CASE STUDY A

Carcinogenic Effect Level for Methylene Chloride

Summary

- Based on the dose-response data in this example, it would be reasonable to expect a carcinogenic response in some individuals exposed chronically to around 650 to 4,100 ppm or 1,150 to 4,100 ppm MC. This range is 6,500 to 41,000 times higher than TCEQ's long-term health-based comparison value for ambient air of 0.1 ppm (TCEQ 2011 http://www.tceq.state.tx.us/implementation/tox/dsd/final.html).
- The results of this process to determine carcinogenic effect levels may fit well with relevant human data for the chemical. For example, concentrations in excess of 500 ppm may be needed to saturate the high-affinity MFO metabolic pathway, and MC is not known to cause cancer even in workers exposed to high MC concentrations (e.g., no excess risk of death from malignant neoplasms has been detected in workers exposed to MC at levels up to 475 ppm) (ATSDR 2000).

Carcinogenic Effect Level: For animal studies, assuming relevance to humans (e.g., MOA, sufficiently similar PK), an air concentration corresponding to the detected increase in cancer incidence/mortality over background can be used as a starting point to determine a likely human carcinogenic effect level. Not extrapolating far below the actual dose-response data increases confidence that tumorigenesis will occur in some chronically-exposed individuals when a carcinogenic point of departure (POD) in animals is converted to a human equivalent concentration (POD_{HEC}), assuming human relevance or that interspecies differences leading to differences in sensitivity have been accounted for (e.g., PBPK). For this example, the following summary of mouse data was used to estimate human carcinogenic effects levels assuming human relevance and chronic exposure to methylene chloride (MC).

Mouse Tumor Incidence Data from NTP (1986) [a]

Gender	Target Tissue	Administered Dose (ppm)	Adenoma	Carcinoma	Combined
Female	Lung [b]	0	2/50	1/50	3/50
		2,000	23/48	13/48	**30/48 (57%↑)**
		4,000	28/48	29/48	41/48
	Liver [c]	0	2/50	1/50	3/50
		2,000	**6/48 (8.5%↑)**	11/48	**16/48 (27%↑)**
		4,000	22/48	32/48	40/48
Male	Lung [b]	0	3/50	2/50	5/50
		2,000	19/50	10/50	27/50
		4,000	24/50	28/50	40/50
	Liver [c]	0	10/50	13/50	22/50
		2,000	14/49	15/49	**24/49 (5%↑)**
		4,000	14/49	26/49	33/49

[a] from Health Canada (1993).

[b] all $p < 0.001$ except for alveolar-bronchiolar carcinoma in males with $p = 0.016$.

[c] generally $p \leq 0.001$ or $p < 0.05$.

Since low-dose extrapolation significantly below the data introduces uncertainty about the probability of a response (e.g., dose-dependent transitions in metabolism), absent human data, the lowest POD corresponding to excess risk observed in a relevant animal study may be the lowest level for which cancer effects in some individuals in the human population would be expected with reasonable certainty if chronically exposed to the corresponding POD_{HEC}. For the above mouse liver and lung tumor data, the tumor HEC doses corresponding to a 5% excess tumor rate for humans ($TD_{HEC\ 0.05}$) were calculated using PBPK modeling (Health Canada 1993). [The modeling accounted for MC metabolism in both humans and mice through both the MFO pathway and GST pathway (putative carcinogenic pathway) using the typical dose metric for MC risk assessments (i.e., internal dose of MC metabolized through the GST pathway (glutathione conjugate) in the liver and lung)].

Environmental $TD_{HEC\ 0.05}$ Values Based on Mouse Liver/Lung Tumor Data [a]

Target Tissue	Tumor Type	$TD_{HEC\ 0.05}$ based on Female Mouse Data (ppm)	$TD_{HEC\ 0.05}$ based on Male Mouse Data (ppm)
Lung	Adenoma	**1155**	1634
	Carcinoma	2651	5257
	Combined	**645**	902
Liver	Adenoma	4092	5590
	Carcinoma	2965	4467
	Combined	**2408**	**4106**

[a] From Health Canada (1993).

The lowest $TD_{HEC\ 0.05}$ values for humans (645 and 2,408 ppm for female mouse lung and liver tumors, respectively) involved extrapolation well below the data, since these tumors were increased 27-56% even at the lowest dose. On the other hand, male mouse liver tumors increased 5% at the lowest dose, so the corresponding $TD_{HEC\ 0.05}$ value for humans (4,106 ppm) did not require extrapolation below the data, and female liver adenomas were increased 8.5% at the low dose ($TD_{HEC\ 0.05}$ of 1,155 ppm). Thus, based on the dose-response data in this example, it would be reasonable to expect a carcinogenic response in some individuals exposed chronically to around 650 to 4,100 ppm or 1,150 to 4,100 ppm MC. This range is 6,500 to 41,000 times higher than the TCEQ 1E-05 excess risk air concentration of 0.1 ppm calculated based on TCEQ's inhalation unit risk factor (URF) of 9.8E-08 per ppb (2.8E-08 per $\mu g/m^3$) (TCEQ 2010 http://www.tceq.state.tx.us/implementation/tox/dsd/final.html).

[The results of this process to determine carcinogenic effect levels may fit well with relevant human data for the chemical. For example, concentrations in excess of 500 ppm may be needed to saturate the high-affinity MFO metabolic pathway, and MC is not known to cause cancer even in workers exposed to high MC concentrations (e.g., no excess risk of death from malignant neoplasms has been detected in workers exposed to MC at levels up to 475 ppm) (ATSDR 2000).]

CASE STUDY B

Acute Effect Levels and Chronic Carcinogenic Effect Levels for 1,3-Butadiene (BD)

B-1 Acute adverse effect level using benchmark concentration (BMC) modeling (threshold MOA)

SUMMARY

- **3,700 µg/m^3 (1,700 ppb):** The health-protective acute reference value (ReV) is based on critical effects of reduction in extragestational weight gain and fetal body weight in a multi-day developmental study in mice. The ReV is based on the $BMCL_{1\ SD}$ of 51.3 ppm for reduction in extragestational weight gain converted to an HEC concentration of 51.3 ppm and divided by total UFs of 30 (Table B-1A) (TCEQ 2008 http://www.tceq.state.tx.us/implementation/tox/dsd/final.html)) [Note: The $BMCL_{05}$ for reduction in fetal body weight was 54.7 ppm]

- **150,000 µg/m^3 (66,000 ppb):** This adverse effect level is based on the critical effect of decreased fetal body weight observed in a developmental study in mice, an animal species known to be more sensitive than humans. The value is based on the BMC_{05} of 65.8 ppm converted to an HEC concentration of 65.8 ppm (Table B-1A). [Note: The $BMC_{1\ SD}$ for reduction in extragestational weight gain was 723 ppm]

- **3,300,000 µg/m^3 (1,500,000 ppb):** This adverse effect level is based on persistent reductions in body weight parameters in F0 and F1 males and females rats, an animal species known to be more similar to humans. The study is a multi-day exposure study. The LOAEL is 1500 ppm and the HEC concentration is 1500 ppm (Table B-1A).

- **4,420,000 µg/m^3 (2,000,000 ppb):** This value is the lowest known effect level in humans for slight smarting of the eyes and difficulty in focusing on instrument scales after two humans were exposed for seven hours to BD (Carpenter *et al.* 1944; TCEQ 2008) (Table B-1B).

Acute Adverse Effect Levels

Determinations of actual effect levels should be based on actual dose-response data as the foundation for any expectation of effects in some exposed individuals. Potential human effects levels are best estimated based on human data. Available human data show that BD produces slight smarting of the eyes and difficulty in focusing (mild neurological effects) at high concentrations. The acute toxicity of BD is of low order.

Reproductive/developmental effects occur in animals at lower concentrations than mild neurological effects in humans. There has been one epidemiology study that investigated reproductive/developmental effects in humans and no effects were noted (Albertini *et al.* (2007). However, the ability of the study to detect differences in the evaluated endpoints may be limited because there were few subjects evaluated. Therefore, reproductive/developmental effects observed in mice after exposure to BD were chosen as the critical effects for determining the acute ReV. Reproductive/developmental effects are also used to provide a range of effect levels for mice, a more sensitive species than humans, and rats, a species thought to be similar to humans based on the metabolism of BD to the reactive diepoxide. For mice data, BMC modeling was conducted, so a BMC level was used as the POD_{HEC} for the effect level whereas for rat data, a $LOAEL_{HEC}$ was used.

DEB is the BD metabolite responsible for ovarian effects and possibly the reproductive/developmental effects (USEPA 2002; TCEQ 2008). Humans produce much lower levels of DEB than mice as demonstrated by experimental data on urinary metabolites (Sabourin *et al.* 1992). 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.

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.

BMC_{HEC} values estimated from mice data represent concentrations at which it is possible that similar effects could occur in some humans exposed to these levels over the same or longer durations as used in the animal studies, although mice produce more of the reactive diepoxide than rats or humans. However, it must be assumed that effects in some humans are possible at the BMC_{HEC} in the most sensitive species. However, effects in some humans are not a certainty at the BMC_{HEC} estimated based on the mice data. In such circumstances, as humans could be more similar to the rat, the determination of possible effect levels needs to be put into the context of study results for rats which did not show effects at similar or higher levels/durations as mice. Therefore, information on effect levels in rats is provided.

The animal POD is not divided by an uncertainty factor in consideration of unknown potential species differences as this may very well negate the expectation of a response even in the animal species (much less humans) based on the dose-response data (i.e., dividing the appropriate POD_{HEC} by an uncertainty factor likely has an unknown effect on the probability of response observed in the study). That is, for example, it interjects uncertainty about the expectation of a human response occurring in some individuals when assuming that the human dose-response is similar to that demonstrated for the most sensitive species.

Table B-1A is a summary of the development of the acute ReV (TCEQ 2008) and acute effect levels in mice, a species believed to be more sensitive than humans. Also shown is the acute level in rats, a species believed to be more similar to humans. Table B-1B is a summary of acute effects of BD in humans. Please refer to TCEQ (2008) http://www.tceq.state.tx.us/implementation/tox/dsd/final.html for additional details.

Table B-1A: Derivation of the Acute ReV and Acute Effect Level			
Study	Hackett *et al.* 1987b		ACC 2003
Study population	CD-1 mice (18-21 pregnant mice per dose group)		Crl:CD® (Sprague-Dawley) IGS BR rats (12 male and 12 female rats per dose group)
Study quality	High		High
Exposure Methods	0, 40, 200, and 1,000 ppm on gestation days (GD) 6-15 for 6 h/day		0, 300, 1,500, and 6,000 ppm (14 days prior to breeding, during gestation, and lactation) for 6 h/day
Critical Effects	Reduction in extragestational weight gain and fetal body weight; developmental toxicity		Persistent reductions in body weight parameters in F_0 and F_1 males and females
	Acute ReV Mice	**Acute Effect Level** **Mice (species more sensitive than humans)**	**Acute Effect Level** **Rat (species more similar to humans)**
POD	51.3 ppm ($BMCL_{1\ SD}$) Reduction in extragestational weight gain * 54.7 ppm ($BMCL_{05}$) reduction in fetal body weight *	723 ppm ($BMC_{1\ SD}$) Reduction in extragestational weight gain * 65.8 ppm (BMC_{05}) reduction in fetal body weight *	300 ppm (NOAEL) 1500 ppm (LOAEL)
Exposure Duration	6 h	6 h	6 h
Extrapolation to 1 h	No adjustment because the critical effect was a maternal/ developmental endpoint	Not applicable (NA)	NA
6 h POD_{animal}	51.3 ppm ppm ($BMCL_{1\ SD}$)	65.8 ppm (BMC_{05}) *	1500 ppm (LOAEL)
6-h POD_{HEC}	51.3 ppm (gas with systemic effects, based on default RGDR = 1.0)	65.8 ppm (gas with systemic effects, based on default RGDR = 1.0)	1500 ppm (gas with systemic effects, based on default RGDR = 1.0)
Total uncertainty factors (UFs)	30	NA	NA
Interspecies UF	1	NA	NA
Intraspecies UF	10	NA	NA
LOAEL UF	Not applicable	NA	NA
Incomplete Database UF *Database Quality*	3 High	NA	NA
acute ReV [6 hr]	**3,700 µg/m^3 (1,700 ppb)**	**150,000 µg/m^3 (66,000 ppb)**	**3,300,000 µg/m^3 (1,500,000 ppb)**

* The BMC_{05} was used as the basis of the effect level since reduction in fetal body weight of 65.8 ppm was lower than the BMC_{1SD} for reduction in extragestational weight gain. The LOAEL was 200 ppm LOAEL for both endpoints.

Table B-1B. Acute Effects of BD in Humans			
Study	**Concentration (Exposure Duration)**	**Subjective Symptoms**	**Differences Observed**
Carpenter *et al.* 1944 2 males 1-hour (h) lunch break Nominal Concentrations	2,000 ppm [1] (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 [2]	
Larionov *et al.* (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

[1] 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.

[2] No subjective complaints because of slight anxiety of subjects concerning the possibility of an explosion

B-2 Chronic carcinogenic effect level using occupational epidemiological study (nonthreshold MOA)

SUMMARY:

- **20 µg/m^3 (9.1 ppb):** 10^{-5} Excess Cancer Risk for leukemia mortality based on a URF of 1.050E-03/ppm (95% UCL) calculated for the general population and including a Age-Dependent Adjustment Factor (Table B-2B) (Grant et al. 2009; TCEQ 2008 http://www.tceq.state.tx.us/implementation/tox/dsd/final.html)
- **290 µg/m^3 (130 ppb):** 10^{-4} Excess Cancer Risk Range for leukemia mortality based on a URF of 7.471E-04/ppm (maximum likelihood estimate (MLE)) (Table B-2B)
- **1800 µg/m^3 (800 ppb):** A free standing NOAEL for biomarkers of effect (hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutations and chromosome aberrations) at mean BD exposure concentrations of 800 ppb has been demonstrated by Albertini et al. (2001) in a small initial study of workers in the Czech Republic.
- **2,900 µg/m^3 (1300) ppb:** 10^{-3} Excess Cancer Risk Range for leukemia mortality based on a URF of 7.471E-04/ppm (MLE) calculated for the general population (Table B-2B)
- **6000 µg/m^3 (2700 ppb):** ≤ 300-ppm-years was the lowest cumulative ppm-years interval (occupational exposure) where the likelihood ratio test that slope = 0 was *not* statistically significant ($p < 0.0591$) for different maximum levels of cumulative BD ppm-years for deaths in which leukemia is the primary cause of death or a contributing cause of death: total leukemia (Sielken et al. 2011) calculated for the general population (Table B-2A)
- **8000 µg/m^3 (3600 ppb):** ≤ 400-ppm-years was the lowest cumulative ppm-years interval (occupational exposure) where the likelihood ratio test that slope = 0 *was* statistically significant ($p < 0.0156$) for different maximum levels of cumulative BD ppm-years for deaths in which leukemia is the primary cause of death or a contributing cause of death: total leukemia (Sielken et al. 2011) calculated for the general population (Table B-2A)

Development of Carcinogenic Effect Levels

A carcinogenic effects level may be estimated based on an evaluation of the dose-response data. More specifically, the lowest air concentration/exposure corresponding to excess risk observed in the key epidemiological study can be considered the lowest level for which cancer effects in some individuals in the human population would be expected with reasonable certainty if exposed over a similar (or longer) exposure duration than those in the epidemiological study. Sielken investigated the statistical significance for different maximum levels of cumulative BD ppm-years for deaths in which leukemia is the primary cause of death or a contributing cause of death for total leukemia (Sielken et al. 2011). The lowest cumulative BD ppm-years for which risk was significant was ≤ 400 ppm-years occupational BD exposure which equates to a lifetime environmental exposure level relevant to the general population of 8000 µg/m^3 (3600 ppb) (refer to footnote 2 of Table B-2A for information on conversion). However, if dose rate plays a role in BD carcinogenicity, additional uncertainty is associated with the occupational-to-environmental exposure downward duration adjustment used to derive this value. For example, 400 ppm-years of occupational exposure would be associated with actual worker exposure concentrations of > 10,000 ppb (i.e., 400 ppm-years/40 years) which may be more predictive of a potential carcinogenic response than the 3600 ppb resulting from adjustment to an environmental concentration applicable to the general population if a significant dose-rate effect exists (e.g., dose-related changes in carcinogenic metabolic pathways such that a higher dose over a short period results in greater risk than the same total dose spread out over a longer period of time). For comparison to the estimated 3600 ppb chronic carcinogenic effect level, TCEQ's long-term comparison value, set at 10^{-5} excess total leukemia risk based on the more conservative 95% UCL URF value, is 9.1 ppb (Table B-2B).

Table B-2A. Statistical significance for different maximum levels of cumulative BD ppm-year for deaths in which leukemia is the primary cause of death or a contributing cause of death: Total leukemia (reproduced from Sielken et al. (2011) [2]		
Cumulative BD intervals included in the estimation (occupational exposure) ppm-years	Cumulative BD intervals included in the estimation (general population) [2] ppm	Likelihood ratio test that slope = 0 p-value
All		0.0263 *
≤ 1338	≤ 11.9	0.0024 **
≤ 1000	≤ 8.93	0.0045 **
≤ 500	≤ 4.46	0.0014 **
≤ 400	≤ 3.57	0.0156 *
≤ 300	≤ 2.68	0.0591
≤ 200	≤ 1.79	0.7415
≤ 100	≤ 0.893	0.6793

[1] Although thresholds were not explicitly considered in the exposure-response modeling, restrictions to the lower exposure range indicate that there is little to no risk at low exposure levels and certainly no statistically significant increasing exposure-response relationship (Sielken et al. 2011)

[2] concentration applicable to the general population (ppm) = [occupational concentration (ppm-years) x ((10 m^3/day) / (20 m^3/day)) x (5 days/7 days))] /40 years)

* Statistically significant at the 5% significance level

** Statistically significant at the 1% significance level

The 3600 ppb is preferred as a carcinogenic effect level for this example as it is based on the worker exposure associated with increased cancer risk for this cohort (i.e., consideration of study-specific dose-response data). However, for comparison, a value will be derived based on the general consideration of the lowest excess risk an epidemiology study may be able to observe/identify. For a well-conducted epidemiology study with adequate number of subjects and statistical power, it may be possible to detect an increase in background cancer incidence/mortality at the 10^{-3} risk level (Grant et al. 2007) or lower. If the study indeed detects such an excess risk level, an air concentration corresponding to around 10^{-3} excess risk could be the lowest level for which effects in some individuals in the human population may be expected with reasonable certainty if exposed over a similar (or longer) exposure duration. This should preferably be based on the statistical best estimate of the potency factor (i.e., the MLE) since this may be most predictive (i.e., it is the best statistical estimate of potency). Based on the best-fitting linear model for BD-induced leukemia in the synthetic rubber production workers (Cheng et al. 2007) and TX or US background rates in a life table analysis, the air concentration associated with 10^{-3} excess risk is 1300 ppb using the URF (MLE) of 7.471E-04/ppm (Table B-2B). Thus, carcinogenic effect levels in the range of 1300-3600 ppb were calculated to demonstrate potentially applicable methodologies for this example for comparison to TCEQ's long-term, cancer-based health-protective value of 9.1 ppb.

Table B-2B. Derivation of the URF and 10^{-5} Air Concentration and Carcinogenic Effect Level(s)		
Study	Cheng et al. (2007)	
Study Population	Synthetic rubber production workers exposed to BD in a retrospective cohort mortality study	
Study Quality	High	
Exposure Method	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, Louisiana, Kentucky and Canada	
Critical Effects	Leukemia mortality data	
Preferred model	Cox log-linear (restricted to lower 95% of exposure range) ppm-years continuous [c]	
β ± SE Maximum likelihood estimate (MLE) p-Value	1.58E-03 ± 3.9E-04 ppm-years continuous < 0.001	
β (95% UCL)	2.221E-03 ppm-years continuous	
	URF and 10^{-5} Air Concentration	**Carcinogenic Effect Level**
URF	1.050E-03/ppm 95% UCL	7.471E-04/ppm MLE
10^{-5} $concentration_{(HEC)}$ excess cancer risk	9.523 ppb	13.39 ppb
10^{-4} to 10^{-3} excess cancer risk	95.23 to 952.3 ppb $concentration_{(HEC)}$	133.9 to 1339 ppb [1] $concentration_{(HEC)}$
10^{-5} $concentration_{(HEC)}$ excess cancer risk *ADAF*	9.1 ppb	Not applicable
Database Quality	High	high
Final Values	**20 µg/m^3 (9.1 ppb)** **$^{chronic}ESL_{linear(c)}$** **$10^{-5}$ Excess Cancer Risk**	**290 µg/m^3 (130 ppb) to 2,900 µg/m^3 (1300) ppb [1]** **Carcinogenic Effect Level** **10^{-4} to 10^{-3} Excess Cancer Risk Range**

[1] A free standing NOAEL for biomarkers of effect (hypoxanthine-guanine phosphoribosyltransferase (HPRT) mutations and chromosome aberrations) at mean BD exposure concentrations of 800 ppb has been demonstrated by Albertini et al. (2001) in a small initial study of workers in the Czech Republic.

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CASE STUDY C

Subacute and Carcinogenic Effect Levels for Benzene

Summary

- **Subacute Effect Levels ≈10-100 ppm**: Based on dose-response data for hematotoxicity from subacute mouse studies, the range for human effects levels may be around 10-100 ppm for subacute exposure (e.g., 6 h per day, 5-6 day). For comparison, TCEQ's 1-hour health-based comparison value for ambient air is 0.18 ppm (56-556 times lower) (TCEQ 2007 http://www.tceq.state.tx.us/implementation/tox/dsd/final.html). [This effect level range may be viewed as reasonable given long-term worker exposure levels that result in blood cell decrements. For example, 7.2-13.6 ppm resulted in lymphocyte depression in the Rothman et al. (1996) study (with lymphocyte count still within the normal range according to information in USDHHS 2005).]
- **Carcinogenic Effect Levels ≈120-230 ppb**: Based on USEPA's (1998) confidence that the risk of leukemia increases at 40 ppm-years of occupational benzene exposure based on epidemiological studies, which equates to a lifetime environmental exposure level of approximately 120 ppb, as well as a 1E-03 excess risk level (lower bound rule-of-thumb detectable excess risk for a well-conducted study) of 230 ppb. For comparison, TCEQ's long-term health-based comparison value for ambient air is 1.4 ppb (86-164 times lower) (TCEQ 2007 http://www.tceq.state.tx.us/implementation/tox/dsd/final.html).

Subacute Effect Levels: Determinations of actual effect levels should be based on actual dose-response data as the foundation for any expectation of effects in some exposed individuals. Potential human effect levels are best estimated based on human data, but if necessary may be estimated based on animal dose-response data (e.g., LOAELs). $LOAEL_{HEC}$ values estimated from animal data represent concentrations at which it is possible that similar effects could occur in some humans exposed to these levels over the same or longer durations as used in the animal studies. Assuming no data are available on the sensitivity of animals versus humans, it must be assumed that effects in some humans are possible at the $LOAEL_{HEC}$ in the most sensitive species. However, effects in some humans are not a certainty at the $LOAEL_{HEC}$ estimated based on the most sensitive species, particularly when information on potential species differences in sensitivity is lacking. In such circumstances, as humans could be more similar to other species, the determination of possible effect levels needs to be put into the context of study results for other species which did not show effects at similar or higher levels/durations. [Furthermore, when an animal LOAEL is relied upon and there is no information on the sensitivity of animals versus humans, the animal LOAEL is not divided by an uncertainty factor in consideration of unknown potential species differences as this may very well negate the expectation of a response even in the animal species (much less humans) based on the dose-response data (i.e., dividing a LOAEL by an uncertainty factor likely has an unknown effect on the probability of response observed in the study). That is, for example, it interjects uncertainty about (i.e., essentially negates) the expectation of a human response occurring in some individuals when assuming that the human dose-response is similar to that demonstrated for the most sensitive species.]

For this example, the following summary of subacute animal LOAEL/NOAEL (as opposed to BMD) data demonstrating benzene-induced hematological effects (e.g., blood cell decreases) was used to estimate possible human effects levels assuming a similar multi-day exposure scenario.

Summary of Subacute Mouse Inhalation Studies					
Study	**Mouse Strain**	**Exposure Duration**	**NOAEL (ppm)**	**LOAEL (ppm)**	**Response at LOAEL**
Green et al. (1981a,b)	CD-1 (male)	6 hs per day for 5 days	9.9	103	granulocytopenia, lymphocytopenia, and decreased marrow cellularity and polymorphonucleucytes
Dempster and Snyder (1991) [2]	DBA/2J (male)	6 hs per day for 5 days	---	10.3	decreased erythroid progenitor cell colony forming units
Rozen et al. (1984) [1]	C57BL/6J (male)	6 hs per day for 6 days	---	10.2	depressed blood lymphocytes, depressed mitogen-induced blastogenesis of femoral B-lymphocytes
Corti and Snyder (1996) [2, 3]	Swiss Webster (male)	6 hs per day for gestational days (GD) 6-15	---	10.2	decreased erythroid progenitor cell colony forming units
Rosenthal and Snyder (1985)	C57BL/6 (male)	6 hs per day for 1-12 days	10	30	T- and B-lymphocyte depression and increased *Listeria monocytogenes* infection bacterial counts
Cronkite et al. (1985)	C57B1/6BNL	6 hs per day, 5 days per week, for 2 weeks	10	25	lymphopenia
Toft et al. (1982)	NMRI (male)	8 hs per day, 5 days per week, for 2 weeks	10.5	21	increased micronucleated polychromatic erythrocytes and decreased granulopoietic stem cells
Cronkite (1986)	CBA/Ca and C57B1/6BNL	6 hs per day, 5 days per week, for 2 weeks	10	25	lymphopenia
Farris et al. (1997a,b)	B6C3F1/CrlBR (male)	6 hs per day, 5 days per week, for 1-8 weeks	10	100	lymphopenia and other blood effects

Again, the $LOAEL_{HEC}$ represents a concentration at which it is possible that similar effects could occur in some humans exposed to this level over the same duration as used in the study or longer, although effects are not a certainty (e.g., potential species differences in sensitivity). Based on a study in the most sensitive mouse species/gender (Rozen et al. 1984), the estimated $LOAEL_{HEC}$ for a 6 h per day, 6 day exposure is 10.2 ppm (the decreased lymphocyte count appears to be within the normal range according to Jackson Laboratory 2007). However, less sensitive mouse species give an estimated $LOAEL_{HEC}$ for a 6 h per day, 5 day for 1-8 weeks as high as 100 ppm (Green et al. 1981a,b, Farris et al. 1997a,b). Thus, based on mouse studies the range for effects levels may be around 10-100 ppm for subacute exposure (e.g., 6 h per day, 5-6 day). This range may be viewed as reasonable given long-term worker exposure levels that result in blood cell decrements. For example, 7.2-13.6 ppm resulted in lymphocyte depression in the Rothman et al. (1996) study (with lymphocyte count still within the normal range according to information in USDHHS 2005).

Derivation of the Acute ReV and Subacute Effect Level			
Study	Rozen et al. (1984), supported by Dempster and Snyder (1991) and Corti and Snyder (1996)		Green et al. (1981a,b) and Farris et al. (1997a,b)
Study Population	C57BL/6J mice (male)		CD-1 (male) and B6C3F1/CrlBR (male) mice
Exposure Methods	6 h per day for 6 days		6 h per day for 5 days, 1-8 weeks
Critical Effects	Hematopoietic: depressed peripheral lymphocytes and depressed mitogen-induced blastogenesis of femoral B-lymphocytes		Hematopoietic: granulocytopenia, lymphocytopenia, and decreased marrow cellularity and polymorphonucleucytes; lymphopenia and other blood effects
	Acute ReV	**Subacute Effect Level (possible lower end)**	**Subacute Effect Level (possible higher end)**
POD	10.2 ppm (LOAEL)	10.2 ppm (LOAEL)	100 ppm (LOAEL)
Exposure Duration	6 h	6 h per day for 6 days	6 h per day for 5 days, 1-8 weeks
Extrapolation to 1 h	TCEQ (2006) default procedures using only 1 day of exposure with n=3	Not applicable	Not applicable
POD	18.5 ppm (1 h)	10.2 ppm (6 h per day for 6 days)	100 ppm (6 h per day for 5 days, 1-8 weeks)
POD_{HEC}	18.5 ppm (gas with systemic effects, based on default RGDR = 1.0)	10.2 ppm (gas with systemic effects, based on default RGDR = 1.0)	100 ppm (gas with systemic effects, based on default RGDR = 1.0)
Total Uncertainty Factors (UFs)	100	Not applicable	Not applicable
Interspecies UF	*3*	Not applicable	Not applicable
Intraspecies UF	*10*	Not applicable	Not applicable
LOAEL UF	*3*	Not applicable	Not applicable
Incomplete Database UF *Database Quality*	*1* *high*	Not applicable	Not applicable
Comparison Value	**580 µg/m^3 (180 ppb)**	**3,150 µg/m^3 (10,200 ppb)**	**32,400 µg/m^3 (100,000 ppb)**

Carcinogenic Effect Levels: A carcinogenic effect level may be estimated based on an evaluation of the dose-response data. More specifically, the lowest air concentration/exposure corresponding to excess risk observed in the key epidemiological study can be considered the lowest level for which cancer effects in some individuals in the human population would be expected with reasonable certainty if exposed over a similar (or longer) exposure duration than those in the epidemiological study. USEPA (1998) states that the agency is fairly confident that the risk of leukemia increases at 40 ppm-years of occupational benzene exposure based on Rinsky et al. (1981, 1987) and recent studies. This equates to a lifetime environmental (i.e., 24 hs per day for 76 years) exposure level of 120 ppb. However, if dose rate plays a role in benzene carcinogenicity, additional uncertainty is associated with the occupational-to-environmental exposure downward duration adjustment used to derive this value. For example, 40 ppm-years of occupational exposure would be associated with actual worker exposure concentrations of > 1,000 ppb, which may be more predictive of a potential carcinogenic response than the 120 ppb resulting from adjustment to an environmental concentration if a significant dose-rate effect exists (e.g., dose-related changes in carcinogenic metabolic pathways such that a higher dose over a short period results in greater risk than the same total dose spread out over a longer period of time). For comparison to the estimated 120 ppb chronic carcinogenic effect level, TCEQ's long-term ESL and comparison value, set at 10^{-5} excess acute myelogenous leukemia (AML) risk based on the more conservative 95% UCL β value, is 1.4 ppb (TCEQ 2007 http://www.tceq.state.tx.us/implementation/tox/dsd/final.html).

The 120 ppb is preferred as a carcinogenic effect level for this example as it is based on the worker exposure associated with increased cancer risk for this cohort (i.e., consideration of study-specific dose-response data). However, for comparison, a value will be derived based on the general consideration of the lowest excess risk an epidemiology study may be able to observe/identify. For a well-conducted epidemiology study with adequate number of subjects and statistical power, it may be possible to detect an increase in background cancer incidence/mortality at the 10^{-3} risk level (Grant et al. 2007) or lower. If the study indeed detects such an excess risk level, an air concentration corresponding to around 10^{-3} excess risk could be the lowest level for which effects in some individuals in the human population may be expected with reasonable certainty if exposed over a similar (or longer) exposure duration. This should preferably be based on the statistical best estimate of the potency factor since this may be most predictive (i.e., it is the best statistical estimate of potency). Based on the best-fitting linear model for benzene-induced AML in the Pliofilm cohort (Crump 1994) and TX or US background rates in a lifetable analysis, the air concentration associated with 10^{-3} excess risk is 230 ppb (see Table 8 of TCEQ 2007). Thus, carcinogenic effect levels in the range of 120-230 ppb were calculated to demonstrate potentially applicable methodologies for this example for comparison to TCEQ's long-term, cancer-based ESL of 1.4 ppb (TCEQ 2007 http://www.tceq.state.tx.us/implementation/tox/dsd/final.html).

Derivation of the URF and 10^{-5} Air Concentration and Carcinogenic Effect Level(s) (Nonthreshold MOA)		
Study	Crump (1994)	
Study Population	rubber hydrochloride "Pliofilm" workers exposed to benzene (1-14 yr) in a retrospective cohort mortality study	
Exposure Method	1,165 white males employed between 1940 and 1965 at three Ohio facilities and followed through 1981	
Critical Effects	acute myelogenous leukemia (AML) mortality data	
Preferred Model	linear multiplicative risk model with weighted cumulative exposure	
β (MLE)	2.3 ppm-years	
β (95% UCL)	3.8 ppm-years	
	Derivation of the URF and 10^{-5} Air Concentration	**Derivation of the Carcinogenic Effect Levels**
URF	7.1E-06 per ppb (2.2E-06 per µg/m^3) 95% UCL	4.3E-06 per ppb (1.3E-06 per µg/m^3) central estimate
10^{-5} Excess Cancer Risk Air Concentration	1.4 ppb	Not applicable
Worker Exposure associated with Excess Cancer Risk	Not applicable	40 ppm-years occupational converted to 120 ppb environmental
10^{-3} Excess Cancer Risk Air Concentration	Not applicable	230 ppb
Comparison Values	**1.4 ppb (4.5 µg/m^3)** **$^{chronic}ESL_{linear(c)}$** **$10^{-5}$ Excess Cancer Risk**	**120 ppb (389 µg/m^3)** **based on** **Worker Exposure associated with Excess Risk**

CASE STUDY D

Acute Effect Level for Acrolein (Case Study D-1)

SubChronic/Chronic Effect Level for Acrolein (Case Study D-2)

D-1 Acute (1-hour) adverse effect level using human data (threshold MOA)

Summary

- **11 µg/m^3 (4.8 ppb):** The health-protective reference value (ReV) is based on the critical effects of eye, nose and throat irritation and decreased respiratory rate in a study with both male and female human volunteers. There was no NOAEL. At the LOAEL of 0.3 ppm, the authors reported a significant after 40 min of exposure to 0.3 ppm number of volunteers experienced a 10 percent decrease in respiratory rate acrolein ($p<0.01$). The ReV is based on that LOAEL divided by a total UFs of 63 (Table D-1A) (TCEQ 2010 http://www.tceq.state.tx.us/implementation/tox/dsd/final.html).
- **687 µg/m^3 (300 ppb):** This adverse effect level is the LOAEL of 0.3 ppm at which 47 percent of subjects experienced a 10 percent decrease in respiratory rate after 10 min and 60 percent of subjects experienced a 10 percent decrease in respiratory rate after 20 minutes. (Table D-1A) (TCEQ 2010).

Acrolein is a high-interest chemical in Texas (and nationwide) due to several studies, including a local Austin study that measured acrolein in ambient air, USEPA's National-Scale Air Toxics Assessment (NATA), and the USEPA's recent School Air Toxics study. In addition, acrolein is very reactive and thus, is challenging to analyze in air samples. During the School Air Toxics study, EPA conducted a separate study designed to improve the analytical techniques used to measure acrolein. Ten additional canister air samples were collected in November and December 2011 at a school in Texas (one of two schools in the nation to be selected for follow-up acrolein sampling) to determine if the modified analytical techniques improved the accuracy of measuring acrolein. Those results are still being evaluated. A respiratory irritant at a fairly low concentration in air, combined with high public interest and analytical difficulties make acrolein a relevant example for effect levels. The TCEQ completed the Acrolein Development Support Document in November 2010.

A**cute Effect Levels:** Determinations of actual effect levels should be based on actual dose-response data as the foundation for any expectation of effects in some exposed individuals. This case study uses data from a study in human volunteers exposed to acrolein for one hour. Since a human LOAEL is available for acrolein, it is a straightforward example of using that value to determine an effect level. Effects in all humans are not a certainty at the LOAEL for acrolein, because not every subject experienced adverse effects after exposure to 0.3 ppm acrolein. The human study used to develop the TCEQ's acute ReV was conducted by Weber-Tschopp et al. (1977). In that study, 46 healthy college students (21 males and 35 females) were exposed in groups of three for 60 minutes to a constant concentration of 0.3 ppm acrolein. Annoyance increased during the first 20-30 min and then remained constant throughout the remainder of the 1-hour exposure period. Eye, nose, and throat irritation and blink rate increased with increased exposure time to acrolein, with eye irritation recorded as being the most sensitive. Eye irritation was described by subjects as between "a little" and "medium" irritation. The highest level of eye irritation occurred after about 40 min. The authors reported a significant number of volunteers experienced a 10 percent decrease in respiratory rate after 40 min of exposure ($p<0.01$). However, the study does not include the number of participants experiencing the decreased respiratory rate.

According to the American Society for Testing and Materials (ASTM 1991 as cited in NRC 2009), a 12-20 percent decrease in respiratory rate corresponds to slight irritation and respiratory rate decreases in the range of 20 to 50 percent correspond to moderate irritation. A LOAEL of 0.3 ppm acrolein was identified based on eye, nose, and throat irritation and decreased respiratory rate.

Table D-1A Derivation of the Acute ReV and Acute Effect Level		
Study	Weber-Tschopp et al. 1977	
Study population	46 healthy college students; 21 males and 25 females	
Study quality	High (human subjects of both genders, three sub-studies)	
Exposure Methods	1 h via inhalation, 0.3 ppm only	
Critical Effects	Eye, nose and throat irritation and decreased respiratory rate (after 40 min of exposure to 0.3 ppm acrolein, a significant number of volunteers experienced a 10 percent decrease in respiratory rate).	
	Acute ReV	**Acute Effect Level**
POD	0.3 ppm (LOAEL)	0.3 ppm
Exposure Duration	1 h	1 h
Extrapolation to 1 h	No adjustment	Not applicable
POD_{HEC}	0.3 ppm	0.3 ppm
Total uncertainty factors (UFs)	63	Not applicable
Interspecies UF	NA	Not applicable
Intraspecies UF	10	Not applicable
LOAEL UF	6.3	Not applicable
Incomplete Database UF *Database Quality*	1 High	Not applicable
acute ReV [1 hr]	**11 µg/m^3 (4.8 ppb)**	**687 µg/m^3 (0.3 ppm or 300 ppb)**

D-2 Subchronic/chronic adverse effect levels using an animal study (threshold MOA)

Summary:

- **0.5 µg/m^3 (0.22 ppb):** The health-protective chronic reference value (ReV) is based on the critical effect of mild hyperplasia of the respiratory epithelium of male F344 rats, without recovery, in a subchronic study. The ReV is based on the NOAEL of 0.2 ppm converted to a duration-adjusted HEC concentration of 0.006678 ppm and divided by total UFs of 30 (Table D-2A) (TCEQ 2010 http://www.tceq.state.tx.us/implementation/tox/dsd/final.html).
- **252 µg/m^3 (110 ppb):** This subchronic effect level is based the critical effect of mild hyperplasia of the respiratory epithelium of male F344 rats, without recovery, in a subchronic study (65 d). The LOAEL was 0.6 ppm and the HEC concentration was 0.11 ppm (Table D-2A).
- **600 µg/m^3 (260 ppb):** This subchronic effect level is based on the critical effect of bronchiolar epithelial necrosis in male and female F344 rats in a subchronic study (62 d). The LOAEL was 1.4 ppm and the HEC concentration was 0.26 ppm (Table D-2A).
- **2500 µg/m^3 (1100 ppb):** This chronic effect level is based on the critical effects of inflammation, hyper- and metaplastic changes in the nasal cavity of male and female Syrian hamsters in a chronic (1-yr) study. The LOAEL was 4 ppm and the HEC concentration was 1.14 ppm (Table D-2A).

Chronic Effect Levels: Determinations of actual effect levels should be based on actual dose-response data as the foundation for any expectation of effects in some exposed individuals. Potential human effect levels are best estimated based on human data, but can be estimated based on animal dose-response data (e.g., LOAELs). $LOAEL_{HEC}$ values estimated from animal data represent concentrations at which it is possible that similar effects could occur in some humans exposed to these levels over the same or longer durations as used in the animal studies. Effects in some humans are not a certainty at the $LOAEL_{HEC}$ estimated based on the most sensitive species, particularly when information on potential species differences in sensitivity is lacking. In such circumstances, as humans could be more similar to other species, the determination of possible effect levels needs to be put into the context of LOAELs and/or NOAELs for other species at similar or higher levels/durations.

The key study for development of a chronic effect level was a subchronic study conducted by Dorman et al. (2008) in which male F344 rats were exposed whole-body to concentrations of 0., 0.02, 0.06, 0.2, 0.6, or 1.8 ppm acrolein for 6 h/d, 5 d/wk for up to 65 d. Nasal respiratory epithelial hyperplasia and squamous metaplasia were the most sensitive endpoints, with a NOAEL of 0.2 ppm and a LOAEL of 0.6 ppm. The $LOAEL_{HEC}$ represents a concentration at which it is possible that similar effects could occur in some humans exposed to this level over the same duration as used in the study or longer, although effects are not a certainty (e.g., potential species differences in sensitivity). Based on two studies in rats, the estimated $LOAEL_{HEC}$ for a 6 h per day, approximate 65 day exposure ranges from 0.11 to 0.26 ppm. However, a study with hamsters exposed to a higher concentration of acrolein, provided an estimated $LOAEL_{HEC}$ for a 6 h per day, 5 day for 52 weeks as high as 1.14 ppm. The range of effect levels may be around 110 to 1100 ppb for subchronic to chronic exposure. The adverse effects resulting from exposure to acrolein are primarily concentration-dependent.

Derivation of the Chronic ReV and Subchronic/Chronic Effect Levels				
Study	Dorman et al. 2008		Kutzman et al. (1981, 1985)	Feron and Kruysse (1977)
Study population	360 adult male F344 rats, 12 rats/exposure concentration (whole-body exposure)		Male and female F344 rats (whole-body exposure)	Male and female Syrian golden hamsters
Study quality	High		Medium	Medium
Exposure Methods	Discontinuous whole body at 0, 0.02, 0.06, 0.2, 0.6, or 1.8 ppm, 6 h/d, 5 d/wk for up to 65 d (13 wk)		Discontinuous whole body at 0, 0.4, 1.4, or 4 ppm, 6 h/d, 5 d/wk for 62 d	0 or 4 ppm, 7 hr/d, 5 d/wk for 52 wk
Critical Effects	Mild hyperplasia of the respiratory epithelium, without recovery		Bronchiolar epithelial necrosis. No nasal pathology.	Inflammation, hyper- and metaplastic changes in the nasal cavity (reversible after 6 mo withdrawal period), other effects included growth retardation, rhinitis, and temporary behavioral disturbance.
	Chronic ReV	**Subchronic Effect Level (possible lower end)**	**Subchronic Effect Level (possible lower end)**	**Chronic Effect Level (possible higher end)**
POD	0.2 ppm (NOAEL)	0.6 ppm (LOAEL)	1.4 ppm (LOAEL)	4 ppm (LOAEL)
Exposure Duration	6 h/day, 5 d/wk for 65 d (13 wk)	Not adjusted	Not adjusted	Not adjusted
POD_{HEC}	0.006678 ppm	0.1122 ppm	0.2618 ppm	1.1396 ppm
Total uncertainty factors (UFs)	30	Not applicable	Not applicable	Not applicable
Interspecies UF	3	Not applicable	Not applicable	Not applicable
Intraspecies UF	10	Not applicable	Not applicable	Not applicable
LOAEL UF	NA	Not applicable	Not applicable	Not applicable
Incomplete Database UF *Database Quality*	1 High	Not applicable High	Not applicable High	Not applicable High
	0.5 µg/m^3 (0.22 ppb)	**252 µg/m^3 (110 ppb)**	**600 µg/m^3 (262 ppb)**	**2500 µg/m^3 (1100 ppb)**