



Development Support Document  
Proposed, March 2014

## **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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TEXAS COMMISSION ON ENVIRONMENTAL QUALITY

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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
° C	degrees Celsius centigrade
BMR	benchmark response
bw	body weight
ConA	Concanavalin A
CRO	crotonaldehyde
DSD	development support document
EC <sub>50</sub>	Effective concentration at a 50% response level
ESL	Effects Screening Level
<sup>acute</sup> ESL	acute health-based Effects Screening Level for chemicals meeting minimum database requirements
<sup>acute</sup> ESL <sub>generic</sub>	acute health-based Effects Screening Level for chemicals not meeting minimum database requirements
<sup>acute</sup> ESL <sub>odor</sub>	acute odor-based Effects Screening Level
<sup>acute</sup> ESL <sub>veg</sub>	acute vegetation-based Effects Screening Level
<sup>chronic</sup> ESL <sub>threshold(c)</sub>	chronic health-based Effects Screening Level for threshold dose response cancer effect
<sup>chronic</sup> ESL <sub>threshold(nc)</sub>	chronic health-based Effects Screening Level for threshold dose response noncancer effects
<sup>chronic</sup> ESL <sub>nonthreshold(c)</sub>	chronic health-based Effects Screening Level for nonthreshold dose response cancer effects
<sup>chronic</sup> ESL <sub>nonthreshold(nc)</sub>	chronic health-based Effects Screening Level for nonthreshold dose response noncancer effects
<sup>chronic</sup> ESL <sub>veg</sub>	chronic vegetation-based Effects Screening Level

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**Acronyms and Abbreviations Definition**


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<sup>chronic</sup> ESL <sub>generic</sub>	chronic health-based Effects Screening Level for chemicals not meeting minimum database requirements
GC	gas chromatography
h	hour
H <sub>b/g</sub>	blood:gas partition coefficient
(H <sub>b/g</sub> ) <sub>A</sub>	blood:gas partition coefficient, animal
(H <sub>b/g</sub> ) <sub>H</sub>	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 <sub>50</sub>	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
LC <sub>50</sub>	concentration causing lethality in 50% of test animals
LD <sub>50</sub>	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
µg/m <sup>3</sup>	micrograms per cubic meter of air
mg	milligrams
mg/m <sup>3</sup>	milligrams per cubic meter of air
min	minute

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<b>Acronyms and Abbreviations Definition</b>	
MOA	mode of action
n	number
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
PBPK	physiologically based pharmacokinetic
POD	point of departure
POD <sub>ADJ</sub>	point of departure adjusted for exposure duration
POD <sub>HEC</sub>	point of departure adjusted for human equivalent concentration
ppb	parts per billion
ppm	parts per million
RD <sub>50</sub>	50% reduction in respiration rate
ReV	reference value
ROS	reactive oxygen species
RP <sub>GM</sub>	geometric mean of relative potency
SA	surface area
SCOEL	Scientific Committee on Occupational Exposure Limits
SD	Sprague-Dawley
TCEQ	Texas Commission on Environmental Quality
TD	Toxicology Division
UF	uncertainty factor
UF <sub>H</sub>	interindividual or intraspecies human uncertainty factor
UF <sub>A</sub>	animal to human uncertainty factor
UF <sub>Sub</sub>	subchronic to chronic exposure uncertainty factor
UF <sub>L</sub>	LOAEL to NOAEL uncertainty factor

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**Acronyms and Abbreviations Definition**

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UF <sub>D</sub>	incomplete database uncertainty factor
USEPA	United States Environmental Protection Agency
V <sub>E</sub>	minute volume

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1 **Chapter 1 Summary Tables**

2 Table 1 for air monitoring and Table 2 for air permitting provide a summary of health- and  
3 welfare-based values from an acute and chronic evaluation of crotonaldehyde (CRO). Please  
4 refer to Section 1.6.2 of the *TCEQ Guidelines to Develop Toxicity Factors* (TCEQ 2012) for an  
5 explanation of air monitoring comparison values (AMCVs), reference values (ReVs) and effects  
6 screening levels (ESLs) used for review of ambient air monitoring data and air permitting. Table  
7 3 provides summary information on CRO's physical/chemical data.

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

Short-Term Values	Concentration	Notes
Acute ReV	<b>Short-Term Health</b> <b>29 µg/m<sup>3</sup> (10 ppb)</b>	<b>Critical Effect:</b> minor eye irritation in occupational workers
<sup>acute</sup> ESL <sub>odor</sub>	<b>Odor</b> 66 µg/m <sup>3</sup> (23 ppb)	50% detection threshold
<sup>acute</sup> ESL <sub>veg</sub>	- - -	No data on vegetative effect levels; concentrations producing no observed effects were significantly above other values
Long-Term Values	Concentration	Notes
ReV	- - -	Chronic toxicity studies were not available, so a <sup>chronic</sup> ESL <sub>generic</sub> was developed
<sup>chronic</sup> ESL <sub>generic</sub>	<b>Long-Term Health</b> <b>3.1 µg/m<sup>3</sup> (1.1 ppb)<sup>a</sup></b>	<b>Critical Effect(s):</b> Relative potency approach using acrolein: mild hyperplasia and lack of recovery of the respiratory epithelium in Fisher 344 rats
<sup>chronic</sup> ESL <sub>nonthreshold(c)</sub> <sup>chronic</sup> ESL <sub>threshold(c)</sub>	- - -	Data are inadequate for an assessment of human carcinogenic potential via the inhalation route
<sup>chronic</sup> ESL <sub>veg</sub>	- - -	No data found

9 <sup>a</sup> Based on the <sup>chronic</sup>ESL<sub>threshold(nc)</sub> for acrolein of 0.36 ppb multiplied by the geometric mean of the relative  
10 potency (RP<sub>GM</sub>) of 2.96

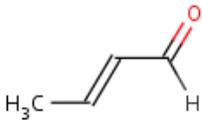
1 **Table 2. Air Permitting Effects Screening Levels (ESLs)**

<b>Short-Term Values</b>	<b>Concentration</b>	<b>Notes</b>
<sup>acute</sup> ESL [1 h] (HQ = 0.3)	<b>Short-Term ESL for Air Permit Reviews</b> <b>8.6 µg/m<sup>3</sup> (3 ppb)</b> <sup>a</sup>	<b>Critical Effect:</b> minor eye irritation in occupational workers
<sup>acute</sup> ESL <sub>odor</sub>	66 µg/m <sup>3</sup> (23 ppb)	50% detection threshold
<sup>acute</sup> ESL <sub>veg</sub>	---	Concentrations producing vegetative effects were significantly above other ESLs
<b>Long-Term Values</b>	<b>Concentration</b>	<b>Notes</b>
<sup>chronic</sup> ESL <sub>generic</sub>	<b>Long-Term ESL for Air Permit Reviews</b> <b>3.1 µg/m<sup>3</sup> (1.1 ppb)</b> <sup>b</sup>	<b>Critical Effect(s):</b> Relative Potency approach using acrolein: mild hyperplasia and lack of recovery of the respiratory epithelium in Fisher 344 rats
<sup>chronic</sup> ESL <sub>nonthreshold(c)</sub> <sup>chronic</sup> ESL <sub>threshold(c)</sub>	---	Data are inadequate for an assessment of human carcinogenic potential via the inhalation route
<sup>chronic</sup> ESL <sub>veg</sub>	---	No data found

2 <sup>a</sup> Based on the acute ReV of 29 µg/m<sup>3</sup> (10 ppb) multiplied by 0.3 to account for cumulative and aggregate  
3 risk during the air permit review.

4 <sup>b</sup> Based on the <sup>chronic</sup>ESL<sub>threshold(nc)</sub> for acrolein of 0.36 ppb multiplied by a RP<sub>GM</sub> of 2.96  
5

1 **Table 3. Chemical and Physical Data**

Parameter	Value	Reference
Chemical Structure		ChemID Plus (2013)
Molecular Formula	CH <sub>3</sub> CH = 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 <i>trans</i> and <i>cis</i> isomers CAS 123-73-9 ( <i>trans</i> isomer); CAS 15798-64-8 ( <i>cis</i> 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 <sub>ow</sub>	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 <sup>3</sup> 1 mg/m <sup>3</sup> = 0.349 ppm	NRC (2007)

## 1 **Chapter 2 Major Sources or Uses and Ambient Air Concentrations**

### 2 ***2.1 Major Sources or Uses***

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

4 Human exposure to crotonaldehyde occurs from both man-made and natural sources.  
5 Crotonaldehyde has been identified in exhaust from jet, gasoline; and diesel engines;  
6 from tobacco smoke; and from the combustion of polymers and wood (IARC 1995).  
7 Crotonaldehyde occurs naturally in meat, fish, many fruits (apples, grapes, strawberries,  
8 tomatoes) and vegetables (cabbage, cauliflower, Brussels sprouts, carrots), bread,  
9 cheese, milk, beer, wine, and liquors (IARC 1995). It is emitted from volcanoes, from  
10 the Chinese arbor vitae plant, and from pine and deciduous forests (IARC 1995; HSDB  
11 2005). Crotonaldehyde has been detected in drinking water, wastewater, human milk,  
12 and expired air from nonsmokers.

13 Crotonaldehyde is a very flammable liquid (Budavari et al. 1996). It is manufactured  
14 commercially by adding aldol to a boiling dilute acid solution and removing the crotonaldehyde  
15 by distillation. Crotonaldehyde is used primarily for the production of sorbic acid; it is also  
16 used for the synthesis of butyl alcohol, butyraldehyde, quinaldine, thiophenes, pyridenes, dyes,  
17 pesticides, pharmaceuticals, rubber antioxidants, and chemical warfare agents and as a warning  
18 agent in locating breaks and leaks in pipes (IARC 1995, Budavari et al. 1996; Verschueren  
19 1996). Crotonaldehyde degrades in the atmosphere by reacting with photochemically produced  
20 hydroxyl radicals (half-life of about 11 h) or ozone (half-life of about 15.5 days; HSDB 2005).

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

25 Recent U.S. production data are not available. U.S. production of crotonaldehyde in 1975 was  
26 > 2,000 pounds, and about 463 pounds was imported into the United States in 1984 (HSDB  
27 2005).

### 28 ***2.2 Background Levels of Crotonaldehyde in Ambient Air***

29 There are six locations in Texas that monitor for CRO using 24-h canister samplers that collect  
30 samples every 6<sup>th</sup> day. The 2011 annual average concentration for CRO at these sites ranged  
31 from 0.04 to 0.08  $\mu\text{g}/\text{m}^3$ . IPCS (2008) provides measured environmental levels for CRO for the  
32 United States (refer to IPCS 2008 for references):

- 33 • Average concentrations in 1983 in the direct vicinity of a United States highway in rush  
34 hour traffic (1 meter from the roadside at a height of 1.5 meters) were 1.1 to 2.1  $\mu\text{g}/\text{m}^3$ .

- 1       • The concentrations measured in air samples at the Tuscarora Mountain tunnel in  
2       Pennsylvania ranged from 0.12 to 0.44  $\mu\text{g}/\text{m}^3$ , whereas at the Caldecott tunnel near San  
3       Francisco, the range was 0.12 up to 0.76  $\mu\text{g}/\text{m}^3$ .
  
- 4       • The mean concentration detected in ambient air at the Oakland-San Francisco Bay Bridge  
5       toll booth plaza ranged from 0.061 to 0.147  $\mu\text{g}/\text{m}^3$ .
  
- 6       • The mean concentrations detected outside of 87 residences in Elizabeth, New Jersey,  
7       throughout 1999 – 2001 were as follows: 0.2  $\mu\text{g}/\text{m}^3$  (spring), 0.5  $\mu\text{g}/\text{m}^3$  (summer), 0.3  
8        $\mu\text{g}/\text{m}^3$  (autumn), and 0.4  $\mu\text{g}/\text{m}^3$  (winter).

## 9       **Chapter 3 Acute Evaluation**

### 10      **3.1 Health-Based Acute ReV**

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

14      CRO is a reactive compound and can cause eye, skin, and respiratory irritation. When high  
15      enough concentrations are inhaled for a sufficient duration, CRO can cause a burning sensation  
16      in the nasal and upper respiratory tract, lacrimation, coughing, bronchoconstriction, pulmonary  
17      edema, and deep lung damage (NRC 2007). Since CRO has strong odorous and irritative  
18      properties, exposure to higher concentrations may be limited, thereby avoiding other toxic effects  
19      (Henschler 1981).

#### 20      **3.1.1 Physical/Chemical Properties**

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

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

## 1 **3.1.2 Key Studies**

### 2 **3.1.2.1 Key Human Study (Fannick 1982)**

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

7 NIOSH conducted a Health Hazard Evaluation in a chemical plant (Sandoz Colors and  
8 Chemicals) in East Hanover, New Jersey, at the request of workers at the plant, some of  
9 whom complained of occasional minor eye irritation (Fannick 1982). NIOSH measured  
10 crotonaldehyde air concentrations using midjet impingers; analysis was performed  
11 using gas chromatography with flame ionization detection. Eight air samplers were  
12 placed near the vats of chemicals and two were worn by the NIOSH industrial  
13 hygienist, who was near the vats most of the time. These measurements likely  
14 overestimated the actual exposure concentrations because workers were allowed to  
15 move about and were not near the vats during an entire 8-h work shift. NIOSH  
16 determined that the average crotonaldehyde concentration of general air samples was  
17  $1.6 \text{ mg/m}^3$  (0.56 ppm; range, <0.35 to 1.1 ppm; 0.35 ppm was the limit of quantitation).  
18 The two personal samples were 0.66 and 0.73 ppm. These workers were also  
19 simultaneously exposed to acetic acid and small amounts of acetaldehyde (which  
20 occasionally caused a perceptible sweet odor), 3-hydroxybutyraldehyde, and  
21 dimethoxane.

22 Crotonaldehyde was probably the most potent irritant among these chemicals, based on  
23 its greater quantity and its much lower  $RD_{50}$  (reference dose—the concentration that  
24 decreases the respiration rate of mice by 50% due to respiratory irritation [Schaper,  
25 1993; Fannick 1982]).

### 26 **3.1.2.2 Supporting Human Studies**

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

1 **Table 4. Human CRO Inhalation Toxicity<sup>a</sup>**

<b>Exposure Concentration</b>	<b>Exposure Time</b>	<b>End Point and Confounding Factors</b>	<b>Reference</b>
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) <sup>b</sup>
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)

2 <sup>a</sup> Table reproduced from NRC (2007). Appendix A contains descriptions of these supporting studies taken  
3 from NRC (2007).

4 <sup>b</sup> Key study

1 **3.1.2.3 Supporting Animal Studies**

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

6 Table 5 is a summary of animal CRO exposure data taken from NRC (2007) arranged in order  
7 from adverse effects observed at low concentrations to higher concentrations. Appendix B  
8 contains a discussion of these supporting animal studies obtained from NRC (2007).

9 Concentrations that produced irritation in humans (Table 4) were similar to concentrations  
10 causing irritation in animals (Table 5). NRC (2007) stated “LC<sub>50</sub> values for several species  
11 varied by a factor of  $\leq 2.5$  for several exposure durations, indicating that interspecies variability  
12 was minor.”

1 **Table 5. Animal CRO Inhalation Toxicity <sup>a</sup>**

<b>Exposure Concentrations (Species)</b>	<b>Exposure Time</b>	<b>Relevant Values</b>	<b>End Point (Reference)</b>
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 <sub>50</sub> = 3.53 ppm RD <sub>50</sub> = 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 <sub>50</sub> = 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 <sup>3</sup> [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 ≥ 2,000 ppm-min <sup>b</sup>	Decrease in carbon monoxide or ether absorption (indicates reduced pulmonary ventilation rate) Rinehart (1967)

2 <sup>a</sup> Appendix B contains a discussion of these supporting animal studies obtained from NRC (2007).

3 <sup>b</sup> The individual concentrations and exposure times were not given. Test responses were presented for five  
4 ranges of concentration times time (C x T) due to variations found among animals within any given  
5 exposure scenario.

#### 6 **3.1.2.4 Reproductive/Developmental Studies**

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

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

3 Jha and Kumar (2006) and Jha et al. (2007) investigated the genotoxicity of CRO in Swiss albino  
4 mice treated with CRO via IP injection at 8, 16, and 32  $\mu\text{l}/\text{kg}$  body weight (bw). These doses  
5 correspond to inhalation concentrations of 2.4, 4.7, and 9.5 ppm, respectively, using route to  
6 route extrapolation (TCEQ 2012). This assumes 100% absorption from the respiratory tract, a  
7 conservative assumption. These concentrations are above those that cause sensory and  
8 respiratory irritation in humans after inhalation exposure (Table 4). Although these studies were  
9 designed to investigate genotoxicity through an exposure route which does not consider  
10 pulmonary absorption, they indicate that if CRO reaches germ cells, it may affect fertility due to  
11 its DNA reactivity. Bone marrow cells could also be affected.

12 The study conducted by Jha and Kumar (2006) reported abnormal sperm morphology after  
13 exposure to CRO, indicative of germ cell mutagens. The effect reached statistical significance  
14 one and three weeks after exposure at doses of 16 and 32  $\mu\text{l}/\text{kg}$  bw and 5 weeks after exposure at  
15 the highest dose of 32  $\mu\text{l}/\text{kg}$ .

16 In the study conducted by Jha et al. (2007), single IP doses of CRO at 8, 16 and 32  $\mu\text{l}/\text{kg}$  bw in  
17 olive oil caused the following:

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

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

### 31 **3.1.3 Mode of Action (MOA) Analysis**

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

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

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

### 9 **3.1.4 Dose Metric**

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

### 13 **3.1.5 POD for the Key Study and Dosimetric Adjustments**

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

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

### 20 **3.1.6 Adjustments of the $POD_{HEC}$**

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

- 22 • A  $UF_H$  of 3 for intraspecies variability was used because the critical effect is minor eye  
23 irritation (i.e., stimulation of the trigeminal nerve), and toxicokinetic differences  
24 between humans would be minimal (even sensitive subpopulations such as children).  
25 However, toxicodynamic differences need to be accounted for.
- 26 • A  $UF_L$  of 3 for the uncertainty of extrapolating from the minimal LOAEL rather than a  
27 NOAEL. The LOAEL observed in the key study was based on minor eye irritation.
- 28 • A  $UF_D$  of 6 was used because the database is limited due to the lack of high quality  
29 human or animal studies. However, the dose-response health effects for CRO from  
30 low concentration to high concentrations were defined qualitatively in human and in  
31 different animal species (mice, rats, and rabbits). There were no human or animal  
32 reproductive/ developmental studies, but since CRO is a water soluble, reactive  
33 compound, it is scrubbed efficiently in the upper respiratory tract. Systemic absorption

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

- 3 • The total UF = 54

$$\begin{aligned} 4 \quad \text{acute ReV} &= \text{POD}_{\text{HEC}} / (\text{UF}_{\text{H}} \times \text{UF}_{\text{L}} \times \text{UF}_{\text{D}}) \\ 5 &= 560 \text{ ppb} / (3 \times 3 \times 6) \\ 6 &= 560 \text{ ppb} / 54 \\ 7 &= 10.37 \text{ ppb} \\ 8 &= 10 \text{ ppb (rounded to two significant figures)} \end{aligned}$$

### 9 **3.1.7 Health-Based Acute ReV**

10 In deriving the acute ReV, no numbers were rounded between equations until the ReV was  
11 calculated. Once the ReV was calculated, it was rounded to two significant figures. The rounded  
12 ReV of 10 ppb ( $29 \mu\text{g}/\text{m}^3$ ) was then used to calculate the ESL. The <sup>acute</sup>ESL of 3 ppb ( $8.6 \mu\text{g}/\text{m}^3$ )  
13 is based on the acute ReV multiplied by a HQ of 0.3, then rounded to two significant figures at  
14 the end of all calculations (Table 6).

15

1 **Table 6. Derivation of the Acute ReV and <sup>acute</sup>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 <sup>a</sup>
POD <sub>ADJ</sub>	560 ppb (no adjustment – effects were concentration dependent)
POD <sub>HEC</sub>	560 ppb <sup>a</sup>
Total uncertainty factors (UFs)	54
<i>Interspecies UF</i>	3
<i>Intraspecies UF</i>	Not applicable
<i>LOAEL-to-NOAEL UF</i>	3
<i>Incomplete Database UF</i>	6
<i>Database Quality</i>	Medium
<b>Acute ReV [1 h] (HQ = 1)</b>	<b>29 µg/m<sup>3</sup> (10 ppb)</b>
<b><sup>acute</sup>ESL [1 h] (HQ = 0.3)</b>	<b>8.6 µg/m<sup>3</sup> (3 ppb)</b>

2 <sup>a</sup> Inhalation observed adverse effect level (Section 3.4)

3 **3.2 Welfare-Based Acute ESLs**

4 **3.2.1 Odor Perception**

5 NRC (2007) reviewed the odor detection data for CRO (Appendix A). As shown in Table 5,  
6 there have been a wide range of concentrations reported for human odor detection for CRO (i.e.,  
7 0.035 to 0.2 ppm), in some cases due to analytical measurement errors (Verschuieren 1996; Ruth  
8 1986; Amooore and Hautala 1983; Tepikina et al. 1997 Trofimov 1962) (Appendix A).

1 However, only one study listed as a Level 1 acceptable source (highest quality level of odor  
2 thresholds) for odor threshold values in the guidelines (TCEQ 2012) was identified. A 50% odor  
3 detection threshold value of  $65.9 \mu\text{g}/\text{m}^3$  (23 ppb) was reported for CRO by Nagata (2003)  
4 utilizing the Japanese triangular odor bag method. The CRO  $^{\text{acute}}\text{ESL}_{\text{odor}}$  was set at the 50% odor  
5 detection threshold of  $66 \mu\text{g}/\text{m}^3$  (23 ppb) determined by Nagata (2003).

### 6 **3.2.2 Vegetation Effects**

7 CRO has been used as a fungicide, with effective concentrations at the 50% response level  
8 ( $\text{EC}_{50\text{s}}$ ) from one experiment being reported as  $80 \text{ mg}/\text{m}^3$ . In this experiment, the host plants,  
9 wheat and barley, had  $\text{EC}_{50\text{s}}$  of  $400 \text{ mg}/\text{m}^3$  (i.e., the parasitic fungi were about 5 times more  
10 sensitive). Bean, tomato, cucumber, and begonia were reported to be more sensitive, but no  
11 details were provided (reported in IPCS 2008). Exposure of plants to CRO at a concentration of  
12 1 ppm did not cause any damage to the leaves of the following plants: 10-day-old oat seedlings  
13 and 30-day-old alfalfa, endive, sugar beet, and spinach plants (Haagen-Smit et al. 1952). Since  
14 concentrations producing vegetative effects (approximately  $> 1 \text{ ppm}$ ) are significantly above  
15 other health- and odor-based concentrations and a LOAEL for vegetative effects was not  
16 identified, an  $^{\text{acute}}\text{ESL}_{\text{veg}}$  was not developed for CRO.

### 17 **3.3 Short-Term ESL and Values for Air Monitoring Data Evaluations**

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

- 19 • acute ReV =  $29 \mu\text{g}/\text{m}^3$  (10 ppb)
- 20 •  $^{\text{acute}}\text{ESL} = 8.6 \mu\text{g}/\text{m}^3$  (3 ppb)
- 21 •  $^{\text{acute}}\text{ESL}_{\text{odor}} = 66 \mu\text{g}/\text{m}^3$  (23 ppb)

22 The short-term ESL for air permit evaluations is the  $^{\text{acute}}\text{ESL}$  of  $8.6 \mu\text{g}/\text{m}^3$  (3 ppb) (Table 2). For  
23 evaluation of ambient air monitoring data, the acute ReV of  $29 \mu\text{g}/\text{m}^3$  (10 ppb) is lower than the  
24  $^{\text{acute}}\text{ESL}_{\text{odor}}$  of  $66 \mu\text{g}/\text{m}^3$  (23 ppb), although both values will be used for the evaluation of ambient  
25 air monitoring data (Table 1).

### 26 **3.4 Acute Inhalation Observed Adverse Effect Level**

27 The acute inhalation observed adverse effect level would be the LOAEL from the key human  
28 study of  $1,600 \mu\text{g}/\text{m}^3$  (560 ppb). The  $\text{LOAEL}_{\text{HEC}}$  determined from human studies, where eye  
29 irritation occurred in some individuals, represents a concentration at which it is probable that  
30 similar effects could occur in some individuals exposed to this level over the same or longer  
31 durations as those used in the study. Importantly, effects are not a certainty due to potential  
32 intraspecies differences in sensitivity. The inhalation observed adverse effect level is provided  
33 for informational purposes only (TCEQ 2012). As the basis for development of inhalation  
34 observed adverse effect levels is limited to available data, future studies could possibly identify a  
35 lower POD for this purpose.

1 The margin of exposure between the observed adverse effect level and the ReV is a factor of 54.  
2 There is uncertainty in this value because the quality of the key study was low, although other  
3 human studies and animal studies reported irritation at higher concentrations (Tables 4 and 5).

### 4 ***3.5 Evaluation of Toxicity Assessment***

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

- 12 • CRO had a range of 3-4 fold higher for the concentration causing 50% depression in  
13 respiration (RD<sub>50</sub>) values (Babiuk et al. 1985; Steinhagen and Barrow (1984)
- 14 • CRO was approximately 13-fold higher for an inhalation LC<sub>50</sub> value (Skog 1950)
- 15 • CRO had a range of 3-5 fold higher for subcutaneous LD<sub>50</sub> values (Skog 1950).

16 Generally, CRO was less toxic than formaldehyde, except for mouse RD<sub>50</sub> values (Steinhagen  
17 and Barrow 1984) and LC<sub>50</sub> studies (Skog 1950).

18

1 **Table 7. Comparison of Acute Sensory and Lethality Data**

Test	Rank Order	Value	Reference
50% odor detection threshold humans	Acrolein CRO Formaldehyde	8.2 $\mu\text{g}/\text{m}^3$ (3.6 ppb) 66 $\mu\text{g}/\text{m}^3$ (23 ppb) 620 $\mu\text{g}/\text{m}^3$ (500 ppb)	Nagata (2003)
RD <sub>50</sub> Fisher-344 rats	Acrolein CRO Formaldehyde	6 ppm 23.2 ppm Not available	Babiuk et al. (1985) <sup>a</sup>
RD <sub>50</sub> Male Swiss-Webster and B6C3F1 mice	Acrolein CRO Formaldehyde	1.41 ppm 4.88 ppm 4.90 ppm	Steinhagen and Barrow (1984) <sup>a</sup>
LC <sub>50</sub> Rat	Acrolein CRO formaldehyde	0.3 mg/L 4 mg/L 1 mg/L	Skog (1950) <sup>a</sup>
LD <sub>50</sub> Rat (subcutaneous injection)	Acrolein CR Formaldehyde	0.05 g/kg 0.14 g/kg 0.42 g/kg	Skog (1950) <sup>a</sup>
LD <sub>50</sub> Mouse (subcutaneous injection)	Acrolein CRO Formaldehyde	0.03 g/kg 0.16 g/kg 0.30 g/kg	Skog (1950) <sup>a</sup>

2 <sup>a</sup> See Appendix B for a description on the Babiuk et al. (1985) and Steinhagen and Barrow (1984) studies and  
3 Appendix C for a description of the Skog (1950) study  
4

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

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

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

## 9 Chapter 4 Chronic Evaluation

### 10 *4.1 Noncarcinogenic Potential – Relative Potency Approach*

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

17 Wolfe et al. (1987) conducted oral toxicity studies in rats and mice. Wolfe et al. (1987) treated  
 18 ten male and ten female F344 rats per dose group with CRO via oral gavage in corn oil to 2.5, 5,  
 19 10, 20, or 40  $\text{mg}/\text{kg}$  body weight for 13 weeks. They also treated ten male and female B6C3F1  
 20 mice with oral doses of 2.5, 5, 10, 20, or 40  $\text{mg}/\text{kg}$  body weight for 13 weeks. Body weight  
 21 decreases occurred in rats at 40  $\text{mg}/\text{kg}$ . Both rats and mice displayed mainly point-of-entry  
 22 effects at 20 or 40  $\text{mg}/\text{kg}$  (hyperplasia of the epithelia lining of the stomach, forestomach  
 23 hyperkeratosis, ulcers, etc.). Refer to IPCS (2008) for additional information. Because CRO is a  
 24 highly reactive compound and causes point-of-entry effects, route-to-route extrapolation using  
 25 the Wolfe et al. (1987) study was not conducted (TCEQ 2012).

26 Since CRO has limited chronic toxicity data (LTD), a relative potency approach was followed to  
 27 determine a chronic generic ESL (i.e., <sup>chronic</sup>ESL<sub>generic</sub> Tier III generic approach (TCEQ 2012)).  
 28 Relative potency can be defined as a procedure to estimate the “toxicity” of a LTD chemical in

1 relation to a reference or an index chemical(s) for which toxicity has been well defined. The  
2 concept of relative potency has been used to derive toxicity values for PAHs with limited  
3 toxicity information based on the toxicity information of benzo[a]pyrene, for which there is a  
4 wealth of information (Collins et al. 1998). The following procedures outlined in TCEQ (2012)  
5 can be employed when similar chemical categories or an analog chemical approach is used:

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

#### 18 **4.1.1 Identify Potential Index Chemical(s)**

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

- 22 • the TCEQ has developed toxicity factors for acrolein (TCEQ 2014);
- 23 • there are numerous studies that compare the toxicity of acrolein and CRO within the  
24 same study for relevant endpoints, although the health effects database for acrolein is  
25 more extensive than the database for CRO (Section 4.1.2.2);
- 26 • they have similar MOAs (Section 4.1.3);
- 27 • they have similar physical-chemical parameters (Section 4.1.2.1);
- 28 • they have similar structures and reactivity. Both are  $\alpha,\beta$ -unsaturated carbonyl compounds.
- 29 • They both produce similar acute adverse health effects in humans (i.e., sensory irritation  
30 to the eye and respiratory tract) and animal studies (respiratory tract effects) (NRC 2007,

1           2010). It is unknown whether chronic health effects for acrolein and CRO are similar  
2           because chronic inhalation studies for CRO are not available. However, similar chronic  
3           effects would be expected based on their similar MOAs.

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

## 12          **4.1.2 Toxicity Data for Acrolein and CRO**

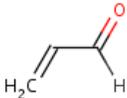
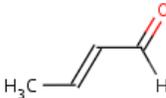
13          The TD gathered data on physical/chemical properties, toxicity, etc., for the potential index  
14          chemical(s) and the LTD chemical.

### 15          ***4.1.2.1 Physical/Chemical Properties***

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

23

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

Parameter	Acrolein <sup>a</sup>	CRO	Formaldehyde <sup>b</sup>
Chemical Structure			
Molecular Formula	CH <sub>2</sub> =CH-CHO ATSDR (2007)	CH <sub>3</sub> -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 <sub>ow</sub>	-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 <sup>3</sup> 1 mg/m <sup>3</sup> = 0.44 ppm Toxicology Staff	1 ppm = 2.87 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.349 ppm NRC (2007)	1 ppm = 1.23 mg/m <sup>3</sup> 1 mg/m <sup>3</sup> = 0.813 ppm ATSDR (1999)

2 <sup>a</sup> Refer to the Acrolein DSD (TCEQ 2014) for references for acrolein's physical/chemical  
3 parameters

4 <sup>b</sup> Refer to the Formaldehyde DSD (TCEQ 2008) for references for formaldehyde's  
5 physical/chemical parameters

## 1 **4.1.2.2 Toxicity Studies Evaluating both Acrolein and CRO**

### 2 **4.1.2.2.1 In Vivo Studies**

3 The only available *in vivo* toxicity studies that evaluated CRO and acrolein in the same study via  
4 inhalation using similar methods are acute studies that determined RD<sub>50</sub> and LC<sub>50</sub> data (Table 7).  
5 Acrolein was consistently more toxic than CRO.

#### 6 **4.1.2.2.1.1 RD<sub>50</sub> Data**

7 RD<sub>50</sub> data for CRO was 3-4 fold higher than acrolein in both rats and mice (Babiuk et al. 1985;  
8 Steinhagen and Barrow (1984) (Table 7)

#### 9 **4.1.2.2.1.2 LC<sub>50</sub> Data**

10 Skog (1950) determined 30-min LC<sub>50</sub> data for CRO of 1400 ppm and for acrolein of 131 ppm  
11 (nominal concentrations), a CRO to acrolein ratio of 13. Rinehart (1967) also determined a 30-  
12 min LC<sub>50</sub> for CRO of 593 ppm (analytical concentrations). The Rinehart (1967) LC<sub>50</sub> data was  
13 about 2-fold lower than that obtained by Skog. Rinehart suggested this difference may have been  
14 due to a loss of CRO between the point of vapor generation and the animal breathing zone. If the  
15 30-min LC<sub>50</sub> value determined by Rinehart for CRO was used to calculate the ratio of CRO to  
16 acrolein, the ratio would be 4.53. There are two other LC<sub>50</sub> studies in rats available for acrolein  
17 and CRO for similar exposure durations (i.e., 10 min and 4 h), although the CRO LC<sub>50</sub> and the  
18 acrolein LC<sub>50</sub> were determined by different researchers:

- 19 • the 10 min LC<sub>50</sub> for CRO was 1480 ppm (Rinehart (1967) and for acrolein it was 374  
20 ppm (Catalina et al. 1966), a ratio of CRO to acrolein of 3.95.
- 21 • the 4-h LC<sub>50</sub> for CRO was 70 ppm (Voronii et al. 1982) to 88 ppm (Rinehart 1967)  
22 whereas the 4-h LC<sub>50</sub> for acrolein was 8 ppm (Carpenter et al. (1949). The ratio of CRO  
23 to acrolein ranged from 8.75-11.

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

### 30 **4.1.2.2.2 In Vitro Studies**

#### 31 **4.1.2.2.2.1 Meacher and Menzel (1999)**

32 Meacher and Menzel (1999) conducted *in vitro* studies in adult rat lung cells to compare the  
33 effective aldehyde concentration that depleted GSH by 50% (EC<sub>50</sub>). Cells were treated for 20  
34 min with a range of aldehyde concentrations and then glutathione levels were evaluated using

1 glutathione-monochlorobimane fluorescence intensity measured using laser cytometry. Results  
2 were reported only for aldehyde concentrations that caused no changes in cell morphology as  
3 observed by phase-contrast microscopy. One of the proposed MOAs for aldehydes, especially  
4 acrolein and CRO, is depletion of cellular GSH, leading to oxidative stress and cellular damage  
5 (Section 4.3). An *in vitro* assay that ranks GSH depletion may be used to rank the potency of  
6 aldehydes within a class.

7 The EC<sub>50</sub>s for the n-alkanals (formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde)  
8 ranged from 110-400 mmol/L, approximately 1000 less potent when compared to the 2-alkenals,  
9 acrolein and CRO. Acrolein was the most potent 2-alkenal studied as it had the lowest EC<sub>50</sub>,  
10 followed by CRO

- 11 • Acrolein (2 µmol/L)
- 12 • CRO (130 µmol/L)
- 13 • trans-2-Hexenal (160 µmol/L)
- 14 • trans -2-pentenal (180 µmol/L).

15 *The ratio of EC<sub>50</sub>s for GSH depletion for CRO compared to acrolein was 65 (Meacher and*  
16 *Menzel 1999).*

#### 17 **4.1.2.2.2 Moretto et al. 2009**

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

23 Acrolein or CRO (3–60 µM) concentration-dependently stimulated the release of IL-8 from both  
24 SAEC and NHLF.

- 25 • In SAEC cultures, acrolein (171.7 ± 5.2% of basal release, n = 4) and CRO (195.5 ±  
26 6.2% of basal release, n = 4) elicited their maximal effect at 30 µM .
- 27 • In NHLF cells, acrolein elicited its maximal effect at 10 µM (258.4 ± 23.5% of basal  
28 release, n = 4) and CRO at 30 µM (202.1 ± 13.6% of basal release, n = 4).

29 Moretto et al. (2009) also evaluated cell viability using the MTT test in SAEC and NHLF cells  
30 (Table 10). There were no statistical differences in cell viability after treatment with acrolein and  
31 CRO compared to control SAEC cells (no statistical differences at concentrations of 3, 10, 30  
32 and 60 µM). However in NHLF cells, acrolein decreased cell viability at 60 µM whereas CRO  
33 did not.

1 **Table 10. Acrolein and CRO viability evaluated in NHLF cells (MTT Test <sup>a</sup>)**

Concentrations	3 $\mu$ M	10 $\mu$ M	30 $\mu$ M	60 $\mu$ M
acrolein	97 $\pm$ 3	99 $\pm$ 3	76 $\pm$ 6	25 $\pm$ 2 <sup>b</sup>
CRO	99 $\pm$ 1	98 $\pm$ 1	94 $\pm$ 2	91 $\pm$ 2

2 <sup>a</sup> MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide3 <sup>b</sup> Statistically different from control viability, P < 0.01.

4 *Cell viability was evaluated by percent decrease in absorbance in the MTT assay and showed the*  
5 *ratio of absorbance for CRO compared to acrolein at 60  $\mu$ M was a ratio of 3.64 (i.e., 91*  
6 *percent/25 percent) (Moretto et al. 2009).*

7 4.1.2.2.3.2.3 Poirier et al. 2002

8 Poirier et al. (2002) assessed thirteen chemicals present in tobacco smoke, including acrolein and  
9 CRO, for their effect on viability and proliferation of mouse lymphocytes *in vitro*. Cell viability  
10 was assessed with propidium iodide, with subsequent analyses by flow cytometry. For cell  
11 proliferation, control and treated cells were exposed to Concanavalin A (ConA), a T-cell  
12 mitogen, and lipopolysaccharide (LPS), a B-cell mitogen. After a 48-h incubation period, 0.5  $\mu$ Ci  
13 of [<sup>3</sup>H]methylthymidine was added to each well. The incubation was resumed for another 18 hr  
14 under the same conditions. Cells were then collected on filters and counted in a  $\beta$  counter.

15 For the viability assay, only acrolein and CRO induced a cytotoxic effect. The other 11  
16 compounds produced no cytotoxic effects on splenocytes. Both aldehydes produced a  
17 concentration- and time-dependent significant effect on splenocyte viability as determined by  
18 propidium iodide dye exclusion. At 10<sup>-5</sup> M and higher concentrations, the significant suppressive  
19 effect was already observed after 3 h of exposure. A longer incubation period with acrolein and  
20 CRO at the highest concentrations resulted in the death of almost all cells. The concentration  
21 causing 50% inhibition (IC<sub>50</sub>) for viability and the mitogenic assay after a 3-h exposure are  
22 shown in Table 11. Acrolein and CRO inhibited both T-cell and B-cell proliferation (Table 12).  
23 The antiproliferative effect of CRO and acrolein could partly be explained by their cytotoxic  
24 effects, with the IC<sub>50</sub> values for viability and the mitogenic assays being within the same range.

25

1 **Table 11. Comparison of IC<sub>50</sub> values for Acrolein and CRO (3-h Exposure)**

	IC <sub>50</sub> viability (M)	IC <sub>50</sub> ConA (M)	IC <sub>50</sub> LPS (M)
acrolein	2.70 x 10 <sup>-5</sup>	2.06 x 10 <sup>-5</sup>	3.16 x 10 <sup>-5</sup>
CRO	4.26 x 10 <sup>-5</sup>	2.01 x 10 <sup>-5</sup>	2.47 x 10 <sup>-5</sup>

2 *The ratio of IC<sub>50</sub> values for cell viability in splenocytes for CRO compared to acrolein was 1.58*  
3 *(Poirier et al. 2002).*

#### 4 **4.1.3 MOA Analysis**

5 A MOA analysis was performed to determine the relevant endpoints that can be used for a  
6 relative potency approach. Relevant endpoints for both acrolein and CRO should be closely tied  
7 to the expected critical effect for the index and LTD chemical and should be determined using  
8 similar testing techniques, exposure durations, and species. The critical effects are  
9 noncarcinogenic and assumed to have a threshold MOA.

##### 10 **4.1.3.1 CRO MOA**

11 Because CRO is an  $\alpha,\beta$ -unsaturated carbonyl, it is highly reactive. It reacts with cellular  
12 components and forms protein adducts and histone–DNA crosslinks (Kurtz & Lloyd, 2003).

13 The general metabolic pathway for aldehydes is oxidation by aldehyde dehydrogenase (ADH).  
14 However, the major detoxification pathway of CRO is with glutathione (GSH) to form  
15 glutathione conjugates.

16 Liu et al. (2010a) investigated the MOA for cell death in a human bronchial epithelial cell line  
17 (BEAS-2B cells) after exposure to CRO. CRO induced cytotoxicity through induction of cellular  
18 oxidative stress with the depletion of intracellular GSH and increase of reactive oxygen species.  
19 CRO caused both apoptosis and necrosis, and there was a transition from apoptosis to necrosis  
20 with increasing CRO concentrations. Apoptosis was mediated via cytochrome c release and  
21 caspases cascade. Liu et al. (2010a) could not rule out the possibility that CRO could induce  
22 apoptosis through another caspase-independent pathway, such as apoptosis-inducing factor.

23 In another study, Liu et al. (2010b) used microarray analysis to study the gene expression profile  
24 of BEAS-2B cells after exposure to CRO. Cytotoxicity and cell cycle arrest caused by CRO were  
25 also investigated in the study. This investigation showed that CRO at low doses caused BEAS-  
26 2B cells to undergo apoptosis, while high doses of CRO caused cells to undergo necrosis. A  
27 large number of inflammation responsive genes were suppressed by CRO, although HMOX1  
28 (antioxidant response) and ALDH1A3 (ADH metabolism) were induced in three treatments.  
29 Taken into account HMOX1 mediating cellular pathways and ALDH1A3 detoxifying toxicants,  
30 HMOX1 and ALDH1A3 were considered as novel transcriptional markers for CRO toxicity.

1 Moretto et al. (2009) showed that CRO produced proinflammatory cytokine production. Yang et  
2 al. (2013) showed that CRO is capable of directly stimulating the production of IL-8 in both  
3 macrophages and airway epithelial cells. CRO-stimulated macrophages also amplify the  
4 inflammatory response by enhancing IL-8 release from airway epithelial cells and can cause lung  
5 inflammatory response via multiple mechanisms.

#### 6 **4.1.3.2 Acrolein MOA (TCEQ 2014)**

7 The following information is in the Acrolein DSD (TCEQ 2014), but was obtained from NRC  
8 (2010):

9 Many of the effects of acrolein are caused by reaction with sulfhydryl groups. Acrolein is  
10 the most toxic of the 2-alkenals (including crotonaldehyde, pentenal, and hexenal) and is  
11 also the most reactive toward sulfhydryl groups. Deactivation of the cellular protein  
12 sulfhydryl groups could result in disruption of intermediary metabolism, inhibition of cell  
13 growth or division, and cell death. The respiratory irritancy of acrolein may be due to  
14 reactivity toward sulfhydryl groups in receptor proteins in the nasal mucosa (Beauchamp  
15 et al., 1985). Li et al. (1997) investigated the effects of acrolein on isolated human  
16 alveolar macrophage function and response *in vitro*. Acrolein induced dose-dependent  
17 cytotoxicity as evidenced by the induction of apoptosis and necrosis. At lower doses, the  
18 heme oxygenase protein was induced; however, stress protein was not induced. These  
19 data suggest that acrolein caused a dose-dependent selective induction of a stress  
20 response, apoptosis, and necrosis. Macrophage function was examined by cytokine  
21 release in response to acrolein exposure. Acrolein caused a dose-dependent inhibition of  
22 IL-1 $\beta$ , TNF- $\alpha$ , and IL-12 release.

#### 23 **4.1.3.3 Comparison of the MOA for Acrolein and CRO**

24 There are differences between the MOAs of acrolein and CRO involving mechanisms affecting  
25 apoptosis and necrosis as well as differences in gene expression profiles as discussed by Liu et  
26 al. (2010a, b). However, the primary mechanisms of toxicity are similar. Both CRO and acrolein  
27 are highly reactive and can induce toxicity in a variety of ways. An increase in reactive oxygen  
28 species resulting from reaction with and depletion of glutathione is considered to be the primary  
29 mechanism of toxicity. Reactions with cell membrane proteins and inhibition of regulatory  
30 proteins may also play a role.”

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

#### 34 **4.1.4 Matrix of Data and Pattern of Relative Toxicity**

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

- 1 • odor potential was not considered to be predictive of chronic adverse effects;
- 2 • LD<sub>50</sub> data was not considered relevant to predict inhalation exposure;
- 3 • Depletion of GSH as evaluated by Meacher and Menzel (1999), although an important  
4 step in the MOA, is an early event and may not lead to cytotoxicity, so this endpoint was  
5 not considered relevant;
- 6 • LC<sub>50</sub> data would be a relevant endpoint because the primary effect observed in animals in  
7 lethality studies was respiratory failure. However, LC<sub>50</sub> data were not used for CRO, as  
8 discussed in Section 4.1.2.2.1 *In Vivo Studies*.

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

- 12 • RD<sub>50</sub> values, although a measure of sensory irritation, were considered relevant for both  
13 acrolein and CRO, because exposed animals likely experienced respiratory tissue damage  
14 at the same concentrations used to calculate RD<sub>50</sub> values (see Table 5 for CRO and  
15 Buckley et al. 1984 for acrolein);
- 16 • *In vitro* results evaluating cell viability or cytotoxicity (Tables 10 and 11).

17 Relevant endpoints in Table 13 were determined using similar testing techniques, exposure  
18 durations, and species. The relative potency of the pertinent endpoints based on a MOA analysis  
19 of the index chemical (acrolein) to the pertinent endpoint of the LTD chemical (CRO) was  
20 calculated as follows:

$$\text{Relative Potency} = \frac{\text{Relevant Endpoint}_{\text{LTD Chemical}}}{\text{Relevant Endpoint}_{\text{Index Chemical}}}$$

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

24

1 **Table 12. Comparison of Relevant Endpoints for Acrolein and CRO**

Endpoint	Acrolein	CRO	Relative Potency
RD <sub>50</sub> Fisher-344 rats Babiuk et al. (1985)	6 ppm	23.2 ppm	3.87
RD <sub>50</sub> (Male Swiss-Webster and B6C3F1 mice) Steinhagen and Barrow (1984)	1.41 ppm	4.88 ppm	3.46
IC <sub>50</sub> values for viability (in vitro) (Poirier et al. 2002).	2.70 x 10 <sup>-5</sup>	4.26 x 10 <sup>-5</sup>	1.58
Cell viability (in vitro) (% control MTT absorbance) (Moretto et al. 2009)	25	91	3.64
		<b>Geometric mean</b>	<b>2.96</b>

2

### 3 **4.1.5 Geometric Mean of Relative Potency**

4 If multiple relative potency values, based on the same or different relevant endpoints, are  
5 available, a geometric mean of the relative potency ( $R_{GM}$ ) is calculated (TCEQ 2012). The value  
6 for the LTD chemical can then be calculated by multiplying the  $R_{GM}$  by the value of the  
7 structurally-similar index chemical. The geometric mean of applicable relative potency ratios for  
8 relevant endpoints was 2.96 (Table 12).

### 9 **4.1.6 <sup>chronic</sup>ESL<sub>generic</sub> for CRO**

10 Table 13 shows a summary of the derivation of the <sup>chronic</sup>ESL<sub>threshold(nc)</sub> for acrolein based on the  
11 Dorman et al. (2008) study (TCEQ 2014) and the calculated <sup>chronic</sup>ESL<sub>generic</sub> for CRO. Details on  
12 the Dorman et al. (2008) study are in Appendix E. The index chemical's <sup>chronic</sup>ESL<sub>threshold(nc)</sub> is  
13 adjusted by the relative potency factor to calculate the <sup>chronic</sup>ESL<sub>generic</sub> for CRO:

14 
$$\text{<sup>chronic</sup>ESL}_{\text{generic}} = \text{ESL}_{\text{Index Chemical}} \times R_{GM}$$

15 
$$\text{<sup>chronic</sup>ESL}_{\text{generic}} \text{ for CRO} = \text{<sup>chronic</sup>ESL}_{\text{threshold(nc)}} (\text{acrolein}) \times R_{GM}$$

16 
$$\text{<sup>chronic</sup>ESL}_{\text{generic}} \text{ for CRO} = 0.36 \text{ ppb} \times 2.96$$

17 
$$\text{<sup>chronic</sup>ESL}_{\text{generic}} \text{ for CRO} = 1.06 \text{ ppb or } 1.1 \text{ ppb (rounded to two significant figures)}$$

18 
$$\text{<sup>chronic</sup>ESL}_{\text{generic}} \text{ for CRO} = 1.1 \text{ ppb (} 3.1 \mu\text{g/m}^3 \text{ using CRO molecular weight)}$$

1 **Table 13. Derivation of the <sup>chronic</sup>ESL<sub>generic</sub> for CRO based on Relative Potency**

<b>Chemical</b>	<b>Acrolein (TCEQ 2014)</b>
<b>Parameter</b>	<b>Summary</b>
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 <sub>ADJ</sub>	0.03571 ppm
POD <sub>HEC</sub>	0.006678 ppm
Total UFs	30
<i>Interspecies UF</i>	3
<i>Intraspecies UF</i>	10
<i>LOAEL UF</i>	NA
<i>Subchronic to chronic UF</i>	1
<i>Incomplete Database UF</i> <i>Database Quality</i>	1 High
<b>Acrolein Chronic ReV (HQ = 1)</b>	<b>2.7 µg/m<sup>3</sup> (1.2 ppb)</b>
<b>Acrolein <sup>chronic</sup>ESL<sub>threshold(nc)</sub> (HQ = 0.3)</b>	<b>0.82 µg/m<sup>3</sup> (0.36 ppb)</b>
<b>Chemical</b>	<b>CRO RP<sub>GM</sub> = 2.96</b>
<b>CRO <sup>chronic</sup>ESL<sub>generic</sub> (HQ = 0.3)</b>	<b>3.1 µg/m<sup>3</sup> (1.1 ppb)<sup>a</sup></b>

2 <sup>a</sup> 3.1 µg/m<sup>3</sup> using CRO molecular weight

3

1 **4.1.7 Health-Based <sup>chronic</sup>ESL<sub>generic</sub> for CRO**

2 The <sup>chronic</sup>ESL<sub>generic</sub> is 3.1 µg/m<sup>3</sup> (1.1 ppb).

3 **4.2 Carcinogenic Potential**

4 **4.2.1 Relevant Data**

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

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

16 There is limited data for carcinogenic potential from oral exposure studies (Chung et al. 1986).  
17 Since CRO causes point-of-entry effects, the TCEQ did not consider route-to-route extrapolation  
18 using the Chung et al. (1986) study as valid (TCEQ 2012). Information on oral studies are  
19 provided for informational purposes only. Results from the Chung et al. 1986 study are  
20 summarized by IPCS 2008):

21 One chronic oral bioassay was located in which male F344 rats were given 0, 0.6, or 6.0  
22 mM of crotonaldehyde in drinking water for 113 weeks (Chung et al. 1986). This is  
23 equivalent to inhalation exposure to 0, 7.2, and 72 ppm, respectively, by route-to-route  
24 extrapolation, as described in Appendix D. The high-dose group had approximately  
25 10% lower body weight gain starting at week 8, and 10 of 23 rats developed moderate  
26 to severe liver damage (fatty metamorphosis, focal necrosis, fibrosis, cholestasis,  
27 mononuclear cell infiltration). The incidence of hepatic neoplastic nodules and  
28 hepatocellular carcinomas combined was 0 of 23, 11 of 27 (p < .01), and 1 of 23 at 0,  
29 0.6, and 6.0 mM, respectively (carcinoma: 0 of 23, 2 of 27, 0 of 23, respectively). The  
30 incidence of enzyme-altered liver foci, considered to be precursors to neoplasms, was 1  
31 of 23, 23 of 27 (p < .01), and 13 of 23 (p < .01) at 0, 0.6 and 6.0 mM, respectively. No  
32 explanation was offered for the lack of a neoplastic dose-response. Interestingly, the 10  
33 high-dose animals that had severe liver toxicity had no liver neoplasms, but the  
34 remaining 13 high dose rats were found to have hepatocellular carcinomas. The authors  
35 state “it is worth noting” that two low-dose rats had urinary bladder papillomas (none in  
36 controls or high-dose group) but did not indicate whether they considered these tumors  
37 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) <sup>1</sup>
ACGIH (1998)	A3, animal carcinogen <sup>2</sup>
USEPA (2005b)	Group C (possible human) carcinogen <sup>3</sup>

<sup>1</sup> 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.

<sup>2</sup> 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).

<sup>3</sup> 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  $\alpha,\beta$ -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

1 hepatocytes, crotyl alcohol undergoes alcohol dehydrogenase-catalysed conversion to  
2 2-butenal, the formation of which was accompanied by marked glutathione depletion,  
3 protein carbonylation, and cell death (Fontaine et al., 2002).

#### 4 **4.2.4 URF Developed by NRC (2007)**

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

#### 10 **4.3 Welfare-Based Chronic ESL**

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

#### 13 **4.4 Long-Term ESL and Values for Air Monitoring Evaluation**

14 This chronic evaluation resulted in the derivation of the following chronic value:

- 15 •  $\text{chronicESL}_{\text{generic}} = 3.1 \mu\text{g}/\text{m}^3$  (1.1 ppb)

16 For the long-term evaluation of air permit evaluations, the  $\text{chronicESL}_{\text{generic}}$  of  $3.1 \mu\text{g}/\text{m}^3$  (1.1 ppb)  
17 is used (Tables 1 and 2). This  $\text{chronicESL}_{\text{generic}}$  will also be used for evaluation of air monitoring  
18 data in lieu of a ReV until data are available to derive a CRO-specific ReV.

#### 19 **4.5 Chronic Inhalation Observed Adverse Effect Level**

20 A chronic inhalation observed adverse effect level was not determined for CRO since a relative  
21 potency approach was used to determine the  $\text{chronicESL}_{\text{generic}}$  for CRO (i.e., CRO had limited  
22 toxicity data).

### 23 **Chapter 5 References**

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

### 2 **2.2.1. Odor Threshold and Odor Awareness**

3 A wide range of concentrations have been reported for the human odor detection and irritation  
4 thresholds for crotonaldehyde, perhaps in some cases due to analytical measurement errors  
5 (Steinhagen and Barrow 1984). Amooore and Hautala (1983) reported the odor threshold to be  
6 0.12 ppm for *trans*-crotonaldehyde, whereas the irritation threshold was 14 ppm and 19 ppm for  
7 the nose and eyes, respectively. In several secondary sources, the odor detection threshold for  
8 crotonaldehyde was given as 0.035-1.05 ppm and the irritation threshold was 8.0 ppm (Ruth  
9 1986; Verschueren 1996). In a study in which 25 volunteers were exposed to 0.02-2.3 mg/m<sup>3</sup>  
10 (0.007-0.8 ppm) of crotonaldehyde, the odor was detected by several persons at the lowest  
11 concentration tested, and roughly half the people were able to detect the odor at 0.11 mg/m<sup>3</sup>  
12 (0.038 ppm; Tepikina et al. 1997). The test subjects were exposed to each concentration  
13 repeatedly (about 2-4 times) to eliminate guessing and also to “pure air” to give a point of  
14 reference (i.e., incidence of false positives). An unpublished source (van Doorn et al. 2002)  
15 reported 0.069 ppm and 0.063-0.2 ppm as the *trans*-crotonaldehyde and *cis*-crotonaldehyde odor  
16 detection thresholds, respectively (OT<sub>50</sub>; i.e., concentration at which 50% of the odor panel  
17 observed an odor without necessarily recognizing it).

### 18 **2.2. Experimental Studies**

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

30 The threshold for crotonaldehyde irritation in humans was reported as 0.0005 mg/liter (L) (0.17  
31 ppm; Trofimov 1962). In this experiment, volunteers inhaled crotonaldehyde vapor through a  
32 mask for 1 min; it was not specified how the vapor was generated or how the concentrations  
33 were measured. Factors taken into account were odor detection and irritation of the eyes and  
34 mucous membranes of the nose and trachea; it was not specified on which of these end points the  
35 estimated irritation threshold was actually based. Trofimov suggested that the maximum  
36 permissible concentration of crotonaldehyde in air should be limited to 0.0005-0.0007 mg/L  
37 (0.17-0.24 ppm) to prevent irritation.

1 **2.2.3. Occupational and Other Exposures**

2 Laboratory personnel (two or three people) who “sniffed” 15 ppm of crotonaldehyde vapor for a  
3 few seconds (<30 s) during brief openings of animal chambers reported that the odor was very  
4 strong but not intolerable and that there was no eye discomfort. The personnel who “sniffed” 45-  
5 50 ppm of crotonaldehyde vapor only momentarily noted that the odor was “very strong,  
6 pungent, and disagreeable, but not particularly biting to nasal passages” (Rinehart 1967, 1998).  
7 Lacrimation was not induced in the subjects, although they experienced a burning sensation of  
8 the conjunctivae and a strong desire to blink repeatedly.

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

15

## 1 **Appendix B: Nonlethal Animal Studies (from NRC 2007)**

### 2 **3.2.1. Rats**

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

24 The concentration of crotonaldehyde calculated to reduce the respiration rate of male F344 rats  
25 by 50% upon exposure for 10 min (RD<sub>50</sub>) was 23.2 ppm (Babiuk et al. 1985). Rats (four per  
26 concentration) were exposed to five to eight different concentrations (not specified).  
27 Crotonaldehyde vapor was generated in a modified impinger and was carried to the inlet of a  
28 head-only exposure chamber by a nitrogen stream; chamber concentrations were continuously  
29 monitored with an infrared gas spectrophotometer. Rats that were exposed 6 h/day for 9 days to  
30 15 ppm of formaldehyde, followed by challenge on day 10 with crotonaldehyde, had a similar  
31 RD<sub>50</sub> (20.5 ppm), indicating desensitization was not caused by prior formaldehyde inhalation  
32 (Babiuk et al. 1985).

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

1

### 3.2.2. Mice

2 The RD<sub>50</sub> (i.e., 50% reduction in respiration rate) values for crotonaldehyde vapor in male Swiss-  
3 Webster mice and B6C3F1 mice were 3.53 and 4.88 ppm, respectively (Steinhagen and Barrow  
4 1984). Mice were exposed to crotonaldehyde for 10 min in a head-only exposure chamber, and  
5 their breathing rates were measured using plethysmographic techniques (Alarie 1966). The  
6 crotonaldehyde chamber concentrations were continuously monitored with an infrared gas  
7 spectrophotometer (Steinhagen and Barrow 1984).

8

### 3.2.3. Rabbits

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

13

### 3.2.4. Cats

14 The threshold concentration of crotonaldehyde in air that was irritating to the mucosa of cats was  
15 0.009 mg/L (3.15 ppm; Trofimov 1962).

## 1 **Appendix C: LC<sub>50</sub> Studies (from NRC 2007)**

### 2 ***Skog (1950)***

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

### 14 ***Rinehart (1967)***

15 Rinehart (1967) conducted an extensive series of experiments to assess the acute toxicity of  
16 crotonaldehyde in male Wistar rats. The rats were exposed for 5 min to 4 h and observed for 2  
17 weeks; exposure concentrations and durations are given in Table 5-5. Crotonaldehyde vapors  
18 were generated by bubbling nitrogen gas through liquid crotonaldehyde (90% pure) and mixing  
19 this with air; the oxygen concentration was maintained at ≥17.8%. Exposure was in either a 20-L  
20 glass chamber or a 1,700-L wooden chamber (the latter was used for lower concentrations;  
21 which were not specified). Crotonaldehyde concentrations were measured two to five times over  
22 the exposure period using a colorimetric reaction with modified Schiff-Elvove reagent; the  
23 analytical concentrations were about 42% of the nominal concentration (range: 29-61%).  
24 Rinehart suggested that the discrepancy between the nominal and analytical concentrations was  
25 due to crotonaldehyde absorption on chamber walls, oxidation, and/or polymerization. The 30-  
26 min LC<sub>50</sub> obtained by Rinehart (600 ppm) was about 2-fold lower than that obtained by Skog;  
27 1950; 1,400 ppm). Rinehart suggested this difference may have been due to a loss of  
28 crotonaldehyde between the point of vapor generation and the animal breathing zone.

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

1 ***Voronii et al. (1982)***

2 Voronii et al. (1982) reported a 4-h LC<sub>50</sub> of 200 mg/m<sup>3</sup> (70 ppm) for white rats during an  
3 observation period of 2 weeks. In preliminary acute toxicity studies, groups of three or four rats  
4 (sex and strain not specified) were exposed to nominal crotonaldehyde concentrations of 2,094-  
5 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  
6 h/day on days 1, 2, and 4 (Eastman Kodak Corp. 1992). Many animals died, as shown in Table  
7 5-4. Symptoms included gasping, labored breathing, pink extremities, tremors, convulsions,  
8 salivation, and prostration. Microscopic examination of unspecified animals revealed lung  
9 congestion.

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

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

13 Human equivalent concentration =

$$14 \frac{2.06 \text{ mg crotonaldehyde/day} \times 70 \text{ kg body weight}}{20 \text{ m}^3 \text{ air/day} \times 0.35 \text{ kg of body weight}} = 20.6 \text{ mg/m}^3$$

15

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

22

## 1 **Appendix E: Dorman et al. (2008)**

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

4 The key study, Dorman et al. (2008), exposed male F344 rats (whole-body exposure) to  
5 concentrations of 0, 0.02, 0.06, 0.2, 0.6, or 1.8 ppm acrolein (analytical concentrations) for 6  
6 h/d, five d/wk for up to 65 exposure days (13 wk). Neither mortality nor a significant increase  
7 in incidence of observable clinical signs occurred following exposure to acrolein at any  
8 concentration. After 5-8 wk of exposure, the authors reported rats exposed to 0.06, 0.2, or 0.6  
9 ppm developed significantly depressed (~3-5%) body weight gains compared to air-exposed  
10 controls after 5-8 wk of exposure. At 1.8 ppm, body weight gains were reduced by ~ 20 percent  
11 compared to air-exposed controls. Histopathology of the respiratory tract was evaluated after 4,  
12 14, 30, and 65 exposure days and a 60-day recovery period after the 13-wk exposure period.

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

23 Dorman et al. (2008) examined animals 60 days following cessation of acrolein exposure: At  
24 the LOAEL of 0.6 ppm for nasal respiratory epithelial hyperplasia (Table 2 of Dorman et al.  
25 2008), hyperplasia of the lateral wall (level II) and septum (level I) did not show recovery  
26 compared to air controls as shown below in Table 10.

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

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

3 <sup>a</sup> statistically significant increase in the incidence of the lesion was seen (versus air-exposed controls, p <  
4 0.05, Pearson's)

5 <sup>b</sup> number in parentheses indicates average severity of the lesion seen in animals with a statistically  
6 significant lesion incidence. Unaffected animals were excluded from this calculation. 1= minimal,  
7 2 = light/mild, 3 = moderate, 4= moderately severe

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

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

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