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Provisional Peer-Reviewed Toxicity Values for

trans-Crotonaldehyde
(CASRN 123-73-9)

U.S. EPA Office of Research and Development
Center for Public Health and Environmental Assessment

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Center for Public Health and Environmental Assessment
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COMMONLY USED ABBREVIATIONS AND ACRONYMS

α2u-g	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AR	androgen receptor
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service registry number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPHEA	Center for Public Health and Environmental Assessment
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	estrogen receptor
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood-gas partition coefficient
Hb/g-H	human blood-gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NAG	*N*-acetyl-β-D-glucosaminidase
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RBC	red blood cell
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase, also known as AST
SGPT	serum glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	database uncertainty factor
UF_H	intraspecies uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
U.S.	United States of America
WBC	white blood cell

Abbreviations and acronyms not listed on this page are defined upon first use in the PPRTV document.

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR *TRANS*-CROTONALDEHYDE (CASRN 123-73-9)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

Currently available PPRTV assessments can be accessed on the U.S. Environmental Protection Agency's (EPA's) PPRTV website at https://www.epa.gov/pprtv. PPRTV assessments are eligible to be updated on a 5-year cycle and revised as appropriate to incorporate new data or methodologies that might impact the toxicity values or affect the characterization of the chemical's potential for causing adverse human-health effects. Questions regarding nomination of chemicals for update can be sent to the appropriate U.S. EPA Superfund and Technology Liaison (https://www.epa.gov/research/fact-sheets-regional-science).

QUALITY ASSURANCE

This work was conducted under the U.S. EPA Quality Assurance (QA) program to ensure data are of known and acceptable quality to support their intended use. Surveillance of the work by the assessment managers and programmatic scientific leads ensured adherence to QA processes and criteria, as well as quick and effective resolution of any problems. The QA manager, assessment managers, and programmatic scientific leads have determined under the QA program that this work meets all U.S. EPA quality requirements. This PPRTV was written with guidance from the CPHEA Program Quality Assurance Project Plan (PQAPP), the QAPP titled *Program Quality Assurance Project Plan (PQAPP) for the Provisional Peer-Reviewed Toxicity Values (PPRTVs) and Related Assessments/Documents (L-CPAD-0032718-QP),* and the PPRTV development contractor QAPP titled *Quality Assurance Project Plan—Preparation of Provisional Toxicity Value (PTV) Documents (L-CPAD-0031971-QP)*. As part of the QA system, a quality product review is done prior to management clearance. A Technical Systems Audit may be performed at the discretion of the QA staff.

All PPRTV assessments receive internal peer review by at least two Center for Public Health and Environmental Assessment (CPHEA) scientists and an independent external peer review by at least three scientific experts. The reviews focus on whether all studies have been correctly selected, interpreted, and adequately described for the purposes of deriving a provisional reference value. The reviews also cover quantitative and qualitative aspects of the provisional value development and address whether uncertainties associated with the assessment have been adequately characterized.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. EPA programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

This document has been reviewed in accordance with U.S. EPA policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

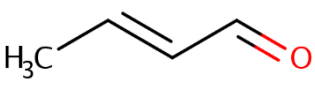
QUESTIONS REGARDING PPRTVS

Questions regarding the content of this PPRTV assessment should be directed to the U.S. EPA Office of Research and Development (ORD) CPHEA website at https://ecomments.epa.gov/pprtv.

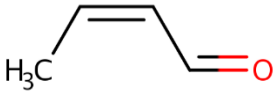
INTRODUCTION

trans-Crotonaldehyde, CASRN 123-73-9, is an α,β-unsaturated aldehyde with defined stereochemistry (WHO, 2008). The commercial product crotonaldehyde is represented by CASRN 4170-30-3 and consists of >95% *trans*-isomer (CASRN 123-73-9). *cis*-Crotonaldehyde is represented by CASRN 15798-64-8. *trans*-Crotonaldehyde is primarily used in chemical manufacturing as a precursor, intermediate, or solvent (WHO, 2008; HSDB, 2005). It is synthesized by aldol condensation of acetaldehyde and a dehydration step (HSDB, 2005). Commercial crotonaldehyde and *trans*-crotonaldehyde are both listed on the U.S. EPA's Toxic Substances Control Act (TSCA) public inventory (U.S. EPA, 2018b), and crotonaldehyde is registered with Europe's Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) program (ECHA, 2018).

The empirical formula for *trans*-crotonaldehyde is C_4H_6O (see Figure 1). Its physicochemical properties are shown in Table 1. *trans*-Crotonaldehyde is a reactive, clear liquid, with high water solubility. In the air, it will exist in the vapor phase based on its vapor pressure of 30.0 mm Hg. *trans*-Crotonaldehyde will be degraded in the atmosphere by reacting with photochemically produced hydroxyl radicals. Direct photolysis of *trans*-crotonaldehyde in the atmosphere does not occur [BUA (1993) as cited in WHO (2018)]. Volatilization of *trans*-crotonaldehyde from dry soil surfaces is expected based on the compound's vapor pressure, and moderate volatilization from water or moist soil surfaces is expected based on its estimated Henry's law constant of 1.45×10^{-5} atm-m^3/mole. The estimated K_{oc} for *trans*-crotonaldehyde indicates potential for mobility in soil and negligible potential to adsorb to suspended solids and sediment in aquatic environments; however, *trans*-crotonaldehyde polymerizes readily and may react when released in the environment (HSDB, 2005). Hydrolysis is not expected to be an important fate process under environmental conditions, although *trans*-crotonaldehyde will undergo hydrolysis in low or high pH conditions (WHO, 2018). In dilute acid aqueous solutions, *trans*-crotonaldehyde reversibly hydrates to form the aldol, 3-hydroxybutanal (ECHA, 2018).



trans-Crotonaldehyde



cis-Crotonaldehyde

Figure 1. *trans*- (CASRN 123-73-9) and *cis*-Crotonaldehyde (CASRN 15798-64-8) Structures

Table 1. Physicochemical Properties of *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95% *trans*-; CASRN 4170-30-3)

Property (unit)	*trans*-Crotonaldehyde Value[a]	Commercial Crotonaldehyde Value[a]
Physical state	Liquid	Liquid
Boiling point (°C)	103	103 (predicted average)
Melting point (°C)	−75.8	−76.0 (predicted average)
Density (g/cm^3)	0.845 (predicted average)	0.820 (predicted average)
Vapor pressure (mm Hg)	30.0	30.9 (predicted average)
pH (unitless)	NA	NA
pKa (unitless)	NA	NA
Solubility in water (mol/L)	2.09	4.54 (predicted average)
Octanol-water partition constant (log K_{ow})	0.573 (predicted average)	0.564 (predicted average)
Henry's law constant (atm-m^3/mol)	1.45×10^{-5} (predicted average)	1.48×10^{-5} (predicted average)
Soil adsorption coefficient K_{oc} (L/kg)	10.7 (predicted average)	10.7 (predicted average)
Atmospheric OH rate constant (cm^3/molecule-sec)	3.61×10^{-11}	3.61×10^{-11} (predicted average)
Molecular weight (g/mol)	70.091	70.091
Flash point (°C)	12.1	4.58 (predicted average)

[a]Data were extracted from the U.S. EPA CompTox Chemicals Dashboard (*trans*-crotonaldehyde, CASRN 123-73-9; https://comptox.epa.gov/dashboard/dsstoxdb/results?search=DTXSID6020351#properties; accessed February 4, 2021 and commercial crotonaldehyde, CASRN 15798-64-8; DTXSID 6020351). All values are experimental averages unless otherwise specified.

NA = not applicable; SMILES = simplified molecular-input line-entry system.

A summary of available toxicity values for *trans*-crotonaldehyde from U.S. EPA and other agencies/organizations is provided in Table 2. Toxicity values for commercial crotonaldehyde (>95% *trans*-isomer) are also included in Table 2. A 2010 PPRTV assessment from the U.S. EPA was previously available for "crotonaldehyde." The assessment herein provides an updated evaluation of *trans*-crotonaldehyde based on recent scientific literature and current PPRTV assessment practices. Information pertaining to commercial crotonaldehyde mixtures (CASRN 15798-64-8) was also considered as this mixture is defined as containing at least 95% *trans*-crotonaldehyde isomer. To promote evaluation of the *trans*-isomer of crotonaldehyde in the context of human health, studies using unspecified isomers, or other isomers of crotonaldehyde (i.e., *cis*-) are mentioned only for comparison, where necessary.

Table 2. Summary of Available Toxicity Values for *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95% *trans*-; CASRN 4170-30-3)

Source (parameter)[a, b]	Value (applicability)	Compound(s)	Notes	Reference[c]
Noncancer				
IRIS	NV	NA	NA	U.S. EPA (2020)
HEAST	NV	NA	NA	U.S. EPA (2011a)
DWSHA	NV	NA	NA	U.S. EPA (2018a)
ATSDR	NV	NA	NA	ATSDR (2019)
IPCS	NV	NA	NA	IPCS (2020)
CalEPA	NV	NA	NA	CalEPA (2019)
OSHA (PEL)	2 ppm	123-73-9 4170-30-3	8-hr TWA for general industry, construction, and shipyard employment	OSHA (2018a); OSHA (2018b); OSHA (2020)
NIOSH (REL)	2 ppm	123-73-9 4170-30-3	TWA for up to a 10-hr workday during a 40-hr workweek	NIOSH (2016); NIOSH (1994)
NIOSH (IDLH)	50 ppm	123-73-9	Based on acute inhalation toxicity data in humans and animals	NIOSH (1994)
ACGIH (TLV-ceiling)	0.3 ppm	4170-30-3	Concentration that should not be exceeded during any part of the working exposure; based on eye and upper respiratory tract irritation; skin notation	ACGIH (2018)
AEGL (AEGL 1)	10 min: 0.19 ppm 30 min: 0.19 ppm 60 min: 0.19 ppm 4 hr: 0.19 ppm 8 hr: 0.19 ppm	123-73-9 4170-30-3	Based on mild eye irritation in humans	U.S. EPA (2016); NRC (2008)
AEGL (AEGL 2)	10 min: 27 ppm 30 min: 8.9 ppm 60 min: 4.4 ppm 4 hr: 1.1 ppm 8 hr: 0.56 ppm	123-73-9 4170-30-3	Based on impaired pulmonary function in rats	U.S. EPA (2016); NRC (2008)
AEGL (AEGL 3)	10 min: 44 ppm 30 min: 27 ppm 60 min: 14 ppm 4 hr: 2.6 ppm 8 hr: 1.5 ppm	123-73-9 4170-30-3	Based on lethality in rats	U.S. EPA (2016); NRC (2008)

Table 2. Summary of Available Toxicity Values for *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95% *trans*-; CASRN 4170-30-3)

Source (parameter)[a, b]	Value (applicability)	Compound(s)	Notes	Reference[c]
Cancer				
IRIS (WOE)	Classification C: possibly carcinogenic to humans	123-73-9	Based on an increased incidence of hepatocellular carcinomas and hepatic neoplastic nodules (combined) in male rats in an oral chronic study	U.S. EPA (2005)
HEAST (OSF)	1.9 $(mg/kg\text{-}d)^{-1}$	123-73-9	Based on liver tumors in rats in an oral chronic study	U.S. EPA (2011a)
DWSHA	NV	NA	NA	U.S. EPA (2018a)
NTP	NV	NA	NA	NTP (2016)
IARC	Group 3: not classifiable as to its carcinogenicity to humans	4170-30-3	Based on inadequate evidence in human and experimental animals	IARC (1995)
CalEPA	NV	NA	NA	CalEPA (2019)
ACGIH (WOE)	A3: confirmed animal carcinogen with unknown relevance to humans	4170-30-3	Based on induction of hepatocellular carcinomas and neoplastic nodules in rats in an oral chronic study	ACGIH (2018)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Registry; CalEPA = California Environmental Protection Agency; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; IARC = International Agency for Research on Cancer; IPCS = International Programme on Chemical Safety; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration.

[b]Parameters: AEGL = acute exposure guideline level; IDLH = immediately dangerous to life or health concentrations; OSF = oral slope factor; PEL = permissible exposure limit; REL = recommended exposure limit; TLV = threshold limit value; TWA = time-weighted average; WOE = weight of evidence.

[c]Reference date is the publication date for the database and not the date the source was accessed.

NA = not applicable; NV = not available.

METHODS

Literature Search

Four online scientific databases (PubMed, Web of Science [WOS], TOXLINE, and Toxic Substances Control Act Test Submissions [TSCATS] via TOXLINE) were searched by U.S. EPA's Health and Environmental Research Online (HERO) staff and stored in the HERO database.[1] The literature search focused on chemical name and synonyms (identified as "valid/validated" or "good" via the U.S. EPA's Chemistry Dashboard[2] and ChemSpider[3]) with no limitations on publication type, evidence stream (i.e., human, animal, in vitro, in silico), or health outcomes. Full details of the search strategy for each database are presented in Appendix A. The initial database searches were conducted in May 2019 and updated in July 2020.

Screening Process

Two screeners independently conducted a title and abstract screen of the search results using DistillerSR[4] to identify study records that met the Population, Exposure, Comparator, Outcome (PECO) eligibility criteria (see Appendix B for a more detailed summary):

- Population: Humans, laboratory mammals, and other animal models of established relevance to human health (e.g., *Xenopus* embryos); mammalian organs, tissues, and cell lines; and bacterial and eukaryote models of genetic toxicity.
- Exposure: In vivo (all routes), ex vivo, and in vitro exposure to the chemical of interest, including mixtures to which the chemical of interest may contribute significantly to exposure or observed effects.
- Comparison: Any comparison (across dose, duration, or route) or no comparison (e.g., case reports without controls).
- Outcome: Any endpoint suggestive of a toxic effect on any bodily system or mechanistic change associated with such effects. Any endpoint relating to disposition of the chemical within the body.

Records that were not excluded based on title and abstract screening advanced to full-text review using the same PECO eligibility criteria. Studies that have not undergone peer review were included if the information could be made public and sufficient details of study methods and findings were included in the report. Full-text copies of potentially relevant records identified from title and abstract screening were retrieved, stored in the HERO database, and independently assessed by two screeners using DistillerSR to confirm eligibility. At both title/abstract and full-text review levels, screening conflicts were resolved by discussion between the primary screeners with consultation by a third reviewer to resolve any remaining disagreements. During title/abstract or full-text level screening, studies that were not directly relevant to the PECO, but could provide supplemental information, were categorized (or "tagged") relative to the type of supplemental information they provided (e.g., review, commentary, or letter with no original data; conference abstract; toxicokinetics and mechanistic

[1]U.S. EPA's HERO database provides access to the scientific literature supporting U.S. EPA science assessments. The database includes more than 2,500,000 scientific references and data from the peer-reviewed literature used by U.S. EPA to develop its regulations.
[2]U.S. EPA's Chemistry Dashboard: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=123-73-9.
[3]ChemSpider: http://www.chemspider.com/Chemical-Structure.394562.html?rid=330d45ef-b664-44c4-b920-67d738e55957.
[4]DistillerSR, a web-based systematic review software used to screen studies, is available at https://www.evidencepartners.com/products/distillersr-systematic-review-software.

information aside from in vitro genotoxicity studies; studies on routes of exposure other than oral and inhalation; acute exposure studies only; etc.). Conflict resolution was not required during the screening process to identify supplemental information (i.e., tagging by a single screener was sufficient to identify the study as potential supplemental information).

LITERATURE SEARCH AND SCREENING RESULTS

The database searches yielded 992 unique records. Of the 992 studies identified, 818 were excluded during title and abstract screening, 174 were reviewed at the full-text level, and 49 were considered relevant to the PECO eligibility criteria (see Figure 2). This included 2 human health effect studies, 22 in vivo animal studies, and 12 in vitro genotoxicity studies. Thirteen additional studies were tagged for inclusion as "supplemental/other." The detailed search approach, including the query strings and PECO criteria, are provided in Appendix A and Appendix B, respectively.

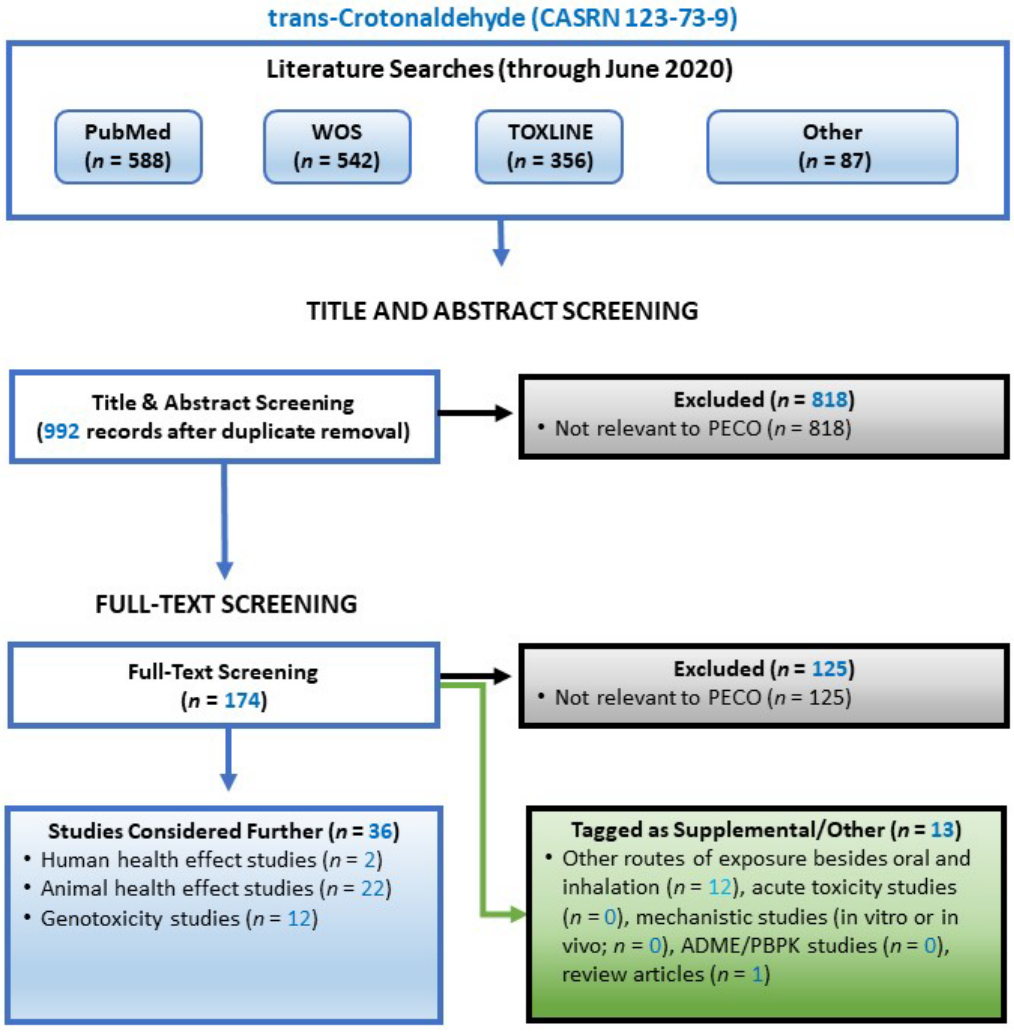


Figure 2. Literature Search and Screening Flow Diagram for *trans*-Crotonaldehyde (CASRN 123-73-9)

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Tables 3A and 3B provide overviews of the relevant noncancer and cancer evidence bases, respectively, for *trans*-crotonaldehyde and commercial crotonaldehyde, and include all potentially relevant repeated short-term, subchronic, and chronic studies, as well as reproductive and developmental toxicity studies. Principal studies are identified in bold. The phrase "statistical significance" and the term "significant," used throughout the document, indicates a *p*-value of < 0.05 unless otherwise specified.

Table 3A. Summary of Potentially Relevant Noncancer Data for *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95% *trans*-; CASRN 4170-30-3)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Human							
ND							
Animal							
1. Oral (mg/kg-d)							
Short term	5 M/5 F, S-D albino rat, diet, 14 d Reported target doses: 0, 22, 44, 88, or 175 mg commercial crotonaldehyde/kg-d	M: 0, 19, 36, 73, 139 F: 0, 17, 36, 68, 136	No exposure-related changes in clinical signs, body weight, organ weight, or gross necropsy	139	NDr	Borriston (1980a)	NPR
Subchronic	**10 M/10 F, F344 rat, gavage in corn oil, 5 d/wk, 13 wk** **Reported doses: 0, 2.5, 5, 10, 20, or 40 mg commercial crotonaldehyde/kg-d**	**0, 1.8, 4, 7.1, 14, 29**	**Epithelial hyperplasia of the forestomach in male and female rats. Thickened forestomach in female rats. Decreased absolute and relative thymus weight in female rats at 13 wk**	**7.1**	**14**	**Hazleton Laboratories (1986b); NTP-PWG (1987)**	**NPR, PS**
Subchronic	10 M/10 F, B6C3F1 mice, gavage in corn oil, 5 d/wk, 13 wk Reported doses: 0, 2.5, 5, 10, 20, or 40 mg commercial crotonaldehyde/kg-d	0, 1.8, 4, 7.1, 14, 29	Epithelial hyperplasia of the forestomach (forestomach thickening and nodules) in male and female mice	NDr	NDr	Hazelton Laboratories (1986a) as summarized by NTP-PWG (1987)	NPR

Table 3A. Summary of Potentially Relevant Noncancer Data for *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95% *trans*-; CASRN 4170-30-3)

Category[a]	Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration	Dosimetry[b]	Critical Effects	NOAEL[b]	LOAEL[b]	Reference (comments)	Notes[c]
Reproductive/ Developmental	20 M/20 F, F344 rat, gavage in corn oil, 9 wk (males exposed 61 d prior to mating and during 7-d mating period; females exposed 30 d prior to mating, through mating and gestation to PND 5) Reported doses: 0, 2.5, 5, or 10 mg commercial crotonaldehyde/kg-d	0, 2.5, 5, 10	No adverse reproductive or developmental effects	10	NDr	Hazleton Laboratories (1987)	NPR
2. Inhalation (mg/m^3)							
ND							

[a]Duration categories are defined as follows: acute = exposure for ≤24 hours; short term = repeated exposure for 24 hours to ≤30 days; long term (subchronic) = repeated exposure for >30 days ≤10% lifespan for humans (>30 days up to approximately 90 days in typically used laboratory animal species); and chronic = repeated exposure for >10% lifespan for humans (>~90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).
[b]Dosimetry: Doses are presented as ADDs (mg/kg-day) for oral noncancer effects and as HECs (mg/m^3) for inhalation noncancer effects. ADD (mg/kg-day) = reported dose × (days treated per week/7 days per week).
[c]Notes: NPR = not peer reviewed; PS = principal study.

ADD = adjusted daily dose; F = female(s); HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; M = male(s); ND = no data; NDr = not determined; NOAEL = no-observed-adverse-effect level; PND = postnatal day; S-D = Sprague-Dawley.

Table 3B. Summary of Potentially Relevant Cancer Data for *trans*-Crotonaldehyde (CASRN 123-73-9)

Category	Number of Male/Female, Strain, Species, Study Type, Reported Doses, Study Duration	Dosimetry[a]	Critical Effects	Reference (comments)	Notes[b]
Human					
ND					
Animal					
1. Oral (mg/kg-d)					
Carcinogenicity	23−27 M, F344 rat, drinking water, 113 wk Reported doses: 0, 0.6, or 6.0 mM *trans*-crotonaldehyde	0, 0.6, 4.6	Elevated incidence of liver neoplastic nodules at low dose, but not high dose	Chung et al. (1986)	PR, IRIS
2. Inhalation (mg/m^3)					
ND					

[a]Dosimetry: Oral exposures are expressed as HEDs (mg/kg-day); HEDs are calculated using DAFs, as recommended by U.S. EPA (2011b):
HED = ADD (mg/kg-day) × DAF. The DAF is calculated as follows: DAF = $(BW_a \div BW_h)^{1/4}$, where BW_a = animal body weight and BW_h = human body weight, using study body-weight values for BW_a and the reference value of 70 kg for BW_h.
[b]Notes: IRIS = used by IRIS (U.S. EPA, 2005); PR = peer reviewed.

ADD = adjusted daily dose; BW = body weight; DAF = dosimetric adjustment factor; HED = human equivalent dose; IRIS = Integrated Risk Information System; M = male(s); ND = no data.

HUMAN STUDIES

Crotonaldehyde is irritating to the skin, eyes, and mucous membranes (ATSDR, 2014; WHO, 2008; ATSDR, 2002; IARC, 1995). Sensitization reactions have been reported in humans following repeated skin exposure to commercial crotonaldehyde (Mellon Institute of Industrial Research, 1942). Irritation of the eyes, mucous membranes, and respiratory tract were reported in a small number of workers exposed to crotonaldehyde (isomer not specified [NS]), along with several other chemicals, during the incineration of polypropylene syringes (Mehta and Liveright, 1986). Headache and irritation of the eyes, nose, and throat were also reported in the majority of workers exposed to crotonaldehyde (NS) (along with several other chemicals) at a small printing and finishing company; some workers also reported difficulty breathing, wheezing, and nausea (Rosensteel and Tanaka, 1976). The measured levels of unspecified isomers of crotanaldehyde ranged from 0.7 to 2.1 mg/m^3. However, due to multiple chemical exposures, including other known irritants such as formaldehyde, the potential irritative effects of *trans*-crotonaldehyde cannot be adequately assessed in either of these health surveys.

Concern for increased cancer incidence in workers from an aldehyde factory was reported in an English-language abstract of a German-language study by Bittersohl (1974). Evaluation of this study by WHO (2008) indicated that no conclusions regarding the carcinogenicity of crotonaldehyde (NS) could be made from this study because all workers were smokers and were exposed to several different aldehydes. IARC (1995) also concluded that the data from this study were too sparse to be conclusive.

In a study examining the role of oxidative stress in the etiology of Alzheimer's disease, Kawaguchi-Niida et al. (2006) used immunohistochemical analysis to measure levels of protein-bound *trans*-crotonaldehyde in hippocampi obtained at autopsy of Alzheimer's disease patients and age-matched controls. Intracellularly, crotonaldehyde (NS) is formed during lipid peroxidation and reacts with proteins to form stable adducts with nucleic acids (WHO, 2008). In Alzheimer's disease patients, statistically significant ($p < 0.01$) higher levels of protein-bound *trans*-crotonaldehyde were observed when compared with age-matched controls. In addition, the protein-bound crotonaldehyde was localized in reactive astrocytes and microglia around senile plaques in Alzheimer's patients.

ANIMAL STUDIES

Oral Exposures

Short-Term Studies

Borriston (1980a)

In a non-peer-reviewed study, groups of albino Sprague-Dawley (S-D) rats (5/sex/group) were exposed to commercial crotonaldehyde (purity not reported) in the diet at target doses of 0, 22, 44, 88, or 175 mg/kg-day for 14 days (Borriston, 1980a). Daily observations and measurements of food consumption were performed, and body weight was recorded weekly. Based on food consumption and body-weight data, the study authors calculated doses of 19 ± 4, 36 ± 7, 73 ± 14, and 139 ± 21 mg/kg-day in males, and 17 ± 4, 36 ± 7, 68 ± 11, and 136 ± 27 mg/kg-day in females. At sacrifice, all animals were subjected to gross necropsy, and the liver and kidney weights were recorded. No organs were examined microscopically.

As described by the study authors, all animals survived until scheduled sacrifice. No clinical signs of toxicity were observed. Body weights, food consumption, and organ weights were comparable among groups. No exposure-related changes were observed at gross necropsy.

The study authors identified a no-observed-adverse-effect level (NOAEL) of 139 mg/kg-day based on a lack of exposure-related effects. A lowest-observed-adverse-effect level (LOAEL) was not identified.

Subchronic Studies

Hazleton Laboratories (1986b); NTP-PWG (1987)

In a non-peer-reviewed study, groups of F344 rats (10/sex/group) were administered commercial crotonaldehyde (CASRN 4170-30-3) at doses of 0, 2.5, 5, 10, 20, or 40 mg/kg-day via gavage in corn oil, 5 days/week, for up to 13 weeks (Hazleton Laboratories, 1986b). Adjusted daily doses (ADDs)[5] calculated for this review were 0, 1.8, 4, 7.1, 14, or 29 mg/kg-day. The animals were subjected to twice daily mortality/morbidity checks; food consumption and body weight were recorded weekly. Blood samples were collected on Days 4, 16, and at the beginning of Week 13 for hematology (hemoglobin [Hb], hematocrit [Hct], red blood cell [RBC] count, mean cell volume [MCV], mean cell hemoglobin [MCH], mean cell hemoglobin concentration [MCHC], total and differential white blood cell [WBC] count, platelet count, and RBC, WBC, and platelet morphology) and serum chemistry (sorbitol dehydrogenase [SDH], gamma glutamyl transferase [GGT], alanine aminotransferase [ALT], alkaline phosphatase [ALP], blood urea nitrogen [BUN], and creatinine) analyses. The report suggested that urine samples were also collected but analysis of the samples was not discussed. An assessment of sperm morphology and vaginal cytology was performed at the end of the study on animals in the three lower dose groups and control animals. At sacrifice, all animals were subjected to gross necropsy, and the brain, heart, liver, right kidney, lung, and thymus were weighed. A complete histopathological examination was performed on all gross lesions and tissue masses, all control rats, all rats in the highest dose group with at least 60% survivors at time of sacrifice, and all rats in the higher dose groups in which death occurred prior to study termination. Target organs (nasal cavities and forestomach) from all rats in all groups were also examined microscopically. The National Toxicology Program (NTP) convened a Pathology Working Group (PWG) (NTP-PWG, 1987) to review selected data and slides from this study.

Early deaths (death prior to cessation of the study) occurred at the following incidences in the control through highest dose groups: 0/10, 0/10, 0/10, 3/10, 3/10, and 5/10 for males, and 1/10, 0/10, 1/10, 1/10, 7/10, and 5/10 for females. The NTP-PWG (1987) concluded that nearly all early deaths were associated with gavage trauma and/or oil in the lungs, and that the early deaths should not be used as criteria for selecting doses for a chronic study. The study authors reported that the highest dose males showed a decrease in body-weight gain from Week 11−13 (data not shown in the original report) and a statistically significant 9% decrease in terminal body weight, compared to control. Terminal body weights were comparable to control in other male groups and in females (see Table D-1 and Table D-2). The study authors indicated that statistically significant changes occasionally occurred in hematological and clinical chemistry measures, but no dose- or time-related trends were observed (quantitative data not available). Therefore, these changes were not considered toxicologically relevant by the study authors or the NTP-PWG (1987). Sporadic (not dose dependent) statistically significant differences were also observed in organ weights in some exposed groups (i.e., relative and absolute thymus weights, relative liver weight, relative brain weight, and relative testicle weight as depicted in Table D-1 and Table D-2). Upon review, organ-weight changes were not considered toxicologically significant by the NTP-PWG (1987), with no further explanation provided. Although the sporadic nature of these changes was apparent for most of the above endpoints, it is unclear why

[5]ADD (mg/kg-day) = reported dose × (5 ÷ 7).

the NTP determined that the decreased absolute and relative thymus-weight changes observed in female rats were not toxicologically relevant. Statistically significant results were seen in these animals only at the highest (29 mg/kg-day) dose. No exposure-related changes were observed in male sperm morphology or female estrous cycle.

At gross necropsy, exposure-related lesions were observed only in the forestomach of rats of both sexes in the two highest dose groups; these lesions included thickened forestomach and/or forestomach hyperplasia (see Table D-3). Microscopic examination showed epithelial hyperplasia in the forestomach at ≥7.1 mg/kg-day (adjusted daily dose [ADD]) in male rats (see Table D-3) although the results were not statistically significant. The NTP-PWG (1987) concluded that no-effect levels for forestomach lesions were 5 and 10 mg/kg-day (ADDs: 4 and 7.1 mg/kg-day) for males and females, respectively, noting that the lesion was equivocal in the single affected female in the 7.1-mg/kg-day group. The study authors reported that statistical significance was only reached at 14 mg/kg-day for females and 29 mg/kg-day for males. Thickened forestomach was observed in male rats at 14 mg/kg-day and 29 mg/kg-day, but only reached statistical significance in female rats at 29 mg/kg-day. The only other exposure-related microscopic change reported was described as nasal inflammation in males at ≥7.1 mg/kg-day and females at ≥14 mg/kg-day, but statistical significance was reached only at 29 mg/kg-day in both sexes (see Table D-3). However, the NTP-PWG (1987) concluded that the nasal lesions were serous exudation and not acute inflammation as reported by Hazleton Laboratories (1986b) and that the effect was likely a localized effect from exhaled crotonaldehyde rather than an effect from blood-circulated crotonaldehyde. Importantly, after histological review of lung tissues in early-death rodents, it was determined that gavage error was the likely cause of early mortality in these animals. NTP-PWG (1987) also observed that the serous exudation was usually present in these early-death rats and may have been exacerbated by postmortem change.

In summary, a NOAEL of 7.1 mg/kg-day and a LOAEL of 14 mg/kg-day were identified by the NTP-PWG based on a statistically significant increase in the incidence of forestomach lesions in female rats.

Hazleton Laboratories (1986a) as summarized by NTP-PWG (1987); NTP (2018)

Hazleton Laboratories (1986a) is a companion study to the 13-week study in F344 rats by Hazleton Laboratories (1986b) that is available only as a summary in a report by NTP-PWG (1987). The Hazleton studies are non-peer-reviewed study reports but were reviewed by the NTP-PWG. Selected data tables are also on the NTP Chemical Effects in Biological Systems (CEBS) database (NTP, 2018). In this study, groups of B6C3F1 mice (10/sex/group) were administered commercial crotonaldehyde (CASRN 4170-30-3) at doses of 0, 2.5, 5, 10, 20, or 40 mg/kg-day via gavage in corn oil, 5 days/week for up to 13 weeks. ADDs calculated for this review were 0, 1.8, 4, 7.1, 14, or 29 mg/kg-day. The study followed the same protocol as the companion study in F344 rats (Hazleton Laboratories, 1986b) reported above, but it excluded the hematology and serum chemistry analyses. The forestomach, designated as the target organ based on lesions observed at initial histopathological examination, was examined microscopically in all mice in the control and treated groups. The NTP-PWG (1987) reviewed selected slides from this study.

All mice survived treatment to terminal sacrifice, no clinical signs were observed by the study authors, and they reported no statistically significant differences between treated and control groups for body-weight gain. The study authors reported statistically significant changes in absolute organ weights and organ-/body-weight ratios between the treated and control groups

(quantitative data not available), but NTP-PWG (1987) did not consider these differences toxicologically significant because of a lack of consistency or dose-related trend. Treatment-related lesions were not observed at gross necropsy. Microscopic examination showed hyperplasia of the forestomach mucosa in males and females from the highest dose group only. Neither NTP-PWG (1987) nor NTP (2018) reported quantitative data for forestomach lesions. No significant pathological findings were reported for lower dose groups. The NTP-PWG (1987) identified the highest dose as a LOAEL (29 mg/kg-day) for epithelial hyperplasia of the forestomach in both sexes of B6C3F1 mice exposed to commercial crotonaldehyde. Due to the lack of available quantitative data, it is not possible for the purposes of this PPRTV assessment to identify NOAELs and LOAELs for this study.

Reproductive/Developmental Studies

Hazleton Laboratories (1987)

In a non-peer-reviewed one-generation reproductive study, groups of F344 rats (20/sex/group) were administered commercial crotonaldehyde (CASRN 4170-30-3) via daily gavage in corn oil at doses of 0, 2.5, 5, or 10 mg/kg-day (Hazleton Laboratories, 1987). Males were dosed for 61 days prior to mating and during the 7-day cohabitation period and then sacrificed. Females were dosed for 30 days prior to mating, during mating, and through gestation to Postnatal Day (PND) 5, when they and their surviving pups were sacrificed. Females that did not get pregnant were sacrificed 30 days after the cohabitation period. Mortality checks were performed twice daily. Body weights were recorded weekly, and pregnant females were weighed on Gestation Days (GDs) 0, 7, 14, and 20; at parturition; and again, at sacrifice on PND 5. All females were subjected to a vaginal cytology evaluation prior to mating and again just prior to termination for nonpregnant females. At study termination, all males were subjected to a sperm morphology evaluation, and weights of the right testes and epididymis were recorded. Blood was collected from all animals at termination for possible hormone evaluations. Gross examination was conducted on all animals at necropsy, and histopathology was performed on the reproductive tissues (testes, epididymis, vagina, uterus, cervix, oviducts, and ovaries). Pups were weighed, counted, sexed, and examined at birth and on PND 5.

All animals except for one mid-dose male survived to study termination; the cause of death was reported as not treatment-related. As stated by the study authors, compound-related clinical signs were not evident in any group at any phase of the study. No significant changes in body weights, testis weights, or epididymis weights were observed among any treated male rats compared with control (see Table D-4). In pregnant females, no significant differences from controls in body weights were observed through gestation to PND 5 (see Table D-4). Among female rats that did not get pregnant, there was a significant 20−21% decrease in body-weight gain (and 10−14% decrease in absolute body weight) in the low- and high-dose groups relative to controls during the postmating period (see Table D-4). However, the control group for this analysis included only two animals, and the treated groups also included small numbers (n = 3−5), which limits interpretation of these results and confounds interpretation of the decreased body-weight effect described above. The study authors suggested that this effect was probably not related to treatment due to “the limited number of non-pregnant females and lack of significant findings in the pregnant females.” The data for reproductive and litter parameters showed no effect of treatment. No exposure-related histological lesions in reproductive tissues were observed in males or females. In total, no treatment-related effects were observed for survival or body weight data (parents and pups). Results of the sperm morphology and vaginal cytology studies were not presented.

The high dose of 10 mg/kg-day is identified by the study authors as a NOAEL for parental and reproductive toxicity in this study. No LOAEL is identified.

Chronic/Carcinogenicity Studies

Chung et al. (1986)

In a peer-reviewed study, groups of male F344 rats (23−27/group) were exposed to *trans*-crotonaldehyde (>99% purity) in drinking water for 113 weeks at concentrations of 0, 0.6, or 6.0 mM (0, 40, or 420 mg/L, based on molecular weight = 70.09 mg/mmol) beginning at 6 weeks of age (Chung et al., 1986). Average daily doses of 0, 2, and 17 mg/kg-day (HEDs: 0, 0.6, and 4.6 mg/kg-day) were calculated for this review using reported body weights and water consumption. Drinking water consumption was measured twice weekly, and body weight was measured weekly for 40 weeks and then biweekly. Upon sacrifice at the end of exposure, gross necropsy was performed on all animals, and histopathology was assessed on gross lesions and major organs (not specified). Statistics were performed by the study authors for histopathological findings only.

Survival percentages were >95% for the exposed and control groups through 70 weeks of exposure but began to decline thereafter (see Table D-5). At 110 weeks, survival for the control, low-, and high-dose groups was 70% (16/23), 63% (17/27), and 57% (13/23), respectively. These differences in survival rate were not statistically significant. Body weight was decreased in the high-dose group beginning at the 8th week of exposure and continuing throughout the study. Based on estimates derived from graphically presented data, body weights in high-dose animals were more than 10% lower than controls over the latter 6 months of the study, suggesting that the high dose of 4.6 mg/kg-day is at or approaching the maximum tolerated dose (MTD). Body weight in the low-dose group remained similar to controls throughout the study.

Incidence of neoplastic nodules in the liver was significantly elevated at the low dose (9/27), but not the high dose (1/23) compared with controls (0/23) (see Table D-5). Two rats (7% of the 27 animals in the dose group) with neoplastic nodules in the low-dose group also showed hepatocellular carcinomas. This finding was not significant as determined by the study authors. Incidences of altered liver foci, considered to be a preneoplastic lesion by the study authors, were significantly elevated at the low dose (23/27) and the high dose (13/23) compared with controls (1/23) (see Table D-5). The number of altered liver foci per square centimeter was also significantly increased at both doses, with greater increases at the low dose (see Table D-5). Among high-dose rats, the 10/23 individuals that did not have preneoplastic (altered foci) or neoplastic lesions in the liver instead showed moderate to severe degenerative liver damage (fatty metamorphosis, focal liver necrosis, fibrosis, cholestasis, and mononuclear cell infiltration). The report did not discuss whether these 10 rats were the ones that died prior to study termination. The degenerative liver lesions described at the high dose were not reported in the control or low-dose groups. Incidences of neoplastic lesions in tissues other than the liver were not significantly affected by treatment, although it may be noteworthy that bladder tumors were observed in two rats in the low-dose group but not in the control or high-dose rats. There were no reports of non-neoplastic lesions in tissues other than the liver.

A NOAEL and LOAEL cannot be identified from this study due to the limited assessment and reporting of noncancer endpoints and confounding due to the elevated incidence of liver tumors at the low dose, but not at the high dose. Although the reduced body weight in high-dose animals was considered as a potential LOAEL, no mention was made of degenerative liver lesions (a potential precursor effect) in the control and low-dose groups, which were

observed to accompany the decreased body weight in the high-dose animals. Due to this lack of reporting on potential precursor effects, it is uncertain that attributing a LOAEL to these non-neoplastic effects would be health protective. Therefore, this study was not included in the "Summary of Potentially Relevant Noncancer Data" table (see Table 3A) above. The study is also of limited value as a quantitative cancer bioassay due to the small group sizes, use of a single sex and species, and the lack of the expected dose-response relationship in the observed tumor data. The latter may reflect that the high dose in this study exceeded the MTD, which is suggested by both the decrease in body weight and the occurrence of degenerative liver lesions in the high-dose group.

Inhalation Exposures

No adequate inhalation studies have been identified.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Genotoxicity Studies

The genotoxicity of *trans*-crotonaldehyde (CASRN 123-73-9) and commercial crotonaldehyde (CASRN 4170-30-3) has been evaluated primarily in vitro, with a limited number of in vivo studies. Available studies are summarized below (see Table 4A for more details). Based on available data, *trans*-crotonaldehyde is clastogenic and forms deoxyribonucleic acid (DNA) adducts, both in vitro and in vivo. It is also mutagenic under certain conditions.

Table 4A. Summary of *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95 *trans*-; CASRN 4170-30-3) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Genotoxicity studies—prokaryotic organisms						
Reverse mutation	*Salmonella typhimurium* TA98, TA1537, TA7001, TA7002, TA7003, TA7004, TA7005, TA7006, and a mix of TA7000 series	50−1,000 µg commercial crotonaldehyde	+ (TA98, TA7002, TA7004, TA7005, TA7006, mix) − (TA1537, TA7001, TA7003)	NDr	Liquid preincubation study. The TA7000 series contains base-specific tester strains. Compound tested up to a toxic dose.	Gee et al. (1998)
Reverse mutation	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	0.005−10 µL commercial crotonaldehyde/plate	−	−	Plate incorporation assay. Cytotoxicity observed at ≥1 µL/plate.	Litton Bionetics (1979)
DNA damage	*Escherichia coli* PQ37	100 mM commercial crotonaldehyde	−	−	SOS chromotest.	von der Hude et al. (1988)
Genotoxicity studies—mammalian cells in vitro						
Mutation	L5178Y/*Tk*+/− mouse lymphoma cells	0, 1, 10, 25, 50 µM commercial crotonaldehyde	+	NDr	Thymidine-kinase mutation assay.	Demir et al. (2011)
CA	Human lymphoblastoid cells (Namalwa cell line)	0, 5, 10, 20, 40, 50, 100, 150, 200, 250 µM *trans*-crotonaldehyde	+	NDr	The total number of structural aberrations was increased at ≥100 µM.	Dittberner et al. (1995)
CA	Primary human blood lymphocytes	0, 5, 10, 20, 40, 50, 100, 150, 200, 250 µM *trans*-crotonaldehyde	+	NDr	The total number of structural aberrations was increased at ≥10 µM.	Dittberner et al. (1995)
CA	CHO cells	0.5−16 µg commercial crotonaldehyde/mL	+	+	The total number of structural aberrations was increased at 1.6 µg/mL (−S9) and 16 µg/mL (+S9).	Galloway et al. (1987)

Table 4A. Summary of *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95 *trans*-; CASRN 4170-30-3) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
SCE	Human lymphoblastoid cells (Namalwa cell line)	0, 5, 10, 20, 40, 50, 100, 150, 200, 250 µM *trans*-crotonaldehyde	+	NDr	SCE induced at ≥20 µM.	Dittberner et al. (1995)
SCE	Primary human blood lymphocytes	0, 5, 10, 20, 40, 50, 100, 150, 200, 250 µM *trans*-crotonaldehyde	+	NDr	SCE induced at ≥10 µM.	Dittberner et al. (1995)
SCE	CHO cells	1.6−160 µg commercial crotonaldehyde/mL	+	+	The total number of SCE was increased at ≥0.5 µg/mL (−S9) and ≥1.6 µg/mL (+S9).	Galloway et al. (1987)
MN	Human lymphoblastoid cells (Namalwa cell line)	0, 5, 10, 20, 40, 50, 100, 150, 200, 250 µM *trans*-crotonaldehyde	+	NDr	MN induced at ≥40 µM. Centromere-positive MN were not significantly increased with exposure.	Dittberner et al. (1995)
MN	Primary human blood lymphocytes	0, 5, 10, 20, 40, 50, 100, 150, 200, 250 µM *trans*-crotonaldehyde	+	NDr	MN induced at ≥40 µM. Centromere-positive MN were not significantly increased with exposure.	Dittberner et al. (1995)
Aneuploidy	Primary human blood lymphocytes	0, 5, 10, 20, 40, 50, 100, 150, 200, 250 µM *trans*-crotonaldehyde	−	NDr	The number of aneuploid metaphases was not increased at any concentration.	Dittberner et al. (1995)
Genotoxicity studies—mammalian species in vivo						
Dominant lethal mutation	Swiss albino mice (20 M) were exposed via i.p. injection in olive oil for 5 d. After final treatment, males were mated with unexposed females for 5 wk. Dams were sacrificed on GDs 14−16 and examined for live and dead implants.	0, 8, 16, 32 µL *trans*-crotonaldehyde/kg Positive control: 40 mg cyclophosphamide	+	NA	Significant increase in the number of dominant lethal mutations in all treated groups. Maximum lethality was observed in the group exposed to 32 µL/kg and mated 15−21 d post-treatment.	Jha et al. (2007)

Table 4A. Summary of *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95 *trans*-; CASRN 4170-30-3) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
CA	Swiss albino mice (3 M, 2 F/group) were exposed once via i.p. injection in olive oil. Mice were sacrificed 6, 13, and 24 hr after treatment. Bone marrow was evaluated for CAs.	0, 8, 16, 32 µL *trans*-crotonaldehyde/kg Positive control: 1.5 mg/kg mitomycin C	+	NA	Significant increase in CAs/cell and percent abnormal cells in all treated groups at all time points.	Jha et al. (2007)
CA	Swiss albino mice (5 M/group) were exposed once via i.p. injection in olive oil. Mice were sacrificed 24 hr after treatment. Spermatocytes were evaluated for CAs.	0, 8, 16, 32 µL *trans*-crotonaldehyde/kg Positive control: 25 mg cyclophosphamide	+	NA	Significant increase in percent abnormal cells at ≥16 µL/kg.	Jha et al. (2007)
MN	B6C3F1 mice (10/sex/group) were exposed via gavage in corn oil for 90 d. Erythrocytes were evaluated for MN.	0, 2.5, 5, 10, 20, 40 mg commercial crotonaldehyde/kg-d	−	NA	NA	Witt et al. (2000)
DNA adducts	F344 rats (4 F/group) were exposed once via gavage in corn oil. Rats were sacrificed 12 or 20 hr after exposure. Major organs were evaluated for adducts.	0, 200, 300 mg *trans*-crotonaldehyde/kg	+	NA	1,N^2-Propanodeoxyguanosine adducts were identified in treated animals at both dose levels. The highest levels were detected in liver, followed by lung, kidney, and large intestine. Adduct levels at 200 and 300 mg/kg were 2.9 and 3.4 adducts per 10^8 nucleotides, respectively. No adducts were detected in controls (detection limit 3 adducts per 10^9 nucleotides).	Budiawan and Eder (2000); Budiawan et al. (2000)

Table 4A. Summary of *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95 *trans*-; CASRN 4170-30-3) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
DNA adducts	F344 rats (4 F/group) were exposed for 6 wk (5 d/wk) via gavage in corn oil. Rats were sacrificed 20 hr after final exposure.	1, 10 mg *trans*-crotonaldehyde/kg-d	+	NA	1,N^2-Propanodeoxyguanosine adducts were identified in treated animals at both dose levels. Adduct levels at 1 and 10 mg/kg were 2.0 and 6.2 adducts per 10^8 nucleotides, respectively. No adducts were detected in controls (detection limit 3 adducts per 10^9 nucleotides).	Eder and Budiawan (2001); Budiawan and Eder (2000)
DNA adducts	F344 rats (F, NS) were exposed for 4 wk (5 d/wk) via gavage in corn oil. Rats were sacrificed 24 hr, 1 wk, or 2 wk after final exposure.	10 mg *trans*-crotonaldehyde/kg-d	+	NA	1,N^2-Propanodeoxyguanosine adducts were identified in treated animals at all time points. The numbers of adducts at 1 and 2 wk postexposure were 69 and 18%, respectively, of the number of adducts 24 hr after final exposure. No adducts were detected in controls (detection limit 3 adducts per 10^9 nucleotides).	Eder and Budiawan (2001); Budiawan and Eder (2000)
Genotoxicity studies—invertebrates in vivo						
Sex-linked recessive lethal	*Drosophila melanogaster*; males were exposed for 3 d via feeding prior to mating to unexposed females.	4,000 ppm commercial crotonaldehyde	−	NA	NA	Woodruff et al. (1985)
Sex-linked recessive lethal	*D. melanogaster*; males were injected with 0.2−0.3 µL 24−48 hr prior to mating to unexposed females.	3,500 ppm commercial crotonaldehyde	+	NA	3,500 ppm dose promoted 15% mortality and 4% increase in male sterility.	Woodruff et al. (1985)

Table 4A. Summary of *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95 *trans*-; CASRN 4170-30-3) Genotoxicity

Endpoint	Test System	Doses/ Concentrations Tested	Results without Activation[a]	Results with Activation[a]	Comments	References
Reciprocal translocation	*D. melanogaster*; males were injected with 0.2−0.3 µL 24−48 hr prior to mating to unexposed females.	3,500 ppm commercial crotonaldehyde	+	NA	Increased reciprocal translocations observed in sperm after 3 d of storage.	Woodruff et al. (1985)
Mitotic recombination (white/white$^+$ eye mosaic bioassay)	*D. melanogaster* larvae were exposed via feeding for 48 hr.	0, 10, 25, 50 mM commercial crotonaldehyde	+	NA	Mitotic recombination observed at ≥25 mM.	Demir et al. (2013)
Genotoxicity studies—subcellular systems						
DNA adduct	Calf thymus DNA; 8−48-hr incubation.	0, 90 µL *trans*-crotonaldehyde	+	NDr	Formed cyclic 1,N^2-propanodeoxy-guanosine adducts. More adducts formed with longer incubation.	Budiawan and Eder (2000); Budiawan et al. (2000)

[a]+ = positive; ± = weakly positive; − = negative.

CA = chromosomal aberration; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; F = female(s); GD = gestation day; i.p. = intraperitoneal; M = male(s); MN = micronuclei; NA = not applicable; NDr = not determined; SCE = sister chromatid exchange.

Mutagenicity

Results of *Salmonella typhimurium* mutation assays for commercial crotonaldehyde in nonmammalian species are mixed (no study specifically indicated that it was testing *trans*-crotonaldehyde). A study by Gee et al. (1998) using the liquid suspension method reported that commercial crotonaldehyde was mutagenic without metabolic activation in *S. typhimurium*, and was not tested under conditions of external metabolic activation. A single study using the plate incorporation method reported that commercial crotonaldehyde was not mutagenic, both with and without metabolic activation (Litton Bionetics, 1979). Research has suggested that contradictory results in bacterial assays may be associated with the high cellular toxicity of crotonaldehyde, particularly in standard plate assays using lower bacterial cell densities (Demir et al., 2011).

In mammals, increased dominant lethal mutations were observed in mice following male exposure to *trans*-crotonaldehyde via intraperitoneal (i.p.) injection for 5 days prior to mating (Jha et al., 2007). In an in vitro mutation study in mammalian cells, commercial crotonaldehyde induced mutations in mouse lymphoma cells without metabolic activation (Demir et al., 2011). In *Drosophila melanogaster*, sex-linked recessive mutations were induced following exposure to commercial crotonaldehyde via injection, but not oral exposure (Woodruff et al., 1985).

Clastogenicity

Chromosomal aberrations (CAs) were induced in both bone marrow cells and spermatocytes in mice exposed once to *trans*-crotonaldehyde via i.p. injection (Jha et al., 2007). Micronuclei (MN) were not induced in erythrocytes of mice following exposure to commercial crotonaldehyde via gavage for 90 days (Witt et al., 2000). CAs, sister chromatid exchanges (SCEs), and MN were all induced by *trans*-crotonaldehyde in primary human blood lymphocytes and cultured human lymphoblastoid cells (Dittberner et al., 1995). However, *trans*-crotonaldehyde did not induce aneuploidy or centromere-positive MN, indicating that observed effects were clastogenic in nature, rather than aneugenic (Dittberner et al., 1995). CAs and SCEs were also induced in Chinese hamster ovary (CHO) cells following in vitro exposure to commercial crotonaldehyde (Galloway et al., 1987). A significant increase in reciprocal translocations and mitotic recombinations were also observed in *D. melanogaster* following exposure to commercial crotonaldehyde (Demir et al., 2013; Woodruff et al., 1985).

DNA Adducts, Damage, and Repair

Numerous studies report that *trans*-crotonaldehyde can directly bind to DNA, forming DNA adducts. 1,N^2-Propanodeoxyguanosine DNA adducts were observed in multiple tissues (the highest levels of adducts were detected in the liver, lung, kidney, and large intestine) of F344 rats exposed to *trans*-crotonaldehyde via a single gavage exposure to doses ≥200 mg/kg or repeated gavage exposures to doses ≥1 mg/kg-day (Eder and Budiawan, 2001; Budiawan and Eder, 2000; Budiawan et al., 2000). DNA adducts persisted several weeks after exposure. 1,N^2-Propanodeoxyguanosine DNA adducts were also observed in CHO cells, human fibroblast cells, and isolated calf DNA exposed to *trans*-crotonaldehyde in vitro (Budiawan and Eder, 2000; Budiawan et al., 2000).

DNA damage was not induced in *Escherichia coli* exposed to commercial crotonaldehyde (with and without activation) in the SOS chromotest (von der Hude et al., 1988).

Supporting Animal Toxicity Studies

Numerous acute oral and inhalation studies, studies available only from secondary sources or as abstracts, and studies via other routes (e.g., dermal, injection) were identified. The relevant studies are summarized below (see Table 4B for additional details).

Table 4B. Other *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95% *trans*-; CASRN 4170-30-3) Studies

Test[a]	Materials and Methods	Results	Conclusions	References
Supporting evidence—noncancer effects in animals following oral exposure				
Acute (oral)	In a range-finding study, S-D rats (2/sex/group) were exposed to 50, 160, 500, 1,600, or 5,000 mg commercial crotonaldehyde/kg via gavage. Rats were observed for 3 d after dosing.	Convulsions observed in all rats exposed to ≥500 mg/kg immediately after dosing, and all rats in these dose groups died within 0.5 hr of dosing. One female died at 160 mg/kg within 1 hr of dosing. No rats died at 50 mg/kg.	FEL: 160 mg/kg (death)	Borriston (1980b); Borriston (1980c)
Acute (oral)	In an LD_{50} study, S-D rats (5/sex/group) were exposed once to 64.5, 107.5, 180, 300, or 500 mg commercial crotonaldehyde/kg via gavage. At the end of the 14-d observation period, all surviving rats were sacrificed. Endpoints evaluated included mortality, clinical signs, body weight, and gross necropsy.	All rats exposed to ≥300 mg/kg died, and 4/5 males and 3/5 females at 180 mg/kg died. All rats survived at ≤107.5 mg/kg. All observed deaths occurred within 24 hr of dosing. Clinical signs of toxicity (salivation, lacrimation, ataxia, lethargy, and convulsions) and decreased body weight were observed prior to death. Gross lesions were observed in lungs (discoloration, mottling, and congestion) and stomach and intestines (distended with gas or fluid) of dead animals. No exposure-related changes were observed in surviving animals.	Rat LD_{50} (95% CI) Male: 165 (107−254) mg/kg Female: 175 (105−292) mg/kg Combined: 174 (131−231) mg/kg FEL: 180 mg/kg (death)	Borriston (1980b); Borriston (1980c)
Acute (oral)	The LD_{50} for commercial crotonaldehyde was determined in groups of rats. No further details were provided.	The reported LD_{50} (95% CI) was 0.22 (0.20−0.25) g/kg.	Rat LD_{50} (95% CI) = 220 (200−250) mg/kg	Mellon Institute of Industrial Research (1948)
Acute (oral)	Albino rats (6 M/group) were exposed once to 0.1 or 1 g commercial crotonaldehyde/kg via gavage in water plus 1% Tergitol. Animals were observed for mortality and clinical signs. Gross necropsy was conducted.	At 1 g/kg, death occurred within 10 min. Prior to death, animals exposed to 1 g/kg showed clinical signs of toxicity (pain, jumping around). Gross necropsy showed pale kidney, excessive peritoneal fluid, and congestion in the liver, stomach, and intestine. The LD_{50} was reported to be approximately 0.3 g/kg.	Rat LD_{50} = 300 mg/kg	Mellon Institute of Industrial Research (1942)

Table 4B. Other *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95% *trans*-; CASRN 4170-30-3) Studies

Test[a]	Materials and Methods	Results	Conclusions	References
Acute (oral)	The LD_{50} for commercial crotonaldehyde was determined in groups of rats. No further details were provided.	The study authors classified crotonaldehyde as having moderate toxicity via the oral route.	Rat LD_{50} = 300 mg/kg	Kennedy and Graepel (1991)
Supporting evidence—noncancer effects in animals following inhalation exposure				
Acute (inhalation)	The LC_{50} for commercial crotonaldehyde was determined in groups of rats. No further details were provided.	The 4-hr rat LC_{50} was 100 ppm. The study authors classified crotonaldehyde as having moderate toxicity via the inhalation route.	4-hr rat LC_{50} = 286 mg/m^3 [b]	Kennedy and Graepel (1991)
Acute (inhalation)	In an LC_{50} study, rats (3/group; sex and strain not reported) were exposed to 0.099, 0.16−0.28, 0.38, 0.48, 1.03, 2.6, 3.6, 6, 37, or 46.5 mg/L of commercial crotonaldehyde for up to 6 hr. Mortality, clinical signs, and body weight were recorded over 2-wk observation period.	Reported LC_{50} was <0.48 mg/L but >0.16−0.28 mg/L. All rats exposed to ≥1.03 mg/L died. At ≥2.6 mg/L, rats died during the first 43−120 min of exposure; at 1.03 mg/L, death occurred 3.5−24 hr after exposure. At 0.48 mg/L, 2/3 rats died within 1 d; surviving rats lost weight. At 0.38 mg/L, all rats died within 48 hr. No deaths were observed in groups exposed to 0.099 or 0.16−0.28 mg/L. Clinical signs were observed in all exposure groups (gasping, nasal irritation, pink extremities, and labored breathing), with serious signs (tremors, prostration, and convulsions) at lethal doses.	6-hr rat LC_{50} (range) = 280−480 mg/m^3 [c] FEL: 380 mg/m^3 (death)	Eastman Kodak (1961)
Acute (inhalation)	Rats (4/group; strain and sex not specified) were exposed to air saturated with commercial crotonaldehyde for 1 or 10 min. Rats were held for 7-d observation period.	All rats exposed for 10 min died on the same day of exposure; all rats exposed for 1 min survived. The LT_{50} was determined to be 3 min.	Exposure to air saturated with commercial crotonaldehyde will kill 50% of animals after approximately 3 min.	Mellon Institute of Industrial Research (1942)
Acute (inhalation)	Groups of guinea pigs were exposed to 1,000 or 2,000 ppm commercial crotonaldehyde for up to 30 min. No further details were provided.	Mortality at 1,000 ppm was 0% at 5 min, 20% at 10 min, and 50% at 30 min. Mortality at 2,000 ppm was 50% at 15 min and 100% at 30 min.	FEL: 2,870 mg/m^3 (death)[b]	Mellon Institute of Industrial Research (1942)

Table 4B. Other *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95% *trans*-; CASRN 4170-30-3) Studies

Test[a]	Materials and Methods	Results	Conclusions	References
Supporting evidence—noncancer effects in animals following dermal or ocular exposure				
Acute (dermal lethality)	The LD_{50} for commercial crotonaldehyde was determined in groups of rabbits. No further details were provided.	Rabbit LD_{50} (95% CI) = 0.38 (0.27−0.52) mL/kg	Rabbit LD_{50} (95% CI) = 330 (230−450) mg/kg[d]	Mellon Institute of Industrial Research (1948)
Acute (dermal lethality)	Male and female guinea pigs (6−12/group; strain and sex not specified) were exposed to 0.1 or 1 g/kg commercial crotonaldehyde under occluded conditions for 2 or 24 hr.	Mortality was 6/6 at 1 g/kg and 0/6 at 0.1 g/kg for both durations. The LD_{50} was reported to be approximately 0.3 g/kg. Skin was tanned brown.	Guinea pig LD_{50} values: 2-hr LD_{50} = 300 mg/kg 24-hr LD_{50} = 300 mg/kg	Mellon Institute of Industrial Research (1942)
Short term (dermal lethality)	Male and female guinea pigs (6−12/group; strain and sex not specified) were exposed to 0.01, 0.1, or 1 g/kg commercial crotonaldehyde under occluded conditions for 4 d.	Mortality was 1/12 at 0.01 g/kg and 6/6 at ≥0.1 g/kg. The LD_{50} was reported to be approximately 0.03 g/kg. Skin was tanned a dark brown with slight necrosis.	4-d guinea pig LD_{50} = 30 mg/kg	Mellon Institute of Industrial Research (1942)
Acute (ocular irritation)	An eye irritation study with commercial crotonaldehyde was conducted in rabbits. No further details were provided.	Very severe necrosis was observed at 0.001 mL.	Crotonaldehyde is a severe eye irritant.	Mellon Institute of Industrial Research (1942)
Supporting evidence—noncancer effects in animals following exposure via other routes				
Acute (i.p.)	Swiss albino mice (5 M/group) were exposed to *trans*-crotonaldehyde once via i.p. injections of 0, 8, 16, or 32 µL/kg (0, 7, 14, or 27 mg/kg). The frequency of abnormal sperm heads was determined at 1, 3, or 5 wk in spermatozoa, spermatid, and preleptotene spermatogonia, respectively.	Sperm head abnormalities were observed in spermatozoa and spermatid at ≥14 mg/kg and in preleptotene spermatogonia at 27 mg/kg. Common types observed included short hooked, without hook, giant amorphous, and banana.	NA	Jha and Kumar (2006)

Table 4B. Other *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95% *trans*-; CASRN 4170-30-3) Studies

Test[a]	Materials and Methods	Results	Conclusions	References
Short term (i.p.)	Swiss albino mice (5 M/group) were exposed to *trans*-crotonaldehyde via daily i.p. injections of 0, 8, 16, or 32 µL/kg-d (0, 7, 14, or 28 mg/kg-d) for 5 d. Males were then mated with groups of 40 unexposed females for 5 wk.	A significant decrease in fertility index was observed at ≥14 mg/kg-d during the 2nd and 3rd wk of mating and at 28 mg/kg-d during the 4th wk of mating.	NA	Jha et al. (2007)

[a]Acute = exposure for ≤24 hours; short term = repeated exposure for >24 hours, ≤30 days; subchronic = repeated exposure for >30 days, ≤10% lifespan (>30 days up to approximately 90 days in typically used laboratory animal species); chronic = repeated exposure for >10% lifespan for humans (more than approximately 90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).
[b]Concentration in mg/m^3 = concentration in ppm × molecular weight (70.09 g/mol) ÷ 24.45.
[c]Concentration in mg/m^3 = concentration in mg/L × 1,000 L/m^3.
[d]Dose in mg/kg = dose in mL/kg × density (0.869 g/mL) × 1,000 mg/g.
[e]Dose in mg = dose in nmol × molecular weight (70.09 ng/nmol) × 1 µg/1,000 ng × 1 mg/1,000 µg.

CI = confidence interval; FEL = frank effect level; i.p. = intraperitoneal; LC_{50} = median lethal concentration; LD_{50} = median lethal dose; LT_{50} = medial lethal time; M = male(s); NDr = not determined; S-D = Sprague-Dawley.

Supporting Studies for Noncarcinogenic Effects in Animals

Acute Oral Toxicity

Acute oral lethality studies with commercial crotonaldehyde reported median lethal dose (LD_{50}) values in rats ranging from 165 to 300 mg/kg (Kennedy and Graepel, 1991; Borriston, 1980b, c; Mellon Institute of Industrial Research, 1948, 1942). Mortality was observed at acute oral doses as low as 160 mg/kg, with no mortality observed at doses ≤107.5 mg/kg (Borriston, 1980b, c). Clinical signs observed at lethal doses included salivation, lacrimation, ataxia, excitability followed by lethargy, and convulsions. In the animals that died, gross lesions were observed in the lungs (discoloration, mottling, congestion), stomachs, and intestines (distended with gas or fluid).

Acute Inhalation Toxicity

trans-Crotonaldehyde is a potent respiratory irritant. Acute inhalation lethality studies with commercial crotonaldehyde reported a 6-hour median lethal concentration (LC_{50}) in rats of 280−480 mg/m^3 (Eastman Kodak, 1961) and a 4-hour LC_{50} in rats of 286 mg/m^3 (Kennedy and Graepel, 1991). Exposure to air saturated with commercial crotonaldehyde resulted in the death of 50% of exposed rats within 3 minutes; all rats died within 10 minutes (Mellon Institute of Industrial Research, 1942). Thirty-minute exposure to 2,870 or 5,740 mg/m^3 killed 50% and 100% of exposed guinea pigs, respectively (Mellon Institute of Industrial Research, 1942). Clinical signs observed at lethal concentrations in these studies included excitation, tremors, convulsions, marked respiratory distress (labored breathing, gasping), lacrimation, nasal irritation, pink extremities, and weight loss. Mild clinical signs of respiratory distress, nasal irritation, and lacrimation were also observed at nonlethal concentrations as low as 99 mg/m^3. Hemorrhage and hyperemia were observed in the lungs, heart, liver, and kidneys of some animals that died.

Ocular and Dermal Toxicity

Commercial crotonaldehyde is a severe eye irritant in rabbits and is classified as a corrosive substance (Mellon Institute of Industrial Research, 1942). In dermal lethality studies, the LD_{50} values in rabbits or guinea pigs following exposure up to 24 hours were 300−330 mg/kg; the 4-day LD_{50} value in guinea pigs was 30 mg/kg (Mellon Institute of Industrial Research, 1948, 1942).

Other Route Toxicity

Available injection studies include acute and short-term studies primarily focused on acute lethality or toxicity to the reproductive, hematological, or immune systems.

Decreased male fertility was observed when male mice were given i.p. injections ≥14 mg/kg-day for 5 days prior to mating with unexposed females; fertility was comparable to control at 7 mg/kg-day (Jha et al., 2007). Damage to male germ cells at all stages of spermatogenesis were also observed in mice following single i.p. injections to *trans*-crotonaldehyde at a dose of 14 mg/kg, but sperm abnormalities were not observed at 7 mg/kg (Jha and Kumar, 2006).

Absorption, Distribution, Metabolism, and Excretion Studies

No studies evaluating absorption, distribution, or excretion of *trans*-crotonaldehyde and commercial crotonaldehyde have been identified.

Available studies indicate that *trans*-crotonaldehyde can be metabolized via oxidation and conjugation with thiols. Specifically, *trans*-crotonaldehyde has been shown to conjugate with thiols in vitro, including glutathione (GSH) and *N*-acetylcysteine, and is a substrate for glutathione S-transferase (GST) (van Iersel et al., 1996; Wang et al., 1992). The reaction rates of thiols with crotonaldehyde were 2-mercaptoethanesulfonate > GSH > *N*-acetylcysteine (Wang et al., 1992).

Mode-of-Action/Mechanistic Studies

A target of non-neoplastic toxicity in rodents following gavage exposure is the forestomach [Hazleton Laboratories (1986a) as cited in NTP-PWG (1987); Hazleton Laboratories (1986b)]. Observed lesions at this portal of entry are likely due to the irritative and corrosive nature of crotonaldehyde (TRL, 1986). This mechanism is also relevant to observed nasal lesions in the subchronic rat study by Hazleton Laboratories (1986b) because nasal effects were considered to be a localized effect from exhaled crotonaldehyde rather than an effect from blood-circulated crotonaldehyde (NTP-PWG, 1987).

Several in vitro studies in cells from the human or mammalian respiratory tract report alterations following exposure to *trans*-crotonaldehyde, including cytotoxicity, apoptosis, alterations in immune parameters (e.g., increased cytokine secretion, decreased phagocytic activity of alveolar macrophages), and induction of genes associated with inflammation and oxidative stress (Yang et al., 2013b; Yang et al., 2013a).

As discussed in the "Genotoxicity Studies" section and Table 4A, *trans*-crotonaldehyde is mutagenic under certain conditions. It has been proposed that cyclic propano DNA adducts associated with crotonaldehyde exposure are highly stable promutagenic lesions and may underlie mutagenicity and subsequent tumor formation following exposure to *trans*-crotonaldehyde (Voulgaridou et al., 2011). In support, DNA-containing *trans*-crotonaldehyde-induced adducts was transfected into mammalian COS-7 cells, and replication in the presence of these adducts resulted in the induction of mutations (Fernandes et al., 2005). Known endogenous production of aldehyde cyclic adducts makes the potential role of *trans*-crotonaldehyde-mediated adduct formation in carcinogenicity unclear. To this point, the liver, which is the site of tumor formation following *trans*-crotonaldehyde exposure (Chung et al., 1986), shows the highest number of DNA adducts in rats following oral exposure (Budiawan and Eder, 2000; Budiawan et al., 2000).

DERIVATION OF PROVISIONAL VALUES

DERIVATION OF PROVISIONAL ORAL REFERENCE DOSES

The database of potentially relevant studies for deriving oral reference values for *trans*-crotonaldehyde includes one subchronic gavage study in rats (Hazleton Laboratories, 1986b), a subchronic gavage study in mice [Hazleton Laboratories (1986a) as cited in NTP-PWG (1987)], a reproductive study in rats (Hazleton Laboratories, 1987), and a chronic drinking water study in rats (Chung et al., 1986). Of the available studies, only Chung et al. (1986) is peer reviewed; however, data reporting for non-neoplastic endpoints in this study is inadequate for evaluation. The two subchronic gavage studies in mice and rats, while not peer reviewed, were performed by a contract research laboratory with extensive documentation of the experimental conditions and methodologies employed. A comprehensive array of endpoints was evaluated in each study, and a review of the pathology results was conducted and confirmed by the NTP-PWG. As part of this review, the NTP-PWG examined the pathology results reported by two independent pathologists, the pathology quality assessment report, and other toxicological data and determined each to be of sufficient scientific quality. Therefore, although there is no explicit indication that these studies were performed under Good Laboratory Practice (GLP) guidelines, review of the study design and data report by the NTP-PWG sufficiently increases confidence in the ability to use these data to derive provisional reference dose (p-RfD) values.

Derivation of a Subchronic Provisional Reference Dose

Available unpublished repeated-dose subchronic gavage studies in rats and mice identify the forestomach as the most sensitive target of toxicity [Hazelton Laboratories (1986a) as cited in NTP-PWG (1987); Hazleton Laboratories (1986b)]. The lowest identified LOAEL for forestomach lesions is 14 mg/kg-day in female rats. As described above, although not peer reviewed, the Hazleton Laboratories [(1986a) as cited in NTP-PWG (1987); (1986b)] studies were performed by a contract laboratory with extensive documentation of experimental and laboratory conditions. Furthermore, the accompanying data and histological results were evaluated by the NTP-PWG for accuracy. Because forestomach lesions were observed in both sexes of mice and rats and the data were reviewed by an independent party (NTP-PWG), the database supporting the identification of forestomach lesions as a viable critical effect is of sufficient confidence to warrant the development of p-RfDs within the main body of this assessment.

In addition to the significantly elevated rates of forestomach lesions, several other pathologies were observed to be significantly altered relative to control-treated animals. An increased rate of nasal inflammation was reported in male and female rats (Hazleton Laboratories, 1986b). Upon review of the reported data, the NTP-PWG concluded that the observed nasal changes should be considered serous exudation (clear, thin, and watery exudate associated with tissue repair and inflammation), and not acute inflammation as concluded in the laboratory report. The exudates were thought to be reactions to the highly volatile *trans*-crotonaldehyde. As such, exudates were usually present in the early-death rats, which were also observed to have significant levels of oil droplets within the lungs, indicating that these early deaths were likely the result of gavage errors (accidental administration into the lung). Taken together, these data suggest that early-death animals likely had *trans*-crotonaldehyde deposited into the lungs, where it could readily volatilize into nasal passages. The NTP-PWG suggested that the apparent serous exudations may have been exacerbated by postmortem change in these animals. Combined, these results suggest that the observed nasal effects may be the

result of gavage error and may have increased in severity after death of the rats, precluding the use of nasal lesions/serous nasal exudations as a potential critical effect.

Hazleton Laboratories (1986b) also identified significant absolute and relative thymus weight decreases in female rats at the highest dose examined. This was the only evidence to suggest effects in the thymus. Because these effects were observed only in female rats (and not male rats or male or female mice) and because there was 50% mortality in this dose group (only five rats alive at the end of the study), evidence of effects within the thymus are not sufficiently supported and were, therefore, not considered as a potential POD.

Data for forestomach lesions in male and female F344 rats (see Appendix D) reported by Hazleton Laboratories (1986b) were modeled using all available dichotomous models, as appropriate, in the U.S. EPA's Benchmark Dose Software (BMDS, Version 2.6) (see Appendix E). Data for forestomach lesions in mice were not modeled because incidence data are not available [Hazelton Laboratories (1986a) as cited in NTP-PWG (1987)]. The modeled data for the F344 rat study are shown in Table D-3. ADDs were used for modeling. In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), the Agency endorses body-weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans (calculation of HED) for the purpose of deriving an RfD from effects that are not portal-of-entry effects or effects resulting from direct exposure of neonatal or juvenile animals. Because forestomach lesions following gavage exposure may be portal-of-entry effects, doses were not converted into HEDs prior to modeling. The standard reporting value benchmark response (BMR) for dichotomous data of 10% extra risk was used.

The benchmark dose lower confidence limit (BMDL) (ADD) of 3 mg/kg-day, based on incidence of forestomach hyperplasia in male rats exposed to crotonaldehyde via gavage 5 days/week for 13 weeks (Hazleton Laboratories, 1986b), provides the lowest candidate point of departure (POD value), and was selected as the POD for deriving the subchronic p-RfD. Forestomach lesions are generally believed to be a manifestation of chronic irritation at the portal of entry associated with oral exposure to corrosive chemicals. The forestomach thickening, nodule formation, and hyperplasia reported in male and female rats [Hazelton Laboratories (1986a) as cited in NTP-PWG (1987); Hazleton Laboratories (1986b)] and forestomach hyperplasia reported in male and female mice [Hazelton Laboratories (1986a) as cited in NTP-PWG (1987)] are consistent with the potential consequence of portal-of-entry exposure to an agent promoting chronic irritation.

The subchronic p-RfD is derived by applying a composite uncertainty factor (UF_C) of 300 (reflecting an interspecies uncertainty factor [UF_A] of 10, an intraspecies uncertainty factor [UF_H] of 10, and a database uncertainty factor [UF_D] of 3) to the selected POD of 3 mg/kg-day.

$$\begin{aligned} \textbf{Subchronic p-RfD} &= \text{POD (ADD)} \div UF_C \\ &= 3 \text{ mg/kg-day} \div 300 \\ &= \mathbf{1 \times 10^{-2} \text{ mg/kg-day}} \end{aligned}$$

Table 5 summarizes the uncertainty factors for the subchronic p-RfD for *trans*-crotonaldehyde.

Table 5. Uncertainty Factors for the Subchronic p-RfD for *trans*-Crotonaldehyde (CASRN 123-73-9)

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied to account for uncertainty associated with extrapolating from animals to humans. Cross-species dosimetric adjustment (HED calculation) was not performed because the critical endpoint may be a portal-of-entry effect.
UF_D	3	A UF_D of 3 ($10^{0.5}$) is applied to account for deficiencies and uncertainties in the database. The database for oral exposure to *trans*-crotonaldehyde consists of subchronic toxicity studies in two species, a single chronic carcinogenicity study in male rats, and a 1-generation reproduction study in rats. There are no developmental toxicity studies available following exposure via any route.
UF_H	10	A UF_H of 10 is applied to account for human variability in susceptibility, in the absence of information to assess toxicokinetic and toxicodynamic variability of *trans*-crotonaldehyde in humans.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because the subchronic POD was derived from a 13-wk study.
UF_C	300	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

BMDL = benchmark dose lower confidence limit; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Confidence in the subchronic p-RfD for *trans*-crotonaldehyde is medium, as described in Table 6.

Table 6. Confidence Descriptors for the Subchronic p-RfD for *trans*-Crotonaldehyde (CASRN 123-73-9)

Confidence Categories	Designation	Discussion
Confidence in study	M	Confidence in the principal study (Hazleton Laboratories, 1986b) is medium. This study is not peer reviewed; however, it used an adequate number of animals (10 rats/sex) at 6 dose levels (including controls). Furthermore, despite a lack of access to the complete data set, a comprehensive suite of endpoints was examined (clinical symptoms, body and organ weights, hematological and clinical chemistry, and pathology), and the data was subsequently reviewed by the NTP-PWG (NTP-PWG, 1987).
Confidence in database	M	Confidence in the database for *trans*-crotonaldehyde is medium. The relevant database consists of one short-term dietary study in rats (Borriston, 1980a), as well as subchronic gavage studies in rats (Hazleton Laboratories, 1986b) and mice [Hazelton Laboratories (1986a), as cited in NTP-PWG (1987)], one single-generation reproduction study (Hazleton Laboratories, 1987), and one chronic carcinogenicity study (Chung et al., 1986). None of the subchronic studies are peer reviewed; however, the NTP-PWG reviewed and confirmed the data and pathology in the subchronic gavage studies (NTP-PWG, 1987). Generally, data from the mouse study corroborated the effects observed in the similarly designed rat gavage study identified as the principal study. Neurotoxicity and teratogenicity were not evaluated.
Confidence in subchronic p-RfD[a]	M	The overall confidence in the subchronic p-RfD is medium.

[a]The overall confidence cannot be greater than the lowest entry in the table (medium).

M = medium; NTP-PWG = National Toxicology Program's Pathology Working Group; p-RfD = provisional reference dose.

Derivation of a Chronic Provisional Reference Dose

No adequate chronic oral non-neoplastic data are available. Data from the Chung et al. (1986) study were not considered as the basis for deriving a chronic p-RfD due to incomplete reporting of relevant data as well as inconsistencies in the dose-response of observed liver tumors and inconsistent reporting of degenerative liver lesions accompanying body-weight changes observed in the high-dose male rats. This lack of reporting precludes an analysis of potential precursor effects at the low dose that could underly the chronic toxicity manifested through reduced body weight at the high dose (Chung et al., 1986). Therefore, the $BMDL_{10}$ (ADD) of 3 mg/kg-day for increased incidence of forestomach hyperplasia in male rats exposed to crotonaldehyde via gavage 5 days/week for 13 weeks (Hazleton Laboratories, 1986b) was also selected as the POD for derivation of the chronic p-RfD.

The chronic p-RfD is derived by applying a UF_C of 3,000 (reflecting a UF_A of 10, a UF_H of 10, a UF_D of 3, and a subchronic-to-chronic extrapolation uncertainty factor [UF_S] of 10) to the selected POD of 3 mg/kg-day.

Chronic p-RfD = POD (ADD) ÷ UF_C
= 3 mg/kg-day ÷ 3,000
= **1×10^{-3} mg/kg-day**

Table 7 summarizes the uncertainty factors for the chronic p-RfD for *trans*-crotonaldehyde.

Table 7. Uncertainty Factors for the Chronic p-RfD for *trans*-Crotonaldehyde (CASRN 123-73-9)

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied to account for uncertainty associated with extrapolating from animals to humans. Cross-species dosimetric adjustment (HED calculation) was not performed because the critical endpoint may be a portal-of-entry effect.
UF_D	3	A UF_D of 3 ($10^{0.5}$) is applied to account for deficiencies and uncertainties in the database. The database for oral exposure to *trans*-crotonaldehyde consists of subchronic toxicity studies in two species, one chronic carcinogenicity study in rats, and a one-generation reproduction study in rats. There are no developmental toxicity studies available following exposure via any route.
UF_H	10	A UF_H of 10 is applied to account for human variability in susceptibility, in the absence of information to assess toxicokinetic and toxicodynamic variability of *trans*-crotonaldehyde in humans.
UF_L	1	A UF_L of 1 is applied because the POD is a BMDL.
UF_S	10	A UF_S of 10 is applied because the chronic POD was derived from a 13-wk study.
UF_C	3,000	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$.

BMDL = benchmark dose lower confidence limit; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point of departure; p-RfD = provisional reference dose; UF = uncertainty factor; UF_A = interspecies uncertainty factor; UF_C = composite uncertainty factor; UF_D = database uncertainty factor; UF_H = intraspecies uncertainty factor; UF_L = LOAEL-to-NOAEL uncertainty factor; UF_S = subchronic-to-chronic uncertainty factor.

Confidence in the chronic p-RfD for *trans*-crotonaldehyde is low, as described in Table 8.

Table 8. Confidence Descriptors for the Chronic p-RfD for *trans*-Crotonaldehyde (CASRN 123-73-9)

Confidence Categories	Designation	Discussion
Confidence in study	M	Confidence in the principal study (Hazleton Laboratories, 1986b) is medium. This study is not peer reviewed; however, it used a standard number of animals (10 rats/sex) at 6 dose levels (including controls). Furthermore, despite a lack of access to the complete data set, a comprehensive suite of endpoints was examined (clinical symptoms, body and organ weights, hematological and clinical chemistry, and pathology), and the data was subsequently reviewed by the NTP-PWG (NTP-PWG, 1987).
Confidence in database	L	There were no chronic studies identified that investigated non-neoplastic endpoints, so confidence in the chronic database for *trans*-crotonaldehyde is low. The relevant database consists of one short-term dietary study in rats (Borriston, 1980a), as well as subchronic gavage studies in rats (Hazleton Laboratories, 1986b) and mice [Hazelton Laboratories (1986a) as cited in NTP-PWG (1987)] and one single-generation reproduction study (Hazleton Laboratories, 1987) which found no effects. None of these studies are peer reviewed; however, the NTP-PWG reviewed and confirmed the data and pathology in the subchronic gavage studies (NTP-PWG, 1987). Generally, data from this mouse study corroborated the effects observed in the similarly designed rat gavage study identified as the principal study. Neurotoxicity and teratogenicity were not evaluated.
Confidence in chronic p-RfD[a]	L	The overall confidence in the chronic p-RfD is low.

[a]The overall confidence cannot be greater than the lowest entry in the table (low).

L = low; M = medium; NTP-PWG = National Toxicology Program's Pathology Working Group; p-RfD = provisional reference dose.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No studies have been identified that were adequate for deriving inhalation toxicity values.

SUMMARY OF NONCANCER PROVISIONAL REFERENCE VALUES

A summary of the noncancer provisional reference values is shown in Table 9.

Table 9. Summary of Noncancer Reference Values for *trans*-Crotonaldehyde (CASRN 123-73-9)

Toxicity Type (units)	Species/ Sex	Critical Effect	p-Reference Value	POD Method	POD (ADD)	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/M	Forestomach hyperplasia	1×10^{-2}	$BMDL_{10}$	3	300	Hazleton Laboratories (1986b)
Chronic p-RfD (mg/kg-d)	Rat/M	Forestomach hyperplasia	1×10^{-3}	$BMDL_{10}$	3	3,000	Hazleton Laboratories (1986b)
Subchronic p-RfC (mg/m^3)	NDr						
Chronic p-RfC (mg/m^3)	NDr						

ADD = adjusted daily dose; BMDL = benchmark dose lower confidence limit (subscripts denote benchmark response: i.e., 10 = dose associated with a 10% extra risk in parameter); M = male(s); NDr = not derived; POD = point of departure; p-RfC = provisional reference concentration; p-RfD = provisional reference dose; UF_C = composite uncertainty factor.

PROVISIONAL CARCINOGENICITY ASSESSMENT

A provisional cancer assessment was not prepared for *trans*-crotonaldehyde. Although IRIS (U.S. EPA, 2005) conducted a cancer assessment for this compound (weight of evidence [WOE] = "C; possible human carcinogen"), the data were not adequate for deriving quantitative estimates of carcinogenic risk by oral or inhalation exposure. Additionally, liver tumor incidence data reported in Chung et al. (1986) could not be modeled due to a lack of dose-response as indicated by a lack of tumorigenesis in the high-dose group (see Table 10).

Table 10. Summary of Cancer Risk Estimates for *trans*-Crotonaldehyde (CASRN 123-73-9)

Toxicity Type (units)	Species/ Sex	Tumor Type	Cancer Risk Estimate	Principal Study
p-OSF $(mg/kg\text{-}d)^{-1}$	NDr			
p-IUR $(mg/m^3)^{-1}$	NDr			

NDr = not derived; p-IUR = provisional inhalation unit risk; p-OSF = provisional oral slope factor.

APPENDIX A. LITERATURE SEARCH STRATEGY

Non-date-limited literature searches were conducted in May 2019 and updated in July 2020 for studies relevant to the derivation of provisional toxicity values for *trans*-crotonaldehyde (CASRN 123-73-9) and the commercial crotonaldehyde mixture (CASRN 4170-30-3; >95% *trans*-isomer). Searches were conducted using U.S. EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: PubMed, TOXLINE (including TSCATS1), and Web of Science. The following databases were searched outside of HERO for health-related values: American Conference of Governmental Industrial Hygienists (ACGIH), Agency for Toxic Substances and Disease Registry (ATSDR), California Environmental Protection Agency (CalEPA), Defense Technical Information Center (DTIC), European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), European Chemicals Agency (ECHA), U.S. EPA Chemical Data Access Tool (CDAT), U.S. EPA ChemView, U.S. EPA Integrated Risk Information System (IRIS), U.S. EPA Health Effects Assessment Summary Tables (HEAST), U.S. EPA Office of Water (OW), International Agency for Research on Cancer (IARC), U.S. EPA TSCATS2/TSCATS8e, U.S. EPA High Production Volume (HPV), Chemicals via IPCS INCHEM, European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC), Japan Existing Chemical Data Base (JECDB), European Chemicals Agency (ECHA), Organisation for Economic Cooperation and Development (OECD) Screening Information Data Sets (SIDS), OECD International Uniform Chemical Information Database (IUCLID), OECD HPV, National Institute for Occupational Safety and Health (NIOSH), National Toxicology Program (NTP), Occupational Safety and Health Administration (OSHA), and World Health Organization (WHO).

LITERATURE SEARCH STRINGS

Pubmed

"123-73-9" OR "4170-30-3" OR "2 butenal"[tw] OR "2 E butenal"[tw] OR "2E but 2 enal"[tw] OR "b methylacrolein"[tw] OR "but 2 enal, E"[tw] OR "crotonal"[tw] OR "crotonaldehyde"[tw] OR "crotonaldehydes"[tw] OR "croton aldehyde"[tw] OR "crotonic aldehyde"[tw] OR "crotylaldehyde"[tw] OR "E but 2 en 1 al"[tw] OR "E but 2 enal"[tw] OR "E crotonaldehyd"[tw] OR "trans 2 buten 1 al"[tw] OR "trans but 2 enal"[tw] OR "topanel CA"[tw] OR "UN 1143"[tw]

WOS

TS="123 73 9" OR TS="4170 30 3" OR TS="2 butenal" OR TS="2 E butenal" OR TS="2E but 2 enal" OR TS="b methylacrolein" OR TS="but 2 enal E" OR TS="crotonal" OR TS="crotonaldehyde" OR TS="crotonaldehydes" OR TS="croton aldehyde" OR TS="crotonic aldehyde" OR TS="crotylaldehyde" OR TS="E but 2 en 1 al" OR TS="E but 2 enal" OR TS="E crotonaldehyd" OR TS="trans 2 buten 1 al" OR TS="trans but 2 enal" OR TS="topanel CA" OR TS="UN 1143") AND ((WC=("Toxicology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Neurosciences" OR "Obstetrics & Gynecology" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Respiratory System" OR "Urology & Nephrology" OR "Anatomy & Morphology" OR "Andrology" OR "Pathology" OR "Otorhinolaryngology" OR "Ophthalmology" OR "Pediatrics" OR "Oncology" OR "Reproductive Biology" OR "Developmental Biology" OR "Biology" OR "Dermatology" OR "Allergy" OR "Public, Environmental & Occupational Health") OR SU=("Anatomy & Morphology" OR "Cardiovascular System & Cardiology" OR "Developmental Biology" OR "Endocrinology &

Metabolism” OR “Gastroenterology & Hepatology” OR “Hematology” OR “Immunology” OR “Neurosciences & Neurology” OR “Obstetrics & Gynecology” OR “Oncology” OR “Ophthalmology” OR “Pathology” OR “Pediatrics” OR “Pharmacology & Pharmacy” OR “Physiology” OR “Public, Environmental & Occupational Health” OR “Respiratory System” OR “Toxicology” OR “Urology & Nephrology” OR “Reproductive Biology” OR “Dermatology” OR “Allergy”)) OR (TS=“rat” OR TS=“rats” OR TS=“mouse” OR TS=“murine” OR TS=“mice” OR TS=“guinea” OR TS=“muridae” OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS=“dog” OR TS=“dogs” OR TS=beagle* OR TS=“canine” OR TS=“cats” OR TS=“feline” OR TS=“pig” OR TS=“pigs” OR TS=“swine” OR TS=“porcine” OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset* OR TS=“child” OR TS=“children” OR TS=adolescen* OR TS=infant* OR TS=“worker” OR TS=“workers” OR TS=“human” OR TS=patient* OR TS=“mother” OR TS=“fetal” OR TS=“fetus” OR TS=“citizens” OR TS=“milk” OR TS=“formula” OR TS=epidemio* OR TS=population* OR TS=exposure* OR TS=“questionnaire” OR SO=epidemio*) OR TI=toxic*

TOXLINE

@SYN0+@OR+(“2+butenal”+”2+E+butenal”+”2E+but+2+enal”+”b+methylacrolein”+”but+2+enal+E”+crotonal+crotonaldehyde+crotonaldehydes+”croton+aldehyde”+”crotonic+aldehyde”+crotylaldehyde+”E+but+2+en+1+al”+”E+but+2+enal”+”E+crotonaldehyd”+”trans+2+buten+1+al”+”trans+but+2+enal”+”topanel+CA”+”UN+1143”+@TERM+@rn+123-73-9+@TERM+@rn+4170-30-3)+@NOT+@org+pubmed+pubdart+nih

TSCATS

@TERM+@rn+”123-73-9”+@org+TSCATS

APPENDIX B. DETAILED PECO CRITERIA

Table B-1. Population, Exposure, Comparison, and Outcome (PECO) Criteria

PECO Element	Evidence
Population	Humans, laboratory mammals, and other animal models of established relevance to human health (e.g., *Xenopus* embryos); mammalian organs, tissues, and cell lines; and bacterial and eukaryote models of genetic toxicity.
Exposure	In vivo (all routes), ex vivo, and in vitro exposure to the chemical of interest, including mixtures to which the chemical of interest may contribute significantly to exposure or observed effects.
Comparison	Any comparison (across dose, duration, or route) or no comparison (e.g., case reports without controls).
Outcome	Any endpoint suggestive of a toxic effect on any bodily system, or mechanistic change associated with such effects. Any endpoint relating to disposition of the chemical within the body.

APPENDIX C. SCREENING PROVISIONAL VALUES

No screening provisional values are derived for *trans*-crotonaldehyde.

APPENDIX D. DATA TABLES

Table D-1. Terminal Body Weight and Select Organ Weights in Male F344 Rats Exposed to Commercial Crotonaldehyde (CASRN 4170-30-3) via Gavage in Corn Oil for 13 Weeks (5 Days/Week)[a]

	Dose Group, mg/kg-d (ADD)					
Endpoint	**0**	**2.5 (1.8)**	**5 (4)**	**10 (7.1)**	**20 (14)**	**40 (29)**
Mortality[b]	0/10 (0%)	0/10 (0%)	0/10 (0%)	3/10 (30%)	3/10 (30%)	5/10 (50%)
Terminal body weight (g)[c, d]	320.8 ± 18.7	323.3 ± 13.6 (+1%)	336.5 ± 20.9 (+5%)	333.7 ± 7.2 (+4%)	318.4 ± 17.8 (−0.7%)	290.4 ± 15.5* (−9%)
Liver weight[c, d]						
Absolute (g)	13.35 ± 1.00	12.98 ± 0.53 (−3%)	13.03 ± 1.70 (−2%)	14.03 ± 1.03 (+5%)	13.21 ± 1.19 (−1%)	12.46 ± 1.01 (−7%)
Relative (%)	4.163 ± 0.217	4.023 ± 0.259 (−3%)	3.859 ± 0.284 (−7%)	4.205 ± 0.291 (+1%)	4.147 ± 0.259 (−0.4%)	4.294 ± 0.314 (+3%)
Kidney weight[c, d]						
Absolute (g)	1.14 ± 0.07	1.11 ± 0.09 (−3%)	1.18 ± 0.13 (+4%)	1.13 ± 0.11 (−0.9%)	1.15 ± 0.12 (+0.9%)	1.09 ± 0.11 (−4%)
Relative (%)	0.355 ± 0.016	0.344 ± 0.020 (−3%)	0.349 ± 0.019 (−2%)	0.340 ± 0.036 (−4%)	0.360 ± 0.027 (+1%)	0.378 ± 0.049 (+6%)
Thymus weight[c, d]						
Absolute (g)	0.308 ± 0.023	0.303 ± 0.039 (−2%)	0.299 ± 0.046 (−3%)	0.314 ± 0.030 (+2%)	0.287 ± 0.068 (−7%)	0.272 ± 0.049 (−12%)
Relative (%)	0.0966 ± 0.0102	0.0939 ± 0.0118 (−3%)	0.0888 ± 0.0140 (−8%)	0.0941 ± 0.0102 (−3%)	0.0895 ± 0.0168 (−7%)	0.0933 ± 0.0130 (−3%)
Brain weight[c, d]						
Absolute (g)	2.15 ± 0.09	2.10 ± 0.06 (−2%)	2.13 ± 0.09 (−0.9%)	2.15 ± 0.03 (0%)	2.09 ± 0.11 (−3%)	2.08 ± 0.09 (−3%)
Relative (%)	0.671 ± 0.033	0.649 ± 0.015 (−3%)	0.633 ± 0.026* (−6%)	0.645 ± 0.009 (−4%)	0.658 ± 0.042 (−2%)	0.718 ± 0.045 (+7%)

Table D-1. Terminal Body Weight and Select Organ Weights in Male F344 Rats Exposed to Commercial Crotonaldehyde (CASRN 4170-30-3) via Gavage in Corn Oil for 13 Weeks (5 Days/Week)[a]

Endpoint	Dose Group, mg/kg-d (ADD)					
	0	**2.5 (1.8)**	**5 (4)**	**10 (7.1)**	**20 (14)**	**40 (29)**
Testicle weight[c, d]						
Absolute (g)	1.532 ± 0.095	1.476 ± 0.114 (−4%)	1.509 ± 0.128 (−2%)	1.450 ± 0.080 (−5%)	1.469 ± 0.131 (−4%)	1.452 ± 0.106 (−5%)
Relative (%)	0.4784 ± 0.0288	0.4560 ± 0.0245 (−5%)	0.4490 ± 0.0211 (−6%)	0.4347 ± 0.0257* (−9%)	0.4618 ± 0.0376 (−3%)	0.5001 ± 0.0214 (+5%)

[a]Hazleton Laboratories (1986b).
[b]Values denote number of animals showing changes ÷ total number of animals examined (% incidence).
[c]Data are mean ± SD; n = 5−10/group.
[d]Value in parentheses is % change relative to control = [(treatment mean − control mean) ÷ control mean] × 100.
*Significantly different from control ($p < 0.05$), as reported by the study authors.

ADD = adjusted daily dose; SD = standard deviation.

Table D-2. Terminal Body Weight and Select Organ Weights in Female F344 Rats Exposed to Commercial Crotonaldehyde (CASRN 4170-30-3) via Gavage in Corn Oil for 13 Weeks (5 Days/Week)[a]

Endpoint	Dose Group, mg/kg-d (ADD)					
	0	**2.5 (1.8)**	**5 (4)**	**10 (7.1)**	**20 (14)**	**40 (29)**
Mortality[b]	1/10 (10%)	0/10 (0%)	1/10 (10%)	1/10 (10%)	7/10 (70%)	5/10 (50%)
Terminal body weight (g)[c, d]	198.9 ± 7.7	200.1 ± 6.6 (+0.6%)	202.8 ± 3.3 (+2%)	200.8 ± 13.1 (+1%)	201.9 ± 4.4 (+2%)	194.3 ± 8.2 (−2%)
Liver weight[c, d]						
Absolute (g)	6.80 ± 0.35	7.05 ± 0.48 (+4%)	7.20 ± 0.47 (+6%)	6.48 ± 0.63 (−5%)	7.24 ± 0.12 (+6%)	7.13 ± 1.11 (+5%)
Relative (%)	3.419 ± 0.142	3.525 ± 0.198 (+3%)	3.556 ± 0.264 (+4%)	3.226 ± 0.186 (−6%)	3.587 ± 0.039* (+5%)	3.666 ± 0.542 (+7%)
Kidney weight[c, d]						
Absolute (g)	0.74 ± 0.06	0.73 ± 0.05 (−1%)	0.76 ± 0.05 (+3%)	0.75 ± 0.05 (+1%)	0.77 ± 0.03 (+4%)	0.77 ± 0.04 (+4%)
Relative (%)	0.371 ± 0.029	0.366 ± 0.024 (−1%)	0.373 ± 0.026 (+0.5%)	0.376 ± 0.018 (+1%)	0.381 ± 0.011 (+3%)	0.396 ± 0.240 (+7%)
Thymus weight[c, d]						
Absolute (g)	0.261 ± 0.029	0.259 ± 0.024 (−0.8%)	0.251 ± 0.026 (−4%)	0.245 ± 0.022 (−6%)	0.260 ± 0.062 (−0.4%)	0.206 ± 0.023* (−21%)
Relative (%)	0.1310 ± 0.0131	0.1295 ± 0.0098 (−1%)	0.1239 ± 0.0131 (−5%)	0.1219 ± 0.0087 (−7%)	0.1283 ± 0.0293 (−2%)	0.1059 ± 0.0102* (−19%)
Brain weight[c, d]						
Absolute (g)	2.00 ± 0.10	1.98 ± 0.05 (−1%)	2.01 ± 0.06 (+0.2%)	2.01 ± 0.08 (+0.5%)	2.04 ± 0.07 (+2%)	1.99 ± 0.07 (−0.5%)
Relative (%)	1.004 ± 0.055	0.992 ± 0.035 (−1%)	0.990 ± 0.038 (−1%)	1.005 ± 0.057 (+0.1%)	1.011 ± 0.046 (+0.7%)	1.026 ± 0.054 (+2%)

[a]Hazleton Laboratories (1986b).
[b]Values denote number of animals showing changes ÷ total number of animals examined (% incidence).
[c]Data are mean ± SD; n = 3−10/group.
[d]Value in parentheses is % change relative to control = [(treatment mean − control mean) ÷ control mean] × 100.
*Significantly different from control ($p < 0.05$), as reported by the study authors.

ADD = adjusted daily dose; SD = standard deviation.

Table D-3. Forestomach and Nasal Lesions in F344 Rats Exposed to Commercial Crotonaldehyde (CASRN 4170-30-3) via Gavage in Corn Oil for 13 Weeks (5 Days/Week)[a]

Endpoint[b]	Dose Group, mg/kg-d (ADD)					
	0	2.5 (1.8)	5 (4)	10 (7.1)	20 (14)	40 (29)
Thickened forestomach						
Males	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	2/10 (20%)	1/10 (10%)
Females	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	5/10* (50%)
Forestomach nodule						
Males	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	3/10 (30%)
Females	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	2/10 (20%)
Forestomach hyperplasia						
Males	0/10 (0%)	0/10 (0%)	0/10 (0%)	3/10 (30%)	3/10 (30%)	8/10* (80%)
Females	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10[c] (10%)	4/10* (40%)	8/10* (80%)
Nasal inflammation[d]						
Males	0/10 (0%)	0/10 (0%)	0/10 (0%)	3/10 (30%)	3/10 (30%)	7/10* (70%)
Females	1/10 (10%)	0/10 (0%)	1/10 (10%)	1/10 (10%)	4/10 (40%)	6/10* (60%)

[a]Hazleton Laboratories (1986b); NTP-PWG (1987).
[b]Values denote number of animals showing changes ÷ total number of animals examined (% incidence).
[c]Lesion reported as "equivocal" by NTP-PWG (1987).
[d]Reported as nasal inflammation by Hazleton Laboratories (1986b), reclassified as serous exudation by NTP-PWG (1987).
*Significantly different from control by Fisher's exact probability test ($p < 0.05$), conducted for this review.

ADD = adjusted daily dose.

Table D-4. Body-Weight Data in F344 Rats Exposed to Commercial Crotonaldehyde (CASRN 4170-30-3) via Gavage in Corn Oil for 9 Weeks (Premating through PND 5)[a]

Endpoint[b, c]	Dose Group, mg/kg-d			
	0	2.5	5	10
Males				
Body weight (g)				
Initial	115.7 ± 8.17	115.8 ± 7.38 (+0.1%)	119.9 ± 6.22 (+4%)	115.6 ± 8.99 (−0.1%)
Wk 9	305.8 ± 20.24	301.1 ± 18.64 (−2%)	307.3 ± 21.78 (+0.5%)	305.6 ± 16.83 (−0.1%)
Body-weight gain (g)				
Wk 0−9	190.1 ± 16.08	185.4 ± 16.50 (−2%)	187.5 ± 17.00 (−1%)	190.0 ± 14.71 (0%)
Females				
Body weight (g)				
Initial (all)	106.3 ± 5.74	108.0 ± 4.69 (+2%)	107.8 ± 7.39 (+1%)	106.6 ± 4.49 (+0.3%)
GD 0 (pregnant)	158 ± 6.0	159 ± 7.9 (+0.6%)	161 ± 8.9 (+2%)	162 ± 4.4 (+3%)
GD 20 (pregnant)	240 ± 16.9	244 ± 14.2 (+2%)	250 ± 16.4 (+4%)	249 ± 14.0 (+4%)
PND 0 (pregnant)	195.1 ± 9.73	196.9 ± 10.78 (+0.9%)	197.6 ± 13.57 (+1%)	196.6 ± 9.64 (+0.8%)
PND 5 (pregnant)	196.2 ± 11.00	198.2 ± 9.77 (+1%)	201.0 ± 12.08 (+2%)	199.7 ± 11.06 (+2%)
Wk 9 (nonpregnant)	202.9 ± 0.21	183.6 ± 8.35 (−10%)	188.9 ± 10.72 (−7%)	175.2 ± 7.43 (−14%)
Body-weight gain (g)				
GDs 0−20 (pregnant)	82.0 ± 14.48	85.4 ± 9.08 (+4%)	88.6 ± 12.24 (+8%)	87.8 ± 13.58 (+7%)
Wk 0−9 (nonpregnant)	90.6 ± 3.32	72.6 ± 6.05* (−20%)	82.1 ± 4.46 (−9%)	72.0 ± 2.91* (−21%)

[a]Hazleton Laboratories (1987).
[b]Data are mean ± SD; *n* =19−20 males/group, 20 total females/group (14−16 pregnant females/group, 2−5 nonpregnant females/group).
[c]Value in parentheses is % change relative to control = [(treatment mean − control mean) ÷ control mean] × 100.
*Significantly different from control ($p < 0.05$), as reported by the study authors.

GD = gestation day; PND = postnatal day; SD = standard deviation.

Table D-5. Survival and Preneoplastic and Neoplastic Lesions in Male F344 Rats Exposed to *trans*-Crotonaldehyde (CASRN 123-73-9) in Drinking Water for 113 Weeks[a]

Endpoint	Dose Group, mg/kg-d (HED)		
	0	2 (0.6)	17 (4.6)
Survival[b]			
70 wk	23/23 (100%)	27/27 (100%)	22/23 (96%)
90 wk	21/23 (91%)	25/27 (93%)	18/23 (78%)
110 wk	16/23 (70%)	17/27 (63%)	13/23 (57%)
Liver tumors[b]			
Neoplastic nodule	0/23 (0%)	9/27* (33%)	1/23 (4%)
Hepatocellular carcinoma	0/23 (0%)	2/27 (7%)	0/23 (0%)
Neoplastic nodule or hepatocellular carcinoma	0/23 (0%)	9/27* (33%)	1/23 (4%)
Preneoplastic lesions			
Altered liver foci[b]	1/23 (4%)	23/27* (85%)	13/23* (57%)
Number of altered foci/cm^2 [c]	0.1 ± 0.4	12.4 ± 7.4* (+124-fold)	3.5 ± 3.8* (+35-fold)

[a]Chung et al. (1986).
[b]Values denote number of animals showing changes ÷ total number of animals examined (% incidence). The differences in terminal survival across groups were not significant based on Fisher's exact tests conducted for this review.
[c]Data are mean ± SD for 23−27 rats; value in parentheses is fold-change relative to control = treatment mean ÷ control mean.
*Significantly different from control ($p < 0.001$), as reported by the study authors.

HED = human equivalent dose; SD = standard deviation.

APPENDIX E. BENCHMARK DOSE MODELING RESULTS

MODELING PROCEDURE

Dichotomous Noncancer Data

The benchmark dose (BMD) modeling of dichotomous data is conducted with the U.S. EPA's Benchmark Dose Software (BMDS; Version 2.6 was used for this document). For these data, the Gamma, Logistic, Log-Logistic, Log-Probit, Multistage, Probit, and Weibull dichotomous models available within the software are fit using a benchmark response (BMR) of 10% extra risk. Alternative BMRs may also be used where appropriate, as outlined in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). In general, the BMR should be near the low end of the observable range of increased risk in the study. BMRs that are too low can result in widely disparate benchmark dose lower confidence limit (BMDL) estimates from different models (high model-dependence). Adequacy of model fit is judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of scaled residuals (absolute value < 2.0), and visual inspection of the model fit. Among all models providing adequate fit, the BMDL from the model with the lowest Akaike's information criterion (AIC) is selected as a potential point of departure (POD), if the BMDLs are sufficiently close (<threefold); if the BMDLs are not sufficiently close (>threefold), model-dependence is indicated, and the model with the lowest reliable BMDL is selected.

BMD MODELING TO IDENTIFY POTENTIAL POINTS OF DEPARTURE FOR DERIVATION OF SCREENING PROVISIONAL REFERENCE DOSES

The data sets for forestomach lesions in male and female rats exposed to crotonaldehyde for 13 weeks via gavage (Hazleton Laboratories, 1986b) were modeled to determine potential PODs for the screening subchronic and chronic provisional reference dose (p-RfD), using BMD analysis. Table D-3 shows the data that were modeled. Summaries of modeling approaches and results (see Tables E-1 and E-2 and Figures E-1 and E-2) for each data set follow.

Increased Incidence of Forestomach Hyperplasia in Male F344 Rats Exposed to Commercial Crotonaldehyde via Gavage for 13 Weeks (Hazleton Laboratories, 1986b)

The procedure outlined above for dichotomous data was applied to the data for forestomach hyperplasia in male F344 rats exposed to commercial crotonaldehyde (>95% *trans*-isomer) via gavage in corn oil 5 days/week for 13 weeks (see Table D-3). Table E-1 summarizes the BMD modeling results. All models provided an adequate fit to the data. BMDL values were sufficiently close (differed by <threefold), so the model with the lowest AIC was selected (Log-Probit). Figure E-1 shows the fit of the Log-Probit model to the data, using the BMR of 10% extra risk. Based on adjusted daily doses (ADDs), the BMD_{10} and $BMDL_{10}$ for increased incidence of forestomach hyperplasia in male rats were 6 and 3 mg/kg-day, respectively.

Table E-1. BMD Modeling Results for Increased Incidence of Forestomach Hyperplasia in Male F344 Rats Exposed to Commercial Crotonaldehyde (CASRN 4170-30-3) via Gavage for 13 Weeks (5 Days/Week)[a]

Model	DF	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residual at Dose Nearest BMD	AIC	BMD_{10} (ADD) (mg/kg-d)	$BMDL_{10}$ (ADD) (mg/kg-d)
Gamma[c]	4	2.99	0.5587	1.39	41.7056	5.7	2.7
Logistic	4	5.22	0.2656	1.854	44.3109	8.2	5.7
LogLogistic[d]	4	3.08	0.5439	1.347	41.8037	5.7	2.9
Log-Probit[d, *]	**4**	**2.83**	**0.5865**	**1.259**	**41.4867**	**5.71482**	**3.46158**
Multistage (2-degree)[e]	4	3.28	0.5121	1.498	42.0748	5.7	2.5
Multistage (3-degree)[e]	4	3.28	0.5121	1.498	42.0748	5.7	2.5
Probit	4	4.95	0.2922	1.86	43.7931	7.7	5.4
Weibull[c]	4	3.03	0.5532	1.405	41.8359	5.6	2.6

[a]Hazleton Laboratories (1986b).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Betas restricted to ≥0.
*Selected model. All models provided adequate fit. BMDLs were sufficiently close (differed by <threefold), so the model with the lowest AIC was selected (Log-Probit).

ADD = adjusted daily dose; AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., 10 = dose associated with 10% extra risk); BMR = benchmark response; DF = degree(s) of freedom.

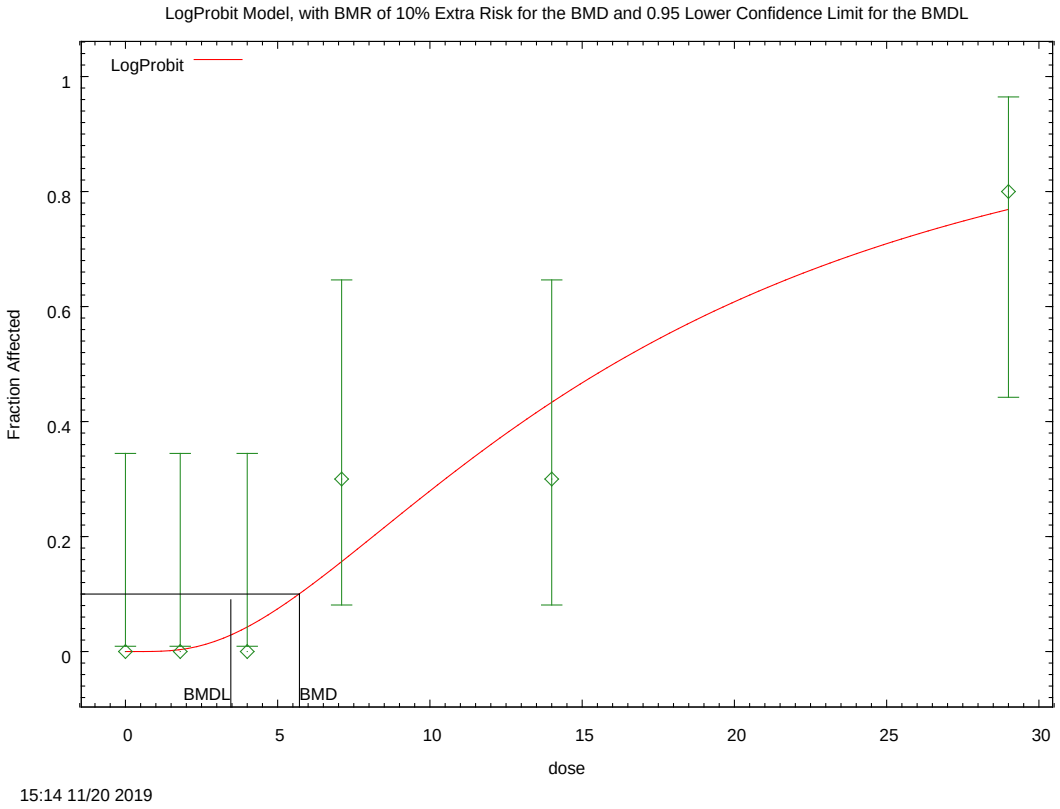


Figure E-1. Fit of Log-Probit Model to Data for Increased Incidence of Forestomach Hyperplasia in Male F344 Rats Exposed to Commercial Crotonaldehyde (CASRN 4170-30-3) via Gavage for 13 Weeks (Hazleton Laboratories, 1986b)

BMD Model Output for Figure E-1:

```
================================================================
        Probit Model. (Version: 3.4;  Date: 5/21/2017)
        Input Data File: C:/Users/jdean04/BMDS2704/Data/lnp_Crotonaldehyde-hazleton
male forestomach hyperplasia_Lnp-BMR10-Restrict.(d)
        Gnuplot Plotting File:
C:/Users/jdean04/BMDS2704/Data/lnp_Crotonaldehyde-hazleton male forestomach
hyperplasia_Lnp-BMR10-Restrict.plt
                                          Wed Nov 20 15:14:54 2019
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = Background
               + (1-Background) * CumNorm(Intercept+Slope*Log(Dose)),

   where CumNorm(.) is the cumulative normal distribution function


   Dependent variable = Effect
   Independent variable = Dose
   Slope parameter is restricted as slope >= 1
```

```
   Total number of observations = 6
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial (and Specified) Parameter Values
                    background =            0
                     intercept =     -2.76936
                         slope =            1


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -background
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

              intercept        slope

 intercept            1        -0.96

     slope        -0.96            1



                                Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     background                0              NA
      intercept         -3.44396
0.889948            -5.18822           -1.69969
          slope          1.24058          0.34207           0.570132
1.91102

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -17.2213         6
   Fitted model        -18.7434         2       3.04412      4          0.5505
  Reduced model        -32.5964         1       30.7501      5         <.0001

           AIC:         41.4867


                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000      10.000        0.000
    1.8000     0.0033         0.033     0.000      10.000       -0.182
```

```
    4.0000     0.0423         0.423     0.000      10.000       -0.665
    7.1000     0.1557         1.557     3.000      10.000        1.259
   14.0000     0.4325         4.325     3.000      10.000       -0.846
   29.0000     0.7684         7.684     8.000      10.000        0.237

 Chi^2 = 2.83      d.f. = 4        P-value = 0.5865


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        5.71482

            BMDL =        3.46158

            BMDU =         8.4939
```

Increased Incidence of Forestomach Hyperplasia in Female F344 Rats Exposed to Commercial Crotonaldehyde via Gavage for 13 Weeks (Hazleton Laboratories, 1986b)

The procedure outlined above for dichotomous data was applied to the data for forestomach hyperplasia in female F344 rats exposed to crotonaldehyde via gavage in corn oil 5 days/week for 13 weeks (see Table D-3). Table E-2 summarizes the BMD modeling results. All models provided adequate statistical fit to the data; however, based on visual inspection, scaled residuals, relatively poor statistical fit, and an outlier result relative to the other models, the Multistage 1-degree model was not considered an adequate fit to the data. Remaining BMDLs differed by <threefold, so the model with the lowest AIC was selected (Multistage 2-degree; Multistage 3- to 5-degree models converged to Multistage 2-degree). Figure E-2 shows the fit of the Multistage 2-degree model to the data, using the BMR of 10% extra risk. Based on ADDs, the BMD_{10} and $BMDL_{10}$ for increased incidence of forestomach hyperplasia in female rats were 7 and 4 mg/kg-day, respectively.

Table E-2. BMD Modeling Results for Increased Incidence of Forestomach Hyperplasia in Female F344 Rats Exposed to Commercial Crotonaldehyde (CASRN 4170-30-3) via Gavage for 13 Weeks (5 Days/Week)[a]

Model	DF	χ^2	χ^2 Goodness-of-Fit *p*-Value[b]	Scaled Residual at Dose Nearest BMD	AIC	BMD_{10} (ADD) (mg/kg-d)	$BMDL_{10}$ (ADD) (mg/kg-d)
Gamma[c]	4	0.39	0.983	0.161	34.5698	7.6	4.3
Logistic	4	2.55	0.635	0.219	37.3479	9.3	6.4
LogLogistic[d]	4	0.29	0.990	0.192	34.4614	7.6	4.5
Log-Probit[d]	4	0.16	0.997	0.179	34.2354	7.5	4.6
Multistage (2-degree)[e, *]	**5**	**0.65**	**0.986**	**0.018**	**32.9994**	**7.166**	**3.8462**
Multistage (3-degree)[e]	5	0.65	0.986	0.018	32.9994	7.2	3.9
Probit	4	2.08	0.721	0.272	36.6479	8.9	6.1
Weibull[c]	4	0.68	0.954	0.076	34.9882	7.4	4.0

[a]Hazleton Laboratories (1986b).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Power restricted to ≥1.
[d]Slope restricted to ≥1.
[e]Betas restricted to ≥0.
*Selected model. All models provided adequate statistical fit to the data; however, based on visual inspection, scaled residuals, relatively poor statistical fit, and an outlier result relative to the other models, the Multistage 1-degree model was not considered an adequate fit to the data. Remaining BMDLs differed by <threefold, so the model with the lowest AIC was selected (Multistage 2-degree; Multistage 3- to 5-degree models converged to Multistage 2-degree).

ADD = adjusted daily dose; AIC = Akaike's information criterion; BMD = maximum likelihood estimate of the dose associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., 10 = dose associated with 10% extra risk); BMR = benchmark response; DF = degree(s) of freedom.

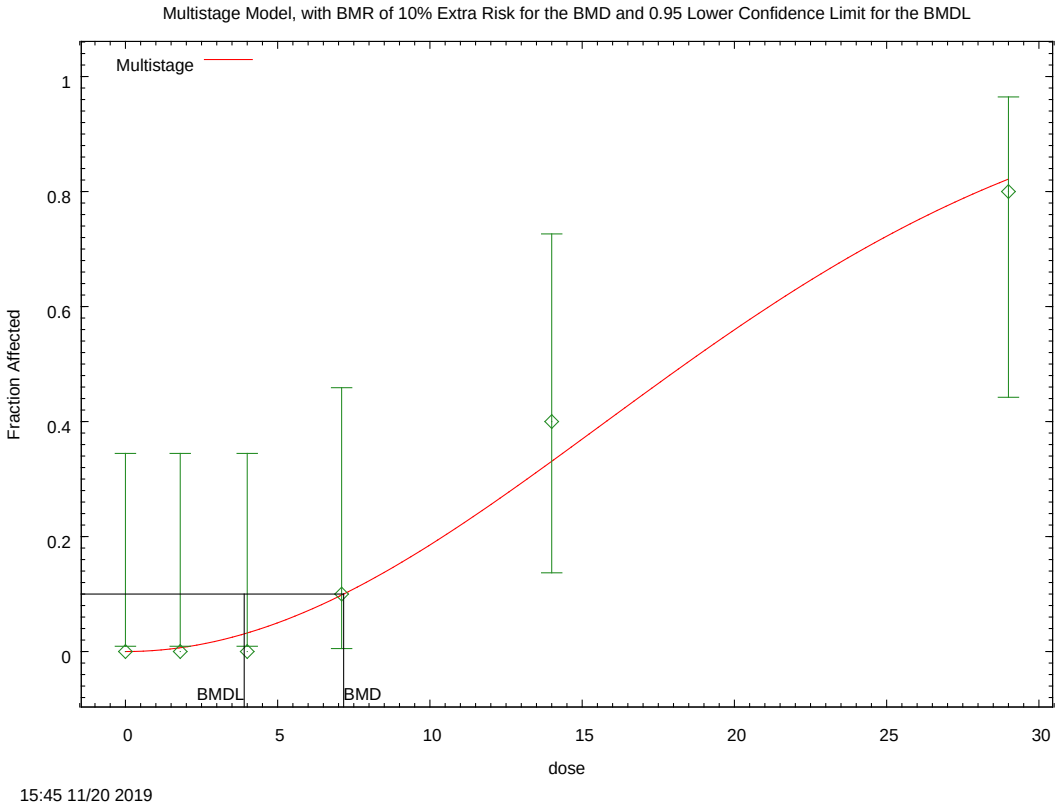


Figure E-2. Fit of Multistage 2-Degree Model to Data for Increased Incidence of Forestomach Hyperplasia in Female F344 Rats Exposed to Commercial Crotonaldehyde (CASRN 4170-30-3) via Gavage for 13 Weeks (Hazleton Laboratories, 1986b)

BMD Model Output for Figure E-2:

```
================================================================
        Multistage Model. (Version: 3.4;  Date: 05/02/2014)
        Input Data File: C:/Users/jdean04/BMDS2704/Data/mst_Crotonaldehyde-hazleton
female forestomach hyperplasia_Mst3-BMR10-Restrict.(d)
        Gnuplot Plotting File:
C:/Users/jdean04/BMDS2704/Data/mst_Crotonaldehyde-hazleton female forestomach
hyperplasia_Mst3-BMR10-Restrict.plt
                                          Wed Nov 20 15:45:42 2019
 ================================================================

 BMDS_Model_Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2-beta3*dose^3)]

   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose

 Total number of observations = 6
```

```
 Total number of records with missing values = 0
 Total number of parameters in model = 4
 Total number of specified parameters = 0
 Degree of polynomial = 3


 Maximum number of iterations = 500
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =           0
                        Beta(1) =     0.015664
                        Beta(2) =    0.0014296
                        Beta(3) =           0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)    -Beta(3)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(2)

   Beta(2)           1



                                Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background               0              NA
        Beta(1)               0              NA
        Beta(2)       0.00205175      0.000612406         0.000851457
0.00325204
        Beta(3)               0              NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -14.985         6
   Fitted model        -15.4997         1       1.02949      5          0.9602
  Reduced model        -31.3594         1       32.7488      5         <.0001

           AIC:         32.9994


                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000      10.000        0.000
    1.8000     0.0066         0.066     0.000      10.000       -0.258
```

```
    4.0000     0.0323         0.323     0.000      10.000       -0.578
    7.1000     0.0983         0.983     1.000      10.000        0.018
   14.0000     0.3311         3.311     4.000      10.000        0.463
   29.0000     0.8219         8.219     8.000      10.000       -0.181

 Chi^2 = 0.65      d.f. = 5        P-value = 0.9857


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =          7.166

             BMDL =         3.8462

             BMDU =        11.1204

Taken together, (3.8462 , 11.1204) is a 90     % two-sided confidence
interval for the BMD
```

Table E-3 summarizes the BMD best-fit modeling results for the modeled endpoints.

Table E-3. BMD and BMDL Values from Best-Fitting Models for Forestomach Hyperplasia in F344 Rats Exposed to *trans*-Crotonaldehyde (CASRN 123-73-9) and Commercial Crotonaldehyde (>95% *trans*-; CASRN 4170-30-3) via Gavage for 13 Weeks (5 Days/Week)[a]

Sex	Best Fitting Model	BMR	BMD (ADD) (mg/kg-d)	BMDL (ADD) (mg/kg-d)
Male	Log-Probit	10% extra risk	6	3
Female	Multistage 2-degree	10% extra risk	7	4

[a]Hazleton Laboratories (1986b).

ADD = adjusted daily dose; BMD = benchmark dose; BMDL = benchmark dose lower confidence limit; BMR = benchmark response.

APPENDIX F. REFERENCES

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