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Methanol

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Acronyms and Abbreviations

Acronyms and Abbreviations	Definition
ACGIH	American Conference of Governmental Industrial Hygienists
ADH	alcohol dehydrogenase
AEGL	Acute Exposure Guideline Levels
^{0} C	degrees Celsius
BMR	benchmark response
CBF	ciliary beat frequency
CNS	central nervous system
DSD	development support document
ESL	Effects Screening Level
$^{acute}ESL$	acute health-based Effects Screening Level for chemicals meeting minimum database requirements
$^{acute}ESL_{odor}$	acute odor-based Effects Screening Level
$^{acute}ESL_{veg}$	acute vegetation-based Effects Screening Level
$^{chronic}ESL_{threshold(c)}$	chronic health-based Effects Screening Level for threshold dose response cancer effect
$^{chronic}ESL_{threshold(nc)}$	chronic health-based Effects Screening Level for threshold dose response noncancer effects
$^{chronic}ESL_{nonthreshold(c)}$	chronic health-based Effects Screening Level for nonthreshold dose response cancer effects
$^{chronic}ESL_{nonthreshold(nc)}$	chronic health-based Effects Screening Level for nonthreshold dose response noncancer effects
$^{chronic}ESL_{veg}$	chronic vegetation-based Effects Screening Level
EEG	electroencephalogram
EU	European Union
GC	gas chromatography
GD	gestation day
h	hour

Acronyms and Abbreviations	Definition
$H_{b/g}$	blood:gas partition coefficient
$(H_{b/g})_A$	blood:gas partition coefficient, animal
$(H_{b/g})_H$	blood:gas partition coefficient, human
Hg	mercury
HEC	human equivalent concentration
HQ	hazard quotient
IPCS	International Programme on Chemical Society
IRIS	USEPA Integrated Risk Information System
kg	kilogram
LOAEL	lowest-observed-adverse-effect-level
MW	molecular weight
µg	microgram
$\mu g/m^3$	micrograms per cubic meter of air
mg	milligrams
mg/m^3	milligrams per cubic meter of air
min	minute
MOA	mode of action
n	number
NOAEL	no-observed-adverse-effect-level
OAEL	observed adverse effect level
OSHA	Occupational Safety and Health Administration
PBPK	physiologically based pharmacokinetic
POD	point of departure
POD_{ADJ}	point of departure adjusted for exposure duration
POD_{HEC}	point of departure adjusted for human equivalent concentration
ppb	parts per billion
ppm	parts per million

Acronyms and Abbreviations	Definition
ReV	reference value
RGDR	regional gas dose ratio
ROS	Reactive oxygen species
SA	surface area
SD	Sprague-Dawley
STT	saccharin transport time
TCEQ	Texas Commission on Environmental Quality
UF	uncertainty factor
UF_H	interindividual or intraspecies human uncertainty factor
UF_A	animal to human uncertainty factor
UF_{Sub}	subchronic to chronic exposure uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_D	incomplete database uncertainty factor
USEPA	United States Environmental Protection Agency
V_E	minute volume

Chapter 1 Summary Tables and Figure

Table 1 for air monitoring and Table 2 for air permitting provide a summary of health- and welfare-based values from an acute and chronic evaluation of methanol. Please refer to Section 1.6.2 of the *TCEQ Guidelines to Develop Toxicty Factors* (TCEQ 2012) for an explanation of air monitoring comparison values (AMCVs), reference values (ReVs) and effects screening levels (ESLs) used for review of ambient air monitoring data and air permitting. Table 3 provides summary information on methanol's physical/chemical data.

Table 1. Air Monitoring Comparison Values (AMCVs) for Ambient Air [a]

Short-Term Values	Concentration	Notes
Acute ReV	**Short-Term Health** 13,000 µg/m^3 (10,000 ppb)	**Critical Effect:** Minimal subclinical nasal epithelial inflammation and neurobehavioral effects in the absence of subjective irritative symptoms
$^{acute}ESL_{odor}$	**Odor** 43,000 µg/m^3 (33,000 ppb)	50% detection threshold
$^{acute}ESL_{veg}$	- - -	No data found
Long-Term Values	**Concentration**	**Notes**
Chronic ReV	**Long-Term Health** 7,200 µg/m^3 (5,500 ppb)	**Critical Effect(s):** Nasal irritation
$^{chronic}ESL_{nonthreshold(c)}$	- - -	Data are inadequate for an assessment of human carcinogenic potential
$^{chronic}ESL_{veg}$	- - -	No data found

[a] Methanol is not monitored for by the TCEQ's ambient air monitoring program, so currently no ambient air data (i.e., peaks, annual averages, trends, etc.) are available to assess methanol concentrations in Texas ambient air.

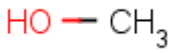
Table 2. Air Permitting Effects Screening Levels (ESLs)

Short-Term Values	Concentration	Notes
$^{acute}ESL$ [1 h] (HQ = 0.3)	**Short-Term ESL for Air Permit Reviews** 3,900 µg/m^3 (3,000 ppb) [a]	**Critical Effect:** Minimal subclinical nasal epithelial inflammation and neurobehavioral effects in the absence of subjective irritative symptoms
$^{acute}ESL_{odor}$	43,000 µg/m^3 (33,000 ppb)	50% detection threshold
$^{acute}ESL_{veg}$	---	No data found
Long-Term Values	**Concentration**	**Notes**
$^{chronic}ESL_{threshold\ (nc)}$ (HQ = 0.3)	**Long-Term ESL for Air Permit Reviews** 2,100 µg/m^3 (1,600 ppb) [b]	**Critical Effect:** Nasal irritation
$^{chronic}ESL_{nonthreshold(c)}$	---	Data are inadequate for an assessment of human carcinogenic potential
$^{chronic}ESL_{veg}$	---	No data found

[a] Based on the acute ReV of 13,000 µg/m^3 (10,000 ppb) multiplied by 0.3 to account for cumulative and aggregate risk during the air permit review.

[b] Based on the chronic ReV of 7,200 µg/m^3 (5,500 ppb) multiplied by 0.3 to account for cumulative and aggregate risks during the air permit review.

Table 3. Chemical and Physical Data

Parameter	**Value**	**Reference**
Chemical Structure		ChemID Plus 2012
Molecular Formula	CH_4O	ACGIH 2001
Molecular Weight	32.04	ACGIH 2001
Physical State at 25°C	Liquid	ACGIH 2001
Color	Clear, colorless	ACGIH 2001
Odor	Alcoholic odor; pungent odor in crude form	AEGL 2005
CAS Registry Number	67-56-1	ACGIH 2001
Synonyms	Methyl alcohol; carbinol; Methylalkohol; wood alcohol; methyl hydrate; methyl hydroxide; monohydroxymethane; methylol	ChemIDplus 2012
Solubility in water	Miscible	ACGIH 2001
Log K_{ow}	-0.77	ChemIDplus 2012
Vapor Pressure	127 mm Hg (25 °C)	ChemIDplus 2012
Relative Vapor Density (air = 1)	1.11	ACGIH 2001
Density/Specific Gravity (water = 1)	0.7866 @ 25 °C	ChemIDplus 2012
Melting Point	-97.6 °C	ChemIDplus 2012
Boiling Point	64.6 °C	ChemIDplus 2012
Conversion Factors	1 ppm = 1.31 mg/m^3 (25 °C) 1 mg/m^3 = 0.764 ppm (25 °C)	ACGIH 2001

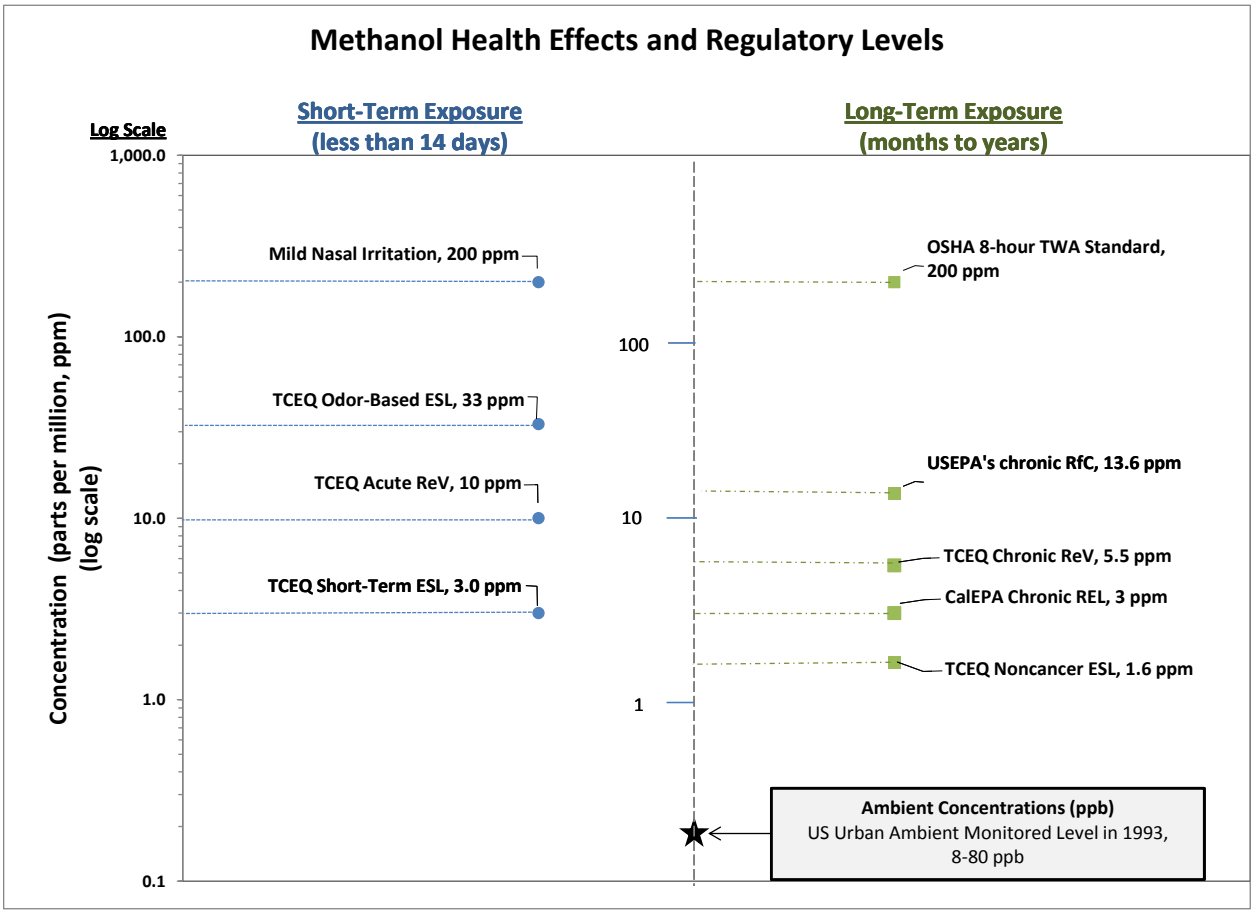


Figure 1. Methanol Health Effects and Regulatory Levels

Figure 1 compares methanol'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 Tables 1 and 2 to the air concentrations associated with nasal inflammation, the California Environmental Protection Agency's (CalEPA) chronic reference exposure level (REL) (OEHHA 2001), USEPA's chronic RfC (USEPA 2013a), and time-weighted average (TWA) permissible exposure level set by the Occupational Safety and Health Administration (OSHA).

Chapter 2 Major Sources or Uses and Ambient Air Concentrations

2.1 Major Sources or Uses

Methanol occurs naturally in humans, animals and plants. It is a normal byproduct of body metabolism and is found in the exhaled air, urine, blood and saliva (AEGL 2005; IPCS 1997). Commercially, it is used in paint removers, windshield washer fluid, automotive fuel, stove fuels, antifreeze, embalming fluids, some paints and as a softening agent for pyroxylin plastics (OEHHA 2003). Prospective commercial uses include using methanol directly as a fuel or in a gasoline blend. In the human diet, methanol is present in fruits, vegetables, fruit juices, fermented beverages and via the artificial sweetener aspartame (IPCS 1997).

Methanol is also used frequently as an industrial solvent and is utilized as a raw material in the production of many organic compounds, most notably, methyl tertiary butyl ether, acetic acid and formaldehyde (IPCS 1997, AEGL 2005). According to the United States Toxics Release Inventory (TRI), 116.8 million pounds (53,000 metric tons) of methanol was released on- and off-site from facilities in all industries in the U.S. in 2009. Approximately 87% of the emissions were released into the air and less than 2.5% into surface waters. The largest emitter in 2009 was the pulp and paper industry (Methanol Institute 2012). In the 2002 TRI Report, Texas ranked 8th in total methanol air releases from industry with 7.3 million pounds. Nationwide, Harris County, Texas ranked highest with 1.7 million pounds released in air.

2.2 Background Levels of Methanol in Ambient Air

Typical environmental exposures to methanol in the air in rural areas are below 0.8 ppb and may approach 30 ppb in urban areas (Methanol Institute 2012).

Methanol was detected at mean ambient atmospheric concentrations of 10 and 3 µg/m^3 (7.9 and 2.6 ppb) at Tucson and two remote Arizona locations, respectively, during monitoring in 1982 (Snider and Dawson 1985, as cited in IPCS 1997). USEPA (1993, as cited in IPCS 1997) reported that median methanol concentrations of 6-60 µg/m^3 (7.8-79 ppb) were measured in 52 samples from Boston, Massachusetts, Houston, Texas and Lima, Ohio.

Chapter 3 Acute Evaluation

3.1 Health-Based Acute ReV and ^{acute}ESL

Human inhalation studies have shown that acute exposure of healthy human subjects to lower concentrations (e.g., ≤ 200 ppm) of methanol did not have adverse effects on central nervous system (CNS), ocular and mucous membrane irritation, and subjective symptoms (Mann et al. 2002, Cook et al. 1991, Chuwers et al. 1995, Muttray et al. 2001, Ernstgard et al. 2005). The only effects were mild and transient subclinical nasal inflammation observed at 200 ppm in the Mann et al. (2002) study. Acute effects in animals, including reproductive/developmental effects, occurred at higher concentrations (i.e., 1,000 ppm). These animal studies were not used to develop a ReV.

3.1.1 Physical/Chemical Properties

Methanol is a volatile, flammable, clear, colorless liquid with a mild alcoholic odor. It is miscible with water and many organic solvents (IPCS 1997, ACGIH 2001, OEHHA 2003). Other physical/chemical properties of methanol can be found in Table 3.

3.1.2 Key and Supporting Studies

This section is based on a review of current literature as well as background readings in the International Programme on Chemical Society (IPCS 1997), the Acute Exposure Guideline Levels (AEGL 2005) and the USEPA Integrated Risk Information System (IRIS) (USEPA 2013a) which describe in detail the acute toxicity of methanol.

3.1.2.1 Key Human Studies

3.1.2.1.1 Mann et al. (2002)

In a study by Mann et al. (2002), 12 healthy non-smoking male volunteers (average age ± SD: 26.8 ± 2.1 years) were exposed to both 20 ppm and 200 ppm of methanol (nominal concentrations) for 4 hours (h). The investigator used the 20 ppm as a control. The exposures were conducted in an 18-m^3 exposure chamber and the interval between the two exposures was one week. Individuals were at rest during exposure. The corresponding mean analytical concentrations ± SD were 20.3 ± 3.8 and 203.5 ± 2.5 ppm. Proinflammatory mediators, such as interleukin (IL)-8, IL-1β, IL-6 and prostaglandin (PGE2), as well as mucociliary clearance parameters such as the saccharin transport time (STT) and the ciliary beat frequency (CBF) were measured in nasal secretions. Additionally, subjective symptoms were assessed with a 17-item questionnaire before and after exposures which asked participants to rate symptoms of local irritation, breathing difficulty and pre-narcotic symptoms on a scale from 0 (no symptoms) to 5 (severe symptoms). The symptom questionnaire showed no difference between "before exposure" and "after exposure" symptoms at either the 20 ppm or 200 ppm exposure level. However, the median concentrations of IL-8 and IL-1β (cytokines involved in nasal epithelial inflammatory reactions) were significantly higher after the 200 ppm exposure versus the 20 ppm exposure. No significant changes were observed for the IL-6, PGE2, STT and CBF. Thus, a Lowest-observed-effects-level (LOEL) was 203.5 ppm for mild and transient subclinical nasal inflammatory reactions was identified from this study. Since the subclinical nasal effects were minimal (only two of the six biomarkers were increased) and no subjective clinical irritations were detected, the level of 203.5 ppm is more appropriately considered a free-standing no-observed-adverse-effect-level (NOAEL).

3.1.2.1.2 Cook et al. (1991)

In a study of neurobehavioral functions on humans by Cook et al. (1991), 12 healthy non-smoking young male volunteers were exposed to a nominal concentration of 250 mg/m^3 (191 ppm) [mean analytical concentration ± SD (249 ± 7 mg/m^3)] methanol vapors for 75 minutes. The study design included two methanol and two sham exposures (filtered room air) with each participant serving as his own control. A number of endpoints were measured before, during and after exposures. Among those measured were blood and urinary methanol, plasma formate, subjective mood, alertness, fatigue, visual- and auditory-event-related potentials, symptom scales and a series of neurobehavioral test batteries. The results showed that the methanol levels were increased approximately 3-fold in blood and urine, but no changes in plasma formate level after methanol exposure. Most of the neurobehavioral endpoints were unaffected by exposure to methanol. Though statistical significance and trend were found for a cluster of variables, including the latency of P200 components of event-related potentials, performance on the Sternberg memory task (memory scanning test) and subjective measures of fatigue and concentration, the effects were subtle and within normal ranges. The authors indicated that these changes did not affect the tested subjects' ability to maintain vigilance or to respond quickly to stimuli. A LOAEL of 191 ppm for subtle effects was identified from this study. Because only

minimal effects were observed, the level of 191 ppm is more appropriately considered a free-standing NOAEL for neurobehavioral effects.

3.1.2.1.3 Chuwers et al. (1995)

This study exposed 26 healthy volunteers (15 male and 11 female) for 4 h to either water vapor or 200 ppm methanol vapor. The subjects served as their own controls and were at rest during exposure. Endpoints including serum and urine methanol and formate levels, visual (color discrimination and contrast sensitivity), neurophysiological (auditory evoked potentials) and neurobehavioral performances were assessed. The results show that formate, the toxic metabolite of methanol, does not increase after 4 h of 200 ppm exposure to methanol. Participant outcomes of these tests did not differ significantly between the methanol and water vapor exposure groups. Slight inter-subject differences were noted in the amplitudes of P-300 (an event related potential wave) and the symbol digit test, though these outcomes were suspected by the authors to be the result of an unknown bias. The findings of this study demonstrate that acute exposure of healthy human subjects to 200 ppm methanol does not affect neurobehavioral, neurophysiological and visual performance in a healthy normal population. The findings in this study are similar to findings in the Cook et al. (1991) study. A free-standing NOAEL of 200 ppm for neurobehavioral effects was identified from this study.

3.1.2.1.4 Muttray et al. (2001)

In a similar study by Muttray *et al.* (2001), 12 healthy non-smoking male volunteers were exposed to 20 ppm (control) and 200 ppm of methanol over 4 h in an 18-m^3 exposure chamber. The subjects served as their own controls. The interval between the two exposure sessions was 1 week. The monitored concentrations were 20.3 ± 3.8 ppm (mean ± SD) and 203.5 ± 2.5, respectively. Brain electrical activity was measured via an electroencephalogram (EEG) and subjective pre-narcotic and irritative symptoms were assessed via questionnaire. The exposed subjects' EEG and questionnaire results did not differ from controls with the exception of a decrease in the spectral power of the theta and delta bands. The theta band changes are indicative of a slight excitatory effect at 200 ppm, though this effect was termed "weak" by the authors, as scores of acute symptoms did not change. The authors indicate that the effect was reversible and does not represent an intolerable loss of sense of wellbeing and thus, is not considered adverse. A NOAEL of 200 ppm was identified from this study. This subtle physiological response in the absence of acute symptoms is in accordance with the 191 ppm NOAEL for neurobehavioral effects established by Cook et al. (1991).

3.1.2.1.5 Ernstgard et al. (2005)

In a study by Ernstgard *et al.* (2005), eight healthy volunteers (4 male and 4 female) were exposed 3 times for 2 h to clean air (control), 100 and 200 ppm (nominal concentrations) methanol in a 20-m^3 exposure chamber. The mean analytical methanol concentrations were 98.4 (range 97.2-101.2) and 192.4 ppm (range 183.8-205.3). Exposure order was randomized among participants and exposure sessions were separated by at least two weeks. Additionally, volunteers performed light physical exercise (50W on a bicycle ergometer) during the exposure period. Perceived discomfort (i.e. irritating symptoms, CNS symptoms, breathing difficulty and odor) was rated by participants before, during and after exposure sessions. There were no significant differences in symptoms ratings found between methanol exposure and control. The average ratings of irritation and CNS symptoms are at the "somewhat" level or below. Female participants ranked certain symptoms (headache, fatigue and nausea) significantly higher than the men at the 200 ppm methanol level. A free-standing NOAEL of 200 ppm was also identified from this study.

3.1.2.1.6 Summary of Key Human Studies

Table 4 (below) summarized the aforementioned five acute human studies. The results showed that a free-standing NOAEL from 191 to 203.5 ppm for absence of subjective symptoms and/or neurobehavioral, neurophysiological and visual performance effects was identified from all five studies. The only observed effects level (LOEL at 203.5 ppm) for subclinical nasal inflammatory reactions was identified from the Mann et al. (2002) study. However, the subclinical nasal effects were mild and no subjective clinical irritations were detected. The LOEL may be too conservative to use as the point of departure (POD) to develop the acute ReV. Therefore, the highest free-standing NOAEL of 203.5 ppm for absence of irritation and subjective symptoms from the Mann et al. study was used as the POD to develop the acute ReV. The NOAEL was consistent with those identified from other human studies.

Table 4. Acute Human Inhalation Exposure Studies

Exposure Concentrations (Reference)	Exposure Time	NOAEL	LOAEL	Effects	Reference
20.3 and 203.5 ppm (12 healthy men)	4 h		203.5 ppm (Free-standing LOEL)	Mild subclinical nasal inflammatory reactions	Mann et al. 2002
Same as above	Same as above	203.5 ppm (Free-standing)		Absence of irritation and other subjective symptoms	Same as above
191 ppm (12 healthy men)	75 min	191 ppm (Free-standing)		Minimal neurobehavioral effects	Cook et al. 1991
0 and 200 ppm (15 male and 11 female)	4 h	200 ppm (Free-standing)		Absence of neuro-behavioral, neuro-physiological and visual performance	Chuwers et al. 1995
20.3 and 203.5 ppm (12 healthy men)	4 h	203.5 ppm (Free-standing)		Subtle physiological response in the absence of subjective irritative symptoms	Muttray *et al.* 2001
0, 100, and 200 ppm (4 healthy male and healthy4 female)	4 h	200 ppm (Free-standing)		Absence of subjective irritation and CNS symptoms	Ernstgard *et al.* 2005

3.1.2.2 Supporting Animal Study

3.1.2.2.1 NEDO (1987) Acute Monkey Study

In an unpublished report- *"Methanol Studies in Monkeys, Rats and Mice"*, performed by the Japanese New Energy Development Organization (NEDO1987, as cited in USEPA 2009, 2013a, monkeys (*M. fascicularis*) were exposed to methanol 21 h/day (d) at concentrations of 3,000 (20 d), 5,000 (5 d), 5,000 (14 d), 7,000 (6 d) and 10,000 ppm (6 d). These are unpublished studies, however, they were externally peer reviewed by USEPA in 2009 (USEPA 2009). There were no clinical or histopathological effects observed to the visual system, however, treatment-related effects were observed in the CNS (fibrosis of stellate cells) and in liver tissue (fatty degeneration) at 3,000-ppm and higher exposure groups. A LOAEL of 3,000 ppm was identified from these studies.

3.1.3 Developmental/Reproductive Studies

Developmental effects following methanol exposures at high concentrations (i.e., > 1,000 ppm) have been noted in both rats and mice (NEDO 1987, Nelson et al. 1985, Rogers et al. 1993, IPCS 1997), but are not as evident or clear in primate exposure studies. No developmental data are available in humans (Andrews et al. 1987, Nelson et al. 1985, Rogers et al. 1993; Clary 2003; Burbacher et al. 2004, as cited in USEPA 2013a).

Table 5 is a summary of acute and subacute developmental/reproductive studies conducted in animals. Appendix A provides detailed information on reproductive/developmental studies. Inhalation exposure of pregnant mice to 1,000 ppm methanol resulted in no developmental effects while exposure to 2,000 ppm resulted in a significant increase in cervical ribs in the fetuses. Higher exposures significantly increased the incidence of cleft palates, exencephaly and skeletal malformations (CERHR 2002, 2003). Reproductive effect studies showed inhalation exposure of sexually mature male rats to methanol at up to 800 ppm did not affect the structure of the male reproductive system. Other studies showed exposures up to 1,500 ppm did not consistently alter male rat sex hormone levels. The Cameron et al (1985) study showed methanol can cause a transient reduction in the formation of testosterone at the 200 ppm (Table 4 and Appendix A.1.5); however, given the rapid recovery (no significant effects observed after 18 h), this is not considered a critical adverse endpoint.

An acute ReV based on developmental/reproductive studies was not developed for the following reasons:

- The lowest relevant LOAEL in acute developmental/reproductive studies was 2,000 ppm, more than 10 times the NOAEL of 200 ppm based on nasal irritation from the key human study).

- Differences in metabolism between rodents and humans indicate rodents are more susceptible based on differences in metabolism between rodents and humans (Appendix A.2.1). Rodents develop higher blood methanol levels after inhalation exposure compared to primates (Perkins et al. 1995), which favors development of methanol-induced CNS and developmental toxicity. The mouse is considerably more susceptible for the developmental toxic effects than the rat.

- Laboratory animal studies reviewed by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (CERHR) Methanol Expert Panel, show that methanol has the potential to adversely affect development in humans (CERHR 2002, 2003). However, given the high blood level of methanol associated with these developmental effects, the CERHR indicated that there was minimal concern for developmental effects in humans (refer to Weight-of Evidence discussion, Appendix A.2.2).

Table 5. Acute and Subacute Animal Inhalation, Developmental/Reproductive Studies

Species	Concentration (ppm)	Duration	NOAEL (ppm)	LOAEL (ppm)	Effect	Reference
Mice CD-1	0, 5,000, 10,000, 15,000	6 h/d on GD 6-15	---	5,000	cleft palate, hydronephrosis	Bolon et al. 1993
Mice CD-1	(I)5,000, 15,000; (II) 2,000, 5,000	7 h/d on GD 6-15	---	(I) 5, 000 (II) 2,000	less weight gain than controls	Rogers 1991
Mice CD-1	1000, 2000, 5000, 7500 10,000, 15,000	7 h/d on GD 6-15	1000	2,000	increase in cervical ribs, ossification sites lateral to the 7th cervical vertebra	Rogers et al. 1993
Mice CD-1	0, 10,000	single day on GD 5-9; or two consecutive days on GD 6-13	---	10,000	cleft palate, exencephaly, skeletal malformations	Rogers and Mole 1997
Mice CD-1	10,000 , 15,000	6 h on GD 8	---	10,000	increase in the incidence of fetuses with open neural tubes; transient decrease in maternal RBC	Dorman et al. 1995
Rat Long-Evans	0, 15000	7 h/d on GD 7-19	---	15,000	reduced pup weight	Stanton et al. 1995
Rat SD	0, 5000, 10,000, 20,000	7 h/d on GD 1-19; 7 h/d on GD 7-15	5000 develop-mental 10,000 maternal	10,000 develop-mental 20,000 maternal	decreased fetal body weight, exencephaly, encephaloceles, rudimentary and extra cervical ribs maternal: unsteady gait	Nelson et al 1985
Rat SD	0, 200,1000, 5000	22.7 h/d on GD 7-17	1000	5,000	reduced body weight gain and food/water intake reproductive-decrease live fetus, late term resorptions	NEDO 1987
Rat SD	200	1 d or 1 week, post-fetal	---	200	reduction in circulation testosterone	Cameron et al. 1985

3.1.4 Mode of Action (MOA) Analysis and Dose Metric

Methanol is rapidly absorbed after inhalation, ingestion, or dermal contact. The major route of methanol elimination is through a series of oxidation reactions that form formaldehyde, formate and carbon dioxide. Acute methanol toxicity varies greatly between species, primarily as a result of differential metabolism. Methanol is converted to formaldehyde by alcohol dehydrogenase (ADH) in primates (humans and monkeys) and by catalase in rodents (sees Figure 2 below). Toxicity is higher in species with a relatively poor ability to metabolize formate. Primates are uniquely more susceptible than non-primate animals to the toxic effects of methanol because of the greater accumulation of formate and there is no evidence of formate accumulation in rodents (Clary 2003). For example, the clearance of formate from the blood of exposed primates is at least 50% slower than for rodents (IPCS 1997). The toxic effects of acute methanol exposure in humans are exhibited in three phases: 1) an inebriation phase with mild eye and mucous membrane irritation, 2) a latent period of 10-48 h; and 3) finally resulting in more severe CNS effects (IPCS 1997, AEGL 2005). The initial phase is likely a direct result of the parent chemical while later responses are a reaction to the methanol metabolite, formic acid (AEGL 2005).

Primates		**Rodents**
	CH_3OH **(Methanol)**	
Alcohol dehydrogenase	↓	Catalase
	HCHO (Formaldehyde)	
Formaldehyde dehydrogenase	↓	Formaldehyde dehydrogenase
	HCOO (Formate)	
Folate-dependent pathway	↓	Folate-dependent pathway
	CO_2 (Carbon dioxide)	

Figure 2 Major enzymes for primate (left) and rodent (right) metabolism of methanol (IPCS 1997)

Human data indicate that methanol inhalation can elicit mucous membrane irritation, headache, nausea, drowsiness, blurred vision, blindness, coma and death (Mallinckrodt 2001, as cited in OEHHA 2003). The MOA for these effects, though not fully elucidated, has been attributed to the methanol metabolite, formic acid or formate. The major metabolic route for methanol in primates is oxidation to form formaldehyde, which is eliminated rapidly by the body, primarily by metabolism to formic acid (Cook et al. 1991). Clinical and experimental evidence indicate that formic acid is responsible for the metabolic acidosis and ocular toxicity observed in humans, non-human primates and folate-depleted rodents following methanol exposure (IPCS 1997).

Since the key study is based on human volunteers exposed to the parent chemical and information on other more appropriate dose metrics was not available, exposure concentration of the parent chemical will be used as the dose metric. Based on the toxicokinetics of methanol, the subclinical nasal effects observed in human volunteers were determined to both concentration and duration dependent.

3.1.5 POD for the Key Study

The 4-h acute NOAEL of 203.5 ppm for absence of irritation and subjective symptoms identified by Mann et al. (2002) was used as the POD to derive the acute ReV. Since respiratory irritation effects are only concentration dependent, so an exposure duration adjustment from 4 h to 1 h for the 4-h NOAEL was not conducted (TCEQ 2012). Thus, the 4-h NOAEL of 203.5 ppm was used as a 1-h concentration POD_{ADJ}.

3.1.6 Dosimetric Adjustments

3.1.6.1 POD Human Equivalent Concentration (POD_{HEC})

Since the POD_{ADJ} was based on human volunteer exposure, the POD_{ADJ} of 203.5 ppm was directly used as a human equivalent concentration (POD_{HEC}) to set the acute ReV.

3.1.7 Adjustments of the POD_{HEC}

The following uncertainty factors (UFs) were applied to the POD_{HEC} of 203.5 ppm:

- a UF_H of 10 for intraspecies variability;
- a UF_D of 2 was used because the acute database for methanol includes five acute inhalation study in humans; more than two animal inhalation exposure supporting studies in multiple species; and more than ten reproductive/developmental toxicity studies in different species (see Section 3.2). However, only NOAELs and some LOAELs with no observed dose-response were identified from available controlled human studies. Furthermore, no more than two doses were administered in these human studies. The quality of the key study is considered medium; however, the confidence in the acute database is medium to high.
- The total UF = 20.

acute ReV= POD_{HEC} / (UF_H x UF_D)
= 203.5 ppm / (10 x 2)
= 203.5 ppm / 20
= 10.18 ppm
=10 ppm (rounded to 2 significant figures)
=10,000 ppb

3.1.8 Health-Based Acute ReV and ^{acute}ESL

In deriving the acute ReV, no numbers were rounded between equations until the ReV was calculated. Once the ReV was calculated, it was rounded to two significant figures. The rounded ReV of 10,000 ppb **(**13,000 µg/m^3) was then used to calculate the ESL. The ^{acute}ESL of 3,000 ppb **(**3,900 µg/m^3) is based on the acute ReV multiplied by a HQ of 0.3 and rounded to two significant figures at the end of all calculations (Table 5).

Table 6. Derivation of the Acute ReV and ^{acute}ESL

Parameter	**Summary**
Study	Mann et al. 2002
Study Population	12 male human volunteers
Study Quality	High
Exposure Method	18-m^3 inhalation chamber exposure to 20.3 and 203.5 ppm (analytical concentrations)
Exposure Duration	4 h
Critical Effects	Absence of irritation and subjective symptoms
NOAEL	203.5 ppm (free-standing)
LOEL	203.5 ppm (subtle subclinical nasal inflammatory response)
POD	203.5 ppm
Extrapolation to 1 h (POD_{ADJ})	203.5 ppm
POD_{HEC}	203.5 ppm
Total uncertainty factors (UFs)	20
Interspecies UF	10
Intraspecies UF	Not applicable
LOAEL-to-NOAEL UF	NA
Incomplete Database UF Database Quality	2 Medium to high
Acute ReV [1 h] (HQ = 1)	**13,000 µg/m^3 (10,000 ppb)**
^{acute}ESL [1 h] (HQ = 0.3)	**3,900 µg/m^3 (3,000 ppb)**

3.2 Welfare-Based Acute ESLs

3.2.1 Odor Perception

Five studies listed as acceptable sources for odor threshold values in the guidelines (TCEQ 2012) were identified: May (1966), Hellman (1974), Cometto-Muniz (1990) and Nagata (2003). A 50% odor detection threshold value of 43,000 µg/m^3 (33,000 ppb, rounded to two significant figures) was reported for methanol by Nagata (2003) utilizing the Japanese triangular odor bag method. According to the guidelines for setting odor-based ESLs (TCEQ 2012), odor detection values defined as the highest quality level of odor thresholds (Level 1) will be considered first in setting the $^{acute}ESL_{odor}$ values. If no Level 1 values are available, Level 2 quality data will be considered.

If no Level 1 or 2 odor thresholds are available, then Level 3 quality data that meet the criteria from the AIHA (1989) and USEPA (1992) may be used. The odor detection thresholds reported by Nagata (2003) were determined by the standardized methods of measuring odor; the odor detection value is defined as Level 1 (TCEQ 2012). The odor threshold reported by May (1966), Hellman (1974) and Cometto-Muniz (1990) (Table 6), however, are defined as Level 3 quality data. Therefore, only the standardized odor detection threshold determined by Nagata (2003) was used to set the $^{acute}ESL_{odor}$. Accordingly, the $^{acute}ESL_{odor}$ for methanol was set at the 50% odor detection threshold of 43,000 µg/m^3 (33,000 ppb) determined by Nagata (2003) (Table 6).

Table 7. Accepted Odor Studies Conducted for Methanol

Investigator	**Odor Detection Threshold Value (µg/m3)**	**Quality Level**
Hellman 1974	5,600	3
May 1966	7,800,000	3
Nagata 2003	43,000	1
May 1966	12,000,000	3
Cometto-Muniz 1990	2,100,000	3
$^{acute}ESL_{odor}$	**43,000 µg/m^3 (33,000 ppb)**	1

3.2.2 Vegetation Effects

No information was found to indicate that special consideration should be given to possible vegetation effects from exposure to methanol.

3.3 Acute ReV and $^{acute}ESL$

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

- acute ReV = 13,000 µg/m^3 (10,000 ppb)

- $^{acute}ESL$ = 3,900 µg/m^3 (3,000 ppb)
- $^{acute}ESL_{odor}$ = 43,000 µg/m^3 (33,000 ppb)

The short-term ESL for air permit evaluations is the $^{acute}ESL$ of 3,900 µg/m^3 (3,000 ppb) Table 2). For evaluation of ambient air monitoring data, the $^{acute}ESL_{odor}$ of 43,000 µg/m^3 (33,000 ppb) is higher than the ReV of 21,000 µg/m^3 (16,000 ppb), although both values will be used for the evaluation of air data (Table 1).

3.4 Acute Inhalation Observed Adverse Effect Level

Since no LOAEL was identified, an acute inhalation observed adverse effect level was not derived.

Chapter 4 Chronic Evaluation

4.1 Noncarcinogenic Potential

Chronic exposure to methanol may cause effects similar to those from relatively high levels of acute exposure, including CNS and visual disorders (IPCS1997). Data for subchronic or chronic human exposures are limited and inconclusive. Nasal irritation and dimmed vision have been reported by workers exposed to methanol at 459 ppm (Kawai et al. 1991). Chronic exposure to methanol at levels of 365-3,080 ppm (mean concentration = 1,060 ppm) can cause headache, dizziness, nausea and blurred vision (Frederick et al. 1984). In animal studies, a dose-dependent formation of nodes in the lung of male rats has been reported after exposure at 1,000 ppm for 19.5 h/d for 12 months (NEDO 1987, as cited in AEGL 2005 and USEPA 2013a), while no histopathological effects were reported at 5,000 ppm for 6 h/d, 5 d/week for 4 weeks (Andrews et al. 1987). NEDO (1987) also conducted a two-generation reproduction study that evaluated the effects of pre- and post-natal methanol exposure (22 h/d) on reproductive and other organ systems of SD rats. The results showed that brain weights were significantly reduced in rats exposed to ≥ 1,000 ppm. The decrease in brain weight was the most sensitive effects among other endpoints examined.

4.1.1 Physical/Chemical Properties

For physical/chemical properties, refer to Section 3.1 and Table 3.

4.1.2 Key and Supporting Studies

The Kawai et al. (1991) occupational exposure study, NEDO (1987) chronic rat bioassay study and NEDO (1987) two-generation reproductive study in rats were used as key studies to derive potential chronic ReVs.

4.1.2.1 Key Occupational Study, Kawai et al. (1991)

Kawai et al. (1991) carried out a study on methanol biomarkers and exposure-related health effects in 33 occupationally exposed workers (16 men and 17 women) in a factory making

methanol fuel. All exposure durations lasted 7-8 h daily for 0.3-7.8 years. However, while the highest exposure duration of 7.8 years is slightly higher than 10% of lifetime exposure of 70 years (TCEQ 2012), the occupational exposure for some workers may not be considered chronic. Information of the duration of service of these workers was not provided. These workers were exposed to time-weighted-average concentrations of methanol of up to 3,577 ppm, as determined by personal sampler measurements of breathing zone air. Workers were grouped into high-exposure (8 male and 14 female productions workers, geometric mean ± GSD of 459 ± 4.1ppm) and low-exposure (8 male and 3 female administrative/clerical workers, geometric mean ± GSD of 31 ± 1.7 ppm) concentration groups. Prevalence of subjective complaints was only observed among workers in the high-exposure group during working hours. No significant differences between the two groups were found for the following symptoms: dimmed vision off work, unusual feeling in the throat, headache off work, forgetfulness off work, fainting after suddenly standing up off work and chill sensation in the extremities off work. Statistically significant differences of incidences of dimmed visions ($p<0.01$) and nasal irritation ($p<0.05$) were observed among workers in the high-exposure group compared to those in the low-exposure group. However, the dimmed vision was found to be related to humidity and production levels-noting that it served as an indication of opacity due to dense methanol vapor. The reported “dimmed vision” is considered most likely not to be a methanol-caused health effect. As such, a LOAEL of 459 ppm for nasal irritation was derived from this repeated exposure study. The LOAEL was used as the POD to derive chronic ReV.

4.1.2.2 Key Animal Study, Rat Two-Generational Reproduction Studies (NEDO 1987)

4.1.2.2.1 Study I

NEDO (1987) conducted a two-generation reproduction study that evaluated the effects of pre- and postnatal methanol exposure (22 h/d) on reproductive and other organ systems of Sprague Dawley (SD) rats. The F_0 generation (30 animals/sex/group) was exposed to 0, 10, 100 and 1,000 ppm from 8 weeks old to the end of mating (males) or to the end of lactation period (females). The F_1 generation was exposed to the same concentrations from birth to the end of mating (males) or to weaning of F_2 pups 21 d after delivery (females). Males and females of the F_2 generation were exposed from birth to 21-d old (one animal/sex/litter was exposed to 8 weeks of age). NEDO (1987) noted reduced brain, pituitary and thymus weights and early testicular descent in the offspring of F_0 and F_1 rats exposed to 1,000 ppm methanol. The early testicular descent is believed to be an indication of earlier fetal development as indicated by the fact that it was correlated with increased pup body weight. However, no histopathologic effects of methanol were observed. A LOAEL of 1,000 ppm for a decrease in weight of brain, pituitary and thymus at 8, 16 and 24 week postnatal in F_1 and at 8 week in F_2. The decrease in brain weight was the most sensitive effects among other endpoints examined.

4.1.2.2.2 Study II

NEDO (1987) conducted a follow-up study in which SD rats were exposed to 0, 500, 1,000 and 2,000 ppm methanol from the first day of gestation through the F_1 generation. Brain weights

were evaluated in 10-14 offspring/sex/group at 3, 6 and 8 weeks of age. Brain weights were significantly reduced in 3-week-old males and females exposed to ≥ 1,000 ppm. At 6 and 8 weeks of age, brain weights were significantly reduced in males exposed to ≥ 1,000 ppm and females exposed to 2,000 ppm. A NOAEL and LOAEL of 500 and 1,000 ppm were identified, respectively. The decrease in brain weights which showed a clear dose-response was considered a biologically significant effect and relevant to humans. The decreases in brain weight observed at 6 weeks, rather than those seen at 3 and 8 weeks, were chosen by USEPA IRIS to derive its reference concentration (RfC) because they resulted in lower estimated BMDs and BMDLs (USEPA 2013a). A rat physiologically based pharmacokinetic (PBPK) model was used to calculate the daily blood methanol area under the blood concentration time curve (AUC) blood methanol internal dose (USEPA 2013a, b). USEPA further performed a BMD modeling to predict 95% lower confidence limits on the benchmark dose (BMDL) (see Section 4.1.4.2). Because the LOAEL (1,000 ppm) in the NEDO (1987) chronic developmental/reproductive studies was only two times the LOAEL (459 ppm) based on nasal irritation from the key human study (Kawai et al. 1991); and because the blood methanol internal dose is available, the TCEQ also developed a potential chronic ReV for developmental effects based on the NEDO (1987) two-generation rat studies. The TCEQ then used the internal BMDL ($POD_{internal}$) estimated by USEPA to derive a potential chronic ReV for developmental/reproductive effects.

4.1.2.3 Supporting Studies

4.1.2.3.1 Frederick et al. (1984) Occupational Study

Frederick et al. (1984) studied the exposure relationship and possible health effects of methanol exposure from spirit duplicators (aka “Ditto” machines) in 66 female teacher aides (mean age 39.8 years, range 24-60). Exposure times varied widely from 1 h/d for 1 d/week to 8 h/d for 5 d/week during about 3 years. Fifteen-minute breathing zone methanol samples from 21 of 58 duplicators in 12 schools were measured. Methanol concentrations ranged from 365 to 3,080 ppm (mean 1,060 ppm, median 1,040 ppm). Of the 21 measurements, 15 measurements exceeded the NIOSH-recommended short-term exposure limit of 800 ppm and 11 measurements were between 1,000 and 1,500 ppm and only one was above this range. Sixty-six females (mean age 37.5 years, range 24-59) from the same schools were randomly selected to serve as a comparison group.

Among the aides, 4 of the 22 symptoms listed in the questionnaire were reported significantly ($p<0.05$) more frequently: headache, dizziness, blurred vision and nausea/upset stomach. No information on the exact exposure duration and the time between start of exposure and occurrence of symptoms was provided. The data indicated that the prevalence of methanol toxicity cases increased with the percentage of time spent at duplicators per week. A LOAEL of 1,060 ppm was identified from this study.4.1.2.4.2 Andrews et al. (1987) Animal Study

Andrews et al. (1987) exposed 5 male and 5 female SD rats/group and 3 male and female cynomolgus monkeys (*M. fascicularis*) at 500, 2,000 or 5,000 ppm methanol (nominal concentrations) for 6 h/d, 5 d/week for 4 weeks. Monitoring of clinical toxicity symptoms,

physical assessments and ophthalmoscopy examinations were performed. Body and organ weights were recorded as well. No overt toxicity was observed in monkeys. No effects on body or organ weights were found, except that female rats exposed to 2,000 ppm had significantly higher relative spleen weights than controls. The authors considered this difference as not having any apparent biological significance. In all methanol-treated groups increased discharges around the nose and eyes, lacrimation, mucoid nasal discharges, red nasal discharge, dried red nasal discharge were observed. The frequency of these symptoms was increased in the treated groups, but only the incidence of mucoid nasal discharges appeared to be concentration related. Gross and histological examination of 35 different tissues of control and high-dose rats revealed no effects. No ocular abnormalities were observed. The study authors concluded that the study identified no target organs and body weight and ocular damage effects. The level of 2,000 ppm could be considered a LOAEL for spleen weight gains in female rats.

4.1.2.3.2 NEDO (1987) Noncancer Toxicity Studies

NEDO (1987) conducted chronic noncancer toxicity of inhalation exposure to methanol on monkeys, rats and mice. Although these are unpublished studies, they were externally peer reviewed by USEPA (2009). The peer review concluded that the rodent studies had good experimental designs and were consistent with the Organization for Economic Cooperation and Development (OECD) guidelines.

4.1.2.3.2.1 Rat Studies

NEDO (1987) exposed groups of 20 Fischer-344 rats/sex/group 19.5 h/d for 12 months to 0, 10, 100, or 1,000 ppm. No alterations in general conditions and behavior were observed. The highest exposure group showed a slightly reduced body weight increase. In clinical, hematological and biochemical examinations, no significant alterations compared to controls were observed. Pathological analysis revealed a slight, dose-dependent increase in liver and spleen weights.

In another bioassay, 52 Fischer-344 rats/sex/group were exposed to 0, 10, 100, or 1,000 ppm methanol vapor for 19.5 h/d for 24 months. No significant difference was observed in urinary, hematology and clinical chemistry bioassay parameters in any exposure groups. There was little change in absolute or relative weights of the major organs or tissues. When the animals were examined grossly at necropsy, however, there was a dose-dependent formation of nodes in the lung of males (2/52, 4/52, 5/52 and 10/52 [$p < 0.01$]) for control, low-, mid- and high-concentration groups, respectively. Histopathologic examination pointed to a possible association of these nodes with the appearance of pulmonary adenoma (1/52, 5/52, 2/52, and 6/52 for control, low-, mid- and high-concentration groups, respectively) and a single pulmonary adenocarcinoma in the high-dose group (1/52). A NOAEL and LOAEL of 100 and 1,000 ppm, respectively, for formation of nodes in the lung were identified from this rat study. However, USEPA (2013a) indicates that peer reviewers of these studies have expressed reservations about the dose-response data quality (e.g., histopathology was only performed on the 10 and 100 ppm groups if the 1,000 ppm group demonstrated statistically significant difference from controls) and interpretation (e.g., statistical methods were incompletely described and, in some cases, improperly applied) (USEPA 2009). USEPA (2013a) further indicates that the evidence for dose-

related effects at 1,000 ppm was weak for both the mouse and rat studies and assigns a low weight-of evidence determination to the 1,000 ppm LOAEL identified for these chronic studies.

For comparison purpose, the TCEQ has conducted benchmark dose (BMD) modeling for incidence data for formation of nodes in the lung observed in male rats to predict 95% lower confidence limits on the BMCL. The estimated BMCL value for formation of nodes in the lung and subsequent derivation of ReV are included in Appendix B. The derived POD_{HEC} of 729.72 ppm for formation of nodes in the lung was similar to that derived based on the NEDO (1987) two-generation rat developmental study (762.891 ppm) (see Section 4.1.6.2).

4.1.2.3.2.2 Mouse and Monkey Studies

4.1.2.3.2.2.1 Mice

NEDO (1987) studied groups of 30 B6C3F1 mice/sex/group continuously exposed 21 h/d for 12 months to 0, 10, 100 or 1,000 ppm. Groups of 10 animals were sacrificed for analysis after 6 months. No alterations in general conditions or behavior were observed.

The body weights of male mice and female mice were decreased after 6 and 9 months, respectively. This difference (4 % and 6 % relative to controls) was significant only in the groups exposed to 1,000 ppm. A significantly reduced food uptake without any effect on body weight was found for the female mice of the 1,000-ppm group during the first two months and after 7 months; no correlation with body weight changes was found. In male mice exposed at 1,000 ppm an increase in liver weight was observed after 6 months and increased kidney and spleen weights were found after 12 months, but the dose-dependency of these effects was unclear. After 12 months a fatty degeneration of hepatocytes was observed in higher frequency in male mice of the high exposure group, but was also reported in lower frequency in the control group. The body weight changes in the 1,000 ppm exposure group are < 10% and were considered a non-adverse effect (TCEQ 2012).

4.1.2.3.2.2.2 Monkeys

In the NEDO (1987) studies, 8 monkeys (*M. fascicularis*) were exposed to 10, 100, or 1,000 ppm methanol, 21 h/d, for 7 months (2 animals), 19 months (3 animals), or 29 months (3 animals). However, there was no control group. Clinical signs, body weight changes and food consumption were monitored. Blood was collected for hematological and clinical chemistry tests and all animals were subject to a histopathologic examination of the major organs and tissues. No compound-related histopathologic lesions were reported in these experiments. However, there were signs of incipient fibrosis and round cell infiltration of the liver in monkeys exposed to 1,000 ppm for 29 months. Slight myocardial disorder and mild respiratory irritation were observed in the 100 (one monkey) and 1,000 ppm (all 3 monkeys) groups. The results also showed dose-dependent changes in the kidney with the appearance of Sudan-positive granules in the renal tubular epithelium at 100 and 1,000 ppm and hyalinization of the glomerulus and penetration of round cells into the renal tubule stroma of monkeys exposed to methanol at 1,000 ppm. However, USEPA indicated that confidence in these determinations is considerably

weakened by uncertainty over whether a concurrent control group was used in the monkey chronic study.

4.1.2.3.2.3 Rat Teratology Study

NEDO (1987) exposed groups of 36 pregnant SD rats to 0, 200, 1,000 or 5,000 ppm 22.7 h/d during GD 7-17. Maternal toxicity was observed at 5,000 ppm: one animal died and another had to be sacrificed; body weight was significantly reduced compared to controls; uptake of food and water was reduced during GD 7-12. At 5,000 ppm, increased embryo lethality in the later period of pregnancy and a reduced birth weight was reported. The F_1 generation showed an increased incidence of deaths, which occurred during the first 4 d after birth and body weights of females were still reduced at weaning. Morphological changes included early dentition, eye lid opening and testes descent. At 8 weeks of age, reduced relative weights of brain, thyroid, thymus and testes as well as an increased relative weight of the pituitary gland were found. No histopathological changes were recorded. No effects on the reproduction of the F_1 generation were found. In groups exposed at 200 or 1,000 ppm, no developmental toxicity was observed. A NOAEL (1,000 ppm) and LOAEL (5,000 ppm) for adverse reproductive and fetal effects were identified.

4.1.2.3.3 White et al. (1983, as cited in USEPA 2013a) Animal Study

White et al. (1983) reported no signs of pulmonary toxicity in male SD rats exposed to 0, 260, 2,600 or 13,000 mg/m^3 (0, 200, 2,000 or 10,000 ppm) methanol for 6 h/d, 5 d/week for 1, 2, 4 and 6 weeks. Lung weight and biochemical and cytological parameters of the lung lavage supernatant, such as lung weight, DNA content, protein content and acid ribonuclease and protease activity were evaluated. None of the examined parameters showed significant changes compared to the control.

4.1.2.3.4 Poon et al. (1995, as cited in USEPA 2013a)

As cited by USEPA, Poon et al. (1995) exposed 15 SD rats/sex/group 6 h/d, 5 d/week for 4 weeks to 0 or 2,500 ppm to methanol. No clinical signs of toxicity were observed. Body weight gain and food consumption did not differ from controls and no histopathologic changes were seen in the lungs or lower respiratory tract of rats exposed to methanol.

4.1.2.3.5 Supporting Reproductive/Developmental Animal Studies

4.1.2.3.5.1 Burbacher et al. (1999a, b, as cited in Clary 2003 and USEPA 2013a)

Burbacher et al (1999) conducted a reproductive and developmental study by exposing *M. fascicularis* monkeys (9-12 monkeys/group) to air, 200, 600, or 1,800 ppm methanol for 2.5 h/d and 7 d/week during pre-mating and mating period (~ 180 d) and through the entire gestation period (~ 168 d). Maternal body weight and health-assessments as well as new born health assessments were routinely conducted during the study. No maternal or neurobehavioral effects were observed. The most sensitive developmental endpoint reported by this study was a reduced gestation length for all exposed groups when compared to the control suggesting a LOAEL of

200 ppm for reproductive effects. However, gestation time in this study was within the normal range and no effect on birth weights was observed.

Further scrutiny from the CERHR expert panel showed these conclusions to be the result of statistical bias. Namely, the control group was influenced by an outlier male offspring whose gestation term fell two standard deviations beyond the mean (NTP-CERHR 2002, 2003, 2004). Additionally, there was no dose response for the reduced gestation length in the treated monkeys (the greatest gestational period decrease having occurred at the lowest exposure level) and the exposure group's gestations were shortened by an increased number of C-sections in several exposed monkeys. CERHR panel concluded that when the outlier in the control group was removed from the analysis, there was no statistical difference in the gestation length between controls and exposed groups and thus, no treatment related reproductive or developmental effect was observed.

4.1.2.3.5.2 Stern et al Studies (1996, 1997, as cited in USEPA 2013a)

Stern et al. (1996, 1997) exposed 4 cohorts of ~ 30 pregnant Long-Evans rats to 0 or 4,500 ppm methanol for 6 h/d beginning on GD 6. After birth, both dams and pups were exposed through postnatal day (PND) 21. Maternal blood methanol concentrations (mean ± SD) were constant during gestation (0.55 ± 0.07 mg/ml) and lactation (0.56 ± 0.09 mg/ml). Before weaning, pups exhibited blood concentrations (mean ± SD) approximately twice those attained by their dams (1.26 ± 0.23 mg/ml). When exposure was continued after weaning on PND 21, blood concentration in pups slowly declined and reached the level of the dams about 48 d after birth. A panel of neurobehavioral tests was performed on the pups. No effects of methanol exposure on suckling or olfactory conditioned behavior were found. In motor activity tests, methanol-exposed neonates were less active on PND 18, but more active on PND 25 than the equivalent control group pups. Very subtle non-adverse effects were also seen in two operant behavior tests. A NOAEL of 4,500 ppm was identified from this study.

Table 8 (below) summarizes the chronic and subchronic animal developmental/reproductive studies.

Table 8. Chronic/Subchronic Animal Inhalation, Developmental/Reproductive Studies

Species	Concen-tration (ppm)	Duration	NOAEL (ppm)	LOAEL (ppm)	Effect	Reference
SD Rats 36/pregnant /group	0, 200, 1,000, or 5,000	22.7 h/d, on GD7-17	1,000	5,000	Late-term resorptions, reduced fetal viability, increased frequency of fetal malformations, variations and delayed ossifications.	NEDO (1987)[a]
SD Rats F1 and F2 generations of a two-generation study	0, 10, 100, or 1,000	20 h/d; F1-birth to end of mating (M) or weaning (F); F2-birth to 8	100	1,000	Reduced weight of brain, pituitary and thymus at 8, 16 and 24 wk postnatal in F1 and at 8 wk in F1.	NEDO (1987)[a]
Follow-up study of brain weights 10-14 SD rats/sex/group in F1 generation[a]	0, 500, 1,000, or 2,000	GD 0 through F1 generation	500	1,000	Reduced brain weight at 3 and 6 wk (males only). Reduced brain and cerebrum weight at 8 wk (males only).	NEDO (1987)[a]
12 Monkeys (M. fascicularis) /group	0, 200, 600, or 1,800	2.5 h/d, 7d/wk, during premating, mating and gestation	ND	ND	Shortened period of gestation; may be related to exposure (no doseresponse), neurotoxicological deficits including reduced performance in the VDR test; may be related to premature births.	Burbacher et al (1999a, b)
~ 30 pregnant Long-Evans rats	0 or 4,500	6 h/d beginning on GD 6; and dams and pups through PND21	---	4,500	No effects of on suckling and olfactory conditioned behavior; subtle effects in perant behavior tests	Stern et al. (1996, 1997)

[a] The NEDO (1987) two-generation rat studies was selected as key study for developmental effects.

4.1.3 MOA Analysis and Dose Metric

4.1.3.1 MOA for Chronic Noncancer Toxicity

While it is well established that the toxic consequences of acute methanol poisoning arise from the action of formate, there is less certainty on how the toxicological impacts of longer-term exposure to lower levels of methanol are brought about. As described in Section 4.1, chronic exposure to methanol may cause effects similar to those from relatively high levels of acute exposure, including CNS and visual disorders (IPCS1997). Therefore, the MOA for nasal irritation observed in workers (Kawai et al. 1991) was presumably attributed to the methanol metabolite formic acid. Since the key study was based on workers exposed to the parent chemical and information on other more appropriate dose metrics were not available, exposure concentration of the parent chemical was used as the dose metric.

4.1.3.2 MOA for Two-Generation Developmental Effects

As described in Section 3.1.4, methanol, not formic acid (or formate), is responsible for the developmental/and reproductive toxicity and blood methanol level is a useful biomarker of exposure (NTP-CERHR 2004). As such, blood methanol concentration is more appropriate to be used as the internal dose metric for developmental/and reproductive effects. A PBPK inhalation model that estimated the rat blood concentration level (internal dose metrics) (USEPA 2013 a, b) is available to use as the dose metric for BMD analysis (see Section 4.1.4.3 below).

4.1.4 POD for the Key Studies

4.1.4.1POD for Nasal Irritation Effects

The chronic LOAEL of 459 ppm for nasal irritation identified in humans in the Kawai et al. (1991) key study was used as the POD to derive a potential chronic ReV.

4.1.4.2 POD for Developmental/Reproductive Effects

NEDO (1987) reports that brain weights decrease in a dose-dependent manner in male rats exposed to methanol throughout gestation and the F_1 generation. USEPA (2013a) considers a change in brain weight to be a biologically significant effect.

USEPA (2013a, b) developed a methanol PBPK inhalation model to estimate the mouse, rat and human blood concentration level (internal dose metrics). The rat PBPK model was used to calculate the daily blood methanol AUC (mg-h/L) associated with 22 h/d continuous exposure in the NEDO (1987) male rats study. The predicted blood methanol AUC values (adjusted for background (control) AUC) for rat dams exposed to methanol at 0, 500, 1,000, or 2,000 ppm are summarized in Table 9.

Table 8. PBPK model estimates of methanol blood levels (USEPA 2013a, b)

Exposure level (ppm)	Blood methanol AUC (mg-h/L)[a] in rat dams	Blood methanol AUC – control AUC (mg-h/L) [a] in rat dams	Mean male rat (F_1 generation) brain weight at 6 week [b]
0	72	0	1.78 ± 0.07 (N=12)
500	619	547	1.74 ± 0.09 (N= 12)
1,000	2,380	2,310	1.69 ± 0.06 [c] (N=11)
2,000	17,600	17,500	1.52 ± 0.07 [d] (N=14)

[a] AUC values were obtained by simulating 22 h/d exposures for 5 d and calculated for the last 24 h of that period; AUCs above background were obtained by subtracting the estimated AUC for controls of 72 mg-h/L.

[b] Exposed throughout gestation and F1 generation. Values are means ± S.D.

[c] $p < 0.01$

[d] $p < 0.001$.

USEPA then performed BMD modeling using USEPA BMD software (version 2.2) to predict BMDLs using the above modeled AUC above background of blood methanol (AUC – control) (Table 9) as the dose metric. A change in the mean response equal to one control standard deviation from the control mean for continuous data was selected as the benchmark response (BMR) to predict internal BMD_{1SD}/$BMDL_{1SD}$. A 5 % change relative to estimated control mean for quantal data was also selected as BMR to predict internal BMD_{05}/$BMDL_{05}$. The results of USEPA BMD modeling analyses for decreased brain weight at 6 weeks in male rats exposed to methanol throughout gestation and continuing into the F_1 generation showed that the fit of the Hill model is better than the other models in the dose region of interest. The BMD_{05} and $BMDL_{05}$ were estimated to be 2,323 and 1,183 mg-h/L, respectively. The BMD_{1SD} and $BMDL_{1SD}$ were estimated to be 1,730 and 858 mg-h/L, respectively, from the Hill model results in the lowest BMDs/BMDLs from among a broad range of BMDs/BMDLs. The $BMDL_{1SD}$ provides a superior fit in the low dose region nearest the BMD. The lower internal BMDL ($BMDL_{1SD}$ of 858 mg-h/L) from the Hill model was used as the $POD_{internal}$ to derive a potential chronic ReV.

4.1.5 Dosimetric Adjustments

4.1.5.1 Default Exposure Duration Adjustments

4.1.5.1.1 Duration Adjustments for Occupational Study

The LOAEL of 459 ppm identified from the Kawai et al. (1991) occupational study was used as POD (POD_{OC}). To convert from occupational exposure to continuous exposure relevant to the

general population (POD_{ADJ}), the POD_{OC} of 459 ppm was multiplied by a dosimetric adjustment factor for exposure continuity using default occupational and nonoccupational ventilation rates and exposure frequencies (TCEQ 2012):

$$POD_{ADJ} = POD_{OC} \times (VE_{ho}/VE_{h}) \times (\text{days per wk}_{oc}/\text{d per wk}_{res})$$

Where:

VE_{ho} = occupational ventilation rate for an 8-h/d (10 m^3/d)
VE_{h} = non-occupational ventilation rate for a 24-h/d (20 m^3/d)
days per wk_{oc} = occupational weekly exposure frequency (study specific)
days per wk_{res} = residential weekly exposure frequency (7 d per week)

$$POD_{ADJ} = 459 \text{ ppm} \times [10/20 \text{ m}^3 \text{ day}] \times [5 \text{ d}/7 \text{ d}]) = 163.93 \text{ ppm}$$

4.1.5.1.2 Duration Adjustments for Rat Developmental Study

The SD rats were exposed for 22 h/d from the GD 1 through the F_1 generation. The $POD_{internal}$ ($BMDL_{1SD}$ of 858 mg-h/L) was predicted by BMD modeling using AUC above background (AUC - control) values, which were obtained by simulating 22 h/d exposure for 5 d and calculated for the last 24 h of that period. Therefore, no duration adjustment was needed.

4.1.6 POD Human Equivalent Concentration (POD_{HEC})

4.1.6.1 POD_{HEC} for Nasal Irritation Effects

As described in Section 4.1.5.1.1, the POD_{OC} of 459 ppm was multiplied by a duration adjustment factor for general population exposure to calculate the POD_{ADJ}. Since the POD for nasal irritation was based on human study, the POD_{ADJ} of 163.93 ppm is the human equivalent concentration (POD_{HEC}).

4.1.6.2 POD_{HEC} for Developmental/Reproductive Effects

The POD_{HEC} was calculated from the $POD_{internal}$ (AUC or $BMDL_{1SD}$) of 858 mg-h/L using an algebraic equation as described in Equation 2 of Appendix B in USEPA (2013b). The equation, based on the human PBPK model, describes the relationship between predicted methanol AUC (adjusted for endogenous background) and the POD_{HEC} in ppm.

$$POD_{HEC} = 0.02308 \times AUC + (1734 \times AUC) \div (1094 + AUC)$$

Since the daily AUC ($POD_{internal}$ or $BMDL_{1SD}$) of 858 mg-h/L estimated from the BMD modeling is above endogenous background, the estimated AUC was adjusted by an endogenous background AUC blood concentration of 36 mg-h/L (1.5 (mg/L)×24 (h)) set by USEPA (2013b). The target AUC ($POD_{internal}$ or $BMDL_{1SD}$) of 822 (858-36) mg-h/L was plugged into Equation 2 below:

$$POD_{HEC} = 0.02308 \times 822 + (1734 \times 822) \div (1094 + 822) = 762.891 \text{ ppm}$$

The POD_{HEC} of 762.891 ppm is above an inhalation concentration of 500 ppm, a level which is considered uncertain according to the human PBPK model developed for methanol by USEPA (2013a), since the blood levels predicted rise above those for which there are model calibration data.

4.1.7 Selection of the Critical Effects

The TCEQ identifies the relevant, adverse health effect observed at the lowest POD_{HEC} in appropriate sensitive (i.e., human relevant) species as the critical adverse effect (TCEQ 2012). Thus, POD_{HEC}s corresponding to effect levels (e.g., LOAELs, BMCs) are needed to make direct comparisons in order to identify the critical effect, since comparing NOAEL-type PODs or comparing PODs that are incomparable in regard to the occurrence of effects (e.g., NOAEL-based versus LOAEL-based POD_{HEC} values) cannot generally be relied upon to be informative regarding the first effect which may be expected to occur as concentrations rise (i.e., the critical effect).

The POD_{HEC} of 762.891 ppm is a NOAEL so the LOAEL would be higher. This NOAEL is higher than the POD_{HEC} of 163.92 ppm, which is a LOAEL. So the LOAEL for nasal irritation in occupational workers was chosen as the critical effect for derivation of the chronic ReV and ESL. The POD_{HEC} of 163.93 ppm is associated with a LOAEL for nasal irritation (459 ppm) and is appropriate for comparison with similar values for determination of the critical effect.

4.1.8 Adjustments of the POD_{HEC}

The POD_{HEC} of 163.93 ppm for nasal irritation based on Kawai et al. (1991) occupational study was used for the extrapolation by applying the following UFs:

- a UF_H of 10 for intraspecies variability;
- a UF_L of 3 was used for extrapolation from LOAEL to NOAEL because the critical effect was local nasal irritation;
- a UF_D of 1 was used because the chronic database for methanol is quite extensive: one occupational key study; one supporting occupational study; more than four animal inhalation exposure supporting studies in multiple species. Additionally, there are developmental toxicity studies in rats, mice, or monkeys; a two-generation reproductive toxicity study in rats and neurotoxicity and immunotoxicity studies. While the confidence in the chronic database is medium to high, the quality of this key study is considered medium.
- The total UF = 30

$$\text{Chronic ReV} = POD_{HEC} / (UF_H \times UF_L \times UF_D)$$
$$= 163.93 \text{ ppm} / (10 \times 3 \times 1)$$
$$= 163.93 \text{ ppm} / 30$$

= 5.464 ppm
=5.5 ppm (rounded to 2 significant figures)
=5,500 ppb

For comparison purpose, the TCEQ also derived a potential chronic ReV (8.4 ppm or 11 mg/m^3) based on the POD_{HEC} of 762.891 ppm calculated from the $POD_{internal}$ of 858 mg-h/L (see Appendix C for details). The derived potential chronic ReV (8.4 ppm or 11 mg/m^3) is compatible to the ReV of 5.5 ppm (7.2 mg/m^3) derived based on the Kawai et al. (1991) study. USEPA (2013a), using the same $POD_{internal}$ value of 858 mg-h/L and a human PBPK model, derived a RfC of 13.6 ppm (17.8 mg/m^3) (before rounded to 1 significant figure) (see Appendix C).

The derived ReV of 5.5 ppm (7.2 mg/m^3) based on nasal irritation from the Kawai et al. (1991) occupational study is the lowest chronic toxicity value and was chose for chronic ReV. Therefore, if the derived chronic ReV protects against critical effect of nasal irritation, reproductive/developmental effects will be protected.

4.1.9 Possible Child/Adult Differences

This section is quoted from Section 4.9.1 of the USEPA IRIS (USEPA 2013a) which describes in detail the possible child/adult differences.

> *"Studies in animals have identified the fetus as being more sensitive than adults to the toxic effects of methanol; the greatest susceptibility occurs during gastrulation and early organogenesis (CERHR, 2004). Human fetuses have limited ability to metabolize methanol as ADH1 activity in 2-month-old and 4–5 month-old fetuses is 3–4% and 10% of adult activity, respectively (Pikkarainen and Raiha, 1967). ADH1 activity in 9–22 week old fetal livers was found to be 30% of adult activity (Smith et al., 1971). Likewise, ADH1 activity is ~20–50% of adult activity during infancy (Smith et al., 1971; Pikkarainen and Raiha, 1967). Activity continues to increase until reaching adult levels at 5 years of age (Pikkarainen 4 and Raiha, 1967). However, no difference between blood methanol levels in 1-year-old infants and adults was observed following ingesting the same doses of aspartame, which releases 10% methanol by weight during metabolism (Stegink et al., 1983). Given that the exposure was aspartame as opposed to methanol, it is difficult to draw any conclusions from this study vis-à-vis ontogeny data and potential influences of age differences in aspartame disposition. With regard to inhalation exposure, increased breathing rates relative to adults may result in higher blood methanol levels in children compared to adults (CERHR, 2004). It is also possible that metabolic variations resulting in increased methanol blood levels in pregnant women could increase the fetus' risk from exposure to methanol. In all, unresolved issues regarding the identification of the toxic moiety increase the uncertainty with regards to the extent and pathologic basis for early life susceptibility to methanol exposure."*

While there are possible child/adult differences, no other information warrants an extra UF is needed. The TCEQ believes that a UF_H of 10 is sufficient to account for human variation including possible child/adult differences.

4.1.10 Health-Based Chronic ReV and $^{chronic}ESL_{threshold(nc)}$.

The ReV of 5,500 ppb (7,200 µg/m^3) was then used to calculate the ESL. The $^{chronic}ESL_{threshold(nc)}$ of 1,600 ppb (2,100 µg/m^3 or) is based on the chronic ReV multiplied by a HQ of 0.3 and rounded to two significant figures at the end of all calculations (Table 10 below).

Table 9. Derivation of the Chronic ReV and $^{chronic}ESL_{threshold(nc)}$

Parameter	**Summary**
Study	Kawai et al. (1991) Occupational Study
Study Population	33 occupationally exposed workers (16 men and 17 women) and 11 administrative/clerical workers (8 male and 3 female)
Study Quality	Medium
Exposure Method	Occupational exposure (geometric mean ± GSD) to: administrative/clerical workers at 31 ± 1.7 ppm (low-exposure group); and high-exposure workers at 459 ± 4.1ppm
Critical Effects	Nasal irritation
LOAEL	459 ppm
NOAEL	Not applicable
POD	459 ppm (free-standing LOAEL)
Exposure Duration	7-8 h/d for 0.3-7.8 years
Extrapolation to continuous exposure (POD_{ADJ})	163.93 ppm
POD_{HEC}	163.93 ppm
Total UFs	30
Interspecies UF	1
Intraspecies UF	10
LOAEL UF	3
Subchronic to chronic UF	Not applicable
Incomplete Database UF *Database Quality*	1 Medium to high
Chronic ReV (HQ = 1)	**7,200 µg/m^3 (5,500 ppb)**
$^{chronic}ESL_{threshold(nc)}$ (HQ = 0.3)	**2,100 µg/m^3 (1,600 ppb)**

4.2 Carcinogenic Potential

Methanol is not currently listed by the International Agency for Research on Cancer (IARC) or other government agencies (e.g., NTP, or ACGIH) as carcinogenic. No studies have been

reported on chromosomal/mutagenic or carcinogenic effects of methanol in humans (IPCS 1997). Methanol has shown no mutagenicity in bacteria (*Salmonella Typhimurium*), increased frequencies of micronuclei in blood cells and of sister chromatic exchanges, chromosome aberrations or micronuclei in lung cells in mice exposed by inhalation to 800 or 4,000 ppm methanol 6 h/d for 5 d (De Flora et al. 1984 and Campbell et al. 1991, as cited in IPCS 1997). It increased the mutation frequency in mouse lymphoma cells only in the presence of S-9 and high methanol concentration (7.9 mg/ml) (McGregor et al. 1985, as cited in IPCS 1997). The only carcinogenicity study in animals was conducted by NEDO (1987), in this study, Fischer-344 rats and B6C3F1 mice were exposed at 10, 100 or 1,000 ppm for 20 h/d for 24 and 18 months, respectively. Male rats exposed at 1,000 ppm showed a higher frequency of papillary adenomas than controls, which was not significantly different from controls. Female rats exposed at 1,000 ppm methanol showed a higher number of adrenal pheochromocytoma (neuroendocrine tumor), which was not significantly different from controls. The results did not show evidence of carcinogenic effects in a lifetime bioassay in rats and mice exposed at 1,000 ppm. NEDO (1987) concluded that exposure to methanol at 1,000 ppm or lower does not cause cancer (USEPA 2009). Because data are inadequate for assessment of human carcinogenic potential via the inhalation route, the ${}^{chronic}ESL_{nonthreshold(c)}$ was not developed.

4.3 Welfare-Based Chronic ESL

No information was found to indicate that special consideration should be given to possible chronic vegetation effects from methanol.

4.4 Long-Term ESL and Values for Air Monitoring Evaluation

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

- chronic ReV = 7,200 µg/m^3 (5,500 ppb)
- ${}^{chronic}ESL_{threshold(nc)}$ = 2,100 µg/m^3 (1,600 ppb)

For the evaluation of ambient air monitoring data, the chronic ReV of 7,200 µg/m^3 (5,500 ppb) is used (Table 1). The long-term ESL for air permit evaluations is the ${}^{chronic}ESL_{threshold(nc)}$ of 2,100 µg/m^3 (1,600 ppb) (Table 2). The ${}^{chronic}ESL_{threshold(nc)}$ (HQ = 0.3) is not used for evaluation of air monitoring data (TCEQ 2012).

4.5 Chronic Inhalation Observed Adverse Effect Level

The POD_{HEC} based on the Kawai et al. (1991) occupational study resulted in the lowest $POD_{HEC.}$ The LOAEL of 459 ppm for nasal irritation identified from the Kawai et al. (1991) study was directly used as the chronic inhalation observed adverse effect level. No duration adjustment was made (TCEQ 2012). The chronic inhalation observed adverse effect level of 459 ppm is provided for informational purposes only (TCEQ 2012). As the basis for development of inhalation observed adverse effect levels is limited to available data, future studies could possibly identify a lower POD for this purpose.

The margin of exposure between the chronic inhalation observed adverse effect level of 459 ppm to the chronic ReV of 5.5 ppm is a factor of 85.

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Appendix A: Acute Reproductive Developmental Effects

A.1 Animal Studies

A.1.1 Rogers et al. (1993)

This study was conducted to provide information on the potential adverse effects of inhaled methanol on a developing fetus. To test this, pregnant CD-1 mice were exposed for 7 h/day (d) on gestation days (GD) 6-15 to air, 1,000, 2,000, 5,000, 7,500, 10,000, or 15,000 ppm methanol vapors. Each exposure group consisted of 114, 40, 80, 79, 30, 30 and 44 mice, respectively. To control for exposure chamber effects, a group of 88 mice were left unhandled (left in their cage) and an additional 30 mice were unhandled and food deprived for 7 h/d on GD 6-15.

Maternal effects: Neither maternal toxicity nor maternal weight change was observed when compared with the sham-exposed group.

Developmental effects: Following mice sacrifice on GD 17, fetuses were weighed, assessed for viability and checked for skeletal and visceral defects. No methanol-related reductions in maternal body weight gain or overt signs of toxicity were observed. The developmental toxicity manifested as a statistically significant increase in cervical ribs/litter at 2,000 ppm and of cleft palate and exencephaly at 5,000 ppm. A significant reduction in live pups/litter was noted at ≥ 7,500 ppm, with a significant increase in fully resorbed litters occurring at ≥ 10,000 ppm. Fetal weight was significantly reduced at ≥ 10,000 ppm. The lowest concentration of observable effects was 2,000 ppm and no effects were observed at 1,000 ppm exposure. These represented LOAEL and NOAEL values, respectively and identified the most sensitive developmental endpoint to be an increase in cervical ribs/litter.

Rogers et al. (1993) conducted BMD modeling for the critical endpoints of cleft palate, exencephaly, resorptions and increased cervical rib. A log-logistic model was used for dose-response modeling and exposure concentration was chosen as the dose metric. The most sensitive benchmark concentration for 5% extra risk (BMC_{05}) of 824 ppm and estimates of the lower 95% confidence limit on the BMC for 5% extra risk ($BMCL_{05}$) of 305 ppm for increased cervical rib were predicted.

A.1.2 Rogers and Mole (1997)

This study was conducted to provide information on the potential adverse effects of inhaled methanol during critical periods of fetal development. To test this, 12-17 pregnant CD-1 mice/group were exposed for 7 h/d on either two consecutive days or a single day during GD 6-13 to filtered air or 10,000 ppm methanol vapors. Mice were sacrificed on GD 17 and live, dead and resorbed fetuses were counted. Live fetuses were weighed and examined for cleft palate and other skeletal defects. Exposure periods overlapping with GD 7 showed increased incidence of resorbed fetuses and peak incidences of cleft palate. Several skeletal malformations were also observed to the exoccipital, atlas, axis and cervical vertebra. The results of this study indicated

that gestation and early organogenesis are very sensitive stages for the induction of developmental toxicity by maternal exposure to methanol. A LOAEL of 10,000 ppm was derived from this study and supports the LOAEL and NOAEL concentrations derived from the Rogers et al. (1993) study.

A.1.3. Nelson et al. (1985)

In a teratology study, Nelson et al. (1985) exposed 15 pregnant SD rats/group to 0, 5,000, 10,000, or 20,000 ppm methanol for 7 h/d. Exposure groups at the 5,000 and 10,000 ppm concentration level were exposed on GD 1-19 while the 20,000 ppm exposure group was exposed on GD 7-15.

Maternal effects: Maternal toxicity was initiated at the 20,000 ppm group as evidenced by a slightly unsteady gait during the first 4 d of exposure. Maternal bodyweight gain and food intake were unaffected by methanol. Thus, the LOAEL and NOAEL for maternal toxicity was 20,000 and 10,000 ppm, respectively.

Developmental effects: The most sensitive endpoint identified from this study was a decrease in fetal body weight. Fetal body weight was significantly reduced at concentrations of 10,000 and 20,000 ppm by 7-10 and 12–16%, respectively, compared to controls. Numbers of litters with skeletal malformations were 0/15, 2/15 and 14/15 at 0, 10,000 and 20,000 ppm, respectively. Increased rudimentary and extra cervical ribs (the most frequently observed skeletal malformations) were observed at 10,000 ppm; however, this increase did not reach statistical significance until 20,000 ppm. As a result, a LOAEL and NOAEL of 10,000 ppm and 5,000 ppm, respectively, were determined for congenital malformations. The markedly lower NOAEL and LOAEL determined in the Rogers *et al.* (1993) study suggest mice are more sensitive to the teratogenic effects of methanol than rats.

A.1.4 NEDO (1987)

NEDO (1987) exposed 36 pregnant SD rats/group to 0, 200, 1,000 and 5,000 ppm methanol vapors for 22.7 h/d on GD 7-17. Adverse developmental effects included reduced fetal weight, fetal malformations (ribs), delayed ossifications, decreased live fetuses and increased late-term resorptions. Additionally, offspring of the 5,000 ppm-exposed groups (at 8 weeks old) had reduced organ weights in the brain, thyroid, testes and thymus. All adverse reproductive and fetal effects were limited to the 5,000 ppm group, indicating a LOAEL and NOAEL of 5,000 and 1,000 ppm, respectively.

A.1.5 Cameron et al. (1985)

Reproductive effects of methanol exposure were assessed by Cameron et al. (1985). In this study, five male SD rats were exposed to 200 ppm methanol vapor for 6 h/d, for either 1 d or 1 week. Assessments were conducted on dams sacrificed either immediately after exposure or following an 18-h recovery period. The effects were evaluated via circulating serum concentrations of testosterone, luteinizing hormone and corticosterone.

A significant decrease in testosterone was observed after a 1-d 6-h exposure, though levels were nearly restored after an 18-h recovery period. There was no significant change in testosterone levels following a one-week exposure for groups with or without a recovery period. These results show that methanol can cause a transient reduction in the formation of testosterone at the 200 ppm exposure level; however, given the rapid recovery (no significant effects observed after 18 h) this is not considered a more critical endpoint than the Rogers *et al*. (1993) study.

A.1.6 Bolon et al. (1993) Study

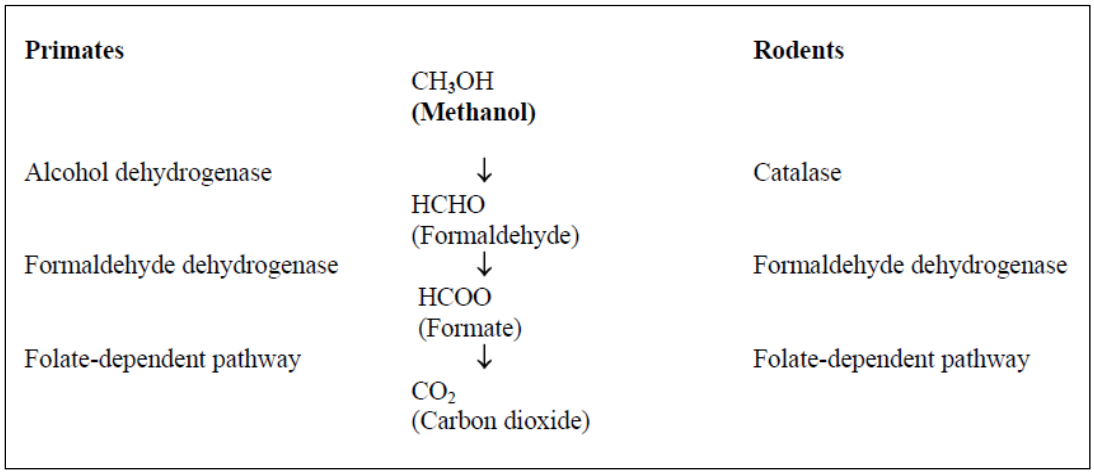
As cited in USEPA (2013a), Bolon et al. (1993) investigated the phase-specific developmental toxicity of methanol in pregnant CD-1 mice. The concentration-response relationship for neural tube defects was determined in a subsequent experiment by exposing dams (20-27 animals/group) at 0, 5,000 (GD 7-9), 10,000 (GD 6-15, 7-9, or 9-11) or 15,000 ppm (GD 7-9 or 9-11). The critical periods of susceptibility to neural tube defects were further narrowed by exposing mice (8-15 animals/group) for 1 (GD 7, 8 or 9) or 2 d (GD 7-8 or 8-9) at 15,000 ppm for 8 h/d. Transient maternal neuronal toxicity was observed at 15,000 ppm after the first exposure in 20 % of dams, after the second exposure in 10% and after the third exposure in 5 %. Signs including ataxia, circling, tilting heads and depressed motor activity were observed. Clinical signs were not apparent at 5,000 or 10,000 ppm. Dams were sacrificed at GD 17. There was a significant increase in resorptions/litter after exposure to 15,000 ppm on GD 7-9. Exposure to 5,000 ppm or higher on GD 7-9 significantly induced in renal pelvic cavitation. Exposure at 10,000 ppm or higher additionally resulted in significantly increased percentages of ocular defects, cleft palate, hydronephrosis and deformed tails and exposure at 15,000 ppm increased in neural tube defects. Neural tube defects and ocular lesions occurred after methanol inhalation between GD 7 and 9, while limb anomalies only occurred after exposure during GD 9 and 11.

A.2 Metabolism of Methanol

A.2.1 Differences in Methanol's Metabolism between Rodents and Humans

While formic acid is responsible for the acute toxicity of methanol, it has been identified that the parent compound, not the metabolites, is the proximate teratogen in laboratory animal studies (Dorman et al. 1995, CERHR 2002, 2003). By exposure of mouse and rat embryos to methanol, Andrews et al. (1993) demonstrated that maternal metabolism of methanol is not required for developmental toxicity and that mouse embryos are intrinsically more sensitive to methanol than rat embryos. Rodents develop higher blood methanol levels after inhalation exposure compared to primates (Perkins et al. 1995), which favors development of methanol-induced CNS and developmental toxicity. Perkins et al. (1995) used a phamarcokinetic model of inhaled methanol in humans to compare to methanol disposition in mice and rats. The results indicated that following an 8-h exposure to 5,000 ppm methanol, blood methanol concentrations in rats and mice were 5-fold and 13-18-fold higher than in humans. The mouse is considerably more susceptible for the developmental toxic effects than the rat. As shown in Figure A-1, rodents and primates utilize different enzymes for key steps in methanol's metabolism. Namely, rodents metabolize methanol to formaldehyde primarily via the enzyme catalase.

Figure A-1. Major enzymes for primate (left) and rodent (right) metabolism of methanol (IPCS 1997)



Developmental effects of methanol are associated with high levels of exposure, which result in the saturation of catalase. When catalase becomes saturated, blood methanol levels exponentially increase, at which point developmental effects are observed (Clary 2003). Bauman et al. 1996 and Poon et al. 1998, as cited in Clary (2003) reported that inhibition of catalase produced a significant increase in malformation in cultured mouse embryos. Wells (2010) indicated that, in rodents, methanol is metabolized primarily by catalase, which produces hydrogen peroxide and other reactive oxygen species (ROS). Wells (2010) further suggested another possible MOA of developmental toxicity in mice involving enhanced oxidative stress and the formation of toxic ROS. Sweeting et al. (2011, as cited in USEPA 2013b) reported that mouse embryo tissue may have a high sensitivity to oxidative damage relative to other species due to a strong reliance on catalase over alcohol dehydrogenase (ADH) to metabolize methanol. Sweeting et al. (2011)suggested that the low ADH activity in mouse embryo relative to rats, combined with the preference of catalase to metabolize methanol over hydrogen peroxide, could lead to a greater depletion of catalase and a higher level of ROS in mouse versus rat embryos, partially explaining the higher sensitivity of mice to the embryotoxic effects of methanol. Therefore, Sweeting et al. (2011) further suggested that, assuming human fetuses do not rely on catalase for methanol metabolism, mouse embryos in sensitive mouse strains may not be a suitable endpoint for assessing human risk.

A.2.2 Weight-of-Evidence Review

Developmental toxicity was the most sensitive endpoint of concern in rodents. Mice are more sensitive than rats to the developmental toxicity of inhaled methanol (Roger et al. 1993). Laboratory animal studies reviewed by the National Toxicology Program Center for the Evaluation of Risks to Human Reproduction (CERHR) Methanol Expert Panel, show that methanol has the potential to adversely affect development in humans (NTP-CERHR 2002, 2003). However, given the high blood level of methanol associated with these developmental

effects, the CERHR indicated that there was minimal concern for developmental effects in humans unless the blood methanol exceeded the “safe level” (10 mg/L). The safe level was established by the CERHR panel following human exposure to methanol at 200 ppm for 6 h. Franzblau et al. (1995, as cited in Clary 2003) reported that blood methanol levels are above the safe level following exposure at 400 ppm for 8 h. Batterman et al. (1998) reported that the baseline or endogenous concentrations of blood averaged 1.8 ± 0.7 mg/L (mean ± standard deviation) and the blood methanol concentration in 4 volunteers exposed to 800 ppm methanol for 1- , 2- and 8-h were 6.6 ± 1.2,14 ± 1.5 and 30.7 ± 6.9 mg/L, respectively. Human blood methanol levels are not expected to exceed 10 mg/L when exposure to methanol at 200 ppm for 6 h or up to 800 ppm for 2 h. Thus, the acute ReV and ESL derived based on a human POD of 203.5 ppm (a 4-h LOAEL for mild and transient subclinical nasal inflammatory reactions) is also expected to be protective of developmental/reproductive effects in humans.

As described in Section 3.2.3.2, the MOA of developmental toxicity in rodents is associated with methanol, not its metabolites. Rodents and primates utilize different enzymes for key steps in methanol’s metabolism, i.e., rodents metabolize methanol to formaldehyde primarily via the enzyme catalase, humans metabolize methanol to formats via the enzyme ADH. Because rodents develop higher blood methanol levels after inhalation exposure compared to primates, the critical developmental effect (an increase in cervical ribs/litter) identified from the rodent studies may not be a human-relevant endpoint. Furthermore, Clary (2003) reevaluated those rodent studies data and suggested that inhaled methanol should not be considered a developmental risk to humans. The Toxicology Excellence for Risk Assessment (TERA 2011) indicates that an increase in rudimentary and extra cervical ribs, as observed by Rogers et al. (1993), should not be considered indicative of developmental toxicity. They should not be suggestive of causing harm to the developing embryo and the findings present no foundation for expecting to cause developmental toxicity in humans. Therefore, the existing animal data were not used to derive acute toxicity values for reproductive/developmental effects. However, the critical effects of decrease in brain weights observed in male rats exposed to methanol (NEDO 1987) were considered biologically significant and relevant to humans. The TCEQ will develop a potential chronic ReV for developmental effects based on decreased brain weight identified in the NEDO (1987) two-generation rat studies (see Section 4.2.1.3).

Appendix B: BMD Modeling and Dosimetric Adjustments for Formation of Nodes in Rat Lung

B.1 BMD Modeling

The TCEQ performed BMD modeling using USEPA BMD software (version 2.2) for the incidences of formation of nodes in rat lungs reported from the NEDO (1987) supporting rat study (Section 4.1.2.4.2). Data was used to predict 95% lower confidence limits on the BMC using dichotomous models. A default BMR of 10% was selected for extra risk (BMC_{10}) and $BMCL_{10}$.

Table A.1 below provides BMD modeling results for formation of nodes in the male rat lung 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). After running all available models, the Log-Logistic model resulted in the lowest AIC (133.95), an acceptable p-value that was greater than 0.1 (i.e., 0.6388) and a BMCL and $BMCL_{10}$ of 660.63 and 319.84 ppm. The $BMCL_{10}$ of 319.84 ppm was used as the POD to derive a potential chronic ReV.

Table B.1 BMD Modeling Results Based on Incidence Data (NEDO 1987)

Dichotomous Model	AIC	P-value	Scaled residuals	BMC_{10}	$BMCL_{10}$
Gamma Multi-Hit	133.98	0.6305	< |2|	683.35	356
Logistic	134.16	0.581	< |2|	806	556
Log-Logistic	133.95	0.6388	< |2|	660.63	319.84
LogProbit	135	0.6774	< |2|	259	7.813
Multistage	133.98	0.6305	< |2|	683.35	356
Probit	134.14	0.587	< |2|	790	527
Weilbull	133.98	0.6305	< |2|	683.35	356
Quantal	133.98	0.6305	< |2|	683.35	356

χ^2 P-Values >0.1 indicate a significant fit

B.2 Dosimetric Adjustments

B.2.1 Duration Adjustments for Rat Noncancer Toxicity Study

The POD based on a $BMCL_{10}$ estimated using BMC modeling of incidence data for formation of nodes in the lung from the NEDO (1987) male rat study was 319.84 ppm. The animals were exposed for 19.5 h/d, 7 d/week, thus the following calculation was applied to adjust for continuous exposure to obtain an adjusted POD based on respiratory effects (POD_{ADJ}):

$$POD_{ADJ} = 319.84 \text{ ppm} \times 19.5 \text{ h}/24 \text{ h} \times 7 \text{ d}/7 \text{ d} = 259.87 \text{ ppm}$$

B.2.2 POD_{HEC} for Formation of Nodes in the Lung

Since the POD for the NEDO (1987) rat study was based on Fischer-344 rat models, a rat-to-human adjustment was applied to calculate the POD_{HEC}. The critical effects (formation of nodes) were seen in the lung and were considered to be respiratory effects. It is appropriately adjusted as a Category 1 gas. Thus, dosimetric adjustments were performed as a Category 1 gas according to TCEQ (2012) guidelines. Based on Equation 4-18 in USEPA (1994), the regional gas dose ratio for the pulmonary region ($RGDR_{PU}$) was calculated based on the default body weight of 0.380 kg for Fischer-344 male rats.

$$RGDR_{PU} = (V_E/SA_{PU})_A / (V_E/SA_{PU})_H$$

where:

$RGDR_{PU}$ = regional gas deposition ratio in the pulmonary region

V_E (ml/min) = min volume in humans $(V_E)_H$ of 13.8 L/min from page 4-26 in USEPA (1994) and in rats $(V_E)_A$ of 0.253 L/min calculated from Equation 4-4 USEPA (1994);

SA_{PU} (m^2) = pulmonary surface area in rats $(SA_{PU})_A$ and humans $(SA_{ET})_H$ from Table 4-4 in USEPA (1994)

$$RGDR_{PU} = (0.253/0.34)_A / (13.800/52)_H = 2.808$$

For Category 1 gases, the default dosimetric adjustment from animal-to-human exposure is conducted using the following equation:

$$POD_{HEC} = POD_{ADJ} \times RGDR_{PU}$$

$$= 259.87 \text{ ppm} \times 2.808 = 729.72 \text{ ppm}$$

Appendix C: Potential ReV Based on Developmental/Reproductive Effects (NEDO 1987)

The POD_{HEC} of 762.891 ppm (Section 4.1.6.2) for decreases in brain weight at 6 weeks of age in male rats observed in the NEDO (1987) study was used for the extrapolation by applying the following UFs:

- a UF_H of 10 for intraspecies variability;
- a UF_A of 3 for the uncertainty of interspecies toxicodynamic variability because the animal-human differences in toxicokinetics were largely accounted for through the use of of PBPK-estimated maternal blood methanol levels for the estimation of POD_{HEC};
- A UF_{Sub} of 1 was used for extrapolation from less than chronic results because the two-generation developmental toxicity (decreased brain weight) was used as the critical effect;
- a UF_L of 1 for the uncertainty of extrapolating of LOAEL to NOAEL because a BMR for BMD modeling was used as POD ($BMCL_{1SD}$);
- a UF_D of 3 was used despite the chronic database for methanol is quite extensive: one occupational key study; one supporting occupational study; more than four animal inhalation exposure supporting studies in multiple species. Additionally, there are developmental toxicity studies in rats, mice, or monkeys; a two-generation reproductive toxicity study in rats and neurotoxicity and immunotoxicity studies. A UF_D of 3 was suggested by USEPA (2013a) to account for uncertain adversity from available reproductive and developmental studies that warrant further research. The quality of this key study is considered high; however, the confidence in the chronic database is medium to high;
- The total UF = 90

Potential Chronic ReV = POD_{HEC} / (UF_H x UF_A x UF_{Sub} x UF_L x UF_D)
= 762.891 ppm / (10 x3 x 1 x 1 x 3)
= 762.891 ppm / 90
= 8.477 ppm
=8.4 ppm or 11 mg/m^3 (rounded to 2 significant figures)

The derived potential chronic ReV (8.4 ppm or 11 mg/m^3) is compatible to the ReV of 5.5 ppm (7.2 mg/m^3) derived based on the Kawai et al. (1991) study but is lower than the RFC of 13.6 ppm (17.8 mg/m^3) (before rounded to 1 significant figure) derived by USEPA (2013a). USEPA (2013a) derived its RfC based on the internal $BMDL_{1SD}$ ($POD_{internal}$) value of 858 mg-h/L estimated from the same data NEDO (1987) study. The $POD_{internal}$ was divided by a total UF of

100 (UF_H of 10, UF_A of 3 and a UF_D of 3) to yield an $RfC_{internal}$, which was converted to a candidate RfC using the human PBPK model. USEPA (2013a) indicates that to apply the UFs to the $POD_{internal}$ prior to the POD_{HEC} derivation results in more scientifically reliable model predictions by lowering the BMDLs to within the more linear, calibrated range of the human PBPK model.

$$RfC = POD_{internal} \div UFs = 858 \text{ mg-h/L} \div 100 = 8.58 \text{ mg-hr/L } (RfC_{internal}) \Rightarrow \text{PBPK Model} \Rightarrow 17.8 \text{ mg/m}^3 \Rightarrow 20 \text{ mg/m}^3 \text{ (rounded to 1 significant figure)}$$

The derived ReV of 5.5 ppm (7.2 mg/m^3) based on nasal irritation from the Kawai et al. (1991) occupational study is the lowest chronic toxicity value and was chose for the chronic ReV.