54.43 ppb
<b>4<sup>th</sup> Alternate</b> Sathiakumar Overall 10% Correction	2.620E-04 $\pm$ 9.26E-05	4.14E-04	80.72 ppb	51.05 ppb

Table 21 shows a summary of results from Appendix 7 for the above mentioned alternate data sets. The 10<sup>-5</sup>-risk air concentration using the URF (LEC<sub>001</sub>) was 45.94 ppb based on the original JEM values and

increased for all alternative data sets. The increased risk-based values ranged from 90.93 ppb for the 1<sup>st</sup> alternative data set to 51.05 ppb for the 4<sup>th</sup> alternative data set. The 10<sup>-5</sup>-risk air concentration using the URF (EC<sub>001</sub>) was 72.65 ppb based on the original JEM values and increased for all alternative data set. The increases ranged from 143.97 ppb for the 1<sup>st</sup> alternative data set to 80.72 ppb for the 4<sup>th</sup> alternative data set.

There was a pattern reversal in exposure estimates before and after 1984. However, exposures before 1984 were higher (in absolute value) and contributed more to the estimation of the slopes in the dose-response models. Increasing the exposure estimates before 1984 tended to decrease the estimated slopes and increase the estimated concentrations (ppb) corresponding to specified risk levels. This indicates that the  $\beta$  and SE calculated by Cheng *et al.* (2007) and Sielken *et al.* (2007) were conservative and did not underestimate potency estimates based on concerns about exposure estimation error.

Sielken and Associates (Appendix 7) also considered two additional alternative data sets. The 5<sup>th</sup> and 6<sup>th</sup> alternative data sets replaced the average exposure estimated JEM values by the 5<sup>th</sup> or the 95<sup>th</sup> percentiles of these distributions, respectively. Then the modeling was done as before except the cumulative ppm-years were calculated using these 5<sup>th</sup> or 95<sup>th</sup> percentile JEM values instead of the average JEM values. The 10<sup>-5</sup>-risk air concentration using the URF (LEC<sub>001</sub>) was 45.94 ppb based on the original average exposure estimate JEM values and ranged from 20.41 ppb for the 5<sup>th</sup> percentile to 86.69 ppb for the 95<sup>th</sup> percentile (Appendix 7), only a four-fold difference. The validation study of Sathiakumar *et al.* (2007) on the updated exposure estimates of Macaluso *et al.* (2004) and the sensitivity study conducted by Sielken and Associates (2008) (Appendix 7) demonstrate the potency estimates derived by the TCEQ based on modeling by Cheng *et al.* (2007) and Sielken *et al.* (2007) have a higher confidence than potency estimates determined by USEPA (2002) using the old 1995 exposure estimates, fewer leukemia deaths, and fewer years of follow-up.

#### **4.2.5.4 Dose-Response Modeling**

Modeling results from several different models were presented and both  $\beta$  and upper 95% UCL estimates were reported in order to provide information on the residual uncertainty in the relative risk estimates based on different dose-response modeling:

- For the preferred model (Section 4.2.4), there was approximately a 1.4-fold difference between the 10<sup>-5</sup>-risk air concentrations of 13.39 ppb calculated with URFs (MLE) versus 9.523 ppb for the URFs (95% UCL) (Table 17).
- The cancer potency estimates and 10<sup>-5</sup>-risk air concentrations from the log-linear Cox regression model and the linear Poisson regression model using URFs (MLE) in Table 17 range from 7.471E-04 per ppm (13.39 ppb) to 1.371E-04 per ppm (72.93 ppb), a 5.4 fold difference.
- The cancer potency estimates and 10<sup>-5</sup>-risk air concentrations using URFs (95% UCL) in Table 17 range from 1.193E-03 per ppm (8.381 ppb) to 2.149E-04 per ppm (46.53 ppb), a 5.5 fold difference. The preferred potency estimate based on the URF (95% UCL) of 1.050E-03 per ppm (9.523 ppb) (Table 17) is at the lower, conservative end of the range.

Linear Poisson regression and log-linear Cox regression models are commonly used to investigate dose-response relationships derived from occupational cohort epidemiologic studies based on mortality and are generally considered to be biologically-plausible models for cancer. As discussed previously, models using untransformed continuous (ungrouped) data are preferred over models using grouped data, so the potency estimates from the models using mean-scored deciles were not preferred. The Cox regression

analysis using data restricted to the lower 95% of the exposure range was used because it is more conservative and to address concerns about sparse data and an erratic exposure-response relationship at high exposure concentrations. The results from the Cox regression model using restricted data are the most conservative among the Cox regression models analyzed here and slightly less conservative than the results from the Poisson regression using mean-scored deciles when only age is included as a covariate.

Cheng *et al.* (2007) also examined the BD ppm-years exposure-response relationships using natural logarithm (ln)-transformed and square-root transformed continuous BD ppm-years. These models each have advantages as discussed by Cheng *et al.* (2007). The models using ln-transformed and square-root transformed continuous BD ppm-years are not standard models and since the mechanism of action of BD is not sufficiently understood to justify the use of these models, the TS preferred the log-linear Cox regression model. In addition, the ln-transformed model may provide an unrealistically high slope in the low dose region and is, therefore, not preferred. One of the advantages of the ln-transformed and square-root transformed data is they may reduce the influence of data at extreme exposure values. The log-linear Cox regression models using restricted data are more conservative and may address concerns for the influence of data at extreme exposure values at the high exposure range (Section 4.2.5.3).

#### ***4.2.5.5 Use of Mortality Rates to Predict Incidence***

The potency estimate for BD was calculated from mortality data because incidence data were not available. When using the BEIR IV methodology to calculate URFs and corresponding  $10^{-5}$ -risk air concentrations based on mortality potency estimates, total leukemia mortality rates were used. Total leukemia incidence is higher than leukemia mortality because the survival rate for leukemia has improved through the years. In 1996-2003, the overall relative survival rate was nearly 50 percent (Leukemia & Lymphoma Society 2008). USEPA (2002) used leukemia incidence rates instead of mortality rates to calculate air concentrations based on a life-table analyses using the BEIR IV approach (NRC 1988) in an attempt to account for the uncertainty that potency estimates were based on mortality and not incidence.

The BEIR IV methodology for calculating excess risk is mathematically correct when the specified response is mortality and mortality rates are used but not when the specified response is mortality and incidence rates are used as was done by USEPA (2002). This error is demonstrated in Appendix 8 *Issues in Quantitative Epidemiology: Calculating Excess Risk When Specified Response is Mortality versus Incidence*. Appendix 8 shows that if the specified response is incidence, then the BEIR IV methodology for mortality cannot be used correctly. Teta *et al.* (2004) investigated the validity and implications of using a mortality-based leukemia relative rate model with background leukemia incidence rates, rather than mortality rates. They concluded that a biased estimate of excess lifetime risk will result, and the direction of the bias will vary by potency and the type of leukemia being modeled. Therefore, the TS did not use leukemia incidence rates to account for the uncertainty of calculating potency estimates for BD from mortality data. If the specified response is incidence and incidence rates are used, the BEIR IV methodology can be altered to account for incidence as demonstrated in Appendix 8.

Table 22 contains URFs and  $10^{-5}$ -risk air concentrations calculated using restricted data,  $\beta$  (95% UCL) of 2.221E-03, Texas-specific mortality or incidence rates for 1999-2003 for all leukemia and Texas-specific survival rates for 2003 (Appendix 4), and ADAFs. If leukemia mortality rates are used in the Beir IV model for mortality, the  $10^{-5}$ -risk air concentration is 9.112 ppb compared to 5.011 ppb using incidence rates, approximately 1.8 fold higher. Similar results are obtained when the Beir IV model for incidence is used (adjusted to correctly account for incidence dose-response based on equations in Appendix 8) (Table

22). The uncertainty in using mortality potency factors and mortality rates to predict incidence rates (i.e., to protect against developing leukemia) is approximately 1.8 fold, although the amount and direction of the bias may vary (Teta *et al.* 2004). The TS will not use leukemia incidence rates to calculate air concentrations using mortality potency factors and the BEIR IV approach (NRC 1988) because it is mathematically incorrect. Given the inherent conservatism when calculating potency estimates, a less-biased estimate of risk based on mortality is better than a more-biased estimate based on incidence. The URF is considered to be sufficiently health-protective because the following conservative default procedures were followed in the calculation of the preferred URF of 1.1E-03 per ppm:

- A linear default was used to extrapolate to lower concentrations instead of using the log-linear Cox regression model to calculate the 10<sup>-5</sup>-risk air concentrations, approximately 1.2 fold more conservative (Table 17);
- The URF (95% UCL) was used instead of the URF (MLE), approximately 1.4 fold more conservative (Table 17). As mentioned previously, the confidence intervals are indicators of the variability, and to some extent the uncertainty, in the dose-response curve for mortality. The risk of incidence will be lowered since using the URF (95% UCL) adds conservatism to the estimate;
- Data restricted to the lower 95% of the exposure range was used, ranging from 4-5 fold more conservative when compared to unrestricted data (Section 4.2.5.3);
- Model did not adjust for the number of HITs > 100 ppm that occur in occupational exposure but not in environmental exposures (Section 4.2.5.2). The URF would be 1.1 fold less conservative if number of HITs > 100 were adjusted for; and
- Model was based on the average BD concentration estimated by Macaluso *et al.* (2004) and did not incorporate the correction to the exposure estimates suggested by Sathiakumar *et al.* (2007) (Section 4.2.5.3 and Appendix 7).

Therefore, the total conservatism is much greater than the possible bias of 1.8-fold.

	<b>Dose-Response Model (mortality or incidence rates)</b>	<b>URF</b>	<b>10<sup>-5</sup>-Risk Air Concentration</b>	<b>Effect on 10<sup>-5</sup>-Risk Air Concentration</b>
BEIR IV methodology for mortality	Mortality potency factors (mortality rates)	1.097E-03/ppm	9.112 ppb	1.8 fold higher 10 <sup>-5</sup> risk air concentration when using mortality rates, and not incidence rates
	Mortality potency factors (incidence rates) <sup>c</sup>	1.996E-03/ppm	5.011 ppb	
BEIR IV methodology for incidence <sup>b</sup>	Mortality potency factors (mortality rates) <sup>c</sup>	1.096E-03/ppm	9.126 ppb	1.8 fold higher 10 <sup>-5</sup> risk air concentration when using mortality rates, and not incidence rates
	Mortality potency factors (incidence rates) <sup>c</sup>	1.989E-03/ppm	5.028 ppb	

<sup>a</sup> Calculations were performed using restricted data, 95% UCL on  $\beta$  of 2.221E-03, Texas-specific mortality or incidence rates for 1999-2003 for all leukemia and Texas-specific survival rates for 2003 (Appendix 4), and ADAFs

<sup>b</sup> The BEIR IV methodology was altered to account for incidence if the specified response is incidence and incidence rates are used as demonstrated in Appendix 8

<sup>c</sup> Incorrect use of The BEIR IV methodology (Appendix 8)

#### 4.2.6 Comparison of TCEQ's URF to USEPA's URF

USEPA published an inhalation URF of 0.08 per ppm in 2002. The URF is based on a Health Canada analysis of data from Delzell *et al.* (1995, 1996) using a linear relative rate model and was calculated for up to 85 years. Relative risks were evaluated with leukemia incidence rates, which is not mathematically correct as demonstrated in Appendix 8. Using the  $LEC_{01}$  (i.e., the 95% lower confidence limit of the exposure concentration associated with a 1% increased risk) of 0.254 ppm as the POD and a linear extrapolation to zero yielded a URF of 0.04 per ppm. An adjustment factor of 2 was applied to the URF to yield a final URF of 0.08 per ppm. This adjustment was applied to reflect evidence from studies in mice which suggest that extrapolating leukemia risks from a male-only occupational cohort may underestimate the cancer risks for the general public. The TCEQ derived an inhalation URF of 0.0011 per ppm based on the most current exposure estimates and updated epidemiological study conducted by the UAB group (Macaluso *et al.* 2004; Sathiakumar *et al.* 2005; Graff *et al.* 2005; HEI 2006). As mentioned previously, based on the validation study of Sathiakumar *et al.* (2007), the updated exposure estimates of Macaluso *et al.* (2004) have a higher confidence than original exposure estimates used by USEPA. Relative risks were evaluated with Texas-specific leukemia mortality rates and survival rates and were calculated for up to 70 years, the default used by the TCEQ in an exposure analysis. The URF is based on the 95% UCL estimate derived with a log-linear Cox regression model, age implicitly included as covariate, and data restricted to the lower 95% of the exposure range (a more conservative value was chosen as a policy decision to address concerns about possible exposure misclassification at the high end of the exposure range) (Cheng *et al.* 2007). Using the  $LEC_{001}$  (i.e., the 95% lower confidence limit of the exposure concentration associated with a 0.1% increased risk) as the POD, a linear extrapolation to zero, and adjusting for the increased susceptibility of children using a life-table approach and applying ADAFs (Appendix 5) yields a URF of 0.0011 per ppm. USEPA's URF is approximately 70 times higher (i.e., more conservative) due to the following reasons:

- The updated and validated exposure estimates of the UAB group were approximately five times higher than the original estimates. The updated median ppm-years for all employees was 71 ppm-years versus 15 ppm-years for original estimates (Table VII, Macaluso *et al.* 2004) which makes USEPA's URF approximately five times higher;
- The TCEQ used a default exposure duration of 70 years (TCEQ 2006) whereas USEPA used an exposure duration of 85 years, which makes USEPA's URF approximately three times higher. The TCEQ will use the 70-year default to be consistent between evaluations for different chemicals (i.e., the risk from different chemicals will be more comparable if the dose-response was evaluated using a consistent 70-year exposure analysis). The use of 85 years instead of 70 years has been criticized for a variety of reasons. The dose-response modeling was not done based on person-years corresponding to older ages. The dose-response model based on early ages and older ages may be very different. Furthermore, the relevance of the dose metric (cumulative BD ppm-years) may differ for older ages;
- The TCEQ used an  $LEC_{001}$  to calculate the URF because it was within the observable range of the data whereas USEPA used an  $LEC_{01}$ , which is above the observable range of the data. This makes USEPA's URF approximately two times higher;
- The TCEQ used total leukemia mortality rates to calculate the URF whereas USEPA used total leukemia incidence rates, which makes USEPA's URF approximately 1.8 times higher; and
- The TCEQ did not apply an adjustment factor of two to the URF to reflect evidence from studies in mice which suggest that extrapolating leukemia risks from a male-only occupational cohort may underestimate the cancer risks for the general public because data on females workers

exposed to BD did not indicate they were more sensitive. This adjustment makes USEPA's URF two times higher.

Consideration of the above differences accounts for approximately a 110-fold difference ( $5 \times 3 \times 2 \times 1.8 \times 2$ ), more than the 70-fold difference when comparing URFs. This indicates that the TCEQ assessment was more conservative than USEPA's assessment in some regards. Minor differences between the TCEQ values and USEPA's values may relate to the use of the following:

- USEPA used potency estimates from Health Canada \*, which appears to be a linear Poisson model with categorical variables, and the full range of the exposure data whereas TCEQ used the log-linear Cox regression model using continuous, untransformed data restricted to the lower 95% of the exposure range. This may account for the TCEQ's URF being only 70-fold lower rather than 110-fold lower.
- TCEQ used a longer follow-up in the current UAB study and TCEQ used 5 days/7 days as opposed to 240 days/364 days to convert from an occupational exposure to the general population.

While an exact partitioning of the 110-fold difference may not be possible, there are science-based and logical explanations accounting for most of the differences.

### ***4.3. Welfare-Based Chronic ESL***

No data were found regarding long-term vegetative effects.

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\* In the Health Canada analyses that USEPA relied upon, the effects of age, years since hire, calendar year, race, and styrene exposure (ppm-years) were incorporated into the Poisson regression modeling using a flawed non-standard methodology which implicitly ignores most of the available data. The statistical procedure used by Health Canada stratified the data into a large number (29,403) of very fine strata. 29,352 strata (99.8% of the 29,403 strata) contain zero leukemia responses at all dose levels. These 29,352 strata contain 97.83% of the person years (i.e., most of the data in the study). The strata with zero leukemias at every dose had zero slope. The slope estimation procedure used by Health Canada did not include the strata with zero leukemias at every dose and hence did not include the corresponding zero slopes. The result is that the slope estimation procedure used by Health Canada biased the slope estimate for 1,3-butadiene toward higher values (i.e., overestimated the slope).

A general discussion of the problem of overly stratifying the covariates is given by N. E. Breslow and N. E. Day in Chapter 6 in *Statistical Methods in Cancer Research, Volume I, The Analysis of Case-Control Studies*, IARC, Lyon, France, 1980. Numerical examples are given by N. E. Breslow and N. E. Day in Chapters 4 and 5 in *Statistical Methods in Cancer Research, Volume II, The Design and Analysis of Cohort Studies*, IARC, Lyon, France, 1987.

#### 4.4 Long-Term ESL and Values for Air Monitoring Evaluation

The chronic evaluation resulted in the derivation of the following values:

- $\text{chronicESL}_{\text{nonlinear(nc)}}$  = 9.9  $\mu\text{g}/\text{m}^3$  (4.5 ppb)
- Chronic ReV = 33  $\mu\text{g}/\text{m}^3$  (15 ppb)
- $\text{chronicESL}_{\text{linear(c)}}$  = 20  $\mu\text{g}/\text{m}^3$  (9.1 ppb)
- URF = 5.0E-04 per  $\text{mg}/\text{m}^3$  (1.1E-03 per ppm)  
= 5.0E-07 per  $\mu\text{g}/\text{m}^3$  (1.1E-06 per ppb).

The long-term ESL for air permit reviews is the  $\text{chronicESL}_{\text{nonlinear(nc)}}$  of 9.9  $\mu\text{g}/\text{m}^3$  (4.5 ppb) because it is lower than the  $\text{chronicESL}_{\text{linear(c)}}$  of 20  $\mu\text{g}/\text{m}^3$  (9.1 ppb) (Table 1). For evaluation of long-term ambient air monitoring data, the  $\text{chronicESL}_{\text{linear(c)}}$  of 20  $\mu\text{g}/\text{m}^3$  (9.1 ppb) is lower than the chronic ReV of 33  $\mu\text{g}/\text{m}^3$  (15 ppb), although both values may be used for the evaluation of air data as well as the URF of 5.0E-04 per  $\text{mg}/\text{m}^3$  (1.1E-03 per ppm) or 5.0E-07 per  $\mu\text{g}/\text{m}^3$  (1.1E-06 per ppb). The  $\text{chronicESL}_{\text{nonlinear(nc)}}$  (HQ = 0.3) is not used to evaluate ambient air monitoring data.

#### 4.5 Other Relevant Information

The proceedings of the International Symposium on Evaluation of Butadiene and Chloroprene Health Risks, held in Charleston, South Carolina on September 20-22, 2005 have recently been published, and the findings and results from many of these articles have been cited in the Development Support Document (DSD). Refer to Himmelstein *et al.* (2007), which provides an excellent summary of the main findings of the symposium. A summary of the molecular epidemiology findings from Albertini *et al.* (2007) as summarized by Himmelstein *et al.* (2007) is reproduced here because of the significance of their findings. The references, which are in numerical format in the journal, have been supplemented with the author(s) names and year of publication.

##### “1.1.3. Molecular epidemiology

Albertini [9 (*Albertini et al. 2001*)] reported that the initial study of workers in the Czech Republic demonstrated a clear no-observed-adverse-effect level (NOAEL) for biomarkers of effect (hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutations and chromosome aberrations) at mean BD exposure concentrations of 0.800 ppm.

This NOAEL reflects the maximum average exposure level experienced by these workers and was based on extensive external exposure assessments and a comprehensive series of biomarker responses, which included urine metabolites (M1 and M2) and hemoglobin adducts of epoxybutene and EBD (N-[2-dihydroxy-3-butenyl]valine = HB-Val and N-[2,3,4-trihydroxybutyl]valine = THB-Val, respectively), HPRT mutations, sister-chromatid-exchange frequencies and chromosomal aberrations determined by traditional methods and chromosome painting (fluorescence in situ hybridization). Both the urine metabolite and hemoglobin adduct concentrations proved to be excellent biomarkers of exposure. A second study of Czech workers was conducted at this same facility to compare biomarker responses in female and male employees [10 (*Albertini et al. 2007*)]. Mean BD exposure concentrations were lower in this second study than in the first, being 0.180 ppm and 0.370 ppm for females and males,

respectively. Again, there were no BD-associated elevations of HPRT mutation or chromosome aberration frequencies above background in either sex. Similarly, there was no difference between genders in the pattern of BD detoxification, as evidenced by urinary M1 and M2 levels. Females, however, appeared to absorb less BD per unit of exposure, as reflected by urine metabolite concentrations. Concentrations of the N,N-(2,3-dihydroxy-1,4-butadiyl)valine (pyr-Val) hemoglobin adduct, which is specific for the highly genotoxic 1,2:3,4-diepoxybutane (DEB) metabolite of BD, were measured in this second study and found to be below the level of quantification for all workers. Later presentations by Swenberg [11 (*Swenberg et al. 2007*)] and Boysen [12 (*Boysen et al. 2007*)] in this Symposium described extensive studies of pyr-Val concentrations in BD exposed rodents that, coupled with the results of this Czech worker study, indicate that DEB production in humans is below levels produced in mice or rats exposed to as little as 1.0 ppm BD by inhalation.”

## Chapter 5. References

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## Appendix 1. Statistical Analyses of Developmental Endpoints

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The analyses performed by Hackett *et al.* (1987) did have some important statistical flaws that needed to be corrected. The statistical analyses reported by Green (2003) are valid and correct the flaws of Hackett *et al.* analyses. We have focused on the analyses of fetal body weights. The NOAEL based on the fetal body weights for this study is 40 ppm.

Hackett *et al.* (1987) conducted analyses of variance (ANOVA) on the average pup weight followed up by Student's t-tests comparing the average pup weight for different treatment groups. Their pairwise comparisons using Student's t-test do not adjust significance levels that occur for the number of multiple tests. In addition, their analyses did not adjust for well-known important covariate effects such as litter size. Hackett *et al.* analyses were based on dam's average pup weights instead of analyzing the individual pup weights and treating the dam as a random effect, which would result in a more powerful statistical test.

The Green (2003) reanalysis was based on analysis of covariance (ANCOVA) on the average pup weight and adjusting for covariates. In this context, Green used the Dunnett-Hsu test to compare the mean weights for each of the exposed groups to the mean weight for the control group after both are adjusted for the effects of the covariates. This is the specific situation for which the Dunnett-Hsu test was designed. Furthermore, the Dunnett-Hsu test is the appropriate test to use here to determine a NOAEL. Green considered the p-values in the Dunnett-Hsu test to draw his conclusions of significant effects. Green's discussion in A. Evaluation of Earlier Methods and B. Method of Re-Analysis is appropriate.

Green's analyses were based on dam's average pup weights instead of analyzing the individual pup weights and treating the dam as a random effect, which would result in a more powerful statistical test. The statistical conclusions reached by Green (2003) hold even when the more powerful statistical analyses where the individual pup weights are analyzed and the dams are treated as random effects.

Thus, the Green (2003) conclusions are reasonable and based on standard statistical analyses practices that were overlooked by Hackett *et al.* (2003). The NOAEL based on the fetal body weights for this study is 40 ppm.

### **Statistical Analyses Performed by Sielken & Associates**

In addition to reviewing the methodology used in Hackett *et al.* (1987) and Green (2003), Sielken & Associates re-analyzed the fetal body weight data. This was to confirm the numerical results obtained by Green, do a sensitivity analysis with respect to the effects of covariates, and determine the outcome of the more powerful statistical analyses where the individual pup weights are analyzed and the dams are treated as random effects. These analyses support the finding that the NOAEL based on the fetal body weights for this study is 40 ppm.

Table 1 contains an overview of the results in Tables 2 to 10 which contain the detailed analyses. The raw data used are given in Table 12. The statistical analyses were done in SAS Ver. 9. In the overview in Table 1, all comparisons to control were based on Dunnett-Hsu tests and were one-sided tests for a decrease in fetal body weight compared to control. The outcomes of the more powerful statistical analyses where the individual pup weights are analyzed and the dams are treated as random effects were comparable to the outcomes obtained with the Green ANCOVA model. The results for 1 Covariate (Litter Size) are highlighted since this covariate was always statistically significant at the 5% significance level – the 2nd Covariate (% Males in Litter) was significant for the Males Only analyses.

Table 1. Overview of Statistical Analyses of Fetal body weight Data: The results for 1 Covariate (Litter Size) are highlighted since this covariate was always statistically significant at the 5% significance level – the 2nd Covariate (% Males in Litter) was significant for the Males Only analyses

Table #	Model: Mixed Model: (1) Based on Mean Data (2) Based on Individual Data and Dam as Random Effect	Sex	# of Covariates	Covariates (1) Litter Size (2) % Males in Litter	p-value in Dunnett-Hsu one-sided comparison to control		
					dose=40	200	1,000
2	(1)	M&F	2	(1) & (2)	0.1354	<0.0001	<0.0001
2	(2)	M&F	2	(1) & (2)	0.1383	<0.0001	<0.0001
3	(1)	M&F	1	(1)	0.1120	<0.0001	<0.0001
3	(2)	M&F	1	(1)	0.1184	<0.0001	<0.0001
4	(1)	M&F	0	None	0.0832	<0.0001	<0.0001
4	(2)	M&F	0	None	0.0849	<0.0001	<0.0001
5	(1)	F	2	(1) & (2)	0.2091	<0.0001	<0.0001
5	(2)	F	2	(1) & (2)	0.2373	<0.0001	<0.0001
6	(1)	F	1	(1)	0.1919	<0.0001	<0.0001
6	(2)	F	1	(1)	0.2298	<0.0001	<0.0001
7	(1)	F	0	None	0.1427	<0.0001	<0.0001
7	(2)	F	0	None	0.1854	<0.0001	<0.0001
8	(1)	M	2	(1) & (2)	0.0687	<0.0001	<0.0001
8	(2)	M	2	(1) & (2)	0.0795	<0.0001	<0.0001
9	(1)	M	1	(1)	0.0603	<0.0001	<0.0001
9	(2)	M	1	(1)	0.0695	<0.0001	<0.0001
10	(1)	M	0	None	0.0408	<0.0001	<0.0001
10	(2)	M	0	None	0.0479	<0.0001	<0.0001

**Table 2.**  
Males & Females Combined  
Litter Size & %Males as Covariates  
Mixed Model Based on Mean Data

Data Set		WORK.MEANDATA								
Type 3 Tests of Fixed Effects										
	Effect		Num	Den	F Value	Pr > F				
	dose		DF	DF						
	dose		3	72	50.29	<.0001				
	PercMales		1	72	3.54	0.0640				
	LitterSize		1	72	19.10	<.0001				
Least Squares Means										
	Effect	dose	Estimate	Standard Error	DF	t Value	Pr >  t			
	dose	0	1.3348	0.02034	72	65.63	<.0001			
	dose	40	1.2898	0.01984	72	65.02	<.0001			
	dose	200	1.1243	0.01879	72	59.83	<.0001			
	dose	1000	1.0378	0.01926	72	53.88	<.0001			
	Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr >  t	Adjustment	Adj P
	dose	40	0	-0.04497	0.02849	72	-1.58	0.1188	Dunnett-Hsu	0.2701
	dose	200	0	-0.2104	0.02767	72	-7.60	<.0001	Dunnett-Hsu	<.0001
	dose	1000	0	-0.2969	0.02801	72	-10.60	<.0001	Dunnett-Hsu	<.0001
	Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P
	dose	0	40	0.04497	0.02849	72	1.58	0.0594	Dunnett-Hsu	0.1354
	dose	0	200	0.2104	0.02767	72	7.60	<.0001	Dunnett-Hsu	<.0001
	dose	0	1000	0.2969	0.02801	72	10.60	<.0001	Dunnett-Hsu	<.0001

Mixed Model Based on Individual Data and Dam as Random Effect

Data Set		WORK.ANDATA								
Type 3 Tests of Fixed Effects										
	Effect		Num	Den	F Value	Pr > F				
	dose		DF	DF						
	dose		3	69.3	52.75	<.0001				
	PercMales		1	72.9	4.17	0.0448				
	LitterSize		1	83.5	14.69	0.0002				
Least Squares Means										
	Effect	dose	Estimate	Standard Error	DF	t Value	Pr >  t			
	dose	0	1.3265	0.02009	69.5	66.04	<.0001			
	dose	40	1.2829	0.01930	68.6	66.47	<.0001			
	dose	200	1.1145	0.01847	69	60.34	<.0001			
	dose	1000	1.0306	0.01886	68.9	54.64	<.0001			
	Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr >  t	Adjustment	Adj P
	dose	40	0	-0.04357	0.02782	69.1	-1.57	0.1218	Dunnett-Hsu	0.2759
	dose	200	0	-0.2120	0.02714	70	-7.81	<.0001	Dunnett-Hsu	<.0001
	dose	1000	0	-0.2959	0.02739	69.5	-10.81	<.0001	Dunnett-Hsu	<.0001
	Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P
	dose	0	40	0.04357	0.02782	69.1	1.57	0.0609	Dunnett-Hsu	0.1383
	dose	0	200	0.2120	0.02714	70	7.81	<.0001	Dunnett-Hsu	<.0001
	dose	0	1000	0.2959	0.02739	69.5	10.81	<.0001	Dunnett-Hsu	<.0001

**Table 3.**

Males & Females Combined  
LitterSize as Covariate (%Males not included as a covariate)  
Mixed Model Based on Mean Data

Data Set		WORK.MEANDATA							
Type 3 Tests of Fixed Effects									
Effect		Num	Den	F Value	Pr > F				
	DF	DF							
dose	3	73	48.14	<.0001					
LitterSize	1	73	16.36	0.0001					
Least Squares Means									
Standard									
Effect	dose	Estimate	Error	DF	t Value	Pr >  t			
dose	0	1.3358	0.02068	73	64.60	<.0001			
dose	40	1.2871	0.02013	73	63.95	<.0001			
dose	200	1.1249	0.01911	73	58.85	<.0001			
dose	1000	1.0388	0.01959	73	53.03	<.0001			
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr >  t	Adjustment	Adj P
dose	40	0	-0.04870	0.02891	73	-1.68	0.0963	Dunnett-Hsu	0.2237
dose	200	0	-0.2109	0.02815	73	-7.49	<.0001	Dunnett-Hsu	<.0001
dose	1000	0	-0.2971	0.02849	73	-10.43	<.0001	Dunnett-Hsu	<.0001
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr > t	Adjustment	Adj P
dose	0	40	0.04870	0.02891	73	1.68	0.0482	Dunnett-Hsu	0.1120
dose	0	200	0.2109	0.02815	73	7.49	<.0001	Dunnett-Hsu	<.0001
dose	0	1000	0.2971	0.02849	73	10.43	<.0001	Dunnett-Hsu	<.0001

Mixed Model Based on Individual Data and Dam as Random Effect

Data Set		WORK.ANDATA							
Type 3 Tests of Fixed Effects									
Effect		Num	Den	F Value	Pr > F				
	DF	DF							
dose	3	71	49.56	<.0001					
LitterSize	1	86.7	12.60	0.0006					
Least Squares Means									
Standard									
Effect	dose	Estimate	Error	DF	t Value	Pr >  t			
dose	0	1.3274	0.02058	71.3	64.50	<.0001			
dose	40	1.2803	0.01974	70.4	64.86	<.0001			
dose	200	1.1162	0.01892	70.7	59.01	<.0001			
dose	1000	1.0318	0.01932	70.7	53.40	<.0001			
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr >  t	Adjustment	Adj P
dose	40	0	-0.04706	0.02846	71	-1.65	0.1026	Dunnett-Hsu	0.2365
dose	200	0	-0.2112	0.02781	71.7	-7.60	<.0001	Dunnett-Hsu	<.0001
dose	1000	0	-0.2956	0.02807	71.2	-10.53	<.0001	Dunnett-Hsu	<.0001
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr > t	Adjustment	Adj P
dose	0	40	0.04706	0.02846	71	1.65	0.0513	Dunnett-Hsu	0.1184
dose	0	200	0.2112	0.02781	71.7	7.60	<.0001	Dunnett-Hsu	<.0001
dose	0	1000	0.2956	0.02807	71.2	10.53	<.0001	Dunnett-Hsu	<.0001

**Table 4.**

Males & Females Combined  
No Covariates  
Model Based on Mean Data

Data Set		WORK.MEANDATA								
Type 3 Tests of Fixed Effects										
Effect		Num	Den	F Value	Pr > F					
dose		DF	DF							
		3	74	40.30	<.0001					
Least Squares Means										
Effect		dose	Estimate	Standard Error	DF	t Value	Pr >  t			
dose		0	1.3407	0.02269	74	59.10	<.0001			
dose		40	1.2824	0.02208	74	58.08	<.0001			
dose		200	1.1259	0.02100	74	53.60	<.0001			
dose		1000	1.0379	0.02152	74	48.22	<.0001			
Standard										
Effect	dose	dose	Estimate	Error	DF	t Value	Pr >  t	Adjustment	Adj P	
dose		40	0	-0.05832	0.03166	74	-1.84	0.0695	Dunnett	0.1664
dose		200	0	-0.2148	0.03092	74	-6.95	<.0001	Dunnett	<.0001
dose		1000	0	-0.3028	0.03127	74	-9.68	<.0001	Dunnett	<.0001
Standard										
Effect	dose	dose	Estimate	Error	DF	t Value	Pr > t	Adjustment	Adj P	
dose		0	40	0.05832	0.03166	74	1.84	0.0347	Dunnett	0.0832
dose		0	200	0.2148	0.03092	74	6.95	<.0001	Dunnett	<.0001
dose		0	1000	0.3028	0.03127	74	9.68	<.0001	Dunnett	<.0001

Mixed Model Based on Individual Data and Dam as Random Effect

Data Set		WORK.ANDATA								
Type 3 Tests of Fixed Effects										
Effect		Num	Den	F Value	Pr > F					
dose		DF	DF							
		3	68.5	44.45	<.0001					
Least Squares Means										
Effect		dose	Estimate	Standard Error	DF	t Value	Pr >  t			
dose		0	1.3377	0.02163	69.1	61.84	<.0001			
dose		40	1.2825	0.02095	67.9	61.23	<.0001			
dose		200	1.1217	0.02001	68.7	56.04	<.0001			
dose		1000	1.0377	0.02044	68.2	50.78	<.0001			
Standard										
Effect	dose	dose	Estimate	Error	DF	t Value	Pr >  t	Adjustment	Adj P	
dose		40	0	-0.05520	0.03011	68.5	-1.83	0.0711	Dunnett	0.1696
dose		200	0	-0.2160	0.02947	68.9	-7.33	<.0001	Dunnett	<.0001
dose		1000	0	-0.3001	0.02976	68.6	-10.08	<.0001	Dunnett	<.0001
Standard										
Effect	dose	dose	Estimate	Error	DF	t Value	Pr > t	Adjustment	Adj P	
dose		0	40	0.05520	0.03011	68.5	1.83	0.0355	Dunnett	0.0849
dose		0	200	0.2160	0.02947	68.9	7.33	<.0001	Dunnett	<.0001
dose		0	1000	0.3001	0.02976	68.6	10.08	<.0001	Dunnett	<.0001

**Table 5.**  
Females Only  
LitterSize & %Males as Covariates  
Mixed Model Based on Mean Data

Data Set		WORK.MEANDATABYSEX								
Type 3 Tests of Fixed Effects										
	Effect		Num	Den		F Value		Pr > F		
	dose		DF	DF						
	dose		3	72		45.71		<.0001		
	PercMales		1	72		0.47		0.4936		
	LitterSize		1	72		13.89		0.0004		
Least Squares Means										
	Effect	dose	Estimate	Standard Error	DF	t Value		Pr >  t		
	dose	0	1.2949	0.02020	72	64.09		<.0001		
	dose	40	1.2579	0.01971	72	63.83		<.0001		
	dose	200	1.0991	0.01867	72	58.87		<.0001		
	dose	1000	1.0155	0.01913	72	53.07		<.0001		
	Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr >  t	Adjustment	Adj P
	dose	40	0	-0.03706	0.02830	72	-1.31	0.1945	Dunnett-Hsu	0.4148
	dose	200	0	-0.1958	0.02749	72	-7.12	<.0001	Dunnett-Hsu	<.0001
	dose	1000	0	-0.2794	0.02783	72	-10.04	<.0001	Dunnett-Hsu	<.0001
	Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P
	dose	0	40	0.03706	0.02830	72	1.31	0.0973	Dunnett-Hsu	0.2091
	dose	0	200	0.1958	0.02749	72	7.12	<.0001	Dunnett-Hsu	<.0001
	dose	0	1000	0.2794	0.02783	72	10.04	<.0001	Dunnett-Hsu	<.0001

Mixed Model Based on Individual Data and Dam as Random Effect

Data Set		WORK.ANDATA								
Type 3 Tests of Fixed Effects										
	Effect		Num	Den		F Value		Pr > F		
	dose		DF	DF						
	dose		3	67.9		48.10		<.0001		
	PercMales		1	77.9		0.65		0.4228		
	LitterSize		1	85.2		10.64		0.0016		
Least Squares Means										
	Effect	dose	Estimate	Standard Error	DF	t Value		Pr >  t		
	dose	0	1.2850	0.02019	67.3	63.66		<.0001		
	dose	40	1.2514	0.01897	65.2	65.97		<.0001		
	dose	200	1.0881	0.01853	67.3	58.72		<.0001		
	dose	1000	1.0063	0.01889	66.8	53.27		<.0001		
	Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr >  t	Adjustment	Adj P
	dose	40	0	-0.03367	0.02761	67.1	-1.22	0.2269	Dunnett-Hsu	0.4692
	dose	200	0	-0.1969	0.02714	69.4	-7.26	<.0001	Dunnett-Hsu	<.0001
	dose	1000	0	-0.2788	0.02738	68.9	-10.18	<.0001	Dunnett-Hsu	<.0001
	Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P
	dose	0	40	0.03367	0.02761	67.1	1.22	0.1134	Dunnett-Hsu	0.2373
	dose	0	200	0.1969	0.02714	69.4	7.26	<.0001	Dunnett-Hsu	<.0001
	dose	0	1000	0.2788	0.02738	68.9	10.18	<.0001	Dunnett-Hsu	<.0001

**Table 6.**  
Females Only  
LitterSize as Covariate (%Males not included as a covariate)  
Mixed Model Based on Mean Data

Data Set			WORK.MEANDATABYSEX							
Type 3 Tests of Fixed Effects										
Effect		DF	DF	F Value	Pr > F					
dose		3	73	45.91	<.0001					
LitterSize		1	73	13.51	0.0004					
Least Squares Means										
Effect		dose	Estimate	Standard Error	DF	t Value	Pr >  t			
dose		0	1.2953	0.02012	73	64.37	<.0001			
dose		40	1.2569	0.01959	73	64.17	<.0001			
dose		200	1.0993	0.01860	73	59.10	<.0001			
dose		1000	1.0159	0.01906	73	53.30	<.0001			
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr >  t	Adjustment	Adj P	
dose	40	0	-0.03841	0.02813	73	-1.37	0.1763	Dunnett-Hsu	0.3813	
dose	200	0	-0.1960	0.02739	73	-7.15	<.0001	Dunnett-Hsu	<.0001	
dose	1000	0	-0.2795	0.02773	73	-10.08	<.0001	Dunnett-Hsu	<.0001	
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P	
dose	0	40	0.03841	0.02813	73	1.37	0.0881	Dunnett-Hsu	0.1919	
dose	0	200	0.1960	0.02739	73	7.15	<.0001	Dunnett-Hsu	<.0001	
dose	0	1000	0.2795	0.02773	73	10.08	<.0001	Dunnett-Hsu	<.0001	

Mixed Model Based on Individual Data and Dam as Random Effect

Data Set			WORK.ANDATA							
Type 3 Tests of Fixed Effects										
Effect		DF	DF	F Value	Pr > F					
dose		3	69.2	47.97	<.0001					
LitterSize		1	87.8	10.11	0.0020					
Least Squares Means										
Effect		dose	Estimate	Standard Error	DF	t Value	Pr >  t			
dose		0	1.2864	0.02010	70	63.99	<.0001			
dose		40	1.2522	0.01893	66.9	66.15	<.0001			
dose		200	1.0904	0.01830	69.3	59.58	<.0001			
dose		1000	1.0084	0.01869	69.2	53.96	<.0001			
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr >  t	Adjustment	Adj P	
dose	40	0	-0.03424	0.02758	68.7	-1.24	0.2186	Dunnett-Hsu	0.4550	
dose	200	0	-0.1960	0.02709	70.5	-7.23	<.0001	Dunnett-Hsu	<.0001	
dose	1000	0	-0.2780	0.02734	70.1	-10.17	<.0001	Dunnett-Hsu	<.0001	
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P	
dose	0	40	0.03424	0.02758	68.7	1.24	0.1093	Dunnett-Hsu	0.2298	
dose	0	200	0.1960	0.02709	70.5	7.23	<.0001	Dunnett-Hsu	<.0001	
dose	0	1000	0.2780	0.02734	70.1	10.17	<.0001	Dunnett-Hsu	<.0001	

**Table 7.**

Females Only  
No Covariates  
Model Based on Mean Data

Data Set		WORK.MEANDATABYSEX							
Type 3 Tests of Fixed Effects									
Effect		Num	Den	F Value	Pr > F				
dose		DF	DF						
		3	74	39.62	<.0001				
Least Squares Means									
Standard									
Effect	dose	Estimate	Error	DF	t Value	Pr >  t			
dose	0	1.2996	0.02172	74	59.83	<.0001			
dose	40	1.2527	0.02114	74	59.25	<.0001			
dose	200	1.1001	0.02011	74	54.71	<.0001			
dose	1000	1.0151	0.02061	74	49.26	<.0001			
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr >  t	Adjustment	Adj P
dose	40	0	-0.04692	0.03031	74	-1.55	0.1259	Dunnett	0.2846
dose	200	0	-0.1995	0.02960	74	-6.74	<.0001	Dunnett	<.0001
dose	1000	0	-0.2846	0.02994	74	-9.50	<.0001	Dunnett	<.0001
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr > t	Adjustment	Adj P
dose	0	40	0.04692	0.03031	74	1.55	0.0630	Dunnett	0.1427
dose	0	200	0.1995	0.02960	74	6.74	<.0001	Dunnett	<.0001
dose	0	1000	0.2846	0.02994	74	9.50	<.0001	Dunnett	<.0001

Mixed Model Based on Individual Data and Dam as Random Effect

Data Set		WORK.ANDATA							
Type 3 Tests of Fixed Effects									
Effect		Num	Den	F Value	Pr > F				
dose		DF	DF						
		3	67.1	43.81	<.0001				
Least Squares Means									
Standard									
Effect	dose	Estimate	Error	DF	t Value	Pr >  t			
dose	0	1.2935	0.02092	68.7	61.84	<.0001			
dose	40	1.2536	0.01982	64.9	63.24	<.0001			
dose	200	1.0947	0.01911	67.7	57.28	<.0001			
dose	1000	1.0130	0.01951	67.2	51.92	<.0001			
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr >  t	Adjustment	Adj P
dose	40	0	-0.03992	0.02882	66.9	-1.39	0.1706	Dunnett	0.3688
dose	200	0	-0.1988	0.02833	68.3	-7.02	<.0001	Dunnett	<.0001
dose	1000	0	-0.2805	0.02860	68	-9.81	<.0001	Dunnett	<.0001
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr > t	Adjustment	Adj P
dose	0	40	0.03992	0.02882	66.9	1.39	0.0853	Dunnett	0.1854
dose	0	200	0.1988	0.02833	68.3	7.02	<.0001	Dunnett	<.0001
dose	0	1000	0.2805	0.02860	68	9.81	<.0001	Dunnett	<.0001

**Table 8.**  
Males Only  
LitterSize & %Males as Covariates  
Mixed Model Based on Mean Data

Data Set		WORK.MEANDATABYSEX							
Type 3 Tests of Fixed Effects									
Effect	DF	DF	F Value	Pr > F					
dose	3	71	51.81	<.0001					
PercMales	1	71	6.19	0.0152					
LitterSize	1	71	5.31	0.0241					
Least Squares Means									
Effect	dose	Estimate	Standard Error	DF	t Value	Pr >  t			
dose	0	1.3704	0.02113	71	64.86	<.0001			
dose	40	1.3131	0.02053	71	63.95	<.0001			
dose	200	1.1321	0.02011	71	56.30	<.0001			
dose	1000	1.0582	0.01993	71	53.09	<.0001			
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr >  t	Adjustment	Adj P
dose	40	0	-0.05724	0.02950	71	-1.94	0.0563	Dunnett-Hsu	0.1372
dose	200	0	-0.2382	0.02934	71	-8.12	<.0001	Dunnett-Hsu	<.0001
dose	1000	0	-0.3122	0.02901	71	-10.76	<.0001	Dunnett-Hsu	<.0001
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P
dose	0	40	0.05724	0.02950	71	1.94	0.0282	Dunnett-Hsu	0.0687
dose	0	200	0.2382	0.02934	71	8.12	<.0001	Dunnett-Hsu	<.0001
dose	0	1000	0.3122	0.02901	71	10.76	<.0001	Dunnett-Hsu	<.0001

Mixed Model Based on Individual Data and Dam as Random Effect

Data Set		WORK.ANDATA							
Type 3 Tests of Fixed Effects									
Effect	DF	DF	F Value	Pr > F					
dose	3	69.5	52.24	<.0001					
PercMales	1	73	5.56	0.0210					
LitterSize	1	74.4	4.65	0.0343					
Least Squares Means									
Effect	dose	Estimate	Standard Error	DF	t Value	Pr >  t			
dose	0	1.3704	0.02132	69.9	64.28	<.0001			
dose	40	1.3158	0.02065	69.2	63.73	<.0001			
dose	200	1.1346	0.01953	67.4	58.09	<.0001			
dose	1000	1.0607	0.01992	69.1	53.25	<.0001			
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr >  t	Adjustment	Adj P
dose	40	0	-0.05466	0.02928	70.7	-1.87	0.0661	Dunnett-Hsu	0.1588
dose	200	0	-0.2359	0.02892	68.9	-8.16	<.0001	Dunnett-Hsu	<.0001
dose	1000	0	-0.3098	0.02879	70.6	-10.76	<.0001	Dunnett-Hsu	<.0001
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P
dose	0	40	0.05466	0.02928	70.7	1.87	0.0330	Dunnett-Hsu	0.0795
dose	0	200	0.2359	0.02892	68.9	8.16	<.0001	Dunnett-Hsu	<.0001
dose	0	1000	0.3098	0.02879	70.6	10.76	<.0001	Dunnett-Hsu	<.0001

**Table 9.**  
Males Only  
LitterSize as Covariate (%Males not included as a covariate)  
Mixed Model Based on Mean Data

Data Set			WORK.MEANDATABYSEX						
Type 3 Tests of Fixed Effects									
Effect		Num	Den	F Value	Pr > F				
		DF	DF						
dose		3	72	47.18	<.0001				
LitterSize		1	72	5.88	0.0178				
Least Squares Means									
Effect		dose	Estimate	Standard Error	DF	t Value	Pr >  t		
dose		0	1.3697	0.02188	72	62.61	<.0001		
dose		40	1.3086	0.02118	72	61.79	<.0001		
dose		200	1.1368	0.02073	72	54.84	<.0001		
dose		1000	1.0583	0.02064	72	51.28	<.0001		
Differences of Least Squares Means									
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr >  t	Adjustment	Adj P
dose	40	0	-0.06107	0.03050	72	-2.00	0.0490	Dunnett-Hsu	0.1206
dose	200	0	-0.2329	0.03029	72	-7.69	<.0001	Dunnett-Hsu	<.0001
dose	1000	0	-0.3114	0.03003	72	-10.37	<.0001	Dunnett-Hsu	<.0001
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P
dose	0	40	0.06107	0.03050	72	2.00	0.0245	Dunnett-Hsu	0.0603
dose	0	200	0.2329	0.03029	72	7.69	<.0001	Dunnett-Hsu	<.0001
dose	0	1000	0.3114	0.03003	72	10.37	<.0001	Dunnett-Hsu	<.0001

Mixed Model Based on Individual Data and Dam as Random Effect

Data Set			WORK.ANDATA						
Type 3 Tests of Fixed Effects									
Effect		Num	Den	F Value	Pr > F				
		DF	DF						
dose		3	69.6	48.38	<.0001				
LitterSize		1	74.2	5.26	0.0246				
Least Squares Means									
Effect		dose	Estimate	Standard Error	DF	t Value	Pr >  t		
dose		0	1.3647	0.02176	70.3	62.71	<.0001		
dose		40	1.3066	0.02084	70.2	62.69	<.0001		
dose		200	1.1334	0.02007	67.5	56.47	<.0001		
dose		1000	1.0560	0.02037	69.6	51.84	<.0001		
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr >  t	Adjustment	Adj P
dose	40	0	-0.05810	0.03005	70.6	-1.93	0.0572	Dunnett-Hsu	0.1389
dose	200	0	-0.2312	0.02965	69	-7.80	<.0001	Dunnett-Hsu	<.0001
dose	1000	0	-0.3087	0.02958	70.5	-10.44	<.0001	Dunnett-Hsu	<.0001
Effect	dose	dose	Estimate	Standard Error	DF	t Value	Pr > t	Adjustment	Adj P
dose	0	40	0.05810	0.03005	70.6	1.93	0.0286	Dunnett-Hsu	0.0695
dose	0	200	0.2312	0.02965	69	7.80	<.0001	Dunnett-Hsu	<.0001
dose	0	1000	0.3087	0.02958	70.5	10.44	<.0001	Dunnett-Hsu	<.0001

**Table 10.**

Males Only  
No Covariates  
Model Based on Mean Data

Data Set			WORK.MEANDATABYSEX						
Type 3 Tests of Fixed Effects									
			Num	Den					
Effect			DF	DF	F Value		Pr > F		
dose			3	73	45.68		<.0001		
Least Squares Means									
			Standard						
Effect	dose		Estimate	Error	DF	t Value	Pr >  t		
dose	0		1.3754	0.02246	73	61.23	<.0001		
dose	40		1.3070	0.02186	73	59.78	<.0001		
dose	200		1.1319	0.02131	73	53.12	<.0001		
dose	1000		1.0596	0.02131	73	49.72	<.0001		
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr >  t	Adjustment	Adj P
dose	40	0	-0.06845	0.03135	73	-2.18	0.0322	Dunnett	0.0815
dose	200	0	-0.2435	0.03096	73	-7.86	<.0001	Dunnett	<.0001
dose	1000	0	-0.3158	0.03096	73	-10.20	<.0001	Dunnett	<.0001
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr > t	Adjustment	Adj P
dose	0	40	0.06845	0.03135	73	2.18	0.0161	Dunnett	0.0408
dose	0	200	0.2435	0.03096	73	7.86	<.0001	Dunnett	<.0001
dose	0	1000	0.3158	0.03096	73	10.20	<.0001	Dunnett	<.0001

Mixed Model Based on Individual Data and Dam as Random Effect

Data Set			WORK.ANDATA						
Type 3 Tests of Fixed Effects									
			Num	Den					
Effect			DF	DF	F Value		Pr > F		
dose			3	69.9	47.22		<.0001		
Least Squares Means									
			Standard						
Effect	dose		Estimate	Error	DF	t Value	Pr >  t		
dose	0		1.3729	0.02202	71.3	62.36	<.0001		
dose	40		1.3081	0.02136	70.5	61.24	<.0001		
dose	200		1.1326	0.02059	67.8	55.00	<.0001		
dose	1000		1.0604	0.02080	70.2	50.98	<.0001		
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr >  t	Adjustment	Adj P
dose	40	0	-0.06478	0.03068	70.9	-2.11	0.0382	Dunnett	0.0957
dose	200	0	-0.2404	0.03015	69.6	-7.97	<.0001	Dunnett	<.0001
dose	1000	0	-0.3125	0.03029	70.8	-10.32	<.0001	Dunnett	<.0001
Standard									
Effect	dose	dose	Estimate	Error	DF	t Value	Pr > t	Adjustment	Adj P
dose	0	40	0.06478	0.03068	70.9	2.11	0.0191	Dunnett	0.0479
dose	0	200	0.2404	0.03015	69.6	7.97	<.0001	Dunnett	<.0001
dose	0	1000	0.3125	0.03029	70.8	10.32	<.0001	Dunnett	<.0001

**Table 11. Fetal body weight Data**

(These data are the same as provided by TCEQ except that a few errors in going from the Hackett *et al.* (1987) data sheets to the electronic copy have been corrected.)

Index	Dam	SITE	Status	FetalSex	dose	fetalwt
1	228	228	1	1	1	0 1.611
2	228	228	2	1	2	0 1.393
3	228	228	3	1	1	0 1.524
4	228	228	4	1	1	0 1.512
5	228	228	5	1	2	0 1.573
6	228	228	6	1	1	0 1.526
7	228	228	7	1	1	0 1.563
8	228	228	8	2		0
9	228	228	9	1	2	0 1.311
10	228	228	10	1	1	0 1.55
11	256	256	1	1	1	0 1.406
12	256	256	2	1	2	0 1.277
13	256	256	3	1	2	0 1.272
14	256	256	4	1	1	0 1.22
15	256	256	5	2		0
16	256	256	6	1	1	0 1.362
17	256	256	7	1	2	0 1.273
18	256	256	8	1	2	0 1.293
19	256	256	9	2		0
20	256	256	10	1	1	0 1.336
21	256	256	11	1	1	0 1.312
22	256	256	12	1	1	0 1.316
23	270	270	1	1	2	0 1.433
24	270	270	2	1	1	0 1.763
25	270	270	3	2		0
26	270	270	4	2		0
27	270	270	5	1	1	0 1.613
28	270	270	6	2		0
29	273	273	1	1	2	0 1.352
30	273	273	2	2		0
31	273	273	3	1	2	0 1.215
32	273	273	4	1	2	0 1.181
33	273	273	5	1	1	0 1.425
34	273	273	6	1	2	0 1.204
35	273	273	7	1	2	0 1.183
36	273	273	8	1	1	0 1.106
37	273	273	9	1	2	0 1.372
38	273	273	10	1	1	0 1.37
39	273	273	11	1	1	0 1.379
40	273	273	12	1	2	0 1.355

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41	273	13	1	2	0	0.664
42	273	14	1	1	0	1.436
43	304	1	1	2	0	1.189
44	304	2	1	1	0	1.165
45	304	3	1	2	0	1.14
46	304	4	1	1	0	1.172
47	304	5	1	1	0	1.289
48	304	6	1	2	0	1.179
49	304	7	1	1	0	1.098
50	304	8	1	1	0	1.105
51	304	9	1	2	0	1.231
52	304	10	1	2	0	1.183
53	304	11	1	1	0	1.349
54	304	12	1	1	0	1.118
55	320	1	1	1	0	1.322
56	320	2	1	2	0	1.132
57	320	3	1	2	0	1.281
58	320	4	1	1	0	1.354
59	320	5	1	1	0	1.383
60	320	6	1	1	0	1.338
61	320	7	1	2	0	1.016
62	320	8	1	2	0	1.273
63	320	9	1	2	0	1.39
64	320	10	1	2	0	1.249
65	320	11	1	1	0	1.444
66	320	12	1	2	0	1.31
67	320	13	1	2	0	1.381
68	321	1	1	2	0	1.294
69	321	2	1	1	0	1.299
70	321	3	1	2	0	1.342
71	321	4	1	2	0	1.294
72	321	5	1	1	0	1.308
73	321	6	1	1	0	1.336
74	321	7	1	1	0	1.285
75	321	8	1	2	0	1.153
76	321	9	1	2	0	1.151
77	321	10	1	1	0	1.3
78	321	11	1	1	0	1.459
79	321	12	1	1	0	1.477
80	321	13	1	2	0	1.259
81	321	14	1	1	0	1.276
82	321	15	1	2	0	1.184
83	341	1	1	1	0	1.493
84	341	2	1	1	0	1.492
85	341	3	1	1	0	1.469
86	341	4	1	2	0	1.379

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87	341	5	1	1	0	1.429
88	341	6	1	2	0	1.361
89	341	7	1	2	0	1.269
90	341	8	1	1	0	1.429
91	341	9	1	2	0	1.381
92	341	10	1	1	0	1.404
93	341	11	1	2	0	1.311
94	341	12	1	2	0	1.403
95	341	13	1	1	0	1.426
96	351	1	1	2	0	1.285
97	351	2	1	2	0	1.18
98	351	3	1	1	0	1.18
99	351	4	1	1	0	1.148
100	351	5	1	2	0	1.117
101	351	6	1	1	0	1.234
102	351	7	1	2	0	1.128
103	351	8	1	2	0	1.218
104	351	9	1	2	0	1.169
105	351	10	1	1	0	0.932
106	351	11	1	2	0	1.214
107	351	12	2		0	
108	351	13	1	2	0	1.158
109	351	14	1	2	0	1.214
110	372	1	1	2	0	1.43
111	372	2	1	2	0	1.252
112	372	3	1	1	0	1.2
113	372	4	1	1	0	1.354
114	372	5	1	2	0	1.322
115	372	6	1	2	0	1.38
116	372	7	1	1	0	1.451
117	372	8	1	1	0	1.316
118	372	9	1	2	0	1.262
119	372	10	1	1	0	1.353
120	372	11	1	2	0	1.24
121	372	12	4		0	
122	372	13	1	2	0	1.305
123	372	14	1	1	0	1.41
124	378	1	1	1	0	1.338
125	378	2	1	1	0	1.402
126	378	3	1	1	0	1.464
127	378	4	1	1	0	1.46
128	378	5	1	2	0	1.348
129	378	6	2		0	
130	378	7	1	1	0	1.35
131	378	8	2		0	
132	378	9	1	1	0	1.346

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133	378	10	1	1	0	1.398
134	378	11	1	1	0	1.4
135	378	12	1	1	0	1.347
136	378	13	1	1	0	1.332
137	378	14	1	1	0	1.245
138	380	1	1	2	0	1.337
139	380	2	1	1	0	1.36
140	380	3	2		0	
141	380	4	1	2	0	1.276
142	380	5	1	1	0	1.429
143	380	6	1	2	0	1.295
144	380	7	1	2	0	1.284
145	380	8	1	1	0	1.482
146	380	9	1	2	0	1.334
147	380	10	1	2	0	1.236
148	380	11	1	1	0	1.365
149	380	12	1	1	0	1.357
150	380	13	1	2	0	1.36
151	380	14	1	1	0	1.275
152	388	1	2		0	
153	388	2	1	1	0	1.511
154	388	3	1	2	0	1.37
155	388	4	1	1	0	1.459
156	388	5	1	2	0	1.428
157	388	6	1	2	0	1.345
158	388	7	1	2	0	1.441
159	388	8	1	2	0	1.376
160	388	9	1	1	0	1.279
161	388	10	1	2	0	1.419
162	391	1	1	2	0	1.206
163	391	2	1	1	0	1.341
164	391	3	1	2	0	1.362
165	391	4	1	1	0	1.482
166	391	5	1	2	0	1.46
167	391	6	1	1	0	1.281
168	391	7	1	1	0	1.179
169	391	8	2		0	
170	391	9	1	2	0	1.07
171	391	10	1	2	0	1.261
172	391	11	1	1	0	1.269
173	391	12	1	1	0	1.344
174	391	13	1	1	0	1.489
175	391	14	1	1	0	1.502
176	415	1	1	1	0	1.459
177	415	2	2		0	
178	415	3	2		0	

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179	415	4	1	1	0	1.364
180	415	5	2		0	
181	415	6	1	2	0	1.288
182	415	7	1	1	0	1.226
183	415	8	1	2	0	1.332
184	415	9	1	2	0	1.137
185	415	10	1	1	0	1.333
186	415	11	1	1	0	1.217
187	415	12	1	1	0	1.456
188	418	1	1	2	0	1.154
189	418	2	1	2	0	1.281
190	418	3	1	2	0	1.383
191	418	4	1	1	0	1.354
192	418	5	1	2	0	1.318
193	418	6	1	2	0	0.957
194	418	7	1	2	0	1.311
195	418	8	1	2	0	1.3
196	418	9	1	1	0	1.37
197	418	10	1	1	0	1.296
198	418	11	1	2	0	1.218
199	418	12	2		0	
200	418	13	1	2	0	1.22
201	418	14	1	2	0	1.328
202	422	1	1	2	0	1.475
203	422	2	1	2	0	1.511
204	422	3	1	1	0	1.49
205	422	4	1	2	0	1.405
206	422	5	1	1	0	1.5
207	422	6	1	2	0	1.413
208	422	7	1	1	0	1.518
209	422	8	1	1	0	1.524
210	422	9	1	1	0	1.498
211	422	10	1	2	0	1.368
212	422	11	1	2	0	1.36
213	422	12	1	2	0	1.351
214	422	13	1	1	0	1.478
215	422	14	2		0	
216	422	15	1	1	0	1.497
217	444	1	1	2	0	1.343
218	444	2	1	2	0	1.347
219	444	3	1	1	0	1.372
220	444	4	1	2	0	1.311
221	444	5	1	1	0	1.357
222	444	6	1	2	0	1.259
223	444	7	1	1	0	1.35
224	444	8	1	2	0	1.275

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225	444	9	1	1	0	1.31
226	444	10	1	1	0	1.138
227	444	11	1	2	0	1.278
228	444	12	1	1	0	1.444
229	444	13	1	2	0	1.304
230	444	14	1	2	0	1.332
231	242	1	1	1	40	1.47
232	242	2	2		40	
233	242	3	1	1	40	1.377
234	242	4	1	1	40	1.429
235	242	5	1	2	40	1.363
236	242	6	1	2	40	1.325
237	242	7	1	1	40	1.269
238	242	8	1	1	40	1.319
239	242	9	1	2	40	1.33
240	242	10	1	1	40	1.381
241	242	11	1	2	40	1.214
242	242	12	1	2	40	1.302
243	242	13	2		40	
244	246	1	1	2	40	1.422
245	246	2	1	2	40	1.394
246	246	3	1	2	40	1.237
247	246	4	1	1	40	1.329
248	246	5	1	2	40	1.372
249	246	6	1	2	40	0.94
250	246	7	1	2	40	1.287
251	246	8	1	1	40	1.356
252	246	9	1	2	40	1.29
253	246	10	1	2	40	1.304
254	246	11	1	1	40	1.168
255	263	1	1	2	40	1.308
256	263	2	1	1	40	1.313
257	263	3	1	2	40	1.373
258	263	4	1	2	40	1.275
259	263	5	1	2	40	1.378
260	263	6	1	2	40	1.295
261	263	7	1	2	40	1.301
262	263	8	1	1	40	1.267
263	263	9	2		40	
264	263	10	1	2	40	1.326
265	263	11	1	1	40	1.363
266	263	12	1	2	40	1.312
267	263	13	1	2	40	1.321
268	263	14	1	2	40	1.048
269	286	1	1	1	40	1.429
270	286	2	1	2	40	1.233

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271	286	3	1	1	40	1.32
272	286	4	1	1	40	1.326
273	286	5	1	1	40	1.359
274	286	6	1	2	40	1.334
275	286	7	1	2	40	1.321
276	286	8	1	1	40	1.426
277	286	9	1	1	40	1.407
278	286	10	1	2	40	1.283
279	286	11	1	2	40	1.356
280	286	12	1	2	40	1.142
281	286	13	1	1	40	1.409
282	286	14	1	1	40	1.313
283	295	1	1	1	40	1.426
284	295	2	1	1	40	1.292
285	295	3	1	1	40	1.25
286	295	4	1	1	40	1.443
287	295	5	1	2	40	1.241
288	295	6	1	2	40	1.23
289	295	7	1	1	40	1.289
290	295	8	4		40	
291	295	9	1	1	40	1.376
292	295	10	1	1	40	1.287
293	295	11	1	2	40	1.157
294	295	12	1	2	40	1.291
295	295	13	1	1	40	1.349
296	295	14	1	2	40	1.264
297	302	1	1	1	40	1.133
298	302	2	1	1	40	1.14
299	302	3	1	2	40	1.065
300	302	4	1	1	40	1.193
301	302	5	1	2	40	1.079
302	302	6	1	1	40	1.108
303	302	7	4		40	
304	302	8	1	1	40	1.183
305	302	9	1	2	40	1.191
306	302	10	1	1	40	1.172
307	302	11	1	2	40	1.121
308	302	12	1	1	40	1.038
309	302	13	1	1	40	1.13
310	302	14	1	1	40	1.22
311	302	15	1	1	40	1.167
312	302	16	1	1	40	1.173
313	307	1	1	2	40	1.343
314	307	2	1	2	40	1.227
315	307	3	1	1	40	1.356
316	307	4	1	1	40	1.423

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317	307	5	1	1	40	1.351
318	307	6	1	2	40	1.179
319	307	7	1	2	40	1.364
320	307	8	1	1	40	1.397
321	307	9	1	1	40	1.362
322	307	10	1	1	40	1.384
323	307	11	1	2	40	1.252
324	307	12	1	2	40	1.265
325	307	13	1	2	40	1.35
326	311	1	1	1	40	1.378
327	311	2	1	1	40	1.337
328	311	3	1	1	40	1.4
329	311	4	1	2	40	1.315
330	311	5	1	2	40	1.297
331	311	6	1	1	40	1.43
332	311	7	1	1	40	1.38
333	311	8	1	2	40	1.294
334	311	9	1	2	40	1.296
335	311	10	1	2	40	1.31
336	311	11	1	1	40	1.28
337	311	12	1	2	40	1.063
338	312	1	1	1	40	1.344
339	312	2	1	1	40	1.239
340	312	3	1	2	40	1.273
341	312	4	1	2	40	1.249
342	312	5	1	2	40	1.259
343	312	6	1	2	40	1.149
344	312	7	1	1	40	1.312
345	312	8	1	2	40	1.217
346	312	9	1	2	40	1.386
347	312	10	1	1	40	1.235
348	312	11	1	2	40	1.151
349	312	12	1	1	40	1.215
350	312	13	1	1	40	1.291
351	312	14	1	1	40	1.146
352	312	15	1	2	40	1.199
353	312	16	4		40	
354	312	17	1	1	40	1.305
355	314	1	1	1	40	1.405
356	314	2	1	2	40	1.184
357	314	3	1	1	40	1.184
358	314	4	1	1	40	1.424
359	314	5	1	2	40	1.3
360	314	6	1	1	40	1.313
361	314	7	1	1	40	1.416
362	314	8	1	1	40	1.437

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363	314	9	1	2	40	1.288
364	314	10	2		40	
365	314	11	1	2	40	1.287
366	314	12	1	2	40	1.321
367	318	1	1	1	40	1.482
368	318	2	1	2	40	1.289
369	318	3	1	1	40	1.245
370	318	4	1	1	40	1.379
371	318	5	1	2	40	1.256
372	318	6	1	1	40	1.217
373	318	7	1	2	40	1.339
374	318	8	1	2	40	1.308
375	318	9	4		40	
376	318	10	1	2	40	1.205
377	318	11	1	1	40	1.49
378	318	12	1	2	40	1.284
379	318	13	1	1	40	1.321
380	346	1	1	2	40	1.092
381	346	2	2		40	
382	346	3	2		40	
383	346	4	2		40	
384	346	5	1	1	40	1.31
385	346	6	1	1	40	1.322
386	346	7	1	1	40	1.048
387	346	8	1	2	40	1.238
388	346	9	1	2	40	1.167
389	349	1	1	2	40	1.015
390	349	2	1	2	40	1.227
391	349	3	1	1	40	1.249
392	349	4	1	2	40	1.394
393	349	5	1	2	40	1.334
394	349	6	1	1	40	1.404
395	349	7	1	1	40	1.344
396	349	8	1	1	40	1.395
397	349	9	1	1	40	1.391
398	349	10	1	1	40	1.246
399	349	11	1	1	40	1.411
400	349	12	1	2	40	1.349
401	349	13	1	2	40	1.354
402	368	1	1	2	40	1.283
403	368	2	1	2	40	1.396
404	368	3	1	1	40	1.421
405	368	4	1	2	40	1.253
406	368	5	1	1	40	1.355
407	368	6	1	1	40	1.391
408	368	7	1	1	40	1.379

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409	368	8	1	1	40	1.48
410	368	9	1	2	40	1.365
411	368	10	1	1	40	1.235
412	368	11	1	1	40	1.369
413	369	1	1	2	40	1.286
414	369	2	1	2	40	1.237
415	369	3	1	1	40	1.292
416	369	4	1	2	40	1.216
417	369	5	1	1	40	1.23
418	369	6	2		40	
419	369	7	1	1	40	1.276
420	369	8	1	1	40	1.127
421	369	9	1	2	40	1.345
422	369	10	2		40	
423	369	11	1	2	40	1.251
424	369	12	1	2	40	1.287
425	373	1	1	1	40	1.421
426	373	2	1	2	40	1.307
427	373	3	1	2	40	1.26
428	373	4	1	1	40	1.342
429	373	5	1	2	40	1.315
430	373	6	1	1	40	1.382
431	373	7	1	1	40	1.391
432	373	8	1	1	40	1.338
433	373	9	1	1	40	1.301
434	373	10	1	1	40	1.289
435	373	11	1	1	40	1.266
436	373	12	1	2	40	1.27
437	373	13	1	2	40	1.308
438	373	14	1	2	40	1.268
439	373	15	1	2	40	1.259
440	381	1	2		40	
441	381	2	1	1	40	1.401
442	381	3	1	2	40	1.243
443	381	4	1	2	40	1.077
444	381	5	1	1	40	1.278
445	381	6	1	1	40	1.283
446	381	7	1	1	40	1.289
447	381	8	1	1	40	1.399
448	381	9	1	2	40	1.238
449	381	10	1	1	40	1.234
450	381	11	1	1	40	1.344
451	381	12	1	2	40	1.41
452	381	13	1	2	40	1.39
453	381	14	1	2	40	0.902
454	381	15	1	2	40	1.37

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455	390	1	1	2	40	1.277
456	390	2	1	1	40	1.338
457	390	3	1	2	40	1.25
458	390	4	1	2	40	1.211
459	390	5	1	2	40	1.215
460	390	6	1	2	40	1.058
461	390	7	1	2	40	1.082
462	390	8	1	2	40	1.078
463	390	9	1	2	40	1.085
464	390	10	1	1	40	1.009
465	390	11	1	1	40	1.187
466	390	12	1	2	40	1.351
467	390	13	1	2	40	1.303
468	390	14	1	2	40	1.298
469	433	1	1	1	40	1.314
470	433	2	1	1	40	1.225
471	433	3	1	2	40	1.115
472	433	4	1	1	40	1.141
473	433	5	1	2	40	1.202
474	433	6	1	2	40	1.214
475	433	7	1	1	40	1.23
476	433	8	1	1	40	1.194
477	433	9	1	1	40	1.293
478	433	10	1	1	40	1.358
479	433	11	1	2	40	1.168
480	433	12	2		40	
481	433	13	4		40	
482	433	14	1	2	40	1.239
483	433	15	1	2	40	1.252
484	251	1	1	2	200	1.124
485	251	2	1	1	200	1.228
486	251	3	1	1	200	1.142
487	251	4	1	1	200	1.183
488	251	5	1	1	200	1.08
489	251	6	1	1	200	1.19
490	251	7	1	1	200	1.061
491	251	8	1	2	200	1.127
492	251	9	1	2	200	1.064
493	251	10	1	1	200	1.123
494	251	11	1	2	200	1
495	251	12	1	2	200	1.068
496	251	13	1	2	200	0.984
497	258	1	1	1	200	1.198
498	258	2	1	2	200	1.122
499	258	3	1	1	200	1.141
500	258	4	1	1	200	1.157

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501	258	5	1	1	200	1.146
502	258	6	2		200	
503	258	7	1	1	200	1.169
504	258	8	1	1	200	1.18
505	258	9	1	2	200	1.127
506	258	10	1	2	200	1.178
507	258	11	1	1	200	1.164
508	258	12	1	1	200	1.121
509	258	13	1	2	200	1.14
510	260	1	1	1	200	1.229
511	260	2	4		200	
512	260	3	1	1	200	1.255
513	260	4	1	2	200	1.224
514	260	5	1	1	200	1.137
515	260	6	2		200	
516	260	7	1	1	200	1.294
517	260	8	1	2	200	1.088
518	260	9	1	2	200	1.223
519	260	10	1	2	200	1.175
520	260	11	1	1	200	1.181
521	260	12	1	2	200	1.13
522	260	13	1	1	200	1.186
523	260	14	1	1	200	1.217
524	265	1	1	2	200	1.075
525	265	2	1	1	200	1.049
526	265	3	1	2	200	1.131
527	265	4	1	1	200	1.139
528	265	5	1	1	200	1.118
529	265	6	1	2	200	1.038
530	265	7	1	2	200	1.078
531	265	8	1	2	200	1.064
532	265	9	1	2	200	0.988
533	265	10	1	1	200	0.974
534	265	11	1	2	200	0.978
535	265	12	1	2	200	0.921
536	265	13	1	2	200	1.051
537	272	1	1	1	200	1.041
538	272	2	1	1	200	0.953
539	272	3	1	2	200	1.051
540	272	4	1	2	200	1.016
541	272	5	1	2	200	1.037
542	272	6	1	2	200	1.01
543	272	7	1	1	200	0.953
544	272	8	1	2	200	0.962
545	272	9	1	2	200	1.026
546	272	10	1	1	200	1.127

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547	272	11	1	1	200	0.993
548	272	12	1	1	200	1.122
549	272	13	1	2	200	0.905
550	272	14	1	2	200	1.06
551	274	1	1	1	200	1.135
552	274	2	1	1	200	1.192
553	274	3	1	1	200	1.13
554	274	4	1	2	200	0.983
555	274	5	1	1	200	1.187
556	274	6	1	2	200	0.995
557	274	7	1	2	200	1.115
558	274	8	1	1	200	0.826
559	274	9	1	2	200	0.967
560	274	10	1	1	200	1.15
561	274	11	1	1	200	1.176
562	274	12	1	2	200	1.106
563	274	13	1	1	200	1.167
564	274	14	1	2	200	1.038
565	274	15	1	1	200	1.138
566	296	1	1	2	200	1.19
567	296	2	1	1	200	1.1
568	296	3	1	1	200	1.159
569	296	4	1	2	200	1.124
570	296	5	1	1	200	1.092
571	296	6	1	1	200	1.18
572	296	7	1	2	200	1.063
573	296	8	1	2	200	1.113
574	296	9	1	2	200	1.097
575	296	10	1	1	200	1.094
576	296	11	1	2	200	1.06
577	319	1	1	2	200	1.071
578	319	2	1	2	200	1.207
579	319	3	1	2	200	1.175
580	319	4	1	2	200	1.139
581	319	5	1	1	200	1.148
582	319	6	1	1	200	1.144
583	319	7	1	2	200	1.092
584	319	8	2		200	
585	319	9	1	2	200	0.951
586	319	10	1	1	200	1.182
587	319	11	1	1	200	1.146
588	319	12	1	1	200	1.186
589	319	13	1	2	200	0.973
590	319	14	1	1	200	1.073
591	319	15	1	1	200	1.121
592	328	1	2		200	

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593	328	2	1	2	200	0.975
594	328	3	1	1	200	1.028
595	328	4	1	2	200	1.007
596	328	5	1	1	200	1.033
597	328	6	4		200	
598	328	7	1	1	200	1.1
599	328	8	1	2	200	0.906
600	328	9	1	1	200	0.843
601	328	10	4		200	
602	328	11	1	1	200	0.99
603	328	12	1	2	200	1.064
604	328	13	1	2	200	1.026
605	328	14	1	1	200	1.002
606	337	1	1	1	200	1.205
607	337	2	1	2	200	1.102
608	337	3	1	1	200	1.231
609	337	4	1	2	200	1.112
610	337	5	1	2	200	1.098
611	337	6	1	2	200	1.087
612	337	7	1	1	200	1.07
613	337	8	1	1	200	1.207
614	337	9	1	2	200	1.048
615	337	10	1	1	200	1.173
616	337	11	1	2	200	0.945
617	337	12	1	1	200	1.141
618	339	1	1	1	200	1.163
619	339	2	1	1	200	1.207
620	339	3	1	2	200	1.072
621	339	4	1	1	200	1.09
622	339	5	1	1	200	0.993
623	339	6	1	2	200	1.049
624	339	7	1	2	200	1.073
625	339	8	1	1	200	1.11
626	339	9	1	2	200	1.034
627	339	10	1	1	200	1.056
628	339	11	1	1	200	0.73
629	342	1	1	2	200	0.979
630	342	2	1	1	200	1.148
631	342	3	1	1	200	1.028
632	342	4	1	1	200	1.116
633	342	5	1	1	200	1.162
634	342	6	1	1	200	1.135
635	342	7	1	2	200	1.026
636	342	8	1	2	200	1.071
637	342	9	1	1	200	1.119
638	342	10	1	1	200	1.116

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639	342	11	1	1	200	1.134
640	343	1	2		200	
641	343	2	1	2	200	1.097
642	343	3	1	2	200	1.118
643	343	4	1	1	200	1.052
644	343	5	1	1	200	1.133
645	343	6	1	2	200	1.087
646	343	7	1	2	200	0.981
647	343	8	1	1	200	1.049
648	343	9	1	1	200	1.077
649	343	10	1	2	200	1.036
650	343	11	1	2	200	1.159
651	343	12	1	2	200	0.94
652	343	13	1	1	200	1.101
653	343	14	1	2	200	1.057
654	348	1	1	2	200	1.257
655	348	2	1	2	200	1.162
656	348	3	1	1	200	1.255
657	348	4	1	2	200	1.181
658	348	5	1	2	200	1.154
659	348	6	1	1	200	1.161
660	348	7	1	2	200	1.167
661	348	8	1	2	200	1.177
662	348	9	1	2	200	1.142
663	348	10	1	1	200	1.186
664	348	11	1	1	200	1.107
665	348	12	1	2	200	1.149
666	348	13	1	1	200	1.242
667	348	14	1	1	200	1.209
668	353	1	1	1	200	1.198
669	353	2	4		200	
670	353	3	1	1	200	1.181
671	353	4	1	1	200	1.236
672	353	5	1	1	200	1.167
673	353	6	1	1	200	1.104
674	353	7	1	2	200	1.182
675	353	8	1	2	200	1.187
676	353	9	1	2	200	1.158
677	353	10	1	1	200	1.167
678	353	11	1	2	200	1.151
679	353	12	1	1	200	1.182
680	353	13	1	1	200	1.226
681	366	1	1	2	200	1.212
682	366	2	1	1	200	1.263
683	366	3	1	1	200	1.378
684	366	4	1	2	200	1.178

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685	366	5	1	2	200	1.27
686	366	6	1	1	200	1.21
687	366	7	1	1	200	1.192
688	366	8	1	1	200	1.226
689	366	9	1	2	200	1.133
690	366	10	2		200	
691	366	11	1	1	200	1.232
692	366	12	1	1	200	1.144
693	366	13	2		200	
694	366	14	1	2	200	1.189
695	371	1	1	1	200	1.091
696	371	2	1	1	200	1.093
697	371	3	1	2	200	0.902
698	371	4	1	2	200	0.976
699	371	5	1	1	200	1.012
700	371	6	1	2	200	0.935
701	371	7	1	2	200	0.987
702	371	8	1	2	200	1.002
703	371	9	1	1	200	1.022
704	371	10	1	2	200	0.984
705	371	11	1	2	200	1.001
706	371	12	1	1	200	1.055
707	371	13	1	1	200	0.973
708	371	14	1	1	200	1.068
709	371	15	1	2	200	0.897
710	382	1	1	2	200	1.548
711	382	2	1	2	200	1.325
712	392	1	1	1	200	1.206
713	392	2	1	1	200	1.253
714	392	3	1	2	200	1.214
715	392	4	1	1	200	1.295
716	392	5	1	2	200	1.087
717	392	6	1	2	200	1.052
718	392	7	1	1	200	1.114
719	392	8	1	2	200	1.135
720	392	9	1	2	200	1.123
721	392	10	1	1	200	1.252
722	392	11	1	1	200	1.134
723	392	12	1	2	200	1.132
724	392	13	1	1	200	1.168
725	392	14	1	2	200	1.176
726	392	15	1	1	200	1.24
727	392	16	1	2	200	1.198
728	402	1	1	2	200	0.903
729	402	2	1	1	200	1.208
730	402	3	1	1	200	1.093

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731	402	4	1	2	200	1.078
732	402	5	1	1	200	1.052
733	402	6	1	2	200	1.098
734	402	7	1	2	200	0.941
735	402	8	1	1	200	1.109
736	402	9	1	2	200	1.051
737	402	10	1	2	200	1.164
738	402	11	1	1	200	1.135
739	402	12	1	2	200	0.933
740	402	13	2		200	
741	402	14	1	2	200	1.061
742	402	15	1	1	200	1.071
743	402	16	1	1	200	1.072
744	420	1	1	1	200	1.294
745	420	2	1	1	200	1.289
746	420	3	2		200	
747	420	4	1	1	200	1.284
748	420	5	2		200	
749	420	6	1	2	200	1.16
750	420	7	1	1	200	1.144
751	420	8	1	1	200	1.263
752	420	9	1	1	200	1.239
753	420	10	1	1	200	1.13
754	420	11	1	2	200	1.143
755	420	12	1	1	200	1.192
756	420	13	1	1	200	1.306
757	231	1	2		1000	
758	231	2	1	1	1000	1.112
759	231	3	1	2	1000	0.932
760	231	4	1	2	1000	1.063
761	231	5	1	2	1000	1.026
762	231	6	1	2	1000	0.955
763	231	7	1	1	1000	1.051
764	231	8	1	2	1000	1.036
765	231	9	1	1	1000	1.038
766	231	10	1	1	1000	1.046
767	243	1	1	2	1000	0.982
768	243	2	1	2	1000	0.96
769	243	3	1	2	1000	1.016
770	243	4	1	1	1000	1.13
771	243	5	1	2	1000	0.949
772	243	6	1	2	1000	1.046
773	243	7	1	1	1000	1.003
774	243	8	1	1	1000	0.998
775	243	9	1	1	1000	1.001
776	243	10	1	2	1000	1.077

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777	243	11	1	2	1000	1.028
778	243	12	1	2	1000	1.041
779	243	13	1	2	1000	1.018
780	243	14	1	1	1000	1.037
781	244	1	1	1	1000	0.934
782	244	2	1	2	1000	0.537
783	244	3	1	2	1000	0.862
784	244	4	1	2	1000	0.746
785	244	5	1	2	1000	0.889
786	244	6	1	1	1000	0.942
787	244	7	1	2	1000	0.948
788	244	8	1	1	1000	0.885
789	244	9	1	2	1000	1
790	244	10	1	1	1000	0.925
791	244	11	1	1	1000	1.08
792	244	12	2		1000	
793	255	1	1	2	1000	1.067
794	255	2	1	2	1000	1.13
795	255	3	1	1	1000	1.081
796	255	4	1	1	1000	1.073
797	255	5	1	1	1000	1.056
798	255	6	1	2	1000	1.034
799	255	7	1	1	1000	1.087
800	255	8	1	2	1000	1.078
801	255	9	1	2	1000	1.058
802	255	10	1	1	1000	1.087
803	255	11	1	1	1000	1.133
804	255	12	1	1	1000	1.131
805	264	1	1	1	1000	1.067
806	264	2	1	1	1000	0.901
807	264	3	1	2	1000	1.057
808	264	4	1	2	1000	0.98
809	264	5	1	2	1000	1.036
810	264	6	1	2	1000	0.856
811	264	7	1	2	1000	0.937
812	264	8	1	1	1000	0.95
813	264	9	1	2	1000	1.09
814	264	10	1	1	1000	1.162
815	264	11	1	2	1000	1.074
816	264	12	1	1	1000	1.004
817	264	13	1	1	1000	1.083
818	264	14	1	2	1000	1.001
819	276	1	1	1	1000	0.862
820	276	2	1	2	1000	0.93
821	276	3	1	2	1000	0.786
822	276	4	1	2	1000	0.783

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823	276	5	4		1000	
824	276	6	1	2	1000	0.809
825	276	7	1	1	1000	0.737
826	276	8	1	1	1000	0.964
827	276	9	1	1	1000	1
828	276	10	1	1	1000	0.84
829	276	11	1	1	1000	0.85
830	276	12	1	1	1000	0.695
831	276	13	1	1	1000	0.934
832	276	14	1	2	1000	0.79
833	276	15	1	1	1000	0.915
834	294	1	1	1	1000	1.047
835	294	2	1	1	1000	1.164
836	294	3	1	1	1000	1.091
837	294	4	1	2	1000	0.917
838	294	5	1	1	1000	1.065
839	294	6	1	1	1000	1.059
840	294	7	1	2	1000	0.934
841	294	8	1	1	1000	1.042
842	294	9	1	2	1000	0.956
843	294	10	1	2	1000	1.029
844	294	11	1	1	1000	0.958
845	294	12	1	2	1000	0.894
846	294	13	1	2	1000	1.032
847	294	14	1	2	1000	0.951
848	294	15	1	2	1000	1.02
849	305	1	1	1	1000	1.197
850	305	2	1	1	1000	1.021
851	305	3	1	2	1000	1.031
852	305	4	1	2	1000	0.924
853	305	5	1	2	1000	1.106
854	305	6	1	1	1000	1.029
855	305	7	1	1	1000	1.127
856	305	8	1	1	1000	1.23
857	305	9	1	1	1000	1.054
858	305	10	1	2	1000	0.996
859	305	11	1	2	1000	0.952
860	305	12	1	1	1000	0.991
861	309	1	1	2	1000	1.045
862	309	2	1	2	1000	1.062
863	309	3	1	1	1000	1.165
864	309	4	1	1	1000	1.076
865	309	5	1	1	1000	1.106
866	309	6	1	2	1000	1.054
867	309	7	1	2	1000	1.097
868	309	8	1	2	1000	1.09

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869	309	9	1	1	1000	1.086
870	309	10	1	1	1000	1.097
871	309	11	1	1	1000	1.153
872	309	12	1	2	1000	1.183
873	309	13	1	2	1000	1.019
874	309	14	1	1	1000	1.072
875	309	15	1	1	1000	1.017
876	309	16	1	1	1000	1.105
877	317	1	1	1	1000	1.046
878	317	2	1	1	1000	1.071
879	317	3	1	1	1000	
880	317	4	1	1	1000	1.055
881	317	5	1	1	1000	1.054
882	317	6	1	1	1000	1.08
883	317	7	1	2	1000	0.902
884	317	8	1	1	1000	0.806
885	317	9	1	2	1000	0.982
886	317	10	1	2	1000	1.034
887	317	11	1	1	1000	
888	317	12	1	1	1000	1.018
889	317	13	1	2	1000	1.031
890	317	14	1	1	1000	1.006
891	325	1	1	1	1000	1.076
892	325	2	2		1000	
893	325	3	1	2	1000	1.156
894	325	4	1	1	1000	1.128
895	325	5	1	2	1000	1.129
896	325	6	1	2	1000	1.082
897	325	7	1	2	1000	1.176
898	325	8	1	1	1000	1.037
899	325	9	1	1	1000	1.187
900	325	10	1	1	1000	1.08
901	325	11	1	1	1000	1.134
902	325	12	1	1	1000	1.068
903	325	13	1	1	1000	1.003
904	325	14	1	1	1000	0.935
905	325	15	1	2	1000	0.985
906	340	1	1	1	1000	1.071
907	340	2	1	1	1000	1.106
908	340	3	1	2	1000	1.078
909	340	4	1	1	1000	1.112
910	340	5	1	2	1000	1.045
911	340	6	1	1	1000	1.017
912	340	7	2		1000	
913	340	8	1	1	1000	1.09
914	340	9	2		1000	

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915	340	10	1	2	1000	1.143
916	340	11	1	1	1000	1.138
917	340	12	1	2	1000	1.069
918	340	13	1	1	1000	1.056
919	365	1	1	1	1000	0.846
920	365	2	1	2	1000	0.829
921	365	3	1	2	1000	0.937
922	365	4	1	2	1000	0.688
923	365	5	1	2	1000	0.868
924	365	6	1	2	1000	0.57
925	365	7	1	1	1000	0.839
926	365	8	1	2	1000	0.945
927	365	9	1	1	1000	0.902
928	365	10	1	1	1000	0.818
929	365	11	2		1000	
930	374	1	2		1000	
931	374	2	1	2	1000	1.022
932	374	3	1	2	1000	1.048
933	374	4	1	1	1000	1.091
934	374	5	1	1	1000	1.048
935	374	6	1	1	1000	1.15
936	374	7	1	1	1000	1.201
937	374	8	1	1	1000	1.068
938	374	9	1	1	1000	1.092
939	374	10	1	1	1000	1.077
940	377	1	1	2	1000	1.189
941	377	2	1	1	1000	1.129
942	377	3	1	2	1000	1.049
943	377	4	1	2	1000	1.127
944	377	5	1	1	1000	0.985
945	377	6	2		1000	
946	377	7	1	1	1000	1.056
947	377	8	1	1	1000	1.248
948	377	9	1	1	1000	1.02
949	377	10	1	2	1000	1.188
950	377	11	1	2	1000	1.082
951	377	12	1	2	1000	1.025
952	377	13	1	2	1000	1.155
953	389	1	1	1	1000	1.087
954	389	2	1	2	1000	1.025
955	389	3	1	2	1000	1.074
956	389	4	1	2	1000	1.14
957	389	5	2		1000	
958	389	6	1	2	1000	1.015
959	389	7	1	2	1000	0.945
960	389	8	1	1	1000	1.191

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961	389	9	1	1	1000	1.182
962	389	10	4		1000	
963	389	11	2		1000	
964	400	1	1	2	1000	1.276
965	400	2	1	2	1000	1.375
966	400	3	1	1	1000	1.341
967	400	4	1	1	1000	1.468
968	400	5	1	2	1000	1.349
969	400	6	2		1000	
970	400	7	1	1	1000	1.249
971	400	8	2		1000	
972	400	9	1	1	1000	1.358
973	400	10	1	1	1000	1.368
974	400	11	1	1	1000	1.415
975	400	12	1	2	1000	1.3
976	427	1	1	2	1000	1.119
977	427	2	1	1	1000	1.235
978	427	3	1	1	1000	1.222
979	427	4	1	1	1000	1.153
980	427	5	1	1	1000	1.078
981	427	6	1	2	1000	1.032
982	427	7	1	1	1000	0.975
983	427	8	1	1	1000	1.092
984	427	9	1	1	1000	1.217
985	427	10	1	2	1000	1.121
986	427	11	1	2	1000	1.105
987	427	12	1	1	1000	1.172
988	427	13	2		1000	
989	427	14	1	1	1000	1.188
990	428	1	1	2	1000	1.017
991	428	2	1	1	1000	0.965
992	428	3	1	2	1000	1.044
993	428	4	1	2	1000	0.993
994	428	5	1	2	1000	0.971
995	428	6	1	1	1000	1.011
996	428	7	1	2	1000	0.928
997	428	8	1	2	1000	0.956
998	428	9	2		1000	
999	428	10	1	2	1000	1.069
1000	428	11	1	2	1000	0.935
1001	428	12	1	2	1000	0.982
1002	445	1	1	1	1000	0.889
1003	445	2	4		1000	
1004	445	3	1	2	1000	0.926
1005	445	4	1	1	1000	1.105
1006	445	5	1	1	1000	1.058

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1007	445	6	1	2	1000	
1008	445	7	1	2	1000	1.039
1009	445	8	1	1	1000	1.067
1010	445	9	1	2	1000	0.983
1011	445	10	1	1	1000	1.056
1012	445	11	1	2	1000	0.977
1013	445	12	1	2	1000	0.975
1014	445	13	1	2	1000	0.993
1015	445	14	2		1000	
1016	445	15	1	2	1000	1.021
1017	445	16	1	2	1000	0.53
1018	445	17	1	1	1000	1.03

Some 6's were 8's and have been corrected for fetalwt  
Animal 445 Pup 14 had status 2 and FetalSex=2 in report,  
it was changed to FetalSex missing

## Appendix 2. BMC Modeling for Acute ReV

**Table 2A. Dose-Response Data for Maternal Toxicity Endpoints**

Dose (ppm)	Mean	Number of Litters	Calculated Standard Deviation **	Standard Error	% Control response	Coefficient of Variation (CV)
<b>Whole-body weight (gm) day 18</b>						
0	54.90	18	5.134	1.21	100%	0.09
40	55.40	19	4.751	1.09	101%	0.09
200	52.50	21	4.628	1.01	96%	0.09
1000	50.80	20	3.846	0.86	93%	0.08
<b>Extragestational weight gain (gm)</b>						
0	7.60	18	2.036	0.48	100%	0.27
40	6.99	19	1.656	0.38	92%	0.24
200	6.20	21	1.741	0.38	82%	0.28
1000	5.91	20	1.252	0.28	78%	0.21
<b>Body weight gain (gm) gestation days 11-16</b>						
0	13.30	18	2.546	0.60	100%	0.19
40	12.70	19	1.744	0.40	95%	0.14
200	11.40	21	2.291	0.50	86%	0.20
1000	10.60	20	1.789	0.40	80%	0.17
<b>Gravid uterine weight (gm)</b>						
0	19.30	18	4.243	1.00	100%	0.22
40	20.30	19	3.487	0.80	105%	0.17
200	18.00	21	3.987	0.87	93%	0.22
1000	16.80	20	2.996	0.67	87%	0.18
<b>Extragestational weight (gm)</b>						
0	35.50	18	2.036	0.48	100%	0.06
40	35.10	19	1.918	0.44	99%	0.05
200	34.50	21	2.108	0.46	97%	0.06
1000	34.10	20	1.610	0.36	96%	0.05
* Hackett et al. (1987b)						
** Standard deviation = standard error x square root of number of litters						

**Table 2B. Dose-Response Data Fetal Toxicity Endpoints**

Dose (ppm)	Mean	Number of Litters	Calculated Standard Deviation **	Standard Error	% Control response	Coefficient of Variation (CV)
<b>Mean placental weight (mg) males and females (mean per litter)</b>						
0	86.80	18	12.685	2.99	100%	0.15
40	85.40	19	9.982	2.29	98%	0.12
200	78.60	21	14.848	3.24	91%	0.19
1000	72.60	20	8.408	1.88	84%	0.12
<b>Mean fetal weight (gm) males and females (mean per litter)</b>						
0	1.34	18	0.127	0.03	100%	0.09
40	1.28	19	0.044	0.01	96%	0.03
200	1.13	21	0.092	0.02	84%	0.08
1000	1.04	20	0.134	0.03	78%	0.13
<b>Abnormal sternebrae (mean percent per litter)</b>						
0	0.60	18	0.900		100%	1.50
40	0.40	19	0.700		67%	1.75
200	0.40	21	0.800		67%	2.00
1000	0.80	20	1.300		133%	1.63
<b>Supernumerary ribs (mean percent per litter)</b>						
0	1.70	18	2.300		100%	1.35
40	1.60	19	2.100		94%	1.31
200	6.00	21	3.600		353%	0.60
1000	9.90	20	3.000		582%	0.30
<b>Reduced ossification (all sites combined) (mean percent per litter)</b>						
0	1.70	18	1.700		100%	1.00
40	1.20	19	1.500		71%	1.25
200	2.70	21	2.700		159%	1.00
1000	3.90	20	2.600		229%	0.67
* Hackett et al. (1987b)						
** Standard deviation = standard error x square root of number of litters						

**Table 2C. Summary of BMC Modeling Results for Maternal Effects**

Linear Model 4 doses		Linear Model 3 doses		Unrestricted Power Model 4 doses	
<b>Whole-body weight (gm) Day 18</b>					
homogeneous variance		homogeneous variance		homogeneous variance	
Test 1 0.02995		Test 1 0.3509	X	Test 1 0.02995	
Test 2 0.6543		Test 2 0.9013		Test 2 0.6543	
Test 3 0.6543		Test 3 0.9013		Test 3 0.6543	
Test 4 0.2575		Test 4 0.4884		Test 4 0.1941	
AIC	320.569569			AIC	321.54225
Scaled residual	< abs value of 2			Scaled residual	< abs value of 2
BMC10 =	1343.75			BMC10 =	1403.64
BMCL10 =	895.747			BMCL10 =	598.977
BMC 1 SD =	1120.78			BMC 1 SD =	962.47
BMCL 1SD =	732.341			BMCL 1SD =	304.564
<b>Extragestational weight gain (gm)</b>					
homogeneous variance		homogeneous variance		homogeneous variance	
Test 1 0.01364		Test 1 0.1505	X	Test 1 0.01364	
Test 2 0.2158		Test 2 0.6481		Test 2 0.2158	
Test 3 0.2158		Test 3 0.6481		Test 3 0.2158	
Test 4 0.0927	X	Test 4 0.5343		Test 4 0.4245	
				AIC	164.106882
				Scaled residual	< abs value of 2
				BMC10 =	31.362
				BMCL10 =	3.46E-05
				BMC 1 SD =	722.796
				BMCL 1SD =	51.3032
<b>Body weight gain (gm) Days 11-16</b>					
homogeneous variance		homogeneous variance		homogeneous variance	
Test 1 0.001187		Test 1 0.03683		Test 1 0.001187	
Test 2 0.2651		Test 2 0.2566		Test 2 0.2651	
Test 3 0.2651		Test 3 0.2566		Test 3 0.2651	
Test 4 0.07957	X	Test 4 0.7342		Test 4 0.339	
		AIC	153.301194	AIC	199.608973
		Scaled residual	< abs value of 2	Scaled residual	< abs value of 2
		BMC10 =	145.382	BMC10 =	108.232
		BMCL10 =	94.2853	BMCL10 =	5.96473
		BMC 1 SD =	237.988	BMC 1 SD =	392.348
		BMCL 1SD =	148.203	BMCL 1SD =	63.495
<b>Gravid uterine weight (gm)</b>					
homogeneous variance		homogeneous variance		homogeneous variance	
Test 1 0.05228	X	Test 1 0.369	X	Test 1 0.05228	X
Test 2 0.4485		Test 2 0.6955		Test 2 0.4485	
Test 3 0.4485		Test 3 0.6955		Test 3 0.4485	
Test 4 0.2653		Test 4 0.2733		Test 4 0.1333	
<b>Extragestational weight (gm)</b>					
homogeneous variance		homogeneous variance		homogeneous variance	
Test 1 0.263	X	Test 1 0.6113	X	Test 1 0.263	X
Test 2 0.6542		Test 2 0.9104		Test 2 0.6542	
Test 3 0.6542		Test 3 0.9104		Test 3 0.6542	
Test 4 0.4253		Test 4 0.7356		Test 4 0.6608	
<p>X = Test 1-4 results unacceptable      <b>Test 1</b> p values &gt; 0.05; <b>Test 2</b> determines whether a homogeneous or nonhomogeneous variance applies (p &gt; 0.1 = homogeneous variance, p &lt; 0.1 = nonhomogeneous variance); <b>Test 3</b> p value &lt; 0.1; <b>Test 4</b> goodness of fit p value &lt; 0.1</p>					

**Table 2D. Summary of BMC Modeling Results for Fetal Effects**

Linear Model 4 doses		Linear Model 3 doses		Unrestricted Power Model 4 doses	
<b>Mean placental weight per litter (mg) males and females</b>					
nonhomogeneous variance *		homogeneous variance		nonhomogeneous variance *	
Test 1 0.0004354		Test 1 0.09712	X	Test 1 0.0004354	
Test 2 0.05768		Test 2 0.215		Test 2 0.05768	
Test 3 0.04312	X	Test 3 0.215		Test 3 0.04312	X
Test 4 0.7669		Test 4 0.9487		Test 4 0.9837	
AIC 466.096041				AIC 467.565607	
Scaled residual < abs value of 2				Scaled residual < abs value of 2	
BMC05 = 344.446				BMC05 = 123.276	
BMCL05 = 255.57				BMCL05 = 4.16675	
BMC 1 SD = 1063.26				BMC 1 SD = 874.047	
BMCL 1SD = 733.771				BMCL 1SD = 233.341	
<b>Mean fetal weight per litter (gm) males and females</b>					
nonhomogeneous variance		nonhomogeneous variance *		nonhomogeneous variance	
Test 1 <.0001		Test 1 <.0001		Test 1 <.0001	
Test 2 <.0001		Test 2 0.0001236		Test 2 <.0001	
Test 3 <.0001	X	Test 3 <.0001	X	Test 3 <.0001	X
Test 4 <.0001	X	Test 4 0.3503		Test 4 0.01814	X
		AIC -212.273267			
		Scaled residual < abs value of 2			
		BMC05 = 65.7926			
		BMCL05 = 54.7521			
		BMC 1 SD = 94.7601			
		BMCL 1SD = 71.78			
<b>Abnormal sternebrae (Mean percent per litter)</b>					
nonhomogeneous variance		homogeneous variance		nonhomogeneous variance	
Test 1 0.07281	X	Test 1 0.7441	X	Test 1 0.07281	X
Test 2 0.02859		Test 2 0.5637		Test 2 0.02859	
Test 3 0.9033		Test 3 0.5637		Test 3 0.9033	
Test 4 0.2526		Test 4 0.4958		Test 4 0.1857	
<b>Supernumerary ribs (Mean percent per litter)</b>					
nonhomogeneous variance		nonhomogeneous variance		nonhomogeneous variance	
Test 1 <.0001		Test 1 <.0001		Test 1 <.0001	
Test 2 0.06411		Test 2 0.02879		Test 2 0.06411	
Test 3 0.364		Test 3 0.8166		Test 3 0.364	
Test 4 <.0001	X	Test 4 0.07209	X	Test 4 0.001961	X
<b>Reduced ossification (all sites combined) (Mean percent per litter)</b>					
nonhomogeneous variance		nonhomogeneous variance		nonhomogeneous variance	
Test 1 0.0002402		Test 1 0.008605		Test 1 0.0002402	
Test 2 0.02047		Test 2 0.01733		Test 2 0.02047	
Test 3 0.6049		Test 3 0.6737		Test 3 0.6049	
Test 4 0.01897	X	Test 4 0.08082	X	Test 4 0.01417	X
<p>X = Test 1-4 results unacceptable      <b>Test 1</b> p values &gt; 0.05; <b>Test 2</b> determines whether a homogeneous or nonhomogeneous variance applies (p &gt; 0.1 = homogeneous variance, p &lt; 0.1 = nonhomogeneous variance); <b>Test 3</b> p value &lt; 0.1; <b>Test 4</b> goodness of fit p value &lt; 0.1</p>					
<p>* Both a nonhomogeneous and homogeneous variance were used to model the data. The scaled residuals for a nonhomogeneous variance produced slightly smaller scaled residuals in the low-dose region of the dose response curve, so the results from a nonhomogeneous variance are reported.</p>					

**Table 2E. Table of Data and Estimated Values of Interest**

<b>Whole-body weight Day 18 Linear Model 4 doses</b>							<b>Mean placental weight per litter Linear Model 4 doses</b>						
Table of Data and Estimated Values of Interest							Table of Data and Estimated Values of Interest						
Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.	Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	18	54.9	54.6	5.13	4.56	0.256	0	18	86.8	84.5	12.7	13	0.746
40	19	55.4	54.5	4.75	4.56	0.897	40	19	85.4	84	9.98	12.8	0.469
200	21	52.5	53.8	4.63	4.56	-1.32	200	21	78.6	82.1	14.9	12.1	-1.31
1000	20	50.8	50.6	3.85	4.56	0.236	1000	20	72.6	72.2	8.41	8.6	0.187
<b>Whole-body weight Day 18 Unrestricted Power Model 4 doses</b>							<b>Mean placental weight per litter Unrestricted Power Model 4 Doses</b>						
Table of Data and Estimated Values of Interest							Table of Data and Estimated Values of Interest						
Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.	Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	18	54.9	55.2	5.13	4.53	-0.324	0	18	86.8	86.3	12.7	13.1	0.156
40	19	55.4	54.4	4.75	4.53	0.961	40	19	85.4	84	9.98	12.4	0.476
200	21	52.5	53.3	4.63	4.53	-0.781	200	21	78.6	80.6	14.9	11.5	-0.814
1000	20	50.8	50.6	3.85	4.53	0.171	1000	20	72.6	72.1	8.41	9.17	0.224
<b>Extragastrational weight gain Unrestricted Power Model 4 doses</b>							<b>Mean fetal weight per litter Linear Model - 3 doses</b>						
Table of Data and Estimated Values of Interest							Table of Data and Estimated Values of Interest						
Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.	Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	18	7.6	7.62	2.04	1.65	-0.052	0	18	1.34	1.33	0.127	0.0958	0.408
40	19	6.99	6.81	1.66	1.65	0.473	40	19	1.28	1.29	0.044	0.0936	-0.481
200	21	6.2	6.42	1.74	1.65	-0.605	200	21	1.13	1.13	0.092	0.0844	0.0803
1000	20	5.91	5.83	1.25	1.65	0.209							
<b>Body weight gain (GD11-16) Linear Model 3 doses</b>							<b>Body weight gain (GD11-16) Unrestricted Power Model 4 doses</b>						
Table of Data and Estimated Values of Interest							Table of Data and Estimated Values of Interest						
Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.	Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	18	13.3	13.2	2.55	2.16	0.213	0	18	13.3	13.4	2.55	2.07	-0.114
40	19	12.7	12.8	1.74	2.16	-0.259	40	19	12.7	12.4	1.74	2.07	0.622
200	21	11.4	11.4	2.29	2.16	0.0494	200	21	11.4	11.7	2.29	2.07	-0.684
							1000	20	10.6	10.5	1.79	2.07	0.202

## **Benchmark Modeling Results Using the Power Model (11/19/07 Email from Bruce Allen)**

**From:** "Bruce Allen" <bruce\_allen@verizon.net>  
**To:** "Roberta Grant" <RGrant@tceq.state.tx.us>  
**Date:** 11/19/2007 8:10:50 AM  
**Subject:** RE: Benchmark modeling results using the power model

Dr. Grant,

Sorry to take so long in getting back to you. Your table is correct on the estimates from the unrestricted power model. Note that the AIC values are all the same, because you are fitting the same model to the same data set each time; changing the definition of the BMR does not change any of that. And, as the full output shows, the fit to the data points is quite good.

As to the differences in the BMC and BMCL - that is totally a product of the curve shape and it can become pronounced when an unrestricted model is used. In such a model, you can get very steep initial (low-dose) slopes and in the search for lower bounds, such a model allows for even greater initial slopes among the candidates that might give the BMCL. So, many people have avoided such models because they can indeed give bigger differences between the BMC and BMCL.

The reason that the restricted model does not give such big differences is the fact the restriction essentially makes the linear fit the worst case (low-dose slope does not get progressively larger). So, as in this case, when the best fit is linear, then the search for lower bounds cannot include anything more extreme than a linear fit and the class of possible model parameter values that gives a good enough likelihood (in the BMCL determination) only includes some slightly steeper (but still linear) dose response shapes.

This, to me, is an arbitrary constraint, especially when the fit to the data is so bad with a restricted model. The results indicate a highly nonlinear dose-response, so why not let the model capture that behavior? That is what the unrestricted model does. Or (probably not an option here) find a better model that does not allow for extreme low-dose shapes but still does capture the observed dose-response pattern.

So, I am left with the impression that the unrestricted model fit to all the data points is still the best - but I would stick with a BMR defined in terms of 1 sd change. As we discussed on the phone, that allows for consistency across endpoints and assessments. And, in this particular case, it does not get you into the region where the low-dose shape is too dominant in determining what your BMCL is. That may not always be the case, but it does help you here. Just my 2 cents.

Bruce

\_\_\_\_\_

From: Roberta Grant [mailto:RGrant@tceq.state.tx.us]  
Sent: Wednesday, October 31, 2007 10:22 AM  
To: bruce\_allen@verizon.net  
Cc: Joan Strawson; Angela Curry; Joseph Haney; Michael Honeycutt

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Subject: Benchmark modeling results using the power model

Dr. Allen, it was a pleasure to participate in the teleconference with you yesterday. Your comments and suggestions were very helpful. There was a question about the output from the unrestricted power model using a BMR of 1 x SD, 0.77 x SD, and a 10% reduction. I've attached the BMCL modeling results using these different BMR rates. Using four doses and an unrestricted power model, these are the values I get:

Decrease in Extragestational Weight Gain 4 Doses  
Bmc bmcl AIC

Unrestricted Power 1 x SD  
722.8 51.3 164.1

Unrestricted Power 0.77 x SD  
250.2 1.89 164.1

Unrestricted Power 10% reduction  
31.36 3.45E-05 164.1

As discussed yesterday during the teleconference, I get exactly the same results as you did when using the BMR of 1 x SD, but very different results when using 0.77 x SD. I notice that there are big differences between the BMC and BMCL values. Is that normal or acceptable? I notice that for the restricted power model, the differences between the BMC and BMCL are generally less than two (see attached "extragestational review table").

Again, thanks for your comments. Roberta

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## Appendix 3. Statistical Analyses of Reproductive Endpoints

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**TCEQ Contract 582-7-81521**

EPA's 2002 final risk assessment for BD (USEPA. 2002. Health Assessment of 1,3-Butadiene. EPA/600/P-98/001F) derived a reference concentration using the ovarian atrophy in female mice exposed to butadiene via inhalation. This animal study was conducted by the NTP in 1993 (NTP. 1993. Toxicology and carcinogenesis studies of 1,3-butadiene (CAS No. 106-99-0) in B6C3F1 mice (inhalation studies). Research Triangle Park, NC: National Toxicology Program, U.S. Public Health Service, U.S. Department of Health and Human Services. TR 434). EPA used a Weibull time-to-tumor dose-response model to fit the time-to-ovarian atrophy data and excluded the highest dose group because of excessive early mortality. The ECs and LECs for ovarian atrophy were calculated at an equivalent human age of 50 years "to reflect only the time before average age at menopause when follicles are no longer present and available for ovulation, because in the mouse studies of ovarian atrophy, the atrophy occurs as a result of follicular failure."

In the NTP 1993 critical study, female mice were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm BD for 6 hours/day, 5 days/week for two years (i.e., equivalent to 0, 1.12, 3.57, 11.2, 35.7, and 111.6 ppm BD of continuous exposure – for example,  $6.25 \times (5/7) \times (6/24) = 1.12$ ). The air concentration 6.25 ppm was identified as a LOAEL for ovarian atrophy. The final 2002 EPA's risk assessment for BD reports several analyses of these data, including application of a log-logistic model, a quantal Weibull model, and a Weibull time-to-response model.

The final Weibull time-to-response model that EPA used is linear in dose with time raised to a power. EPA used TOX\_RISK version 3.5 (Crump *et al.*, ICF Kaiser International, Ruston, LA) for the model fitting and the estimation of the ECs and LECs. In February 2006, the Olefins Panel of the American Chemistry Council asked the Sapphire Group, Inc. to recalculate EPA's ECs and LECs for ovarian atrophy (Kirman, C. R. and M. L. Gargas. 2006. Benchmark Dose Analyses for Reproductive and Developmental Endpoints for 1,3-Butadiene, Submitted to Olefins Panel, American Chemistry Council, Arlington, VA, February 2006). The Sapphire Group, Inc.'s report included the time-to-response data for ovarian atrophy of the NTP 1993 study, and those data are reproduced here in Attachment A.

Sielken & Associates Consulting, Inc. reanalyzed the ovarian atrophy data using the Weibull time-to-response model and the data presented in Attachment A. The linear Weibull time-to-response model had the following form:

$$\text{Probability of a response (ovarian atrophy) by week T at dose d} = 1 - \exp \{ - [ Q_0 + Q_1 \times d ] \times T^Z \}.$$

Tables 1 and 2 list the results of the analyses when the highest exposure group is not included in the estimation of the model and when all exposure groups are included, respectively. The results labeled SA# were calculated using Sielken & Associates, Inc.'s GEN.T software package – however, Sielken & Associates verified that the parameter estimates are identical to those estimated with TOX\_RISK version 3.5. The LEC<sub>10</sub> values for the SA# analyses in the table were estimated using 99 simulated bootstrap data sets. The two analyses in addition to EPA's analyses included in Tables 1 and 2 are:

- 1) Analysis SA1 parallels the analysis performed by EPA. The small discrepancies between the SA1 and EPA analyses may be due to assumptions that EPA may have made and did not describe in their report.
- 2) Analysis SA2 uses a modified data set in which all animals that lived beyond age 521 days (74.3 weeks – which is equivalent to 50 years in a 70-year human lifetime -- (50/70) × 104 weeks) were excluded from the parameter estimation.

In Tables 1 and 2, the range of EC<sub>10</sub> values derived by EPA, SA1, and SA2 analyses is 1.05 to 1.25 ppm whereas the range of the LEC<sub>10</sub> values derived by EPA, SA1, and SA2 analyses is 0.768 to 0.958 ppm.

Table 1 and 2 also show the results for concentrations corresponding to an extra risk of 0.05. Because the Weibull time-to-tumor model in these analyses is linear in dose, the EC<sub>05</sub> and LEC<sub>05</sub> values are approximately half the corresponding EC<sub>10</sub> and LEC<sub>10</sub> values.

**Table 1.** Parameters (Q<sub>0</sub>, Q<sub>1</sub>, and Z) for Weibull time-to-response model for ovarian atrophy and corresponding human benchmark 1,3-butadiene exposure concentrations for extra risks of 0.1 and 0.05 at 50 years of age using different methods of calculation – **excluding** the highest dose group

Analysis	Q <sub>0</sub>	Q <sub>1</sub>	Z	EC <sub>10</sub>	LEC <sub>10</sub>	EC <sub>05</sub>	LEC <sub>05</sub>
EPA	4.86×10 <sup>-6</sup>	7.06×10 <sup>-6</sup>	2.21	1.05	0.878	n/a	n/a
SA1	6.96×10 <sup>-6</sup>	8.62×10 <sup>-6</sup>	2.15	1.15	0.881	0.560	0.429
SA2	6.76×10 <sup>-23</sup>	6.90×10 <sup>-5</sup>	1.66	1.18	0.768	0.573	0.374

**Table 2.** Parameters for Weibull time-to-response model for ovarian atrophy and corresponding human benchmark 1,3-butadiene exposure concentrations for extra risks of 0.1 and 0.05 at 50 years of age using different methods of calculation – **including** the highest dose group

Analysis	Q <sub>0</sub>	Q <sub>1</sub>	Z	EC <sub>10</sub>	LEC <sub>10</sub>	EC <sub>05</sub>	LEC <sub>05</sub>
EPA	9.01×10 <sup>-6</sup>	1.32×10 <sup>-6</sup>	2.58	1.13	0.958	n/a	n/a
SA1	1.68×10 <sup>-6</sup>	2.04×10 <sup>-6</sup>	2.47	1.25	0.949	0.607	0.462
SA2	3.61×10 <sup>-25</sup>	1.95×10 <sup>-6</sup>	2.49	1.17	0.812	0.569	0.396

The estimated values of  $EC_{10}$  and  $LEC_{10}$  are close to the lowest experimental dose (1.12 ppm) while the values of  $EC_{05}$  and  $LEC_{05}$  are approximately half way between the lowest experimental dose and zero. The values of  $EC_{05}$  and  $LEC_{05}$  can be used if the dose-response relationship below the lowest experimental dose is believed to be the linear Weibull time-to-response model fit to the data. The assumption of linearity below the lowest experimental dose is usually conservative and, therefore, health protective. However, the motivation behind the benchmark dose methodology is to identify the point of departure (EC or LEC) to be within the range of the experimental data (the range of the non-zero doses in the experimental data) and to be a dose whose risk can be reasonably reliably estimated without undue sensitivity to the dose-response model selected or the model estimation. Here, the  $EC_{05}$  and  $LEC_{05}$  in the SA1 and SA2 analyses are below the range of the experimental data and, hence, introduce an additional element of uncertainty into the point of departure.

The EPA and SA1 analyses include ovarian atrophy responses beyond the equivalent of age 50 years in humans. These older-age responses in mice may not be relevant to humans and may inappropriately impact the fitted dose-response model used to estimate the risk at age 50. SA2 eliminates all animals that lived beyond the equivalent of age 50. However, it is known that some of these animals did not have an observed response (ovarian atrophy) and this information is ignored/lost and not incorporated into the dose-response modeling as it should be. The fitted models for all the mice (analyses SA1) are very similar to the fitted models for only mice that died on or before week 74.3 (analyses SA2). This suggests that the older-age animals in the SA1 analyses are not distorting those analyses. Therefore, the results for analyses SA1 are preferable to the SA2 analyses because the SA1 analyses include more data (i.e., mice that lived past 74.3 weeks) and the inclusion of mice older than 74.3 weeks does not distort the fit of the model. In other words, the models fit to either all the mice (analyses SA1) or only to mice that died on or before week 74.3 are (analyses SA2) very similar but the confidence limits for analyses SA1 are more reliable because they are based on more animals.

The ovarian atrophy data were analyzed excluding the highest dose group (Table 1) and also including all the data (Table 2). The analyses that exclude the high dose were performed to parallel those analyses used by EPA. Traditionally, EPA drops the highest dose group when the model does not fit the data well due to some biological phenomenon or when quantal data are fit with a quantal model and there is high mortality in the highest dose group. The ovarian atrophy data, however, were modeled with a time-to-response model (i.e., a model that accounts for the time of death) as opposed to a quantal model which do not account for time of death. Furthermore, the model fit to the data that excluded the highest dose group was not better than the model fit to the data that included the highest dose group. Figure 1 shows the fit of analysis SA1 to the lower four dose groups and the control group while Figure 2 shows the fit of analysis SA1 to all dose groups and the control group.

In summary, the SA1 analysis in Table 2 that includes all the exposure groups and all animals in each exposure group is the most statistically sound analysis of the ovarian atrophy study because: 1) the model fit using all animals is similar to the model fit using only animals that died on or before 74.3 weeks of age, 2) the model fit using all dose groups is similar to the model fit to only the four lowest dose groups, and 3) using all the data results in more reliable maximum likelihood estimates and corresponding confidence limits.

Figure 1. Observed versus multistage-Weibull model predicted proportions of mice with ovarian atrophy when only the four lowest dose groups and the control group are used to fit the model

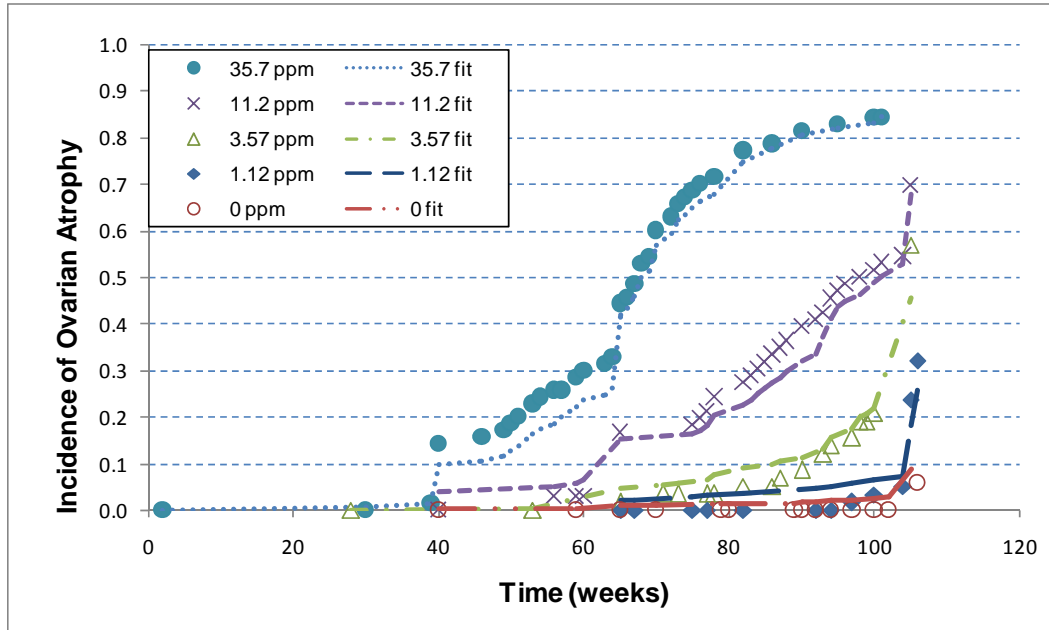
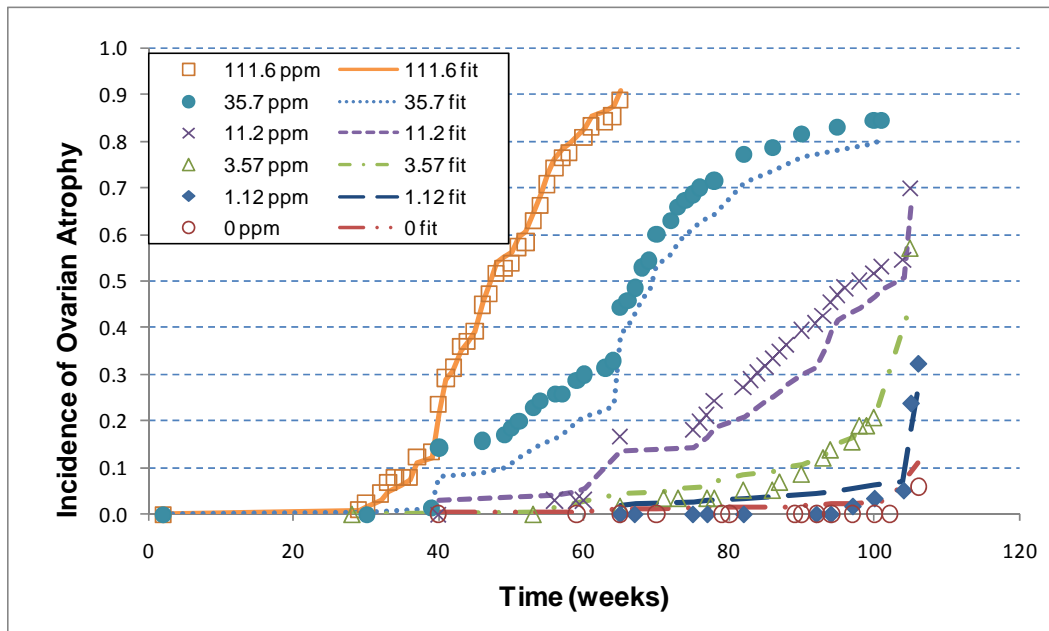


Figure 2. Observed versus multistage-Weibull model predicted proportions of mice with ovarian atrophy when all five dose groups and the control group are used to fit the model.



**Attachment A**

Time-to-response for ovarian atrophy as reported by the Sapphire Group, Inc. of the NTP 1993 study (NTP. 1993. Toxicology and carcinogenesis studies of 1,3-butadiene (CAS No. 106-99-0) in B6C3F1 mice (inhalation studies). Research Triangle Park, NC: National Toxicology Program, U.S. Public Health Service, U.S. Department of Health and Human Services. TR 434).

Concentration (ppm)	Responders	Non-Responders	n	Day	Week
0	0	10	10	280	40
0	0	1	1	413	59
0	0	10	10	455	65
0	0	1	1	490	70
0	0	1	1	553	79
0	0	1	1	560	80
0	0	1	1	623	89
0	0	1	1	630	90
0	0	1	1	644	92
0	0	1	1	658	94
0	0	1	1	679	97
0	0	1	1	700	100
0	0	3	3	714	102
0	4	32	36	742	106
6.25	0	10	10	455	65
6.25	0	1	1	469	67
6.25	0	2	2	525	75
6.25	0	1	1	539	77
6.25	0	1	1	574	82
6.25	0	3	3	644	92
6.25	0	1	1	658	94
6.25	1	0	1	679	97
6.25	1	1	2	700	100
6.25	1	0	1	728	104
6.25	11	10	21	735	105
6.25	5	10	15	742	106
20	0	1	1	196	28
20	0	1	1	371	53
20	1	9	10	455	65
20	1	0	1	497	71
20	0	1	1	511	73
20	0	1	1	539	77

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20	0	2	2	546	78
20	1	1	2	574	82
20	0	1	1	602	86
20	1	0	1	609	87
20	1	0	1	630	90
20	2	0	2	651	93
20	1	2	3	658	94
20	1	1	2	679	97
20	2	1	3	686	98
20	0	1	1	693	99
20	1	0	1	700	100
20	21	3	24	735	105
62.5	0	10	10	280	40
62.5	2	0	2	392	56
62.5	0	1	1	413	59
62.5	0	1	1	420	60
62.5	9	1	10	455	65
62.5	1	0	1	525	75
62.5	1	0	1	532	76
62.5	1	0	1	539	77
62.5	2	0	2	546	78
62.5	2	0	2	574	82
62.5	1	0	1	581	83
62.5	1	0	1	588	84
62.5	1	0	1	595	85
62.5	1	0	1	602	86
62.5	1	0	1	609	87
62.5	1	0	1	616	88
62.5	2	0	2	630	90
62.5	1	0	1	644	92
62.5	1	2	3	651	93
62.5	2	1	3	658	94
62.5	1	1	2	665	95
62.5	1	0	1	672	96
62.5	1	0	1	686	98
62.5	1	1	2	700	100
62.5	1	0	1	707	101
62.5	1	1	2	728	104
62.5	10	1	11	735	105

200	0	1	1	14	2
200	0	1	1	210	30
200	1	0	1	2733*	390.4286
200	9	1	10	280	40
200	1	0	1	322	46
200	1	0	1	343	49
200	1	0	1	350	50
200	1	0	1	357	51
200	2	0	2	371	53
200	1	0	1	378	54
200	1	0	1	392	56
200	0	1	1	399	57
200	2	0	2	413	59
200	1	0	1	420	60
200	1	0	1	441	63
200	1	0	1	448	64
200	8	4	12	455	65
200	1	0	1	462	66
200	2	0	2	469	67
200	3	0	3	476	68
200	1	0	1	483	69
200	4	0	4	490	70
200	2	0	2	504	72
200	2	0	2	511	73
200	1	0	1	518	74
200	1	0	1	525	75
200	1	0	1	532	76
200	1	0	1	546	78
200	4	1	5	574	82
200	1	1	2	602	86
200	2	0	2	630	90
200	1	0	1	665	95
200	1	0	1	700	100
200	0	1	1	707	101
625	0	1	1	14	2
625	1	0	1	203	29
625	1	0	1	210	30
625	2	0	2	224	32
625	2	0	2	231	33

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625	1	0	1	238	34
625	0	1	1	245	35
625	0	1	1	252	36
625	4	0	4	259	37
625	1	0	1	273	39
625	9	1	10	280	40
625	5	2	7	287	41
625	2	0	2	294	42
625	4	0	4	301	43
625	1	1	2	308	44
625	2	0	2	315	45
625	5	1	6	322	46
625	2	2	4	329	47
625	4	0	4	336	48
625	1	0	1	343	49
625	1	0	1	350	50
625	3	0	3	357	51
625	1	0	1	364	52
625	4	0	4	371	53
625	3	0	3	378	54
625	4	0	4	385	55
625	3	0	3	392	56
625	2	0	2	399	57
625	1	0	1	406	58
625	3	0	3	420	60
625	2	0	2	427	61
625	1	0	1	441	63
625	1	0	1	448	64
625	3	0	3	455	65

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\*2733 was replaced by 273 in our analyses

## Appendix 4. Leukemia Mortality/Incidence Rates and Survival Rates

US Total Population 2000-2003		Texas Statewide 1999-2003	Texas Statewide 1999-2003
Total Leukemia Mortality Rates per 100,000 <sup>1</sup>		Total Leukemia Mortality Rates per 100,000 <sup>2</sup>	Total Leukemia Incidence Rates per 100,000 <sup>2</sup>
	Rate	Rate	Rate
00 years	0.7	0.9	5.1
01-04 years	0.9	0.9	8.7
05-09 years	0.7	0.6	3.8
10-14 years	0.8	0.9	3.5
15-19 years	1.1	1.3	3.1
20-24 years	1.2	1.5	2.6
25-29 years	1.1	1.1	2.8
30-34 years	1.3	1.4	2.9
35-39 years	1.6	1.5	3.5
40-44 years	2.0	1.8	4.4
45-49 years	2.9	3.4	6.8
50-54 years	4.4	4.2	10.5
55-59 years	7.5	8.4	16.8
60-64 years	12.9	13.2	24.6
65-69 years	20.8	21.3	35.7
70-74 years	33.0	31.8	48.8
75-79 years	47.0	43.4	62.6
80-84 years	63.2	65.5	82.7
85+ years	81.5	81.3	91.3

<sup>1</sup> Table XIII-8, Seer Cancer Statistics Review 2000-2003 Surveillance, Epidemiology, and End Results database (SEER 2006)

<sup>2</sup> Texas-specific mortality and incidence rates for 1999-2003 for all leukemia and Texas-specific survival rates for 2003 were kindly provided by the Texas Department of State Health Services, Cancer Epidemiology and Surveillance Branch, Texas Cancer Registry.

2000 US All <sup>1</sup>		Total Texas Population 2003 <sup>2</sup>	
Age	Survival	Life Tables	
0	1	0	1
1	0.99307	1	0.99342
5	0.99177	5	0.99191
10	0.99095	10	0.99105
15	0.98992	15	0.99005
20	0.98654	20	0.98659
25	0.98181	25	0.9818
30	0.97696	30	0.9772
35	0.97132	35	0.97192
40	0.96349	40	0.9641
45	0.9521	45	0.95248
50	0.93522	50	0.93546
55	0.91113	55	0.91092
60	0.87498	60	0.87584
65	0.82131	65	0.82385
70	0.74561	70	0.75079
75	0.64244	75+	0.65073
80	0.51037		
85	0.34959		

<sup>1</sup> US survival rates for 2000 (Arias 2002)

<sup>2</sup> Texas-specific survival rates for 2003 were kindly provided by the Texas Department of State Health Services, Cancer Epidemiology and Surveillance Branch, Texas Cancer Registry.

## **Appendix 5. Calculating Excess Risk with Age-Dependent Adjustment Factors**

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**TCEQ Contract 582-7-81521**

### **1. Background:**

When calculating an excess risk, a general guiding principle is that the dose-response model, model parameter, dose metric, response, and population used in the excess risk calculation using the BEIR IV approach (NRC 1988) should be the same as the dose-response model, model parameter, dose metric, response, and population used in the dose-response modeling of the epidemiological study data.

If the population in the dose-response modeling has specific characteristics (e.g., gender, race, and geographic region), then the inference (the calculated excess risk) applies directly to that specified population. It only applies to a more general population under the assumption that the estimated model parameter and dose-response model apply to that population – this is an assumption, not a guarantee, and not something that is necessarily proven or implied by the study data.

### **2. Age-Dependent Adjustment Factor (ADAF): General**

An ADAF is intended to be used when the epidemiological study data do not include exposures at an early age (generally before age 16). According to U.S. EPA (Barton, H., *et al.* Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens, EPA/360/R-03/003F, March 2005, Washington, D.C.), an ADAF is intended to address the "...potential for increased susceptibility to cancer from early-life exposure, relative to comparable exposure later in life...".

ADAFs are age-specific adjustments to the susceptibility (slope) in the dose-response model and are not adjustments to the dose metric itself.

If the epidemiological study data do not include exposures at an early age (e.g., before age 16) such as would generally be the case for occupational epidemiological studies, then ADAFs that are only different than 1 before age 16 do not impact the dose-response modeling and cannot reflect different susceptibilities at early ages relative to later ages. Thus, the fitted model parameter cannot directly reflect different susceptibilities at early ages relative to later ages. Therefore, it is reasonable to do an excess risk calculation using the dose metric and model parameter estimated from the epidemiological data – without

explicitly recomputing the model parameter based on the susceptibilities implied by the ADAFs – since the model parameter won't change anyway.

Although it is somewhat of an aside, it is important to note the treatment of "background doses" needs to be the same in the dose-response modeling and the excess risk calculation. Specifically, if for example, there is a general background exposure of X ppm per year to the chemical of interest, then the excess risk calculation should treat that X ppm per year in the same way as the dose-response model fitting. If the dose-response modeling was done with the dose metric including that X ppm per year, then the excess risk calculation should be done with the dose metric including that X ppm per year. If the dose-response modeling was done with the dose metric excluding that X ppm per year, then the excess risk calculation should be done with the dose metric excluding that X ppm per year. It would be invalid for the dose-response modeling to be done with the dose metric excluding that X ppm per year, and the excess risk calculation to be done with the dose metric including that X ppm per year – or vice versa.

### **3. Age-Dependent Adjustment Factor (ADAF): EPA Guidelines**

EPA guidelines call for the default use of ADAFs be considered only when the chemicals mode of action is mutagenic.

EPA guidelines (pages 32-34) also include the following text:

*The adjustments described below reflect the potential for early-life exposure to make a greater contribution to cancers appearing later in life. The 10-fold adjustment represents an approximation of the weighted geometric mean tumor incidence ratio from juvenile or adult exposures in the repeated dosing studies (see Table 8). This adjustment is applied for the first 2 years of life, when toxicokinetic and toxicodynamic differences between children and adults are greatest (Ginsberg et al., 2002; Renwick, 1998). Toxicokinetic differences from adults, which are greatest at birth, resolve by approximately 6 months to 1 year, while higher growth rates extend for longer periods. The 3-fold adjustment represents an intermediate level of adjustment that is applied after 2 years of age through <16 years of age. This upper age limit represents middle adolescence following the period of rapid developmental changes in puberty and the conclusion of growth in body height in NHANES data (Hattis et al., 2005). Efforts to map the approximate start of mouse and rat bioassays (i.e., 60 days) to equivalent ages in humans ranged from 10.6 to 15.1 years (Hattis et al., 2005). Data are not available to calculate a specific dose response adjustment factor for the 2 to <16-year age range, so EPA selected the 3-fold adjustment because it reflects a midpoint, i.e., approximately half the difference between 1 and 10 on a logarithmic scale ( $10^{1/2}$ ), between the 10-fold adjustment for the first two years of life and no adjustment (i.e., 1-fold) for adult exposure. ...*

*...the Supplemental Guidance emphasizes that chemical-specific data should be used in preference to these default adjustment factors whenever such data are available.*

*The following adjustments represent a practical approach that reflects the results of the preceding analysis, which concluded that cancer risks generally are higher from early-life exposure than from similar exposure durations later in life:*

- For exposures before 2 years of age (i.e., spanning a 2-year time interval from the first day of birth up until a child's second birthday), a 10-fold adjustment.
- For exposures between 2 and <16 years of age (i.e., spanning a 14-year time interval from a child's second birthday up until their sixteenth birthday), a 3-fold adjustment
- For exposures after turning 16 years of age, no adjustment.

...This Supplemental Guidance focuses on carcinogens with a mutagenic mode of action.

...When data, including well established mode of action data, are available that allow specific evaluation of lifestage differences in toxicokinetics or toxicodynamics that would lead to lesser or greater susceptibility from early-life exposures to carcinogens, then those data should be used, as generally discussed in EPA's cancer guidelines (U.S. EPA, 2005), in preference to the default procedures described in this Supplemental Guidance.

The 10-fold and 3-fold **adjustments in slope factor** are to be **combined with age-specific exposure estimates when estimating cancer risks from early life exposure to carcinogens** that act through a mutagenic mode of action. It is important to emphasize that **these adjustments are combined with corresponding age-specific estimates of exposure to assess cancer risk**. For example, for a 70-year lifetime, where there are data showing negligible exposure to children, the estimated cancer risk from childhood exposure would be also negligible and the lifetime cancer risk would be reduced to that resulting from the relevant number of years of adult exposure (in the absence of specific information, 55 years). Where there are data (measured or modeled) for childhood exposures, the **age-group specific exposure values** are used along with the corresponding **adjustments to the slope factor**. Where there are no relevant data or models for childhood exposures and only lifetime average exposure data are available, the lifetime exposure data are used with the **adjustments to the slope factor for each age segment**.

(emphasis added)

**There are several important points/clarifications in this last paragraph. The first is that the ADAF is an adjustment to the slope factor (as opposed to an adjustment to the dose metric). The second is that the ADAF is to be applied on an age-specific basis. That is, the ADAFs are applied to each year in a life and summed to get the lifetime risk, as opposed to calculating a lifetime excess risk without ADAFs and then multiplying this calculated value by a constant ADAF.**

This second point is reinforced in the examples provided by EPA (Sections 6.1 and 6.2, pages 36 to 41). Although EPA's examples do not explicitly refer to cumulative doses, they do refer to age-dependent doses and cumulative doses are age-dependent doses.

#### **4. Age-Dependent Adjustment Factor (ADAF): Recent Implementations by EPA and Others when the Dose Metric is Cumulative Exposure are Inconsistent with EPA Guidelines**

In recent risk assessments (e.g., for ethylene oxide) **when the dose metric is cumulative exposure**, EPA has implemented the ADAF by calculating the excess risk by first calculating the excess risk without any ADAFs and then multiplying this excess risk by a weighted average of the age-specific ADAFs over the "lifetime" (i.e., the period over which the excess risk is calculated, 70, 78, or 85 years). Similarly, the

EPA has implemented the ADAF by calculating the point of departure (POD) by first calculating the POD without an ADAF and then dividing this POD by a weighted average of the age-specific ADAFs over the "lifetime".

In these recent risk assessments (e.g., for ethylene oxide) **when the dose metric is cumulative exposure**, EPA's method of incorporating an ADAF has been inconsistent with EPA's Guidelines, has not properly incorporated age-dependence, and is mathematically incorrect. There is no good scientific/mathematical reason for incorporating an ADAF in the manner in which EPA has attempted to do it. Others (including the first draft assessment of butadiene by NC SAB) have made the same mistake.

For inhalation exposure of a chemical with a mutagenic mode of action, EPA guidelines [Barton, H., *et al.* Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens. EPA/630/R-03/003F, March 2005] suggest that the increased risk caused by early-life exposure be determined through the use of three Age-Dependent Adjustment Factors (ADAFs):

- (1) ADAF(age) = 10 for exposure before 2 years of age
- (2) ADAF(age) = 3 for exposure between ages 2 and < 16 years of age
- (3) ADAF(age) = 1 for exposure after turning 16 years of age.

Furthermore, **assuming that exposure to a mutagenic chemical via inhalation is constant over a 70-year lifetime**, EPA's proposed overall adjustment factor (ADAF) for early-life exposure is:

$$\begin{aligned} \text{ADAF} &= \sum_i (\text{ADAF}(i) \times \text{Age Interval}) / 70 \text{ years} \\ &= [(10 \times 2 \text{ years}) + (3 \times 13 \text{ years}) + (1 \times 55 \text{ years})] / 70 \text{ years} = 1.63. \end{aligned}$$

For a 78-year lifespan, the corresponding ADAF would be 1.56 because

$$[(10 \times 2 \text{ years}) + (3 \times 13 \text{ years}) + (1 \times 63 \text{ years})] / 78 \text{ years} = 1.56.$$

For an 85-year lifespan, the corresponding ADAF would be 1.52 because

$$[(10 \times 2 \text{ years}) + (3 \times 13 \text{ years}) + (1 \times 70 \text{ years})] / 85 \text{ years} = 1.52.$$

Then, the point of departure (POD) is "adjusted for early-life exposure" by dividing the unadjusted POD by ADAF, according to the EPA proposed method of adjustment.

This adjustment is consistent with EPA Guidelines provided that **exposure to a mutagenic chemical via inhalation is constant over the lifetime and the dose metric is the exposure concentration (as opposed to cumulative exposure)**. Here, "lifetime" should be interpreted as the period over which the excess risk is calculated (e.g., 70, 78, or 85 years).

Specifically, in Example 2 (part a) in Section 6.1 of EPA Guidelines, the calculation of excess risk for 70 years exposure to a constant dose (0.0001 mg/kg-d) when the dose metric is exposure concentration (as opposed to cumulative exposure) is as follows:

a. To calculate lifetime risk for a population with average life expectancy of 70 years, sum the risk associated with each of the three relevant time periods:

- Risk during the first 2 years of life (where the ADAF = 10);
- Risk for ages 2 through < 16 (ADAF = 3); and
- Risk for ages 16 until 70 years (ADAF = 1).

Thus, risk equals the sum of:

- Risk for birth through < 2 yr =  
 $(2 \text{ per mg/kg-d}) \times 10 \text{ (ADAF)} \times (0.0001 \text{ mg/kg-d}) \times 2\text{yr}/70\text{yr} = 0.6 \times 10^{-4}$
  - Risk for ages 2 through < 16 =  
 $(2 \text{ per mg/kg-d}) \times 3 \text{ (ADAF)} \times (0.0001 \text{ mg/kg-d}) \times (13\text{yr}/70\text{yr}) = 1.1 \times 10^{-4}$
  - Risk for ages 16 until 70 =  
 $(2 \text{ per mg/kg-d}) \times 1 \text{ (ADAF)} \times (0.0001 \text{ mg/kg-d}) \times (55\text{yr}/70\text{yr}) = 1.6 \times 10^{-4}$
- $$\text{Risk} = 0.6 \times 10^{-4} + 1.1 \times 10^{-4} + 1.6 \times 10^{-4} = 3.3 \times 10^{-4}$$

Here, where the exposure is to a constant dose (0.0001 mg/kg-d) and the dose metric is exposure concentration (as opposed to cumulative exposure), the risk could be calculated as "risk without ADAFs" times a weighted average adjustment factor -- here,

$$\begin{aligned} \text{ADAF} &= \sum_i (\text{ADAF}(i) \times \text{Age Interval}) / 70 \text{ years} \\ &= [ (10 \times 2 \text{ years}) + (3 \times 13 \text{ years}) + (1 \times 55 \text{ years}) ] / 70 \text{ years} = 1.63. \end{aligned}$$

This works only when the age-specific risk per year (before ADAF) is a constant for all ages. Here, the lifetime risk (before ADAF) is

$$(2 \text{ per mg/kg-d}) \times (0.0001 \text{ mg/kg-d}).$$

Here,

$$(2 \text{ per mg/kg-d}) \times (0.0001 \text{ mg/kg-d})$$

is a common term in each of the risks being summed, so it can be factored out and the calculation represented as

$$\begin{aligned} &(2 \text{ per mg/kg-d}) \times (0.0001 \text{ mg/kg-d}) \\ &\times [ (10 \times 2 \text{ years}) + (3 \times 13 \text{ years}) + (1 \times 55 \text{ years}) ] / 70 \text{ years} \\ &= (2 \text{ per mg/kg-d}) \times (0.0001 \text{ mg/kg-d}) \times 1.63. \end{aligned}$$

$$= 0.000326 = 3.3 \times 10^{-4}$$

However, **the ADAFs do not factor out when the dose is not constant for each age in the age-specific calculation.** For example, in Example 2 (part b) in Section 6.1 of EPA Guidelines, the calculation is as follows:

*b. If exposure varies with age, then such differences are also included. Now suppose the same example as immediately above, except that exposure for ages 1 through <12 was twice as high as exposure for all other ages. In this case, sum the risk associated with each of the five relevant time periods in which exposure rates and/or potencies (slope factors) vary:*

*Risk equals the sum of:*

- *Risk for birth through < 1 yr (1yr) =*  
 $(2 \text{ per mg/kg-d}) \times 10 \text{ (ADAF)} \times 0.0001 \text{ mg/kg-d} \times 1\text{yr}/70\text{yr} = 0.3 \times 10^{-4}$
  - *Risk for ages 1 through < 2 (1yr) =*  
 $(2 \text{ per mg/kg-d}) \times 10 \text{ (ADAF)} \times 0.0002 \text{ mg/kg-d} \times 1\text{yr}/70 \text{ yr} = 0.6 \times 10^{-4}$
  - *Risk for ages 2 through < 12 (10yr) =*  
 $(2 \text{ per mg/kg-d}) \times 3 \text{ (ADAF)} \times 0.0002 \text{ mg/kg-d} \times 10\text{yr}/70\text{yr} = 1.7 \times 10^{-4}$
  - *Risk for ages 12 through < 16 (4yr) =*  
 $(2 \text{ per mg/kg-d}) \times 3 \text{ (ADAF)} \times 0.0001 \text{ mg/kg-d} \times 4\text{yr}/70\text{yr} = 0.3 \times 10^{-4}$
  - *Risk for ages 16 until 70 years (55yr) =*  
 $(2 \text{ per mg/kg-d}) \times 1 \text{ (ADAF)} \times 0.0001 \text{ mg/kg-d} \times 55\text{yr}/70\text{yr} = 1.6 \times 10^{-4}$
- $$\text{Risk} = 0.3 \times 10^{-4} + 0.6 \times 10^{-4} + 1.7 \times 10^{-4} + 0.3 \times 10^{-4} + 1.6 \times 10^{-4} = 4.5 \times 10^{-4}$$

Here, the dose x slope does not factor out of the above calculation – even though the slope is constant for all ages (namely, 2 per mg/kg-d) -- since the dose is 0.0002 mg/kg-d for ages between 1 and 12 and 0.0001 mg/kg-d for ages <1 and ages 12 and above. If one calculated the risk without ADAFs, namely,

Risk equals the sum of:

- Risk for birth through < 1 yr (1yr) =  
 $(2 \text{ per mg/kg-d}) \times 0.0001 \text{ mg/kg-d} \times 1\text{yr}/70\text{yr} = 2.9 \times 10^{-6}$
- Risk for ages 1 through < 2 (1yr) =  
 $(2 \text{ per mg/kg-d}) \times 0.0002 \text{ mg/kg-d} \times 1\text{yr}/70 \text{ yr} = 5.7 \times 10^{-6}$

- Risk for ages 2 through < 12 (10yr) =  
 $(2 \text{ per mg/kg-d}) \times 0.0002 \text{ mg/kg-d} \times 10\text{yr}/70\text{yr} = 5.7 \times 10^{-5}$
  - Risk for ages 12 through < 16 (4yr) =  
 $(2 \text{ per mg/kg-d}) \times 0.0001 \text{ mg/kg-d} \times 4\text{yr}/70\text{yr} = 1.1 \times 10^{-5}$
  - Risk for ages 16 until 70 years (55yr) =  
 $(2 \text{ per mg/kg-d}) \times 0.0001 \text{ mg/kg-d} \times 55\text{yr}/70\text{yr} = 1.6 \times 10^{-4}$
- $$\text{Risk} = 2.9 \times 10^{-6} + 5.7 \times 10^{-6} + 5.7 \times 10^{-5} + 1.1 \times 10^{-5} + 1.6 \times 10^{-4}$$
- $$= 2.4 \times 10^{-4}$$

and then multiplied this sum by a weighted average adjustment factor -- here,  
 $\text{ADAF} = [(10 \times 2 \text{ years}) + (3 \times 13 \text{ years}) + (1 \times 55 \text{ years})] / 70 \text{ years} = 1.63$  –  
the result would be

$$(2.4 \times 10^{-4}) \times 1.63 = 3.9 \times 10^{-4}$$

and not  $4.5 \times 10^{-4}$ .

Except for the trivial case in which the exposure concentration is only non-zero in the first year, cumulative exposure changes from year to year and is not constant throughout the period included in the excess risk calculation. Hence, when dose is cumulative exposure, the ADAFs do not factor out of the excess risk calculation and the risk can NOT be calculated as "risk without ADAFs" times a weighted average adjustment factor.

### **5. Age-Dependent Adjustment Factor (ADAF): An Implementation When the Dose Metric is Cumulative Exposure That Is Consistent with EPA Guidelines**

An implementation that is consistent with EPA guidelines when the dose metric is cumulative exposure would be to calculate the excess risk as in Example 2 (part b) in Section 6.1 of EPA Guidelines. That is, calculate the excess risk in each year using the age-specific dose (cumulative dose) for that year and multiplying the slope by the age-specific ADAF for that year (age). This would be consistent with EPA's Guidelines from the point of view of both the excess risk calculation being done using age-specific exposures and also the ADAFs being age-specific modifiers of the slope (potency). This implementation of the ADAF is NOT equivalent to computing the excess risk by first calculating the excess risk without any ADAFs and then multiplying this excess risk by a weighted average of the age-specific ADAFs over the lifetime.

## Appendix 6. Cox Proportional Hazards Models Not Included in Cheng *et al.* (2007)

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Cheng *et al.* presented several analyses with the objective of showing different alternatives they thought could be relevant. For example, they restricted the analyses to include only cumulative ppm-years, average intensity or lagged cumulative ppm-years as the relevant doses. There is no evidence that any of these measures of dose is the relevant dose. They also fit models that adjusted for race, year of birth, race, years since hire, plant and number of high intensity tasks (HITs) and exposures to DMDTC. Cheng *et al.* did not give any biological reasons to include or exclude from the model. Ideally, the final model should adjust for effects that are biologically relevant to the outcome of study. However, there is not enough scientific knowledge to indicate what, if any, covariate effects should be included in a model of leukemia mortality with cumulative exposure to butadiene. The research closest to shedding some light on which covariates to include in the model is that published by Albertini *et al.* (2007), which seems to indicate that leukemia does not occur at low exposure to butadiene.

Although the decision of whether or not to adjust for a confounder should ideally be based on biological knowledge, Sielken *et al.* (2007) adjustment for confounders was determined using a statistically-based approach. The use of statistical methodology instead of biological arguments serves for the purpose of corroborating new biological evidence about possible confounders – specifically the role of the number of high intensity tasks in leukemia rate ratios. That is, the inclusion of the number of HITs as a covariate, although based on statistical arguments, was consistent with the biological findings of Albertini *et al.* (2007). In other words, not only was the number of HITs a plausible explanation of the increase in the number of leukemia deaths from a biological and mechanistic standpoint but also the statistical analysis of the data reached the same conclusion. Other attributes to see in model selection are issues like: consistency with biological expectations (i.e., the model should make biological sense), model parsimony (i.e., include as few variables as necessary to explain the relationship when there is no sufficient biological knowledge to justify the inclusion or exclusion of a variable), etc.

Cheng *et al.* (2007) presented a model that adjusts for age and the number of HITs (BD peaks). That is,  $\beta = 2.5 \times 10^{-4}$ ,  $p = 0.03$  presented in Section 3.5 of the Cheng *et al.* (2007) paper. This results in a S.E. of  $1.2 \times 10^{-4}$ . This model is close to the Poisson regression model in the Sielken *et al.* (2007) paper with the

exceptions that: 1) Sielken *et al.* adjusted for the number of HITS using a nonparametric relation based on quintiles whereas Cheng *et al.* adjusted for the number of HITS using a parametric linear relationship, 2) Cheng *et al.* models assume an log-linear relationship between rate ratios and cumulative BD ppm-years whereas Sielken *et al.* uses a linear relationship, 3) Cheng *et al.* use Cox proportional hazards model and Sielken *et al.* use Poisson regression model, and 4) Cheng *et al.* use continuous cumulative BD ppm-years and Sielken *et al.* uses BD ppm-years mean-scored deciles.

Model	Covariates	Parameter Estimate		URF <sup>a</sup> (ppm <sup>-1</sup> )	
		$\beta$ (S.E.)	95% UCL	Air Concentration for an excess risk of 1 in 100,000 (ppb)	
				URF (MLE)	URF(95% UCL); 95% LCL on Conc.
Cox regression Cheng <i>et al.</i> (2007) ppm-years continuous <sup>b</sup> , # of HITS continuous <sup>c</sup>	Age number of HITS > 100 ppm	2.5E-04 (1.2E-04)	4.474E-04	1.284E-04 77.88	2.298E-04 43.52
Cox regression ppm-years continuous <sup>b</sup> , # of HITS categorical <sup>d</sup>	Age number of HITS > 100 ppm	2.0E-04 (1.3E-04)	4.138E-04	1.027E-04 97.35	2.125E-04 47.05
Cox regression ppm-years mean-scored deciles <sup>e</sup> , # of HITS categorical <sup>d</sup>	Age number of HITS > 100 ppm	2.8E-04 (2.4E-04)	6.748E-04	1.438E-04 69.53	3.466E-04 28.85
Poisson regression (Sielken <i>et al.</i> (2007)) ppm-years mean-scored deciles <sup>e</sup> , # of HITS categorical <sup>d</sup>	Age number of HITS > 100 ppm	1.89E-04 (3.6E-04)	7.812E-04	8.083E-05 123.7	3.314E-04 29.93

<sup>a</sup> URF(MLE) = 0.001 / EC<sub>001</sub> and URF(95% UCL) = 0.001 / LEC<sub>001</sub>

<sup>b</sup> ppm-years is included as a continuous variable (untransformed) in a parametric model of the effect of ppm-years

<sup>c</sup> number of HITS > 100 ppm is included as a continuous variable (untransformed) in a parametric model of the effect of the number of HITS > 100 ppm

<sup>d</sup> number of HITS > 100 ppm is included as a categorical variable (based on quintiles) in a nonparametric model of the effect of the number of HITS > 100 ppm

<sup>e</sup> ppm-years is included as a categorical variable (based on mean-scored deciles, untransformed) in a parametric model of the effect of ppm-years

Despite all these differences, the models are close and converge to very similar results if some of the discrepancies are resolved. For example, if the Cox proportional hazards log-linear model presented by Cheng *et al.* were non-parametrically adjusted for BD peaks, then the estimate of the coefficient for cumulative BD ppm-years would be  $\beta = 0.00020$  (S.E.=0.00013), which is close to the parameter estimates reported in Sielken *et al.* (i.e.,  $\beta = 0.000189$ , S.E.=0.00036) for the Poisson linear model. If, in addition to adjusting for the number of HITS nonparametrically, the Cox proportional hazards log-linear model used BD ppm-years mean-scored deciles instead of continuous exposures, then the coefficient for cumulative BD ppm-years would be  $\beta = 0.00028$  (S.E.=0.00024). This last model differs from Sielken *et al.* model only in that Sielken *et al.* used a Poisson regression model and a linear relationship as opposed to the Cox proportional hazards model and a log-linear relationship. The following table summarizes the results of the Cox proportional hazards model and the Sielken *et al.* Poisson regression model when adjusting for the number of HITS.

In the above discussion, a parametric model is a model that assumes a specified functional form (e.g., linear or log-linear), and a nonparametric model is a model that does not assume a specified functional form. This is analogous to the difference between regression which assumes a specified functional form (e.g., linear or polynomial) and hence is parametric and analysis of variance (ANOVA or AOV) which is nonparametric. Continuing with the analogy, if a treatment can be characterized by a number (e.g., concentration or amount), then in a regression analysis (say, a linear regression) the magnitudes of the different treatment values are important and a treatment with twice the magnitude has twice the effect. On the other hand, in an analysis of variance the different treatments are dealt with nonparametrically (say, as treatments A, B, C, etc.) and the magnitudes (numerical values) are ignored. Therefore, in an analysis of variance there is no functional relationship specified between the effects of the different treatments.

If a variable is said to be treated continuously, then each individual value of that variable is used – the values are not grouped and no representative values for the groups are used. On the other hand, if a variable is treated categorically, then the individual values of that variable are grouped and representative values for the groups replace the individual values in the analysis. Cumulative butadiene ppm-years and cumulative number of HITS > 100 ppm can both be treated either as continuous or categorical variables. Since the categorical (group) values for these variables are numerical, a categorical variable could be included in both parametric and nonparametric models.

In the table above, both the Cox and Poisson regressions assume a parametric model for the effect of cumulative butadiene ppm-years. The model for the effect of ppm-years is log-linear in Cox regression and is linear in Poisson regression. In Cox regression, ppm-years is treated as a continuous variable in the first two models and treated as a categorical variable in the third model. In the Poisson regression, ppm-years is treated as a categorical variable.

In the first model in the table above, the cumulative number of HITS > 100 ppm is treated as a continuous variable and treated parametrically. In the other three models, the cumulative number of HITS > 100 ppm is treated as a categorical variable and treated nonparametrically.

Albertini, R., Sram, R. J., Vacek, P. M., Lynch, J., Rossner, P., Nicklas, J. A., McDonald, J. D., Boysen, G., Georgieva, N., and Swenberg, J. A. (2007). Molecular epidemiological studies in 1,3-butadiene exposed Czech workers: Female-male comparisons. *Chemico-Biological Interactions*, Volume 166, Issues 1-3, 20 March 2007, Pages 63-77.

## **Appendix 7. Sensitivity Analysis: Exposure Estimation Errors**

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Although the UAB epidemiological data are the most comprehensive available epidemiological data for a butadiene cancer risk assessment, an evaluation of the potential quantitative impact of BD exposure uncertainty (including exposure estimation error) is appropriate and informative. This source of uncertainty, like most other sources of uncertainty, is best addressed by explicitly presenting the quantitative results of alternative characterizations. This is consistent with the practice of primarily focusing on the best estimate (maximum likelihood estimate, MLE), and, then, secondarily, characterizing the uncertainty. This practice seems to be the most appropriate course for the development of risk assessments.

Exposure misclassification is a loosely defined term that is oftentimes used to imply exposure estimation error. More precisely, exposure misclassification refers to the assignment of an incorrect exposure estimate to a worker. For example, a worker may work in area A, but the job history incorrectly places him in area B, which has a different exposure level.

Exposure estimation error results when the exposure assigned to a job is different from the true exposure due to the methodology or data used to estimate the exposure. For example, if the air speed at a job may vary depending on the task but the exposure model assumes a constant air flow, the exposure will sometimes underestimate the true exposure concentration and some other times will overestimate the true exposure concentration.

Exposure misclassification and exposure estimation error can be incorporated into the uncertainty analysis accompanying a risk assessment if there is information about the nature of the misclassification and the estimation error.

For butadiene, the primary measure of dose is cumulative exposure (e.g., ppm-years). The cumulative exposure for a worker at any particular point in time is calculated by summing, over all jobs up to that time, the product of the exposure concentration in the job work area and the duration of the job. The duration (but not the calendar years) of any high and low concentration exposures impacts the cumulative exposure. As is the case for the butadiene exposures in the UAB cohort, any high concentration exposures that are part of a cumulative exposure may have occurred early, or late, or anytime in the worker's job history. For example, a cumulative exposure of, say 100 ppm-years may be the result of: 1) 100 ppm for 1

year 20 years ago, 2) 5 ppm during 20 years of work, 3) 90 ppm on the first year and 1 ppm during the next 10 years, 4) 1 ppm during the first 10 years and 90 ppm the last year, etc. These examples indicate that cumulative exposure is a summary measure of dose that does not take into account the timing of the exposures. In particular, the high concentration exposure years may not have occurred at or near the end of a worker's job history. Therefore, for butadiene, dropping the later person years from a worker's job history after the cumulative exposure reaches a specific cut-off value does not necessarily drop the years with high concentration exposures. This also means that, for butadiene, if the concerns about exposure misclassification and/or exposure estimation error relate primarily to high concentration exposures, these concerns cannot be addressed by dropping the later person years from a worker's job history after the cumulative exposure reaches a specific cut-off value. Instead, exposure misclassification and exposure estimation error are addressed directly herein by re-evaluating the dose-response relationship using alternative characterizations of the specific job exposure concentrations in the job-exposure-matrix (JEM) used to calculate cumulative BD ppm-years. This is what is done in the following.

This investigation encompasses both the recent work of Sathiakumar *et al.* (2007) and the distributional characterization of JEM values provided by Macaluso *et al.* (2004).

Sathiakumar *et al.* (2007) provides information on the magnitude of the estimation errors for different calendar years. They investigated the likely magnitude of the difference between the exposure estimates (JEM values) and exposure measurements. In their abstract, they concluded that

“Exposure misclassification may have been more severe for subjects from the validation study plant than for subjects from other plants in the mortality study. BD estimates for typical SBR jobs, which comprise most operations at all but one of the plants in the mortality study, appeared to be useful for ranking workers by cumulative exposure. Uncertainty analyses would enhance the utility of the BD exposure estimates for quantitative risk assessment.”

Figure 1 and Table 1 provide information on the likely magnitude of the difference between the exposure estimates (JEM values) and exposure measurements. (Figure 1 is their Figure 1.) (Table 1 is an amended version of their Table 4 with an additional column for the measurement divided by the estimate.) In each year before 1984 the mean measurement is greater than the mean estimate (JEM value). In 1984 and each year thereafter, the opposite is true (namely, the mean measurement is less than the mean estimate). Hence, 1984 is a convenient partition point. The average of yearly mean measurement divided by the corresponding yearly mean estimate before 1984 is 1.98 and is 0.37 for 1984 and after. Thus, prior to 1984 the exposure estimates were approximately 2-fold too low, and in 1984 and later years the exposure estimates were approximately 3-fold too high.

The magnitude of the exposure measurements before 1984 is greater than the magnitude of the exposure measurements in 1984 and thereafter. Hence, the impact on the cumulative BD ppm-years of the error in the exposure estimates before 1984 is greater than the impact in later years even though prior to 1984 the exposure estimates were approximately 2-fold too low, and in 1984 and later years the exposure estimates were approximately 3-fold too high.

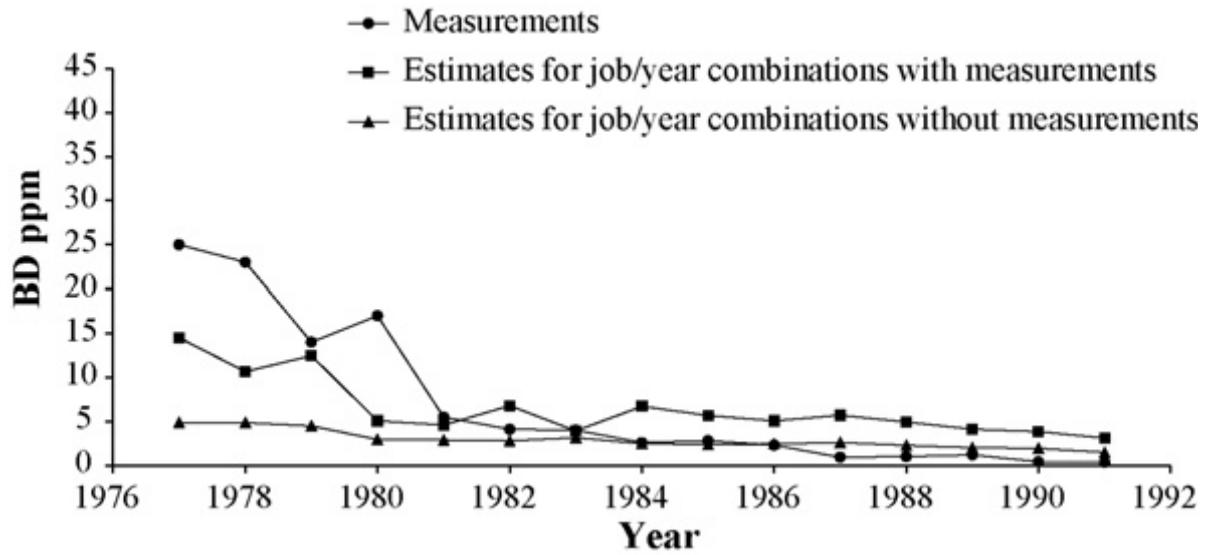


Figure 1. Year-specific mean of measurements and year-specific estimates for 235 job/year combinations appearing in subjects' work histories; year-specific estimates for 1879 job/year combinations that appeared in subjects' work histories but that did not have any BD measurements (reproduced with permission from Sathiakumar *et al.*, 2007)

Table 1. Number of jobs monitored, measurement mean (standard deviation, S.D.), butadiene concentration estimates, and difference, by year of monitoring (reproduced with permission from Sathiakumar *et al.*, 2007, with the last column added)

Year	Jobs	No. of measurements	Mean <sup>a</sup>	S.D.	Mean	S.D.	Mean	S.D.	Measurement / Estimate <sup>c</sup>
			Measurement		Estimate		Difference <sup>b</sup>		
1977	3	56	24.8	-69.9	15.5	-44.7	-9.2	-36.5	1.60
1978	11	527	16	-166.6	9.5	-65.5	-6.6	-128.3	1.68
1979	13	274	10.6	-153.2	6.1	-38.9	-4.5	-141.6	1.74
1980	13	301	14.5	-137.8	3.8	-22.9	-10.7	-130	3.82
1981	15	307	4.8	-38.4	2.2	-19.3	-2.6	-36.1	2.18
1982	21	406	3.8	-28.2	2.9	-24.7	-0.88	-26.4	1.31
1983	13	113	3.9	-19.4	2.5	-9.3	-1.4	-20.5	1.56
1984 <sup>c</sup>	27	658	2.5	-20.3	3.6	-30.3	1.2	-35.6	0.69
1985	27	482	2.6	-18.4	4.4	-31.4	1.8	-32.2	0.59
1986	30	504	2.3	-16.2	3.7	-27.6	1.4	-23.1	0.62
1987	26	310	0.85	-6.3	4.8	-28.6	4	-28.4	0.18
1988	28	417	1	-5.2	5.8	-36.6	4.7	-35.3	0.17
1989	27	238	1.5	-5.5	4.1	-24	2.6	-22.3	0.37
1990	27	223	0.63	-3.3	3.9	-23	3.3	-22.6	0.16
1991	25	162	0.34	-0.61	2.4	-14.1	2	-14.1	0.14

<sup>a</sup> Weighted by the number of measurements for job/year combinations in a year.

<sup>b</sup> Mean estimate minus mean measurement

<sup>c</sup> The average of the yearly mean measurement divided by the corresponding yearly mean estimate before 1984 is 1.98 and is 0.37 for 1984 and after.

In order to characterize the quantitative impact of exposure uncertainty on the dose-response modeling based on Sathiakumar *et al.* (2007), we have determined the maximum likelihood estimate ( $\beta$ ) and the standard error of the estimate (SE) for the following four alternative characterizations of BD exposure data sets:

- 1) The first alternative data set has the JEM values altered so that the exposure estimates (JEM values) are:
  - a) increased approximately 2-fold (1.98-fold) prior to 1984, and
  - b) decreased approximately 3-fold [(1/0.37)-fold] from 1984 through 1991.
  
- 2) The second alternative data set has the JEM values altered so that the exposure estimates (JEM) values are:
  - a) left unchanged prior to 1977,
  - b) increased approximately 2-fold (1.98-fold) from 1977 through 1983, and
  - c) decreased approximately 3-fold [(1/0.37)-fold] from 1984 through 1991

This alternative is the same as the first alternative except that the exposure estimates prior to 1977 are left unchanged because these years are not specifically addressed in Sathiakumar *et al.* (2007).

- 3) The third alternative data set has the JEM values altered so that the exposure estimates prior to 1977 are left unchanged and the exposure estimates (JEM values) for 1977 through 1991 are multiplied by the following calendar-year specific value for “measurement / estimate” rather than values averaged over years:

Year	Measurement / Estimate
1977	1.60
1978	1.68
1979	1.74
1980	3.82
1981	2.18
1982	1.31
1983	1.56
1984	0.69
1985	0.59
1986	0.62
1987	0.18
1988	0.17
1989	0.37
1990	0.16
1991	0.14

This third alternative is the same as the second alternative except that the calendar-year specific findings for 1977 to 1991 in Sathiakumar *et al.* (2007) are used.

- 4) Sathiakumar *et al.* (2007) noted in their conclusions that “On average, estimates were about 10% lower than measurements.” The fourth alternative data set has the JEM values altered so that these estimates are all divided by 0.90 corresponding to

$$\text{estimate (JEM value)} = 0.90 \times \text{measurement.}$$

Table 2 shows the impact of these 4 alternative values of the BD exposures (JEM values) on the estimate of the slope ( $\beta$ ) in the log-linear Cox proportional hazards model and the excess risk of 1 in a 100,000 determined from the model or a linear extrapolation below an excess risk of 1 in 1000.

When Macaluso *et al.* (2004) developed their final distributional characterizations of the specific job exposure concentrations in the job-exposure-matrix (JEM), these distributions indicated the average exposure concentration values used in Sielken *et al.* (2007) and Cheng *et al.* (2007). In addition to the

average values, the 5-th and 95-th percentiles of the exposure concentrations for every job-plant-calendar year combination were available.

Table 2. Impact of alternative exposure values (job exposure matrix (JEM) values) on the characterizations of the model slope parameter ( $\beta$ ) and the excess risk estimates: Based on the exposure estimation error reported by Sathiakumar *et al.* (2007) and the distributional characterization of JEM values in the UAB dataset (Macaluso *et al.*, 2004)

Data Set	Description of JEM Values	$\beta$	Standard Deviation of Estimate of $\beta$	95% UCL on $\beta$	Air Concentration (ppb) Corresponding to 1 in 100,000 excess cancer risk			
					Using model and $\beta$	Using URF and EC <sub>001</sub>	Using model and 95% UCL on $\beta$	Using URF and LEC <sub>001</sub> (95% LCL on EC <sub>001</sub> )
Original	Average in Macaluso Distribution	2.911E-4	1.03E-4	4.60E-4	87.03	72.65	55.03	45.94
1 <sup>st</sup> Alternate	Sathiakumar Average Calendar-Year Correction before 1984 and Average Calendar-Year Correction after 1983	1.469E-4	5.21E-5	2.33E-4	172.46	143.97	108.91	90.93
2 <sup>nd</sup> Alternate	Sathiakumar Average Calendar-Year Correction for 1977 through 1983 and Average Calendar-Year Correction for 1984 through 1991	2.478E-4	8.66E-5	3.90E-4	102.24	85.35	64.92	54.19
3 <sup>rd</sup> Alternate	Sathiakumar Calendar-Year Specific Correction for 1977 through 1991	2.468E-4	8.62E-5	3.89E-4	102.65	85.70	65.19	54.43
4 <sup>th</sup> Alternate	Sathiakumar Overall 10% Correction	2.620E-4	9.26E-5	4.14E-4	96.69	80.72	61.14	51.05
5 <sup>th</sup> Alternate	5-th Percentile Macaluso Distribution	6.438E-4	2.39E-4	1.04E-3	39.35	32.85	24.44	20.41
6 <sup>th</sup> Alternate	95-th Percentile of Macaluso <i>et al.</i> Job and Calendar-Year Specific Distribution	1.576E-4	5.25E-5	2.44E-4	160.75	134.20	103.84	86.69

In order to characterize the quantitative impact of exposure uncertainty on the dose-response modeling based on Macaluso *et al.* (2004), we have determined the maximum likelihood estimate ( $\beta$ ) and the standard error of the estimate (SE) for the following two additional alternative characterizations of BD exposure data sets:

- 5) The fifth alternative data set replaces the average values by the 5-th percentiles of these distributions. Then the Cox regression modeling is done as before except that the cumulative ppm-years are calculated using these 5-th percentile JEM values instead of the average JEM values. This lowers the exposure values and increases the slope in the fitted Cox regression.
- 6) The sixth alternative data set replaces these average values by the 95-th percentiles of these distributions. Then the modeling is done as before except that the cumulative ppm-years are calculated using these 95-th percentile JEM values instead of the average JEM values. This raises the exposure values and decreases the slope in the fitted Cox regression.

Table 2 also shows the impact of these last two alternative values of the BD exposures (JEM values). In general, all of the alternative characterizations of the JEM values, except one, result in higher values of the BD exposure corresponding to an extra risk of 1 in 100,000 than were calculated using the original JEM values. The fifth alternative for JEM values (namely, the 5-th percentiles in the Macaluso *et al.* (2004) JEM distributions) results in exposures from an excess risk of 1 in a 100,000 that are approximately 2.2-fold smaller than the original based on the average JEM exposures. The first alternative exposure JEM measurement (Sathiakumar *et al.* average correction before 1984 and after 1983) results in exposures from an excess risk of 1 in a 100,000 that are approximately 2-fold greater than the original based on the average JEM exposures. The third alternative exposure JEM measurement (Sathiakumar *et al.* year-specific correction) results in exposures from an excess risk of 1 in a 100,000 that are approximately 18% higher than the original based on the average JEM exposures. The third alternative exposure JEM measurement uses all published data on the BD exposure estimation error in the UAB epidemiological data set and is the best estimate of the true exposure concentrations.

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## **APPENDIX 8. CALCULATING EXCESS RISK WHEN SPECIFIED RESPONSE IS MORTALITY VERSUS INCIDENCE**

### **Issues in Quantitative Epidemiology Calculating Excess Risk When Specified Response is Mortality Vs When the Specified Response is Incidence**

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The BEIR IV methodology for calculating excess risk is mathematically correct when the specified response is mortality; however, the BEIR IV methodology is mathematically incorrect when the specified response is incidence (not death).

The following slides are divided into two presentations. The first presentation provides a step-by-step derivation of the BEIR IV methodology when the specified response is mortality. This presentation directly parallels the same derivation in BEIR IV. The second presentation provides a step-by-step derivation that is “parallel” to that in the first presentation except that in the second presentation the specified response is incidence (not death). However, the steps and result are fundamentally different when the specified response is incidence (not death) than when the response is death.

The fact that the “result” (i.e., the mathematical formula for calculating excess risk) is different when the response is mortality than it is when the response is incidence, means that when the response is incidence (not death) the excess risk cannot be validly calculated using the formula (BEIR IV methodology) for death.

### **The First Presentation: Issues in Quantitative Epidemiology: Calculating Excess Risk: When Specified Response is Mortality**

*Calculating Excess Risk using Actuarial Method or Life Table Method.* This way of calculating excess risks from a RR function is the implementation of the methodology described in “BEIR IV. Health Risks of Radon and Other Internally Deposited Alpha-Emitters. Committee on the Biological Effects of Ionizing Radiations. Board on Radiation Effects Research Commission of Life Sciences. National Research Council. National Academy Press, Washington, DC, 1988.”

BEIR IV:

Derivation of Formulas:  
(Using notation in BEIR report)

$i = 1, 2, \dots, T$

$i$  = index for the years for a person's life

year  $i$  is the year from the person's  $(i-1)$ -th birthday  
to his (or her)  $i$ -th birthday

$i=1$  refers to the year from birth to the 1st birthday

$i=1 = \text{age } 0$

...

$i=7$  refers to the year from the 6-th birthday to the 7-th birthday

$i=7 = \text{age } 6$

BEIR IV: Derivation of Formulas:

$i = 1, 2, \dots, T$

$q(i)$  = probability of surviving year  $i$   
when all causes of death are acting  
conditional on the person surviving through year  $i - 1$

$q(7)$  = probability of reaching a person's 7-th birthday  
given that he reached his 6-th birthday

$q(7) = P(\text{Death} \geq 7 \mid \text{Death} \geq 6)$

$h(i)^*$  = mortality rate due to all causes in year  $i$   
conditional on the person surviving through year  $i - 1$

$q(i) = \exp[ - h(i)^* ]$

$1 - q(i)$  = probability of death in year  $i$   
conditional on the person surviving through year  $i - 1$

BEIR IV: Derivation of Formulas:

$$i = 1, 2, \dots, T$$

$q(i)$  = probability of surviving year  $i$   
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$   
conditional on the person surviving through year  $i-1$

$$q(i) = \exp[ - h(i)^* ]$$

$1 - q(i)$  = probability of death in year  $i$   
conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) mortality rate in year  $i$   
conditional on the person not having the response  
through year  $i-1$

BEIR IV: Derivation of Formulas:

$$i = 1, 2, \dots, T$$

$q(i)$  = probability of surviving year  $i$   
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$   
conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) mortality rate in year  $i$   
conditional on the person not having the response  
through year  $i-1$

$S(1,i)$  = probability of surviving up to year  $i$  is the product of  
surviving each prior year:

$$S(1,i) = q(1) \times q(2) \times \dots \times q(i-1) \quad \text{with } S(1,1) = 1.0.$$

$S(1,i) \times [ 1 - q(i) ]$  = probability of surviving up to year  $i$  and  
then dying (from any cause) in year  $i$

BEIR IV: Derivation of Formulas:

$$i = 1, 2, \dots, T$$

$q(i)$  = probability of surviving year  $i$   
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$   
conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) mortality rate in year  $i$   
conditional on the person not having the response  
through year  $i-1$

$S(1,i)$  = probability of surviving up to year  $i$

$S(1,i) \times [1 - q(i)]$  = probability of surviving up to year  $i$  and  
then dying (from any cause) in year  $i$

$h(i)/h(i)^*$  = proportion of deaths in year  $i$  due to the response

$[h(i)/h(i)^*] \times S(1,i) \times [1 - q(i)]$  = probability of surviving  $i-1$  years  
and dying of response in year  $i$

BEIR IV: Derivation of Formulas:

$$i = 1, 2, \dots, T$$

$q(i)$  = probability of surviving year  $i$   
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$   
conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) mortality rate in year  $i$   
conditional on the person not having the response  
through year  $i-1$

$S(1,i)$  = probability of surviving up to year  $i = q(1) \times q(2) \times \dots \times q(i-1)$

$S(1,i) \times [1 - q(i)]$  = probability of surviving up to year  $i$  and  
then dying (from any cause) in year  $i$

$h(i)/h(i)^*$  = proportion of deaths in year  $i$  due to the response

$[h(i)/h(i)^*] \times S(1,i) \times [1 - q(i)]$  = probability of surviving  $i-1$  years  
and dying of response in year  $i$

$R_0 = \sum_{i=1, \dots, T} [h(i)/h(i)^*] \times S(1,i) \times [1 - q(i)]$   
= probability of a response mortality in the first  $T$  years of life  
(i.e., up to the  $T$ -th birthday, age  $T$ ) at dose 0  
(no exposure in addition to background exposure)

BEIR IV: Derivation of Formulas: Risk with exposure

$i=1, 2, \dots, T$

$q(i)$  = probability of surviving year  $i$  without exposure  
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) mortality rate in year  $i$  without exposure  
conditional on the person not having the response through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$  without exposure  
conditional on the person surviving through year  $i-1$

$f(i)$  = proportional effect (multiplier) in year  $i$  assuming a proportional hazards  
model for the effect of exposure of the form  $h(i) \times f(i)$   
 $f(i) = [ 1 + e(i) ]$  if the multiplier is a linear function

$h(i) \times f(i)$  = response (e.g., lung cancer) mortality rate in year  $i$  with exposure  
conditional on the person not having the response  
through year  $i-1$

$h(i) \times [ f(i) - 1 ]$  = increase in response mortality rate in year due to exposure

BEIR IV: Derivation of Formulas: Risk with exposure

$i=1, 2, \dots, T$

$q(i)$  = probability of surviving year  $i$  without exposure  
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) mortality rate in year  $i$  without exposure  
conditional on the person not having the response through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$  without exposure  
conditional on the person surviving through year  $i-1$

$f(i)$  = proportional effect (multiplier) in year  $i$  assuming a proportional hazards  
model for the effect of exposure of the form  $h(i) \times f(i)$   
 $f(i) = [ 1 + e(i) ]$  if multiplier is a linear function

$h(i) \times f(i)$  = response (e.g., lung cancer) mortality rate in year  $i$  with exposure  
conditional on the person not having the response  
through year  $i-1$

$h(i) \times [ f(i) - 1 ]$  = increase in response mortality rate in year due to exposure

$h(i)^* + h(i) \times [ f(i) - 1 ]$  = mortality rate due to all causes in year  $i$  with exposure  
conditional on the person surviving through year  $i-1$

### BEIR IV: Derivation of Formulas: Risk with exposure

$i=1, 2, \dots, T$

$q(i)$  = probability of surviving year  $i$  **without exposure**  
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) mortality rate in year  $i$  **without exposure**  
conditional on the person not having the response through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$  **without exposure**  
conditional on the person surviving through year  $i-1$

$f(i)$  = proportional effect (multiplier) in year  $i$  assuming a proportional hazards  
model for the effect of exposure of the form  $h(i) \times f(i)$   
 $f(i) = [ 1 + e(i) ]$  if multiplier is a linear function

$h(i) \times f(i)$  = response (e.g., lung cancer) mortality rate in year  $i$  **with exposure**  
conditional on the person not having the response  
through year  $i-1$

$h(i) \times [ f(i) - 1 ]$  = increase in response mortality rate in year  $i$  **due to exposure**

$h(i)^* + h(i) \times [ f(i) - 1 ]$  = mortality rate due to all causes in year  $i$  **with exposure**  
conditional on the person surviving through year  $i-1$

$\exp \{ - h(i)^* - h(i) \times [ f(i) - 1 ] \}$  = probability **with exposure** of surviving year  $i$   
conditional on person surviving thru year  $i-1$

$q(i) \times \exp \{ - h(i) \times [ f(i) - 1 ] \}$  = probability **with exposure** of surviving year  $i$   
conditional on person surviving thru year  $i-1$

### BEIR IV: Derivation of Formulas: Risk with exposure

$q(i)$  = probability of surviving year  $i$  **without exposure**  
when all causes of death are acting conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) mortality rate in year  $i$  **without exposure**  
conditional on the person not having the response through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$  **without exposure**  
conditional on the person surviving through year  $i-1$

$f(i)$  = proportional effect (multiplier) in year  $i$  assuming a proportional hazards  
model for the effect of exposure of the form  $h(i) \times f(i)$ ;  $f(i) = [ 1 + e(i) ]$  if multiplier is a linear function

$h(i) \times f(i)$  = response (e.g., lung cancer) mortality rate in year  $i$  **with exposure**  
conditional on the person not having the response through year  $i-1$

$h(i) \times [ f(i) - 1 ]$  = increase in response mortality rate in year **due to exposure**

$h(i)^* + h(i) \times [ f(i) - 1 ]$  = mortality rate due to all causes in year  $i$  **with exposure**  
conditional on the person surviving through year  $i-1$

$\exp \{ - h(i)^* - h(i) \times [ f(i) - 1 ] \}$  = probability **with exposure** of surviving year  $i$   
conditional on person surviving thru year  $i-1$

$q(i) \times \exp \{ - h(i) \times [ f(i) - 1 ] \}$  = probability **with exposure** of surviving year  $i$   
conditional on person surviving thru year  $i-1$

$q(1) \times \exp \{ - h(1) \times [ f(1) - 1 ] \} \times \dots \times q(i-1) \times \exp \{ - h(i-1) \times [ f(i-1) - 1 ] \}$   
=  $S(1,i) \times \exp \{ - \sum_{k=1, \dots, i-1} \{ - h(k) \times [ f(k) - 1 ] \} \}$   
= probability of surviving up to year  $i$  **with exposure**

$S(1,i) \times \exp \{ - \sum_{k=1, \dots, i-1} \{ - h(k) \times [ f(k) - 1 ] \} \} \times (1 - q(i) \times \exp \{ - h(i) \times [ f(i) - 1 ] \})$   
= probability **with exposure** of surviving up to year  $i$   
and then dying (from any cause) in year  $i$

### BEIR IV: Derivation of Formulas: Risk with exposure

$q(i)$  = probability of surviving year  $i$  **without exposure**  
 when all causes of death are acting conditional on the person surviving through year  $i-1$   
 $h(i)$  = response (e.g., lung cancer) mortality rate in year  $i$  **without exposure**  
 conditional on the person not having the response through year  $i-1$   
 $h(i)^*$  = mortality rate due to all causes in year  $i$  **without exposure**  
 conditional on the person surviving through year  $i-1$   
 $f(i)$  = proportional effect (multiplier) in year  $i$  assuming a proportional hazards  
 model for the effect of exposure of the form  $h(i) \times f(i)$ ;  $f(i) = [1 + e(i)]$  if multiplier is a linear function  
 $h(i) \times f(i)$  = response (e.g., lung cancer) mortality rate in year  $i$  **with exposure**  
 conditional on the person not having the response through year  $i-1$   
 $h(i) \times [f(i) - 1]$  = increase in response mortality rate in year **due to exposure**  
 $h(i)^* + h(i) \times [f(i) - 1]$  = mortality rate due to all causes in year  $i$  **with exposure**  
 conditional on the person surviving through year  $i-1$   
 $\exp \{ -h(i)^* - h(i) \times [f(i) - 1] \}$  = probability **with exposure** of surviving year  $i$   
 conditional on person surviving thru year  $i-1$   
 $q(i) \times \exp \{ -h(i) \times [f(i) - 1] \}$  = probability **with exposure** of surviving year  $i$   
 conditional on person surviving thru year  $i-1$   
 $q(1) \times \exp \{ -h(1) \times [f(1) - 1] \} \times \dots \times q(i-1) \times \exp \{ -h(i-1) \times [f(i-1) - 1] \}$   
 $= S(1,i) \times \exp \{ -\sum_{k=1, \dots, i-1} \{ -h(k) \times [f(k) - 1] \} \}$  = probability of surviving up to year  $i$  **with exposure**  
 $S(1,i) \times \exp \{ -\sum_{k=1, \dots, i-1} \{ -h(k) \times [f(k) - 1] \} \} \times (1 - q(i) \times \exp \{ -h(i) \times [f(i) - 1] \} )$   
 = probability **with exposure** of surviving up to year  $i$  and then dying (from any cause) in year  $i$   
 $\{ h(i) \times f(i) \} / \{ h(i)^* + h(i) \times [f(i) - 1] \}$   
 = proportion of deaths in year  $i$  due to the response **with exposure**  
 $(\{ h(i) \times f(i) \} / \{ h(i)^* + h(i) \times [f(i) - 1] \}) \times S(1,i) \times \exp \{ -\sum_{k=1, \dots, i-1} \{ -h(k) \times [f(k) - 1] \} \} \times (1 - q(i) \times \exp \{ -h(i) \times [f(i) - 1] \} )$   
 = probability of surviving  $i-1$  years and dying of response in year  $i$  **with exposure**

### BEIR IV: Derivation of Formulas: Risk with exposure

$( \{ h(i) \times f(i) \} / \{ h(i)^* + h(i) \times [f(i) - 1] \} )$   
 $\times S(1,i) \times \exp \{ -\sum_{k=1, \dots, i-1} \{ -h(k) \times [f(k) - 1] \} \}$   
 $\times (1 - q(i) \times \exp \{ -h(i) \times [f(i) - 1] \} )$   
 = probability of surviving  $i-1$  years  
 and dying of response in year  $i$  **with exposure**

$$R_{\text{exposure}} = \sum_{i=1, \dots, T} ( \{ h(i) \times f(i) \} / \{ h(i)^* + h(i) \times [f(i) - 1] \} ) \times S(1,i) \times \exp \{ -\sum_{k=1, \dots, i-1} \{ -h(k) \times [f(k) - 1] \} \} \times (1 - q(i) \times \exp \{ -h(i) \times [f(i) - 1] \} )$$

= probability of a response mortality in the first  $T$  years of  
 life (i.e., up to the  $T$ -th birthday, age  $T$ ) **with exposure**  
 (with exposure in addition to the background exposure)

### BEIR IV: Risks

$R_0 = \sum_{i=1, \dots, T} [ h(i)/h(i)^* ] \times S(1, i) \times [ 1 - q(i) ]$   
 = probability of a response mortality in the first T years of life (i.e., up to the T-th birthday, age T ) at dose 0  
 (no exposure in addition to background exposure)

$R_{\text{exposure}} = \sum_{i=1, \dots, T} ( \{ h(i) \times f(i) \} / \{ h(i)^* + h(i) \times [ f(i)-1 ] \} )$   
 $\times S(1, i) \times \exp(- \sum_{k=1, \dots, i-1} \{ -h(k) \times [ f(k)-1 ] \} )$   
 $\times ( 1 - q(i) \times \exp \{ - h(i) \times [ f(i) - 1 ] \} )$   
 = probability of a response mortality in the first T years of life (i.e., up to the T-th birthday, age T ) with exposure  
 (with exposure in addition to the background exposure)

$$\text{Added Risk} = R_{\text{exposure}} - R_0$$

$$\text{Extra Risk} = ( R_{\text{exposure}} - R_0 ) / ( 1 - R_0 )$$

Excess Risk = either Added Risk or Extra Risk

### The Second Presentation: 3.1 Issues in Quantitative Epidemiology: Calculating Excess Risk: When Specified Response is Incidence

Calculating Excess Risk using Actuarial Method or Life Table Method. The following derivation for the situation in which the specified response is incidence (not death) “parallels” the derivation in BEIR IV; however, the derivation and result are necessarily different for incidence than for mortality.

“BEIR IV. Health Risks of Radon and Other Internally Deposited Alpha-Emitters. Committee on the Biological Effects of Ionizing Radiations. Board on Radiation Effects Research Commission of Life Sciences. National Research Council. National Academy Press, Washington, DC, 1988.”

Derivation of Formulas:  
(Using notation in BEIR report)

$$i = 1, 2, \dots, T$$

$i$  = index for the years for a person's life

year  $i$  is the year from the person's  $(i-1)$ -th birthday  
to his (or her)  $i$ -th birthday

$i=1$  refers to the year from birth to the 1st birthday

$i=1$  = age 0

...

$i=7$  refers to the year from the 6-th birthday to the 7-th birthday

$i=7$  = age 6

Derivation of Formulas:

$$i = 1, 2, \dots, T$$

$q(i)$  = probability of surviving year  $i$   
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$q(7)$  = probability of reaching a person's 7-th birthday  
given that he reached his 6-th birthday

$$q(7) = P(\text{Death} \geq 7 \mid \text{Death} \geq 6)$$

$h(i)^*$  = mortality rate due to all causes in year  $i$   
conditional on the person surviving through year  $i-1$

$q(i) = \exp[-h(i)^*]$  -- definition of hazard rate

$1 - q(i)$  = probability of death in year  $i$   
conditional on the person surviving through year  $i-1$

Derivation of Formulas:

$$i = 1, 2, \dots, T$$

$q(i)$  = probability of surviving year  $i$   
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$   
conditional on the person surviving through year  $i-1$

$$q(i) = \exp[ - h(i)^* ]$$

$1 - q(i)$  = probability of death in year  $i$   
conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) incidence rate in year  $i$   
conditional on the person not having the response  
through year  $i-1$

Note that  $h(i)$  is NOT part of  $h(i)^*$ ,  
because  $h(i)$  refers to incidence and  $h(i)^*$  refers to death.

Derivation of Formulas:

$$i = 1, 2, \dots, T$$

$q(i)$  = probability of surviving year  $i$   
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$   
conditional on the person surviving through year  $i-1$

$$q(i) = \exp[ - h(i)^* ]$$

$1 - q(i)$  = probability of death in year  $i$   
conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) incidence rate in year  $i$   
conditional on the person not having the response  
through year  $i-1$

$qr(i) = \exp[ - h(i) ]$  = probability of no response in year  $i$   
conditional on the person not responding through year  $i-1$

$1 - qr(i)$  = probability of response (incidence) in year  $i$   
conditional on the person not responding through year  $i-1$

Derivation of Formulas:

$i = 1, 2, \dots, T$

$q(i)$  = probability of surviving year  $i$   
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$   
conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) incidence rate in year  $i$   
conditional on the person not having the response  
through year  $i-1$

$qr(i)$  = probability of no response (incidence) in year  $i$   
conditional on the person not responding through year  $i-1$

$S(1,i)$  = probability of surviving up to year  $i$  is the product of  
surviving each prior year:

$$S(1,i) = q(1) \times q(2) \times \dots \times q(i-1) \quad \text{with } S(1,1) = 1.0.$$

$SR(1,i)$  = probability of no response up to year  $i$  is the product of  
no response in each prior year:

$$SR(1,i) = qr(1) \times qr(2) \times \dots \times qr(i-1) \quad \text{with } SR(1,1) = 1.0.$$

Derivation of Formulas:

$i = 1, 2, \dots, T$

$q(i)$  = probability of surviving year  $i$  when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$   
conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) incidence rate in year  $i$   
conditional on the person not having the response  
through year  $i-1$

$qr(i)$  = probability of no response (incidence) in year  $i$   
conditional on the person not responding through year  $i-1$

$S(1,i)$  = probability of surviving up to year  $i$  is the product of  
surviving each prior year:

$$S(1,i) = q(1) \times q(2) \times \dots \times q(i-1) \quad \text{with } S(1,1) = 1.0.$$

$SR(1,i)$  = probability of no response up to year  $i$  is the product of  
no response in each prior year:

$$SR(1,i) = qr(1) \times qr(2) \times \dots \times qr(i-1) \quad \text{with } SR(1,1) = 1.0.$$

$S(1,i) \times SR(1,i) \times [1 - q(i) \times qr(i)]$  = probability of surviving to year  $i$ ,  
not responding before year  $i$ , and  
then dying (from any cause) or having the response in year  $i$

### Derivation of Formulas:

$i = 1, 2, \dots, T$

$q(i)$  = probability of surviving year  $i$  when all causes of death are acting conditional on the person surviving through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$  conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) incidence rate in year  $i$  conditional on the person not having the response through year  $i-1$

$qr(i)$  = probability of no response (incidence) in year  $i$  conditional on the person not responding through year  $i-1$

$S(1,i)$  = probability of surviving up to year  $i$  is the product of surviving each prior year:  
 $S(1,i) = q(1) \times q(2) \times \dots \times q(i-1)$  with  $S(1,1) = 1.0$ .

$SR(1,i)$  = probability of no response up to year  $i$  is the product of no response in each prior year:  
 $SR(1,i) = qr(1) \times qr(2) \times \dots \times qr(i-1)$  with  $SR(1,1) = 1.0$ .

$S(1,i) \times SR(1,i) \times [1 - q(i) \times qr(i)]$  = probability of surviving to year  $i$ , not responding before year  $i$ , and then dying (from any cause) or having the response in year  $i$

A person is "observed" in year  $i$  if that person either dies in year  $i$  or has the specified response (incidence) in year  $i$ .

$h(i) / [ h(i)^* + h(i) ]$  = proportion of observations (deaths plus incidences) in year  $i$  due to the response

$\{ h(i) / [ h(i)^* + h(i) ] \} \times S(1,i) \times SR(1,i) \times [1 - q(i) \times qr(i)]$  = probability of surviving to year  $i$ , not responding before year  $i$ , and then having the response (incidence) in year  $i$

### Derivation of Formulas:

$i = 1, 2, \dots, T$

$q(i)$  = probability of surviving year  $i$  when all causes of death are acting conditional on the person surviving through year  $i-1$

$h(i)^*$  = mortality rate due to all causes in year  $i$  conditional on the person surviving through year  $i-1$

$h(i)$  = response (e.g., lung cancer) incidence rate in year  $i$  conditional on the person not having the response through year  $i-1$

$qr(i)$  = probability of no response (incidence) in year  $i$  conditional on the person not responding through year  $i-1$

$S(1,i)$  = probability of surviving up to year  $i$  is the product of surviving each prior year:  
 $S(1,i) = q(1) \times q(2) \times \dots \times q(i-1)$  with  $S(1,1) = 1.0$ .

$SR(1,i)$  = probability of no response up to year  $i$  is the product of no response in each prior year:  
 $SR(1,i) = qr(1) \times qr(2) \times \dots \times qr(i-1)$  with  $SR(1,1) = 1.0$ .

$S(1,i) \times SR(1,i) \times [1 - q(i) \times qr(i)]$  = probability of surviving to year  $i$ , not responding before year  $i$ , and then dying (from any cause) or having the response in year  $i$

$h(i) / [ h(i)^* + h(i) ]$  = proportion of observations (deaths plus incidences) in year  $i$  due to the response

$\{ h(i) / [ h(i)^* + h(i) ] \} \times S(1,i) \times SR(1,i) \times [1 - q(i) \times qr(i)]$  = probability of surviving to year  $i$ , not responding before year  $i$ , and then having the response (incidence) in year  $i$

$R_0 = \sum_{i=1, \dots, T} \{ h(i) / [ h(i)^* + h(i) ] \} \times S(1,i) \times SR(1,i) \times [1 - q(i) \times qr(i)]$   
= probability of a response (incidence) in the first  $T$  years of life (i.e., up to the  $T$ -th birthday, age  $T$ ) at dose 0 (no exposure in addition to background exposure)

Derivation of Formulas:

Background Risk of an Incidence:

$$R_0 = \sum_{i=1, \dots, T} \{ h(i) / [ h(i)^* + h(i) ] \} \times S(1, i) \times \mathbf{SR(1, i)} \times [1 - q(i) \times \mathbf{qr(i)}]$$

= probability of a response (incidence) in the first T years of life  
(i.e., up to the T-th birthday, age T ) at dose 0  
(no exposure in addition to background exposure)

Contrast with the form of the calculation for the  
Background Risk of a Mortality  
and that h(i) refers to mortality here and incidence above:

$$R_0 = \sum_{i=1, \dots, T} [ h(i) / h(i)^* ] \times S(1, i) \times [1 - q(i)]$$

= probability of a response mortality in the first T years of  
life (i.e., up to the T-th birthday, age T ) at dose 0  
(no exposure in addition to background exposure)

Derivation of Formulas: Risk with exposure

i=1, 2, ..., T

q(i) =  $\exp [ - h(i)^* ]$  = probability of surviving year i without exposure  
when all causes of death are acting  
conditional on the person surviving through year i-1

h(i)\* = mortality rate due to all causes in year i without exposure  
conditional on the person surviving through year i-1

h(i) = response (e.g., lung cancer) incidence rate in year i without exposure  
conditional on the person not having the response through year i-1

qr(i) =  $\exp [ - h(i) ]$  = probability of no response in year i without exposure  
conditional on the person not responding through year i-1

f(i) = proportional effect (multiplier) in year i assuming a proportional hazards  
model for the effect of exposure of the form  $h(i) \times f(i)$   
 $f(i) = [ 1 + e(i) ]$  if the multiplier is a linear function

$h(i) \times f(i)$  = response (e.g., lung cancer) incidence rate in year i with exposure  
conditional on the person not having the response  
through year i-1

### Derivation of Formulas: Risk with exposure

$i=1, 2, \dots, T$

$q(i) = \exp[-h(i)^*] =$  probability of surviving year  $i$  **without exposure**  
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^* =$  mortality rate due to all causes in year  $i$  **without exposure**  
conditional on the person surviving through year  $i-1$

$h(i) =$  response (e.g., lung cancer) incidence rate in year  $i$  **without exposure**  
conditional on the person not having the response through year  $i-1$

$qr(i) = \exp[-h(i)] =$  probability of no response (incidence) in year  $i$  **without exposure**  
conditional on the person not responding through year  $i-1$

$f(i) =$  proportional effect (multiplier) in year  $i$  assuming a proportional hazards  
model **for the effect of exposure** of the form  $h(i) \times f(i)$   
 $f(i) = [1 + e(i)]$  if the multiplier is a linear function

$h(i) \times f(i) =$  response (e.g., lung cancer) incidence rate in year  $i$  **with exposure**  
conditional on the person not having the response through year  $i-1$

A person is "observed" in year  $i$  if that person either dies in year  $i$   
or has the specified response (incidence) in year  $i$ .

$h(i)^* + h(i) \times f(i) =$  observation rate due to all causes in year  $i$  **with exposure**  
conditional on the person not dying or having the response through year  $i-1$

### Derivation of Formulas: Risk with exposure

$i=1, 2, \dots, T$

$q(i) = \exp[-h(i)^*] =$  probability of surviving year  $i$  **without exposure**  
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^* =$  mortality rate due to all causes in year  $i$  **without exposure**  
conditional on the person surviving through year  $i-1$

$h(i) =$  response (e.g., lung cancer) incidence rate in year  $i$  **without exposure**  
conditional on the person not having the response through year  $i-1$

$qr(i) = \exp[-h(i)] =$  probability of no response (incidence) in year  $i$  **without exposure**  
conditional on the person not responding through year  $i-1$

$f(i) =$  proportional effect (multiplier) in year  $i$  assuming a proportional hazards  
model **for the effect of exposure** of the form  $h(i) \times f(i)$   
 $f(i) = [1 + e(i)]$  if the multiplier is a linear function

$h(i) \times f(i) =$  response (e.g., lung cancer) incidence rate in year  $i$  **with exposure**  
conditional on the person not having the response through year  $i-1$

$h(i)^* + h(i) \times f(i) =$  observation rate due to all causes in year  $i$  **with exposure**  
conditional on the person not dying or having the response through year  $i-1$

$\exp\{-h(i)^* - h(i) \times f(i)\} = q(i) \times \exp\{-h(i) \times f(i)\}$   
 $= q(i) \times \exp\{-h(i) - h(i) \times [f(i) - 1]\} = q(i) \times qr(i) \times \exp\{-h(i) \times [f(i) - 1]\}$   
probability **with exposure** of not dying and not  
responding in year  $i$  conditional on not dying and not responding thru year  $i-1$

Derivation of Formulas: Risk with exposure

$i=1, 2, \dots, T$

$q(i) = \exp[-h(i)^*] =$  probability of surviving year  $i$  **without exposure**  
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^* =$  mortality rate due to all causes in year  $i$  **without exposure**  
conditional on the person surviving through year  $i-1$

$h(i) =$  response (e.g., lung cancer) incidence rate in year  $i$  **without exposure**  
conditional on the person not having the response through year  $i-1$

$qr(i) = \exp[-h(i)] =$  probability of no response (incidence) in year  $i$  **without exposure**  
conditional on the person not responding through year  $i-1$

$S(1,i) =$  probability of surviving up to year  $i$  is the product of surviving each prior year:  
 $S(1,i) = q(1) \times q(2) \times \dots \times q(i-1)$  with  $S(1,1) = 1.0$ .

$SR(1,i) =$  probability of no response up to year  $i$  is the product of no response in each prior year:  
 $SR(1,i) = qr(1) \times qr(2) \times \dots \times qr(i-1)$  with  $SR(1,1) = 1.0$ .

$q(i) \times qr(i) \times \exp\{-h(i) \times [f(i)-1]\} =$  probability with exposure of not dying and not responding in year  $i$  conditional on not dying and not responding thru year  $i-1$

$q(1) \times qr(1) \times \exp\{-h(1) \times [f(1)-1]\} \times \dots \times q(i-1) \times qr(i-1) \times \exp\{-h(i-1) \times [f(i-1)-1]\}$   
 $= S(1,i) \times SR(1,i) \times \exp\left(-\sum_{k=1, \dots, i-1} \{-h(k) \times [f(k) - 1]\}\right)$   
 $=$  probability with exposure of not dying and not responding up to year  $i$

$S(1,i) \times SR(1,i) \times \exp\left(-\sum_{k=1, \dots, i-1} \{-h(k) \times [f(k)-1]\}\right) \times [1-q(i) \times qr(i) \times \exp\{-h(i) \times [f(i)-1]\}]$   
 $=$  probability with exposure of not dying and not responding up to year  $i$   
and then dying (from any cause) or having the response in year  $i$

Derivation of Formulas: Risk with exposure

$i=1, 2, \dots, T$

$q(i) = \exp[-h(i)^*] =$  probability of surviving year  $i$  **without exposure**  
when all causes of death are acting  
conditional on the person surviving through year  $i-1$

$h(i)^* =$  mortality rate due to all causes in year  $i$  **without exposure**  
conditional on the person surviving through year  $i-1$

$h(i) =$  response (e.g., lung cancer) incidence rate in year  $i$  **without exposure**  
conditional on the person not having the response through year  $i-1$

$qr(i) = \exp[-h(i)] =$  probability of no response (incidence) in year  $i$  **without exposure**  
conditional on the person not responding through year  $i-1$

$S(1,i) =$  probability of surviving up to year  $i$  is the product of surviving each prior year:  
 $S(1,i) = q(1) \times q(2) \times \dots \times q(i-1)$  with  $S(1,1) = 1.0$ .

$SR(1,i) =$  probability of no response up to year  $i$  is the product of no response in each prior year:  
 $SR(1,i) = qr(1) \times qr(2) \times \dots \times qr(i-1)$  with  $SR(1,1) = 1.0$ .

$q(i) \times qr(i) \times \exp\{-h(i) \times [f(i)-1]\} =$  probability with exposure of not dying and not responding in year  $i$  conditional on not dying and not responding thru year  $i-1$

$S(1,i) \times SR(1,i) \times \exp\left(-\sum_{k=1, \dots, i-1} \{-h(k) \times [f(k) - 1]\}\right) \times [1 - q(i) \times qr(i) \times \exp\{-h(i) \times [f(i)-1]\}]$   
 $=$  probability with exposure of not dying and not responding up to year  $i$   
and then dying (from any cause) or having the response in year  $i$

$\{h(i) \times f(i)\} / \{h(i)^* + h(i) \times f(i)\} =$  proportion of observations (deaths plus incidences)  
in year  $i$  due to the response with exposure

$\left(\frac{h(i) \times f(i)}{h(i)^* + h(i) \times f(i)}\right) \times S(1,i) \times SR(1,i) \times \exp\left(-\sum_{k=1, \dots, i-1} \{-h(k) \times [f(k)-1]\}\right)$   
 $\times [1-q(i) \times qr(i) \times \exp\{-h(i) \times [f(i)-1]\}] =$  probability with exposure of not dying and not responding  
up to year  $i$  and then having the response in year  $i$

Derivation of Formulas: Risk with exposure

$$\begin{aligned}
 & ( \{ h(i) \times f(i) \} / \{ h(i)^* + h(i) \times f(i) \} ) \\
 & \times S(1,i) \times SR(1,i) \times \exp(- \sum_{k=1, \dots, i-1} \{ -h(k) \times [f(k)-1] \}) \\
 & \times [ 1 - q(i) \times qr(i) \times \exp \{ -h(i) \times [ f(i)-1 ] \} ] \\
 & = \text{probability of not dying and not responding in } i-1 \text{ years} \\
 & \text{and then having the response in year } i \text{ with exposure}
 \end{aligned}$$

$$\begin{aligned}
 R_{\text{exposure}} &= \sum_{i=1, \dots, T} \\
 & ( \{ h(i) \times f(i) \} / \{ h(i)^* + h(i) \times f(i) \} ) \\
 & \times S(1,i) \times SR(1,i) \times \exp(- \sum_{k=1, \dots, i-1} \{ -h(k) \times [ f(k)-1 ] \} ) \\
 & \times [ 1 - q(i) \times qr(i) \times \exp \{ -h(i) \times [ f(i)-1 ] \} ]
 \end{aligned}$$

= probability of a response (incidence) in the first T years of life (i.e., up to the T-th birthday, age T ) with exposure (with exposure in addition to the background exposure)

Derivation of Formulas:

Risk of an Incidence with exposure:

$$\begin{aligned}
 R_{\text{exposure}} &= \sum_{i=1, \dots, T} \\
 & ( \{ h(i) \times f(i) \} / \{ h(i)^* + h(i) \times f(i) \} ) \\
 & \times S(1,i) \times SR(1,i) \times \exp(- \sum_{k=1, \dots, i-1} \{ -h(k) \times [ f(k)-1 ] \} ) \\
 & \times [ 1 - q(i) \times qr(i) \times \exp \{ -h(i) \times [ f(i)-1 ] \} ]
 \end{aligned}$$

Contrast with the form of the calculation for the Risk of a Mortality with exposure

and that h(i) refers to mortality here and incidence above:

$$\begin{aligned}
 R_{\text{exposure}} &= \sum_{i=1, \dots, T} ( \{ h(i) \times f(i) \} / \{ h(i)^* + h(i) \times [ f(i)-1 ] \} ) \\
 & \times S(1,i) \times \exp(- \sum_{k=1, \dots, i-1} \{ -h(k) \times [ f(k)-1 ] \} ) \\
 & \times ( 1 - q(i) \times \exp \{ - h(i) \times [ f(i) - 1 ] \} )
 \end{aligned}$$

### Risks

$R_0 = \sum_{i=1, \dots, T} \{ h(i) / [ h(i)^* + h(i) ] \} \times S(1, i) \times SR(1, i) \times [ 1 - q(i) \times qr(i) ]$   
 = probability of a response (incidence) in the first T years of life  
 (i.e., up to the T-th birthday, age T )

at dose 0 (no exposure in addition to background exposure)

$$R_{\text{exposure}} = \sum_{i=1, \dots, T}$$

$$\left( \frac{h(i) \times f(i)}{h(i)^* + h(i) \times f(i)} \right)$$

$$\times S(1, i) \times SR(1, i) \times \exp(- \sum_{k=1, \dots, i-1} \{ -h(k) \times [ f(k)-1 ] \})$$

$$\times [ 1 - q(i) \times qr(i) \times \exp \{ -h(i) \times [ f(i)-1 ] \} ]$$

= probability of a response (incidence) in the first T years of  
 life (i.e., up to the T-th birthday, age T ) with exposure

(with exposure in addition to the background exposure)

$$\text{Added Risk} = R_{\text{exposure}} - R_0$$

$$\text{Extra Risk} = ( R_{\text{exposure}} - R_0 ) / ( 1 - R_0 )$$

Excess Risk = either Added Risk or Extra Risk