Development Support Document
September 4, 2015

Crotonaldehyde (*Cis* and *Trans*)

CAS Registry Number: 4170-30-3

Trans-Crotonaldehyde

CAS Registry Number: 123-73-9

Cis-Crotonaldehyde

CAS Registry Number: 15798-64-8

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Acronyms and Abbreviations

Acronyms and Abbreviations	Definition
ACGIH	American Conference of Governmental Industrial Hygienists
ADH	aldehyde dehydrogenase
AEGL	Acute Exposure Guideline Levels
ATSDR	Agency for Toxic Substances and Disease Registry
$^{\circ}$C	degrees Celsius centigrade
BMR	benchmark response
bw	body weight
ConA	Concanavalin A
CRO	crotonaldehyde
DSD	development support document
EC_{50}	Effective concentration at a 50% response level
ESL	Effects Screening Level
$^{acute}ESL$	acute health-based Effects Screening Level for chemicals meeting minimum database requirements
$^{acute}ESL_{generic}$	acute health-based Effects Screening Level for chemicals not 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

Acronyms and Abbreviations	Definition
${}^{chronic}ESL_{generic}$	chronic health-based Effects Screening Level for chemicals not meeting minimum database requirements
GC	gas chromatography
GSH	glutathione
h	hour
$H_{b/g}$	blood:gas partition coefficient
$(H_{b/g})_A$	blood:gas partition coefficient, animal
$(H_{b/g})_H$	blood:gas partition coefficient, human
HEC	human equivalent concentration
HQ	hazard quotient
HSDB	Hazardous Substance Data Base
IARC	International Agency for Research on Cancer
IC_{50}	inhibitory concentration at a 50% response level
IL	interleukin
IP	intraperitoneal
IPCS	International Programme on Chemical Society
IRIS	USEPA Integrated Risk Information System
kg	kilogram
K_{ow}	n-octanol-water partition coefficient
LC_{50}	concentration causing lethality in 50% of test animals
LD_{50}	dose causing lethality in 50% of test animals
LPS	lipopolysaccharide
LOAEL	lowest-observed-adverse-effect-level
LTD	limited toxicity data
MW	molecular weight
µg	microgram
$\mu g/m^3$	micrograms per cubic meter of air
mg	milligrams

Acronyms and Abbreviations	Definition
mg/m^3	milligrams per cubic meter of air
min	minute
MOA	mode of action
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
n	number
NHLF	normal human lung fibroblasts
NIOSH	National Institute for Occupational Safety and Health
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NRC	National Research Council
OSHA	Occupational Safety and Health Administration
PAH	polycyclic aromatic hydrocarbon
PBPK	physiologically based pharmacokinetic
PI	propidium iodide
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
RD_{50}	50% reduction in respiration rate
ReV	reference value
RGDR	Regional Gas Dose Ratio
ROS	reactive oxygen species
RPF	relative potency factor
SA	surface area
SAEC	small airway epithelial cells;
SC	subcutaneous

Acronyms and Abbreviations	Definition
SCOEL	Scientific Committee on Occupational Exposure Limits
SD	Sprague-Dawley
TCEQ	Texas Commission on Environmental Quality
TD	Toxicology Division
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

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 crotonaldehyde (CRO). Please refer to Section 1.6.2 of the *TCEQ Guidelines to Develop Toxicity Factors* (TCEQ 2015a) 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 CRO's physical/chemical data.

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

Short-Term Values	Concentration	Notes
Acute ReV	**Short-Term Health** **29 µg/m^3 (10 ppb)**	**Critical Effect:** minor eye irritation in occupational workers
$^{acute}ESL_{odor}$	**Odor** **180 µg/m^3 (63 ppb)**	Pungent, suffocating odor
$^{acute}ESL_{veg}$	- - -	No data on vegetative effect levels; concentrations producing no observed effects were significantly above other values
Long-Term Values	**Concentration**	**Notes**
Generic ReV	**Long-Term Health** **10 µg/m^3 (3.6 ppb)** [a]	Relative potency approach using acrolein: mild hyperplasia and lack of recovery of the respiratory epithelium in Fisher 344 rats
$^{chronic}ESL_{nonthreshold(c)}$ $^{chronic}ESL_{threshold(c)}$	- - -	Data are inadequate for an assessment of human carcinogenic potential via the inhalation route
$^{chronic}ESL_{veg}$	- - -	No data found

[a] Based on the chronic ReV for acrolein of 1.2 ppb multiplied by the median *in vivo* RPF of 3

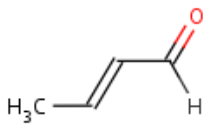
Table 2. Air Permitting Effects Screening Levels (ESLs)

Short-Term Values	Concentration	Notes
$^{acute}ESL$ [1 h] (HQ = 0.3)	**Short-Term ESL for Air Permit Reviews 8.6 µg/m^3 (3 ppb)** [a]	**Critical Effect:** minor eye irritation in occupational workers
$^{acute}ESL_{odor}$	180 µg/m^3 (63 ppb)	Pungent, suffocating odor
$^{acute}ESL_{veg}$	- - -	Concentrations producing vegetative effects were significantly above other ESLs
Long-Term Values	**Concentration**	**Notes**
$^{chronic}ESL_{generic}$	**Long-Term ESL for Air Permit Reviews 3.2 µg/m^3 (1.1 ppb)** [b]	**Critical Effect(s):** Relative Potency approach using acrolein: mild hyperplasia and lack of recovery of the respiratory epithelium in Fisher 344 rats
$^{chronic}ESL_{nonthreshold(c)}$ $^{chronic}ESL_{threshold(c)}$	- - -	Data are inadequate for an assessment of human carcinogenic potential via the inhalation route
$^{chronic}ESL_{veg}$	- - -	No data found

[a] Based on CRO's acute ReV of 29 µg/m^3 (10 ppb) multiplied by 0.3 to account for cumulative and aggregate risk during the air permit review

[b] Based on CRO's chronic generic ReV of 10 µg/m^3 (3.6 ppb) multiplied by 0.3 to account for cumulative and aggregate risk during the air permit review

Table 3. Chemical and Physical Data

Parameter	Value	Reference
Chemical Structure		ChemID Plus (2015)
Molecular Formula	$CH_3CH = CH - CHO$	NRC (2007)
Molecular Weight	70.09	NRC (2007)
Physical State at 25°C	Liquid	NRC (2007)
Color	White liquid; yellows on contact with air	NRC (2007)
Odor	strong, suffocating odor	ATSDR (2002)
CAS Registry Number	CAS 4170-30-3; mixture of *trans* and *cis* isomers CAS 123-73-9 (*trans* isomer); CAS 15798-64-8 (*cis* isomer);	NRC (2007)
Synonyms	2-butenal, crotonal, crotonic aldehyde, 1-formylpropene, β-methylacrolein 123-73-9: (E)-2-butenal, (E)- crotonaldehyde, trans-2-butenal, trans-crotonaldehyde	NRC (2007)
Solubility in water	18.1 g/100 g (20 °C)	NRC (2007)
Log K_{ow}	0.63	IPCS (2008)
Vapor Pressure	19 mm Hg (20 °C)	NRC (2007)
Relative Vapor Density (air = 1)	2.41	NRC (2007)
Density/Specific Gravity (water = 1)	0.853 at 20 °C	NRC (2007)
Melting Point	-76.5 °C	NRC (2007)
Boiling Point	104.0 °C at 760 mm	NRC (2007)
Conversion Factors	1 ppm = 2.87 mg/m^3 1 mg/m^3 = 0.349 ppm	NRC (2007)

Chapter 2 Major Sources or Uses and Ambient Air Concentrations

2.1 Major Sources or Uses

The following information was obtained from National Research Council (NRC 2007):

> Human exposure to crotonaldehyde occurs from both man-made and natural sources. Crotonaldehyde has been identified in exhaust from jet, gasoline; and diesel engines; from tobacco smoke; and from the combustion of polymers and wood (IARC 1995). Crotonaldehyde occurs naturally in meat, fish, many fruits (apples, grapes, strawberries, tomatoes) and vegetables (cabbage, cauliflower, Brussels sprouts, carrots), bread, cheese, milk, beer, wine, and liquors (IARC 1995). It is emitted from volcanoes, from the Chinese arbor vitae plant, and from pine and deciduous forests (IARC 1995; HSDB 2005). Crotonaldehyde has been detected in drinking water, wastewater, human milk, and expired air from nonsmokers.

Crotonaldehyde is a very flammable liquid (Budavari et al. 1996). It is manufactured commercially by adding aldol to a boiling dilute acid solution and removing the crotonaldehyde by distillation. Crotonaldehyde can be produced by aldol condensation of acetaldehyde, followed by dehydration. A process involving direct oxidation of 1,3-butadiene to crotonaldehyde with palladium catalysis has also been reported. Crotonaldehyde is used primarily for the production of sorbic acid; it is also used for the synthesis of butyl alcohol, butyraldehyde, quinaldine, thiophenes, pyridenes, dyes, pesticides, pharmaceuticals, rubber antioxidants, and chemical warfare agents and as a warning agent in locating breaks and leaks in pipes (IARC 1995, Budavari et al. 1996; Verschueren 1996). Crotonaldehyde degrades in the atmosphere by reacting with photochemically produced hydroxyl radicals (half-life of about 11 h) or ozone (half-life of about 15.5 days; HSDB 2005).

CRO and other alkenals may be produced endogenously from lipid peroxidation, a process involving the oxidation of polyunsaturated fatty acids, basic components of biological membranes. The formation of these aldehydes may be causally related to oxidative stress (Ichihash et al. 2001).

Recent U.S. production data are not available. U.S. production of crotonaldehyde in 1975 was > 2,000 pounds, and about 463 pounds was imported into the United States in 1984 (HSDB 2005).

2.2 Background Levels of Crotonaldehye in Ambient Air

There are six locations in Texas that monitor for CRO using 24-h canister samplers that collect samples every 6th day. The 2014 annual average concentration for CRO at these sites ranged from 0.007 to 0.03 ppb, well below the chronic ReV of 3.6 ppb (10 $\mu g/m^3$).

IPCS (2008) provides measured environmental levels for CRO for the United States (refer to IPCS 2008 for references):

- Average concentrations in 1983 in the direct vicinity of a United States highway in rush hour traffic (1 meter from the roadside at a height of 1.5 meters) were 1.1 to 2.1 µg/m^3.

- The concentrations measured in air samples at the Tuscarora Mountain tunnel in Pennsylvania ranged from 0.12 to 0.44 µg/m^3, whereas at the Caldecott tunnel near San Francisco, the range was 0.12 up to 0.76 µg/m^3.

- The mean concentration detected in ambient air at the Oakland-San Francisco Bay Bridge toll booth plaza ranged from 0.061 to 0.147 µg/m^3.

- The mean concentrations detected outside of 87 residences in Elizabeth, New Jersey, throughout 1999 – 2001 were as follows: 0.2 µg/m^3 (spring), 0.5 µg/m^3 (summer), 0.3 µg/m^3 (autumn), and 0.4 µg/m^3 (winter).

Chapter 3 Acute Evaluation

3.1 Health-Based Acute ReV

This section is based on a review of current literature as well as background readings in the International Programme on Chemical Society (IPCS 2008), Acute Exposure Guideline Levels (NRC 2007), and Scientific Committee on Occupational Exposure Limits (SCOEL 2013).

CRO is a reactive compound that is known to produce eye, skin, and respiratory irritation. When sufficient concentrations are inhaled for a sufficient duration, CRO can cause a burning sensation in the nasal and upper respiratory tract, lacrimation, coughing, bronchoconstriction, pulmonary edema, and deep lung damage (NRC 2007). Since CRO possesses potent odorous and irritative properties, exposure to higher concentrations may be limited, thereby avoiding other adverse effects (Henschler 1981).

3.1.1 Physical/Chemical Properties

CRO exists as a *cis* isomer (CASRN 15798-64-8) and a *trans* isomer (CASRN 123-73-9), or as a mixture of the two isomers (CASRN 4170-30-3). Commercial CRO (CASRN 4170-30-3) consists of >95% *trans* isomer and <5% *cis* isomer (Budavari et al. 1996; IARC 1995). A mixture of CRO isomers results in a clear, colorless liquid at room temperature that turns yellow upon contact with air or exposure to light. CRO has a pungent, suffocating odor, which provides warning of hazardous concentrations (ATSDR 2002). It is very flammable and may polymerize violently. CRO is soluble in water, alcohol, ether, acetone, and benzene. Other physical/chemical properties of CRO can be found in Table 3.

Acute toxicity values were not developed separately for cis- and trans-CRO because no apparent studies were available on the individual isomers, although there were studies on mixtures of cis- and trans-CRO. The commercial mixture of CRO consists mainly of trans-CRO.

3.1.2 Key Studies

3.1.2.1 Key Human Study (Fannick 1982)

Fannick (1982) was deemed to be the best available human study to develop an acute ReV, although the study quality was low. NRC (2007) provides the following description of the Fannick (1982) study conducted by the National Institute for Occupational Safety and Health (NIOSH):

> NIOSH conducted a Health Hazard Evaluation in a chemical plant (Sandoz Colors and Chemicals) in East Hanover, New Jersey, at the request of workers at the plant, some of whom complained of occasional minor eye irritation (Fannick 1982). NIOSH measured crotonaldehyde air concentrations using midget impingers; analysis was performed using gas chromatography with flame ionization detection. Eight air samplers were placed near the vats of chemicals and two were worn by the NIOSH industrial hygienist, who was near the vats most of the time. These measurements likely overestimated the actual exposure concentrations because workers were allowed to move about and were not near the vats during an entire 8-h work shift. NIOSH determined that the average crotonaldehyde concentration of general air samples was 1.6 mg/m^3 (0.56 ppm; range, <0.35 to 1.1 ppm; 0.35 ppm was the limit of quantitation). The two personal samples were 0.66 and 0.73 ppm. These workers were also simultaneously exposed to acetic acid and small amounts of acetaldehyde (which occasionally caused a perceptible sweet odor), 3-hydroxybutyraldehyde, and dimethoxane.
>
> Crotonaldehyde was probably the most potent irritant among these chemicals, based on its greater quantity and its much lower RD_{50} (reference dose—the concentration that decreases the respiration rate of mice by 50% due to respiratory irritation [Schaper, 1993; Fannick 1982]).

3.1.2.2 Supporting Human Studies

There were several supporting human studies that provide health effects information for brief exposures. In some cases, the descriptions of the studies were minimal, exposure concentrations were not well-defined, or exposures were for short periods of time. These studies were not considered adequate toxicity studies to develop a ReV. However, they provide useful qualitative dose-response information on the health effects of CRO from low concentrations to higher concentrations. Table 4 is a summary of human CRO exposure data taken from NRC (2007) arranged in order from effects at low concentrations to higher concentrations. Appendix A contains descriptions of these supporting studies taken from NRC (2007).

Table 4. Human CRO Inhalation Toxicity [a]

Exposure Concentration	Exposure Time	End Point and Confounding Factors	Reference
0.035-0.2 ppm 0.037-1.05 ppm 0.12 ppm	Undefined (a few seconds)	Odor thresholds from secondary sources; descriptions of most of the original studies were unavailable.	Verschueren (1996); Ruth (1986); Amoore and Hautala (1983)
0.038 ppm	Undefined (a few seconds)	Subjects were exposed multiple times. Roughly half detected odor at this air concentration.	Tepikina et al. (1997)
0.17 ppm	1 min	Odor detection and/or irritation; exposure through mask; undefined analytical method.	Trofimov (1962)
0.56 ppm (up to 1.1 ppm)	<8 h	Occasional eye irritation; concentration up to 1.1 ppm; coexposure to other chemicals.	Fannick (1982) [b]
4.1 ppm	15 min (10 min)	Marked respiratory irritation; lacrimation in ~30 s; only one concentration evaluated; co-exposure to cigarette smoke (smoking or activity levels were not provided).	Sim and Pattle (1957)
3.5-14 ppm 3.8 ppm	Undefined (10 s)	Irritation sufficient to wake a sleeping person "Irritating within 10 s"; no further details.	Fieldner et al. (1954)
7.3 ppm	Undefined (seconds?)	Very sharp odor and strong irritation to the eye and nose; no experimental details.	Dalla Vale and Dudley (1939)
8 ppm 14 ppm (nose) 19 ppm (eyes)	Undefined (a few seconds)	Irritation threshold; methods used to determine or define "irritation" were not given.	Ruth (1986); Amoore and Hautala (1983)
15 ppm	<30 s	Lab workers "sniffed" crotonaldehyde. Odor strong but not intolerable; no eye discomfort.	Rinehart (1967)
45-50 ppm	<30 s	Lab workers "sniffed" crotonaldehyde. Odor strong, pungent, and disagreeable; burning sensation of conjunctivae but no lacrimation.	Rinehart (1967)

[a] Table reproduced from NRC (2007). Appendix A contains descriptions of these supporting studies taken from NRC (2007).

[b] Key study

3.1.2.3 Supporting Animal Studies

There are few animal studies that describe nonlethal effects of CRO after inhalation. As with human studies, descriptions of the studies were minimal, exposure concentrations were not well-defined, or exposures were for short periods of time. Therefore, these animal studies were not used to develop a ReV.

Table 5 is a summary of animal CRO exposure data taken from NRC (2007) arranged in order from adverse effects observed at low concentrations to higher concentrations. Appendix B contains a discussion of these supporting animal studies obtained from NRC (2007). Concentrations that produced irritation in humans (Table 4) were similar to concentrations causing irritation in animals (Table 5). NRC (2007) stated "LC_{50} values for several species varied by a factor of ≤ 2.5 for several exposure durations, indicating that interspecies variability was minor."

Table 5. Animal CRO Inhalation Toxicity [a]

Exposure Concentrations (Species)	Exposure Time	Relevant Values	End Point (Reference)
unknown (cats)	unknown	3.15 ppm (0.009 mg/L)	Threshold concentration irritating to the mucosa of cats Trofimov (1962)
unknown (Male Swiss-Webster and B6C3F1 mice)	10 min	RD_{50} = 3.53 ppm RD_{50} = 4.88 ppm	50% decrease in respiratory rate Steinhagen and Barrow (1984)
unknown (rabbits)	< 10 min	5 ppm	Significant decrease in respiration and heart rate details Ikeda et al. (1980)
unknown (rabbits)	unknown	17.5 ppm (0.05 mg/L)	Irritation to the mucosa of rabbits Trofimov (1962)
5-8 different concentrations (not specified) (Male F344 rats)	10 min	RD_{50} = 23.2 ppm	50% decrease in respiratory rate Babiuk et al. (1985)
0.02, 0.14, 0.28, 1.3, and 12.7 mg/m^3 [7, 49, 98, 454, 4,430 ppb] (Rats, sex and strain not specified)	30 min	NOAEL 98 ppb LOAEL 454 ppb	Changes in the morphology of the lung and liver tissues Tepikina et al. (1997)
10-580 ppm (Wistar rats)	5 min to 4 h	C x T product $\geq$ 2,000 ppm-min[b]	Decrease in carbon monoxide or ether absorption (indicates reduced pulmonary ventilation rate) Rinehart (1967)

[a] Appendix B contains a discussion of these supporting animal studies obtained from NRC (2007).

[b] The individual concentrations and exposure times were not given. Test responses were presented for five ranges of concentration times time (C x T) due to variations found among animals within any given exposure scenario.

3.1.2.4 Reproductive/Developmental Studies

There were no available inhalation reproductive/developmental studies conducted in animals or humans. Oral (2 g/L for 50 days) or intraperitoneal (IP) administration of CRO to strain Q mice caused production of polyploidy cells at all stages of spermatogenesis, degenerated

spermatogenic cells in the seminiferous tubules, and abnormal pairing of sex chromosomes at diakinesis or metaphase I (Moutshcen-Dahmen et al. 1976; Auerbach et al. 1977).

Jha and Kumar (2006) and Jha et al. (2007) investigated the genotoxicity of CRO in Swiss albino mice treated with CRO via IP injection at 8, 16, and 32 µl/kg body weight (bw). These doses correspond to inhalation concentrations of 2.4, 4.7, and 9.5 ppm, respectively, using route to route extrapolation. This assumes 100% absorption from the respiratory tract, a conservative assumption. These concentrations are above those that cause sensory and respiratory irritation in humans after inhalation exposure (Table 4). Although these studies were designed to investigate genotoxicity through an exposure route which does not consider pulmonary absorption, they indicate that if CRO reaches germ cells, it may affect fertility due to its DNA reactivity. Bone marrow cells could also be affected.

The study conducted by Jha and Kumar (2006) reported abnormal sperm morphology after exposure to CRO, indicative of germ cell mutagens. The effect reached statistical significance one and three weeks after exposure at doses of 16 and 32 µl/kg bw and 5 weeks after exposure at the highest dose of 32 µl/kg.

In the study conducted by Jha et al. (2007), single IP doses of CRO at 8, 16 and 32 µl/kg bw in olive oil caused the following:

- Dose dependent decrease in mitotic index and increase in both chromosome aberrations per cell and the percentage of aberrant metaphases in bone marrow cells;
- A dose-dependent increase in chromosomal aberrations in spermatocytes.
- A dominant lethal mutation study was performed with male mice given the same doses as above once daily for 5 days and then mated with untreated females The treatment resulted in a significant decreases in fertility indices, total number of implants and number of live implants per female, and increased number of dead implants per female which was dose-dependent. Dominant lethality was maximum for mice treated for 5 days at 32 µl/kg body weight.

CRO is a potent point-of-contact respiratory irritant and is efficiently scrubbed in the upper respiratory tract. At low concentrations that protect against mild sensory and respiratory effects in humans, there would be insignificant distribution remote to the respiratory tract, so reproductive/developmental effects would be minimized.

3.1.3 Mode of Action (MOA) Analysis

Similar to the MOA for formaldehyde (TCEQ 2008), the MOA for minor eye or sensory irritation after exposure to CRO may involve interaction with local nerve endings or trigeminal stimulation. Arts et al. (2006) state the free nerve endings of the trigeminal system innervate the

walls of the nasal passages and eyes and respond with nasal pungency or watery/prickly eyes to a large variety of volatile chemicals.

As the concentration of CRO increases, it first causes a perception of odor intensity, then minor eye irritation followed by irritation to the respiratory tract. Chemical stimulation of the vagal or glossopharyngeal nerves may be involved as well as trigeminal stimulation for sensory irritation. Sensory and respiratory irritation are threshold effects which may occur in tissues at sites where CRO is deposited and absorbed (i.e., points of contact). For information on the MOA for respiratory irritation due to cellular damage, refer to Section 4.3.

3.1.4 Dose Metric

The MOA analysis indicates the parent chemical causes sensory and respiratory irritation. Since the key study is based on minor eye irritation in workers exposed to CRO, the most appropriate dose metric is CRO exposure concentration.

3.1.5 POD for the Key Study and Dosimetric Adjustments

The critical effect in the key human study (Fannick 1982) was a minimal LOAEL for minor eye irritation for workers exposed over an 8-h work day to CRO at an average concentration of 0.56 ppm.

Since minor eye sensory irritation is a concentration-dependent effect, a duration adjustment from 8 h to 1 h was not applied. Therefore, the 1-h POD_{HEC} applicable for a 1-h exposure is 0.56 ppm or 560 ppb.

3.1.6 Adjustments of the POD_{HEC}

The following uncertainty factors (UFs) were applied to the POD_{HEC} of 560 ppb:

- A UF_H of 3 for intraspecies variability was used because the critical effect is minor eye irritation (i.e., stimulation of the trigeminal nerve), and toxicokinetic differences between humans would be minimal (even sensitive subpopulations such as children). However, toxicodynamic differences need to be accounted for.

- A UF_L of 3 for the uncertainty of extrapolating from the minimal LOAEL rather than a NOAEL. The LOAEL observed in the key study was based on minor eye irritation.

- A UF_D of 6 was used because the database is limited due to the lack of high quality human or animal studies. However, the dose-response health effects for CRO from low concentration to high concentrations were defined qualitatively in human and in different animal species (mice, rats, and rabbits). There were no human or animal reproductive/ developmental studies, but since CRO is a water soluble, reactive compound, it is scrubbed efficiently in the upper respiratory tract. Systemic absorption

would be minimal. The quality of the key study is considered low; however, the confidence in the acute database is medium.

- The total UF = 54

acute ReV = POD_{HEC} / (UF_H x UF_L x UF_D)
= 560 ppb / (3 x 3 x 6)
= 560 ppb / 54
= 10.37 ppb
= 10 ppb (rounded to two significant figures)

3.1.7 Health-Based Acute ReV

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 ppb **(**29 µg/m^3) was then used to calculate the ESL. The ^{acute}ESL of 3 ppb **(**8.6 µg/m^3) is based on the acute ReV multiplied by a HQ of 0.3, then rounded to two significant figures at the end of all calculations (Table 6).

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

Parameter	Summary
Study	Fannick (1982)
Study Population	Occupationally exposed workers
Study Quality	Low
Exposure Method	Average CRO concentration of general air samples was 0.56 ppm (range from < 0.35 to 1.1 ppm)
Exposure Duration	8 h
Critical Effects	Intermittent minor eye irritation
NOAEL	Not available
LOAEL	560 ppb [a]
POD_{ADJ}	560 ppb (no adjustment – effects were concentration dependent)
POD_{HEC}	560 ppb [a]
Total uncertainty factors (UFs)	54
Interspecies UF	3
Intraspecies UF	Not applicable
LOAEL-to-NOAEL UF	3
Incomplete Database UF *Database Quality*	6 Medium
Acute ReV [1 h] (HQ = 1)	**29 µg/m^3 (10 ppb)**
^{acute}ESL [1 h] (HQ = 0.3)	**8.6 µg/m^3 (3 ppb)**

[a] Inhalation observed adverse effect level (Section 3.4)

3.2 Welfare-Based Acute ESLs

3.2.1 Odor Perception

Crotonaldehyde has a strong, suffocating odor (ACGIH 2001). NRC (2007) reviewed the odor detection data for CRO (Appendix A). There have been a wide range of concentrations reported for human odor detection for CRO (i.e., 0.035 to 0.2 ppm), in some cases due to analytical measurement errors (Verschueren 1996; Ruth 1986; Amoore and Hautala 1983; Tepikina et al. 1997; Trofimov 1962) (Appendix A). Recently, a 50% odor detection threshold value of 65.9

µg/m^3 (23 ppb) was reported for CRO by Nagata (2003) utilizing the Japanese triangular odor bag method. The $^{acute}ESL_{odor}$ for CRO, based on an evidence-integration approach and historical information is 180 µg/m^3 (63 ppb) (TCEQ (2015b).

3.2.2 Vegetation Effects

CRO has been used as a fungicide, with effective concentrations at the 50% response level (EC_{50}s) from one experiment being reported as 80 mg/m^3. In this experiment, the host plants, wheat and barley, had EC_{50}s of 400 mg/m^3 (i.e., the parasitic fungi were about 5 times more sensitive). Bean, tomato, cucumber, and begonia were reported to be more sensitive, but no details were provided (reported in IPCS 2008). Exposure of plants to CRO at a concentration of 1 ppm did not cause any damage to the leaves of the following plants: 10-day-old oat seedlings and 30-day-old alfalfa, endive, sugar beet, and spinach plants (Haagen-Smit et al. 1952). Since concentrations producing vegetative effects (approximately > 1 ppm) are significantly above other health- and odor-based concentrations and a LOAEL for vegetative effects was not identified, an $^{acute}ESL_{veg}$ was not developed for CRO.

3.3 Short-Term ESL and Values for Air Monitoring Data Evaluations

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

- acute ReV = 29 µg/m^3 (10 ppb)
- $^{acute}ESL$ = 8.6 µg/m^3 (3 ppb)
- $^{acute}ESL_{odor}$ = 180 µg/m^3 (63 ppb).

The short-term ESL for air permit evaluations is the $^{acute}ESL$ of 8.6 µg/m^3 (3 ppb) (Table 2). For evaluation of ambient air monitoring data, the acute ReV of 29 µg/m^3 (10 ppb) will be used (Table 1).

3.4 Acute Inhalation Observed Adverse Effect Level

The acute inhalation observed adverse effect level would be the LOAEL from the key human study of 1,600 µg/m^3 (560 ppb). The $LOAEL_{HEC}$ determined from human studies, where eye irritation occurred in some individuals, represents a concentration at which it is probable that similar effects could occur in some individuals exposed to this level over the same or longer durations as those used in the study. Importantly, effects are not a certainty due to potential intraspecies differences in sensitivity. The inhalation observed adverse effect level is provided for informational purposes only (TCEQ 2015a). 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 observed adverse effect level and the ReV is a factor of 54. There is uncertainty in this value because the quality of the key study was low, although other human studies and animal studies reported irritation at higher concentrations (Tables 4 and 5).

3.5 Evaluation of Acute Toxicity Assessment

There is uncertainty for the acute ReV for CRO because of a medium database completeness and low study quality. Therefore, to evaluate the reasonableness of the toxicity assessment for the CRO acute ReV, we compared the acute toxicity data for CRO to the acute toxicity data for acrolein (TCEQ 2014) and formaldehyde (TCEQ 2008), aldehydes similar in structure to CRO (Table 7). This comparison was made using studies that evaluated CRO, acrolein, and formaldehyde using similar testing techniques, exposure durations, and species. In all cases, acrolein showed greater toxicity than CRO:

- CRO had a range of 3-4 fold higher for the concentration causing 50% depression in respiration (RD_{50}) values (Babiuk et al. 1985; Steinhagen and Barrow (1984)
- CRO was approximately 13-fold higher for an inhalation LC_{50} value (Skog 1950)
- CRO had a range of 3-5 fold higher for subcutaneous LD_{50} values (Skog 1950).

Generally, CRO was less toxic than formaldehyde, except for mouse RD_{50} values (Steinhagen and Barrow 1984) and LC_{50} studies (Skog 1950).

Table 7. Comparison of Acute Sensory and Lethality Data

Test (Species)	Rank Order	Value [ratio CRO to acrolein]	Reference
50% odor detection threshold (Humans)	Acrolein	8.2 µg/m^3 (3.6 ppb)	Nagata (2003) Analytical concentrations
	CRO	66 µg/m^3 (23 ppb)	
		[ratio 8.05]	
RD_{50} [a] (Male Fisher-344 rats)	Acrolein	6 ppm	Babiuk et al. (1985) [b] Analytical concentrations
	CRO	23.2 ppm	
		[3.87]	
RD_{50} [a] (Male B6C3F1 mice)	Acrolein	1.41 ppm	Steinhagen and Barrow (1984) [b] Analytical concentrations
	CRO	4.88 ppm	
		[ratio 3.46]	
RD_{50} [a] (Male Swiss-Webster mice)	Acrolein	1.03 ppm	Steinhagen and Barrow (1984) [b] Analytical concentrations
	CRO	3.53 ppm	
		[ratio 3.43]	
LC_{50}, 30 min (Rat)	Acrolein	0.3 mg/L (131 ppm)	Skog (1950) [b, c] Nominal concentrations [b]
	CRO	4 mg/L (1400 ppm)	
		[ratio 13.3]	
LD_{50} subcutaneous injection [a] (Rat)	Acrolein	50 mg/kg	Skog (1950) [b]
	CRO	140 mg/kg	
		[ratio 2.80]	
LD_{50} subcutaneous injection [a] (Mouse)	Acrolein	30 mg/kg	Skog (1950) [b]
	CRO	160 mg/kg	
		[ratio 5.33]	

[a] See Appendix B for a description of the Babiuk et al. (1985) and Steinhagen and Barrow (1984) RD_{50} studies and Appendix C for a description of the Skog (1950) LC_{50} study

The health effects database for acrolein and formaldehyde are more extensive than the database for CRO. The acute ReVs for these three aldehydes are based on minor sensory and respiratory irritation observed in humans. Generally, the toxicity is as follows: acrolein > CRO > formaldehyde. The acute ReV of 10 ppb for CRO is between acrolein's acute ReV of 4.8 ppb (TCEQ 2014) and formaldehyde's acute ReV of 41 ppb (TCEQ 2008) (Table 8). This indicates the acute ReV for CRO is reasonable, based on a comparison of ReVs for aldehydes with similar structures and health effects.

Table 8. Comparison of Acute ReVs for Acrolein, CRO, and Formaldehyde

Chemical	Short-Term ReV	Critical Effect(s)
Acrolein	11 µg/m^3 (4.8 ppb)	eye, nose, and throat irritation and decreased respiratory rate in human volunteers
CRO	29 µg/m^3 (10 ppb)	minor eye irritation in occupational workers
Formaldehyde	50 µg/m^3 (41 ppb)	eye and nose irritation in human volunteers

Chapter 4 Chronic Evaluation

4.1 Noncarcinogenic Potential – Relative Potency Approach

There are no subchronic or chronic inhalation studies appropriate for the development of a chronic ReV for CRO. A poorly reported study conducted by Voronin et al. (1982) is described in IPCS (2008). Rats and mice (strain and number unknown) were treated with CRO for a continuous inhalation exposure for a period of 3 months. Concentrations from 1.2 mg/m^3 (0.419 ppm) led to alterations of motor activity as well as hemoglobin content of blood. The Voronin et al. (1982) study was an abstract - no other information was provided.

Wolfe et al. (1987) conducted oral toxicity studies in rats and mice. Wolfe et al. (1987) treated 10 male and 10 female F344 rats and ten male and ten female B6C3F1 mice per dose group with CRO via oral gavage in corn oil to 2.5, 5, 10, 20, or 40 mg/kg body weight for 13 weeks for five days per week (WHO 1995). At a dose of 5 mg/kg body weight per day and above, compound-related mortality was observed in rats of both sexes and acute inflammation of the nasal cavity was noted in females. At a dose of 10 mg/kg, microscopic lesions (hyperplasia of the forestomach epithelia) were observed in the stomach. At doses of 20 and 40 mg/kg, compound-related gross necropsy lesions (thickened forestomach or nodules) were found in male and female rats and acute inflammation of the nasal cavity was noted in male rats. At a dose of 40 mg/kg, mean body weights were significantly decreased for male rats at termination and forestomach hyperkeratosis, ulcers, moderate necrosis, and acute inflammation were noted. Rats were more sensitive to CRO compared to mice. All mice survived to termination, and no compound-related gross necropsy lesions were noted. At a dose of 40 mg/kg, microscopic

lesions (hyperplasia of the epithelial lining of the stomach) were observed. Because CRO is a highly reactive compound and initiates point-of-entry effects, route-to-route extrapolation using the Wolfe et al. (1987) study was not conducted (TCEQ 2015a).

Since CRO has limited subchronic and chronic toxicity data (LTD), a relative potency approach was followed to determine a chronic generic ReV. Relative potency can be defined as a procedure to estimate the "toxicity" of a LTD chemical in relation to a reference or an index chemical(s) for which toxicity has been well defined. The concept of relative potency has been used to derive toxicity values for polycyclic aromatic hydrocarbons (PAHs) with limited toxicity information based on the toxicity information of benzo[a]pyrene, for which there is a wealth of information (Collins et al. 1998). The following procedures outlined in TCEQ (2015a) can be employed when similar chemical categories or an analog chemical approach is used:

- Identify potential index chemical(s) for which toxicity factors have been developed.
- Gather data on physical/chemical properties, toxicity, etc. for the potential index chemical(s) and the LTD chemical.
- Perform an MOA analysis and determine the relevant endpoints that can be used for a relative potency approach. Relevant endpoints should be determined using similar testing techniques, exposure durations, and species.
- Construct a matrix of data on relevant endpoints for all chemicals.
- Evaluate the data to determine if there is a correlation among chemicals and the endpoints by conducting a simple trend analysis to determine whether a predictable pattern exists amongst the chemicals.
- Calculate the relative potency of the pertinent endpoint based on an MOA analysis of the LTD chemical to the index chemical.

4.1.1 Identify Potential Index Chemical(s)

The Toxicology Division (TD) identified potential index chemical(s) for CRO for which toxicity factors have been developed. Acrolein was selected as the index chemical for CRO for the following reasons:

- the TCEQ has developed toxicity factors for acrolein (TCEQ 2014);
- there are numerous studies that compare the toxicity of acrolein and CRO within the same study for relevant endpoints, although the health effects database for acrolein is more extensive than that for CRO (Section 4.1.2.2);
- they have similar MOAs (Section 4.1.3);

- they have similar physical-chemical parameters (Section 4.1.2.1);
- they have similar structures and reactivity. Both are α,β-unsaturated carbonyl compounds.
- They both produce similar acute adverse health effects in humans (i.e., sensory irritation to the eye and respiratory tract) and animal studies (respiratory tract effects) (NRC 2007, 2010). It is unknown whether chronic health effects for acrolein and CRO are similar because chronic inhalation studies for CRO are not available. However, similar chronic effects would be expected based on similar MOAs.

The use of toxicity information for formaldehyde was initially considered, as the MOA for formaldehyde (TCEQ 2008) is similar to CRO, but there are more available *in vivo* and *in vitro* supporting studies that compare toxicity of CRO to acrolein within the same study than for formaldehyde. Both acrolein and CRO are alkenals whereas formaldehyde is an alkanal. Generally, alkenals are more reactive than alkanals. The chemical/physical parameters for formaldehyde are significantly different than CRO. Therefore, toxicity studies for formaldehyde are not discussed, although a comparison of physical/chemical parameters for acrolein, CRO, and formaldehyde is provided in Table 9.

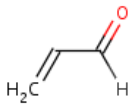
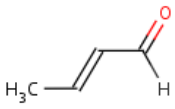
4.1.2 Toxicity Data for Acrolein and CRO

The TD gathered data on physical/chemical properties, toxicity, etc., for the potential index chemical(s) and the LTD chemical.

4.1.2.1 Physical/Chemical Properties

For a complete listing of physical/chemical properties of CRO, refer to Section 3.1 and Table 3. Table 9 shows a comparison of key physical/chemical properties of CRO to acrolein and formaldehyde. CRO is more similar to acrolein in chemical structure and physical/chemical properties than formaldehyde. As mentioned previously in Section 4.1.1, formaldehyde was not considered an appropriate index chemical for CRO. Both acrolein and CRO are soluble in water and have a low K_{ow}, which indicates that bioaccumulation would not occur. The vapor pressure for CRO is lower than acrolein's vapor pressure.

Table 9. Physical Chemical Parameters for Acrolein, CRO and Formaldehyde

Parameter	Acrolein	CRO	Formaldehyde
Chemical Structure			
Molecular Formula	CH_2=CH –CHO ATSDR (2007)	CH3-CH = CH–CHO ChemID Plus (2013)	HCHO ATSDR (1999)
Molecular Weight (g/mole)	56.1 TRRP (2009)	70.09 NRC (2007)	33.03 TRRP (2006)
Physical State	Liquid ATSDR (2007)	Liquid NRC (2007)	Gas ATSDR (1999)
Odor	Disagreeable, choking odor, pungent ATSDR (2007)	Strong, suffocating odor ATSDR (2002)	Pungent, suffocating, highly irritating odor ATSDR (1999)
Solubility in water mg/L	121,000 ATSDR (2007)	181,000 NRC (2007)	550,000 TRRP (2006)
Log K_{ow}	-0.1 TRRP (2009)	0.63 IPCS (2008)	0.35 TRRP (2006)
Vapor Pressure	274 mm Hg ATSDR (2007)	19 mm Hg (20 °C) NRC (2007)	3,880 mm Hg at 25°C TRRP (2006)
Conversion Factors	1 ppm = 2.29 mg/m^3 1 mg/m^3 = 0.44 ppm Toxicology Staff	1 ppm = 2.87 mg/m^3 1 mg/m^3 = 0.349 ppm NRC (2007)	1 ppm = 1.23 mg/m^3 1 mg/m^3 = 0.813 ppm ATSDR (1999)

4.1.2.2 Toxicity Studies Evaluating both Acrolein and CRO

4.1.2.2.1 In Vivo Studies

The only available in vivo toxicity studies that evaluated CRO and acrolein in the same study using similar methods are acute studies that determined 50% odor detection thresholds, 50% inhalation concentration lethality data (LC_{50}), inhalation concentration for 50% respiratory depression (RD_{50}), and 50% lethality data for subcutaneous dose (LD_{50}) (Table 7). Acrolein was consistently more toxic than CRO.

4.1.2.2.1.1 LC_{50} Data

Skog (1950) determined 30-min LC_{50} data for CRO of 1400 ppm and for acrolein of 131 ppm (nominal concentrations), a CRO to acrolein ratio of 13. Rinehart (1967) also determined a 30-min LC_{50} for CRO of 593 ppm (analytical concentrations). The Rinehart (1967) LC_{50} data was approximately 2-fold lower than that obtained by Skog (1950). Rinehart (1967) suggested this difference may have been due to a loss of CRO between point of vapor generation and animal breathing zone. If the 30-min LC_{50} value determined by Rinehart for CRO was used to calculate the ratio of CRO to acrolein, the ratio would be 4.53 .There are two other LC_{50} studies in rats available for acrolein and CRO for similar exposure durations (i.e., 10 min and 4 h), although the CRO LC_{50} and the acrolein LC_{50} were determined by different researchers:

- the 10 min LC_{50} for CRO was 1480 ppm (Rinehart (1967) and for acrolein it was 374 ppm (Catalina et al. 1966), a ratio of CRO to acrolein of 3.95.

- the 4-h LC_{50} for CRO was 70 ppm (Voronii et al. 1982) to 88 ppm (Rinehart 1967) whereas the 4-h LC_{50} for acrolein was 8 ppm (Carpenter et al. (1949). The ratio of CRO to acrolein ranged from 8.75-11.

Rinehart (1967) was a high quality study that reported analytical concentrations. The other LC_{50} studies reported nominal concentrations or were poorly described. Since there may have been a loss of CRO between the point of vapor generation and the animal breathing zone at high concentrations, as suggested by Rinehart (1967), the ratio of LC_{50} data for CRO to acrolein may be unreliable. Therefore, LC_{50} data were not used to determine a ratio of CRO to acrolein for the relative potency approach.

4.1.2.2.1.2 RD_{50} Data

RD_{50} investigations were conducted at lower, more relevant concentrations, and reported analytical concentrations (Table 7). RD_{50} data for CRO ranged from 3.43 to 3.87 fold higher than acrolein in rats (Babiuk et al., 1985) and mice (Steinhagen and Barrow, 1984) (Table 7). Rodents likely experienced cellular damage in the respiratory tract at concentrations used to determine RD_{50} values (Buckley et al. 1984).

- For acrolein, the RD_{50} value is 1.03 ppm in Swiss-Webster mice (Steinhagen and Barrow 1984). Buckley et al. (1984) exposed groups of 16-24 male Swiss-Webster mice to 1.7

ppm acrolein 6 hr/day for 5 days. Acrolein-exposed mice exhibited lesions in the nasal region. There was minimal to moderate recovery after 72 hr.

- For acrolein, the RD_{50} in rats was 6 ppm (Babiuk et al., 1985). Acrolein produced respiratory damage at 1.8 ppm after treatment of rats for 6 hr/day for 4 days (Dorman et al., 2008; TCEQ 2014)

- For CRO, the RD_{50} in rats and mice ranged from 3.53 to 23.2 ppm. Trofimov (1962) reported irritation to the mucosa of rabbits at 17.5 ppm CRO and the threshold concentration irritating to the mucosa of cats was 3.15 ppm CRO (Table 5).

4.1.2.2.1.3 Subcutaneous LD_{50} Data

LD_{50} data were determined by Skog (1950) via the subcutaneous route. Approximately eight animals/group were injected with acrolein at a concentration range of 20-80 mg/kg for mice and 40-60 mg/kg for rats. For CRO, mice were injected at a concentration range of 120-260 mg/kg and rats were injected at a concentration range of 100-180 mg/kg. Rodents were observed for up to three weeks. Histological examinations of lungs, heart, liver, spleen, kidneys and brain were made for each aldehyde from at least four animals. For acrolein, the animals experienced moderate anesthesia with general convulsions of short duration being noted in some animals. Mice seemed to have respiratory trouble more than rats. In the lungs, the following effects were observed: intra-alveolar and perivascular edema, especially perivenously with insignificant hemorrhages. Hyperemia and slight fatty degeneration occurred in the liver whereas focal inflammation changes were observed in the kidney. The subcutaneous LD_{50} for acrolein was 30 mg/kg in mice and 50 mg/kg in rats (Table 7).

For CRO, the animals experienced an intense excitation lasting 10-15 minutes, during which the animals showed signs of distress. The nose, ears, and feet became strongly reddened during the same excitation stage. At larger doses, death occurred during or close to the excitation stage. In the lungs, the following effects were observed: hyperemia, hemorrhages, perivascular edema with slight peribronchial pneumonic changes. Hyperemia was observed in the heart, liver, and kidneys. The subcutaneous LD_{50} for CRO was 160 mg/kg in mice and 140 mg/kg in rats (Table 7). The subcutaneous LD_{50} data were considered relevant for deriving the CRO-to-acrolein RPF.

4.1.2.2.2 In Vitro Studies

4.1.2.2.2.1 Meacher and Menzel (1999)

Meacher and Menzel (1999) conducted *in vitro* studies in adult rat lung cells to compare the effective aldehyde concentration that reduced GSH by 50% (EC_{50}). Cells were treated for 20 min with a range of aldehyde concentrations and then glutathione levels were evaluated using glutathione-monochlorobimane fluorescence intensity measured using laser cytometry. Results were reported for aldehyde concentrations that produced no marked changes in cell morphology as observed by phase-contrast microscopy. One of the proposed MOAs for aldehydes, especially acrolein and CRO, is depletion of cellular GSH, leading to oxidative stress and cellular damage

(Section 4.3). An *in vitro* assay that ranks GSH reduction may be used to rank the potency of aldehydes within a class.

The EC_{50}s for the n-alkanals (formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde) ranged from 110-400 mmol/L, a factor of approximately 1000 times less potent when compared to the 2-alkenals, acrolein and CRO. Acrolein was the most potent 2-alkenal studied as it had the lowest EC_{50}, followed by CRO

- Acrolein (2 µmol/L)
- CRO (130 µmol/L)
- trans-2-Hexenal (160 µmol/L
- trans -2-pentenal (180 µmol/L).

The ratio of EC_{50}s for GSH depletion for CRO compared to acrolein was 65 (Meacher and Menzel 1999).

4.1.2.2.2.2 Moretto et al. 2009

Moretto et al. (2009) examined the acute effects of aqueous cigarette smoke extract (CSE) and of two α, β unsaturated aldehydes (acrolein and CRO) contained in CSE in cultured normal human lung fibroblasts (NHLF) and small airway epithelial cells (SAEC). By examining a panel of 19 cytokines and chemokines, data showed that IL-8 release was elevated by CSE. Acrolein and CRO concentrations mimicked the CSE-evoked IL-8 release induced by CSE.

Cultured cells were treated with 0, 3, 10, 30, and 60 µM acrolein or CRO. Acrolein or CRO (3–60 µM) stimulated the release of IL-8 from both SAEC and NHLF in a concentration-dependent manner.

- In SAEC cultures, acrolein (171.7 ± 5.2% of basal release, n = 4) and CRO (195.5 ± 6.2% of basal release, n = 4) elicited their maximal effect at 30 µM .

- In NHLF cells, acrolein elicited its maximal effect at 10 µM (258.4 ± 23.5% of basal release, n = 4) and CRO at 30 µM (202.1 ± 13.6% of basal release, n = 4).

Moretto et al. (2009) also evaluated cell viability using the MTT test in SAEC and NHLF cells (Table 10). There were no statistical differences in cell viability after treatment with acrolein and CRO compared to control SAEC cells (no statistical differences at concentrations of 3, 10, 30 and 60 µM). However in NHLF cells, acrolein significantly decreased cell viability at 60 µM whereas a numerical lower trend was observed for CRO.

Cell viability was evaluated by percent decrease in absorbance in the MTT assay. The ratio of absorbance for CRO compared to acrolein at 60 µM (a concentration where acrolein produced a significant decrease in cell viability) was a ratio of 3.64 (i.e., 91% reduction/25 % decrease) (Moretto et al. 2009).

Another method to calculate a RPF value from the Moretto et al. (2009) study is to compare the concentration of CRO and acrolein that produce the same reduction in cell viability. The concentration that resulted in a 91% reduction of absorbance for CRO was 60 µM. The concentration corresponding to a 91% reduction in MTT absorbance for acrolein was not provided, but can be estimated using a linear interpolation between 10 µM (99% reduction in absorbance) and 30 µM (76% reduction in absorbance). Based on this interpolation, the concentration of acrolein projected to result in a 91% reduction in cell viability is 17 µM. The RPF for the CRO concentration of 60 µM (91% reduction) to the acrolein concentration of 17 µM (91% reduction) is 3.53. This supports the RPF of 3.64 calculated above.

Table 10. Acrolein and CRO viability evaluated in NHLF cells (MTT Test [a])

Concentrations	3 µM	10 µM	17 µM [b]	30 µM	60 µM
acrolein	97 ± 3	99 ± 3	91	76 ± 6	25 ± 2 [c]
CRO	99 ± 1	98 ± 1	- - -	94 ± 2	91 ± 2

[a] MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

[b] As a sensitivity analysis, the estimated concentration corresponding to a 91% decrease in absorbance for acrolein was calculated. The concentration of 17 µM was calculated using a linear interpolation between 99% decrease in absorbance (10 µM) and 76% decrease in absorbance (30 µM)

[c] Statistically different from control viability, $P < 0.01$.

4.1.2.2.3.2.3 Poirier et al. 2002

Poirier et al. (2002) assessed 13 chemicals present in tobacco smoke, including acrolein and CRO, for their effect on viability and proliferation of mouse lymphocytes *in vitro*. Lymphocytes were obtained from the spleen and were referred to as splenocytes. Cell viability was assessed with propidium iodide (PI), with subsequent analyses by flow cytometry. For cell proliferation, control and treated cells were exposed to Concanavalin A (ConA), a T-cell mitogen, and lipopolysaccharide (LPS), a B-cell mitogen. After a 48-h incubation period, 0.5 µCi of [^{3}H]methylthymidine was added to each well. The incubation was resumed for another 18 hr under the same conditions. Cells were then collected on filters and counted in a β counter.

Only acrolein and CRO induced a cytotoxic effect in the viability assay. The other 11 compounds produced no cytotoxic effects on splenocytes. Both aldehydes produced a concentration- and time-dependent significant effect on splenocyte viability as determined by PI dye exclusion. At 10^{-5} M and higher concentrations, the significant suppressive effect was already observed after 3 h of exposure. A longer incubation period with acrolein and CRO at the highest concentrations resulted in the death of almost all cells. The concentration causing 50% inhibition (IC_{50}) for viability and the mitogenic assay after a 3-h exposure are shown in Table 11. Acrolein and CRO inhibited both T-cell and B-cell proliferation (Table 12). The antiproliferative effect of CRO and acrolein may partly be explained by their cytotoxic effects, with IC_{50} values

for viability and the mitogenic assays being within the same range. The ratio of IC_{50} values for cell viability in splenocytes for CRO compared to acrolein was 1.58.

Table 11. Comparison of IC_{50} values for Acrolein and CRO (3-h Exposure)

	IC_{50} viability (M)	**IC_{50} ConA (M)**	**IC_{50} LPS (M)**
acrolein	2.70×10^{-5}	2.06×10^{-5}	3.16×10^{-5}
CRO	4.26×10^{-5}	2.01×10^{-5}	2.47×10^{-5}

4.1.3 MOA Analysis

A MOA analysis was performed to determine the relevant endpoints that might be used for a RPF approach. Relevant endpoints for both acrolein and CRO need to be closely tied to the expected critical effect for the index chemical and LTD chemical and need to be determined using similar testing techniques, exposure durations, and species. The critical effects are noncarcinogenic and the toxicity of each effect was assumed to have a threshold exposure associated with its MOA (threshold MOA).

4.1.3.1 CRO MOA

Because CRO is an α,β-unsaturated carbonyl, it is highly reactive with cellular components and forms protein adducts and histone–DNA crosslinks (Kurtz & Lloyd, 2003). The general metabolic pathway for aldehydes is oxidation by aldehyde dehydrogenase (ADH). However, the major detoxification pathway of CRO is with (GSH) to form GSH conjugates.

Liu et al. (2010a) investigated the MOA for cell death in a normal human bronchial epithelial cell line (BEAS-2B cells) after exposure to CRO. CRO induced cytotoxicity through induction of cellular oxidative stress with depletion of intracellular GSH and increase in reactive oxygen species. CRO induced both apoptosis and necrosis, and there was a transition from apoptosis to necrosis with increasing CRO concentrations (Liu et al., 2010a). This transition was dependent on decreasing ATP levels, reduction in mitochondrial membrane potential, opening of the mitochondrial permeability transition pore (a critical event), and cytochrome c release from the mitochondria to the cytosol. Apoptosis was mediated via cytochrome c release and caspases cascade (caspase-9 increased, but diminished after prolonged exposure; caspase-3/7 was elevated at higher concentrations). Liu et al. (2010a) could not rule out the possibility that CRO might induce apoptosis through another caspase-independent pathway, such as apoptosis-inducing factor.

In a later study, Liu et al. (2010b) used microarray analysis to study the gene expression profile of BEAS-2B cells after exposure to increasing concentrations of CRO. Cell cycle arrest was also investigated in the study. A large number of inflammation responsive genes were suppressed by CRO. HMOX1 (antioxidant response) and ALDH1A3 (ADH metabolism) were induced at three

different increasing concentrations. Although some cell cycle genes were upregulated, several were down regulated; overall, CRO produced cell cycle arrest in S and G2M phase. Heat shock response related genes were strongly upregulated. Taken into account HMOX1 mediating cellular pathways and ALDH1A3 detoxifying toxicants, HMOX1 and ALDH1A3 were considered as novel transcriptional markers for CRO toxicity.

Both Moretto et al. (2009) and Yang et al. (2013) investigated inflammatory mechanisms after exposure of cultured cells to CRO. Morretto et al (2009) demonstrated CRO increased interleukin (IL)-8 release in cultured normal human lung fibroblasts and small airway epithelial cells. Phosphorylation of both ERK1/2 (extracellularly-regulated kinase-1 and -2) and p38 (38-kDa mitogen-activated protein kinase) underlies the IL_8 release. Yang et al. (2013) showed that CRO treatment is capable of directly stimulating the production of IL-8 in both macrophages and airway epithelial cells (BEAS-2B and A549 cells). In addition, conditioned media from THP-1 cells stimulated after CRO exposure elevated IL-8 production, enhanced nuclear factor (NF)-κB and AP-1 DNA-binding activity in BEAS-2B and A549 cells. CRO-stimulated macrophages also amplify the inflammatory response by enhancing IL-8 release from airway epithelial cells and produce lung inflammatory response via multiple mechanisms which result in chronic airway inflammation in smokers.

4.1.3.2 Acrolein MOA

Similar to CRO, acrolein is highly reactive and rapidly forms conjugates with cellular GSH, cysteine, N-acetylcysteine, and/or thioredoxin (Moghe et al. 2015). Acrolein was found to be cytotoxic to various cells *in vivo* and *in vitro* (Li et al. 1997). Many of the effects of acrolein may be due to saturation of protective cellular mechanisms (e.g., GSH) and reactions with critical sulfhydryl groups in proteins and peptides (WHO 2002). The effects following inhalation exposure to acrolein are qualitatively similar to that of other aldehydes, although, acrolein is the most irritating (NRC 2010). The respiratory irritancy of acrolein may be due to reactivity toward sulfhydryl groups in receptor proteins in the nasal mucosa (Beauchamp et al., 1985). Acrolein was also shown to suppress defenses against infections. In order to study how acrolein may decrease host defense, Li et al. (1997) studied human alveolar macrophage function and response after exposure to acrolein. Macrophages treated with varying concentrations of acrolein displayed a concentration-dependent inhibition in release of IL-1β, IL-12, and TNF-α. Treatment of alveolar macrophages by acrolein also induced concentration-dependent necrosis and apoptosis after 24 hr.

4.1.3.3 Comparison of the MOA for Acrolein and CRO

There are differences between the MOAs of acrolein and CRO involving mechanisms affecting apoptosis and necrosis as well as differences in gene expression profiles as described by Liu et al. (2010a, b). However, the primary mechanisms of toxicity are similar. Both CRO and acrolein are highly reactive and induce toxicity in a variety of ways. An increase in reactive oxygen species resulting from reaction with and depletion of glutathione is considered to be the primary

mechanism underlying toxicity. Reactions with cell membrane proteins and inhibition of regulatory proteins may also play a role.

Based on the comparison of the MOA of acrolein to CRO, respiratory depression at concentrations causing respiratory damage, cytotoxicity, and cellular damage would be the most relevant endpoints to evaluate chronic exposure.

4.1.4 Matrix of Data and Pattern of Relative Toxicity

The next steps are to construct a comparison of CRO to acrolein for relevant endpoints. The following endpoints were not considered relevant for chronic exposure:

- odor potential was not considered to be predictive of chronic adverse effects;
- Depletion of GSH as evaluated by Meacher and Menzel (1999), although an important step in the MOA, is an early event and may not lead to cytotoxicity, so this endpoint was not considered relevant;
- LC_{50} data would be a relevant endpoint because the primary effect observed in animals in lethality studies was respiratory failure. However, LC_{50} data were not used for CRO, as discussed in Section 4.1.2.2.1 *In Vivo Studies*.

Since respiratory depression, cytotoxicity, and cellular damage would be the most relevant endpoints to evaluate chronic exposure based upon MOA, the following endpoints were considered relevant:

- RD_{50} values, although a measure of sensory irritation, were considered relevant for both acrolein and CRO, because exposed animals likely experienced respiratory tissue damage at the same concentrations used to calculate RD_{50} values (see Table 5 for CRO and Buckley et al. 1984 for acrolein). The quality of the RD_{50} studies was high and results were available in both rats and two species of mice.
- Subcutaneous LD_{50} data (Skog 1950), available in both rats and mice, determined for both acrolein and CRO in one study were considered relevant to calculate a RPF (Collins et al., 1998; Glass et al., 1991).
- *In vitro* results evaluating cell viability or cytotoxicity (Tables 10 and 11) were used as supporting studies. The RPF of 3.64 from the Moretto et al. (2009) study is informative because it is based on responses from cultured normal human lung cells

Relevant endpoints in Table 13 were determined using similar testing techniques, exposure durations, and species. The RPF of the pertinent endpoints based on MOA analysis of the LTD chemical (CRO) to the index chemical (acrolein) for pertinent endpoints was calculated as follows:

$$\mathrm{RPF} = \frac{\text{Relevant Endpoint}_{\text{LTD Chemical}}}{\text{Relevant Endpoint}_{\text{Index Chemical}}}$$

The data are evaluated to determine if there is a correlation among chemicals and endpoints to determine whether a predictable pattern exists amongst the chemicals. There was a definite pattern for relevant endpoints (Tables 7 and 12). In all cases, acrolein was more toxic than CRO.

Table 12. Comparison of Relevant Endpoints for Acrolein and CRO

Endpoint	Acrolein	CRO	Relative Potency
RD_{50} Male Fisher-344 rats Babiuk et al. (1985)	6 ppm	23.2 ppm	3.87
RD_{50} Male B6C3F1 mice Steinhagen and Barrow (1984)	1.41 ppm	4.88 ppm	3.46
RD_{50} Male Swiss-Webster mice Steinhagen and Barrow (1984)	1.03 ppm	3.53 ppm	3.43
LD_{50} Rat (subcutaneous injection)	50 mg/kg	140 mg/kg	2.80
LD_{50} Mouse (subcutaneous injection)	30 mg/kg	160 mg/kg	5.33
IC_{50} values for viability (*in vitro*) in mouse lymphocytes Poirier et al. (2002).	2.70×10^{-5} (molar concentrations)	4.26×10^{-5} (molar concentrations)	1.58
Cell viability (*in vitro*) in cultured normal human lung fibroblasts Moretto et al. (2009**)**	25	91	3.64

4.1.5 Median of Relative Potency Factors

If multiple RPF values, based on the same or different relevant endpoints, are available, a median of the RPF is calculated. The median value is the most appropriate summary statistic of the central biologic tendency (Glass et al. 1991; Jones and Easterly 1996). The median of applicable RPF values for *in vivo* endpoints was 3.46 (n = 5). In contrast, the median of applicable RPF values for *in vitro* endpoints was 2.61 (n = 2), which is less than a factor of two compared to the *in vivo* RPF. When rounded to one significant figure, the *in vivo* RPF of 3 and *in vitro* RPF of 3

are identical. The most relevant RPF is based on *in vivo* data since it best represents the response in the intact organism.

4.1.6 Chronic ReV for CRO

Table 13 shows a summary of the derivation of the ReV for acrolein based on the Dorman et al. (2008) study (TCEQ 2014) and the calculated ReV for CRO using the RPF. Details on the Dorman et al. (2008) study are in Appendix E. The animal-to-human dosimetric adjustments for acrolein are relevant to CRO since both aldehydes are water soluble with low K_{ow}s and are expected to produce respiratory damage in the extrathoracic region (USEPA, 1994, 2012). The duration adjustments for acrolein are applicable to CRO since respiratory damage is assumed to be concentration and duration dependent.

The index chemical's chronic ReV of 1.2 ppb is multiplied by the median *in vivo* RPF of 3 to calculate the chronic ReV for CRO of 3.6 ppb (10 μg/m^3), rounded to two significant figures (TCEQ 2015a). A conversion factor of 1 ppm = 2.87 mg/m^3 based on the CRO molecular weight (Table 3) was used to calculate the concentration in μg/m^3.

4.1.7 Health-Based Generic Chronic ReV and $^{chronic}ESL_{generic}$

The generic chronic ReV is 3.6 ppb (10 μg/m^3). The rounded chronic ReV was then used to calculate the $^{chronic}ESL_{generic}$. At the target hazard quotient (HQ) of 0.3, the $^{chronic}ESL_{generic}$ is 1.1 ppb (3.2 μg/m^3) (Table 13).

Table 13. Derivation of the $^{chronic}ESL_{generic}$ for CRO based on Relative Potency

Chemical	**Acrolein (TCEQ 2014)**
Parameter	**Summary**
Study	Dorman et al. 2008
Study Population	360 adult Fischer-344 rats (12 rats/exposure concentration/time point)
Study Quality	High
Exposure Method	Discontinuous whole body at 0, 0.018, 0.052, 0.20, 0.586, or 1.733 ppm
Critical Effects	Mild hyperplasia and lack of recovery of the respiratory epithelium
Exposure Duration	6 h/day, 5 d/wk for 13 wk (65 d)
LOAEL	0.6 ppm
NOAEL	0.2 ppm
POD_{ADJ}	0.03571 ppm
POD_{HEC}	0.006678 ppm
Total UFs	30
Interspecies UF	3
Intraspecies UF	10
LOAEL UF	NA
Subchronic to chronic UF	1
Incomplete Database UF *Database Quality*	1 High
Acrolein Chronic ReV (HQ = 1)	**2.7 µg/m^3 (1.2 ppb)**
Chemical	**CRO Median RPF = 3**
CRO Generic ReV (HQ = 1)	**10 µg/m^3 (3.6 ppb) [a]**
CRO $^{chronic}ESL_{generic}$ (HQ = 0.3)	**3.2 µg/m^3 (1.1 ppb)**

[a] Grant and Jenkins (2015)

4.1.8 Confidence in the CRO Generic ReV

In vivo endpoints were preferred to calculate the final RPF because these endpoints are more appropriate for observing the overall effects on the whole organism. *In vitro* cytotoxicity data were used to support the *in vivo* RPF. The *in vitro* RPF of 3 (rounded to one significant figure) based on decreases in cell viability in cultured normal human lung cells and mouse lymphocytes is the same as the *in vivo* RPF.

The RPFs from *in vivo* and *in vitro* endpoints ranged from 1.58 to 5.33, a three-fold difference. A potential reason the RPF values are consistent was only studies that evaluated CRO and acrolein in the same study using similar testing techniques, exposure durations, and species were used. Only endpoints that were closely tied to the expected critical effect and MOA for the index and LTD chemical were considered. RD_{50} studies in rodents (Babiuk et al. 1985; Steinhagen and Barrow 1984) exposed to concentrations of CRO and acrolein that produce respiratory tract damage (Buckley et al. 1984; TCEQ 2014) were indicative of reactivity and ability to cause cellular damage. Subcutaneous LD_{50} data (Skog 1950) compared lethality for CRO and acrolein. Although LC_{50} studies were preferred to predict toxicity through the inhalation route, subcutaneous LD_{50} studies can be used to calculate RPF values applicable to the inhalation route (Collins et al. 1998; Glass et al. 1991). The TCEQ did not select to use early precursor events, such as GSH depletion (Meacher and Menzel 1999), to calculate a RPF, but instead chose later, more apical endpoints such as decrease in cell viability in different cell lines determined with cytotoxicity assays (Poirier et al. 2002; Moretto et al. 2009).

Even though *in vivo* tests were short-term tests, they are useful to calculate a chronic CRO-to-acrolein RPF. Jones and Easterly (1996) used numerous short-term tests to evaluate carcinogenic potential of chemicals. In addition, they stated "It is desirable for the reference compounds to have been tested extensively in various bioassays so that several relative potency values can be computed for each new compound of interest."

4.2 Carcinogenic Potential

4.2.1 Relevant Data

Among 150 workers exposed to CRO concentrations of 1–7 mg/m^3 (0.3–2.4 mg/m^3) for 20 years, nine malignant tumors, two squamous cell carcinomas of the oral cavity, one adenocarcinoma of the stomach, one adenocarcinoma of the caecum and 5 squamous cell tumours of the lung were reported. However, there were coexposures to acetaldehyde, butyraldehyde and higher aldehydes, to n-butanol and higher alcohols and possibly also to butadiene (Bittersohl 1974). All cases were smokers. These data could not be used to derive a unit risk factor (URF) for CRO.

Chronic human or animal inhalation studies indicating that CRO has carcinogenic potential via the inhalation route are not available, so an inhalation unit risk factor (URF) could not be developed. Data from *in vitro* and *in vivo* mutagenicity assays indicate that CRO may be mutagenic (as reviewed by Foiles et al. 1990, IARC 1995, IPCS 2008, SCOEL 2013).

There is limited data for carcinogenic potential from oral exposure studies (Chung et al. 1986). Since CRO causes point-of-entry effects, the TCEQ did not consider route-to-route extrapolation using the Chung et al. (1986) study as valid (TCEQ 2015a). Information on oral studies is provided for informational purposes only. Results from the Chung et al. 1986 study are summarized by IPCS (2008):

> One chronic oral bioassay was located in which male F344 rats were given 0, 0.6, or 6.0 mM of crotonaldehyde in drinking water for 113 weeks (Chung et al. 1986). This is

equivalent to inhalation exposure to 0, 7.2, and 72 ppm, respectively, by route-to-route extrapolation, as described in Appendix D. The high-dose group had approximately 10% lower body weight gain starting at week 8, and 10 of 23 rats developed moderate to severe liver damage (fatty metamorphosis, focal necrosis, fibrosis, cholestasis, mononuclear cell infiltration). The incidence of hepatic neoplastic nodules and hepatocellular carcinomas combined was 0 of 23, 11 of 27 ($p < .01$), and 1 of 23 at 0, 0.6, and 6.0 mM, respectively (carcinoma: 0 of 23, 2 of 27, 0 of 23, respectively). The incidence of enzyme-altered liver foci, considered to be precursors to neoplasms, was 1 of 23, 23 of 27 ($p < .01$), and 13 of 23 ($p < .01$) at 0, 0.6 and 6.0 mM, respectively. No explanation was offered for the lack of a neoplastic dose-response. Interestingly, the 10 high-dose animals that had severe liver toxicity had no liver neoplasms, but the remaining 13 high dose rats were found to have hepatocellular carcinomas. The authors state "it is worth noting" that two low-dose rats had urinary bladder papillomas (none in controls or high-dose group) but did not indicate whether they considered these tumors to be treatment related.

4.2.2 Carcinogenic Weight of Evidence

Based on the Guidelines for Carcinogen Risk Assessment (USEPA 2005a), the most appropriate cancer classification descriptor for CRO would be "suggestive evidence of carcinogenicity via the oral pathway, but not sufficient to assess human carcinogenic potential via inhalation exposure." Table 14 summarizes cancer classifications from different organizations, based on the Chung et al. (1986) oral exposure study.

Table 14. Carcinogenic Weight of Evidence

International Agency for Research on Cancer (1995)	Group 3 (not classifiable as to its carcinogenicity to humans) [1]
ACGIH (1998)	A3, animal carcinogen [2]
USEPA (2005b)	Group C (possible human) carcinogen [3]

[1] IARC (1995) concluded there was inadequate evidence in both humans and experimental animals to establish the carcinogenicity of CRO. Increased incidences of hepatic neoplastic nodules and altered liver-cell foci in rats in the Chung et al. (1986) study were not seen at the high dose.

[2] Based on the Chung et al. (1986) carcinogenicity oral study in which CRO-treated rats developed liver neoplastic lesions and hepatocellular carcinomas. Also based on positive genotoxicity data (caused mutations, clastogenicity, and DNA adducts).

[3] Based on the increased incidence of hepatic neoplastic nodules and hepatocellular carcinomas (combined) in rats in the Chung et al. (1986) study (despite the lack of a dose-response), a lack of human data, CRO genotoxic activity in some of the short-term tests, the anticipated reactivity of croton oil (a known tumor promoter) and aldehyde with DNA, and the fact that CRO is a suspected metabolite of the probable human carcinogen *N* nitrosopyrrolidine (EPA weight-of-evidence classification B2).

4.2.3 MOA

As mentioned previously, CRO reacts with cellular components and forms protein adducts and histone–DNA crosslinks (Kurtz & Lloyd, 2003). CRO can form DNA adducts and therefore can be a source of DNA damage like other α,β-unsaturated compounds. At higher concentrations, cell necrosis, tissue damage, hyperplasia, etc., may occur at the point of contact.

IPCS (2008) provides the following proposed MOA for cellular damage and injury to DNA:

> There is increasing evidence for the cytotoxicity of 2-butenal *(i.e., CRO)* and other alkenals, which induce cell death by acute exposure of cells to oxidative stress through consumption of the antioxidant glutathione. Metabolically proficient cells rich in glutathione and glutathione *S*-transferase may be efficiently protected against the genotoxic effects of alkenals. However, reductions in glutathione cause a marked carbonylation of a wide range of cellular proteins and trigger carcinogenesis by chronic injury of DNA (Cooper et al., 1987; Eisenbrand et al., 1995). In isolated mouse hepatocytes, crotyl alcohol undergoes alcohol dehydrogenase–catalysed conversion to 2-butenal, the formation of which was accompanied by marked glutathione depletion, protein carbonylation, and cell death (Fontaine et al., 2002).

4.2.4 Unit Risk Factor (URF) Developed by NRC (2007)

Based on the Chung et al. (1986) oral exposure study, NRC (2007) developed a URF for CRO based on route-to-route extrapolation, assuming 100% absorption from the respiratory tract (Appendix D). However, route-to-route extrapolation from the Chung et al. (1986) study was not considered valid because CRO is a highly reactive compound and causes point-of-entry effects. Therefore, the TCEQ did not use the URF for CRO based on oral studies.

4.3 Welfare-Based Chronic ESL

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

4.4 Long-Term ESL and Values for Air Monitoring Evaluation

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

- Chronic generic ReV = 10 µg/m^3 (3.6 ppb)
- $^{chronic}ESL_{generic}$ = 3.2 µg/m^3 (1.1 ppb)

The chronic ReV of 10 µg/m^3 (3.6 ppb) will be used for the evaluation of ambient air monitoring data (Table 1). The $^{chronic}ESL_{generic}$ of 3.2 µg/m^3 (1.1 ppb) is the long-term ESL used for air permit reviews (Table 2). The $^{chronic}ESL_{generic}$ is not used to evaluate ambient air monitoring data.

4.5 Chronic Inhalation Observed Adverse Effect Level

A chronic inhalation observed adverse effect level was not determined for CRO since a relative potency approach was used to determine the chronic ReV and $^{chronic}ESL_{generic}$ for CRO (i.e., CRO had limited toxicity data).

Chapter 5 References

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Appendix A: Supporting Acute Human Studies (from NRC 2007)

2.2.1. Odor Threshold and Odor Awareness

A wide range of concentrations have been reported for the human odor detection and irritation thresholds for crotonaldehyde, perhaps in some cases due to analytical measurement errors (Steinhagen and Barrow 1984). Amoore and Hautala (1983) reported the odor threshold to be 0.12 ppm for *trans*-crotonaldehyde, whereas the irritation threshold was 14 ppm and 19 ppm for the nose and eyes, respectively. In several secondary sources, the odor detection threshold for crotonaldehyde was given as 0.035-1.05 ppm and the irritation threshold was 8.0 ppm (Ruth 1986; Verschueren 1996). In a study in which 25 volunteers were exposed to 0.02-2.3 mg/m^3 (0.007-0.8 ppm) of crotonaldehyde, the odor was detected by several persons at the lowest concentration tested, and roughly half the people were able to detect the odor at 0.11 mg/m^3 (0.038 ppm; Tepikina et al. 1997). The test subjects were exposed to each concentration repeatedly (about 2-4 times) to eliminate guessing and also to "pure air" to give a point of reference (i.e., incidence of false positives). An unpublished source (van Doorn et al. 2002) reported 0.069 ppm and 0.063-0.2 ppm as the *trans*-crotonaldehyde and *cis*-crotonaldehyde odor detection thresholds, respectively (OT_{50}; i.e., concentration at which 50% of the odor panel observed an odor without necessarily recognizing it).

2.2. Experimental Studies

Twelve healthy males ages 18-45 were exposed for 10 or 15 min to 12 mg/m^3 (about 4.1 ppm) in a 100-m^3 chamber at 20-25°C with a wind velocity of 1 mph (exposure duration was unclear from the study text; Sim and Pattle 1957). Crotonaldehyde vapor was produced by bubbling air through a known volume of liquid until all of the liquid evaporated; air samples were analyzed for concentration by using a bubbler containing hydroxylamine hydrochloride solution at pH 4.5 and noting the pH change. The men reported the crotonaldehyde vapor to be highly irritating to all mucosal surfaces, particularly the nose and upper respiratory tract (Sim and Pattle 1957). Lacrimation occurred after an average of 30 s, but eye irritation "did not increase after onset of lacrimation." A confounding factor in the experiment was that there were no restrictions on the men's activities, and they were allowed to smoke tobacco during exposure; smoking or activity levels were not provided.

The threshold for crotonaldehyde irritation in humans was reported as 0.0005 mg/liter (L) (0.17 ppm; Trofimov 1962). In this experiment, volunteers inhaled crotonaldehyde vapor through a mask for 1 min; it was not specified how the vapor was generated or how the concentrations were measured. Factors taken into account were odor detection and irritation of the eyes and mucous membranes of the nose and trachea; it was not specified on which of these end points the estimated irritation threshold was actually based. Trofimov suggested that the maximum permissible concentration of crotonaldehyde in air should be limited to 0.0005-0.0007 mg/L (0.17-0.24 ppm) to prevent irritation.

2.2.3. Occupational and Other Exposures

Laboratory personnel (two or three people) who “sniffed” 15 ppm of crotonaldehyde vapor for a few seconds (<30 s) during brief openings of animal chambers reported that the odor was very strong but not intolerable and that there was no eye discomfort. The personnel who “sniffed” 45-50 ppm of crotonaldehyde vapor only momentarily noted that the odor was “very strong, pungent, and disagreeable, but not particularly biting to nasal passages” (Rinehart 1967, 1998). Lacrimation was not induced in the subjects, although they experienced a burning sensation of the conjunctivae and a strong desire to blink repeatedly.

Fieldner et al. (1954) reported that inhalation exposure to crotonaldehyde at 3.5-14 ppm was sufficiently irritating to wake a sleeping person and that 3.8 ppm was irritating within 10 s. Dalla Vale and Dudley (1939) compiled a list of “threshold values” that produce a noticeable odor in the air. The list included crotonaldehyde at 7.3 ppm, which the authors characterized as an eye and a nose irritant. (Experimental details for these two studies were not available.) A summary of the human studies is presented in Table 5-3.

Appendix B: Nonlethal Animal Studies (from NRC 2007)

3.2.1. Rats

Alterations in pulmonary performance caused by exposure to 10-580 ppm of crotonaldehyde for 5 min to 4 h were investigated using Wistar rats (Rinehart 1967). Pulmonary performance was evaluated by measuring the rates of ether and carbon monoxide (CO) absorption over a 24-h period following crotonaldehyde exposure; typical evaluations were at 1, 2, 6, 10, and 24 h postexposure (Rinehart 1998). A parallel drop in CO and ether uptake implies that the pulmonary ventilation rate was reduced (compared to preexposure levels); a greater drop in CO than ether absorption suggests that the diffusion rate of oxygen from air in the lungs into the blood was reduced (Rinehart and Hatch 1964). The individual concentrations and exposure times were not given; rather test responses were presented for five ranges of concentration times time (Ct) due to variations found among animals within any given exposure scenario. Twelve rats were tested in each exposure range, as shown in Table 5-6. Crotonaldehyde caused a parallel dose-dependent decrease in CO and ether uptake rates that were significant at the 5% or 10% level (for CO and ether, respectively) for Ct of ≥2,000 ppm-min. Death occurred in four animals before 24 h (time not specified) treated with 16,000-32,000 ppm min (geometric mean = 28,900 ppm-min). Concentration and time were stated to be roughly equally important in determining toxicity. The maximal depression in the uptake of the gases occurred 6-10 h after treatment, with subsequent recovery taking 24-72 h. Animals exposed to >8,000 ppm-min and autopsied 3 days after exposure had proliferative lesions of the respiratory bronchioles. Edema was evident only at high Ct values (>16,000 ppm-min), where death occurred within 24 h. Based on these results, Rinehart (1967) concluded that “crotonaldehyde is predominantly a typical deep lung irritant,” with the point of attack being the bronchiole and not the alveolus itself.

The concentration of crotonaldehyde calculated to reduce the respiration rate of male F344 rats by 50% upon exposure for 10 min (RD_{50}) was 23.2 ppm (Babiuk et al. 1985). Rats (four per concentration) were exposed to five to eight different concentrations (not specified). Crotonaldehyde vapor was generated in a modified impinger and was carried to the inlet of a head-only exposure chamber by a nitrogen stream; chamber concentrations were continuously monitored with an infrared gas spectrophotometer. Rats that were exposed 6 h/day for 9 days to 15 ppm of formaldehyde, followed by challenge on day 10 with crotonaldehyde, had a similar RD_{50} (20.5 ppm), indicating desensitization was not caused by prior formaldehyde inhalation (Babiuk et al. 1985).

Rats (sex and strain not specified) were exposed for 30 min to 12.7, 1.3, 0.28, 0.14, or 0.02 mg/m^3 of crotonaldehyde vapor (Tepikina et al. 1997). After 72 h, some animals were necropsied (exposure concentration not specified), and changes were seen in the morphology of the lung and liver tissues of rats exposed to 12.7 or 1.3 mg/m^3. The nature of the changes and the analytical technique used to measure crotonaldehyde in air were not described.

3.2.2. Mice

The RD_{50} (i.e., 50% reduction in respiration rate) values for crotonaldehyde vapor in male Swiss-Webster mice and B6C3F1 mice were 3.53 and 4.88 ppm, respectively (Steinhagen and Barrow 1984). Mice were exposed to crotonaldehyde for 10 min in a head-only exposure chamber, and their breathing rates were measured using plethysmographic techniques (Alarie 1966). The crotonaldehyde chamber concentrations were continuously monitored with an infrared gas spectrophotometer (Steinhagen and Barrow 1984).

3.2.3. Rabbits

The threshold concentration of crotonaldehyde in air that was irritating to the mucosa of rabbits was reported as 0.05 mg/L (17.5 ppm; Trofimov 1962). Respiration and heart rate were significantly decreased in male rabbits that inhaled 5 ppm of crotonaldehyde for <10 min (Ikeda et al. 1980).

3.2.4. Cats

The threshold concentration of crotonaldehyde in air that was irritating to the mucosa of cats was 0.009 mg/L (3.15 ppm; Trofimov 1962).

Appendix C: LC_{50} Studies (from NRC 2007)

Skog (1950)

Skog (1950) obtained a 30-min LC_{50} of 4,000 mg/m^3 (1,400 ppm) for 48 white rats exposed to 100-7,000 mg/m^3 (35-2,450 ppm) of crotonaldehyde vapor (sex, individual concentrations tested, and rats per concentration were not given). Exposure concentrations were not measured analytically but were calculated from the amount of air used to vaporize a measured amount of liquid crotonaldehyde to achieve the target concentration. During treatment the rats gasped and jerked their heads backward at each breath, shut their eyes, lacrimated, and had heavy nose secretion. Exposure was followed by a 3-week observation period; all rats that died did so on or before the second day after treatment. The surviving animals breathed with a “snuffling” sound for 4-5 days after cessation of exposure. Histological examination of the lungs, heart, kidneys, liver, spleen, and brain from at least four rats revealed hyperemia and hemorrhage in the lungs, heart, liver, and kidneys; no edema was evident in the lungs.

Rinehart (1967)

Rinehart (1967) conducted an extensive series of experiments to assess the acute toxicity of crotonaldehyde in male Wistar rats. The rats were exposed for 5 min to 4 h and observed for 2 weeks; exposure concentrations and durations are given in Table 5-5. Crotonaldehyde vapors were generated by bubbling nitrogen gas through liquid crotonaldehyde (90% pure) and mixing this with air; the oxygen concentration was maintained at ≥17.8%. Exposure was in either a 20-L glass chamber or a 1,700-L wooden chamber (the latter was used for lower concentrations; which were not specified). Crotonaldehyde concentrations were measured two to five times over the exposure period using a colorimetric reaction with modified Schiff-Elvove reagent; the analytical concentrations were about 42% of the nominal concentration (range: 29-61%). Rinehart suggested that the discrepancy between the nominal and analytical concentrations was due to crotonaldehyde absorption on chamber walls, oxidation, and/or polymerization. The 30-min LC50 obtained by Rinehart (600 ppm) was about 2-fold lower than that obtained by Skog; 1950; 1,400 ppm). Rinehart suggested this difference may have been due to a loss of crotonaldehyde between the point of vapor generation and the animal breathing zone.

During exposure, rats inhaling ≥ 1,000 ppm developed an excitatory stage, and all treated animals had signs of respiratory distress (gasping and lowered respiratory rate) that persisted for several days in some cases. Treated rats lost up to 25% of their body weight within the first 3 days, roughly in proportion to their exposure concentration. Most deaths occurred within 4 days after exposure; these animals had clear or slightly blood-stained nasal discharge; rats that died within a day had terminal convulsions. Death from days 5-14 were attributed to secondary infections. Necropsy showed that a few animals had pulmonary congestion but that other organs were grossly normal. Rinehart visually estimated LC_{50} values from log-probit plots and obtained values similar to those that can be obtained by probit analysis using the method of Litchfield and Wilcoxon (the estimated and calculated LC50 values are shown in Table 5-5).

Voronii et al. (1982)

Voronii et al. (1982) reported a 4-h LC_{50} of 200 mg/m^3 (70 ppm) for white rats during an observation period of 2 weeks. In preliminary acute toxicity studies, groups of three or four rats (sex and strain not specified) were exposed to nominal crotonaldehyde concentrations of 2,094-16,229 ppm for 30-43 min, 907 or 1,256 ppm for 2 h, 133-359 ppm for 6 h, or 94-108 ppm for 6 h/day on days 1, 2, and 4 (Eastman Kodak Corp. 1992). Many animals died, as shown in Table 5-4. Symptoms included gasping, labored breathing, pink extremities, tremors, convulsions, salivation, and prostration. Microscopic examination of unspecified animals revealed lung congestion.

Appendix D: Cancer Assessment of CRO (from NRC 2007)

A preliminary cancer assessment of crotonaldehyde was performed using data from Chung et al. (1986). In this study, male F344 rats were treated with 0, 0.6, or 6.0 mM of crotonaldehyde in their drinking water for 113 weeks. The high-dose group had approximately 10% lower body weight gain starting at week 8. The incidence of hepatic neoplastic nodules and hepatocellular carcinomas (combined) was 0/23, 11/27*, and 1/23 at 0, 0.6, and 6.0 mM, respectively (*$p < .01$; carcinoma: 0/23, 2/27, 0/23, respectively). The oral dose can be extrapolated to an air concentration that results in an equivalent human inhaled dose when assuming 100% lung absorption (NRC 1993). The extrapolation uses a rat intake of 2.06 mg of crotonaldehyde/day from the drinking water at the low dose (0.049 L/day (default) × 0.6 mmol/L × 70.09 g/mol crotonaldehyde), default body weights (BW) of 70 kg for humans and 0.35 kg for rats, and an inhalation rate of 20 m^3/day for humans. The calculation is performed as follows:

Human equivalent concentration =

$$\frac{2.06 \text{ mg crotonaldehyde/day} \times 70 \text{ kg body weight}}{20 \text{ m}^3 \text{ air/day} \times 0.35 \text{ kg of body weight}} = 20.6 \text{ mg/m}^3$$

This yields air concentrations of 20.6 mg/m^3 (7.2 ppm) and 206 mg/m^3 (72 ppm), respectively, for 0.6 and 6.0 mM crotonaldehyde in water. Using the linearized multistage model (GLOBAL86 program; Howe et al. 1986), the inhalation unit risk (or slope factor; i.e., q1*) was calculated to be 0.0327 per (mg/m^3). Note that the high dose was excluded from the unit risk calculation by the GLOBAL86 program due to lack of fit. For a lifetime theoretical cancer risk of 10^{-4}, crotonaldehyde air concentration is $10^{-4}/0.0327 \text{ (mg/m}^3)^{-1} = 3.06 \times 10^{-3}$ mg/m^3.

Appendix E: Dorman et al. (2008)

The following information describing the Dorman et al. (2008) study (the key study for the chronic ReV for acrolein) was taken from the Acrolein DSD (TCEQ 2014).

The key study, Dorman et al. (2008), exposed male F344 rats (whole-body exposure) to concentrations of 0, 0.02, 0.06, 0.2, 0.6, or 1.8 ppm acrolein (analytical concentrations) for 6 h/d, five d/wk for up to 65 exposure days (13 wk). Neither mortality nor a significant increase in incidence of observable clinical signs occurred following exposure to acrolein at any concentration. After 5-8 wk of exposure, the authors reported rats exposed to 0.06, 0.2, or 0.6 ppm developed significantly depressed (~3-5%) body weight gains compared to air-exposed controls after 5-8 wk of exposure. At 1.8 ppm, body weight gains were reduced by ~ 20 percent compared to air-exposed controls. Histopathology of the respiratory tract was evaluated after 4, 14, 30, and 65 exposure days and a 60-day recovery period after the 13-wk exposure period.

Nasal respiratory epithelial hyperplasia and squamous metaplasia were more sensitive endpoints, both with a NOAEL of 0.2 ppm and a minimal LOAEL of 0.6 ppm (minimal to slight/mild hyperplasia in the dorsal meatus and the lateral wall and squamous metaplasia in the septum and the larynx). In rats exposed to $\geq$0.6 ppm acrolein, mild/moderate respiratory epithelial hyperplasia was observed following 4 or more days of exposure. As the concentration of acrolein increased, more severe effects were observed. A higher NOAEL of 0.6 ppm and a LOAEL of 1.8 ppm were identified for olfactory epithelial inflammation and atrophy. Because hyperplasia and squamous metaplasia of the respiratory epithelium were associated with exposure to acrolein at lower concentrations than olfactory epithelium atrophy, they were considered the critical effects.

Dorman et al. (2008) examined animals 60 days following cessation of acrolein exposure: At the LOAEL of 0.6 ppm for nasal respiratory epithelial hyperplasia(Table 2 of Dorman et al. 2008), hyperplasia of the lateral wall (level II) and septum (level I) did not show recovery compared to air controls as shown below in Table 10.

Lack of Recovery for Nasal Respiratory Epithelial Hyperplasia at the LOAEL of 0.6 ppm (number of affected/number examined)

Exposure Day	4	14	30	65	+60 recovery
Lateral wall (level II)	12/12[a] (2.0)[b]	12/12[a] (1.0)[b]	12/12[a] (2.0)[b]	12/12[a] (1.0)[b]	11/12[a] (1.0)[b]
Septum (level I)	0/12	0/12	0/12	0/12	10/12[a] (2)[b]

[a] statistically significant increase in the incidence of the lesion was seen (versus air-exposed controls, $p < 0.05$, Pearson's)

[b] number in parentheses indicates average severity of the lesion seen in animals with a statistically significant lesion incidence. Unaffected animals were excluded from this calculation. 1= minimal, 2 = light/mild, 3 = moderate, 4= moderately severe

At the LOAEL of 1.8 ppm for olfactory epithelial atrophy (Table 4 of Dorman et al. 2008), they found partial recovery of the olfactory epithelium and stated, "Areas where recovery occurred were generally the more caudal regions of the nose where lesions developed more slowly." They further state, "…subchronic exposure to relatively high levels (1.8 ppm) of acrolein inhibited regeneration of the olfactory epithelium. It remains unknown whether the remainder of the olfactory epithelium would recover over time."

The Dorman et al. (2008) study was selected as the key study because it investigated both duration and concentration effects including several exposure groups, evaluated recovery, evaluated histopathology of the respiratory tract, and identified both a LOAEL and NOAEL. The critical effects are minimal to light/mild nasal respiratory epithelial hyperplasia in areas that did not show signs of recovery (i.e., lateral wall (level II) and septum (level I)).

The POD identified from the key study was the NOAEL of 0.2 ppm for nonreversible hyperplasia of nasal respiratory epithelial (Dorman et al. 2008). These effects were not amenable to benchmark dose modeling because incidences were either 0% at lower concentrations or 100% at the LOAEL and above.