Overview information for

Crotonaldehyde

CROTONALDEHYDE
(Group 3)

For definition of Groups, see Preamble Evaluation.

VOL.: 63 (1995) (p. 373)

CAS No.: 4170-30-3
Chem. Abstr. Name: 2-Butenal

CAS No.: 15798-64-8
Chem. Abstr. Name: (*Z*)-2-Butenal (*cis*-isomer)

CAS No.: 123-73-9
Chem. Abstr. Name: (*E*)-2-Butenal (*trans*-isomer)

5. Summary and Evaluation

5.1 Exposure data

Crotonaldehyde is produced principally as an intermediate for the production of sorbic acid. It was formerly used in large amounts in the production of *n*-butanol.

Crotonaldehyde occurs naturally in foods and is formed during the combustion of fossil fuels (including engine exhausts), wood and tobacco and in heated cooking oils. Human exposure occurs from these sources and may occur during its production and use.

5.2 Human carcinogenicity data

The available data were too limited to form the basis for an evaluation of the carcinogenicity of crotonaldehyde to humans.

5.3 Animal carcinogenicity data

Crotonaldehyde was tested for carcinogenicity in one study in male rats by administration in the drinking-water. Increased incidences of hepatic neoplastic nodules and altered liver-cell foci were seen, but these were not dose-related.

5.4 Other relevant data

Crotonaldehyde is a substrate for aldehyde dehydrogenase and forms conjugates with glutathione, in the presence or absence of glutathione transferase. Mercapturic acid metabolites have been identified in urine.

Crotonaldehyde is a potent irritant, and it has been reported to interfere with immune function.

Crotonaldehyde did not induce DNA damage in rat hepatocytes *in vitro* in a single study. It was mutagenic to insects and bacteria. It bound to DNA of mouse skin *in vivo* after topical application and to DNA *in vitro* and caused formation of DNA-protein cross-links.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of crotonaldehyde.

There is *inadequate evidence* in experimental animals for the carcinogenicity of crotonaldehyde.

Overall evaluation

Crotonaldehyde *is not classifiable as to its carcinogenicity to humans (Group 3)*.

For definition of the italicized terms, see Preamble Evaluation.

Synonyms for Crotonaldehyde

- 2-Butenaldehyde
- Crotonal
- Crotonic aldehyde
- Crotylaldehyde
- 1-Formylpropene
- β-Methylacrolein
- Propylene aldehyde

Synonyms for *cis* Isomer

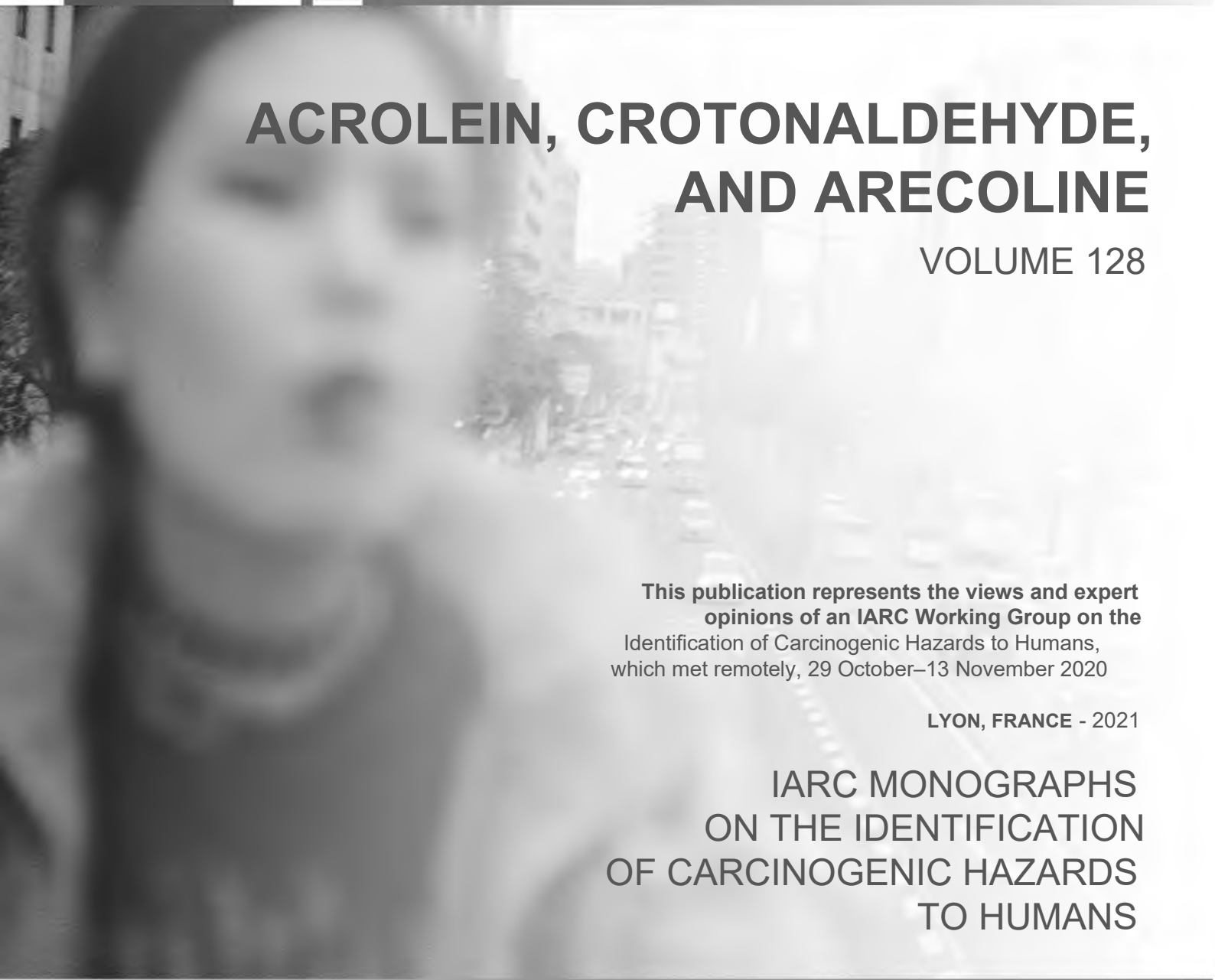
- *cis*-2-Butenal
- *cis*-Crotonaldehyde

Synonyms for *trans* Isomer

- 2(*E*)-Butenal
- *trans*-2-Butenal
- *trans*2-Buten-1-al
- *trans*-Crotonal
- *trans*-Crotonaldehyde
- Topanel CA

Last updated 05/27/1997

IARC MONOGRAPHS



ACROLEIN, CROTONALDEHYDE, AND ARECOLINE

VOLUME 128

This publication represents the views and expert opinions of an IARC Working Group on the Identification of Carcinogenic Hazards to Humans, which met remotely, 29 October–13 November 2020

LYON, FRANCE - 2021

IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

1.1 Identification of the agent

1.1.1 Nomenclature

Chem. Abstr. Serv. Reg. No.: 107-02-8

Deleted Chem. Abstr. Serv. Reg. No.: 25314-61-8

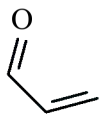
EC/List No.: 203-453-4

Chem. Abstr. Serv. name: 2-propenal

IUPAC systematic name: prop-2-enal

Synonyms: acraldehyde; acrylaldehyde; acryl - ic aldehyde; allyl aldehyde; ethylene aldehyde; propenal; 2-propenal; prop-2-en-l-al (IARC, 1995; O'Neil, 2013; ECHA, 2020).

1.1.2 Structural and molecular formulae, and relative molecular mass *Structural formula:*



Molecular formula: C_3H_4O

Relative molecular mass: 56.06 (O'Neil, 2013).

1.1.3 Chemical and physical properties

Description: colourless to yellowish liquid with extremely acrid, pungent, and irritating odour, causing lachrymation (Verschueren, 1983; IARC, 1985; O'Neil, 2013)

Boiling point: 52.5–53.5 °C (Lide, 1993)

Melting-point: –86.9 °C (Lide, 1993)

Relative density: 0.8410 at 20 °C/4 °C (Lide, 1993)

Solubility: soluble in water (206 g/L at 20 °C), ethanol, diethyl ether, and acetone (IPCS, 1992; Lide, 1993)

Volatility: vapour pressure 29.3 kPa (220 mm Hg) at 20 °C (IPCS, 1992)

Flash-point: −26 °C (IPCS, 1992)

Stability: unstable in the absence of an inhibitor (IPCS, 1992); polymerizes, especially under light or in the presence of alkali or strong acid, to form disacryl, a plastic solid (O'Neil, 2013). Inhibited acrolein undergoes dimerization above 150 °C and highly exothermic polymerization also occurs in the presence of traces of acids or strong bases even when an inhibitor is present (IPCS, 1992) *Reactivity*: reactions shown by acrolein include Diels–Alder condensation, dimerization and polymerization, additions to the carbon–carbon double bond, carbonyl additions, oxidation, and reduction (IPCS, 1992; see also Section 4.2.1)

*Octanol/water partition coefficient (*P*)*: log K_{ow}, –0.01 (O'Neil, 2013)

Odour perception threshold: 0.07 mg/m^3 (IPCS, 1992)

Conversion factor: 1 ppm = 2.29 mg/m^3 (IARC, 1995).

1.1.4 Technical products and impurities

Hydroquinone (IARC Group 3; IARC, 1999) at a concentration of 0.1–0.25% is typically used to stabilize commercially available preparations of acrolein (Etzkorn, 2009). Hydroquinone protects acrolein from polymerization, and also from hydrolysis in aqueous solutions (Kächele et al., 2014). Acrolein is available commercially with purities in the range of 90–98% and as solutions to be used as reference materials in water, methanol, and acetone (Chemical Abstracts Service, 2020). Impurities include water (up to 3.0% by weight; IPCS, 1992), acetaldehyde, and, depending on the production process, small amounts of propionaldehyde, acetone, propene oxide, and methanol, and traces of allyl alcohol and ethanol (Arntz et al., 2007).

1.2 Production and use

1.2.1 Production process

Acrolein was first prepared in 1843 by the dry distillation of fat (Redtenbacher, 1843). Commercial production of acrolein began in Germany in 1942, by a process based on the vapour-phase condensation of acetaldehyde and formaldehyde. This method was used until 1959, when a process was introduced for producing acrolein by vapour-phase oxidation of propene (Arntz et al., 2007). Several catalysts have been used in this process, including bismuth molybdate (Etzkorn, 2009). Propene oxidation is still the commercially dominant production process (Etzkorn, 2009), while research on more environmentally friendly methods of acrolein production from renewable feedstock such as glycerol, methanol, or ethanol is ongoing (Arntz et al., 2007; Etzkorn, 2009; Lilić et al., 2017). The oxidation of propene produces acrolein, acrylic acid, acetaldehyde, and carbon oxides.

1.2.2 Production volume

In 1975, global production of acrolein was approximately 59 000 tonnes (Hess et al., 1978). Worldwide production of acrolein in 1977 was estimated to have been 100–120 000 tonnes (IARC, 1979). The worldwide capacity for production of refined acrolein was estimated in the 1990s to be about 113 000 tonnes per year (Etzkorn et al., 1991). In 2007, the production capacity for acrolein in western Europe, USA, and Japan was estimated to total 425 000 tonnes per year (Arntz et al., 2007). In 2009, worldwide estimated acrolein production capacity was about 350 000 tonnes per year, which included acrolein made for captive use in methionine production (Etzkorn, 2009). Estimated global demand in 2018 was 620 000 tonnes (Zion Market Research, 2019).

Acrolein was listed by the Organisation for Economic Co-operation and Development (OECD) and the United States Environmental Protection Agency (US EPA) as a High Production Volume chemical for 2007 (IARC, 2019). About 100–1000 tonnes per year are manufactured and/or imported in the European Economic Area (ECHA, 2020).

The Chem Sources database lists 27 manufacturing companies worldwide, of which 12 are located in the USA and 5 in China (including Hong Kong Special Administrative Region) (Chem Sources, 2020).

1.2.3 Uses

Acrolein is an α,β-unsaturated aldehyde and a highly reactive, volatile organic chemical (see also Section 4.2.1). These properties contribute to the many reactions of acrolein and its commercial usefulness, either directly or (for the most part) as a chemical intermediate for the production of numerous chemical products. These include acrylic acid, which is used to make acrylates, and DL-methionine, an essential amino acid used as a feed supplement for livestock (Arntz et al., 2007; Faroon et al., 2008). Other important derivatives of acrolein are glutaraldehyde, pyridines, tetrahydrobenzaldehyde, allyl alcohol and glycerol, 1,4-butanedial and 1,4-butenediol, 1,3-propanediol, DL-glyceraldehyde, flavours and fragrances, and polyurethane and polyester resins (Sax & Lewis, 1987; Arntz et al., 2007).

The most important direct use of acrolein is as a biocide. It is used as an herbicide and to control algae, aquatic weeds, and molluscs in recirculating process water systems (at a concentration of 6–10 mg/L). It is also used to control the growth of microorganisms in liquid fuel, the growth of algae in oil fields, and the formation of slime in paper manufacture. Acrolein has been used in leather tanning and as a tissue fixative in histology (IPCS, 1992; IARC, 1995; Arntz et al., 2007; Etzkorn, 2009). Acrolein has also been used as a warning agent in methyl chloride refrigerants and other gases, in poison gas mixtures for military use, in the manufacture of colloidal forms of metals, and as a test gas for gas masks (IARC, 1979; Neumüller, 1979; O'Neil, 2013).

The market share for global acrolein production in 2017 was methionine use (61.2%), pesticide use (17.4%), glutaraldehyde use (7.3%), water treatment use (9.0%), and other applications (5.1%), with this distribution being stable (within 1%) for several consecutive years (Regal Intelligence, 2020).

1.3 Methods of measurement and analysis

Methods for the analysis of acrolein in air, water, biological media including tissue, and food have been reviewed (IPCS, 1992; IARC, 1995; Shibamoto, 2008). Representative analytical methods for a variety of sampling matrices (air, water, cigarettes, foods and beverages, and biological specimens) are presented in Table S1.1 (Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc.fr/602).

1.3.1 Air

Several reference procedures are available for the analysis of acrolein in air or gaseous emissions. These include ISO 19 701 (ISO, 2013) and ISO 19 702 (ISO, 2015) for the analysis of fire effluents, JIS K0089 (JIS, 1998) and VDI 3862 Part 5 (VDI, 2008) for the analysis of gaseous emissions, and MAK Air Monitoring Methods (Hahn, 1993). Official analytical methods for air analysis by the United States (US) National Institute for Occupational Safety and Health (NIOSH) (NIOSH 2501, NIOSH 2539) and Occupational Safety and Health Administration (OSHA) (OSHA 52) are available (NCBI, 2020). Methods for the analysis of mainstream cigarette smoke (see Section 1.4.2(b)), ISO 21 160 (ISO, 2018) and Health Canada Official machine smoking regime methods are also available. Protocols are required to standardize measurements of the emissions of toxic chemicals in mainstream cigarette smoke for regulatory purposes. Although ISO methods (from the International Organization for Standardization) have been widely used for decades, Health Canada and WHO have developed more intensive smoking conditions.

The key differences between these protocols are that the ISO regime sets the machine to take 35 mL puffs every 60 seconds with ventilation holes left open, whereas the intensive regimes prescribe 50 mL puffs every 30 seconds, and, importantly, all filter ventilation holes are blocked (WHO, 2012).

[The Working Group noted that the higher values provided by the Health Canada Official method correspond better to human exposure during smoking.]

High-performance liquid chromatography (HPLC) is the routine method to quantify acrolein derivatives obtained from sorbent matrix samplers, which may be used in conjunction with ultraviolet (UV), ion trap mass spectrometry (MS), and fluorescence detectors (Alberta Environment, 2011). Gas chromatography (GC) is the routine method to quantify acrolein pre-concentrated in pressurized sampling canisters and can be used with MS (GC-MS), flame ionization, and electron capture detectors (Alberta Environment, 2011).

1.3.2 Water

Similar chromatographic methods to those used for air analysis are applied to water. Several official analytical methods for water analysis are available from the US EPA (EPA-EAD 603: US EPA, 1984a; EPA-EAD 624: US EPA, 1984b; EPA-EAD 1624: US EPA, 1984c; EPA-RCA 5030C: US EPA, 2003; EPA-RCA 8015C: US EPA, 2007; EPA-RCA 8316: US EPA, 1994) and the United States Geological Survey (USGS) National Water Quality Laboratory (USGS-NWQL O-4127-96, Connor et al., 1996) (NCBI, 2020).

1.3.3 Soil

Standardized methods for analysing acrolein in soil were not identified. However, given the extent to which acrolein is expected to volatilize from soil based on its high vapour pressure and the irreversible binding of acrolein in soil, the lifetime of acrolein in soil may be too short for concern in the context of human exposure (ATSDR, 2007).

1.3.4 Food, beverages, and consumer products

Due to its high reactivity, direct analytical determination of acrolein is difficult, specifically in complex matrices such as foods and beverages (Kächele et al., 2014). Standardized methods for analysing acrolein in foods and beverages were not identified, but several methods with a focus on analysing alcoholic beverages and fat-based products are available (Table S1.1, Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc. fr/602). Several different analytical approaches that mostly include derivatization have been suggested, typically based on HPLC or GC with various detectors including MS (Shibamoto, 2008). Several methods for acrolein analysis have applied solid-phase microextraction (SPME) for sample extraction and enrichment (Wardencki et al., 2003; Curylo & Wardencki, 2005; Saison et al., 2009; Osório & de Lourdes Cardeal, 2011; Lim & Shin, 2012; Kächele et al., 2014).

According to Kächele et al. (2014), acrolein standard solutions for calibrations should be stabilized by a suitable agent such as hydroquinone. The original hydroquinone content found in some commercial acrolein preparations as a stabilizer is not sufficient to prevent degradation if aqueous dilutions for trace analysis are prepared (Kächele et al., 2014).

1.3.5 Biological specimens

Several methods are available for the direct analysis of acrolein in saliva, urine and serum (Table S1.1, Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc.fr/602) as well as the analysis of its metabolites or DNA and protein adducts (Table S1.2, Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc.fr/602). Of these, the urinary biomarkers *N*-acetyl-*S*-(3hydroxypropyl)-L-cysteine (3-hydroxypropyl- mercapturic acid, HPMA) and *N*-acetyl-*S*(carboxyethyl)-L-cysteine (2-carboxyethylmer- capturic acid, CEMA) appear to be most commonly determined, and can be detected using liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods (see Table S1.2, Annex 1, Supplementary material for Section 1, web only; available from: https://www. publications.iarc.fr/602).

Information regarding an internationally accepted validated biomarker for acrolein exposure was not available to the Working Group.

1.4 Occurrence of and exposure to acrolein

1.4.1 Environmental and natural occurrence

The incomplete combustion and heating of cooking oils produce acrolein, as does the photochemical degradation of 1,3-butadiene in the environment. Acrolein may also be formed endogenously (Faroon et al., 2008; see also Nath & Chung, 1994). Zhang et al. (2018) cited several sources of endogenous acrolein formation, the most important of which include the reactions of myeloperoxidase on hydroxyl-amino acids such as threonine, and the oxidation of spermine and spermidine by amine oxidase (Stevens & Maier, 2008), while other endogenous sources include peroxidation of polyunsaturated fatty acids (Uchida et al., 1998a) and oxidative ring opening of the anticancer drug cyclophosphamide and other oxazaphosphorine drugs such as ifosfamide (Brock et al., 1979). No quantitative data on endogenous production of acrolein were available to the Working Group.

Landfill leachate contained acrolein at a concentration of 0.07–2.1 ppm [0.07–2.1 mg/L] (Faroon et al., 2008). The US EPA lists acrolein as a pollutant in National Priority Superfund sites in at least 16 USA states; acrolein was detected at a concentration of 0.006–1.3 ppm [0.006–1.3 mg/L] in groundwater at half of these sites (Faroon et al., 2008). Because acrolein is highly reactive, it is not expected to bioaccumulate, but it can be formed in the environment as a breakdown product of other chemicals, in addition to occurring as a result of the direct emission of acrolein as a combustion product (Faroon et al., 2008).

1.4.2 Exposure in the general population

The most important sources of acrolein exposure in the general population include tobacco use and cooking with oil at high temperatures. Forest and residential fires, vehicle exhaust, and incinerators are other significant sources of acrolein exposure.

(a) Food, beverages, and cooking emissions

Acrolein concentrations measured in food, beverages, and cooking emissions are presented in Table 1.1.

Most food items are not considered to be major sources of acrolein in the general population. However, higher concentrations have been reported in certain food items, including frying

fats and oils (mean acrolein concentration, 276 µg/L; maximum, 1389 µg/L; *n* = 15; see Table 1.1), and cooking food in hot oil has been shown to produce emissions containing acrolein, which can be a significant source of exposure.

An analysis by Umano & Shibamoto (1987) revealed that the two most important factors in the production of acrolein during cooking were cooking duration and cooking temperature, both of which were positively associated with acrolein production; the type of oil (i.e. sunflower, beef fat, soybean, corn, sesame, and olive, in increasing order of acrolein production) was less important. While little acrolein was formed under 240 °C, emissions increased 10-fold when the temperature was increased from 280 to 300 °C, and 3-fold from 300 to 320 °C. Temperatures in home cooking were reported to rarely exceed 200 °C.

Table 1.1 Concentrations of acrolein in food, beverages, and cooking emissions

Item	Acrolein concentration (mean or range)	Country of study or purchase	Reference
Food			
French fries	1.97–4.85 mg/kg	Brazil	Osório & de Lourdes Cardeal (2011)
Domiati cheese	0.29–1.3 mg/kg	Egypt	Collin et al. (1993)
Doughnuts	0.1–0.9 mg/kg	USA	Lane & Smathers (1991)
Fried fish coating	0.1 mg/kg	USA	Lane & Smathers (1991)
Fruits	< 0.01–0.05 mg/kg	NR	Feron et al. (1991)
Vegetables	≤ 0.59 mg/kg	NR	Feron et al. (1991)
Frying fats and oils (15 tested)	Mean, 276; max., 1389 mg/kg	Germany	Kächele et al. (2014)
Beverages			
Lager beer, fresh (3 bottlings tested)	Mean, 1.6 µg/L	UK	Greenhoff & Wheeler (1981)
Lager beer, force aged (3 bottlings tested)	Mean, 5.05 µg/L	UK	Greenhoff & Wheeler (1981)
Lager beer (22 tested)	< 2.5–5.4 µg/L	Brazil	Hernandes et al. (2019)
Beer (9 tested)	All < 14 µg/L (LOD)	Germany	Kächele et al. (2014)
Wine (23 tested)	Mean, 0.7; max., 8.8 µg/L	Germany	Kächele et al. (2014)
Merlot wine	Mean, 15.9; max., 29.8 µg/L	Brazil	Ferreira et al. (2018)
Brandy/cognac (11 tested)	1.42–1.5 mg/L [1420–1500 µg/L]	Armenia	Panosyan et al. (2001)
Whiskey/bourbon (3 tested)	0.67–11.1 ppm [670–1110 µg/L]	USA	Miller & Danielson (1988)
Whiskey (15 tested)	Mean, 252; max., 915 µg/L	Germany	Kächele et al. (2014)
Vodka (4 tested)	All < 14 µg/L (LOD)	Germany	Kächele et al. (2014)
Absinthe (5 tested)	All < 14 µg/L (LOD)	Germany	Kächele et al. (2014)
Fruit spirits (28 tested)	Mean, 591; max., 2394 µg/L	Germany	Kächele et al. (2014)
Tequila (7 tested)	Mean, 404; max., 1205 µg/L	Germany	Kächele et al. (2014)
Asian spirits (16 tested)	Mean, 54; max., 477 µg/L	Germany	Kächele et al. (2014)
Grape marc (10 tested)	Mean, 487; max., 1808 µg/L	Germany	Kächele et al. (2014)
Mineral & table water (10 bottles)	All < 14 µg/L (LOD)	Germany	Kächele et al. (2014)
Water stored in cisterns	< 3–115 µg/L	Brazil	de Oliveira Moura et al. (2019)

Item (cooking oil)	Acrolein concentration in air (µg/m^3)	Emission rate of acrolein (mg/kg food per hour)	Reference
Emissions during cooking (for 5 minutes)			
Oil only (soybean)	57.9	26.67	Seaman et al. (2009)
French fries (soybean)	41.8	17.81	Seaman et al. (2009)
Chicken strips (soybean)	40	16.06	Seaman et al. (2009)
Battered fish (soybean)	64.5	27.04	Seaman et al. (2009)
Doughnuts (soybean)	32.4	12.9	Seaman et al. (2009)
Doughnuts (canola)	31.6	13.15	Seaman et al. (2009)
Doughnuts (corn)	26.4	10.68	Seaman et al. (2009)
Doughnuts (olive)	29.2	11.79	Seaman et al. (2009)
Doughnuts (no oil)	1.83	0.19	Seaman et al. (2009)

LOD, limit of detection; max., maximum; NR, not reported; ppm, parts per million.

However, Hecht et al. reported that, among non-smoking Chinese women in Singapore who cook at much higher temperatures or cook more frequently than controls (women randomly selected from the Chinese Health Study), concentrations of urinary acrolein metabolites were about 50% higher than among women who cooked less frequently (see Table 1.2; Hecht et al., 2010, 2015).

Beer typically contains acrolein at a concentration of 1–5 µg/L, although higher concentrations (up to 25 µg/L) are found in the early stages of beer making, before processing to make the final product; the acrolein in other alcoholic drinks ranges from 0.02 to 11 µg/L, (Greenhoff & Wheeler, 1981; Ferreira et al., 2018; Hernandes et al., 2019). A study of 117 alcoholic beverages found that over half had detectable levels of acrolein (limit of detection, 14 µg/L), some at much higher concentrations (Kächele et al., 2014). None of 9 beers, 4 vodkas, and 5 absinthes tested had detectable concentrations, nor did only 21 out of 23 wines tested. However, over 85% of the 15 whiskey samples, 7 tequilas, 28 fruit spirits, and 10 grape marc samples tested were positive; the average acrolein concentration in all the samples was 276 µg/L, but some tequilas, fruit spirits, and grape marc were over 1000 µg/L (Kächele et al., 2014). Rainwater to be used as drinking-water and stored in polyethylene cisterns in Brazil was found to contain acrolein in 75% of the 36 cisterns tested, with concentrations up to 115 µg/L (de Oliveira Moura et al., 2019). No acrolein was detected in 10 bottles of mineral and table water in Germany (Kächele et al., 2014).

(b) Tobacco products and tobacco-related products

Acrolein is present in smoke from cigarettes, cigars, bidis, and hookahs, as well as in emissions from electronic cigarettes and "heatsticks" (Table 1.3). Average concentrations in mainstream smoke from bidis and small cigars are slightly higher than in cigarette smoke. The apparent variability in acrolein yield in mainstream smoke from cigarettes smoked according to the outdated ISO 3308 method is greatly reduced when using the Health Canada Intensive method recommended by WHO, with most products producing 100–200 µg of acrolein/rod. In general, sugars (which are natural components of tobacco and which may also be added during the manufacturing process) increase the emissions of acrolein in tobacco smoke by 20–70% (Talhout et al., 2006). Hookahs (waterpipes, narghile) produce approximately 900 µg of acrolein in mainstream smoke and 1100 µg of acrolein in sidestream smoke per session, which lasts for approximately

1 hour, meaning that secondhand acrolein exposure from waterpipes may exceed that from cigarettes, at 140 µg/rod (Al Rashidi et al., 2008; Daher et al., 2010). Although the fluid in electronic cigarettes ("e-liquid") does not contain acrolein, it is apparently formed during the heating of the fluid, at an amount that is dependent on the composition of the fluid and the temperature of the coil (Conklin et al., 2018); a single puff contains 3–15 ng of acrolein (Herrington & Myers, 2015). Increasing voltage from 3.8 V to 4.8 V increased the acrolein yield more than 4-fold (Kosmider et al., 2014), and the addition of humectants, sweeteners, and flavourings increased the production of acrolein from nondetectable to several hundred micrograms per gram of e-liquid (Khlystov & Samburova, 2016). [The Working Group noted that newer devices contain voltage/temperature controls that can increase the delivery of nicotine and also enhance acrolein production, indicating that acrolein exposures among current users may be much greater than reflected in the recent literature.] Heatsticks, which have been available in over 40 countries for the past 5 years, each discharge about 5 µg of acrolein in mainstream and 0.7 µg of acrolein in sidestream emissions (Cancelada et al., 2019). The acrolein exposure from the heatsticks is reduced by a factor of

Study, Group (if No. of samples country applicable) HPMA/CEMA

Table 1.2 Levels of acrolein metabolite biomarkers measured in human urine

				HPMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		CEMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		Reference
		Unexposed[a]	Exposed	Unexposed	Exposed	Unexposed	Exposed	
Cigarette smoking								
NHANES 2005–2006, USA		2467/NR	601/NR	219 (140, 353)[b]	1089 (469, 2012)	78.8 (51.8, 121)	203 (111, 338)	Alwis et al. (2015)
PATH Study, USA	Cigarettes only	1571/1517	2284/2176	272.4	1143.5	98.14	271.5	Goniewicz et al. (2018)
European multicentre observational study, Germany, Switzerland, and UK	< 10 cigarettes/day		467/NR		1.12 mg/24 h			Lindner et al. (2011)
	10–19 cigarettes/day		557/NR		2.10 mg/24 h			
	≥ 20 cigarettes/day		135/NR		2.98 mg/24 h			
German university study		54/NR	40/NR	146[b]	884[b]			Eckert et al. (2011)
					4123 (2341, 6808) [911 (517, 1505)][c,d]			Park et al. (2015)
					6007 (3947, 9606) [1328 (872, 2123)][c,d]			
					6738 (3885, 1057) [1489 (859, 2422)][c,d]			
					3480 (186, 5908) [769 (412, 1306)][c,d]			
					5344 (3163, 8596) [11 851 (699, 1900)][c,d]			

Multiethnic cohort study, USA	African American	362/NR			
	Native Hawaiian	329/NR			
	White	438/NR	Latino	449/NR	
	Japanese American	704/NR			

Table 1.2 (continued)

Study, Group (if country		No. of samples HPMA/CEMA		HPMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		CEMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		Reference
		Unexposed[a]	Exposed	Unexposed	Exposed	Unexposed	Exposed	
Betel-quid chewing								
Healthy subjects in a study of smoking, betel quid chewing and oral cancer, Taiwan, China	Cigarettes only		111/NR		5.8 [1282][c]			Tsou et al. (2019)
	Betel quid only		12/NR		3.6 [796][c]			
	Cigarettes + betel quid		107/NR		8.9 [1967][c]			
E-cigarettes								
PATH Study, USA	E-cigarettes only	1571/1517	212/198	272	315	98	108	Goniewicz et al. (2018)
	Cigarettes only				1144		272	
	E-cigarettes + cigarettes		767/739		1318		302	
Cooking								
Study of Chinese female regular home cookers, Singapore	Frequent home cooking vs random	50/NR	54/NR	1370 [303][d]	1959 [433][d]			Hecht et al. (2010)
	Cook > 7×/wk vs < 1×/wk	90/NR	95/NR	1901 [420][d]	2600 [575][d]			
Non-source–related								
Shanghai cohort Study, China	Control participants		392/NR		6712 (5845, 7707) [1483 (1292, 1703)][d]			Yuan et al. (2012)
National Children's Study, USA	Pregnant women	488/NR		240 µg/L[b]		71.8 µg/L[b]		Boyle et al. (2016)

applicable)

Table 1.2 (continued)

Study, country	Group (if applicable)	No. of samples HPMA/CEMA		HPMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		CEMA Geometric mean (25th, 75th percentile) µg/g creatinine (unless otherwise stated)		Reference
		Unexposed[a]	Exposed	Unexposed	Exposed	Unexposed	Exposed	
Pregnant women, Guatemala	Fasting	23/NR		268 (178, 399)[c]				Weinstein et al. (2017)
	After sauna	23/NR		572 (429, 1041)[c]				

CEMA, *N*-acetyl-S-(2-carboxyethyl)-L-cysteine (2-carboxyethylmercapturic acid); e-cigarette, electronic cigarette; HPMA, *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine (3-hydroxypropyl-mercapturic acid); NR, not reported; vs, versus.

[a] Unexposed/exposed applies to exposures in subheadings (e.g. *Cigarette smoking*).

[b] Median.

[c] Median (interquartile range).

[d] pmol/mg creatinine [converted to µg/g creatinine].

[e] µmol/g creatinine [converted to µg/g creatinine].

Table 1.3 Concentrations of acrolein in smoke from tobacco products

Product and method details	Reported measurements				Reference
	Method: ISO 3308 (µg/product)		Method: Health Canada Intensive (µg/product)		
	Range	Median	Range	Median	
Cigarettes					
12 brands, mainstream smoke			51–223[a]	163[a]	Borgerding et al. (2000)
12 brands, sidestream smoke			342–523[b]	412[b]	Borgerding et al. (2000)
6 Thai & 2 US brands (90% market share)	79.9–181				Mitacek et al. (2002)
35 brands	30.8–82.6		139–213		Cecil et al. (2017)
3 brands + 1 reference cigarette	24.9–52.2	48.5	100–125	117	Eldridge et al. (2015)
Cigars					
Sheet-wrapped cigars (15 brands)	34.3–105		105–185		Cecil et al. (2017)
Bidis					
76 mm unfiltered bidi – one selected sample	67 µg				Hoffmann et al. (1974)
	Mean total yield	**Mean mainstream yield**	**Mean sidestream yield**	**Sidestream/mainstream yield ratio**	
Narghile/hookah (waterpipe)					
Narghile	145.5 µg/g tobacco				Al Rashidi et al. (2008)
Narghile, per session	892 µg				Al Rashidi et al. (2008)
Narghile, per session		1135 µg	0.7		Daher et al. (2010)
	Various metrics				
Electronic cigarettes					
Aerosol	0.003–0.015 µg/mL (≈20–230 g of acrolein per cigarette assuming 400–500 × 40 mL puffs)				Herrington & Myers (2015)

Aerosol from neat PG	< LOD (0.03×10^{-3} µg/puff)	Conklin et al. (2018)
Aerosol from neat VG	0.08 ± 0.002 µg/puff	Conklin et al. (2018)
Aerosol from 25–75% PG in VG	0.04 µg/puff	Conklin et al. (2018)
Aerosol – 'brand I' (unflavoured)	ND	Khlystov & Samburova (2016)
Aerosol – 'brand III' (unflavoured)	ND	Khlystov & Samburova (2016)
Aerosol – 'brand I' (flavoured)	172 ± 27 to 347 ± 37 µg/g of e-liquid	Khlystov & Samburova (2016)
Aerosol – 'brand II' (flavoured)	ND	Khlystov & Samburova (2016)
Aerosol – 'brand II' (flavoured)	ND to 237 ± 61 µg/g of e-liquid	Khlystov & Samburova (2016)

Table 1.3 (continued)

Product and method details	Reported measurements					Reference
	Mean mainstream emissions	Mean sidestream	Mean emissions concentration	environmental	Range of % of conventional cigarette	
"Heatsticks"						
Heated tobacco device: "iQOS blue"[c]	5.4 ± 0.7 µg per heatstick	0.6 ± 0.3 µg per heatstick				Cancelada et al. (2019)
Heated tobacco device: "iQOS amber"	4.9 ± 0.6 µg per heatstick	0.8 ± 0.3 µg per heatstick				Cancelada et al. (2019)
Heated tobacco device: "iQOS yellow"	5.3 ± 0.7 µg per heatstick	0.7 ± 0.3 µg per heatstick				Cancelada et al. (2019)
Heated tobacco device: "iQOS"			4.6 ± 3.2 µg/m^3		1.8–2.3%	Ruprecht et al. (2017)

LOD, limit of detection; ND, not detected; PG, propylene glycol; VG, vegetable glycerin.

[a] Massachusetts machine smoking protocol. [b] Sidestream smoke, Massachusetts machine smoking protocol. The median value was calculated by multiplying the median value for mainstream smoke by the median value for the sidestream/mainstream smoke ratios for the 12 commercial cigarette brands, which was 2.53. [c] iQOS is a brand name.

about 10 compared with conventional cigarettes (Lachenmeier et al., 2018).

While acrolein metabolites (the mercapturic acids HPMA and CEMA) have been detected in the urine of 99% of Americans (Alwis et al., 2015), concentrations of these metabolites were three to five times higher in smokers than in non-smokers (Eckert et al., 2011; Lindner et al., 2011; Alwis et al., 2015; Goniewicz et al., 2018; Table 1.2), with concentrations increasing with the number of cigarettes smoked per day (Lindner et al., 2011) and with increasing urinary concentration of cotinine (a metabolite of nicotine) (Alwis et al., 2015). Acrolein metabolite concentrations were slightly higher in electronic-cigarette smokers than in non-smokers, but four times higher in dual users of cigarettes and electronic cigarettes (Goniewicz et al., 2018). Passive exposure to secondhand smoke led to comparable increases in urinary acrolein metabolites among hookah smokers and non-smokers alike after visiting a hookah lounge or attending a hookah social event at home (Kassem et al., 2018), probably due to the abovementioned high sidestream emission of acrolein from hookahs. Levels of urinary acrolein metabolites were significantly higher in children living with daily hookah smokers than in children from non-smoking homes (Kassem et al., 2014).

Park et al. (2015) reported significantly different concentrations of acrolein metabolites for smokers from different racial and ethnic groups. Similarly, the National Health and Nutrition Examination Survey (NHANES) found that the 25th percentile of the HPMA concentrations for tobacco smokers was greater than the 75th percentile for non-tobacco users, for all age groups, and that HPMA concentrations among non-tobacco users were similar for both sexes, and were lower for non-Hispanic White people and non-Hispanic Black people than for Mexican Americans or for people of other Hispanic origins or for other or multiple ethnicities. However, among Mexican Americans, metabolite concentrations for smokers were much lower (36%) than those of non-Hispanic White people (Alwis et al., 2015).

In Taiwan, China, healthy subjects who chewed betel quid had HPMA concentrations that were significantly elevated, but significantly lower than in cigarette smokers, and those who both smoked cigarettes and chewed betel quid had the highest urinary HPMA levels (3600, 5800, and 8900 pmol/mg creatinine [796, 1282, and 1967 µg/g creatinine], respectively, see Table 1.2 (see also Table 2.1); Tsou et al., 2019). In contrast, in patients with oral squamous cell carcinoma who both smoked cigarettes and chewed betel quid, urinary levels of HPMA were only 7% those of healthy people with matched smoking and betel-quid use history, despite the fact that their NNAL (4-(methylnitrosamino)-1- (3-pyridyl)-1-butanol)/creatinine levels were com - parable.

(c) Indoor air (i) In the home

Activities in the home, especially tobacco smoking and cooking with oils and fats heated to high temperatures, are the primary indoor source of acrolein (see Section 1.4.2(a)). Cooking can increase air concentrations of acrolein by 26 to 64 µg/m^3 (Seaman et al., 2009). Other indoor sources of acrolein include gas stoves, woodburning fireplaces and stoves, burning candles, and incense. When indoor air in the home and outdoor air are measured simultaneously, the indoor concentration of acrolein is usually 2–10 times greater than the outdoor concentration. Azuma et al. (2016) reviewed surveys of Japanese homes and reported an average indoor concentration of acrolein of 0.267 µg/m^3, which was three times higher than the outdoor concentration, but much lower than that found in homes in other countries.

A survey of 130 homes in Beijing, China, reported much higher concentrations, with an average of 2.1 µg/m^3, although neither smoking nor cooking occurred during the sampling period (Liu et al., 2014). A study of acrolein concentrations outdoors and inside occupied homes and unoccupied, newly constructed, model homes (expected to have high emissions from construction materials) reported morning indoor concentrations in occupied homes (2–8 µg/m^3) that were generally more than 10 times higher than the outdoor concentrations (0.1–0.3 µg/m^3) in Davis and surrounding towns in California, USA. Similarly, in new model homes, the indoor concentrations were also 10 times higher than the outdoor concentrations. The outdoor concentrations in occupied homes in Los Angeles averaged 5–10 times higher than those around Davis (0.8–1.7 µg/m^3), but indoor concentrations were comparable (Seaman et al., 2007). The greatest increases in indoor acrolein concentrations in occupied homes in all three counties studied (Los Angeles, Placer, Yolo) were associated with cooking with fats and oils. Homes with frequent, regular cooking activity had the highest morning acrolein levels. In four unoccupied new houses, indoor acrolein concentrations were increased by 10-fold compared with those outdoors, although no cooking or smoking had taken place. However, the particle board and lumber used to construct these houses was found to emit acrolein (1–8 ng acrolein/g). The Relationship of Indoor, Outdoor and Personal Air (RIOPA) study of 398 homes in the USA found quite different average acrolein concentrations in the three cities studied. The average concentration in these cities ranged from 1.0 µg/ m^3 in Elizabeth, New Jersey, and 1.2 µg/m^3 in Los Angeles, California, to 3.1 µg/m^3 in Houston, Texas (Weisel et al., 2005).

In Prince Edward Island, Canada, acrolein concentrations were consistently two and a half times higher in homes with smokers than in homes without. Similarly, concentrations were higher in homes with new carpets than in those without new carpets. No significance was found for the presence of wood stoves, the type of heating, or painting (Gilbert et al., 2005). Subsequent studies of over 250 homes in Edmonton, Halifax, Regina, and Windsor, Canada, also found that homes with smokers had distinctly higher concentrations of acrolein than homes without, and also that indoor concentrations of acrolein were higher than outdoor concentrations; median indoor concentrations ranged from 1.3 to 8.1 µg/m^3, while paired outdoor concentrations were more than 60% lower (ranging from 0.2 to 2.2 µg/m^3) (Health Canada, 2020).

Other sources of acrolein in homes include burning incense and using kilns. Burning incense increases acrolein concentrations by 2.67–8.14 ppm/g [6000–19 000 µg/m^3 per g] burned (Lin & Wang, 1994). Hirtle et al. (1998) measured acrolein concentrations greater than 20 ppb [46 µg/m^3] in three homes with kilns.

Overall, the acrolein concentrations in homes ranged from less than 0.01 to 39 µg/m^3, with median concentrations of 1 to 8 µg/m^3.

(ii) Primary schools

In a study of 408 primary schools (attended by 6590 students) in France, 14% of the children were found to be exposed to acrolein at concentrations greater than 1.55 µg/m^3 in their classrooms (Annesi-Maesano et al., 2012). [The Working Group noted that the aldehyde (acrolein, formaldehyde, and acetaldehyde) concentrations inside the classrooms in this study were greater than the outdoor concentrations in the same cities, which indicates that there might be indoor sources, but these were not identified. Possibilities include smoking by staff or emissions from building materials.] Similarly, a study of seven schoolrooms in Mira Loma, California, USA, reported that acrolein concentrations in the

classroom were greater than outdoor concentrations. The authors attributed the higher indoor acrolein concentrations to building elements such as carpet, drywall, and adhesives (Sawant et al., 2004).

(iii) Hospitality sites

Hospitality sites where smoking was permitted had higher indoor concentrations of acrolein. Measurements made in the 1970s and early 1980s in France found acrolein concentrations in cafés to be between 12 and 43 µg/ m^3. Acrolein concentrations in restaurants and taverns in the Netherlands were between 1 and 8 µg/m^3, and concentrations in a car with three smokers increased from 13 µg/m^3 with the windows open to ten times that level when the windows were closed (Triebig & Zober, 1984). Löfroth et al. (1989) reported acrolein concentrations on two evenings to be 21 and 24 µg/m^3 in a tavern in the USA. [The Working Group noted that the advent of smoke-free regulations has presumably lowered these concentrations substantially.]

(d) Outdoor air pollution

The major sources of acrolein in the outdoor environment are forest fires and exhaust from motor vehicles and aircraft. Acrolein is released directly into the ambient air from vehicle exhaust and is also formed by photo-oxidation of 1,3-butadiene and other hydrocarbons (Faroon et al., 2008,). These reactions comprised an estimated 39% of total acrolein emissions in California, USA, in 2012 (OEHHA, 2018). Other sources of acrolein, which may be important in nearby local areas, include emissions from manufacturing processes such as pulp and paper, coal/gas/oil-fired power plants, waste-disposal emission, and the volatilization of biocides.

The seasonal effect for acrolein is opposite to that for many other pollutants in that concentrations decrease in winter. For example, the median summer concentration measured in several European cites was 2 µg/m^3, while the median winter concentration was 0.6 µg/m^3 (Campagno- lo et al., 2017), which may be partially attributable to the decline in frequency of photochemical reactions with seasonal reduction in solar intensity.

Outdoor concentrations of acrolein in the USA are typically 0.5–3.2 ppb [1–7 µg/m^3] (Faroon et al., 2008), although acrolein concentrations measured outside 124 homes in Houston, Texas, averaged 17.9 µg/m^3 (Weisel et al., 2005). Median concentrations in California were 0.041 µg/m^3 in coastal areas, 0.068 µg/m^3 in intermediate areas, 0.101 µg/m^3 in the San Francisco Bay area, and 0.32 µg/m^3 in the Los Angeles air basin (Cahill, 2014); concentrations outside 15 homes averaged 0.60 µg/m^3 (Seaman et al., 2007). Based on measurements throughout the state, acrolein exposures in California increased between 2004 (0.51 ppb) [1.2 µg/m^3] and 2014 (0.66 ppb) [1.5 µg/ m^3], although concentrations of volatile organic compounds other than aldehydes have declined, and acrolein emissions from gasoline-related sources decreased by two thirds between 1996 and 2012. The increase in acrolein emissions from non-gasoline related sources in 2012 was attributed primarily to a higher estimate of emissions from waste disposal (OEHHA, 2018).

Exhaust from gasoline- and diesel-powered vehicles is one of the most important, ubiquitous sources of acrolein in outdoor air. With the introduction of engine and fuel improvements due to stricter regulations to reduce exhaust emissions, this contribution has declined in North America and Europe. Schauer et al. (2002) reported that tailpipe emissions of acrolein from several gasoline-powered vehicles equipped with early catalytic converters (1981–1994) were greatly reduced compared with those from vehicles without these converters (1969–1970), from 3800 to 60 µg/km. The estimated acrolein emissions

from on-road vehicles in the 48 contiguous states of the USA in 2007 were less than half the estimated emissions in 1996 (10 185 versus 21 266 metric tonnes/year). This decrease was almost entirely due to reductions from gasoline-powered vehicles and was attributed to changes in gasoline formulation and implementation of stricter Tier 2 emission standards for light-duty vehicles (IARC, 2013).

(i) Local sources

Local sources may increase acrolein concentrations. The importance of nearby industry and traffic is illustrated by the results of 2 years of sampling in the Pittsburgh area, Pennsylvania, USA. Four locations were sampled every sixth day: one near downtown (near the city centre) with heavy traffic; one remote from both traffic and industry; and two in residential areas within 0.8 km of heavy industry. In the two residential areas near industry, acrolein concentrations were approximately double those in the rural area, while the downtown area had the highest average and 95th percentile concentrations (Logue et al., 2010). Other evidence of the importance of local sources included measurements made in the vicinity of a petrochemical plant: acrolein concentrations were 640 µg/m^3 at a distance of 1 km, and 2000 µg/m^3 at 100 m. Concentrations measured 50 m from a perfume factory ranged from 40 to 480 µg/m^3 (Izmerov, 1984).

Acrolein is used as a biocide in irrigation canals and volatilizes quickly after application. In the San Joaquin Valley of California, USA, a major agricultural area through which pass the 640 km California Aqueduct and numerous irrigation canals, an estimated 90 tonnes of acrolein were volatilized into the air in 2001 (CEPA, 2002). The estimate for 2012 was 33 tonnes (OEHHA, 2018). [The Working Group noted that no measurements of ambient acrolein concentrations were made while acrolein was in use, but these could affect local concentrations.]

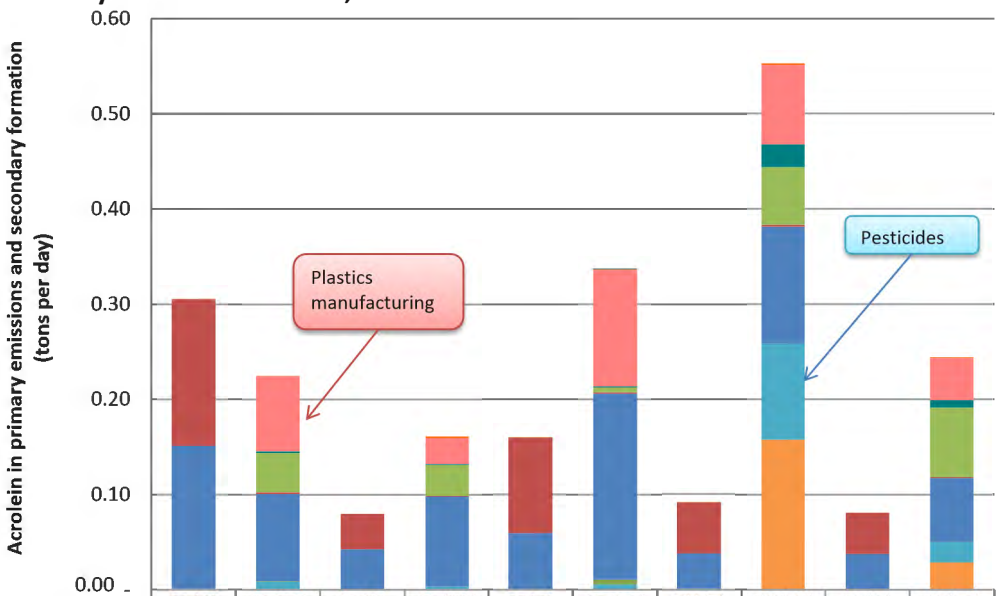
(ii) Diesel and biodiesel

A study of emissions from the engines of two heavy-duty trucks found that both pollution-control technology and fuel were major determinants of acrolein emissions (Cahill & Okamoto, 2012). The truck engine built in 2008 was equipped with a diesel oxidation catalyst/diesel particulate filter, while the truck engine built in 2000 was not, although it complied with the environmental regulations of the time. Emissions from the truck engine without pollution controls (without the catalyst/filter) were 2–10 times greater than those from the engine with these controls, depending on the fuel type used; the difference was least for ultra-low sulfur diesel fuel (ULSD; a petroleum product) and greatest for soy biodiesel blend (50% soy biodiesel and 50% ULSD). More fuels were tested with the 2008 engine than with the 2000 engine. These included: ULSD, a soy biofuel, an animal biofuel, a "renewable" fuel (hydrotreated biofuel), and 50:50 blends of each of the biofuels and ULSD. Acrolein emissions from the renewable fuels (hydrotreated biofuel, and 50:50 hydrotreated biofuel and ULSD) were comparable to those from the petroleum-based fuel (ULSD); the animal biofuel and blend emitted 40% more acrolein than the ULSD fuel, and the soy biofuel emitted the most acrolein (two and a half to three times that of the ULSD).

(iii) Gasoline and other sources of acrolein

Gasoline- and diesel-powered road motor vehicles are the major quantified source of acrolein in outdoor air in Canada. Annual releases from these were estimated to be 209 000 to 2 730 000 kg. However, unquantified but possi - bly greater sources of acrolein are other vehicles that are not fitted with pollution-control devices, such as aircraft, railway and marine vehicles, as well as off-road motor vehicles, lawnmowers, and snowblowers. Other major anthropogenic sources include the

Fig. 1.1 Primary and secondary sources of acrolein, 2012



	SC (G)	SC (NG)	SD (G)	SD (NG)	SF (G)	SF (NG)	SJV (G)	SJV (NG)	SV (G)	SV (NG)
CLEANING AND SURFACE COATINGS	-	-	-	-	-	< 0.01	-	-	-	-
FUEL COMBUSTION	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
INDUSTRIAL PROCESSES	-	0.08	-	0.03	-	0.12	-	0.08	-	0.04
MISCELLANEOUS PROCESSES	-	< 0.01	-	< 0.01	-	< 0.01	-	0.02	-	< 0.01
NATURAL SOURCES	-	0.04	-	0.03	-	< 0.01	-	0.06	-	0.07
ON-ROAD MOTOR VEHICLES	0.15	< 0.01	0.04	< 0.01	0.10	< 0.01	0.05	< 0.01	0.04	< 0.01
OTHER MOBILE SOURCES	0.15	0.09	0.04	0.09	0.06	0.20	0.04	0.12	0.04	0.07
PETROLEUM PRODUCTION AND DISTRIBUTION	-	< 0.01	-	-	-	< 0.01	-	< 0.01	-	< 0.01
SOLVENT EVAPORATION	-	< 0.01	-	< 0.01	-	< 0.01	-	0.10	-	0.02
WASTE DISPOSAL	-	-	-	-	-	-	-	0.16	-	0.03

G, gasoline sources; NG, non-gasoline sources; SC, South Coast (Los Angeles and surrounding counties); SD, San Diego County; SF, San Francisco Bay Area; SJV, San Joaquin Valley, southern part of Central Valley, a hot, dry agricultural region with major irrigation canals; SV, Sacramento Valley, northern part of Central Valley, also agricultural.
"Other mobile sources" of gasoline-attributed emissions include recreation boats, off-road equipment including garden and lawn, (each responsible for about 40%), off-road recreational vehicles such as motorcycles and all-terrain vehicles, and agricultural equipment and fuel storage.
Each region has two bars, the first for the gasoline sources and the second for all other sources. The first bar in each region represents the portion attributable to gasoline; especially notable here is that on-road motor vehicles, which have been tightly regulated, now contribute approximately half the gasoline-attributable acrolein, while the other half comes from "other mobile sources," such as recreational boats, off-road equipment for gardens and lawns (each contributes about 40% of this category), and off-road motorcycles and all-terrain vehicles. The second bar in each set illustrates the contribution of industrial processes, natural sources, such as wildfires, waste disposal, and "solvent evaporation," which refers to the volatilization of acrolein used as a biocide. This latter contribution appears significant only in the two agricultural regions of the Central Valley, the San Joaquin Valley, and the Sacramento Valley.
From OEHHA (2018), with permission.

oriented strand board industry (25 664 kg/year), pulp and paper mills (18 735 kg/year), waste incineration (2435 kg/year), and coal-based power plants (467–17 504 kg/year) (Environment Canada and Health Canada, 2000).

A recent evaluation of the sources of acrolein emissions in outdoor air in California, USA, reported that the contribution of gasoline, as both a primary and secondary pollutant, declined significantly from 52% in 1996 to 28% in 2012. Despite this decline, the average exposure to ambient acrolein in California increased

from 0.51 to 0.66 ppb [1.2 to 1.5 µg/m^3] (OEHHA, 2018). Fig. 1.1 presents the dominant sources of acrolein in three urban and two agricultural areas of California in 2012.

1.4.3 Occupational exposure

Workers may be exposed occupationally to acrolein during its manufacture and use as a chemical intermediate (see Section 1.2). However, as for the population at large, workplace exposures to acrolein occur primarily from the formation of acrolein during the incomplete combustion of organic material such as tobacco, cooking oils, gasoline and diesel fuel, and forest and residential fires.

The National Occupational Exposure Survey estimated that approximately 1300 workers were potentially exposed to acrolein in the USA when the study was conducted in 1981–1983. Approximately one third of these workers were mechanics and repairers. Other occupations identified with potential exposure to acrolein included painters and spray painters, machinists, sheet metal workers, chemical technicians, janitors, and water and sewage treatment plant operators (NIOSH, 1990). [The Working Group noted that the survey did not include agricultural production, mining activity, or railroad transportation. During the subsequent 40 years, occupational exposures in manufacturing in the USA have evolved significantly and these numbers have probably changed substantially due to changes in product usage, export of chemical manufacturing, and automation, to name a few examples.] Between 1993 and 2009, 8 cases of acrolein-related illness from pesticide usage were identified in Washington State and California, USA (Rodriguez et al., 2013).

Occupational exposure to acrolein in firefighting, manufacturing, welding, food processing, and traffic-related occupations is presented in Table 1.4 and detailed below.

(a) Firefighting

Firefighters are exposed to high concentrations of acrolein produced during the incomplete combustion of burning materials. Structural fires and wildland fires are fought by distinctly different crews who have different exposure profiles. The exposures of wildland firefighters and urban firefighters are presented in Table 1.4.

The two distinct phases of fighting structural fires are: (i) knockdown, when the visible flames are extinguished; and (ii) overhaul, during which smouldering material is searched for embers and hidden flames. Jankovic et al. (1991) collected short-term personal samples from 22 fires, mostly residential, in the USA and reported that half the samples from during knockdown exceeded the short-term exposure limit (STEL) for acrolein at the time – 300 ppb [690 µg/m^3] – and that the maximum value was 3200 ppb [7330 µg/m^3]. Their data were similar to those reported by Burgess et al. in 1979 and plotted in the Jankovic publication. Together, these data provided a median of 500 ppb [1100 µg/m^3], with a 95th percentile of 5000 ppb [11 000 µg/m^3] and a maximum of 15 000 ppb [34 000 µg/m^3]. During knockdown, firefighters wear a self-contained breathing apparatus; some samples collected inside the breathing mask measured as high as 900 ppb [2000 µg/m^3]. During overhaul, when a self-contained breathing apparatus is not generally worn, measured acrolein concentrations were as high as 200 ppb [500 µg/m^3] in the Jankovic publication and 300 ppb [700 µg/m^3] in a study of 25 fires in the USA by Bolstad-Johnson et al. (2000). Of the 96 30-minute samples collected by BolstadJohnson et al. (2000), only 7 exceeded the limit of detection (11 ppb [25 µg/m^3]). The mean for these 7 samples was 123 ppb [282 µg/m^3].

Wildland firefighters do not wear respiratory protection. The three types of wildland firefighting are: (i) initial attack – the first day of a fire, during which all but 5% of

fires are extinguished; (ii) project fires – the second and successive days of fighting those few fires that continue past the first day; and (iii) prescribed burns – intentionally set and controlled fires in an established area. In the USA, Reinhardt & Ottmar (2004) reported geometric mean (GM) acrolein concentrations of 1 ppb [2 µg/m^3] during 13–14 hour shifts for the initial attack day (45 samples) and also for the subsequent days (84 samples), while the GM during prescribed burns was 9 ppb [21 µg/m^3] (11.5-hour average shift, 200 samples), and the maximum concentrations were 11, 15, and 60 ppb [25, 34, and 140 µg/m^3], respectively. Similar results for prescribed burns in the USA

Table 1.4 Occupational exposure to acrolein

Job, task or industry	Country	No. of sites	No. of samples	Acrolein air concentration	Reference
Firefighting					
Overhaul (structure fires)	USA	25	96	Mean, 0.123 ppm [282 µg/m³] Max., 0.3 ppm [687 µg/m³]	Bolstad-Johnson et al. (2000)
Initial attack – fireline (wildfires)	USA	NR	45	Geometric mean, 5 ppb [11.5 µg/m³] Max., 11 ppb [25 µg/m³]	Reinhardt & Ottmar (2004)
Project fires – fireline (wildfires)		NR	84	Geometric mean, 2 ppb [4.6 µg/m³] Max., 16 ppb [34 µg/m³]	
Prescribed burns – fireline (wildfires)		NR	200	Geometric mean, 15 ppb [34.4 µg/m³] Max., 98 ppb [225 µg/m³]	
Prescribed burns – pre- to post-shift timeweighted averages	USA	NR	65	Mean, 0.01 ppm [22.9 µg/m³] Max., 0.041 ppm [94 µg/m³]	Slaughter et al. (2004)
Manufacturing					
Phenol-formaldehyde resins (abrasive materials)	Poland	13	NR	Range, 0–0.003 mg/m³ [0–3 µg/m³]	Pośniak et al. (2001)
Phenol-formaldehyde resins (friction linings)		11	NR	Range, 0–0.01 mg/m³ [0–10 µg/m³]	
Plastics	USA	130[a]	23[b]	Mean[c], 39 ppb [89 µg/m³] Max., 240 ppb [550 µg/m³]	OSHA (2020)
Tyres and inner tubes		1	1 [b]	Max: 11 ppb [25 µg/m³]	
Copper foundries		17 [a]	6 [b]	Mean[c], 12 ppb [27 µg/m³] Max., 45 ppb [103 µg/m³]	
Photographic equipment		4 [a]	3 [b]	Mean[c], 1.2 ppb [2.7 µg/m³] Max., 1.8 ppb [4.1 µg/m³]	
Packing and crating		3[a]	3[b]	Mean[c], 6.7 ppb [15 µg/m³] Max., 8.5 ppb [19 µg/m³]	
Potters	Canada	10	50	Range, < 28–110 ppb [< 64–252 µg/m³]	Hirtle et al. (1998)
Welding and flame cutting					
Welding (unspecified)	USA	3[a]	1[b]	Max., 21 ppb [48 µg/m³]	OSHA (2020)
Food production					
Tortilla manufacturing	USA	8[a]	6[b]	Mean[c], 14 ppb [32 µg/m³] Max., 26 ppb [60 µg/m³]	OSHA (2020)

Food production including tortilla	USA	22[a]	13[b]	Mean[c], 29 ppb [66 µg/m³] Max., 74 ppb [169 µg/m³]	OSHA (2020)
Restaurants					
University catering kitchen	Iran	16	NR	Mean, 670 ppb [1534 µg/m³] Range, 210–910 ppb [481–2084 µg/m³]	Neghab et al. (2017)

Table 1.4 (continued)

Job, task or industry	Country	No. of sites	No. of samples	Acrolein air concentration	Reference
Restaurants, hotels and burger chains	Norway	44	NR	Mean, 10 ppb [23 µg/m³] Max., 32 ppb [73 µg/m³]	Svendsen et al. (2002)
Gasoline and diesel exhaust-related exposures					
Bus drivers	Poland	10 drivers serving 5 bus lines	NR	Range, 0.01–0.035 mg/m³ [10–35 µg/m³]	Brzeźnicki & Gromiec (2002)
Toll station operators	USA	NR	6	Range, 0.031–0.14 µg/m³	Destaillats et al. (2002)
Toll station operators	Spain	15 attendants at 2 toll stations	17	Range, < 0.5–2.75 µg/m³]	Belloc-Santaliestra et al. (2015)
Highway construction	USA	12[a]	3[b]	Mean[c], 91 ppb [208 µg/m³] Max., 155 ppb [355 µg/m³]	OSHA (2020)
Transportation	USA	12[a]	2[b]	Mean[c], 9 ppb [21 µg/m³] Max, 20 ppb [46 µg/m³]	OSHA (2020)
Waste management and incineration					
Waste management	USA	3[a]	1[b]	Max, 13 ppb [29.3 µg/m³]	OSHA (2020)
Working near burn pit and incinerator operations at an airfield	Afghanistan	3 sites within close proximity	78	Site means, 9–19 ppb [21–44 µg/m³] Site maxima, 39–140 ppb [89–321 µg/m³]	Blasch et al. (2016)

NR, not reported; ppb, parts per billion. [a] Number of measurements.

[b] Number of measurements above the limit of detection. [c] Mean value of measurements above the limit of detection was calculated by the Working Group.

were reported by Slaughter et al. (2004): a timeweighted average (TWA) mean of 10 ppb [23 µg/ m³] and a maximum of 41 ppb [94 µg/m³] for 65 samples. Task-specific (~2 hours) concentrations ranged from < 1 ppb [< 2.3 µg/m³] at the engine and 5 ppb [11 µg/m³] while igniting the fire to 30 ppb [69 µg/m³] for the holding boss and 18 ppb [41 µg/m³] for others holding the fire within prescribed boundaries. A 30-minute exposure during direct attack to extinguish flames that had escaped these boundaries was 62 ppb [140 µg/m³] (Reinhardt & Ottmar, 2004).

(b) Manufacturing operations

The manufacture of acrolein can lead to very high exposures of 43–3526 ppb [98–4075 µg/m³] (Izmerov, 1984). Various plastic-manufacturing processes use or produce acrolein. Polyethylene extrusion operations and phenol–formaldehyde resins led to exposures under 13 ppb [< 30 µg/m³] (Tikuisis et al., 1995; Pośniak et al., 2001).

(c) Welding

In a study in Ukraine, Protsenko et al. (1973) found that, while metal untreated with primer emitted no measurable acrolein, some primers coated onto metals resulted in significant acrolein emissions during both gas cutting and automatic submerged arc welding, with acrolein concentrations reaching 447 ppb [1024 µg/m³]. While exposures during welding in new ship outfitting averaged 9 ppb [21 µg/m³], with maximum values reaching 28 ppb [64 µg/m³], exceeding the occupational exposure limit (OEL) for the European Union (EU), exposures during ship repair were even higher, reaching 64 ppb [150 µg/m³], and over half the shipbreaking samples exceeded the EU OEL, with one sample at 600 ppb [1400 µg/m³]. Although in most short-term (15-minute) samples collected in engine and garage repair shops acrolein was not detectable (i.e. < 65 ppb [< 150 µg/m³]), one sample contained acrolein at 260 ppb [595 µg/m³].

(d) Food processing, traffic-related, and other occupations

Exposures (summarized in Table 1.4) mea - sured in restaurant kitchens are highly variable, probably reflecting emissions from cooking fuels. Similarly, those who work near gasoline exhaust, such as bus drivers, garage workers, and highway construction workers, and those who work at or near incineration facilities, have highly variable and significant exposures, from 10 ppb to > 100 ppb [~23 to > 230 µg/m³]. Klochkovskii et al. (1981) reported that 37% of 800 samples collected in quarry operations in an area of the former Soviet Union exceeded permissible limits, and that acrolein concentrations in exhaust gases and workplace air averaged 900–3100 ppb [2100–7100 µg/m³].

(e) Occupational exposure to acrolein from secondhand smoke

Workers, especially hospitality workers, may also be subject to significant exposures to acrolein in places where smoking is permitted. Acrolein concentrations in a tavern in North Carolina, USA, with moderately high levels of secondhand smoke (on average, particles, 430 µg/m³; and nicotine, 66 µg/m³) were measured at 21 µg/m³ and 24 µg/m³ on two sampling trips of 3–4 hours each (Löfroth et al., 1989). In open offices where smoking was allowed in Massachusetts, USA, the 90th percentile of weekly average concentrations of nicotine was 34 µg/m³ (Hammond et al., 1995), so office exposures may exceed 5 ppb [11 µg/m³] acrolein (Mitova et al., 2016). Ayer & Yeager (1982) reported that acrolein concentrations reached > 50 ppm [114 000 µg/m³] in the smoke plume of cigarettes. Thus, secondhand smoke can

be an important source of both peak and TWA exposure to acrolein.

(f) Occupational Safety and Health Administration compliance data

OSHA maintains a publicly available database of industrial hygiene samples collected in the USA as part of its compliance monitoring programme, the Chemical Exposure Health Data (OSHA, 2020). The results for 1220 samples and blanks collected by OSHA inspectors and analysed for acrolein between 1984 and 2019 provide some information from inspections for those 35 years (OSHA, 2020). These values should be compared with the 8-hour TWA OSHA permissible exposure limit of 100 ppb [250 µg/m^3], the EU OEL of 20 ppb [50 µg/m^3] for 8-hour TWA and 50 ppb [114 µg/m^3] STEL (for 15 minutes) as well as the American Conference of Governmental Industrial Hygienists (ACGIH) ceiling value of 100 ppb [250 µg/m^3]. Only about 10% of the samples were above the limit of detection, and only 3 of the nearly 200 samples collected for less than 1 hour had detectable concentrations of acrolein, but of these 2 were of concern: the 15-minute sample was 115 ppb [263 µg/m^3] acrolein and the 24-minute sample was 69 ppb [158 µg/m^3], both in excess of the EU short-term limit of 50 ppb [120 µg/m^3]; the limit of detection in air for these shorter-timed samples would have been higher than that for the 8 hour samples, but these values were not in the database. Because of the intense irritation caused by acrolein, the ACGIH recommends neither an 8 hour nor a 15-minute STEL, but, rather, a ceiling of 100 ppb [250 µg/m^3] that should never be exceeded. [The Working Group noted that, while none of the OSHA samples contained detectable levels of acrolein after such short exposures, the higher concentrations clearly indicated that this recommendation was exceeded for many samples.] Of the samples with detectable levels of acrolein, 40% exceeded the EU OEL of 20 ppb [50 µg/m^3], and half of these samples contained acrolein at more than twice that OEL (Table 1.4).

The highest acrolein concentration reported was from samples collected in late 2018 at a company that manufactured plastic pipes and pipe fittings. Four workers wore the sampling equipment for 90–180 minutes and their exposure concentrations were less than detectable, 17, 25, and 240 ppb [39, 57, and 550 µg/m^3] (sampling times were 90, 140, 180, and 170 minutes, respectively). Only 17% of the 134 personal samples collected at approximately three dozen plastic-manufacturing establishments were above the limit of detection. Those samples that were detectable ranged from 3 to 240 ppb [7 to 550 µg/m^3] acrolein, with an average of 39 ppb [89 µg/m^3] and a median of 21 ppb [48 µg/m^3]; one 24-minute sample averaged 69 ppb [158 µg/m^3], above the EU STEL of 50 ppb [120 µg/m^3] (Table 1.4; OSHA, 2020).

Over half of the personal samples collected from food production workers had detectable concentrations of acrolein, and both the mean and median values of those samples (29 and 25 ppb [66 and 57 µg/m^3], respectively) exceeded the EU OEL of 20 ppb [50 µg/m^3] (OSHA, 2020).

1.5 Regulations and guidelines

1.5.1 Exposure limits and guidelines

(a) Occupational exposure limits

Acrolein is a severe irritant to the eyes, mucous membranes, and the respiratory tract at concentrations lower than 1 ppm, and this is the basis for OELs. At higher concentrations, acrolein can cause pulmonary oedema and death (10 ppm; 23.3 mg/m^3) (ATSDR, 2014; ACGIH, 2019).

In 1946, the ACGIH recommended that 8-hour TWA exposure to acrolein should not

exceed 0.5 ppm [1100 µg/m³]. This value was lowered to 0.1 ppm [230 µg/m³] in 1963. In 1976, a STEL of 0.3 ppm [690 µg/m³] was added to this recommendation, and in 1998 both the TWA and the STEL were replaced by a ceiling value of 0.1 ppm [230 µg/m³] that should not be exceeded for any duration. These ACGIH Threshold Limit Values® were intended as recommendations to industrial hygienists but have been adopted by many countries as OELs directly, by reference, or as the basis upon which national OELs were developed. Currently the EU has an 8-hour TWA OEL of 0.02 ppm or 0.05 mg/m³ and a STEL of 0.05 ppm or 0.12 mg/m³ (European Commission, 2017). Within the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) registration of acrolein, the derived no-effect level (DNEL) of long-term exposed workers was set at 0.2 mg/m³ for both local and systemic effects, and the DNEL for long-term skin exposure at 0.08 mg/kg body weight (bw) per day (ECHA, 2020).

Table 1.5 presents the OELs for various countries. Many countries use the EU OEL of 0.02 ppm [0.05 mg/m³], or the older ACGIH OEL (TWA, 0.1; STEL, 0.3) or the current ACGIH ceiling value of 0.1 ppm [0.23 mg/m³].

(b) Environmental exposure limits

The US EPA reference concentration for inhalation exposures is 2×10^{-5} mg/m³, and the reference dose for oral exposures is 0.5 µg/kg per day (US EPA, 2003). The US Agency for Toxic Substances and Disease Registry (ATSDR) set the minimal risk level for ingestion of acrolein at 4 µg/kg per day for 15–364 days on the basis of forestomach squamous epithelial hyperplasia in mice (ATSDR, 2007). The International Programme on Chemical Safety tolerable intake levels are 0.17 ppb [0.4 µg/m³] for inhalation exposures and 1.5 µg/mL (corresponding to 7.5 µg/kg bw per day) for drinking-water exposures (IPCS, 1992).

For subchronic exposures, e.g. 8 hours, environmental guidelines were 0.03–4.8 ppb [0.07–11 µg/m³], whereas OELs were 20–100 ppb [0.05–0.23 mg/m³], although some guidelines suggested ceiling values of 100 ppb [230 µg/m³] that should never be exceeded.

The occupational guidelines for acute exposures (50–100 ppb [120–250 µg/m³]) are approximately 10–100 times the environmental guidelines for acute exposures. Acute Exposure Guideline Levels (AEGLs) have been established for acrolein (National Research Council, 2010). The lethal level of exposure (AEGL-3) is reached after 10 minutes of exposure to acrolein at 6.2 ppm [14 000 µg/m³], whereas exposure to acrolein for any duration from 10 minutes to 8 hours at 30 ppb [69 µg/m³] leads to slight eye irritation and discomfort.

Table S1.3 (Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc.fr/602) presents some guidelines for acrolein concentrations in the air.

1.5.2 Reference values for biological monitoring of exposure

A metabolite of acrolein (the mercapturic acid HPMA) has been measured as an indicator of exposure. The German Committee for the determination of occupational exposure limits (the "MAK-Commission") suggests a biological reference value for workplace substances (BAR) for HPMA of 600 µg/g creatinine in the urine in non-smokers (Jäger, 2019).

1.6 Quality of exposure assessment in key epidemiological studies

Table S1.4 and Table S1.5 (Annex 1, Supplementary material for Section 1, web only; available from: https://www.publications.iarc.fr/602) provide a detailed overview and critique of the methods used for exposure assessment in cancer epidemiology studies and mechanistic studies in humans that have been included in the evaluation of acrolein. Methods for the exposure assessment varied according to type of study. In the cancer studies in humans, two occupational cohort studies assigned exposure to acrolein, on the basis of expert evaluation of company

Table 1.5 Occupational exposure limits for acrolein in various countries

Country or agency	8-hour TWA		Short-term (15 minutes)		Ceiling		Reference
	ppm	mg/m³	ppm	mg/m³	ppm	mg/m³	
Argentina					0.1	0.23	ACGIH (2019), IOHA (2018)
Australia	0.1	0.23	0.3	0.69			IFA (2020)
Austria	0.02	0.05	0.05	0.12			IFA (2020)
Belgium	0.02	0.05	0.05	0.12			IFA (2020)
Brazil					0.1	0.23	ACGIH (2019)
Canada – Ontario					0.1		Government of Ontario (2020)
Canada – Québec	0.1	0.23	0.3	0.69			IFA (2020)
Chile					0.1	0.23	ACGIH (2019), IOHA (2018)
China						0.3	IFA (2020)
Columbia					0.1	0.23	ACGIH (2019), IOHA (2018)
Denmark	0.02	0.05	0.04	0.1			IFA (2020)
European Union	0.02	0.05	0.05	0.12			IFA (2020)
Finland	0.02	0.05	0.05	0.12			IFA (2020)
France	0.02	0.05	0.05	0.12			IFA (2020)
Germany – AGS	0.09	0.2	0.18	0.4			IFA (2020)
Hungary		0.23		0.23			IFA (2020)
India	0.1	0.25	0.3	0.8			Government of India (2015)
Ireland	0.02	0.05	0.05	0.12			IFA (2020)
Japan – JSOH	0.1	0.23					IFA (2020)
Latvia	0.02	0.05	0.05	0.12			IFA (2020)
Mexico					0.1	0.23	ACGIH (2019), IOHA (2018)
New Zealand	0.1	0.23					IFA (2020)
Poland		0.05		0.1			IFA (2020)
Romania	0.02	0.05	0.05	0.12			IFA (2020)
Singapore	0.1	0.23	0.3	0.69			IFA (2020)

South Africa	0.1	0.25	0.3	0.8			South Africa Department of Labour (1995)
Republic of Korea	0.1	0.25	0.3	0.8			IFA (2020)
Spain	0.02	0.05	0.05	0.12			IFA (2020)
Sweden	0.02	0.05	0.05	0.12			IFA (2020)
Switzerland	0.02	0.05	0.05	0.12			IFA (2020)
USA – ACGIH					0.1	0.23	ACGIH (2019)
USA – NIOSH	0.1	0.25	0.3	0.8			IFA (2020)
USA – OSHA	0.1	0.25					IFA (2020)

Table 1.5 (continued)

Country or agency	8-hour TWA		Short-term (15 minutes)		Ceiling		Reference
	ppm	mg/m³	ppm	mg/m³	ppm	mg/m³	
USA – Cal/OSHA					0.1	0.25	State of California (2020)
United Kingdom	0.02	0.05	0.05	0.12			IFA (2020)
Venezuela					0.1	0.23	ACGIH (2019), IOHA (2018)

ACGIH, American Conference of Governmental Industrial Hygienists; AGS, Ausschuss für Gefahrstoffe (German Committee on Hazardous Substances); Cal/OSHA, California Division of Occupational Safety and Health; JSOH, Japan Society for Occupational Health; NIOSH, National Institute for Occupational Safety and Health; OSHA, Occupational Safety and Health Administration; ppm, parts per million; TWA, time-weighted average.

records on the use of chemicals and also on job history information. No quantitative exposure assessment methods were applied. Other studies used internal markers of exposure to acrolein, based on urinary acrolein metabolites (HPMA), acrolein–DNA adducts from buccal cells, or analyses of acrolein–protein conjugates in serum samples (see Section 4.2.1 for further discussion of acrolein-derived DNA and protein adducts). In these studies, some information on possible external sources of exposure (e.g. smoking, betel-quid chewing, air pollution) was collected through questionnaires. The mechanistic studies in humans showed a partial overlap with the cancer studies in humans, applying internal markers of exposure showing similar limitations regarding assessment of external exposure.

1.6.1 Quality of exposure assessment in key cancer epidemiology studies

Two studies of occupational exposure identified workers exposed to acrolein using information from available records (Bittersohl, 1975; Ott et al., 1989a). No quantitative assessment of exposure was carried out in these studies.

Bittersohl (1975) investigated cancer fre - quency in an aldehyde factory in Germany and reported that the derivatives produced contained traces of acrolein; however, no evidence was provided that this resulted in any exposure of the workforce to acrolein. Employees were exposed to other chemicals at higher levels than acrolein.

Ott et al. (1989a) investigated risk of lymphohaematopoietic cancer in a complex chemical-manufacturing facility in the USA and assessed the potential for exposure of workers to 21 specific chemicals, including acrolein. Workers were assigned as having been exposed to acrolein if they worked in an area where acrolein was used for 1 day or more. This assessment was based on linking information on job histories with records that contained information on the historical use of chemicals in each department. Intensity of exposure was not assessed, but duration of exposure was estimated. There was no evidence provided of the airborne levels of acrolein in these production facilities. Among 200 production workers, 25 (12.5%) were judged to have been exposed to acrolein for at least 1 day and 3% were exposed to acrolein for 5 years or more (Ott et al., 1989b). Workers were likely to be exposed simultaneously to other chemical agents.

Four other studies assessed exposure to acrolein using internal markers. Yuan et al. (2012, 2014) estimated exposure to acrolein in two lung cancer case–control studies of smokers and non-smokers, respectively, nested within a cohort study of men in Shanghai, China. A single void urine sample was collected from each participant at baseline and analysed to determine the concentration of HPMA, and a range of other urinary biomarkers (including cotin - ine). Information on smoking was available. No assessment of external exposure to acrolein was carried out.

Tsou et al. (2019) investigated the role of acrolein in oral cancer and estimated exposure to acrolein through analyses of urinary HPMA and of acrolein–DNA adducts in buccal cells collected from cases and controls in Taiwan, China. Information was also collected on smoking history and betel-quid chewing. Buccal cells and urine samples were collected after diagnosis. There was no statistically significant difference in buccal acrolein–DNA adduct levels between healthy controls with different smoking and betel-quid chewing histories. The urinary HPMA concentration was statistically significantly correlated with smoking years and betel-quid chewing years. [The Working Group noted that it was not clear from the data to what extent the levels of buccal

acrolein–DNA adducts and urinary HPMA levels are representative of historical exposure attributable to smoking and betel-quid chewing. The Working Group was not certain whether acrolein–DNA adducts can be considered as a marker of exposure or effect, particularly since samples were collected and analyses carried out after diagnosis.]

Finally, Hong et al. (2020) investigated the role of endogenous exposure to acrolein in a case–control study of urothelial carcinoma patients with chronic kidney disease and healthy controls in Taiwan, China. Endogenous exposure to acrolein was estimated using acrolein–DNA adducts in DNA from tumour or normal urothelial cells, HPMA in urine, and acrolein–protein conjugates in serum samples. [The Working Group noted that information on smoking and air pollution was collected, but these exposures were considered only as confounders in the analyses. The Working Group was not certain whether acrolein–DNA adducts can be considered as a marker of exposure or effect. Markers of acrolein exposure were estimated in samples collected from cases and controls after diagnosis; hence it is not clear whether endogenous exposure to acrolein preceded tumour development or was a consequence of the urothelial carcinoma.]

1.6.2 Quality of exposure assessment in mechanistic studies in humans

Common elements of the human mechanistic studies were their cross-sectional nature, the small sample size (typically 10–20 participants) and the method-development design (e.g. to facilitate and optimize the measurement of certain acrolein adducts in various human tissues) (e.g. Nath & Chung, 1994; Chen & Lin, 2011; Alamil et al., 2020).

The majority of the studies investigated smokers (mainly relying on self-reports), assuming that smoking is the predominant source of exposure to acrolein in humans (Nath et al., 1998; Zhang et al., 2007; Bessette et al., 2009; Zhang et al., 2011; Weng et al., 2018; Yang et al., 2019b). Tsou et al. (2019) included other factors besides smoking, such as alcohol consumption or betel-quid chewing (also see Section 1.6.1 above for a detailed critique of Tsou et al. (2019) in the Acrolein context of studies of cancer in humans). Wang et al. (2019) included fried food consumption in non-smokers, but insufficiently defined other external exposures.

Another large subset of studies investigated acrolein adducts in tumour tissues without considering any potential external exposure of the patients (Liu et al., 2005; Chen & Lin, 2011; Chung et al., 2012; Yin et al., 2013; Lee et al., 2014; Fu et al., 2018). Hence it is not clear whether external or internal exposure caused adduct formation, or adduct formation was a consequence of tumour development.

Several studies researched treatment with cyclophosphamide or other medicinal products of which acrolein is the principal metabolite (McDiarmid et al., 1991; Al-Rawithi et al., 1998; Takamoto et al., 2004). While external exposure attributable to the medicines is well characterized, all these studies failed to consider other external exposures except smoking.

Interestingly, endogenous exposure was not defined or assessed in most of the studies. Yang et al. (1999b) suggested that both endogenous and exogenous sources may contribute to the formation of acrolein–DNA adducts. [The Working Group noted that it was unclear whether the background exposure comes from endogenous formation or from a low external exposure such as air pollution, secondhand smoke, or consumption of fried food.] Noteworthy regarding endogenous exposure is the study of Ruenz et al. (2019), which placed non-smoking participants in defined living conditions, adhering to a defined diet, and which

provided convincing evidence for substantial background exposure to acrolein that was independent of smoking, ingestion of heat-processed food, or other nearby environmental exposures such as exhaust gases or open fires.

2. Cancer in Humans

2.1 Descriptions of individual studies

See Table 2.1.

Six studies – one cohort study, two case–control studies, and three nested case–control studies in cohorts – have been published on the relationship between cancer and exposure to acrolein. Five other studies (mainly case reports) described bladder cancers or leukaemia occurring after use of the pharmaceutical cyclophosphamide (classified in IARC Group 1, *carcinogenic to humans*) or ifosfamide to treat cancer or autoimmune disease. These studies on pharmaceutical agents were determined by the Working Group to be uninformative because the role of acrolein in causing these cancers could not be distinguished from that of other metabolites. The quality of the exposure assessment in the six studies described below is detailed in Section 1.6.

Bittersohl (1975) reported on a small cohort of 220 workers exposed to multiple aldehydes or aldehyde derivatives including acrolein (in trace amounts) in a factory in the former German Democratic Republic, who were followed up from 1967 to 1972. There were 9 cases of cancer in men (5 squamous cell lung carcinomas, 2 squamous cell carcinomas of the oral cavity, 1 adenocarcinoma of the stomach, and 1 adenocarcinoma of the colon) and 2 cases in women (1 leukaemia and 1 cancer of ovary). There was no formal comparison group except a narrative comparison with incidence rates in the general population, source unspecified. [The Working Group noted that although cancer rates were reported to be higher in the cohort than in the population of the German Democratic Republic, the study did not quantify any excess, nor specify the population rate in the German Democratic Republic. Exposure was poorly defined, and no attempt was made to assess exposure (semi-) quantitatively by measurements of duration. No

inference can be made regarding the association between acrolein exposure and cancer risk.]

In an occupational nested case–control study among male chemical workers in the USA, Ott et al. (1989a) reported on 129 workers who died from lymphohaematopoietic cancer and their controls (matched on hire decades), with the time scale being time since hire. Information on multiple chemical exposures was available (Ott et al., 1989b), with expert assessment of individual exposures based on jobs, including acrolein. Positive associations between acrolein exposure and non-Hodgkin lymphoma (NHL), multiple myeloma, and leukaemia were reported, based on small numbers of exposed cases (n = 6). Given the small sample size and multiple exposures, no inference was possible. [The Working Group noted that matching was based on hire decades. Implications for potential bias were not discussed in the paper. In addition, the exposure assessment was insufficient, limited to dichotomous (ever/never) classification, based on production records and not measured exposure, and exposure encompassed multiple chemicals in addition to acrolein.]

Yuan et al. (2012, 2014) published the results of two nested case–control studies within a cohort of 18 244 Chinese men enrolled in 1986–1989 in Shanghai, China. Besides in-person interviews, a spot urine sample was taken from each

participant at baseline and stored until laboratory analysis. Incident cases of and deaths from lung cancer were identified through annual in-person interviews of all surviving participants, the local cancer registry, and the vital statistics office. The first study (Yuan et al., 2012) was a nested case– control study on lung cancer, limited to current smokers at enrolment, and based on follow-up through 2006. Urinary biomarkers related to smoking habits were measured at enrolment, including HPMA (an acrolein-derived, mercapturic acid metabolite), NNAL, cotinine and others. Overall, 343 cases and 392 controls were included in the analysis, after exclusion of

Table 2.1 Epidemiological studies of cancer in humans exposed to acrolein

Reference, location, enrolment/ Exposed level	Population size, description, exposure assessment method deaths follow-up period, study design	Cancer type	Exposure category or	cases or	Risk estimate	Covariates	Comments (95% CI) controlled
Bittersohl (1975) Former German Democratic Republic 1967–1972 Cohort	220 workers in the chemical industry for dimerization of aldehydes. Workers were exposed to acetaldehyde, crotonaldehyde, butyraldehyde, and/or acrolein (traces). Exposure assessment method: records; exposure was assumed based on employment within the aldehyde factory	Lung (squamous cell carcinoma), incidence	Men: NR	5	NR	None	*Exposure assessment critique*: Poorly defined exposure. No evidence of acrolein exposure provided. No separate exposure assessment for different chemical agents present in the factory. *Limitations*: no inference possible for lack of comparator.
		Oral cavity, incidence	Men: NR	2	NR	None	
		Stomach, incidence	Men: NR	1	NR	None	
		Colon, incidence	Men: NR	1	NR	None	
		Leukaemia, incidence	Women: NR	1	NR	None	
		Ovary, incidence	Women: NR	1	NR	None	

Table 2.1 (continued)

Reference, location, period, study design	Population size, description, enrolment/ cases or deaths	Cancer type or category	Exposure level	Exposed	Risk estimate (95% CI)	Covariates controlled	Comments
Ott et al. (1989a) USA 1940–1978 Nested case–control	Cases: 52 cases of NHL, 20 cases of multiple myeloma, 39 cases of nonlymphocytic leukaemia, 18 cases of lymphocytic leukaemia; 129 deaths from lymphohaematopoietic cancers; in two chemical manufacturing plants; 29 139 men in the cohort Controls: 5 controls randomly selected per case (N not reported); incidence sampling design from the cohort. Exposure assessment method: expert judgement; 1020 substances, including acrolein, associated with different working areas; exposure was assumed based on whether a chemical substance was used at all in a production unit; no assessment of the intensity of exposure or estimation of cumulative exposure	NHL, mortality	Acrolein exposure (OR): Never Ever	 NR 2	 NR 2.6	Decade of hire (by matching in design)	*Exposure assessment critique*: No (semi-) quantitative exposure assessment carried out. Exposure was assumed based on assignment to production unit within factory. Exposure was not based on measurement of personal exposure. 21 chemicals were included, and workers are likely exposed to multiple agents (see Ott et al., 1989b). *Limitations* : cases had died, controls alive; small number of subjects exposed to acrolein.
		Multiple myeloma, mortality	Acrolein exposure (OR): Never Ever	 NR 1	 NR 1.7		
		Nonlymphocytic leukaemia, mortality	Acrolein exposure (OR): Never Ever	 NR 3	 NR 2.6		

Table 2.1 (continued)

Reference, location, enrolment/ period, study design	Population size, description, category or level	Cancer type or cases or deaths	Exposure assessment method	Exposed cases or controls	Risk estimate (95% CI)	Covariates controlled for follow-up	Comments
Yuan et al. (2012) Shanghai, China enrolment, 1986–1989/ follow-up, 2006 Nested case– control	Cases: 343 cases of incident lung cancers and deaths, current smokers at enrolment, identified through annual in-person interviews and reviewed through Shanghai Cancer Registry and Shanghai Municipal Vital Statistics Office; lung cancer cases and matched controls from within a cohort of 18 244 Chinese men in Shanghai	Lung, incidence	Quartile of urinary HPMA (l creatinine), current smokers (OR):		1.39 (0.86–2.23)	Age at baseline, neighbourhood of residence, duration of sample storage, number of cigarettes smoked per day, years of cigarette smoking at baseline	*Exposure assessment critique*: Internal exposure assessment only. All study subjects were smokers. Smoking history collected and included in the models. Urine samples were collected at baseline, so clearly preceded the health outcome; however, only one urine sample was collected. *Strengths*: study design; relatively large sample and long follow-up (20 yr); few losses to follow-up (4.6%); urinary biomarker was collected before disease occurrence; self-reported smoking status was verified by urinary cotinine. *Limitations*: intraindividual variation in exposure not captured; 35% of cases were not histologically confirmed.
			First quartile	49	1.60 (1.00–2.58)		
			Second quartile	74	2.00 (1.25–3.20)		
			Third quartile	92			
			Fourth quartile	128	(pmol/mg rs ıt enrolment		
			Trend-test *P* value, 0.004				
	Controls: 392 participants in the Shanghai Cohort Study; one control was selected from the same cohort, current smoker at enrolment, alive and free of cancer and matched to the index case by age (± 2 yr), date of specimen collection (± 1 month) and neighbourhood of residence at enrolment. Exposure assessment method: exposure to acrolein was determined based on measurement of urinary metabolites of acrolein (HPMA); urine samples were collected at baseline survey of the cohort, in which the case– control study was nested; smoking information was also collected	Lung, incidence	Quartile of urinary HPMA creatinine), current smoke (OR):	1	1.26 (0.77–2.05)	Age at baseline, neighbourhood of residence, duration of sample storage, number of cigarettes smoked per day, years of cigarette smoking at baseline, urinary total NNAL and PheT	
			First quartile	49	1.38 (0.84–2.26)		
			Second quartile	74	1.64 (1.01–2.66)		
			Third quartile	92			
			Fourth quartile	128			
			Trend-test *P* value, 0.046				

Table 2.1 (continued)

Reference, location, exposure assessment method	Population size, description, category or cases or deaths	Cancer type	Exposure level	Exposed (95% CI)	Risk estimate	Covariates controlled	Comments
Yuan et al. (2012) (cont.)		Lung, incidence	Quartile of urinary HPMA (pmol/mg creatinine), current smokers at enrolment (OR):			Age at baseline, neighbourhood of residence, duration of sample storage, number of cigarettes smoked per day, years of cigarette smoking at baseline, urinary total NNAL and PheT, total cotinine	
			First quartile	49	1		
			Second quartile	74	0.98 (0.59–1.65)		
			Third quartile	92	1.02 (0.61–1.72)		
			Fourth quartile	128	1.06 (0.62–1.8)		
			Trend-test *P* value, 0.772				
		Lung (squamous cell carcinoma), incidence	Quartile of urinary HPMA (pmol/mg creatinine), current smokers at enrolment (OR):			Age at baseline, neighbourhood of residence, duration of sample storage, number of cigarettes smoked per day, years of cigarette smoking at baseline	
			First tertile	NR	1		
			Second	NR	NR tertile		
			Third tertile	NR	2.56 (1.30–5.05)		
			Trend-test *P* value, < 0.05				
		Lung (squamous cell carcinoma), incidence	Quartile of urinary HPMA (pmol/mg creatinine), current smokers at enrolment (OR):			Age at baseline, neighbourhood of residence, duration of sample storage, number of cigarettes smoked per day, years of cigarette smoking at baseline, total cotinine	
			First tertile	NR	1		
			Second	NR	NR tertile		
			Third tertile	NR	NR		
			Trend-test *P* value: > 0.10				

Table 2.1 (continued)

Reference, location	Population size, description, period, study design	Cancer type	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments enrolment/ follow-up, exposure assessment method
Yuan et al. (2014) Shanghai, China enrolment, 1986–1989/ follow-up, 2008 Nested case–control	Cases: 82 cases of incident lung cancer in men, lifelong non-smokers aged 45–64 yr at enrolment; Shanghai Cohort Study consisted of 18 244 men (80% of eligible) who were aged between 45 and 64 yr at enrolment in 1986–1989 and resided in one of four small geographically defined communities in Shanghai, China. Controls: 83 members of the Shanghai Cohort Study without cancer, non-smokers and alive at the time of cancer diagnosis of the case; matched by age at enrolment (± 2 yr), year and month of urine sample collection (± 1 month) and neighbourhood of residence at recruitment. Exposure assessment method: exposure was determined based on measurement of urinary metabolites of acrolein (HPMA); urine samples were collected at baseline survey of the cohort, in which the case–control study was nested; there was no assessment of external exposure	Lung, incidence	Quartile of urinary HPMA, never smokers (OR):			Age at baseline, neighbourhood of residence at enrolment, years of sample storage and urinary cotinine level	*Exposure assessment critique*: Internal exposure assessment only. No evidence of external exposure. Smokers were excluded. Urine samples were collected at baseline, so clearly preceded the health outcome; however, only one urine sample at baseline was collected. *Strengths*: active follow-up with annual in-person interviews; after 22 yr loss to follow-up low, only 5%; urinary cotinine was also quantified to confirm non-smoking status. *Limitations*: relatively small sample size; 26% of cases not histologically confirmed; small number of cases of squamous cell cancer ($n = 16$); intraindividual variation in exposure not captured.
			First quartile	21	1		
			Second quartile	19	0.97 (0.40–2.34)		
			Third quartile	19	0.98 (0.40–2.36)		
			Fourth quartile	21	1.13 (0.47–2.75)		
			Trend-test *P* value, 0.79				

Table 2.1 (continued)

Reference, location, enrolment/ period, study design	Population size, description, category or cases or deaths	Cancer type level	Exposure (95% CI)	Exposed controlled	Risk estimate follow-up	Covariates	Comments
Tsou et al. (2019) Taiwan, China 2016–2018 Case–control	Cases: 97 cases of cancer of the oral cavity; hospital-based Controls: 230 healthy controls, not further described Exposure assessment method: questionnaire; information on smoking and betel-quid chewing history was collected during interviews with participants or relatives; urine samples were analysed for HPMA; acrolein–DNA adducts were measured in buccal cells or tumour tissues	Oral cavity, incidence	Acrolein–DNA adduct: Controls Ratio of vs controls	222 80	1 1.4 ($P < 0.001$) cases	None	*Exposure assessment critique*: Acrolein–DNA adducts and urinary HPMA were measured in samples from cases and controls; however, it is not clear to what extent acrolein–DNA adducts are a marker of effect, or of exposure. Both were measured at the time of cancer treatment (and similar period for controls). The authors indicated that urinary HPMA was significantly correlated with smoking history but no correlation coefficient was given and the correlation appeared weak in the graph. Acrolein–DNA adduct levels were higher in the tumour tissues than in the buccal swabs, but HPMA levels were lower. There was no indication whether and for how long the cases had stopped smoking or chewing before their samples were collected. *Strengths*: DNA adducts in buccal swabs for exposure assessment. *Limitations*: small sample size; controls not described.
		Oral cavity, incidence	Acrolein–DNA, cigarette smokers + betelquid chewers: Controls Ratio of vs controls	101 51	1 1.3 (P < 0.05) cases	None	
		Oral cavity, incidence	Acrolein–DNA, cases: Buccal tissue Ratio of tissue to buccal tissue	NR NR	1.8 ($P < 0.01$) tumour	None	

Table 2.1 (continued)

Reference, location	Population size, description, period, study design	Cancer type	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates	Comments	Exposure assessment method
Tsou et al. (2019) (cont.)		Oral cavity, incidence	Mean urinary HPMA (μmol/g creatinine): Controls, all	230	7.1	None		
			Controls, cigarette smokers only	111	5.8			
			Controls, betel-quid chewers only	12	3.6			
			Controls, cigarette smokers and betel-quid chewers	107	8.9			
			Cases	97	0.7 ($P < 0.001$, compared with all controls)			
Hong et al. (2020) Taiwan, China 2016–2019 Case–control	Cases: 62 cases of urothelial cancer; hospital-based, Taiwan, China; patients with CKD; no treatment with cyclofosfamide or ifosfamide Controls: 43 healthy controls; Biomarkers CKD or other diseases. Controls had not related to	Urinary bladder (urothelial cancer), incidence	Acrolein–DNA, cases: Normal urothelial cells	62	1	None	*Exposure assessment critique*: Only considered endogenous exposure due to kidney failure. External exposure to smoking was considered but only as a not described but did not have compared confounder.	
			Ratio in tumour cells with normal cells	62	1.2 ($P < 0.001$)			
			Acrolein–DNA, non-smoking cases: Normal urothelial cells	48	1	None		
			Ratio in tumour cells with normal cells	48	1.2 ($P < 0.001$)			

Reference, location	Population size, description, period, study design	Cancer type	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates	Comments	Exposure assessment method
	questionnaire; exposure of interest was endogenous exposure considered.	Urinary bladder (urothelial cancer), urothelial	Acrolein–DNA, non-smoking cases: Normal urothelial cells	48	1	None	higher levels of HPMA. Air pollution was to chronic kidney failure, HPMA. No other external exposure to acrolein due	
			Ratio in tumour cells with normal cells	48	1.2 ($P < 0.001$)			
			Acrolein–DNA, smoking cases: Normal urothelial cells	14	1	None		
			Ratio in tumour cells with normal cells	14	1.2 ($P < 0.001$)			
			Acrolein–DNA, cases: Normal urothelial cells	62	1	None		
			Ratio in tumour cells with normal cells	62	1.2 ($P < 0.001$)			
			Acrolein–DNA, non-smoking cases: Normal urothelial cells	48	1	None		
			Ratio in tumour cells with normal cells	48	1.2 ($P < 0.001$)			
			Acrolein–DNA, smoking cases: Normal urothelial cells	14	1	None		
			Ratio in tumour cells with normal cells	14	1.2 ($P < 0.001$)			

Table 2.1 (continued)

Reference, location, enrolment/ exposure assessment method	Population size, description, category or cases or controls	Cancer type level	Exposure deaths	Exposed follow-up	Risk estimate (95% CI)	Covariates	Comments
		measured through Acr–PC, acrolein–DNA adducts, acrolein, but it is not if endogenous acrolein contribute to higher levels measured, but not	Ratio in information on smoking and Smoking did not appear to Urinary bladder (urothelial Controls incidence	48 43	1.2 ($P < 0.001$) 0.26	were appropriate for and by HPMA in urine; clear from the results air pollution was collected. compared with normal cells levels are a result or a consequence of urothelial cancer), Cases of acrolein–DNA adducts or GST activity.	tumour cells
		Urinary bladder (urothelial Controls incidence	Mean plasma Acr–PC (mM) 43		0.26	None carcinomas. GSH levels Acr–PC	*Strengths*: measurement of specific DNA adducts cancer), Cases with early-stage CKD
				37	0.51 ($P < 0.001$)	were	
				25	0.48 ($P < 0.001$)	by acrolein and *TP53* mutations.	
were				12	0.56 ($P < 0.001$)	*Limitations*not described; small sample : controls	size; there is a serious flaw in the disproportion of non-smokers: all controls and 79% of cases.

Table 2.1 (continued)

Reference, location, exposure assessment method	Population size, description, enrolment/ follow-up	Cancer type	Exposure category or level	Exposed cases or deaths	Risk estimate (95% CI)	Covariates controlled	Comments

cases and controls for whom urine samples were depleted or values for one or more mercapturic acid metabolites were missing. One control per case was selected from among cohort members who were current smokers at enrolment, free of cancer, and alive at the time of the cancer diagnosis of the index case, and further matched on age at enrolment, date of biological specimen collection, and neighbourhood of residence at recruitment. Comparing the highest with the lowest quartiles, risk of lung cancer associated with HPMA levels doubled in models adjusting for matching factors and number of cigarettes smoked per day and years of cigarette smoking at baseline. In models with further adjustment for metabolites of polycyclic aromatic hydrocarbons and tobacco-specific nitrosamines (NNAL) and/or cotinine, no association was found between HPMA and lung cancer. [The Working Group noted that there were multiple correlated exposures (biomarkers). Strengths of the study included: a relatively large sample and long follow-up (20 years); few losses to follow-up (4.6%); urinary biomarkers collected before disease occurrence; and self-reported smoking status verified by urinary cotinine. The 2-fold increase in risk of lung cancer was associated with the highest quartile of HPMA concentration, adjusted for only intensity and duration of smoking. However, this effect disappeared with further adjustment for other smoking biomarkers, indicating that acrolein represented a biomarker of smoking. The Working Group judged that this study was uninformative for an evaluation of the carcinogenicity of acrolein.]

The second study (Yuan et al., 2014) had a similar study design but extended follow-up through 2008 and included only never-smokers at baseline (82 cases of lung cancer and 83 controls; same design as in the Yuan et al., 2012). The same urinary biomarkers as in the previous paper were measured. There was no association between quartile of urinary HPMA concentration and lung cancer in never-smokers (fourth quartile versus first quartile: OR, 1.13; 95% CI, 0.47–2.75) in analysis adjusting for matching factors and urinary cotinine level. [The Working Group noted that only internal exposure was assessed, and since the participants were all non-smokers, the source of external exposure to acrolein was unclear. The Working Group also noted that urinary cotinine represents a shortterm biomarker of passive smoking and therefore may not fully adjust for long-term secondhand smoke exposure.]

Tsou et al. (2019) measured acrolein–DNA adducts in buccal swabs from patients (n = 97) with cancer of the oral cavity. Acrolein–DNA adducts were also measured in buccal swabs from 230 healthy controls. Additionally, HPMA and NNAL were measured in the urine of the same 97 patients with cancer of the oral cavity and 230 healthy controls. For the patients with cancer, Tsou et al. (2019) also compared DNA– adduct levels in cancer biopsies with those in adjacent normal tissue collected from buccal swabs. Levels of acrolein–DNA adducts in buccal cells were 1.4 times higher in cases than in controls ($P < 0.001$). The ratio was 1.3 among smokers and betel-quid chewers only ($P < 0.05$). Levels of acrolein–DNA adducts were 1.8 times higher in cancer biopsy specimens than in buccal swabs from adjacent normal tissue ($P < 0.01$). However, there was no significant difference in levels of acrolein–DNA adducts among healthy controls with different cigarette smoking or betel-quid chewing histories. Smoking and betelquid chewing were associated with significantly higher levels of HPMA. Levels of urinary HPMA were lower among cases (0.7 µmol/g creatinine) than among controls (7.1 µmol/g creatinine) ($P < 0.001$), with a similar difference observed when only smokers and chewers were considered. There was no

adjustment for covariates. [The Working Group noted that, overall, the paper suggests that HPMA (but not acrolein–DNA adducts) is associated with smoking and betelquid chewing, and acrolein–DNA adducts are associated with oral cancer (cross-sectionally). There were lower levels of HPMA in the urine of cases than in controls (irrespective of smoking/ chewing status). The cross-sectional nature of the study and the fact that specimens were collected after cancer diagnosis in cases make causal inference difficult.]

Hong et al. (2020) in a case–control study in Taiwan, China, included 62 patients with urothelial carcinoma and 43 healthy controls. All cases and none of the controls had chronic kidney disease (CKD), the rationale being that CKD patients have a high risk of bladder cancer and altered metabolism that increases susceptibility to chemical exposures. Urinary HPMA, plasma acrolein–protein conjugates, DNA adducts formed by acrolein, and *TP53* mutations in frozen tissue samples were measured. Tumour biopsies showed levels of acrolein–DNA adducts that were 1.2 times higher than those in adjacent normal tissue in urothelial carcinoma patients overall ($P < 0.005$). The same ratio and *P* value were also found in cases and controls who were non-smokers. Levels of acrolein–DNA adducts were correlated with CKD severity. Also, levels of plasma acrolein–protein conjugates were twice as high in cases as in controls ($P < 0.001$). Similar results were observed for acrolein–protein conjugates in plasma in study participants with different degrees of severity of CKD and in non-smokers. Urinary HPMA levels were lower in cases (0.83 µmol/g creatinine) than in controls (1.16 µmol/g creatinine) ($P = 0.023$), this observation being attributed to binding of HPMA to glutathione (GSH) as a cellular defence mechanism. [The Working Group noted that controls were not described, and cases were all affected by CKD. The only endogenous exposure considered was due to kidney failure, while external exposure to smoking was considered only as a confounder. The study also had a small sample size, considerable age difference between cases and controls, and short follow-up period. There appeared to be a disproportionate number

of non-smokers included: all controls and 79% of cases.]

2.2 Evidence synthesis for cancer in humans

The epidemiological evidence available on acrolein in relation to cancer in humans included one occupational cohort study (Bittersohl, 1975), three nested case–control studies in occupational or population-based cohorts (Ott et al., 1989a; Yuan et al., 2012, 2014), and two hospital-based case–control studies (Tsou et al., 2019; Hong et al., 2020). There was little consistency in the cancer sites evaluated across these studies, with studies variously examining cancers of the lung (Bittersohl, 1975; Yuan et al., 2012, 2014), oral cavity (Bittersohl, 1975; Tsou et al., 2019), bladder (Hong et al., 2020), or lymphohaematopoietic cancers (Ott et al., 1989a).

2.2.1 Exposure assessment

The quality of the exposure assessment carried out within the available studies was of concern, as detailed in Section 1.6. For the studies that considered occupational exposure to acrolein (Bittersohl, 1975; Ott et al., 1989a, b), no quantitative exposure assessment was carried out, and therefore no exposure–response analyses could be performed. In addition, study participants were simultaneously exposed to multiple,

undifferentiated chemical agents, reducing the informativeness of a comparison of cancer risk between exposed and unexposed groups.

The remaining studies investigated acrolein mainly from a mechanistic point of view and looked at urinary metabolites (mercapturic acids) (Yuan et al., 2012, 2014; Tsou et al., 2019; Hong et al., 2020), acrolein–DNA adducts (Tsou et al., 2019; Hong et al., 2020), and/or acrolein–protein conjugates measured in serum (Hong et al., 2020). These studies did not consider external exposure to acrolein explicitly. Although information on smoking was available in some studies and may have been an important source of acrolein exposure, these studies adjusted for smoking through restriction or statistical adjustment (Yuan et al., 2012, 2014; Hong et al., 2020).

2.2.2 Cancers of the lung, oral cavity, and other sites

Two case–control studies (Yuan et al., 2012, 2014) nested in a population-based cohort studied several biomarkers in relation to lung cancer (one among current smokers, one among non-smokers). There was matching by smoking habits and adjustment for markers of smoking (NNAL, cotinine, and urinary HPMA) but the study did not investigate the etiological involvement of acrolein per se.

One case–control investigation (Tsou et al., 2019) studied acrolein–DNA adducts in buccal swabs of patients with cancer of the oral cavity compared with healthy controls and found higher levels in cancer cases. However, adducts were not associated with tobacco smoking or betel-quid chewing, and thus were unlikely to be markers of those exposures. Urinary HPMA (a metabolite of acrolein) was associated with smoking and betel chewing. There were lower levels of HPMA in the urine of cases than in controls (irrespective of smoking/chewing status), attributed by the authors to HPMA binding to GSH as a cellular defence mechanism.

The three other studies were considered uninformative – one occupational cohort (Bittersohl, 1975), one nested case–control study on lymphohaematopoietic cancer in an occupational cohort (Ott et al., 1989a), and a case–control study on urothelial carcinoma in patients with CKD (Hong et al., 2020) – due to small numbers, poor external exposure assessment, and flaws in design.

Taken together, these studies provide little evidence of a positive association between acrolein exposure and cancer in humans. Some of the available studies were of a mechanistic nature, i.e. they investigated the role of a urinary mercapturic acid metabolite of acrolein in smokers with null results after controlling for other smoking-related biomarkers. In other studies, the design, including external exposure assessment, was poor.

3. Cancer in Experimental Animals

In previous evaluations, the *IARC Monographs* programme concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of acrolein (e.g. IARC, 1995).

Studies of carcinogenicity with acrolein in experimental animals are summarized in Table 3.1.

3.1 Mouse

3.1.1 Inhalation

In a study that complied with Good Laboratory Practice (GLP), groups of 50 male and 50 female B6D2F$_1$/Crlj mice (age, 6 weeks) were treated with acrolein (purity, > 98.3%; 1.42% acetaldehyde identified by GC-MS) by inhalation with whole-body exposure for 6 hours per day, 5 days per week, for up to 99 weeks. (JBRC, 2016a, b, c). The concentration in the exposure chambers was set to 0 (clean air, control), 0.1, 0.4, or 1.6 ppm (v/v) for males and females. The mean air concentrations, based on monitoring every 15 minutes, were the target values and the coefficients of variation were within 0.6%. The survival rates for all groups (including both male and female control groups) were decreased due to the development of renal lesions and/or amyloid deposition but were not affected by exposure to acrolein. When the survival rates for the male and female control groups were lower than 25%, the study was terminated by

Table 3.1 Studies of carcinogenicity with acrolein in experimental animals

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Full carcinogenicity Mouse, B6D2F$_1$/Crlj (M) 6 wk 93 wk JBRC (2016a, b)	Inhalation (whole-body) > 98.3% Clean air 0, 0.1, 0.4, 1.6 ppm 6 /day, 5 days/wk 50, 50, 50, 50 11, 15, 14, 15	*Nasal cavity*: adenoma Incidence: 0/50, 0/50, 0/50, 1/50 (2%) *Lymph node*: malignant lymphoma Incidence: 1/50, 3/50, 2/50, 4/50	NS NS	Principal strengths: multiple dose study; use of males and females; study complied with GLP. Historical control data in B6D2F$_1$/Crlj male mice for nasal cavity adenoma: 1/499 (0.2%; range, 0–2%); the incidence of hyperplasia of the respiratory tract (nasal cavity) was significantly increased in treated animals compared with controls; the Working Group considered hyperplasia of the respiratory tract to be a pre-neoplastic lesion.
Full carcinogenicity Mouse, B6D2F$_1$/Crlj (F) 6 wk 99 wk JBRC (2016a, b)	Inhalation (whole-body) Clean air 0, 0.1, 0.4, 1.6 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 11, 18, 14, 19	*Nasal cavity*: adenoma Incidence: 0/50, 0/50, 16/50 (32%)* *Lymph node*: malignant lymphoma Incidence: 12/50 (24%), 8/50 (16%), 6/50 (12%), 17/50 (34%) *Uterus* Principal strengths: multiple dose study; use of males and females; study complied with GLP. Histiocytic sarcoma	carcinogenicity > 98.3% *P < 0.0001, Fischer exact test; *P* < 0.0001, Peto trend test (prevalence method) and Cochran–Armitage trend test *P* = 0.0347, Cochran–Armitage trend test	Historical control data in B6D2F$_1$/Crlj female mice for malignant lymphoma: 169/500 (33.8%; range, 28–46%); uterus histiocytic sarcoma: 114/500 (22.8%; range, 18–34%); nasal cavity adenoma: 0/500; the incidence of hyperplasia of the respiratory tract (nasal cavity) was significantly increased in treated animals compared with controls; the Working Group considered hyperplasia of the respiratory tract to be a pre-neoplastic lesion.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of	Significance	Comments
Study design **(sex)** **Age at start**	**Vehicle** **Dose(s)**	**tumours**		
		Incidence: 6/50 (12%), 13/50 (26%), 14/50 (28%)*, 6/50 (12%) Endometrial stromal polyp Incidence: 1/50, 1/50, 2/50, 3/50 *Liver*: histiocytic sarcoma Incidence: 0/50, 2/50, 0/50, 3/50	*P = 0.0392, Fischer exact test NS NS	
Duration **Reference**	**No. of animals at start** **No. of surviving animals**			
Full carcinogenicity Mouse, CD-1 (M) 8 wk 18 mo Parent et al. (1991a)	Oral administration (gavage) 94.9–98.5% (hydroquinone, 0.25–0.31%) Deionized water 0, 0.5, 2.0, 4.5 mg/kg bw per day 1×/day 70, 70, 70, 75 NR	*All sites*: no significant increase in the incidence of tumours		Principal strengths: use of males and females; use of multiple doses; large number of animals per group. Principal limitations: all major tissues and gross lesions from the control and high-dose groups were examined microscopically; only the lungs, liver, kidneys, and gross lesions from the groups at the low and intermediate dose were examined microscopically; histopathological data from mice found dead or killed because moribund were to have been collected according to the protocol, but data were not reported; dosing volume not reported; trend towards reduced survival, and decreased survival in the group at the highest dose.
Full carcinogenicity Mouse, CD-1 (F) 8 wk 18 mo Parent et al. (1991a)	Oral administration (gavage) 94.9–98.5% (hydroquinone, 0–25–0.31%) Deionized water 0, 0.5, 2.0, 4.5 mg/kg bw per day 1×/day 70, 70, 70, 75 NR	*All sites*: no significant increase in the incidence of tumours		Principal strengths: use of males and females; use of multiple doses; large number of animals per group. Principal limitations: microscopic examination was reported for all major tissues and gross lesions from the control and high-dose groups, but only for lungs, liver, kidneys, and gross lesions from the groups at the low and intermediate dose; histopathological data from mice found dead or killed because moribund were to have been collected according to the protocol, but data were not reported; dosing volume not reported.

Table 3.1 (continued)

Route Study design Species, strain (sex) Age at start	Purity Vehicle Dose(s)	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Full carcinogenicity Mouse, B6C3F$_1$ (M) Neonatal (8 days) 12 mo Von Tungeln et al. (2002)	Intraperitoneal injection NR DMSO 0, 150 nmol Injections with one-third and two-thirds of the total dose in 30 µL DMSO at age 8 and 15 days, respectively 24, 23 24, 23	*Liver* Adenoma Incidence: 0/24, 1/23 Carcinoma Incidence: 0/24, 0/23 Adenoma or carcinoma (combined) Incidence: 0/24, 1/23 Multiplicity: 0, 2.0	 NS NA NS NR	Principal strength: use of males and females. Principal limitations: use of single dose; lack of bodyweight data; rationale for dose not given, only data regarding liver tumours were reported.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of	Significance	Comments
Study design **(sex)** **Age at start**	**Vehicle** **Dose(s)**	**tumours**		
Duration **Reference**	**No. of animals at start** **No. of surviving animals**			
Full carcinogenicity Mouse, B6C3F$_1$ (F) Neonatal (8 days) 12 mo Von Tungeln et al. (2002)	Intraperitoneal injection NR DMSO 0, 150 nmol Injections with one-third and two-thirds of the total dose in 30 µL DMSO at age 8 and 15 days, respectively 23, 24 23, 23	*Liver* Adenoma Incidence: 0/23, 0/23 Carcinoma Incidence: 0/23, 0/23	 NA NA	Principal strength: use of males and females. Principal limitations: use of single dose; lack of body-weight data; rationale for dose not given; only data regarding liver tumours were reported.
Full carcinogenicity Mouse, B6C3F$_1$ (M) Neonatal (8 days) 15 mo Von Tungeln et al. (2002)	Intraperitoneal injection NR DMSO 0, 75 nmol Injections with one-third and two-thirds of the total dose in 30 µL DMSO at age 8 and 15 days, respectively 24, 24 24, 24	*Liver* Adenoma Incidence: 4/24, 5/24 Carcinoma Tumour incidence: 0/24, 0/24 Adenoma or carcinoma (combined) Incidence: 4/24, 5/24 Multiplicity: 1.3, 1.0	 NS NA NS NR	Principal strength: use of males and females. Principal limitations: use of single dose; lack of body-weight data; rationale for dose not given; only data regarding liver tumours were reported.
Full carcinogenicity Mouse, B6C3F$_1$ (F) Neonatal (8 days) 15 mo Von Tungeln et al. (2002)	Intraperitoneal injection NR DMSO 0, 75 nmol Injections with one-third and two-thirds of the total dose in 30 µL DMSO at age 8 and 15 days, respectively 24, 24 24, 24	*Liver* Adenoma Incidence: 0/24, 0/24 Carcinoma Incidence: 0/24, 0/24	 NA NA	Principal strength: use of males and females. Principal limitations: use of single dose; lack of body-weight data; rationale for dose not given; only data regarding liver tumours were reported.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Full carcinogenicity Mouse, NR, "partly inbred albinos" (F) ~3 mo ≤ 21–24 mo Steiner et al. (1943)	Subcutaneous injection NR Sesame oil 0.2 mg 1×/wk in 0.1 mL sesame oil for 24 wk 15 0 (11, 6, 3 and 1, at 12, 15, 18 and 21 mo, respectively)	*Subcutaneous tissue*: sarcoma Incidence: 0/15	NA	Principal limitations: use of females only; small number of mice; use of single dose; lack of body-weight data; limited information on sesame oil control group (see below); histopathological reporting limited to the induction of sarcomas; poor survival; justification of the dose was not provided. The authors stated: "at 12 months, the number of mice [sex distribution unspecified] living that had been injected with unheated sesame oil, used as vehicle for other substances in these experiments, was 61. None developed tumours at the site of injection".
Initiation–promotion (tested the dose was not provided. Mouse, S NR NR 21–22 wk Salaman & Roe (1956)	Skin application NR 0, 12.6 mg (total dose) Untreated (control) or 1×/wk application for 10 wk of 0.5% acrolein (in [presumably] 0.3 mL acetone); 25 days after 1st application, 1×/wk application of 0.17% croton oil for 18 wk (dose reduced to 0.085% for the 2nd and 3rd application) 20, 15 19, 15	*Skin*: papilloma Incidence: 4/19, 2/15 [NS]	Principal limitations: small number of mice per group; use of a single dose; limited reporting; justification of as initiator) Acetone No.: 4, 3	NR

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of	Significance	Comments
Table 3.1 (continued)				
Study design (sex) Age at start	**Vehicle Dose(s)**	**tumours**		
Full carcinogenicity Rat, F344/ DuCrlCrlj (M) 6 wk 104 wk JBRC (2016d, e)	Inhalation (whole-body) > 98.3% Clean air 0, 0.1, 0.5, 2 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 41, 40, 37, 42	*Nasal cavity*: squamous cell carcinoma Incidence: 0/50, 0/50, 0/50, 1/50 (2%)	NS	Principal strengths: multiple dose study; used males and females; study complied with GLP. Historical control data in F344/DuCrlCrlj male rats for nasal cavity squamous cell carcinoma, 0/599; the incidence of hyperplasia of the respiratory tract (nasal cavity) was significantly increased in treated rats compared with controls; the Working Group considered hyperplasia of the respiratory tract to be a pre-neoplastic lesion.

Table 3.1 (continued)

Route		Incidence (%),	Significance	Comments
Study design				
Species, strain (sex)	**Purity**	**multiplicity, or no. of tumours**		
Age at start	**Vehicle**			
Duration	**Dose(s)**			
	No. of animals at start			
				Principal strengths: multiple dose study; used males and females; study complied with GLP.
				Historical control data in F344/DuCrlCrlj female rats for nasal cavity squamous cell carcinoma, 0/600; nasal cavity rhabdomyoma, 0/600; pituitary gland adenoma, 165/599 (27.5%; range, 22–42%); the incidence of hyperplasia of the respiratory tract (nasal cavity) was significantly increased in treated rats compared with controls; the Working Group considered hyperplasia of the respiratory tract to be a pre-neoplastic lesion.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Study design **(sex)** **Age at start** **Reference**	**Vehicle** **Dose(s)** **No. of surviving animals** Full			
Inhalation (whole-body)	*Nasal cavity*			
carcinogenicity Rat, F344/ DuCrlCrlj (F) 6 wk 104 wk JBRC (2016d, e)	> 98.3% Clean air 0, 0.1, 0.5, 2 ppm 6 h/day, 5 days/wk 50, 50, 50, 50 43, 42, 41, 34	Squamous cell carcinoma or rhabdomyoma (combined) Incidence: 0/50, 0/50, 0/50, 6/50 (12%)*	*P = 0.0133, Fischer exact test; P < 0.0001, Peto trend test (prevalence method and combined analysis) and Cochran–Armitage trend test	
		Rhabdomyoma Incidence: 0/50, 0/50, 0/50, 4/50 (8%)	$P \leq 0.0007$, Peto trend test (prevalence method) and Cochran–Armitage trend test	
		Squamous cell carcinoma Incidence: 0/50, 0/50, 0/50, 2/50 (4%)	NS	

Table 3.1 (continued)

Route		Incidence (%),	Significance	Comments
Study design **Species, strain (sex)** **Age at start** **Duration**	**Purity** **Vehicle** **Dose(s)** **No. of animals at start**	**multiplicity, or no. of tumours**		
Full carcinogenicity Rat, F344 (M) 7–8 wk ≤ 124–132 wk Lijinsky & Reuber (1987)	Oral administration (drinking-water) NR, stabilized with hydroquinone (concentration, NR) Tap water 0 (control), 100 (for 124 wk), 250 (for 124 wk), 625 (for 104 wk) mg/L 5×/wk for 104–124 wk 20, 20, 20, 20 NR	*Liver*: tumours Incidence: 2/20, 8/20*, 0/20, 3/20	*[$P < 0.0324$, one-tail Fischer exact test]	Principal strengths: long-term study (> 2 yr); use of males and females; use of multiple doses. Principal limitations: small number of rats per group; variable duration of treatments between groups; body-weight and survival data not reported. Median week of death: 115 (range, 92–124) (control), 119 (83–130), 116 (53–130), and 129 (95–132) wk, respectively; total acrolein consumption: 0, 1.2, 3.1, and 6.5 g, respectively; liver tumours were mainly neoplastic nodules, with a few hepatocellular carcinomas.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Study design **(sex)** **Age at start** **Reference**	**Vehicle** **Dose(s)** **No. of surviving animals**			
Full carcinogenicity DuCrlCrlj (F) 6 wk	Adenoma or adenocarcinoma (combined) Rat, F344/	*Pituitary gland* 21/50, 17/50 Adenoma	Incidence: 14/50, 17/50, *P* = 0.0215, Peto trend test (standard method)	
JBRC (2016d, e) (104 wk cont.)	Incidence: 14/50 (28%), 1517/50 (34%/50 (30%), 20/50 (40%),)			*P*test (standard method) = 0.0115, Peto trend
		Adenocarcinoma Incidence: 0/50, 2/50, 1/50, 0/50	NS	

Table 3.1 (continued)

Route	Study design Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Full 4/20	Oral administration NS	*Liver*: tumours males and females.			Principal strengths: long-term study (> 2 yr); use of carcinogenicity (drinking-water) Incidence: 2/20,
Rat, F344 (F)		NR, stabilized with	*Adrenal gland*: tumours		Principal limitations: small number of rats per group;
7–8 wk hydroquinone (concentration, NR) Incidence: 1/20, 5/20 NS variable duration of treatments between groups; body-weight data and survival not reported; use of a single ≤ 124–132 wk					
Lijinsky & Reuber (1987)		Tap water 0 (control), 625 (for 104 wk) mg/L 5×/wk for 104 wk 20, 20 NR			dose. Median week of death: 118 (range, 82–124) (control), and 117 (58–132) wk, respectively; total acrolein consumption: 0 and 6.5 g, respectively; liver tumours were mainly neoplastic nodules, with a few hepatocellular carcinomas.
Full carcinogenicity Rat, SpragueDawley (M) ~6 wk 102 wk Parent et al. (1992)		Oral administration (gavage) 94.9–98.5% (hydroquinone, 0.25–0.31%) Deionized water 0.0, 0.05, 0.5, 2.5 mg/kg bw 1×/day 70, 70, 70, 70 NR	*Adrenal gland* Cortical adenoma Incidence: 0/60, 4/60, 3/60, 0/60 Cortical carcinoma Incidence: 0/60, 0/60, 1/60, 1/60	NS NS	Principal strengths: use of males and females; multiple dose study; long-term study. Principal limitations: all major tissues and gross lesions from the control and high-dose groups were examined microscopically; only the lungs, liver, kidneys, and gross lesions from the groups at the low and intermediate dose were examined microscopically; reporting of adrenal gland tumours only. 10 rats per dose group were killed after 1 year.
Full carcinogenicity Rat, Sprague-Dawley (F) ~6 wk 102 wk Parent et al. (1992)		administration (gavage) (hydroquinone,	*Adrenal gland*: cortical adenoma Incidence: 2/60, 3/60,	NS	Principal strengths: use of males and females; multiple 94.9–98.5% dose study; long-term study.
Oral					

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Study design **(sex)** **Age at start**	**Vehicle** **Dose(s)**			
Deionized water 0.0170NR0×/day.25–0.31%), 70, 70, , 0.05, 0.5, 2.5 mg/kg bw 70 0/60, 0/60	Principal limitations: trend towards reduced survival, and decreased survival in the high-dose group; all major tissues and gross lesions from the control and high-dose groups were examined microscopically; only the lungs, liver, kidneys and gross lesions from the low- and mid-dose groups were examined microscopically;			reporting of adrenal gland tumours only. 10 rats per dose group were killed after 1 year.
Duration **No. of surviving animals**	**No. of animals at start**	**Reference**		
Initiation–promotion (tested as initiator) Rat, F344 (M) 5 wk 32 wk Cohen et al. (1992)	Intraperitoneal injection 97% (containing 3% water and 200 ppm hydroquinone) Distilled water Acrolein at 2 mg/kg bw followed by control diet (negative control), 0 mg/kg bw followed by uracil (sham control), 2 mg/kg bw followed by uracil, 0.2% FANFT followed by uracil (positive control) Intraperitoneal injection of 2 mg/kg bw acrolein or of distilled water, 2×/wk for 6 wk; followed by 3% uracil in the diet for 20 wk then 6 wk of control diet, or by control diet for 26 wk 30, 30, 30, 30 NR	*Urinary bladder* Papilloma Incidence: 0/30, 8/30, 18/30*, 9/30 Carcinoma Incidence: 0/30, 1/30, 1/30, 21/30	*[$P < 0.02$, Fischer exact test; acrolein + uracil group vs sham control (uracil only)] [NS]	Principal strength: sufficient duration. Principal limitations: use of single dose; data from stomach, lungs, oesophagus, liver, and kidney were not reported.

Table 3.1 (continued)

Route Study design Species, strain (sex) Age at start Duration Reference	Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Initiation–promotion (tested as promoter) Rat, F344 (M) 5 wk 53 wk Cohen et al. (1992)	Intraperitoneal injection 97% (containing 3% water and 200 ppm hydroquinone) Distilled water Untreated (negative control), FANFT followed by distilled water (sham control), 2 mg/kg bw acrolein, followed by acrolein (see comments for regimen), FANFT followed by acrolein (see comments for regimen) Intraperitoneal injection of 2 mg/kg bw acrolein or of distilled water, 2×/wk for 6 wk, or 0.2% FANFT in the diet for 6 wk; followed by intraperitoneal injection of acrolein (see comments for regimen) or of distilled water until experimental wk 53, or by control diet until experimental wk 53 30, 30, 30, 30 NR	*Urinary bladder* Papilloma Incidence: 0/30, 0/30, 0/30, 0/30 Carcinoma Incidence: 0/30, 1/30, 0/30, 0/30 Simple or papillary/nodular (combined) hyperplasia Incidence: 0/30, 14/30, 16/30*, 22/30 Simple hyperplasia Incidence: 0/30, 14/30, 14/30*, 22/30 Papillary/nodular hyperplasia Incidence: 0/30, 0/30, 2/30, 0/30	NA [NS] *$P < 0.001$, Fischer exact test; acrolein + acrolein vs negative (untreated) control *$P < 0.001$, Fischer exact test; acrolein + acrolein vs negative (untreated) control NS	Principal limitations: use of single dose; data from stomach, lungs, oesophagus, liver, and kidney were not reported. The protocol (originally for a 100 wk-study) had to be revised for the two acrolein-treated groups, because of severe toxicity, and the acrolein treatment regimen was revised as follows: intraperitoneal injections of acrolein at 2 mg/kg bw, 2×/wk, during experimental wk 1–9; 1.5 mg/kg bw, 1× at experimental wk 10; 1.5 mg/kg bw, 2×/wk, during experimental wk 11–17; and 1.0 mg/kg bw, 1× at experimental wk 18, 2× at experimental wk 19, and 1× at experimental wk 20 and 21.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of	Significance	Comments
Study design **(sex)** **Age at start** **Duration** **Reference**	**Vehicle** **Dose(s)** **No. of animals at start** **No. of surviving animals**	**tumours**		
Full carcinogenicity Hamster, Syrian golden (M) 6 wk 81 wk Feron & Kruysse (1977)	Inhalation (whole-body) NR Filtered air 0 (unexposed control), 9.2 mg/ m^3 7 h/day, 5 days/wk for 52 wk 30, 30 7 (at 80 wk), 7 (at 80 wk)	*Respiratory tract*: all tumours (nasal cavity, larynx, trachea, bronchi or lung, combined) Incidence: 0/30, 0/30	NA	Principal strengths: use of males and females. Principal limitations: small number of animals per group; short duration of exposure; use of single dose; histopathological data were reported only for respiratory tract tumours; justification of the dose was not provided; lower survival. 15 hamsters per group also received intratracheal instillations of 0.2 mL 0.9% saline 1×/wk for 52 wk; the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined microscopically; in acrolein-treated animals, inflammation and epithelial metaplasia of the nasal cavity were observed.
Full Hamster, Syrian golden (F) 6 wk	Inhalation (whole-body)	*Respiratory tract*: all tumours (nasal cavity, larynx,		Principal strengths: use of males and females.

carcinogenicity NRFiltered air0[m]730 (unexposed control), 9.2 mg/ h/day, 5 days/wk for 52 wk[3], 30
trachea, bronchi or lung, combined)Incidence: 0/30, 1/30 NS
respiratory tract tumours; justification of the dose was not provided.Principal limitations: small number of animals per group; short duration of exposure; use of single dose; histopathological data were reported only for

81 wk

Table 3.1 (continued)

Route	Study design Species, strain (sex) Age at start	Purity Vehicle Dose(s)	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
	Feron & Kruysse (1977)	16 (at 80 wk), 13 (at 80 wk)			15 hamsters per group also received intratracheal instillations of 0.2 mL 0.9% saline 1×/wk for 52 wk; the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined microscopically; in acrolein-treated animals, inflammation and epithelial metaplasia of the nasal cavity were observed.

Table 3.1 (continued)

Route Species, strain	Purity	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Study design **(sex)** **Age at start** **Duration** **No. of surviving animals**	**Vehicle** **Dose(s)** **No. of animals at start Reference**			
Co-carcinogenicity Hamster, Syrian golden (M) 6 wk 81 wk Feron & Kruysse (1977)	Inhalation (whole-body) NR Filtered air 0 mg/m³ acrolein + 0.175% B[*a*]P, 9.2 mg/m³ acrolein + 0.175% B[*a*]P, 0 mg/m³ acrolein + 0.35% B[*a*]P, 9.2 mg/m³ acrolein + 0.35% B[*a*]P, 0 mg/m³ acrolein + NDEA, 9.2 mg/m³ acrolein + NDEA Exposure to acrolein was 7 h/day, 5 days/wk for 52 wk; together with either weekly intratracheal instillations of a suspension of 0.175 or 0.35% B[*a*]P (in 0.2 mL 0.9% saline), or 1×/3 wk subcutaneous injections of 0.0625% NDEA in 0.2 mL saline 30, 30, 30, 30, 30, 30 8 (at 80 wk), 7 (at 80 wk), 11 (at 80 wk), 9 (at 80 wk), 1 (at 80 wk), 7 (at 80 wk)	*Respiratory tract*: all tumours (nasal cavity, larynx, trachea, bronchi or lung, combined) Incidence: 4/29, 7/30, 19/30, 19/29, 12/29, 10/30 No.: 5, 7, 27, 29, 15, 11	NS NS	Principal strengths: use of males and females. Principal limitations: small number of animals per group; short duration of exposure; use of single dose; histopathological data were reported only for respiratory tract tumours; justification of the dose was not provided; lower survival. 15 hamsters per control group also received intratracheal instillations of 0.2 mL 0.9% saline 1×/wk for 52 wk; the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined histologically; in acrolein-treated animals, inflammation and epithelial metaplasia of the nasal cavity were observed.

Table 3.1 (continued)

Study design Species, strain (sex) Age at start Duration Reference	Route Purity Vehicle Dose(s) No. of animals at start No. of surviving animals	Incidence (%), multiplicity, or no. of tumours	Significance	Comments
Co-carcinogenicity Hamster, Syrian golden (F) 6 wk 81 wk Feron & Kruysse (1977)	Inhalation (whole-body) NR Filtered air 0 mg/m³ acrolein + 0.175% B[*a*]P, 9.2 mg/m³ acrolein + 0.175% B[*a*]P, 0 mg/m³ acrolein + 0.35% B[*a*]P, 9.2 mg/m³ acrolein + 0.35% B[*a*]P, 0 mg/m³ acrolein + NDEA, 9.2 mg/m³ acrolein + NDEA Exposure to acrolein was 7 h/day, 5 days/wk for 52 wk; together with either weekly intratracheal instillations of a suspension of 0.175 or 0.35% B[*a*]P (in 0.2 mL 0.9% saline), or 1×/3 wk subcutaneous injections of 0.0625% NDEA in 0.2 mL saline 30, 30, 30, 30, 30, 30 21 (at 80 wk), 17 (at 80 wk), 18 (at 80 wk), 17 (at 80 wk), 11 (at 80 wk), 20 (at 80 wk)	*Respiratory tract*: s (nasal cavity, larynx, trachea, bronchi or lung, con Incidence: 3/27, 8/29, 7/24, 15/30, 11/27, 11/28 No.: 3, 8, 9, 22, 13, 15	s NS NS	Principal strengths: use of males and females. Principal limitations: small number of animals per group; short duration of exposure; use of single dose; histopathological data were reported only for respiratory tract tumours; justification of the dose was not provided. 15 hamsters per control group also received intratracheal instillations of 0.2 mL 0.9% saline 1×/wk for 52 wk; the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined histologically; in acrolein-treated animals, inflammation and epithelial metaplasia of the nasal cavity were observed.

B[*a*]P; benzo[*a*]pyrene; bw, body weight; DMSO, dimethyl sulfoxide; F, female; FANFT, *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide; GLP, Good Laboratory Practice; h, hour; M, male; NA, not applicable; NDEA, *N*-nitrosodiethylamine; NR, not reported; NS, not significant; mo, month; ppm, parts per million; vs, versus; wk, week.

necropsy; this was done at week 93 for males and week 99 for females. Survival in the groups at 0, 0.1, 0.4, and 1.6 ppm was: for males, 11/50, 15/50, 14/50, and 15/50, respectively, at week 93; and for females, 11/50, 18/50, 14/50, and 19/50, respectively, at week 99. Body weights of male mice at 1.6 ppm were significantly decreased from the first week of exposure and throughout the exposure period compared with the control value. The relative final body weight in males at 0.1, 0.4, and 1.6 ppm were 89%, 95%, and 83% of the control value, respectively. Body weights of female mice at 1.6 ppm were significantly decreased from the first week of exposure until week 82, compared with the control value. The relative final body weight in females at 0.4 ppm was slightly but significantly increased. The relative final body weight for females at 0.1, 0.4, and 1.6 ppm was 104%, 111%, and 101% of the control value, respectively. All mice underwent complete necropsy, and all organs and tissues were examined microscopically.

In treated male mice, there was no significant increase in the incidence of any tumour. One (1/50, 2%) adenoma of the nasal cavity was observed in a male at 1.6 ppm; this incidence was at the upper bound of the historical control range (incidence, 1/499 (0.2%); range, 0–2%).

In female mice, the incidence of malignant lymphoma was significantly increased with a positive trend ($P = 0.0347$, Cochran–Armitage test). The incidence of histiocytic sarcoma of the uterus was 6/50 (12%, control), 13/50 (26%, 0.1 ppm), 14/50 (28%, 0.4 ppm), and 6/50 (12%, 1.6 ppm). The incidence in the group at 0.4 ppm (28%) was significantly increased ($P = 0.0392$, Fischer test) compared with the control value. [The Working Group noted that this increase did not indicate a clear dose–response relationship. The Working Group considered that this increase may have been related to treatment.] The incidence of adenoma of the nasal cavity was 0/50 (control), 0/50 (0.1 ppm), 0/50 (0.4 ppm), and 16/50 (32%, 1.6 ppm) and showed a significant positive trend ($P < 0.0001$, Peto test prevalence method and Cochran–Armitage test). The incidence in the group at 1.6 ppm was significantly increased ($P < 0.0001$, Fischer test) compared with the value for the control group and was clearly in excess of the value for historical controls (0/500).

Regarding non-neoplastic lesions in the respiratory tract (see also Section 4 of this monograph), for males at 1.6 ppm there was a significant increase in the incidence and/or severity of: eosinophilic change, inflammation, squamous cell metaplasia, regeneration, and hyperplasia in the respiratory epithelium; respiratory metaplasia and atrophy in the olfactory epithelium; respiratory metaplasia in the nasal glands; hyperplasia in the transitional epithelium; atrophy and adhesion in the turbinate; and exudate in the nasal cavity was observed. For females at 1.6 ppm, there was a significant increase in the incidence and/or severity of: inflammation, squamous cell metaplasia, regeneration, and hyperplasia in the respiratory epithelium; respiratory metaplasia and atrophy in the olfactory epithelium; respiratory metaplasia in the nasal glands; and exudate in the nasal cavity was observed. The incidence of inflammation and hyperplasia in the respiratory epithelium was also increased in female mice at 0.4 ppm. [The Working Group considered the hyperplasias of the respiratory tract observed in both males and females to be pre-neoplastic lesions.]

[The Working Group noted this was a GLP study conducted with multiple doses, and with both males and females.]

3.1.2 Oral administration (gavage)

In a study performed by Parent et al. (1991a), groups of 70–75 male and 70–75 female CD-1 mice (age, 8 weeks) were given acrolein (purity, 94.9–98.5%; containing 0.25–0.31% hydroquinone as a stabilizer) at a dose of 0 (control, deionized water only), 0.5, 2.0, or 4.5 mg/kg body weight (bw) per day by daily gavage [dosing volume not reported] for 18 months. In treated males, there was a significant negative trend in survival, and a significant decrease in survival in the group of males at the highest dose. Excess mortality was reported in all groups and attributed to trauma during gavage dosing, mis-dosing, or reasons unknown. [The number of surviving animals was not provided.] In males treated with the highest dose, a significant reduction in bodyweight gain was observed. Histopathological examination was reported for all major tissues and gross lesions from mice in the control group and at the highest dose, but only for the lungs, liver, kidneys, and gross lesions from mice in the groups receiving the lowest and intermediate dose. In addition, tumour incidence was reported only for about half of the experimental animals (271/570) killed at 18 months. For males, data were reported for 30, 29, 30, and 27 animals for controls and each dose group respectively. For females, data were reported for 42, 30, 40, and 43 animals for controls and each dose group, respectively. [According to the protocol, histopathological data from mice found dead or killed in a moribund state were to be collected, but data were not shown.]

No significant increase in the incidence of tumours was observed.

[The Working Group noted the principal strengths of the study: the use of males and females, the large number of mice per group at start, and the use of multiple doses. The principal limitations of the study were that data were obtained from a limited number of mice assessed for histopathology after killing; that full histopathological examination was performed only for mice in the control group and at the highest dose; and that survival was lower in treated males.]

3.1.3 Intraperitoneal injection

In the first experiment in a carcinogenicity study by Von Tungeln et al. (2002), which focused on the induction of liver and lung tumours in newborn mice, groups of 23 male and 24 female B6C3F$_1$ mice (age, 8 days) were given two intraperitoneal injections of acrolein [purity not reported] at a total dose of 150 nmol. One third [50 nmol] and two thirds [100 nmol] of the total dose were given in dimethyl sulfoxide (DMSO) at age 8 and 15 days, respectively. Control groups of 24 males and 23 females were given intraperitoneal injections of DMSO only. There was no significant effect on survival. The mice were killed at age 12 months and underwent a complete necropsy; livers, lungs, and gross lesions were examined microscopically. Only one male mouse, in the treated group, developed liver adenomas (controls, 0/24; treated, 1/23). No liver tumours were observed in treated females and control females.

In a second experiment in the study by Von Tungeln et al. (2002), groups of 24 male and 24 female B6C3F$_1$ mice (age, 8 days) were given two intraperitoneal injections of acrolein at a total dose of 75 nmol. One third [25 nmol] and two thirds [50 nmol] of the total dose were given in DMSO at age 8 and 15 days, respectively. Control groups of 24 males and 24 females were given intraperitoneal injections of DMSO only. There was no significant effect on survival. Mice were killed at age 15 months. Control and treated males developed liver adenomas (incidence: controls, 4/24; treated, 5/24). No liver tumours were observed in control or treated females. [The Working Group

noted the principal strength of the study: the use of males and females. The principal limitations were that a single dose was used, justification for the dose used was not given, only data regarding liver tumours were reported, and body-weight data were not provided.]

3.1.4 Subcutaneous injection

A group of 15 female mice [strain not reported, referred to as "partly inbred albinos", of unspecified age ("around 3 months")] were given weekly subcutaneous injections of 0.2 mg of acrolein [purity not reported] in sesame oil for 24 weeks (total dose, 4.8 mg) to assess the induction of sarcoma. Survival was poor, with 11, 6, 3, and 1 mice alive at 12, 15, 18, and 21 months, respectively. After 21–24 months, no sarcomas were observed (Steiner et al., 1943). [The Working Group noted the principal limitations of the study: the small number of animals, poor survival, use of females only, use of a single dose, limited reporting on a sesame oil control group, lack of body-weight data, absence of justification for the dose used, and unspecified histopathological assessment for tumours other than sarcoma. The study was considered inadequate for the evaluation of the carcinogenicity of acrolein.]

3.1.5 Initiation–promotion

A group of 15 strain S mice [sex and age not reported] was given 0.5% acrolein [purity not reported] in acetone by skin application, once per week, for 10 weeks (total dose, 12.6 mg). Twentyfive days after the first application, the mice were given 0.17% croton oil [purity not reported] in acetone by skin application, once per week, for 18 weeks (on the second and third week of treatment the dose was reduced to 0.085%). A group of 20 control animals was given croton oil only by skin application following the same schedule: 0.17% croton oil in acetone was applied once per week, for 18 weeks (on the second and third week of treatment, the dose was also reduced to 0.085%). One control mouse died before the end of the study. After experimental weeks 21–22, no increased incidence of papilloma of the skin was observed in mice initiated with acrolein compared with controls (Salaman & Roe, 1956). [The Working Group noted the principal limitations of the study: the limited reporting of the study, absence of justification for the dose used, and the use of a small number of animals and a single dose. The study was considered inadequate for the evaluation of the carcinogenicity of acrolein.]

3.2 Rat

3.2.1 Inhalation

In a study that complied with GLP, groups of 50 male and 50 female F344/DuCrlCrlj rats (age, 6 weeks) were treated by inhalation with acrolein (purity, > 98.3%; 1.42% acetaldehyde identified by GC-MS) by whole-body exposure for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2016d, e, f). The concentration in the exposure chambers was set to 0 (clean air, control), 0.1, 0.5, or 2 ppm for males and females and was monitored every 15 minutes. The mean air concentrations (± standard deviation) for these groups were 0.10 ± 0.00, 0.50 ± 0.00, and 2.01 ± 0.02 ppm, respectively. At 104 weeks, survival of females at 2 ppm was significantly decreased, compared with controls. Survival in the groups at 0, 0.1, 0.5, and 2 ppm was: for males, 41/50, 40/50, 37/50, and 42/50, respectively; and for females, 43/50, 42/50, 41/50, and 34/50, respectively. Male rats at 2 ppm showed a decrease in body-weight gain from the first week of exposure and throughout the exposure period, compared with controls. The relative final body weight in males at 0.1, 0.5, and 2 ppm was 96%, 99%, and 88% of the control value, respectively. Body weights of female rats at

2 ppm were slightly but significantly decreased (maximum, 10%) from the first week of exposure and throughout the exposure period, compared with controls. The relative final body weight in females at 0.1, 0.5, and 2 ppm was 101%, 98%, and 95% of the control value, respectively. All rats underwent complete necropsy, and all organs and tissues were examined microscopically.

In treated male rats, there was no significant increase in the incidence of any tumours. One (1/50, 2%) squamous cell carcinoma of the nasal cavity was observed in the group of males at 2 ppm, which was in excess of the value for historical controls (0/599). [The Working Group considered that this rare squamous cell carcinoma of the nasal cavity may have been related to exposure.]

Of the females, two rats (2/50, 4%) developed squamous cell carcinoma of the nasal cavity in the group at 2 ppm. Although not significantly increased compared with controls, the incidence of this rare tumour exceeded the historical control rate (0/600). The incidence of rhabdomyoma of the nasal cavity was 0/50 (control), 0/50 (0.1 ppm), 0/50 (0.5 ppm), and 4/50 (8%, 2 ppm), and showed a significant positive trend ($P \leq 0.0007$, Peto test (prevalence method) and Cochran–Armitage test), and the incidence in the group at 2 ppm exceeded the historical control rate (0/600). The incidence of squamous cell carcinoma or rhabdomyoma (combined) of the nasal cavity was 0/50 (control), 0/50 (0.1 ppm), 0/50 (0.5 ppm), and 6/50 (12%, 2 ppm), and showed a significant positive trend ($P < 0.0001$, Peto test (prevalence method and combined analysis) and Cochran–Armitage test). The incidence in the group at 2 ppm (12%) was significantly increased ($P = 0.0133$, Fischer test) compared with the control value. [The Working Group considered that rare squamous cell carcinomas and rhabdomyomas of the nasal cavity observed in female rats were related to exposure. The Working Group also noted that these two tumours have different histotypes.] The incidence of adenoma in the pituitary gland was 14/50 (28%, control), 15/50 (30%, 0.1 ppm), 20/50 (40%, 0.5 ppm), and 17/50 (34%, 2 ppm), and showed a significant positive trend ($P = 0.0115$; Peto test, standard method). The incidence of adenocarcinoma of the pituitary gland was 0/50 (control), 2/50 (4%, 0.1 ppm), 1/50 (2%, 0.5 ppm), and 0/50 (0%, 2 ppm). The incidence of adenoma or adenocarcinoma (combined) of the pituitary gland, was 14/50 (28%, control), 17/50 (34%, 0.1 ppm), 21/50 (42%, 0.5 ppm), and 17/50 (34%, 2 ppm), and showed a significant positive trend ($P = 0.0215$; Peto test, standard method); however, the incidence of adenocarcinoma of the pituitary gland was not significantly increased. [The Working Group considered that the occurrence of adenoma and/ or adenocarcinoma of the pituitary gland may not be related to exposure, because of the high background incidence of adenoma of the pituitary gland in ageing rats, because the increased incidence was seen in females only, and because the incidence of adenocarcinoma of the pituitary gland was not significantly increased.]

Regarding non-neoplastic lesions in the respiratory tract (see also Section 4 of the present monograph), for males at 2 ppm there was a significant increase in the incidence and/ or severity of: inflammation and squamous cell metaplasia in the respiratory epithelium; eosinophilic change, respiratory metaplasia, and atrophy in the olfactory epithelium; respiratory metaplasia in the nasal glands; hyperplasia in the transitional epithelium; adhesion in the turbinate; goblet cell hyperplasia; inflammation with foreign body; oedema in the lamina propria; and proliferation of striated muscle was observed in the nasal cavity. For females at 2 ppm, there was a significant increase in the incidence and/ or severity of: inflammation and squamous cell metaplasia in the respiratory epithelium; respiratory metaplasia and atrophy in the

olfactory epithelium; respiratory metaplasia in the nasal glands; hyperplasia in the transitional epithelium; goblet cell hyperplasia; inflammation with foreign body; and oedema in the lamina propria was observed in the nasal cavity. [The Working Group considered that hyperplasias of the respiratory tract observed in both males and females were pre-neoplastic lesions.]

[The Working Group noted this was a GLP study conducted with multiple doses and using males and females.]

3.2.2 Oral administration (drinking-water)

Groups of 20 male and 20 female Fischer 344 rats (age, 7–8 weeks), were given drinking-water containing acrolein at a concentration of 0 mg/L (control), 100 mg/L (males only), 250 mg/L (males only), or 625 mg/L [purity not reported] stabilized with hydroquinone [concentration not reported], for 5 days per week (the other 2 days per week, the rats were given tap water) for 124 weeks (except for 104 weeks for the highest dose) (Lijinsky & Reuber, 1987; Lijinsky, 1988). The rats were killed at age 124–132 weeks There was little or no difference in survival [data were not reported]. [Body-weight and water-consumption data were not reported.] Histopathological examination was performed on all lesions, major tissues, and organs. There was a significant increase in the incidence of liver tumours (mainly neoplastic nodules, and a few hepatocellular carcinomas) in the group of males at the lowest dose compared with controls. [The Working Group noted the principal strengths of the study: this was a long-term study (> 2 years) that used multiple doses in males, and both males and females. The principal limitations were the small number of animals per group, the variable duration of treatments between groups, and the use of a single dose in females.]

3.2.3 Oral administration (gavage)

In a study by Parent et al. (1992), groups of 70 male and 70 female Sprague-Dawley rats (age, about 6 weeks), received acrolein (purity, 94.9–98.5%; stabilized with 0.25–0.31% hydroquinone; in deionized water) at a dose of 0 (control), 0.05, 0.5, or 2.5 mg/kg bw by daily gavage for up to 102 weeks. The dosing volume was 10 mL/kg bw. Excess mortality was reported in all groups; this was attributed to trauma during gavage dosing, mis-dosing, or reasons unknown. Ten rats of each sex per dose group were killed after 1 year, and surviving rats were killed [the number of surviving rats was not reported] after 102 weeks. There was a negative trend in survival and a decrease in survival at the highest dose that was significant among males and females during the first year, but only significant in females throughout the entire treatment period. There was no significant effect on body weight. All major tissues and gross lesions from rats in the control group and at the highest dose were examined microscopically; only the lungs, liver, kidneys, and gross lesions from the groups at the lowest and intermediate dose were examined microscopically. In treated males, the incidence of cortical cell adenoma of the adrenal gland was increased, but the effect was not statistically significant. Cortical cell carcinoma of the adrenal gland was seen in two male rats, one in each group at the intermediate and highest dose. In females, the incidence of cortical cell adenoma of the adrenal gland in treated rats was not significantly increased, and no adrenal gland carcinomas were observed in any group. [The Working Group noted the principal strengths of the study: this was a long-term study (> 2 years) that used multiple doses, and both males and females. The principal limitations were the reduced survival among treated females; that histopathological data

were reported only for adrenal gland tumours; and that full histopathological examination was performed only for rats in the control group and at the highest dose, while only the lungs, liver, kidneys, and gross lesions from rats in the groups at the lowest and intermediate dose were examined microscopically.]

3.2.4 Initiation–promotion

To evaluate the initiating activity of acrolein, two groups of 30 male Fischer 344 rats (age, 5 weeks) were given intraperitoneal injections of acrolein (purity, 97%; containing approximately 3% water and 200 ppm hydroquinone; in distilled water), at a dose of 0 (sham control, distilled water) or 2 mg/kg bw, twice per week, for 6 weeks, followed by feed containing 3% uracil for 20 weeks, and then control feed for 6 weeks. Another group (negative control group) of 30 male rats was given intraperitoneal injections of acrolein at a dose of 2 mg/kg bw, twice per week, for 6 weeks, followed by control feed for 26 weeks. A positive control group of 30 male rats was given feed containing 0.2% *N*-[4-(5nitro-2-furyl)-2-thiazolyl]formamide (FANFT) for 6 weeks, followed by feed containing 3% uracil for 20 weeks, and then control feed for 6 weeks. The rats were killed at experimental week 32. The stomach, lungs, oesophagus, liver, kidneys, and bladder were processed for histopathological examination. A significant increase [$P < 0.02$, Fischer exact test] in the incidence of urinary bladder papilloma was observed in rats initiated with acrolein and then exposed to the promotor uracil, compared with sham controls. The incidence of urinary bladder carcinoma was not significantly increased (Cohen et al., 1992). [The Working Group noted that the principal strength of the study was the sufficient duration. The principal limitations were the use of a single dose, and that data from stomach, lungs, oesophagus, liver, and kidneys were not reported.]

To evaluate the promoting activity of acrolein, two groups of 30 male Fischer 344 rats (age, 5 weeks) were given feed containing 0.2% FANFT for 6 weeks during the first phase, followed by intraperitoneal injections of acrolein (purity, 97%; containing 3% water and 200 ppm hydroquinone; in distilled water) at 0 (sham control, distilled water) or various concentrations (described below) during the second phase. Another group (acrolein-only group) received intraperitoneal injections of acrolein for the first and second phases. The intraperitoneal injections of acrolein were given as follows: 0 or 2 mg/kg bw, twice per week, during experimental week 1–9; 0 or 1.5 mg/kg bw, once at experimental week 10; 0 or 1.5 mg/kg bw, twice per week, during experimental week 11–17; and 0 or 1.0 mg/kg bw, once at experimental week 18, twice at experimental week 19, and once at experimental weeks 20 and 21. A negative control group was given the control feed only. The rats were killed at experimental week 53. The stomach, lungs, oesophagus, liver, kidneys, and bladder were processed for histopathological examination. No papilloma or carcinoma of the urinary bladder developed in any of the four groups of rats, apart from one rat bearing a carcinoma in the FANFT-only group (sham control). Regarding pre-neoplastic lesions, there was a significant increase ($P < 0.001$) in the incidence of simple or papillary/nodular (combined) hyperplasia of the urinary bladder in the acrolein-only group (16/30) compared with the negative (untreated) control group (0/30) (Cohen et al., 1992). [The Working Group noted that the principal limitations of the study were the use of a single dose, and that data from stomach, lungs, oesophagus, liver, and kidneys were not reported.]

3.3 Hamster

3.3.1 Inhalation

In a study by Feron & Kruysse (1977), groups of 30 male and 30 female Syrian golden hamsters (age, 6 weeks), were treated with acrolein at 0 mg/ m^3 (filtered air, control), or 9.2 mg/m^3 [purity not reported] by inhalation with whole-body exposure for 7 hours per day, 5 days per week, for 52 weeks, and the hamsters were then killed at 81 weeks. Half of the hamsters also received intratracheal instillations of 0.2 mL of 0.9% sodium chloride, once per week, for 52 weeks [but mortality and tumour results were reported and combined for all 30 animals of each sex per group]. Survival in males was low, but acrolein exposure did not affect survival rate. Seven male controls, 7 treated males, 16 control females, and 13 treated females were alive at 80 weeks. All hamsters were subject to full necropsy, but only the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined microscopically. No respiratory tract tumours were observed in any group, apart from a single papilloma of the trachea that was found in a treated female. Exposure to acrolein vapour caused inflammation and a slight to moderate degree of epithelial metaplasia in the nasal cavity. [The Working Group noted that the principal strength of the study was the use of males and females. The principal limitations were the small number of animals per group; the short duration of the exposure; absence of justification for the dose used; the lower survival in males; reporting of pathological data only for respiratory tract tumours; and the use of a single dose.]

3.3.2 Administration with known carcinogens

In a study by Feron & Kruysse (1977), groups of 30 male and 30 female Syrian golden hamsters (age, 6 weeks), were treated with acrolein [purity not reported] at a concentration of 0 mg/m^3 (filtered air, control groups), or 9.2 mg/m^3 by inhalation with whole-body exposure for 7 hours per day, 5 days per week, for 52 weeks, together with either weekly intratracheal instillations of a suspension of 0.175% or 0.35% benzo[*a*] pyrene (B[*a*]P, purity > 99%) in 0.9% sodium chloride (B[*a*]P total dose, 18.2 or 36.4 mg/ animal) or subcutaneous injections of 0.0625% *N*-nitrosodiethylamine (NDEA) in 0.2 mL of 0.9% sodium chloride once per 3 weeks (NDEA total dose, 2.1 µL/animal), and the hamsters were then killed at 81 weeks. Half of the control animals received also intratracheal instillations of 0.2 mL of 0.9% sodium chloride, once per week, for 52 weeks [but mortality and tumour results were reported and combined for all 30 control animals per sex]. Survival in males was low, but acrolein exposure did not affect survival rate. All hamsters were subject to full necropsy, but only the entire respiratory tract, grossly visible tumours, and gross lesions suspected of being tumours were examined microscopically.

There were no significant differences in the number or incidence of total respiratory tract tumours, or in the incidence of tumours of the nasal cavity, larynx, trachea, bronchi, or lungs. Tumours appeared slightly earlier in the groups of males and females exposed to acrolein plus NDEA compared with their respective NDEAonly controls. Exposure to acrolein vapour caused inflammation and a slight to moderate degree of epithelial metaplasia in the nasal cavity. [The Working Group noted the principal strength of the study: the use of males and females. The principal

limitations were: the small number of animals per group; the short duration of the exposure; that justification for the dose was not provided; the lower survival in males; that pathological data were reported only for respiratory tract tumours; and the use of a single dose.]

3.4 Evidence synthesis for cancer in experimental animals

The carcinogenicity of acrolein has been assessed in one study that complied with GLP in male and female mice and rats treated by inhalation with whole-body exposure. The carcinogenicity of acrolein in mice and rats was also evaluated by other routes of exposure in studies that did not comply with GLP. Specifically, in mice, there was one study in males and females treated by oral administration (gavage), and there were two studies in newborn males and females treated by intraperitoneal injection. In addition, one study in females treated by subcutaneous injection and one initiation–promotion study (sex not reported) were available, but these studies were judged to be inadequate for the evaluation of the carcinogenicity of acrolein in experimental animals. In rats, there were two studies in males and females treated by oral administration (one drinking-water study and one gavage study), and two initiation–promotion studies in males. The carcinogenicity of acrolein has been assessed in hamsters in one study in males and females treated by inhalation with whole-body exposure, both in the presence and absence of two known carcinogens.

In the inhalation study that complied with GLP in B6D2F1/Crlj mice, acrolein significantly increased the incidence of histiocytic sarcoma of the uterus in treated females, but without a clear dose–response relationship, and caused a significant positive trend in the incidence of malignant lymphoma in treated females. In treated females, there was also a significant positive trend and significant increase in the incidence of nasal cavity adenoma, which is a very rare tumour in the mouse strain used in the study (JBRC, 2016a, b, c). In the inhalation study that complied with GLP in F344/DuCrlCrlj rats, there was a significant positive trend in the incidence of rhabdomyoma of the nasal cavity and of squamous cell carcinoma or rhabdomyoma (combined) of the nasal cavity in treated females. The incidence of squamous cell carcinoma or rhabdomyoma (combined) of the nasal cavity was also significantly increased in treated females. Both tumour types are very rare in the rat strain used in the study (JBRC, 2016d, e, f).

In studies of oral administration, acrolein administered by gavage to male and female CD-1 mice or Sprague-Dawley rats did not cause an increased tumour incidence (Parent et al., 1991a). When administered in drinking-water in a study in male Fischer 344 rats, acrolein increased the incidence of liver tumours (mainly benign) (Lijinsky & Reuber, 1987; Lijinsky, 1988).

When tested by intraperitoneal injection, acrolein did not cause an increased tumour incidence in newborn B6C3F$_1$ mice (Von Tungeln et al., 2002). Acrolein increased the incidence of urinary bladder papilloma in male Fischer 344 rats when administered as a tumour initiator with uracil as a tumour promoter (Cohen et al., 1992). The incidence of urinary bladder tumours was not increased when acrolein was tested as a promoter in male Fischer 344 rats, with FANFT as the initiator (Cohen et al., 1992).

Tumours did not occur in mice treated with acrolein by subcutaneous injection (Steiner et al., 1943), and acrolein did not increase the incidence of skin tumours in an initiation–promotion study in strain S mice, with croton oil as the promoter (Salaman & Roe, 1956). However, these studies were judged to be inadequate for the evaluation of the

carcinogenicity of acrolein in experimental animals.

In Syrian golden hamsters treated by inhalation with whole-body exposure, acrolein did not increase the incidence of tumours, either in the presence or absence of known carcinogens (B[*a*]P or NDEA) (Feron & Kruysse, 1977).

4. Mechanistic Evidence

4.1 Absorption, distribution, metabolism, and excretion

4.1.1 Humans

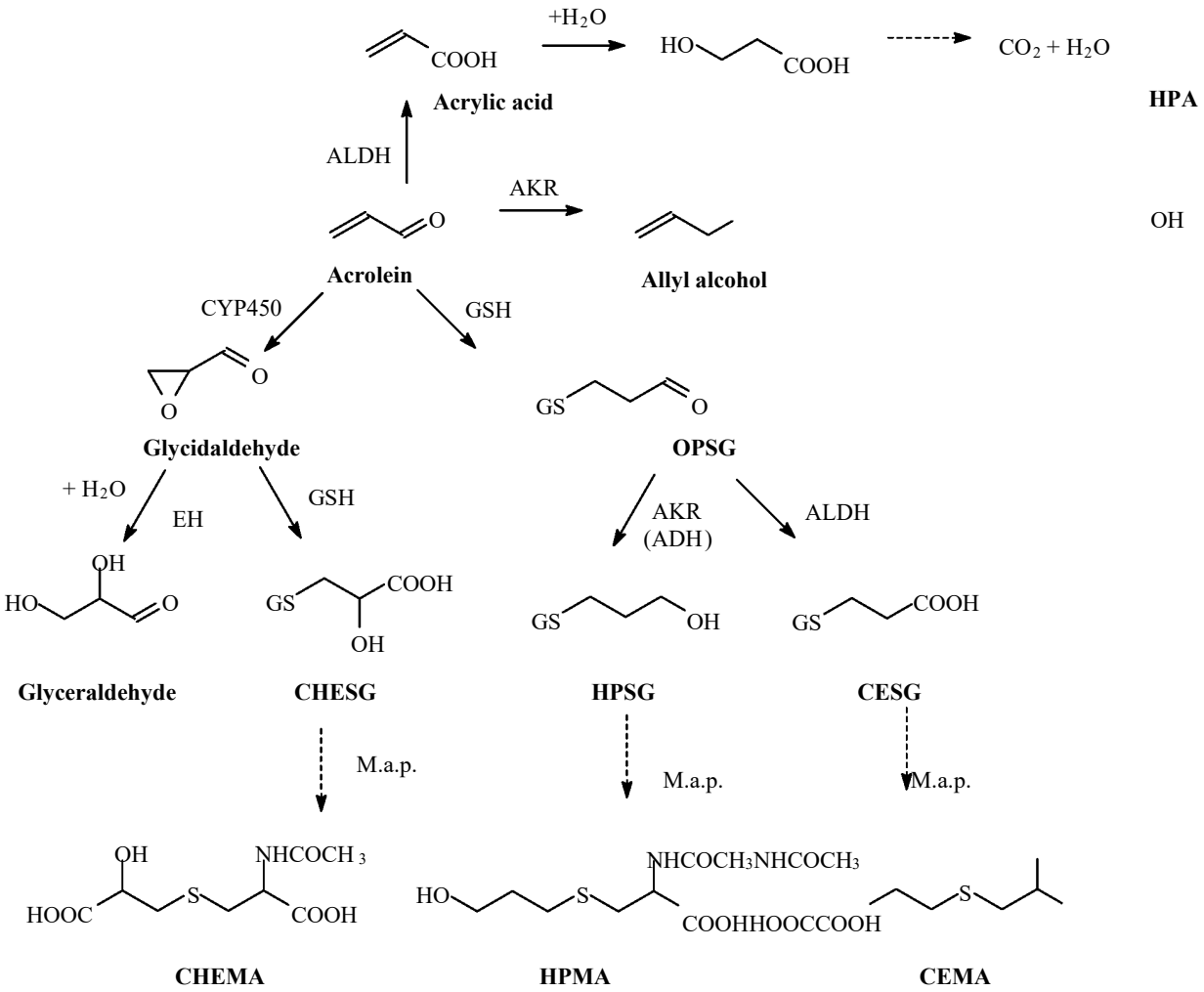
(a) Exposed humans

No data on the absorption or distribution of acrolein by inhalation were available to the Working Group.

The main metabolic pathways of acrolein are depicted in Fig. 4.1.

Two acrolein-derived mercapturic acids, HPMA and CEMA, were found in the urine of both smokers and non-smokers, HPMA being consistently the more common. Tobacco smokers showed significantly higher levels of both HPMA and CEMA (Alwis et al., 2012, 2015). A significant increase in acrolein-derived urinary mercapturic acids was also reported shortly after being served heat-processed food containing acrolein (Wang et al., 2019; Watzek et al., 2012). These mercapturic acids were also found in a limited exploratory toxicokinetic study on a single subject within

Fig. 4.1 The main pathways of acrolein metabolism



ADH, alcohol dehydrogenase; AKR, aldo-keto reductase; ALDH, aldehyde dehydrogenase; CEMA, *N*-acetyl-*S*-(carboxyethyl)-L-cysteine (2-carboxyethylmercapturic acid); CESG, *S*-(2-carboxyethyl)glutathione; CHEMA, *N*-acetyl-*S*-(2-carboxy-2-hydroxyethyl)-L-cysteine (2-carboxy-2-hydroxyethylmercapturic acid); CHESG, *S*-(2-carboxy-2-hydroxyethyl)glutathione; CYP450, cytochrome P450; EH, epoxide hydrolase; GSH, glutathione; HPA, 3-hydroxypropanoic acid; HPMA, *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine (3-hydroxypropylmercapturic acid); HPSG, *S*-(3-hydroxypropyl)glutathione; M.a.p., mercapturic acid pathway; OPSG, *S*-(3-oxopropyl)glutathione. Adapted from Patel et al. (1980), Parent et al. (1998), and Kurahashi et al. (2014).

24 hours after oral uptake of acrolein at a dose of 7.5 µg/kg bw in drinking-water. For HPMA and CEMA, respectively, elimination half-times were 8.9 hours and 11.8 hours, and maximum urinary concentrations reached 2 hours after ingestion were 1.61 and 1.05 µmol/g creatinine (Watzek et al., 2012). A similar elimination half-time for acrolein (9 hours) based on urinary metabolite HPMA profile was reported in a study on subjects who were served fried food containing acrolein (Wang et al., 2019).

Acrolein can be produced endogenously, including as a result of lipid peroxidation (Nath & Chung, 1994; Stevens & Maier, 2008).

Acrolein can be excreted unchanged in exhaled air (Andreoli et al., 2003; Ligor et al., 2008; Ruenz et al., 2019).

Free acrolein was also found in the urine and saliva (Korneva et al., 1991) of patients treated with cyclophosphamide; acrolein is a metabolite of cyclophosphamide. Once formed, acrolein appeared to be rapidly excreted in urine because its urinary concentration peaked shortly (1–12 hours) after treatment with cyclophosphamide (Takamoto et al., 2004).

A role of glutathione *S*-transferases (GSTs) in detoxification of acrolein was demonstrated by a randomized clinical trial in which a significant increase in the excretion of HPMA was observed in individuals who received 2-phenethyl isocyanate, an inducer of GST mu 1 (GSTM1) and GST theta 1 (GSTT1), compared with controls (Yuan et al., 2016). The role of GSH and GSTs is discussed further below (see Section 4.1.2).

(b) In vitro

A low absorption rate of 0.480 ± 0.417 μg/cm^2 in 30 minutes was observed in experiments with human skin in vitro at 153 ppm (351 mg/m^3) of acrolein in air (Thredgold et al., 2020).

The reaction of acrolein with GSH in vitro is efficiently catalysed by human GST ε, μ, and π, the last one isolated from human placenta being the most catalytically active (Berhane & Mannervik, 1990). Significant differences were found in catalytic efficiency (k_{cat}/K_m) between four allelic variants of the π isoenzyme (hGSTP1-1) (Pal et al., 2000). However, in a search for genetic variants related to acrolein metabolism to mercapturic acids (GST polymorphism) by a genome-wide association study, no association with HPMA levels in smokers after adjusting for total nicotine equivalents was found (Park et al., 2015). [The Working Group noted that these results, together with the known high electrophilic reactivity of acrolein, suggest that its conjugation with GSH leading eventually to the excretion of HPMA is mainly a spontaneous non-catalysed process.]

Acrolein can be reduced by human aldo-keto (Al-Rawithi et al., 1998; Takamoto et al., 200 reductases with catalytic efficiencies that vary greatly among the superfamily members. Thus, aldose reductase (EC 1.1.1.21) catalysed acrolein reduction with k_{cat}/K_m = 1.09 μM^{-1} min^{-1} and was significantly induced (7–20-fold) towards a variety of aldehydes by acrolein (Kolb et al., 1994). Human aldo-keto reductase AKR1A showed a much lower catalytic activity (k_{cat}/ K_m = 0.29 × 10^{-3} μM^{-1} min^{-1} (Kurahashi et al., 2014), whereas AKR1B1 and ABR1B10 showed k_{cat}/K_m values of 0.12 and 1.07 μM^{-1} min^{-1}, respectively (Shen et al., 2011). AKR1B1, which is ubiquitously expressed in humans, also efficiently reduced the acrolein–GSH conjugate (*S*-(3-oxopropyl)glutathione, OPSG) with k_{cat}/ K_m = 0.355 μM^{-1} min^{-1}, whereas AKR1B10 expressed mainly in the gastrointestinal tract showed a much lower catalytic efficiency, k_{cat}/ K_m = 0.004 μM^{-1} min^{-1} (Shen et al., 2011). Downregulation of the *AKR1B10* gene increased the susceptibility of a colorectal cancer cell line to cytotoxicity caused by acrolein (Yan et al., 2007).

4.1.2 Experimental systems

(a) In vivo

Due to high electrophilicity and solubility in water, a significant portion of inhaled acrolein is taken up in the upper respiratory tract. Experiments on Fischer 344 rats with surgically isolated upper respiratory tract in vivo showed that the nasal uptake efficiency decreased with increasing acrolein concentration, time of exposure, and inspired air flow rate (Morris, 1996; Struve et al., 2008). At the inspired air flow rate of 100 mL/minute, the uptake efficiency averaged over an 80-minute exposure period was 98%, 68%, and 50% at 0.6, 1.8, and 3.6 ppm, respectively. At 300 mL/minute these values fell to 85%, 48%, and 38%, respectively (Struve et al., 2008).

Somewhat lower time-averaged values were obtained earlier by Morris (1996), namely, 62%, 38%, and 28% at the exposure concentrations 0.87, 4.4, and 8.7 ppm, respectively (inspiratory flow rate, 200 mL/minute).

GSH concentrations in nasal epithelium were markedly lowered in a concentration-dependent manner in rats exposed for 80 minutes. However, when the rats were pre-exposed to acrolein at 3.6 ppm during 14 days (6 hours per day, 5 days per week), the depletion was nearly compensated by an adaptive response (Struve et al., 2008). A marked depletion in rat nasal GSH was also reported earlier by Lam et al. (1985). [The Working Group noted that these results indicate a marked influence of tissue reactivity on uptake in the upper respiratory tract.]

Mercapturic acids, namely, HPMA and CEMA, were identified in the urine of rats dosed subcutaneously (Kaye, 1973; HPMA only) or orally with acrolein (Draminski et al., 1983; CEMA only), as well as in mice after inhalation and intraperitoneal injection (Linhart et al., 1996). Due to its high electrophilic reactivity, acrolein forms protein adducts in vivo (Gan & Ansari, 1989; Kautiainen et al., 1989; see also Section 4.2.1). A gradual accumulation of protein-adducted acrolein was reported in mice exposed to acrolein by inhalation at 1.5 ppm for 30 minutes twice per day for 3 weeks. At the same time, a gradual increase in urinary HPMA excretion was observed (Tully et al., 2014).

The metabolism and disposition of [2,3^{14}C]acrolein was studied in male and female Sprague-Dawley rats treated by oral and intravenous administration (Parent et al., 1996a, 1998). The rats were divided into five groups of 5 males and 5 females and were given a single dose of [2,3-^{14}C]acrolein intravenously at 2.5 mg/ kg bw, or orally by gavage at 2.5 or 15 mg/kg bw. One group of rats was pre-exposed to unlabelled acrolein for 14 days at 2.5 mg/kg-day before oral administration of [2,3-^{14}C]acrolein at 2.5 mg/kg bw. Urine, faeces, and expired air were collected for 7 days. In all exposure groups, about 26–31% of the radiolabel was exhaled as carbon dioxide while < 1.2% was tissue-bound. Rats given a single intravenous injection of [2,3^{14}C]acrolein at 2.5 mg/kg bw excreted 66–69% of the radiolabel in urine and < 2% in faeces. The main urinary metabolites were identified by HPLC/MS analysis using authentic standards as 3-hydroxypropanoic acid, HPMA, CEMA, and *N*-acetyl-*S*-(2-carboxy-2-hydroxyethyl)-Lcysteine (2-carboxy-2-hydroxy ethylmercapturic acid, CHEMA) and traces of malonic acid. After oral doses, less radiolabel was excreted in the urine (lower dose, 52%; higher dose, 36.5%) and more in the faeces (lower dose, 13%; higher dose, 31%). Two additional urinary metabolites, oxalic and malonic acid, were identified (Parent et al., 1998). No significant effect on the excretion pattern was observed after pre-treatment with acrolein. The main portion of radiolabel was excreted within 48 hours after dosing, but excretion was delayed in the group receiving the higher oral dose. The analysis of faeces did not reveal any distinct peaks in the excretion of radiolabel over time. [The Working Group noted that faeces probably contained polymers of acrolein or polysaccharide, or protein adducts resulting from the reaction of acrolein with food components.]

A computational fluid dynamics model was developed to predict nasal dosimetry of acrolein in rats and humans using parameters adjusted to fit experimental uptake efficiency data from Struve et al. (2008) and Morris (1996). In humans, calculated nasal uptake efficiencies for inhaled acrolein were 16% and 28% at exposure concentrations of 3.6 ppm and 0.6 ppm,

respectively, and were consistently lower than those in rats. These predictions capture the overall trend of increased uptake when exposure concentrations decrease (Schroeter et al., 2008). [The Working Group noted that because of oral breathing, delivery of acrolein to the lower respiratory tract could be higher in humans than in rats, which are obligate nasal breathers.]

(b) In vitro

Acrolein reacts spontaneously with GSH to form OPSG (Esterbauer et al., 1975; Mitchell & Petersen, 1989; Horiyama et al., 2016), which is subsequently oxidized by rat liver aldehyde dehydrogenase (ALDH) to *S*-(2-carboxyethyl)glu - tathione (CESG) and, in a lesser extent, reduced by rat liver alcohol dehydrogenase (ADH) to *S*-(3-hydroxypropyl)glutathione (HPSG) as the affinity of ADH (K_m = 877 µM) was low compared with the high-affinity cytosolic (K_m = 310 µM) and mitochondrial (K_m = 198 µM) ALDH forms (Mitchell & Petersen, 1989). Rat AKR7A1 catalysed reduction of both acrolein and its GSH conjugate. Chinese hamster V79 cells expressing rat AKR7A1 were efficiently protected against acrolein-induced mutations (Gardner et al., 2004) (see Section 4.2.2b).

The carbonyl group of acrolein can be oxi - dized by ALDH and reduced by aldo-keto reductases (AKR). Recombinant mouse ALDH1a1 and ALDH 3a1 efficiently oxidized acrolein to acrylic acid, ALDH1a1 showing comparable catalytic efficiency (V_{max}/K_m >> 23) but a higher affinity (K_m = 23.2 µM) than ALDH3a1 (K_m = 464 µM) (Makia et al., 2011). Significant catalytic ALDH activities were found in the microsomes, cytosol, and mitochondria of rat liver (Rikans, 1987). In mitochondria, two different ALDH activities were found: a high-affinity one with K_m = 0.017 mM, V_{max} = 42.2 nmol min^{-1} mg^{-1}, and a low-affinity one with K_m = 0.430 mM, V_{max} = 29.2 nmol min^{-1} mg^{-1}. Similarly, in the cytosolic fraction, there was a high-affinity ALDH form with K_m = 0.026 mM, V_{max} = 14.9 nmol min^{-1} mg^{-1}, and a low-affinity form with K_m = 0.725 mM, V_{max} = 7.1 nmol min^{-1} mg^{-1}. In the microsomes, a single low-affinity ALDH activity with K_m = 1.5 mM and V_{max} = 30.5 nmol min^{-1} mg^{-1} was reported. Hence, the low K_m ALDH in mitochondria was found to have the highest metabolic activity (Rikans, 1987). [The Working Group noted that both oxidation by ALDHs and reduction by AKRs are important detoxication pathways in the metabolism of acrolein.]

Metabolic activation of acrolein to glycidaldehyde and its detoxification to acrylic acid were described in rat liver and lung preparations by Patel et al. (1980). Notably, oxidation to acrylic acid was not observed in the lung preparations. Glycidaldehyde is a substrate for epoxide hydrolases as well as for cytosolic GSTs in rat lung and liver (Patel et al., 1980). However, metabolic activation was not necessary for conjugation with GSH. A weak increase in GSH conjugation as measured by GSH depletion was observed only when cytochrome P450 (CYP) in rat liver microsomes was induced by pre-treatment of rats with phenobarbital (Garle & Fry, 1989). Experiments with [^{14}C]-labelled acrolein proved its covalent association with rat microsomal CYP and further metabolism to an epoxide (Marinello et al., 1984). [The Working Group noted that conjugation of glycidaldehyde with GSH should lead to urinary CHEMA, a confirmed metabolite of acrolein.]

4.2 Evidence relevant to key characteristics of carcinogens

This section summarizes the evidence for the key characteristics of carcinogens (Smith et al., 2016), including whether acrolein is electrophilic or can be metabolically activated to electrophiles; is genotoxic; alters DNA repair or causes genomic instability; induces oxidative

stress; is immunosuppressive; induces chronic inflammation; alters cell proliferation, cell death, or nutrient supply; induces epigenetic alterations; modulates receptor-mediated effects; and causes immortalization.

4.2.1 Is electrophilic or can be metabolically activated to an electrophile

(a) DNA binding (i) Studies in humans

Acrolein is a chemically reactive aldehyde that directly interacts with DNA as a result of its α,β-unsaturated carbonyl function. As further described in Section 4.2.1(b), it forms four isomeric α- and γ-hydroxy-1,N^2- propano-2′-deoxyguanosine adducts (α-OH-PdG and γ-OH-PdG, two of each), and their ringopened precursors (Chung et al., 1999). Acroleininduced DNA adducts have been found in various tissues in studies in humans, including lung, buccal cells, leukocytes, peripheral blood, liver tissues, sputum, brain tissues, bladder, and urothelial mucosa (Weng et al., 2018; Zhang et al., 2007; Yang et al., 2019a; Chung et al., 2012; Nath et al., 1998; Bessette et al., 2009; Wang et al., 2019; Tsou et al., 2019; Zhang et al., 2011; Yin et al., 2013; McDiarmid et al., 1991; Alamil et al., 2020; Nath & Chung, 1994; Fu et al., 2018; Chen & Lin, 2011; Liu et al., 2005; Lee et al., 2014; Hong et al., 2020; see Table 4.1 and Table 2.1). Furthermore, significantly higher levels of acrolein–DNA adducts were found in bladder tumour tissues, hepatocellular carcinoma tissues, and brain tissues from patients with Alzheimer disease (Liu et al., 2005; Lee et al., 2014; Fu et al., 2018; see Table 4.1 and Table 2.1).

Acrolein–DNA adduct formation has been detected in the leukocytes of 40% of a group of patients treated with cyclophosphamide compared with none of the controls (McDiarmid et al., 1991). Several studies reported higher levels of acrolein–dG adducts in the buccal cells and lung tissues of tobacco smokers (Nath et al., 1998; Zhang et al., 2007; Weng et al., 2018). Similar results have been reported using immunochemical, ^{32}P-postlabelling 2D thinlayer chromatography/high-performance liquid chromatography (TLC/HPLC) and LC-MS/MS methods for mea suring acrolein–dG adducts in buccal cells (Nath et al., 1998; Weng et al., 2018; Wang et al., 2019). [The Working Group noted that different methods were used in these studies, which may account for differences in levels detected.] Using the immunochemical method and ^{32}P-postlabelling 2D TLC/HPLC, Weng et al. (2018) reported that γ-OH-PdG accumulated significantly more in smokers than in non-smokers (Weng et al., 2018). Using the LC-MS/MS method, Chung et al. (2012) also confirmed that γ-OH-PdG is the major acrolein– DNA adduct formed in the human lung tissues. Alamil et al. (2020) reported higher levels of acrolein–DNA adducts in peripheral blood in a smoker than in a non-smoker. On the other hand, it has been reported that two isomers, α- and γ-OH-PdG, formed almost equally in the lung tissues of smokers and non-smokers; and that the level of acrolein–DNA adducts in smokers is not significantly different from that in non-smokers (Ma et al., 2019; Yang et al., 2019a). The levels of acrolein–dG adducts detected were about 10–100 times lower than those reported by other laboratories, and the levels of acrolein–DNA adducts in leukocytes and lungs were similar (Chung et al., 2012; Ma et al., 2019; Zhang et al., 2011; Weng et al., 2018; Yang et al., 2019a; Alamil et al., 2020). [Since tobacco smoke contains substantial amounts of acrolein (see Section 1.4.2(b)), the Working Group noted that the lack of differences in acrolein–DNA adduct formation in both lung

tissues and leukocytes of smokers and non-smokers may be explained by other exposure sources.]

(ii) Human cells in vitro

There is ample evidence demonstrating that acrolein can adduct DNA in various primary human cells and in cultured human cell lines in vitro (Wilson et al., 1991; Feng et al., 2006; Pan et al., 2009, 2012, 2016; Greenspan et al., 2012; Wang et al., 2012; see Table 4.2). Feng et al. (2006) reported that acrolein treatment in normal human bronchial epithelial cells and normal human lung fibroblasts induces acrolein– DNA adducts that were preferentially formed at lung cancer *TP53* mutational hotspots, and that acrolein preferentially adducts guanines at cytosine methylation CpG sites. Wang et al. (2009a, 2012), using shuttle vectors containing the s*upF* gene, showed that cytosine methylation at CpG sites enhanced acrolein–DNA adduct formation and mutations at these sites; and that in human lung cells, acrolein induced γ-OH-PdG (95%)

Table 4.1 Acrolein-derived DNA adducts in exposed humans

Biosample	Location, exposure level	Adduct frequency (analytical method)	Response (significance)	Comments	Reference
Lung	Normal lung tissue of tobacco smokers (obtained from marginal tissues during tumour resection) and non-smokers (obtained from the Lung Tissue Research Consortium of the National Heart Lung and Blood Institute)	Smokers (n = 41) Non-smokers (n = 13)	Adducts/10^5 dG (^{32}P postlabelling TLC/HPLC) γ-OH-PdG: 1–24 in smokers vs 1–8 in non-smokers (statistically significant; P value, NR)	Lung, buccal cells, and sputum samples are from different individuals; smoking histories were from < 20 to > 50 packs/year.	Weng et al. (2018)
Lung	Normal tissue obtained at surgery from The Cancer Tissue Procurement Facility, University of Minnesota	Smokers (n = 5) Ex-smokers (n = 23) non-smokers	Adducts/10^9 dG (LC-MS/MS) γ-OH-PdG: 49 in smokers vs 25 in 1 mo to 26 yr; samples were from	Average calculated by the Working Group; ex-smokers quit smoking self-reported smokers; moderately well-defined exposure; other sources of acrolein exposure except smoking not considered.	Zhang et al. (2007) Center
Lung	Tissues obtained during surgery for lung cancer through the Tissue Procurement Facility, University of Minnesota	Smokers (n = 24) Non-smokers (n = 13)	Adducts/10^9 dG (LC-MS/MS) γ-OH-PdG: 20 in smokers vs 15 in non-smokers (NS)	Moderately well-defined exposure; other sources of acrolein exposure except smoking not considered.	Yang et al. (2019a)
Lung	Tissues obtained after surgery from the Histopathology & Tissue Shared Resource of the Lombardi Comprehensive Cancer Center, Georgetown University	n = 5	Adducts/10^9 dG (LC-MS/MS) γ-OH-PdG: 4–10	Poorly defined exposure; unclear whether the adducts were from endogenous exposure or from any unknown external exposure.	Chung et al. (2012)
Buccal cells	Buccal mucosa from subjects free of lung cancer at the time of the initial screening	Smokers (n = 33) Non-smokers (n = 17)	Adducts/10^7 dG (immunochemical method) γ-OH-PdG: 10–250 in smokers vs 5–15 in non-smokers (P < 0.0001)	Lung, buccal cells, and sputum samples are from different individuals; smoking histories were from < 20 to > 50 packs/year.	Weng et al. (2018)
Buccal cells (gingival tissue)	Samples from surgery at a periodontal clinic of New York University Dental Center, New York	11 smokers (4M, 7F); 12 non-smokers (8M, 4F)	Adducts/10^6 dG (^{32}P postlabelling and HPLC) Adduct levels significantly higher in smokers 1.36 ± 0.90 than in non-smokers 0.46 ± 0.26 (P = 0.003)	Small study; self-reported exposure.	Nath et al. (1998)

Table 4.1 (continued)

Biosample	Location, setting	Exposure level and no. of exposed and controls	Adduct frequency (analytical method) Response (significance)	Comments	Reference
Buccal cells	Smokers	$n = 5$	Adducts/10^7 dG (loss-triple stage with linear quadruple ion trap MS) > 5 per 10^7 unmodified DNA bases in buccal cell DNA	Tobacco smokers, smoking > 20 cigarettes per day, and on a noncontrolled diet.	Bessette et al. (2009)
Buccal cells	Healthy subjects after consumption of fried food from three commercial restaurants	$n = 19$	Acr–dG (immunochemical method) Fried food causes a 50% increase in Acr–dG levels, 2–24 hours after meal ($P < 0.005$)	Urinary HPMA, 30% increase; poorly defined exposure.	Wang et al. (2019)
Buccal cells	Healthy subjects Patients with oral squamous cell carcinoma	$n = 222$ $n = 80$	Acr–dG (immunochemical method) 25% increase ($P = 0.001$)	Moderately well-defined exposure. Sources of acrolein exposure other than smoking, alcohol drinking, and betel-quid chewing were not considered.	Tsou et al. (2019)
Leukocytes	Samples from smokers and non-smokers obtained at the University of Minnesota Tobacco Use Research Center	Smokers ($n = 25$) Non-smokers ($n = 25$)	γ-OH-PdG/10^9 nucl (LC-MS/MS) Adduct levels: smokers, 7.4 ± 3.4 adducts/10^9 nucl; non-smokers, 9.8 ± 5.5 adducts/10^9 nucl; (NS)	No difference between smokers and non-smokers; poorly defined exposure; unclear whether the adduct levels are from endogenous exposure or from any unknown external exposure.	Zhang et al. (2011)
Leukocytes	Provided by five subjects	$n = 5$	γ-OH-PdG/10^8 nucl (LC-MS/MS) 7.5–11 adducts/10^8 nucl (mean, ~9.0 ± 1.3 adducts/10^8 nucl)	Acr–dA, Acr–dC and etheno– DNA also detected. Poorly defined exposure; unclear if the adduct levels are from endogenous exposure or from any unknown external exposure.	Yin et al. (2013)
Leukocytes	Patients treated with cyclophosphamide Untreated matched patients	$n = 12$ 6 positive results in $n = 15$	Acr–dG (immunochemical methods) sources of acrolein exposure other cyclophosphamide treated patients vs 0 in matched controls ($P = 0.003$)	Moderately well-defined exposure; than smoking were not considered.	McDiarmid et al. (1991)

Table 4.1 (continued)

Biosample	Location, setting	Exposure level and no. of exposed and controls	Adduct frequency (analytical method) Response (significance)	Comments	Reference
Peripheral blood	Smoker vs non-smoker	$n = 1$ $n = 1$	Acr–dG/10^7 nucl (LC-MS/MS) 4.1 in smokers, NR in non-smokers	Reduced FA–dG and MDA–dG detected; compared single samples, one a heavy smoker, for first-level validation of methods; no measurements supporting smoking extent provided (e.g. cotinine); smoker was said to have regularly smoked 30 cigarettes/day; acrolein adducts not seen in non-smoker.	Alamil et al. (2020)
Liver	Autopsy samples from Colombia University, New York	$n = 5$	γ-OH-PdG/10^6 nucl (^{32}P postlabelling and HPLC) 0.03–0.74 adducts/10^6 dG	The health status of these individuals was unknown; demonstration study.	Nath & Chung (1994)
Liver	Tissues obtained after surgery from the Histopathology & Tissue Shared Resource of the Lombardi comprehensive Cancer Center, Georgetown University	$n = 5$	γ-OH-PdG/10^9 nucl (LC-MS/MS) 1.11 adducts/10^9 dG	Poorly defined exposure; unclear if the adduct levels are from endogenous exposure or from any unknown external exposure.	Chung et al. (2012)
Liver	Liver biopsies or HCC specimens from patients who had liver biopsies or curative resection of HCC as part of standard medical care; Georgetown University Medical Center		γ-OH-PdG (immunostaining)	Biomarker for predicting the risk of human HCC recurrence.	Fu et al. (2018)
	HCC patients	$n = 90$	High γ-OH-PdG levels in the HCC specimens were strongly correlated ($P < 0.0001$) with poorer survival in these patients.		
	HCC recurrence 500 days after surgery	$n = 45$	Patients with tumours with low γ-OH-PdG experienced a significantly longer HCC recurrence-free survival than patients with tumours with high γ-OH-PdG ($P < 0.007$)		

Table 4.1 (continued)

Biosample	Location, setting	Exposure level and no. of exposed and controls	Adduct frequency (analytical method) Response (significance)	Comments	Reference
Sputum	From subjects free of lung cancer at the time of the initial screening	Smokers ($n = 22$) Non-smokers ($n = 8$)	Adducts/10^7 dG (immunochemical method) γ-OH-PdG: 5–240 in smokers vs 5–130 in non-smokers ($P < 0.05$)	Lung, buccal cells, and sputum samples were from different individuals; smoking histories were from < 20 to > 50 packs/year.	Weng et al. (2018)
Saliva	Healthy individuals	$n = 27$	γ-OH-AdG/10^8 nucl (LC-MS/MS) 13–218 adducts/10^8 dG	Etheno–dG detected (68–752 adducts/10^8 nucl); no information on external exposure.	Chen & Lin (2011)
Brain	Brain specimens removed at autopsy from subjects with Alzheimer disease and agematched control subjects	Alzheimer disease, $n = 8$ (4M, 4F) Controls, $n = 5$ (3M, 2F)	γ-OH-PdG/10^6 nucl (LC-MS/MS) γ-OH-PdG: 5.1 in specimens from patients with Alzheimer disease vs 2.8 in healthy participants ($P < 0.025$)	Poorly defined exposure; unclear whether the adduct levels are from endogenous exposure or from any unknown external exposure.	Liu et al. (2005)
Bladder mucosa	Bladder tumours	$n = 10$	63 ± 25/10^7 dG in bladder tumours vs 25 ± 10 in normal urothelial mucosa ($P < 0.001$)	External exposure not defined.	Lee et al. (2014)
	Normal urothelial mucosa	$n = 19$			
	CKD early	$n = 40$	30% increase ($P < 0.01$)		
	CKD late	$n = 22$			
Urothelial tissue (nonsmokers)	Normal tissue Tumour tissue	$n = 48$ $n = 48$	30% increase ($P < 0.005$)		

Acr, acrolein; CKD, chronic kidney disease; dG, deoxyguanosine; F, female; FA, formaldehyde; HCC, hepatocellular carcinoma; LC-MS/MS, liquid chromatography-tandem mass spectrometry; M, male; MDA, malondialdehyde; mo, month; MS, mass spectrometry; NR, not reported; NS, not significant; nucl, nucleotide; γ-OH-PdG, γ-hydroxy-1,N^2-propano-2′deoxyguanosine; TLC/HPLC, thin-layer chromatography/high-performance liquid chromatography; vs, versus; yr, year.

Table 4.2 Acrolein-derived DNA adducts in human cells in vitro

End-point	Tissue, cell line	Result[a]	Concentration (LEC or HIC)	Comments	Reference
DNA adducts (^{32}P-postlabelling)	Xeroderma pigmentosum fibroblasts, GM 5509	+	1 µM		Wilson et al. (1991)
DNA adducts (^{32}P-postlabelling)	Primary normal bronchial fibroblasts, human skin fibroblasts from a cystic fibrosis patient, GM 4539	+	100 µM	Only one concentration tested.	Wilson et al. (1991)

DNA adducts (^{32}P-postlabelling)	Lung epithelial cells and fibroblasts	+	5–20 µM	Acrolein induced DNA damage at *TP53* mutational hotspots and inhibited DNA repair.	Feng et al. (2006)
DNA adducts (^{32}P-postlabelling)	Colon HT-29 cells	+	20 µM		Pan et al. (2009)
DNA adducts (^{32}P-postlabelling)	Colon HT-29 cells	+	200 µM		Pan et al. (2012)
DNA adducts (LC-MS/MS-MRM)	BEAS-2B (bronchial epithelial cells)	+	20 µM		Greenspan et al. (2012)
DNA adducts (TLC/HPLC)	Normal bronchial epithelial cells, normal lung fibroblasts, cultured lung adenocarcinoma cells (A549)	+	25–100 µM		Wang et al. (2012)
	Human colon cancer HCT116 + ch3 cells	+	200 µM		Pan et al. (2016)

HIC, highest ineffective concentration; LC-MS, liquid chromatography-mass spectrometry; LEC, lowest effective concentration; MS-MRM, mass spectrometry multiple reaction monitoring; NT, not tested; TLC/HPLC, thin-layer chromatography/high-performance liquid chromatography. [a] All tests were conducted without metabolic activation.

and α-OH-PdG (5%). These studies also investigated subsequent mutagenesis as well as effects on DNA repair (see Sections 4.2.2 and 4.2.3).

(iii) Experimental systems: reactions with deoxyribonucleosides

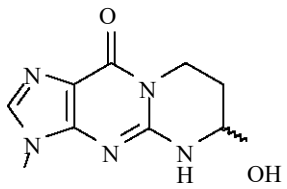
Acrolein is a strong electrophile and readily undergoes reactions with deoxyribonucleosides forming covalent adducts via Michael addition. The reactions of acrolein with deoxyguanosine, deoxyadenosine, and deoxycytidine have been well studied. The electron-rich purine bases are more reactive towards acrolein than are pyrimidine bases. These reactions involve an initial nucleophilic attack of a nitrogen in the bases to the terminal (β) olefinic carbon of acrolein, followed by the addition of a second nitrogen to the aldehydic carbon, leading to the formation of a new ring structure. The end products consist of a class of structurally unique cyclic adducts (Chung et al., 1986). Specifically, upon reaction with deoxyguanosine, acrolein yields cyclic 1,N^2-propano-2′-deoxyguanosine (PdG) adducts as a pair of regioisomers, designated as α and γ-OH-PdG (formerly as Acr–dG 1/2 and 3, respectively), depending on which deoxyguanosine nitrogen is involved in the Michael addition (Chung et al., 1984; Fig. 4.2). The α-isomers are a pair of diastereomers that exist in equilibrium due to interconversion via ring opening. The reaction of acrolein with deoxyadenosine yields cyclic 1,N^6-propano-2′-deoxyadenosine derivatives (1,N^6-PdA) with the possible formation of either 9- or 7-OH substituted regioisomers (Sodum & Shapiro, 1988, Smith et al., 1990a; Pawłowicz et al., 2006a); however, studies were mostly focused on the 9-OH isomer (Fig. 4.2). The 9-OH-1,N^6-PdA adduct can further react with another acrolein molecule, forming a 2:1 adduct (Pawłowicz et al., 2006a); [the Working Group noted that such adducts are unlikely to be formed under physiological conditions in vivo]. In addition to the above-mentioned adducts, the exocyclic amino group in deoxyadenosine can be involved in two Michael additions with two acrolein molecules, followed by intramolecular aldol condensation, which gives rise to a 6-(3-formyl-1,2,5,6-tetrahydropyridyl) substituted adduct (Pawłowicz et al., 2006b). The reaction of acrolein with deoxycytidine forms a 3,N^4-substituted cyclic adduct (7-hydroxy-3,N^4-propano-2′-deoxycytidine) as a pair of diastereomers (Chenna & Iden, 1993). However, only one of the two possible regioisomers, the one resulting from Michael addition of the endocyclic N3 to the acrolein β-carbon, has been described (Fig. 4.2). Alkylated adducts, sometimes 2:1 adducts with deoxyadenosine and thymidine, which result from Michael addition to acrolein without subsequent ring closure, have also been described; these appear to be minor products (Lutz et al., 1982; Chenna et al., 1992; Pawłowicz et al., 2006a; Pawłowicz & Kronberg, 2008). Interestingly, under strenuous conditions (DMSO at 100 °C for 5 days) γ-OH-PdG, one of the cyclic adducts of acrolein with deoxyguanosine, can further react with another molecule of deoxyguanosine forming a cyclic bis-nucleoside, γ-OH-PdG–dG (Kozekov et al., 2001). [The Working Group noted that, despite the somewhat harsh conditions, the identification of the bis-nucleoside adduct suggests the possibility that interstrand dG–acrolein–dG crosslinks can be formed in duplex DNA.] Table 4.3 summarizes the reported reaction conditions between acrolein and deoxyribonucleosides/deoxyribonucleotides and the identity of the resulting adducts.

(iv) Experimental systems: reactions with DNA in vitro

Using synthetic adducts from the reactions with deoxyribonucleosides as reference standards, several studies, mostly with calf thymus DNA, have shown that acrolein can also modify DNA, forming some of the same adducts as with deoxyribonucleosides. Adducts formed in the acrolein-modified DNA have been detected and quantified, mainly after hydrolysis, by a variety of methods, including HPLC with fluorescence detection, ^{32}P-postlabelling, immune-based assays, or LC-MS/MS (Chung et al., 1984; Liu et al., 2005; Pawłowicz et al., 2006a; Pawłowicz & Kronberg, 2008; Pan et al., 2012; Chen et al., 2019a). The levels of adduct modification in these reactions are considerably lower than those with the monomers; however, the levels of modification may be significantly increased using denatured or single-stranded DNA, or oligomers. As the most nucleophilic base in DNA, guanine reacts to the greatest extent, in what constitutes a major pathway of DNA modification by acrolein.

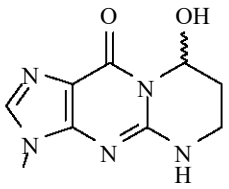
Unlike its reactions with the monomers, the formation of cyclic adducts by acrolein with deoxyguanosine and deoxyadenosi

Fig. 4.2 Structures of the major acrolein–deoxyribonucleoside adducts



dR

7-hydroxy-3,N^4-propano-2′-deoxycytidine

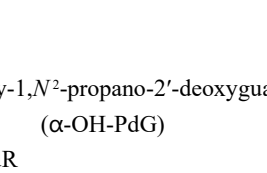


dR

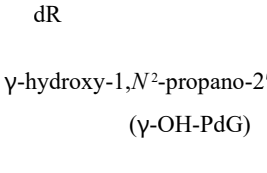
9-hydroxy-3,N^4-propano-2′-deoxycytidine

dR, 2′-deoxyribosyl.
Compiled by the Working Group.

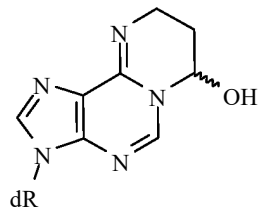
ne in DNA appears to be regioselective. For example, γ-OH-PdG predominates over the α-isomer in DNA (Chung et al., 1984). Similarly, 9-OH-1,N^6-PdA was reported to be the product in acrolein-modified DNA, not the 7-OH isomer (Smith et al., 1990a). Studies were carried out to



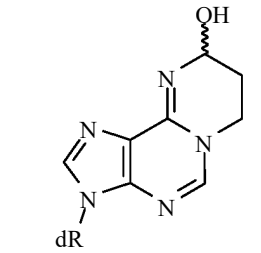
α-hydroxy-1,N^2-propano-2′-deoxyguanosine
(α-OH-PdG)



γ-hydroxy-1,N^2-propano-2′-deoxyguanosine
(γ-OH-PdG)

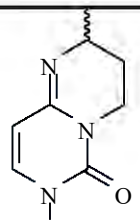


7-hydroxy-1,N^6-propano-2′-deoxyadenosine



9-hydroxy-1,N^6-propano-2′-deoxyadenosine

OH



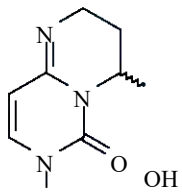


Table 4.3 Detection of acrolein-derived adducts with deoxynucleosides or deoxynucleotides in acellular systems

Nucleoside or deoxynucleotide	Conditions	Adduct	Reference
dG	PBS at 37 °C	γ-OH-PdG & α-OH-PdG	Chung et al. (1984)
dA		N^6-alkylated dA (Michael addition)	Lutz et al. (1982)
dA 5′-mp	PBS at 37 °C	9-OH-1,N^6-PdA-5′p	Smith et al. (1990a)
dA 3′,5′-bp	PBS at 37 °C	9-OH-1,N^6-PdA-3′,5′-bp	Smith et al. (1990a)
T	PBS at 37 °C	N3-alkylated	Chenna et al. (1992)
dC	PBS at 37 °C	7-OH-1,N^6-PdC	Chenna & Iden (1993)
dU	PBS at 37 °C	N3-alkylated	Chenna & Iden (1993)
dG/ γ-OH-PdG	DMSO at 100 °C	γ-OH-PdG–dG	Kozekov et al. (2001)
dG 5′-mp	ω-3 and ω-6 olyunsaturated fatty acids with ferrous sulfate/tris buffer at 37 °C	γ-OH-PdG	Pan & Chung (2002)
dA	PBS at 37 °C	1:1 and 2:1 (acrolein:dA) 1,N^6-PdA 1:1 and 2:1 (acrolein:dA) N^6-alkylated	Pawłowicz et al. (2006a, b)
T	PBS at 37 °C	1:1 N3-alkylated and four 2:1 (acrolein:T) N3-alkylated	Pawłowicz & Kronberg (2008)

1,N^6-PdA, 1,N^6-propano-2′-deoxyadenosine; 7-OH-1,N^6-PdC, 7-hydroxy-1,N^6-propano-2′-deoxycytosine; 9-OH-1,N^6-PdA, 9-hydroxy-1,N^6-PdA; α-, γ-OH-PdG, α-, γ-hydroxy-1,N^2-propano-2′-deoxyguanosine; dA, deoxyadenosine; dA 5′-mp, dA 5′-monophosphate; dA 3′,5′-bp, dA 3′,5′bisphosphate; dC, deoxycytosine; dG, deoxyguanosine; dG 5′-mp, dG 5′-monophosphate; DMSO, dimethyl sulfoxide; dU, deoxyuridine; PBS, phosphate-buffered saline; T, thymidine.

shed light onto the molecular basis for the regioselectivity. Possible explanations involve the tertiary structure of DNA and/or an intermediacy of the Schiff's base between acrolein and amines (Chung et al., 2012). The 2:1 adduct of acrolein with deoxyadenosine, but not thymidine or deoxycytidine, was also observed in the reactions with DNA in vitro (Pawłowicz et al., 2006b; Pawłowicz & Kronberg, 2008). The formation of cyclic adducts of acrolein involves covalent binding with the nitrogens that participate in hydrogen bonding in the double helical structure of DNA.

Interestingly, the cyclic bis-nucleoside adduct of γ-OH-PdG (γ-OH-PdG–dG) described above was also found in a DNA duplex containing γ-OH-PdG in a 5′-CpG sequence context with the exocyclic amino group of deoxyguanosine in the opposite strand, resulting from interstrand crosslinking in oligonucleotide

or DNA (Kozekov et al., 2001, 2010; Minko et al., 2009).

Although the crosslinking product can undergo reversible reaction, it was sufficiently stable to be isolated for structural characterization. Table 4.4 summarizes the reactions of acrolein with oligomers and DNA.

(v) Experimental systems: DNA adduct formation in tissues and cells

See Table 4.5 and Table 4.6.

Most in vivo studies of the acrolein-derived DNA adducts in cells and tissues have focused on γ-OH-PdG. The only acrolein-derived DNA adduct other than γ-OH-PdG reported to be formed in vivo is 9-OH-1,N^6-PdA (Kawai et al., 2003). It has been shown that γ-OH-PdG can be formed in DNA in vivo from acrolein derived from two major sources: environmental exposure, such as tobacco smoke; and endogenous production, such as lipid peroxidation and polyamine oxidation. Although diet may also be a possible source, its importance has been

Table 4.4 Detection of acrolein-derived DNA adducts with oligonucleotides and DNA

Oligomers or DNA	Conditions	Adduct	Detection method	Reference
ct-DNA	PBS pH 7 at 37 °C	γ-OH-PdG	HPLC-fluorescence	Chung et al. (1984)
ct-DNA	Tris pH 8.5 at 37 °C	γ-OH-PdG	CapLC-nanoESIMS/MS	Liu et al. (2005)
ct-DNA	PBS pH 7.4 at 37 °C	1:1 9-OH-1,N^6-PdA; 2:1 1,N^6-PdA; 1:1 N^6-alkylated dA; 2:1 N^6-alkylated dA	LC-ESI-MS/MS	Pawłowicz et al. (2006b)
ct-DNA	PBS pH 7.4 at 37 °C	1:1 N3-alkylated T	LC-ESI-MS/MS	Pawłowicz & Kronberg (2008)
ct-DNA	PBS pH 7.0 at 37 °C	γ-OH-PdG-dG (crosslinking)	LC-ESI-MS/MS	Kozekov et al. (2010)
ct-DNA/plasmid pSP189 ct-DNA	PBS pH 7.0 at 37 °C LPO	γ-OH-PdG α-, γ-OH-PdG, and other LPO-derived cyclic adducts	ELISA/slot blot UHPLC/ESI-IT-MS	Pan et al. (2012) Chen et al. (2019a)

ct, calf thymus; dA, deoxyadenosine; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; LPO, lipid peroxidation; α-, γ-OH-PdG, α-, gamma-hydroxy-1,N^2-propano-2′-deoxyguanosine; 1,N^6-PdA, 1, N^6-propano-2′-deoxyadenosine; 9-OH-1,N^6-PdA, 9-hydroxy-1, N^6-PdA; CapLC-nanoESI-MS/MS, capillary liquid chromatography-nano electrospray ionization-tandem mass spectrometry; PBS, phosphate-buffered saline; UHPLC/ESI-IT-MS, ultra high-HPLC ESI-ion trap multistage mass spectrometry.

Table 4.5 Detection of acrolein-derived DNA adducts in experimental animals in vivo

Adduct	Species	Tissue	Exposure	Method of detection	Reference
γ-OH-PdG	Dog	Lymphocytes	Cyclophosphamide (6.6 mg/kg)	^{32}P-Postlabelling	Wilson et al. (1991)
γ-OH-PdG	Mouse Rat	Liver Liver	None None	^{32}P-Postlabelling	Nath & Chung (1994)

γ-OH-PdG	Mouse Rat	Skin Brain Lung Kidney Colon Prostate Mammary gland Leukocytes	None	^{32}P-Postlabelling	Nath et al. (1996)
γ-OH-PdG	Rat	Liver	None	LC-MS/MS	Fu et al. (2018)
γ-OH-PdG	Rat	Liver	None	UHPLC/ESI-IT-MS	Chen et al. (2019a)
γ-OH-PdG	Cockerel	Aorta	Acrolein inhalation (0, 1, and 10 ppm)	^{32}P-Postlabelling	Penn et al. (2001)
γ-OH-PdG	Mouse	Lung/bladder	Sidestream smoke	Immunoassay/^{32}Ppostlabelling	Lee et al. (2015)
γ-OH-PdG	Mouse	Lung/bladder	Tobacco smoke	Immunoassay/^{32}Ppostlabelling	Weng et al. (2018)
Not identified	Rat	Lung	Diesel-exhaust inhalation	HPLC-MS/MS	Douki et al. (2018)
γ-OH-PdG	Mouse	Liver	High-fat diet	IHC and LC-MS/MS	Coia et al. (2018)
1,N^6-PdA	Rat	Kidney	Ferric nitrilotriacetate	IHC	Kawai et al. (2003)

1,N^6-PdA, 1,N^6-propoanodeoxyadenosine; γ-OH-PdG, γ-hydroxy-1,N^2-propano-2′-deoxyguanosine; IHC, immunohistochemistry; HPLC-MS/MS; high-performance liquid chromatography-tandem mass spectrometry. UHPLC/ESI-IT-MS, ultrahigh-HPLC electrospray ionization-ion trap multistage mass spectrometry.

Table 4.6 Detection of acrolein-derived DNA adducts in experimental systems in vitro

Adduct	Cells	Acrolein concentration	Method of detection	Reference
γ-OH-PdG	*Salmonella typhimurium* TA100 and TA104	1, 4, 7, 10, 13 mM	ELISA	Foiles et al. (1989)
γ-OH-PdG	Chinese hamster ovary	mM	ELISA	Foiles et al. (1990)
1,N^6-PdA	Rat liver epithelial cells	5, 10, 25, 50 µM	Immunoassay	Kawai et al. (2003)
γ-OH-PdG	*Sphingobium* spp. strain KK22	10 mM	LC-ESI-MS/MS	Kanaly et al. (2015)

ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting, flow cytometry; LC-ESI-MS/MS, liquid chromatography-electrospray ionization-tandem mass spectrometry; 1,N^6-PdA, 1,N^6-propano-2′-deoxyadenosine; γ-OH-PdG, gamma-hydroxy-1,N^2-propano-2′-deoxyguanosine.

questioned by a study in which integrated quantitative structure–activity relationship–physiologically based kinetic/dynamic (QSAR-PBK/D) modelling was used to predict formation of γ-OH-PdG (Kiwamoto et al., 2015). As acrolein is an oxidation product of lipid peroxidation from ω-3 and -6 polyunsaturated fatty acids, the acrolein-derived adducts can be formed upon incubation of these fatty acids in the presence of deoxyguanosine under oxidative conditions (Pan & Chung, 2002; Kawai et al., 2003). As lipid peroxidation occurs continuously in vivo as part of normal physiological processes, acrolein- derived DNA adducts are constantly formed in cellular DNA as endogenous background lesions. Several methods have been developed to detect acrolein-derived DNA adducts in vivo, including ^{32}P-postlabelling, LC-MS/MS, and immunohistochemistry. The availability of monoclonal antibodies against acrolein-derived deoxyadenosine and deoxyguanosine adducts has facilitated the development of immune-based methods, such as immunohistochemistry, immunocytochemistry, and dot blot, for

detecting these adducts in cells and tissues (Kawai et al., 2003 and Pan et al., 2012). However, it is generally agreed that LC-MS/MS is by far the most specific and most sensitive method for adduct detection and identification in vivo.

Acrolein-derived DNA adducts, including γ-OH-PdG, have been detected in various experimental animals in vivo (see Table 4.5). γ-OH-PdG was detected by a ^{32}P-postlabelling method in DNA of peripheral blood lymphocytes obtained from a dog given a therapeutic oral dose of cyclophosphamide at 6.6 mg/kg (Wilson et al., 1991). Studies later showed γ-OH-PdG is an endogenous background DNA lesion in livers of rodents and humans without known treatment and exposure (Nath & Chung, 1994; Nath et al., 1996). γ-OH-PdG was also detected in rats and mice given control feed (Fu et al., 2018; Chen et al., 2019a). Exposure of cockerels to acrolein (1 and 10 ppm) for 6 hours via inha lation gave rise to γ-OH-PdG in the aortic DNA (Penn et al., 2001). Exposure to tobacco smoke (mainstream, ~75 mg/m^3, 6 hours per day, 5 days per week, for 12 weeks; or sidestream, 500 μg/m^3, 6 hours per day, 5 days per week, for 8 or 16 weeks) and automobile exhaust was shown to induce γ-OH-PdG formation in the rodent lung (Lee et al., 2015; Weng et al., 2018; Douki et al., 2018). A small, but significant, increase in levels of acrolein-derived DNA adducts was found in the lung DNA of rats exposed to diesel exhaust; however, the data on the specific identity of the adduct were not reported (Douki et al., 2018). The notion that DNA adducts of acrolein can be derived from endogenous sources, such as lipid peroxidation, has been reinforced by recent studies showing that the levels of γ-OH-PdG are significantly increased in liver DNA of mice fed a high-fat diet (Coia et al., 2018). This study further demonstrated that the elevated hepatic formation of γ-OH-PdG in mice fed a high-fat diet parallels the increased risk of developing hepatocellular carcinoma in these mice. The only other acrolein-derived DNA adduct in vivo so far reported is 9-OH-1,*N*^{6}-PdA. This adduct was found in rat kidney, using an iron-induced kidney carcinogenesis model under oxidative stress conditions in which rats were exposed to ferric nitrilotriacetate (Kawai et al., 2003). However, the structural identity of the adduct was not unequivocally established in this study because the adduct was detected by a monoclonal antibody raised against acrolein-modified DNA, not specifically 9-OH-1,*N*^{6}-PdA.

Acrolein-derived DNA adducts, including γ-OH-PdG, have also been assessed in various experimental cell types in vitro (see Table 4.6). Using a monoclonal antibody developed against crotonaldehyde-derived cyclic deoxyguanosine adducts structurally analogous to γ-OH-PdG (Foiles et al., 1987), an early study demonstrated the detection of γ-OH-PdG in *Salmonella typhimurium* strains TA100 and TA104 exposed to acrolein at the concentration range in which mutations were induced (Foiles et al., 1989). The first study detecting γ-OH-PdG in mammalian cells was reported using enzyme-linked immunosorbent assay (ELISA) in Chinese hamster ovary cells exposed to acrolein at a high concentration (1 mM) (Foiles et al., 1990). This concentration, however, was too toxic for scoring mutations. Later, monoclonal antibodies were raised against 1,*N*^{6}-PdA, using acrolein-modified DNA (Kawai et al., 2003), and against γ-OH-PdG, using specifically γ-OH-PdG-conjugated bovine serum albumin (Pan et al., 2012; see Table 4.4). More recently, a DNA adductomics approach was applied to the study of γ-OH-PdG in the soil bacterium *Sphingobium* spp. strain KK22 (Kanaly et al., 2015). This study demonstrated the potential

of LC-MS/MS in DNA adductomics as a promising tool to study γ-OH-PdG and other related adducts in cells.

(b) Interactions with cellular proteins

(i) Reactions with amino acids and proteins in vitro See Table 4.7.

Acrolein shows a strong propensity to react with amino acids or proteins via Michael addition, considerably more so than with DNA bases. Cysteines and the thiols of amino acids and proteins are the major sites for covalent binding with acrolein. Because the thiols are known to play important roles in enzyme activities and redox homeostasis, their facile interactions with acrolein can profoundly alter cellular functions. On the other hand, compounds with the mercapto (-SH) group, like GSH and cysteine, are widely used as effective scavengers of acrolein, with aim of reducing its adverse effects in cells or animals (Rees & Tarlow, 1967; Gurtoo et al., 1981; Wildenauer & Oehlmann, 1982).

The N-alkylation of proteins by acrolein may also occur. These reactions, through the sidechain amino group of lysine or a ring nitrogen of histidine, are kinetically less favourable than conjugation with -SH groups. Unlike reactions with cysteines, N-alkylation is irreversible, and the end products are usually quite stable (Cai et al., 2009). Reactions of acrolein with lysine have been investigated extensively with 3-formyl-3,4-dehydropiperidine (FDP), a 2:1 adduct, as a notable product that may serve as a potential biomarker of acrolein exposure detectable by a monoclonal antibody (Uchida et al., 1998a, b). The formation of FDP lysine adducts in histone has been associated with the inhibition of chromatin assembly mediated by acrolein (Fang et al., 2016). Furthermore, acrolein can form a Schiff base with the amine of lysine, followed by Michael addition yielding *N*-(3-methylpyridium)lysine via 2:1 addition (Furuhata et al., 2003; Kaminskas et al., 2005) and intra- and inter-protein crosslinks (Burcham & Pyke, 2006; Ishii et al., 2007; Minko et al., 2008). In addition to lysine, acrolein can also react with histidine by nucleophilic attack

Table 4.7 Reactions of acrolein with amino acids and proteins

Source	Amino acid or protein	Adduct	Detection method	Reference
Cyclophosphamide and acrolein	Rat hepatic microsomal CYP450	Not identified	Radioactivity with gel electrophoresis	Marinello et al. (1984)
Acrolein	[^{3}H-lysine]albumin	Not identified	Radioactivity	Thakore et al. (1994)
Acrolein	Synthetic peptide	Not identified	HPLC-MS	Carbone et al. (1997)
Acrolein	Lysine and low-density lipoprotein	(3-Formyl-3,4-dehydropiperidino)lysine	HPLC/MS/amino acid analysis IHC (mAb5F6)	Uchida et al. (1998a, b)
Acrolein	Histidine	3-Formylethylhistidine	LC-MS and NMR	Pocker & Janjić (1988), Uchida et al. (1998a)
Acrolein	BSA	Michael adduct	Spectrophotometric method for detection of DNPH derivative	Burcham et al. (2000)
Acrolein	BSA	*N*-(3-Formyl-3,4-dehydropiperidino)lysine	HPLC-MS/amino acid analysis	Furuhata et al. (2002)
Acrolein	Peptide (B chain of insulin)	*N*-(3-Methylpyridinium)lysine	ESI-LC/MS mAb5F6	Furuhata et al. (2003)
Acrolein	BSA	Lysine mono-Michael adduct versus Schiff base and FDP cyclic adduct	ESI-MS	Kaminskas et al. (2005)
Acrolein	Bovine pancrease Ribonuclease A	Crosslinking dimerized proteins	Gel electrophoresis	Burcham & Pyke (2006)
Acrolein	Actin	Cys374	LC-ESI-MS/MS	Dalle-Donne et al. (2007)
Acrolein	Peptide (B chain of insulin)	Crosslinking adducts	LC-ESI-MS/MS	Ishii et al. (2007)
Acrolein–dG or –dA adduct	KWKK peptide	Crosslinking adducts	Gel electrophoresis	Minko et al. (2008)
Acrolein	Insulin peptides	Cys, lysine, histidine, intra-molecular Schiff base	ESI-MS, ESI-MS/MS	Cai et al. (2009)
Acrolein/lipid oxidation	BSA/LDL	*N*-(3-Propanal)histidine	ESI/LC/MS/MS	Maeshima et al. (2012)
Acrolein	Recombinant histone/ H2a and H4	Lysine FDP adduct	LC-MS/MS	Fang et al. (2016)
Acrolein	Human serum albumin	Michael addition adducts	Biotin affinity tag LC-MS/MS	Coffey & Gronert (2016)
Acrolein	Lysozyme and human serum albumin	Histidine/cysteine/lysine adducts	LC-MS/MS	Afonso et al. (2018)

BSA, bovine serum albumin; CYP450, cytochrome P450; DNPH, dinitrophenyl hydrazine; ESI-MS, electrospray ionization-tandem mass spectrometry; FDP, *N*(ε)-3-formyl-3,4dehydropiperidine; HPLC-MS, HPLC, high-performance liquid chromatography-mass spectrometry; LC, liquid chromatography; LDL, low-density lipoprotein; mAb, monoclonal antibody; NMR, nuclear magnetic resonance spectroscopy.

on the imidazole ring nitrogen (Pocker & Janjić, 1988; Maeshima et al., 2012). A recent study demonstrated that acrolein may be one of the aldehydes in tobacco smoke responsible for the inhibition of the enzymes involved in DNA repair by targeting these proteins via direct binding (Weng et al., 2018). Acrolein is a major metabolic product of certain anticancer drugs, such as cyclophosphamide, and early studies showed that the bladder toxicity of cyclophosphamide can be effectively attenuated by GSH or other SH-containing small compounds, whereas its therapeutic efficacy was not affected by GSH (Gurtoo et al., 1981). The reaction products were studied and compared between acrolein versus 1,3-bis-(2-chloroethyl)-1-nitrosourea (BCNU) and a synthetic peptide 128 (Carbone et al., 1997). Ample evidence shows that dithiothreitol and hydralazine also inhibit acrolein-induced cellular toxicity through their interactions with acrolein (Rees & Tarlow, 1967; Cox et al., 1988; Burcham et al., 2000; Burcham & Pyke, 2006; Chen et al., 2016). The chemical basis for the inhibition is the -SH conjugation with the former and formation of a hydrazone derivative with the latter; both reactions can effectively block acrolein's ability to bind cellular target proteins.

The identification of the binding sites of acrolein to protein is important because this knowledge may help understand the molecular basis underlying the toxicity caused by acrolein. To this end, LC-MS/MS-based proteomic methods have been developed in the past decade (Spiess et al., 2011; Coffey & Gronert, 2016; Afonso et al., 2018; Chen, Liu et al., 2019b). The application of proteomics in the determination of protein binding sites has been demonstrated with the use of model proteins as well as in cells treated with acrolein (Table 4.7).

(ii) Protein binding in human cells in vitro

Enhanced protein binding of acrolein has been demonstrated in exposed human bronchial epithelial cells (Caito et al., 2010), in human serum albumin (Colombo et al., 2010) and with the lactate dehydrogenase isozymes in human saliva (Avezov et al., 2014). Further investigations have revealed effects on protein function. For example, acrolein formed Michael adducts with sirtuin 1 (SIRT1) and reduced its activity (Caito et al., 2010). The evidence for protein dysfunction is ample; for example, Biswal et al. (2003) showed that acrolein modified c-JUN, preventing its dimerization and consequently preventing AP-1-promoter binding, and that acrolein modified B[*a*]P-induced TP53 and reduced its transcription transactivation activity. In human T cells, acrolein caused modification at Cys-61 and Arg-307 sites in p50 and IkB phosphorylation, consequently preventing DNA binding of NF-kB and reducing the expression of interleukins IL2 and IL10, interferon gamma (INFγ), tumour necrosis factor α (TNFα), and granulocyte-macrophage colony-stimulating factor. In human lung cells, acrolein at noncytotoxic levels can cause acrolein–Cys binding and consequently Hsp90 crosslinks (Burcham et al., 2007).

(iii) Protein binding in experimental animal cells and tissues

See Table 4.8.

To identify target proteins and binding sites in cells and tissues of rodents exposed to acrolein the methods currently used include immunohistochemistry, immunocytochemistry, Western blot, and LC-MS/MS. Because direct exposure to acrolein can cause overt toxicity, studies in vivo are often carried out with cancer chemotherapeutics, cigarette smoke, ethanol, and diet as indirect sources of acrolein (Gurtoo et al., 1981; Wildenauer & Oehlmann, 1982;

Günther et al., 2008; Conklin et al., 2009; Chen et al., 2016). The availability of a monoclonal antibody to acrolein-modified keyhole limpet haemocyanin, with the lysine binding as an epitope, has greatly facilitated studies of acrolein-bound proteins in cells and tissues (Uchida et al., 1998a, b). The antibody was specifically developed

Table 4.8 Detection of acrolein-derived adducts in proteins in experimental animal cells and tissues

Source	Cell or animal	Protein target	Detection method	Reference
Cyclophosphamide	Mouse/rat	Hepatic proteins	Radioactivity	Gurtoo et al. (1981)
Cyclophosphamide	Rabbit liver microsomes Human erythrocytes	Membrane and cytoplasm	Radioactivity with SDS polyacrylamide Gel electrophoresis	Wildenauer & Oehlmann (1982)
Cyclophosphamide	SCID mouse	Implanted CT26 tumour cells	IHC	Günther et al. (2008)
Tobacco smoke or Acrolein	Mouse lung, plasma, aorta		Western blot	Conklin et al. (2009)
Acrolein	Human lung epithelial cells	Proteome	LC-MS/MS	Spiess et al. (2011)
Acrolein	F344 rat	Cardiac mitochondria	Aldehyde-specific chemical labelling and LC-MS/MS	Chavez et al. (2011)
Endogenous	F344 rat	Cardiac mitochondria	Proteomics NanoLC MALDI-MS/MS	Han et al. (2012)
Alcohol or acetaldehyde	Rat hepatoma H4IIEC cells	FDP-lysine adduct	ICC	Chen et al. (2016)
Diet with 5% ethanol	Male C57BL/6J mouse	Hepatic proteins	IHC	Chen et al. (2016)
Acrolein	Proteomes of human lung cancer H1299 cells	> 2300 proteins > 500 cysteines	Aldehyde-directed aniline-based probe by LC-MS/MS	Chen et al. (2019b)

GST, glutathione-*S*-transferase, ICC; immunocytochemistry; IHC, immunohistochemistry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; MALDI, matrix-assisted laser desorption ionization; MS, mass spectrometry; SDS, sodium dodecyl sulfate.

for lysine-adducted proteins. In recent years, LC-MS/MS-based proteomics has also been used to identify hundreds, if not thousands, of protein targets in cells and tissues (Spiess et al., 2011; Chavez et al., 2011; Han et al., 2012; Chen et al., 2019b; Table 4.8).

4.2.2 Is genotoxic

(a) Humans (i) Exposed humans

No data were available to the Working Group.

(ii) Human cells in vitro

See Table 4.9.

Using the alkaline elution assay, acrolein-induced DNA strand breaks were observed in primary human bronchial epithelial cells (Grafström et al., 1986, 1988), human normal skin fibroblasts (CRL 1508) as well as xeroderma pigmentosum fibroblasts (Dypbukt et al., 1993), human myeloid leukaemia cells (K562) (Crook et al., 1986), and human lymphoblastoid cells (Namalwa) (Eisenbrand et al., 1995). [The Working Group noted that some of these experiments (Grafström et al., 1986; Dypbukt et al., 1993) were carried out at concentrations of acrolein that induced excessive cytotoxicity.] The frequency of phosphorylated H2AX proteins (γH2AX), an indicator of DNA double-strand breaks, was found to be significantly increased in acrolein-treated lung epithelial adenocarcinoma cells (A549) (Zhang et al., 2017) and human bronchial epithelial cells (BEAS-2B) (Zhang et al., 2020), and positive results were obtained in the comet assay for DNA damage in human normal lung fibroblasts (IMR-90) (Luo et al., 2013), A549 cells (Zhang et al., 2018), BEAS-2B cells (Zhang et al., 2020), human Burkitt lymphoma B lymphocytes (Raji) (Yang et al., 1999a), liver hepatoma cells (HepG2) (Li et al., 2008a), and retinal epithelial cells (ARPE-19) (Li et al., 2008b). [The Working Group noted that one experiment (Yang et al., 1999a) was carried out with acrolein at concentrations up to 500 μM with no measure of cytotoxicity.]

Acrolein-induced DNA–protein crosslinks were reported in bronchial epithelial cells (Grafström et al., 1986, 1988), in HepG2 cells (Li et al., 2008b), and in Burkitt lymphoma cells (EBV-BL) (Costa et al., 1997), but a negative result was reported in human promyelocytic leukaemia cells at a concentration (i.e. 100 μM) that resulted in a study-specific cell viability of 58% (Schoenfeld & Witz, 2000). [The Working Group noted that some of these experiments (Grafström et al., 1986; Schoenfeld & Witz, 2000) were carried out at concentrations of acrolein that induced excessive cytotoxicity.] Additionally, a negative result was reported in BEAS-2B cells exposed to acrolein at 7.5 μM; however, at this same concentration acrolein significantly enhanced the level of DNA–protein crosslinks observed when co-administered with formaldehyde (Zhang et al., 2020).

Acrolein induced a dose-dependent increase in *HPRT* mutant frequency in human DNA-repair-deficient xeroderma pigmentosum fibroblasts (Curren et al., 1988) and normal human bronchial epithelial cells (BEAS-2B) (Zhang et al., 2020), but failed to elicit a positive response in normal human fibroblasts when tested up to 2 μM (Curren et al., 1988). A positive result was obtained for micronucleus formation in lung A549 cells (Zhang et al., 2018) and BEAS-2B cells (Zhang et al., 2020), and for sister-chromatid exchanges in human primary lymphocytes (Wilmer et al., 1986). All studies in human cells were carried out in the absence of exogenous metabolic activation.

In eight experiments, plasmids containing the *supF* gene were reacted with acrolein and were

then transfected into various human cell types to allow for repair and replication; the *supF* mutant frequency was subsequently assessed in *Escherichia coli*. Six experiments reported positive results (Feng et al., 2006; Kawanishi et al., 1998; Wang et al., 2009a, 2013a; Lee et al., 2014)

Table 4.9 Genetic and related effects of acrolein in human cells in vitro

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA strand breaks (alkaline elution)	Primary human bronchial epithelial cells	(+)	0.1 mM [5.6 µg/mL]	Concentration tested induced excessive cytotoxicity; single concentration tested.	Grafström et al. (1986)
DNA strand breaks (alkaline elution)	Primary human bronchial epithelial cells	+	30 µM [1.7 µg/mL]	Single concentration tested.	Grafström et al. (1988)
DNA strand breaks (alkaline elution)	Human xeroderma pigmentosum fibroblasts, CRL1223	(+)	100 µM [5.6 µg/mL]	Vehicle not reported; concentrations tested induced excessive cytotoxicity.	Dypbukt et al. (1993)
DNA strand breaks (alkaline elution)	Human normal skin fibroblasts, CRL1508	(+)	300 µM [17 µg/mL]	Vehicle not reported; concentrations tested induced excessive cytotoxicity.	Dypbukt et al. (1993)
DNA strand breaks (alkaline elution)	Human myeloid leukaemia, K562 cells	+	5.4 µM [0.3 µg/mL]		Crook et al. (1986)
DNA strand breaks (alkaline elution)	Human lymphoblastoid, Namalwa cells	+	50 µM [2.8 µg/mL]		Eisenbrand et al. (1995)
DNA double strand breaks (γH2AX)	Human lung epithelial carcinoma, A549	+	80 µM [4.5 µg/mL]		Zhang et al. (2017)
DNA double strand breaks (γH2AX)	Human bronchial epithelial cells, BEAS-2B	+	7.5 µM [0.42 µg/mL]		Zhang et al. (2020)
DNA damage (comet assay)	Human normal lung fibroblasts, IMR-90	+	4 µM [0.22 µg/mL]	Minimal information in description of comet method; single concentration tested.	Luo et al. (2013)
DNA damage (comet assay)	Human, retinal epithelial cells, ARPE-19	+	75 µM [4.2 µg/mL]	Minimal information in description of methods (i.e. pH of lysis etc.); no quantification of the level of DNA damage (binary approach used: nuclei with tails vs those without).	Li et al. (2008b)
DNA damage (comet assay)	Human Burkitt lymphoma B lymphocytes, Raji	(+)	500 µM [28 µg/mL]	No cytotoxicity assessment; minimal information in description of methods (i.e. pH of lysis etc.); comets classified into three size classes; vehicle not reported.	Yang et al. (1999a)
DNA damage (alkaline comet assay)	Human liver hepatoma, HepG2	+	12.5 µM [0.7 µg/mL]		Li et al. (2008a)
DNA damage (alkaline comet assay)	Human lung epithelial carcinoma, A549	+	55 µM [3 µg/mL]	This was the lowest concentration tested.	Zhang et al. (2018)
DNA damage (alkaline comet assay)	Human bronchial epithelial cells, BEAS-2B	+	1 µM [0.056 µg/mL]		Zhang et al. (2020)

DNA–protein crosslinks (method not specified)	Primary human bronchial epithelial cells	(+)	0.1 mM [5.6 µg/mL]	Single concentration tested, which induced excessive toxicity; minimal information in description of methods.	Grafström et al. (1986)

Table 4.9 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
DNA–protein crosslinks (modified alkaline elution assay)	Primary human bronchial epithelial cells	+	30 µM [1.7 µg/mL]	Single concentration tested.	Grafström et al. (1988)
DNA–protein crosslinks (alkaline comet assay, ProtK modified)	Human liver hepatoma, HepG2	+	50 µM [2.8 µg/mL]		Li et al. (2008a)
DNA–protein crosslinks (SDS/KCl precipitation assays)	Human bronchial epithelial cells, BEAS-2B	–	7.5 µM [0.42 µg/mL]	This was the only concentration tested; significantly enhanced DNA–protein crosslinks when co-exposed with formaldehyde.	Zhang et al. (2020)
DNA–protein crosslinks (SDS/KCl precipitation assay)	Human promyelocytic leukaemia cells, HL60	(–)	100 µM [5.6 µg/mL]	Cell viability at this dose was 58%; single concentration tested.	Schoenfeld & Witz (2000)
DNA–protein crosslinks (SDS/KCl precipitation assay)	Human Burkitt lymphoma cells, EBV-BL	+	150 µM [8.4 µg/mL]	Concentrations at which DNA–protein crosslinks were found were highly cytotoxic when assessed 4 days later by trypan blue exclusion.	Costa et al. (1997)
Gene mutation (*HPRT*)	Human xeroderma pigmentosum fibroblasts	+	0.2 µM [0.01 µg/mL]		Curren et al. (1988)
Gene mutation (*HPRT*)	Human normal fibroblasts	–	2 µM [0.1 µg/mL]		Curren et al. (1988)
Gene mutation (*HPRT*)	Human bronchial epithelial cells, BEAS-2B	+	7.5 µM [0.42 µg/mL]	Single concentration tested.	Zhang et al. (2020)
Micronucleus formation (CBMN)	Human bronchial epithelial cells, BEAS-2B	+	4 µM [0.22 µg/mL]		Zhang et al. (2020)
Micronucleus formation (CBMN)	Human lung epithelial carcinoma, A549	+	55 µM [3 µg/mL]	This was the lowest concentration tested.	Zhang et al. (2018)
Micronucleus formation (non-CBMN)	Human bronchial epithelial cells, BEAS-2B	+	7.5 µM [0.42 µg/mL]	Single concentration tested.	Zhang et al. (2020)
Sister-chromatid exchanges	Human primary lymphocytes	+	5 µM [0.28 µg/mL]		Wilmer et al. (1986)
Forward mutation (*supF*)	Plasmid pSP189 (exposed acellularly); transfected into repair.	+	100 µM [5.6 µg/mL] and then transfected into human cells for	Plasmids were reacted with acrolein at 37 °C	Feng et al. (2006) normal human lung fibroblasts replication and

Table 4.9 (continued)

End-point	Tissue, cell line	Results[a]	Concentration (LEC or HIC)	Comments	Reference
Forward mutation (*supF*)	Plasmid pMY189 (exposed acellularly); transfected into a normal human fibroblast cell line (W138-VA13)	+	26 mM [1456 µg/mL]	Plasmids were reacted with acrolein at 37 °C and then transfected into human cells for replication and repair.	Kawanishi et al. (1998)
Forward mutation (*supF*)	Plasmid pSP189 (exposed acellularly); transfected into human repair-proficient fibroblasts (GM637) and human repairdeficient (XPA) fibroblasts (GM4427)	–	1 mM [56 µg/mL]	Plasmids were reacted with acrolein and transfected into human cells for replication and repair.	Kim et al. (2007)
Forward mutation (*supF*)	Plasmid pSP189 (exposed acellularly); transfected into immortalized normal human lung fibroblasts (CCL-202)	+	0.5 mM [28 µg/mL]	Plasmids were reacted with acrolein at 37 °C and then transfected into human cells for replication and repair.	Wang et al. (2009a)
Forward mutation (*supF*)	Plasmid pSP189 (exposed acellularly); transfected into normal human lung fibroblasts	+	0.5 mM [28 µg/mL]	Plasmids were reacted with acrolein at 37 °C and then transfected into human cells for replication and repair.	Wang et al. (2013a)
Forward mutation (*supF*)	Plasmid pSP189 (exposed acellularly); transfected into immortalized normal human bladder cells (UROtsa) or normal human lung fibroblasts (CCL-202)	+	0.5 mM [28 µg/mL]	Plasmids were reacted with acrolein at 37 °C and then transfected into human cells for replication and repair.	Lee et al. (2014)

CBMN, cytokinesis-blocked micronucleus; γH2AX, phosphorylated gamma-histone 2AX; HIC, highest ineffective concentration; HPRT, hypoxanthine-guanine phosphoribosyltransferase; KCl, potassium chloride; LEC, lowest effective concentration; NA, not applicable; NT, not tested; PrtK, protein kinase; SDS, sodium dodecyl sulfate; vs, versus. [a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive/negative in a study of limited quality. All studies in human cells in vitro were carried out in the absence of exogenous metabolic activation.

and two experiments reported negative results (Kim et al., 2007). In one of these studies, Feng et al. (2006) sequenced mutations in the recovered plasmid from normal human lung fibroblasts and found that > 50% of the acrolein-induced base substitutions in the *supF* gene were G→T transversions. In the *supF* gene of acrolein-reacted plasmids recovered from human lung fibroblasts (CCL-202), primarily G→T transversions (53%) were observed, followed by G→A transitions (30%), and G→C transversions (12%); moreover, they found that mutational hotspots occurred in sequences with runs of Gs, and that the mutations across the *supF* gene mapped to the same sequence locations as those where the acrolein-derived adducts formed (Wang et al., 2009a). In another *supF* shuttle vector study, of the acrolein-exposed plasmids recovered from a transformed normal human fibroblast cell line (W138VA13), 76% of mutations were base substitutions (46% single substitutions, 30% tandem or multiple substitutions), 21% were deletions, and 2% were insertions. Of the base substitutions, it was found that G→T predominated (44%), followed by G→A (24%), and G→C (12%) (Kawanishi et al., 1998).

A study in human xeroderma pigmentosum group V (XPV) cells transfected with a plasmid containing the α-OH-PdG adduct found that there was inaccurate translesion synthesis by both polymerases η and κ (Yang et al., 2003). Only marginal miscoding (< 1%) was observed for translesion synthesis across the γ-OH-PdG adduct in normal human fibroblasts, HeLa cells, xeroderma pigmentosum group A (XPA), and group V (XPV) cells (Yang et al., 2002a; Yoon et al., 2018). Another study in XPA cells transfected with plasmids containing either the α- or the γ-OH-PdG adduct found that the α-OH-PdG adduct strongly blocked DNA synthesis and induced base-pair substitutions (predominantly G→T) with an overall miscoding frequency of 10.4–12.5%, whereas the γ-OH-PdG adduct had neither effect (Yang et al., 2002b).

In one acellular study, human DNA polymerase ι was found to replicate past γ-OH-PdG in an error-free manner (Washington et al., 2004a), whereas in another acellular study, γ-OH-PdG was found to cause a significant replication block to human polymerase η (i.e. 100 times lower efficiency than dGTP), and caused misincorporation frequencies of approximately 10^{-2} to 10^{-1} (Minko et al., 2003).

(b) Experimental systems (i) Non-human mammals in vivo

See Table 4.10.

A negative result was obtained for formation of DNA–protein crosslinks in the nasal respiratory mucosa of male Fischer 344 rats exposed to acrolein by inhalation for 6 hours; however, acrolein enhanced the level of DNA–protein crosslinks when rats were co-exposed to both acrolein and formaldehyde (Lam et al., 1985). No significant increase in the frequency of dominant lethal mutations was observed in male ICR/ Ha Swiss mice exposed to acrolein as a single intraperitoneal injection (Epstein et al., 1972). In the micronucleus assay, a significant increase of 1.4-fold in the frequency of micronucleated polychromatic erythrocytes was observed in the bone marrow of male Sprague-Dawley rats treated with acrolein at 5 mg/kg bw per day by gavage, six times per week, for 30 days (Aydın et al., 2018). There was no dose-dependent increase in the frequency of micronucleated normochromatic erythrocytes in male and female B6C3F_1 mice exposed to acrolein at 10 mg/kg bw per day by gavage for 14 weeks. However, a significant increase of 2-

fold in the frequency of micronucleus formation was observed in the female mice at 5 mg/kg bw per day (Irwin, 2006).

(ii) Non-human mammalian cells in vitro

See Table 4.11.

An increase in the frequency of DNA strand breaks was observed via the alkaline elution assay in Chinese hamster ovary (K1) cells (Deaton et

Table 4.10 Genetic and related effects of acrolein in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
DNA–protein crosslinks	Rat, F344 (M)	Nasal respiratory mucosa	–	1.0 mg/m^3 [2 ppm]	Inhalation, 6 h	Acrolein enhanced the level of DNA–protein crosslinks when rats were co-exposed to both acrolein and formaldehyde.	Lam et al. (1985)
Dominant lethal mutation	Mouse, ICR/Ha Swiss (M)	Early fetal death/implants	–	2.2 mg/kg bw	Intraperitoneal 1×		Epstein et al. (1972)
Micronucleus formation	Rat, SprageDawley (M)	Bone marrow (polychromatic erythrocytes)	+	5 mg/kg bw per day	Gavage, 6×/wk for 30 days; killed at day 30.	Significant increase but only 1.4-fold control value; single dose tested.	Aydın et al. (2018)
Micronucleus formation	Mouse, B6C3F$_1$ (M)	Blood (normochromatic erythrocytes)	–	10 mg/kg bw	Gavage daily, 5×/wk for 14 wk		Irwin (2006)
Micronucleus formation	Mouse, B6C3F$_1$ (F)	Blood (normochromatic erythrocytes)	–	10 mg/kg bw	Gavage daily 5×/wk for 14 wk	Positive at a single dose (2-fold, 5 mg/kg bw per day) but no dose trend; no analysis of target tissue exposure was reported.	Irwin (2006)

bw, body weight; d, day; F, female; h, hour; HID, highest ineffective dose; lowest effective dose; M, male; NT, not tested; ppm, parts per million; wk, week.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive/negative in a study of limited quality.

Table 4.11 Genetic and related effects of acrolein in non-human mammalian cells in vitro

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
DNA strand breaks (alkaline elution)	Chinese hamster ovary K1	+	NT	0.022 mM [1.2 µg/mL]		Deaton et al. (1993)
DNA strand breaks (alkaline elution)	Mouse, leukaemia L1210	(+)	NT	NR	Concentration at which a positive response was observed caused substantial cytotoxicity.	Eder et al. (1993)
DNA damage (alkaline comet assay)	Mouse, Leydig cells TM3	+	NT	7.4 µM [0.4 µg/mL]	LEC was the lowest concentration tested; LEC reported was for tail% DNA. LEC for OTM and tail length was 13.6 µM.	Yildizbayrak et al. (2020)

DNA damage (alkaline comet assay)	Rat, primary hepatocytes	(–)	NT	44.1 mM [2500 µg/mL]	Concentrations tested induced excessive cytotoxicity.	Kuchenmeister et al. (1998)
DNA and/or protein crosslinks (alkaline comet assay)	Rat, primary hepatocytes	(+)	NT	11 mM [616 µg/mL]	94% of cells had condensed spot in middle of cell characteristic of DNA and/or protein crosslinks; concentrations tested induced excessive cytotoxicity.	Kuchenmeister et al. (1998)
DNA–protein crosslinks	African green monkey kidney cell, CV-1	(+)	NT	0.5 mM [28 µg/mL]	No cytotoxicity assessment.	Permana & Snapka (1994)
DNA–protein crosslinks	Rat, nasal mucosal cells	(+)	NT	3 mM [168 µg/mL]	No cytotoxicity assessment.	Lam et al. (1985)
Gene mutation (*Hprt*)	Chinese hamster lung fibroblasts V79	+	NT	1 µM [0.056 µg/mL]		Smith et al. (1990b)
Gene mutation (*Hprt*)	Chinese hamster lung fibroblasts V79	+	NT	20 µM [1 µg/mL]	Only concentration tested.	Gardner et al. (2004)
Gene mutation (*Hprt*)	Chinese hamster lung fibroblasts V79 expressing rat AKR7A1	–	NT	20 µM [1 µg/mL]	Only concentration tested.	Gardner et al. (2004)
Gene mutation (*Hprt*)	Chinese hamster ovary (CHO)	+	NT	30 µM [1.7 µg/mL]	Control value not explicitly stated however response appears to be robust.	Cai et al. (1999)
Gene mutation (*Hprt*)	Chinese hamster ovary (CHO)	–	(+)	89 µM [5 µg/mL]	Elevated mutant frequencies observed at some concentrations but no clear concentration–response relationship.	Parent et al. (1991b)
Gene mutation (*Hprt*)	Chinese hamster ovary (CHO)	–	NT	100 µM [5.6 µg/mL]	No methods reported; HIC was cytotoxic.	Foiles et al. (1990)

Table 4.11 (continued)

End-point	Species, tissue, cell line	Results[a]		Concentration (LEC or HIC)	Comments	
		Without metabolic activation	With metabolic activation			
Gene mutation ($Tk^{+/-}$)	Mouse, lymphoma L5178Y/$Tk^{+/-}$	+	NT	10 µM [0.56 µg/mL]		Demir et al. (2011)
Gene mutation (*cII*)	Mouse, embryonic fibroblasts from BigBlue TGR mouse	–	NT	100 µM [5.6 µg/mL]		Kim et al. (2007)
Chromosomal aberrations	Chinese hamster ovary (CHO)	–	NT	10 µM [0.56 µg/mL]	Only concentration tested that was nontoxic.	Au et al. (1980)

						Reference
Chromosomal aberrations	Chinese hamster ovary (CHO)	–	–	17.9 µM [1 µg/mL]		Galloway et al. (1987)
Sister-chromatid exchange	Chinese hamster ovary (CHO)	+	–	10 µM [0.56 µg/mL]		Au et al. (1980)
Sister-chromatid exchange	Chinese hamster ovary (CHO)	+	–	17.9 µM [1 µg/mL]	Reported as a weak positive.	Galloway et al. (1987)

AKR, aldo-keto reductase; Hprt, hypoxanthine-guanine phosphoribosyltransferase; HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested; OTM, olive tail moment; Tk, thymidine kinase.

[a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive/negative in a study of limited quality.

al., 1993) and mouse leukaemia (L1210) cells, although the latter study noted that the tested dose caused substantial toxicity (Eder et al., 1993). An alkaline comet assay in mouse Leydig cells gave a positive result for DNA damage (i.e. comet tail intensity) at the lowest concentration tested (i.e. 7.4 µM) (Yildizbayrak et al., 2020). At a dose that was higher by nearly 6000-fold (i.e. 44.1 mM), an alkaline comet assay in rat primary hepatocytes gave a negative response when cells were analysed for comet tail length/intensity. However, 94% of cells had a condensed nucleus characteristic of compounds that cause DNA and/or protein crosslinks (Kuchenmeister et al., 1998). Acrolein-induced DNA–protein crosslinks were also observed in African green monkey kidney cells (CV-1) (Permana & Snapka, 1994) and in rat nasal mucosal cells (Lam et al., 1985). [The Working Group noted that these experiments were carried out with acrolein at high concentrations that either induced excessive cytotoxicity (Kuchenmeister et al., 1998; Lam et al., 1985), or at which cytotoxicity was not assessed (Permana & Snapka, 1994).]

Acrolein was found to be mutagenic, with a positive result for *Hprt* mutations in two assays in Chinese hamster lung fibroblasts (V79) (Smith et al., 1990b; Gardner et al., 2004). However, a negative result was obtained in V79 cells that express the rat aldo-keto reductase enzyme AKR7A1 (Gardner et al., 2004). [The Working Group noted that AKR7A1 catalyses the reduction of acrolein to alcohols, indicating that rat AKR7A1 protects against acrolein-induced mutagenicity (see Section 4.1.2b).] The frequency of acrolein-induced *Hprt* mutants was also analysed in Chinese hamster ovary cells, with one study reporting a positive response at 30 µM (Cai et al., 1999). Another study reported elevated mutant frequencies at some doses, but with no clear dose–response relationship when acrolein was tested at up to 89 µM with and without metabolic activation (rat liver S9) (Parent et al., 1991b). An additional study reported negative results for *Hprt* mutations in Chinese hamster ovary cells (Foiles et al., 1990). A significant increase in the frequency of $Tk^{+/-}$ mutations was reported in mouse lymphoma (L5178Y) cells (Demir et al., 2011), but a negative response was reported for the induction of *cII* mutations in mouse embryonic fibroblasts from the Big Blue mouse (Kim et al., 2007). Chromosomal aberrations and sister-chromatid exchanges were both assessed in two different studies in Chinese hamster ovary cells, with both reporting a negative response for chromosomal aberrations, and a positive result for sister-chromatid exchanges (Au et al., 1980; Galloway et al., 1987).

Using shuttle vectors containing either adduct isomer, the α- and γ-OH-PdG adducts were found to be mutagenic in African green monkey kidney (COS-7) cells, with a similar percentage mutagenicity observed for both isomers (i.e. 8.3% and 7.4%, respectively) (Sanchez et al., 2003). The γ-OH-PdG adduct was found to be significantly mutagenic in plasmid-transfected COS-7 cells; primarily transversions were observed, but also transition mutations (Kanuri et al., 2002).

(iii) Non-mammalian experimental systems

See Table 4.12.

In *Drosophila melanogaster*, largely positive results were obtained in SMART eye and wing spot mutation studies after exposure to acrolein in feed (Sierra et al., 1991; Demir et al., 2013; Vogel & Nivard, 1993), or via inhalation (Vogel & Nivard, 1993). Acrolein was also tested in *Drosophila* for the ability to induce sex-linked

recessive lethal mutations, with negative results for all four feeding assays, but when acrolein was administered by injection, two out of three assays gave positive results (Zimmering et al., 1985, 1989; Sierra et al., 1991; Barros, et al., 1994a, b). Acrolein did not induce sex chromosome loss in *Drosophila* when administered either by injection or via feed (Sierra et al., 1991).

In *Saccharomyces cerevisiae,* acrolein did not induce DNA strand breaks and interstrand

Table 4.12 Genetic and related effects of acrolein in non-mammalian experimental systems

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Drosophila melanogaster	SMART wing spot mutation	+	NA	10 mM [560 µg/mL] (feed)		Sierra et al. (1991)
Drosophila melanogaster	SMART wing spot mutation	+	NA	10 mM [560 µg/mL] (feed)		Demir et al. (2013)
Drosophila melanogaster	SMART eye spot mutation	+	NA	5 mM [280 µg/mL] (feed)		Sierra et al. (1991)
Drosophila melanogaster	SMART eye spot mutation	+	NA	8.9 mM, 500 ppm [500 µg/mL] (inhalation)	Vehicle was ethanol.	Vogel &Nivard (1993)
Drosophila melanogaster	SMART eye spot mutation	–	NA	80 mM [4480 µg/mL] (feed)	Vehicle was ethanol.	Vogel &Nivard (1993)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	5 mM [280 µg/mL] (feed)		Sierra et al. (1991)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	10 mM [560 µg/mL] (feed)		Barros et al. (1994a, b)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	14.3 mM [800 µg/mL] (feed)		Zimmering et al. (1989)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	53.6 mM [3000 µg/mL] (feed)		Zimmering et al. (1985)
Drosophila melanogaster	Sex-linked recessive lethal mutation	–	NA	3.6 mM [200 µg/mL] (injection)		Zimmering et al. (1985)
Drosophila melanogaster	Sex-linked recessive lethal mutation	+	NA	3 mM [168 µg/mL] (injection)		Sierra et al. (1991)
Drosophila melanogaster	Sex-linked recessive lethal mutation	+	NA	3 mM [168 µg/mL] (injection)		Barros et al. (1994a, b)
Drosophila melanogaster	Sex chromosome loss	–	NA	5 mM [280 µg/mL] (feed)		Sierra et al. (1991)
Drosophila melanogaster	Sex chromosome loss	–	NA	5 mM [280 µg/mL] (injection)		Sierra et al. (1991)
Saccharomyces cerevisiae	DNA strand breaks and interstrand crosslinks	–	NT	0.1 mM [5.6 µg/mL]		Fleer & Brendel (1982)
Saccharomyces	Reverse mutation	–	NT	100 µg/mL		Izard (1973) *cerevisiae* S211 and

						Reference
S138						
		activation	**activation**			
Salmonella typhimurium TA1535 pSK 1002	DNA damage SOS (*umu*) induction assay	–	NT	5.6 µg/mL [0.1 mM]		Benamira & Marnett (1992)
Salmonella typhimurium TA1535	Reverse mutation	–	(+)	0.005 µg/mL		Hales (1982)
Salmonella typhimurium TA1535	Reverse mutation	–	–	13 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA1535	Reverse mutation	–	–	17 µg/plate		Florin et al. (1980)
Salmonella typhimurium TA1535	Reverse mutation	–	–	17 µg/plate		Florin et al. (1980)
Salmonella typhimurium TA1535,	Reverse mutation	–	–	28 µg/plate		Loquet et al. (1981)
Salmonella typhimurium TA1535	Reverse mutation	–	–	43 µg/plate		Lijinsky & Andrews (1980)
Salmonella typhimurium TA1535 (vapour protocol)	Reverse mutation	–	–	0.5 mL/chamber		Irwin (2006)
Salmonella typhimurium TA1535 (preincubation)	Reverse mutation	–	–	16 µg/plate		Irwin (2006)
Salmonella typhimurium TA100	Reverse mutation	+	+	10 µg/plate	Inconsistent dose–response relationship.	Parent et al. (1996b)
Salmonella typhimurium TA100	Reverse mutation	–	–	17 µg/plate		Florin et al. (1980)
Salmonella typhimurium TA100	Reverse mutation	–	–	28 µg/plate		Loquet et al. (1981)

Table 4.12 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			

Table 4.12 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic activation	With metabolic activation			
Salmonella typhimurium TA100	Reverse mutation	–	(+)	38 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA100	Reverse mutation	–	–	43 µg/plate		Lijinsky & Andrews (1980)
Salmonella typhimurium TA100	Reverse mutation	+	NT	224 µg/mL		Foiles et al. (1989)
Salmonella typhimurium TA100	Reverse mutation	–	–	NR		Basu & Marnett (1984)
Salmonella typhimurium TA100	Reverse mutation	+	Toxic	NR		Eder et al. (1993)
Salmonella typhimurium TA100	Reverse mutation	+	NT	NR	Solvent NR.	Khudoley et al. (1987)
Salmonella typhimurium TA100	Reverse mutation	+	NT	NR	Solvent NR.	Eder et al. (1990)
Salmonella typhimurium TA100 (vapour protocol)	Reverse mutation	–	–	1 mL/chamber		Irwin (2006)
Salmonella typhimurium TA100 (pre-incubation)	Reverse mutation	–	–	6 µg/plate without activation, 16 µg/plate with activation	Slight toxicity at highest dose without activation.	Irwin (2006)
Salmonella typhimurium TA100 (liquid suspension)	Reverse mutation	+	–	2.1 µg/mL –S9; HIC, 4.2 µg/mL +S9		Lutz et al. (1982)
Salmonella typhimurium TA104	Reverse mutation	+	NT	224 µg/mL		Foiles et al. (1989)

						Reference
Salmonella typhimurium TA104	Reverse mutation	+	NT	14 µg/plate		Marnett et al. (1985)
Salmonella typhimurium TA1537	Reverse mutation	–	–	13 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA1537	Reverse mutation	–	–	17 µg/plate		Florin et al. (1980)
		activation	**activation**			
Salmonella typhimurium TA1537	Reverse mutation	–	–	43 µg/plate		Lijinsky & Andrews (1980)
Salmonella typhimurium TA1538	Reverse mutation	–	–	43 µg/plate		Lijinsky & Andrews (1980)
Salmonella typhimurium TA1538 (preincubation)	Reverse mutation	NT	–	16 µg/plate		Irwin (2006)
Salmonella typhimurium TA97 (vapour protocol)	Reverse mutation	–	–	0.5 mL/chamber		Irwin (2006)
Salmonella typhimurium TA98	Reverse mutation	+	–	8.4 µg/plate		Lijinsky & Andrews (1980)
Salmonella typhimurium TA98	Reverse mutation	(+)	+	10 µg/plate	Weak positive (2-fold). Inconsistent dose–response relationship.	Parent et al. (1996b)
Salmonella typhimurium TA98	Reverse mutation	–	–	13 µg/mL		Haworth et al. (1983)
Salmonella typhimurium TA98	Reverse mutation	–	–	17 µg/plate		Florin et al. (1980)
Salmonella typhimurium TA98	Reverse mutation	–	–	28 µg/plate		Loquet et al. (1981)
Salmonella typhimurium TA98	Reverse mutation	–	–	NR		Basu & Marnett (1984)
Salmonella typhimurium TA98	Reverse mutation	+	+	NR		Claxton (1985)

Table 4.12 (continued)

Test system (species, strain)	End-point	Results[a]		Concentration (LEC or HIC)	Comments	Reference
		Without metabolic	With metabolic			
Salmonella typhimurium TA98	Reverse mutation	+	NT	NR	Solvent NR.	Khudoley et al. (1987)
Salmonella typhimurium TA98 (vapour protocol)	Reverse mutation	–	–	1 mL/chamber		Irwin (2006)

Table 4.12 (continued)

Test system (species, strain)	End-point (LEC or HIC)	Results[a] metabolic activation	metabolic activation	Concentration	Comments	
		Without	With			
Salmonella typhimurium TA98 (pre-incubation)	Reverse mutation	–	–	16 µg/plate		Irwin (2006)
Salmonella typhimurium TA102	Reverse mutation	–	NT	NR		Marnett et al. (1985)
*Salmonella typhimurium hisD3052/nop*KM101	Reverse mutation	–	–	NR		Basu & Marnett (1984)
Escherichia coli HB101pUC13	DNA–histone crosslinks	+	NT	8.4 µg/mL		Kuykendall & Bogdanffy (1992)
*Escherichia coli PQ*37, SOS chromotest	DNA damage	+	NT	NR	Solvent NR.	Eder et al. (1990)
*Escherichia coli PQ*37, SOS chromotest	DNA damage	+	NT	NR		Eder et al. (1993)
*Escherichia coli PQ*37, SOS chromotest	DNA damage	–	NT	NR		Eder & Deininger (2002)

						Reference
*Escherichia coli PQ*37, SOS chromotest	DNA damage	+	NT	NR	Ethanol used as solvent.	Eder & Deininger (2002)
Escherichia coli AB1157	Reverse mutation	+	NT	56 µg/mL [1 mM]		Nunoshiba &Yamamoto (1999)
Escherichia coli JTG10	Reverse mutation	+	NT	56 µg/mL [1 mM]	Strain lacks glutathione; mutation frequency higher than in AB1157 strain.	Nunoshiba &Yamamoto (1999)
Escherichia coli WP2 (*uvr*A)	Reverse mutation	–	(+)	50 µg/plate	Weak positive (2-fold); inconsistent dose–response relationship.	Parent et al. (1996b)
Escherichia coli WP2 (*uvr*A)	Reverse mutation	(+)	NT	NR	Reported as weak mutagenicity.	Hemminki et al. (1980)
		activation	**activation**			
Plasmid pIF101 (acellular)	Reverse mutation A→C (*lacZ*)	+	NT	0.010 mM [0.56 µg/mL]	Plasmids were reacted with acrolein at 37 °C then transfected into AlkB proficient and deficient *E. coli* for mutation scoring.	Dylewska et al. (2017)
Plasmid pIF105 (acellular)	Reverse mutation A→T (*lacZ*)	+	NT	0.005 mM [0.28 µg/mL]	Plasmids were reacted with acrolein at 37 °C then transfected into AlkB proficient and deficient *E. coli* for mutation scoring.	Dylewska et al. (2017)
Plasmid pIF106 (acellular)	Forward mutation A→G (*lacZ*)	+	NT	0.005 mM [0.28 µg/mL]	Plasmid were reacted with acrolein at 37 °C then transfected into AlkB proficient and deficient *E. coli* for mutation scoring.	Dylewska et al. (2017)
Calf thymus DNA (acellular)	DNA damage (fluorescent screen for changes in DNA melting and annealing behaviour)	+	NA	100 mM [5600 µg/mL]		Kailasam & Rogers (2007)

AlkB, alpha-ketoglutarate B-dependent dioxygenase; A, adenine; C, cytosine; G, guanine; T, thymine; HIC, highest ineffective concentration; LEC, lowest effective concentration; NA, not applicable; NR, not reported; NT, not tested; ppm, parts per million; S9, 9000 × *g* supernatant. [a] +, positive; –, negative; +/–, equivocal (variable response in several experiments within an adequate study); (+) or (–), positive/negative in a study of limited quality.

crosslinks in one study (Fleer & Brendel, 1982), or reverse mutations in another study (Izard, 1973).

Acrolein has been evaluated in multiple assays in several *Salmonella* tester strains sensitive to base-pair substitutions (i.e. TA1535, TA100, TA104) and frameshift mutations (i.e. TA1537, TA1538, TA97, and TA98). However, only one assay was carried out in TA102, a strain that is used specifically for the detection of crosslinking agents. In the TA1535 base-pair substitution strain, a negative response was observed in the SOS induction assay (Benamira & Marnett, 1992) and the results were negative for reverse mutation (Hales, 1982; Haworth et al., 1983; Florin et al., 1980; Loquet et al., 1981; Lijinsky & Andrews, 1980; Irwin, 2006). In the TA100 strain, the results were mixed positive (Parent et al., 1996b; Haworth et al., 1983; Foiles et al., 1989; Eder et al., 1993; Khudoley et al., 1987; Eder et al., 1990; Lutz et al., 1982) or negative (Florin et al., 1980; Loquet et al., 1981; Lijinsky & Andrews, 1980; Basu & Marnett, 1984; Irwin, 2006), with the positive responses mainly occurring without metabolic activation (rat liver S9). Notably, only one pre-incubation assay was carried out with TA100 and a negative result was reported (Irwin, 2006). However, a positive result was obtained in TA100 when acrolein was tested in a liquid suspension assay (Lutz et al., 1982). Of the two assays reported in TA104, both gave positive results without metabolic activation (S9) (Foiles et al., 1989; Marnett et al., 1985). In the frameshift strains, all three TA1537 assays gave negative results (Haworth et al., 1983; Florin et al., 1980; Lijinsky & Andrews, 1980), both results in TA1538 were negative (Lijinsky & Andrews, 1980; Irwin, 2006), the one TA97 experiment gave negative results (using the vapour protocol) (Irwin, 2006), and four positive results (Lijinsky & Andrews, 1980; Parent et al.,1996b; Claxton, 1985; Khudoley et al., 1987) and six negative results were reported in TA98 (Haworth et al., 1983; Florin et al., 1980; Loquet et al., 1981; Basu Acrolein

& Marnett,1984; Irwin, 2006). A negative result was obtained in the crosslink strain TA102, but the highest tested dose was not reported (Marnett et al., 1985). The more sensitive pre-incubation version of the Ames assay was not carried out with any frameshift strains without metabolic activation.

In *E. coli*, a positive result for DNA-histone crosslinks was reported (Kuykendall & Bogdanffy, 1992). Several studies reported positive results for acrolein in the SOS chromotest (Eder et al., 1990, 1993; Eder & Deininger, 2002), whereas a negative response was observed in the SOS chromotest when DMSO was used as the solvent. Additional studies in *E. coli* reported positive results for reverse mutations (Nunoshiba & Yamamoto, 1999; Hemminki et al., 1980), as well as one experiment with a negative result without metabolic activation and a weak positive result with metabolic activation (Parent et al., 1996b). In one study, three different plasmids containing different mutational targets in the *lacZ* gene were reacted with acrolein and then transfected into *E. coli* for mutant frequency assessment; positive results were observed for all three mutation types (i.e. A→C, A→T, and A→G) (Dylewska et al., 2017). An increase in DNA damage, assessed via a fluorescence-based screen quantifying changes in DNA melting/annealing behaviour, was observed in calf thymus DNA reacted with acrolein in an acellular system (Kailasam & Rogers, 2007).

In the study of Kanuri et al. (2002), described above, the γ-OH-PdG adduct was found to be significantly less mutagenic in *E. coli* than in COS-7 cells transfected with the same plasmid (i.e. 0.96% mutations in *E. coli* versus 6.3–7.4% in COS-7). In *E. coli*, a study by Yang et al. (2001) found that DNA polymerase III catalysed

translesion synthesis across the γ-OH-PdG adduct in an error-free manner, but that DNA polymerase I did so in an error-prone manner, with incorporation frequencies opposite the γ-OH-PdG adduct of 93% for deoxyadenosine triphosphate (dATP), 88% for deoxyguanosine triphosphate

(dGTP), 7% for deoxycytidine triphosphate (dCTP), and 5% for deoxythymidine triphosphate (dTTP). Additionally, γ-OH-PdG was found to inhibit DNA synthesis in *E. coli* (Yang et al., 2001). In another study with *E. coli* transformed with bacteriophage vectors containing an 8-hydroxy-1,N^2-propano-2′-deoxyguanosine (OH-PdG) adduct (no stereochemistry specified), the correct base was inserted under all conditions (VanderVeen et al., 2001).

In an acellular study, γ-OH-PdG was found to cause a significant replication block to yeast polymerase η (i.e. 190 times lower efficiency than deoxyguanosine), although incorporation opposite the adduct was relatively accurate (Minko et al., 2003). In an acellular study, both α- and γ-OH-PdG caused a significant replication block to yeast DNA pol η, with α-OH-PdG being a significantly stronger blocking lesion as pairing with dCTP was strongly inhibited (Sanchez et al., 2003). When assayed for nucleotide incorporation frequency, dCTP was primarily incorporated across from both lesions, but extension with other deoxynucleoside triphosphates (dNTPs) was also observed at almost identical ratios for both stereoisomers (Sanchez et al., 2003). In other acellular studies, yeast Rev1 was demonstrated to replicate past γ-OH-PdG in an error-free manner (Washington et al., 2004b; Nair et al., 2008).

In an acellular study with bacteriophage DNA polymerase $T7^-$ and HIV-1 reverse transcriptase, OH-PdG adducts (stereochemistry not specified) were found to be miscoding, with dATP being preferentially incorporated instead of dCTP (Zang et al., 2005). In another acellular study, *Sulfolobus solfataricus* Dpo4, the prototypic Y-family DNA polymerase, was capable of bypassing γ-OH-PdG adducts in a primarily error-free manner (Shanmugam et al., 2013).

4.2.3 Alters DNA repair

(a) Humans

No studies on exposed humans were available to the Working Group.

Acrolein was found to inhibit the DNA repair enzyme O^6-methylguanine-DNA methyltransferase (MGMT) in human bronchial fibroblasts in two studies (Krokan et al., 1985; Grafström et al., 1986). [The Working Group noted that, as aldehydes are highly reactive towards thiols, this inhibition is probably due to acrolein reacting with and inhibiting the methyl-acceptor cysteine residue in MGMT (Grafström et al., 1986).] In a study using human normal skin fibroblasts and DNA-repair deficient XPA fibroblasts, it was concluded that acrolein inhibited nucleotide excision repair since there was an accumulation of DNA single-strand breaks in acrolein-treated normal skin fibroblasts, which only increased after a recovery period in fresh medium (Dypbukt et al., 1993). Indeed, several studies found that acrolein treatment causes concentration-dependent inhibition of nucleotide excision repair in primary normal human lung fibroblasts (NHLFs) (Feng et al., 2006; Wang et al., 2012), primary normal human bronchial epithelial cells (NHBEs), human lung adenocarcinoma cells (A549s) (Wang et al., 2012), and in immortalized human urothelial (UROtsa) cells (Lee et al., 2014). Acrolein also causes concentration-dependent inhibition of base excision repair in NHBEs, NHLFs, A549s (Wang et al., 2012), and UROtsa cells (Lee et al., 2014), and of mismatch repair in HeLa (epithelial adenocarcinoma) cells (Wang et al., 2012). A subgenotoxic concentration of acrolein (i.e. 50 μM) has also been demonstrated to inhibit the repair of gamma-

irradiation–induced DNA damage in human B-lymphoid cells, and the repair inhibition increased with acrolein dose (Yang et al., 1999b).

Acrolein treatment reduced the expression level of certain DNA repair genes in A549 cells (Sarkar, 2019). Other studies did not find an effect on gene expression but showed that acrolein reacts rapidly with and directly inhibits DNA repair proteins (Wang et al., 2012; Lee et al., 2014). More specifically, in NHBE, NHLF, A549, and UROtsa cells, acrolein treatment caused a dose-dependent reduction in the expression of repair proteins (i.e. XPA, XPC, human 8-oxo guanine DNA glycosylase (hOGG1), PMS2, and MLH1) that are crucial for nucleotide excision repair, base excision repair, and mismatch repair (Wang et al., 2012; Lee et al., 2014). Pre-treatment of cells with proteasome inhibitors reduced the level of protein degradation, and pre-treatment with an autophagy inhibitor caused partial reduction in the degradation of DNA repair proteins; however, repair capacity was not rescued (Wang et al., 2012; Lee et al., 2014). [The Working Group noted that these results indicate that acrolein protein modification alone is capable of causing DNA-repair protein dysfunction, and that this modification results in DNA-repair protein degradation both by proteasomes and by autophagy; see also Section 4.2.1(a).]

Wang et al. (2012) found that both α-OH-PdG and γ-OH-PdG adducts were not efficiently repaired in acrolein-exposed NHBEs and NHLFs. A study using HeLa whole-cell extracts found that α-OH-PdG and γ-OH-PdG adducts were not efficiently removed by base excision repair (Yang et al., 2002b). In a study of nuclear extracts from unexposed human normal skin fibroblasts and DNA-repair deficient human XPA cells transfected with acrolein-treated plasmids, it was found that acrolein–dG adducts (i.e. a mixture of α-OH-PdG and γ-OH-PdG) are substrates for nucleotide excision repair proteins, but are repaired at a much slower rate than other similar adducts, and that this is probably because of poor recognition and/or excision of the lesions in DNA (Choudhury et al., 2013).

(b) Experimental systems

No data were available to the Working Group.

4.2.4 Induces oxidative stress

(a) Humans

No in vivo data were available to the Working Group.

In vitro studies using human retinal pigmented epithelial and lung fibroblast cell lines have demonstrated that acrolein induces a variety of biochemical changes, including decreased nuclear protein levels of nuclear factor erythroid 2-related factor 2 (NRF2; [NFE2L2, nuclear factor, erythroid 2-like 2]) (retinal pigmented epithelial cells only), decreased superoxide dismutase and glutathione peroxidase activities, lowered cellular GSH levels, and increased generation of reactive oxygen species (ROS) and protein carbonyls (Jia et al., 2007, 2009; Li et al., 2008a). Haem oxygenase-1 (*HO-1*) gene expression is induced in human bronchial epithelial cells (HBE1 cells) after acrolein exposure, and acrolein-induced HO-1 protein levels are attenuated by pan-protein kinase C (PKC) and phosphatidylinositol 3-kinase (PI3K) inhibitors (Zhang & Forman, 2008). Exposure of human cultured liver (HepG2) or retinal pigment epithelial cells to acrolein results in endoplasmic reticulum stress, mitochondrial disruption, and oxidative stress (Li et al., 2008a, b; Mohammad et al., 2012). Human primary bronchial epithelial cells exposed to acrolein

vapour (0.1 and 0.2 ppm) for 30 minutes had increased IL17 expression (Johanson et al., 2020).

(b) Experimental systems

See Table 4.13.

Multiple in vivo studies in rodents have shown that acrolein administration via multiple routes of exposure, including oral administration, inhalation, and intraperitoneal injection, results in decreased tissue GSH concentrations

Table 4.13 Effects of acrolein on markers of oxidative stress in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
GSH SOD, GPx	Rat, Wistar (M)	Liver	↓	2.5 mg/kg bw per day	Gavage, 45 days		Arumugam et al. (1997)
PCO, TBARS	Rat, Sprague Dawley (M)	Spleen, thymus, PMN	↑	5 mg/kg bw per day	Oral, 6 days/wk for 30 days		Aydın et al. (2018)
GSH		Spleen, thymus, PMN	↓				
GPx NPSH GST	Rat, Wistar (M)	Nasal cavity	– ↑ ↓	0.25 ppm 0.67 ppm 1.4 ppm	Inhalation (noseonly), 6 h/day for 3 days		Cassee et al. (1996)
GSH	Rat, F344 (M)	Nasal cavity Tracheobronchial mucosa	↓	0.2 ppm 0.2 ppm	Inhalation (noseonly), 6 h		Cichocki et al. (2014)
GSH	Rat, F344 (M)	Liver	↓	89 µmol/kg (0.1 mL/100 g bw) [31.5 mg/kg]	Intraperitoneal, 1×		Cooper et al. (1992)
GSH	Mouse (F)	Liver	↓	4.5 mg/kg	Intraperitoneal, 1×	Qualitative statistics only.	Gurtoo et al. (1981)
GSH 8-OHdG	Mouse, C57BL/6 (M)	Lung	↓ ↑	10 ppm 10 ppm	Inhalation (wholebody), 12 h		Kim et al. (2018)
Lipid peroxides, TBARS	Mouse, *ApoE*$^{-/-}$ (M)	Serum, peritoneal macrophages	↑	3 mg/kg per day	Oral (drinkingwater), 1 mo	Genetic background not provided.	Rom et al. (2017)
ROS	Mouse, C57BL/6 (F)	Lung	↑	5 µmol/kg bw [0.06 mg/kg bw]	Intranasal, 1×	Elevated at day 7 postexposure only (not day 28).	Sun et al. (2014)
8-Isoprostane Total antioxidant capacity	Mouse, $gp^{91phox(-/-)}$ (M)	Liver	↑ ↓	0.5 µg/kg per day	Intraperitoneal, 7 days		Yousefipour et al. (2013)
8-isoprostane Total antioxidant capacity	Mouse, $gp^{91phox(+/+)}$ (M)	Liver	↑ ↓	0.5 µg/kg per day	Intraperitoneal, 7 days		Yousefipour et al. (2013)

Table 4.13 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
8-isoprostane Total antioxidant capacity	Mouse, $gp^{91phox(-/-)}$ (M)	Liver	↑ ↓	0.5 mg/kg per day	Intraperitoneal, 7 days		Yousefipour et al. (2017)
8-isoprostane Total antioxidant capacity	Mouse, $gp^{91phox(+/+)}$ (M)	Liver	↑ ↓	0.5 mg/kg per day	Intraperitoneal, 7 days		Yousefipour et al. (2017)

bw, body weight; F, female; h, hour; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione-*S*-transferase; HID, highest ineffective dose; LED, lowest effective dose; M, male; mo, month; NPSH, nonprotein sulfydryl groups; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; PCO, protein carbonyls; PMN, polymorphonuclear leukocytes; ppm, parts per million; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; wk, week. [a]↑, increase; ↓, decrease;–, no effect.

(Arumugam et al., 1997; Aydın et al., 2018; Cassee et al., 1996; Cichocki et al., 2014; Cooper et al., 1992; Gurtoo et al., 1981; Kim et al., 2018). Oral and parenteral rodent studies have shown evidence of lipid peroxidation or protein carbonyl production after short-term (up to 1 month) exposure (Aydın et al., 2018; Rom et al., 2017; Sun et al., 2014; Yousefipour et al., 2013, 2017). A significant increase in levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) has been reported in the mouse lung after inhalation of acrolein (Kim et al., 2018). Intraperitoneal exposure of wildtype and *gp91*^{phox} knockout mice with acrolein at 0.5 µg/kg provided evidence that increased oxygen radical generation occurs via NAD(P)H oxidase activation (Yousefipour et al., 2013). In vitro studies with bovine pulmonary artery endothelial cells have likewise shown that acrolein causes increased generation of oxygen radicals by NAD(P)H oxidase activation (Jaimes et al., 2004).

4.2.5 Is immunosuppressive

(a) Humans

No data in exposed humans were available to the Working Group.

In vitro studies show that acrolein exposure is associated with apoptosis and necrosis in human alveolar macrophages and with inhibition of IL1β, TNF, and IL12 release from cells (Li et al., 1997). Human T cells treated with acrolein also demonstrated suppressed cytokine production and T-cell responses (Lambert et al., 2005). Human alveolar macrophages infected with *Mycobacterium tuberculosis* and exposed to acrolein have a reduced ability to clear these bacteria (Shang et al., 2011).

(b) Experimental systems

See Table 4.14.

Multiple studies in rodents have assessed whether acrolein inhalation alters bacterial-induced mortality, bactericidal activity, or innate immune function (Aranyi et al., 1986; Astry & Jakab, 1983; Jakab, 1993; Danyal et al., 2016; Hristova et al., 2012; Leach et al., 1987). Most of these studies have used short-term exposures (e.g. < 10 days).

Splenic cells isolated from naïve female C57/BL6 mice that were subsequently exposed to acrolein exhibited decreased T- and B-cell proliferation (Poirier et al., 2002). Immunosuppression by acrolein has been attributed to GSH depletion and interactions with redox-sensitive signalling pathways such as NF-κB or JNK (Lambert et al., 2005; Valacchi et al., 2005; Kasahara et al., 2008).

4.2.6 Induces chronic inflammation

(a) Humans

No data were available to the Working Group.

(b) Experimental systems

See Table 4.15.

Chronic inhalation (6 hours per day, 5 days per week, for 104 weeks) of acrolein was associated with mild inflammation in the nasal respiratory epithelium in rats and mice (JBRC, 2016d, e, f; see also Section 3). A 1-year study in hamsters treated with acrolein by inhalation (7 hours per day, 5 days per week, for 52 weeks) was also associated with mild inflammation in the nasal respiratory epithelium (Feron & Kruysse, 1977; see Table 3.1). [The Working Group noted that changes in cell proliferation in response to acrolein exposure have not been evaluated in experimental systems.] Multiple studies in rodents with short-term or subchronic exposures to acrolein via inhalation have shown

that acrolein produces airway inflammation (Johanson et al., 2020; Kasahara et al., 2008; Wang et al., 2009b; Liu et al., 2009a, b; Sithu et al., 2010). Accumulation of monocytes, macrophages, and lymphocytes in the lung interstitium and mucous cell metaplasia are common features seen in many rodent inhalation studies with acrolein (Kutzman et al., 1985; Borchers et

Table 4.15 Inflammatory responses after acrolein exposure in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Respiratory epithelial inflammation	Mouse, Crj:BDF1 (M,F)	Nasal cavity	↑	1.6 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk, 104 wk		JBRC (2016a)

Table 4.14 Immunosuppression after acrolein exposure in non-human mammals in vivo

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Streptococcus zooepidemicus induced mortality	Mouse, CD_1 (F)	Lung	–	0.1 ppm	Inhalation (whole-body); 3 h	Single exposure concentration.	Aranyi et al. (1986)
Streptococcus zooepidemicus induced mortality	Mouse, CD_1 (F)	Lung	↑	0.1 ppm	Inhalation (whole-body); 3 h/day for 5 days	Single exposure concentration.	Aranyi et al. (1986)
35S-*Klebsiella pneumoniae* clearance	Mouse, CD_1 (F)	Lung	–	0.1 ppm	Inhalation (whole-body); 3 h	Single exposure concentration.	Aranyi et al. (1986)
35S-*Klebsiella pneumoniae* clearance	Mouse, CD_1 (F)	Lung	↓	0.1 ppm	Inhalation (whole-body); 3 h/day for 5 days	Single exposure concentration.	Aranyi et al. (1986)
Staphylococcus aureus clearance	Mouse, Swiss (F)	Lung	↓	3 ppm	Inhalation (whole-body); 8 h		Astry & Jakab (1983)
Staphylococcus aureus or *Proteus mirabilis* clearance	Mouse, Swiss (F)	Lung	–	2.5 ppm	Inhalation (nose-only); 4 h/day for 4 days	Single exposure concentration.	Jakab (1993)
Antigen-induced inflammation	Mouse, C57BL/6J (NR)	Lung	↓	5 ppm	Inhalation; 1 or 4 h	Single exposure concentration.	Danyal et al. (2016)
Innate macrophage function	Mouse, C57BL/6J (M)	Lung	↓	5 ppm	Inhalation (whole-body); 4 h	Single exposure concentration.	Hristova et al. (2012)
Listeria monocytogenes-induced mortality	Rat, SpragueDawley (M)	Lung	–	3 ppm	Inhalation (whole-body); 6 h/day, 5 days/wk for 3 wk	Exposure associated with nasal pathology.	Leach et al. (1987)
Antibody plaque-forming cells			–				

F, female; h, hour; HID, highest ineffective dose; LED, lowest effective dose; M, male; NR, not reported; ppm, parts per million; wk, week.

[a] ↑, increase; ↓, decrease; –, no effect.

Respiratory epithelial inflammation	Rat, F344 (M, F)	Nasal cavity	↑	2.0 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk, 104 wk	JBRC (2016d)

CxCl2, IL6, IL17β, and TNF expression	Mouse, 129S1/SvlmJ (F) Mouse, A/J (F) Mouse, BALB/cByJ (F) Mouse, C3H/HeJ (F) Mouse, C57BL/6J (F) Mouse, DBA/2J (F) Mouse, FVB/NJ (F)	Lung	↑ CxCl2: (C57BL/6J and FVB/NJ only) ↑ IL6: (129S1/SvlmJ; BALB/cByJ; C57BL/6J and A/J only) ↑ IL17β: (129S1/SvlmJ; BALB/cByJ; and C57BL/6J only)	1 ppm	Inhalation (whole-body), 6 h/day, 4–5 days/wk, 11 wk	Single exposure concentration; TNF data incompletely reported.	Johanson et al. (2020)
Cell count and cytokine level	Mouse C57BL/6J (M)	BALF	–	5 ppm	Inhalation (whole-body); 6 h/day for 3 days	Single exposure concentration.	Kasahara et al. (2008)
Total cells, macrophages, neutrophils; TNFα, CINC-1	Rat, Sprague–Dawley (M)	BALF Lung	↑ ↑	2.5 ppm 2.5 ppm	Inhalation (whole-body); 6 h/day, 7 day/wk for 2 or 4 wk	Single exposure concentration.	Wang et al. (2009b)
Macrophage, neutrophil, leukocytes, TNFα, KC (IL8 homologue)	Mouse, Kunning (M)	BALF	↑	4 ppm	Inhalation (whole-body), 6 h/day, 7 days/wk, for 21 days	Single exposure concentration.	Liu et al. (2009a)
Mucin, macrophage, neutrophil, TNFα, IL8, IL1β	Rat, Sprague-Dawley (M)	BALF	↑	3 ppm	Inhalation (whole-body), 6 h/day, 7 days/wk, 2 wk	Single exposure concentration.	Liu et al. (2009b)
TNFα, IL6, IL1β	Mouse, C57BL/6 (M)	Lung	–	5 ppm	Inhalation (whole-body), 6 h	Single exposure concentration.	Sithu et al. (2010)
TNFα, IL6, IL1β	Mouse, C57BL/6 (M)	Lung	–	1 ppm	Inhalation (whole-body), 6 h/day for 4 days	Single exposure concentration.	Sithu et al. (2010)
Wet weight, oedema	Rat, F344 (M, F)	Lung	↑	1.4 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for 13 wk		Kutzman et al. (1985)

Table 4.15 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Macrophages, neutrophils	Mouse, FVB/N, (M)	BALF	↑	3 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk, 3 wk	Single exposure concentration.	Borchers et al. (1999)

Macrophages	Mouse, C57BL/6J, (NR) Mouse, MME (+/+), (NR) Mouse, MME (−/−), (NR)	BALF	↑ ↑ ↑	3 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk, 3 wk	Single exposure concentration.	Borchers et al. (1999)
CD8+ lymphocytes, macrophage	Mouse, C57BL/6J (NR)	Lung	↑	2 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for up to 12 wk	Single exposure concentration.	Borchers et al. (2007)
CD8+ lymphocytes, macrophage	Mouse, $Cd8^{-/-}$ (NR)	Lung	↑	2 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for up to 12 wk	Single exposure concentration.	Borchers et al. (2007)
Mucous cell metaplasia Macrophage accumulation	Mouse, C57BL/6J (F)	Lung	↑ ↑	2 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for up to 4 wk	Single exposure concentration.	Borchers et al. (2008)
Mucous cell metaplasia Macrophage accumulation	Mouse, γδ T-cell deficient (F)	Lung	↑ ↑	2 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for up to 4 wk	Single exposure concentration.	Borchers et al. (2008)
Mucous cell metaplasia Macrophage accumulation	Mouse, αβ T-cell deficient (F)	Lung	↑ –	2 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for up to 4 wk	Single exposure concentration.	Borchers et al. (2008)
Interstitial inflammation, neutrophil infiltration, congestion, and oedema	Mouse, C57BL/6 (M)	Lung	↑	10 ppm	Inhalation (whole-body), 12 h	Single exposure concentration.	Kim et al. (2018)
Nasal epithelial inflammation and metaplasia	Rat, Wistar (M, F) Hamster, Golden Syrian (M, F) Rabbit, Dutch (M, F)	Nasal cavity	↑ ↑ ↑	0.4 ppm 1.4 ppm 4.9 ppm	Inhalation (whole-body), 6 h/day, 5 days/wk for 13 wk		Feron et al. (1978)
Olfactory epithelial inflammation	Rat, F344 (M)	Nasal cavity	↑	1.8 ppm	Inhalation (whole-body), 6 h/day 5 days/wk for 13 wk		Dorman et al. (2008)

Table 4.15 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Respiratory epithelial cell inflammation	Rat, F344 (M)	Nasal cavity	↑	1.8 ppm	Inhalation (whole-body), 6 h/day 5 days/wk for 13 wk		Dorman et al. (2008)
Mucus hypersecretion	Rat, Sprague-Dawley (M)	BALF	↑	3 ppm	Inhalation (whole-body), 6 h/day for 12 days	Single exposure concentration.	Chen et al. (2013a)
Inflammatory cells	Mice, C57BL/6 (M)	BALF	–	5 ppm	Inhalation (whole-body), 4 h/day, 4 days/wk for 2 wk	Single exposure concentration.	O'Brien et al. (2016)
Total protein	Mouse, 129S1/SvlmJ (F) Mouse, A/J (F) Mouse, BALB/cByJ (F) Mouse, C3H/HeJ (F) Mouse, C57BL/6J (F) Mouse, DBA/2J (F) Mouse, FVB/NJ (F)	BALF	All strains (↓)	1 ppm	Inhalation (whole-body), 6 h/day, 4–5 days/wk for 11 wk	Single exposure concentration.	Johanson et al. (2020)
Total protein Total cells Total protein	Rat, Wistar, (M)	BALF BALF NALF	↑ ↑ ↑	4 ppm 4 ppm 4 ppm	Inhalation (nose-only), 4 h/day for 2 days		Snow et al. (2017)
Total protein Total cells Total protein	Rat, Goto Kakizaki (M)	BALF BALF NALF	↑ ↑ ↑	2 ppm 4 ppm 4 ppm	Inhalation (nose-only), 4 h/day for 2 days		Snow et al. (2017)
Total cells; neutrophils; TNFα, IL1α; IL1β; KC	Mouse. BALB/c (M)	BALF	↑	1 mg/kg	Oropharyngeal aspiration	Relevance of route of exposure (anaesthetized).	Ong et al. (2012)
Bladder wet weight (oedema)	Mouse, Swiss (M)	Urinary bladder	↑	75 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Batista et al. (2006)
Bladder wet weight (oedema)	Mouse, Swiss (M)	Urinary bladder	↑	75 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Batista et al. (2007)
Bladder wet weight (oedema)	Mouse, C57 (F)	Urinary bladder	↑	6 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Bjorling et al. (2007)
Bladder wet weight (oedema)	Mouse, C3H/HeJ (F)	Urinary bladder	↑	6 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Bjorling et al. (2007)

Table 4.15 (continued)

End-point	Species, strain (sex)	Tissue	Results[a]	Dose (LED or HID)	Route, duration, dosing regimen	Comments	Reference
Bladder wet weight (oedema)	Mouse, C3H/OuJ (F)	Urinary bladder	↑	6 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Bjorling et al. (2007)
Bladder wet weight (oedema)	Rat, Wistar (M)	Urinary bladder	↑	75 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Macedo et al. (2008)
Bladder wet weight (oedema)	Rat, Wistar (F)	Urinary bladder	↑	1 mM (400 µL)/ bladder	Intravesical, 1×	Relevance of exposure route.	Merriam et al. (2011)
Bladder wet weight (oedema)	Rat, Wistar (F)	Urinary bladder	↑	75 µg/bladder	Intravesical, 1×	Relevance of exposure route.	Wang et al. (2013b)

BALF, bronchoalveolar lavage fluid; CINC, cytokine-induced neutrophil chemoattractant; F, female; h, hour; HID, highest ineffective dose; IL, interleukin; KC, mouse homologue for human IL8; LED, lowest effective dose; M, male; NALF, nasal lavage fluid; NR, not reported; TNF, tumour necrosis factor; ppm, parts per million; wk, week. [a] ↑, increase; ↓, decrease; –, no effect.

al., 1999, 2007, 2008). Interstitial inflammation, neutrophil infiltration, congestion, and oedema were reported in mouse lung (Kim et al., 2018). Increased inflammation has also been reported in rat respiratory epithelial cells and in the rat, hamster, and rabbit olfactory epithelium after acrolein inhalation (Feron et al., 1978; Dorman et al., 2008). Mucus hypersecretion has been observed in rats after acrolein inhalation (Chen et al., 2013a), and effects on bronchoalveolar lavage fluid have variously been observed in studies in mice and rats (O'Brien et al., 2016; Johanson et al., 2020; Snow et al., 2017). Oropharyngeal administration of acrolein in mice results in pulmonary inflammation as shown by the associated increase in elevated macrophage and neutrophil counts in the bronchoalveolar lavage fluid, and increased expression of production of cytokines, including interleukins IL1α, IL1β, IL6, IL17, and TNF, IFNγ, and monocyte chemotactic protein 1 (MCP-1) (Ong et al., 2012).

Some in vivo studies in rodents have investigated the role of acrolein in cyclophosphamide-induced inflammation and haemorrhagic cystitis. These studies rely on an injection of acrolein directly into the urinary bladder (Batista et al., 2006, 2007; Bjorling et al., 2007; Macedo et al., 2008; Merriam et al., 2008; Wang et al., 2013b). [The Working Group noted that these studies use a route of exposure that is unlikely to occur in humans and they involve acute exposures.]

4.2.7 Alters cell proliferation, cell death, or nutrient supply

(a) Humans

No data in exposed humans were available to the Working Group.

In human cell lines, several studies suggested that acrolein is capable of inhibiting tumour suppressor genes and activating proto-oncogenes either by directly binding and modulating the protein or by disrupting signalling cascades that promote cell proliferation. Acrolein inhibited both DNA-binding activity and luciferase-reporter activity of the tumour suppressor TP53, in a B[*a*]P induction study using human lung adenocarcinoma cells (A549) (Biswal et al., 2003). The inhibitory effect of acrolein occurred in the absence of altered TP53 protein levels under basal or induced conditions (i.e. a 48-hour pre-treatment with B[*a*]P), which is probably the result of direct binding of the TP53 protein by acrolein, as well as the alteration of the intracellular redox status.

Acrolein was demonstrated to both covalently modify and inhibit phosphatase and tensin homologue (PTEN) in MCF-7 breast ductal carcinoma cancer cells (Covey et al., 2010). Perturbation of Wnt/β-catenin signalling in human embryonic kidney cells (HEK-293), which favour the retention of active protein kinase AKT, was demonstrated in both time- and dose-dependent manners. Acrolein inactivation of PTEN lead to an increase in activity of the proto-oncogene AKT, which can increase cellular proliferation and survival (Covey et al., 2010). AKT activity was further explored in a human colon carcinoma cell line (HCT 116), and in MCF-7 cells: acrolein was generated endogenously as a by-product of myeloperoxidase catalysis (quantified spectrophotometrically) and resulted in the accumulation of higher amounts of phospho-Ser^{473}AKT; when a PI3K inhibitor (wortmannin) or a myeloperoxidase inhibitor (resorcinol) were added, phospho-Ser^{473}AKT formation was suppressed (Al-Salihi et al., 2015).

Acrolein caused differential inhibition and modification (covalent adducts) of pyruvate kinase, the enzyme involved in the last step of anaerobic glycolysis, in MCF-7 cells and in a cell-free system. This change in activity has been reported during the transformation of cells to a

proliferative or tumorigenic phenotype (Sousa et al., 2019).

(b) Experimental systems

A significant increase in the incidence and/or severity of respiratory tract metaplasia and hyperplasia was observed in B6D2F$_1$/Crlj mice and F344/DuCrlCrlj rats exposed to acrolein by inhalation for 2 years (JBRC, 2016a, b, c, d, e, f) and is discussed in Sections 3.1 and 3.2 of the present monograph.

Feron & Kruysse (1977) reported an increase in epithelial metaplasia of the nasal cavity epithelium in Syrian golden hamsters repeatedly inhaling acrolein vapour (4 ppm) for 7 hours per day, 5 days per week, for 52 weeks. In a subacute toxicity study (6 hours per day, 5 days per week, for 13 weeks) in hamsters, rats, and rabbits, squamous metaplasia was only observed in rats treated with the intermediate dose of 1.4 ppm (Feron et al., 1978). [The Working Group noted high mortality in the group at the higher dose.]

Fischer 344 rats treated with acrolein (0.6 or 1.8 ppm) by inhalation for up to 65 days presented with respiratory epithelial hyperplasia and squamous metaplasia (Dorman et al., 2008). In the most sensitive location, the lateral wall, respiratory epithelial cell proliferation occurred in the two highest dose groups as detected by proliferating cell nuclear antigen (PCNA) immunohistochemistry (Dorman et al., 2008). In a separate inhalational study, acrolein (3 ppm) induced goblet cell hyperplasia in the bronchial epithelium in lungs of male Sprague-Dawley rats exposed for 6 hours per day, 7 days per week, for 2 weeks (Liu et al., 2009b). Acrolein (≥ 0.1 μM) elicited a similar increase in goblet cell number in a differentiated lung epithelium model, which mirrored, to some extent, the goblet cell hyperplasia observed in animal inhalation models and after human chronic exposure (Haswell et al., 2010).

In Sprague-Dawley rats, acrolein (3 ppm) inhalation for 3 weeks led to metaplastic, dysplastic, and hyperplastic changes in the mucous, respiratory, and olfactory epithelium of the nasal cavity (Leach et al., 1987). These changes were prominent on the septum and in the anterior and ventral areas.

In Sprague-Dawley rats given a single exposure or a sustained 3-day exposure to acrolein at 0.2 or 0.6 ppm via inhalation, a concentration-dependent increase in the proportion of 5-bromo-2′-deoxyuridine (BrdU)-labelled nasal epithelial, tracheal epithelial, or free lung cells was observed (Roemer et al., 1993). Although significantly increased compared with control at both time-points, the single exposure elicited a stronger proliferative response (Roemer et al., 1993). Similar treatment-related proliferative increases were measured by both BrdU and PCNA labelling in nasal epithelium of albino Wistar rats that were treated (≤ 0.67 ppm) for 6 hours per day, for 3 days (Cassee et al., 1996). These rats also presented with slight disarrangement, necrosis, thickening, and desquamation of respiratory/transitional epithelium (Cassee et al., 1996). [The Working Group noted necrosis associated with the highest dose.]

A single inhalational exposure (5 ppm for 10 minutes) of BALB/c mice to acrolein led to a sustained increase in levels of vascular endothelial growth factor protein that persisted for 8 weeks (Kim et al., 2019). A single inhalation exposure of Sprague-Dawley rats to acrolein (3 ppm for a 12-day period of 5 days of treatment, 2 days of rest, and another 5 days of treatment) significantly activated the Ras/ERK pathway in bronchial epithelial cells, which functions downstream of epidermal growth factor. This finding was accompanied by an increase in goblet cell hyperplasia and metaplasia, which were significantly inhibited

by simvastatin, a Ras inhibitor (Chen et al., 2010).

In oral gavage studies in B6C3F$_1$ mice and F334/N rats, acrolein treatment led to lesions associated with uncontrolled cell growth. Squamous epithelial hyperplasia in the forestomach and hyperplasia of bone marrow cells were observed in rats treated with acrolein at ≤ 10 mg/kg bw 5 days a week for 2 weeks; mice in the dose groups treated with ≤ 10 mg/kg bw
developed squamous epithelial hyperplasia of the forestomach (Irwin, 2006). [The Working Group noted the high mortality in the groups of rats and mice at the highest dose.]

Forestomach epithelial hyperplasia was observed in male and female Fischer 344/N rats and B6C3F$_1$ mice given acrolein (≤ 10 mg/kg bw) by gavage once per day, 5 days per week, for 14 weeks (Auerbach et al., 2008). The pro-toxicants, allyl acetate and allyl alcohol, which are metabolized to acrolein, were also investigated. Periportal hepatocyte hypertrophy was observed in rats treated with allyl acetate and allyl alcohol, but not acrolein. Both species treated with the highest dose of allyl acetate exhibited forestomach epithelial necrosis. [The Working Group noted the 100% mortality of this dose group for all species and sexes; the Working Group also noted low (93.3%) purity of allyl acetate.]

In a mouse model of intestinal cancer, *Apc*$^{min/+}$ mice were either treated with water or dextran sodium sulfate to induce a model of colitis. Colonocytes isolated from mice treated with dextran sodium sulfate were found to have covalent acrolein–protein adducts on the PTEN tumour suppressor from endogenously generated acrolein (myeloperoxidase catalysis), which corresponded with the activation of the *Akt* protooncogene in these samples (Al-Salihi et al., 2015).

In studies in hypertension-resistant and salt-induced rats treated with acrolein (≤ 1.4 ppm) via inhalation for 6 hours per day, 5 days per week, for 62 days, bronchiolar epithelial hyperplasia was reported that was sometimes accompanied by squamous metaplasia and fibrosis (Kutzman et
al., 1984). [The Working Group noted the toxicity associated with the highest dose, and the model of hypertension that was used for this study.]

4.2.8 Other key characteristics of carcinogens

(a) Induces epigenetic alterations

Several studies in experimental systems investigated the effect of acrolein on histone modification. Acrolein inhibited acetylation of the N-terminal tails of cytosolic histones H3 and H4 in vitro, compromising chromatin assembly in immortalized human bronchial epithelial and lung adenocarcinoma cell lines (Chen et al., 2013b). Interestingly, the effect of acrolein was specific to unmodified and newly synthesized histones; post-translational modifications seemed to protect the histone from being targeted. The mechanism behind these phenomena was further investigated by the same research group. Fang et al. (2016) determined that acrolein reacts and forms covalent adducts with lysine residues in an immortalized human bronchial epithelial cell line (BEAS-2B), including those residues important for chromatin assembly, therefore preventing these sites from undergoing physiological modifications (see Section 4.2.1). Promoter histone modifications of the *FasL* gene were enhanced by acrolein in the human liver hepatocarcinoma HepG2 cell line and in primary rat hepatocytes both alone and when co-treated with the HIV antiretroviral zidovudine (Ghare et al., 2016). When the acrolein scavenger hydralazine was added to the experiment, promoter-associated epigenetic changes were inhibited. Global DNA

methylation and accumulation of DNA damage because of silencing of DNA repair genes was observed in acrolein-treated C57BL/6 mouse bladder tissue and in cultured mouse bladder muscle cells (Haldar et al., 2015, 2016). Cox et al. (1988) showed that DNA methylase isolated from the liver and urothelium of rats (strain not reported), treated with acrolein, was inhibited by 30–50% but the mechanism behind the inhibition was unclear.

(b) Modulates receptor-mediated effects

Several receptors appear to be activated or modulated by acrolein, although the studies are limited in both number and specificity. Thyroid hormone co-treatment with acrolein, administered both as a single compound and as a component of cigarette smoke, acts as a partial agonist for the thyroid receptor through recruitment of the nuclear coactivators glucocorticoid receptor interacting protein 1 (GRIP1) and steroid receptor coactivator 1 (SCR1) (Hayashi et al., 2018). Again, independently or as a component of cigarette smoke extract, acrolein was able to recruit GRIP1 or SCR1, but this time to peroxisome proliferator-activated receptor-α (PPARα) to induce transcriptional changes (Matsushita et al., 2019).

In male Fischer 344 rats given acrolein intraperitoneally with phenobarbital, α-, 2β-, 6β-, 16α-, and 16β-hydroxylation of testosterone and androstenedione was decreased (Cooper et al., 1992). This was the result of acrolein impairing the induction of CYP by 45%.

(c) Causes immortalization

Acrolein significantly increased soft agar anchorage-independent-growth colony formation, a characteristic of tumorigenic cell transformation, in immortalized human bronchial epithelial cells (BEAS-2B) and bladder urothelial cells (UROtsa) (Lee et al., 2015).

(d) Multiple characteristics

Gene expression changes in response to acrolein exposure were investigated in several studies. Data suggested a coordination of several of the key characteristics, namely induces oxidative stress, induces chronic inflammation and, to some degree, alters DNA repair in epithelial tissue or cells.

In normal human bronchial epithelial cells treated with acrolein for up to 24 hours, a combination of high-content screening and genome-wide transcriptomics revealed induction of genes associated with cellular stress followed by proliferation, and to a lesser extent, senescence networks (Gonzalez-Suarez et al., 2014). Interestingly, NRF2 was consistently activated despite the lack of observed increases in ROS. Furthermore, an increase in phosphorylation of histone 3 (pH3) levels was not accompanied by changes in cell number, suggesting the presence of cell cycle arrest at G_2/M. Rats exposed for 6 hours to acrolein by inhalation (nose-only) exhibited similar patterns of protein and gene expression (Gonzalez-Suarez et al., 2014). In addition to the nuclear accumulation of Nrf2 protein, antioxidant genes (i.e. NAD(P)H quinone dehydrogenase 1, *Nqo1*; catalytic sub - unit of glutamyl cysteine ligase, *Gclc*; and haem oxygenase 1, *Hmox1*) were upregulated at much lower acrolein concentrations than those required to induce the expression of proinflammatory genes (i.e. chemokine-induced neutrophil chemoattractant-1, *Cinc1*; and interleukin 6, *Il6*) (Cichocki et al., 2014).

Three studies investigated the transcriptional response to acrolein in human adenocarcinoma lung epithelial (A549) cells at various time-points. Over the course of 4 hours, a strong initial downregulation of genes was observed, possibly

in response to DNA damage, followed by an increase in gene upregulation in which pro-inflammatory and pro-apoptotic pathways were dominant (Thompson & Burcham, 2008). Overall, these results indicate a dysregulation in several key characteristics of carcinogens including apoptosis, cell cycle control, and cell signalling. In a 2-hour exposure study in the same cells (A549), acrolein given alone or as a mixture with other short-chain aldehydes resulted in only one upregulated gene, *HMOX1*, a key gene in oxidative stress response (Cheah et al., 2013). A 24-hour treatment of A549 cells with acrolein at half maximal inhibitory concentration (IC_{50}) induced a robust expression of DNA repair genes, but this failed to rescue cells from apoptosis, even after acrolein washout and a recovery period (Sarkar, 2019)

4.3 Data relevant to comparisons across agents and end-points

Acrolein is one of approximately 1000 chemicals tested across the full assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) programmes supported by the US EPA (2020). In vitro assay descriptions to map Tox21 and ToxCast screening data in the context of the 10 key characteristics were previously summarized by Chiu et al. (2018). Results in this data set only include one active hit out of 235 assays. Acrolein was active in the antioxidant response element assay designed to target transcription factor activity, specifically mapping to the *NRF2* gene using a positive control of naphthoflavone. *NRF2* encodes the transcription factor NRF2, which regulates genes containing antioxidant response elements (ARE) in their promoters; this is probably the result of acrolein inducing oxidative stress. [The Working Group noted one flag for < 50% efficiency with this assay.]

5. Summary of Data Reported

5.1 Exposure characterization

Acrolein is a High Production Volume chemical that is used as a reactive intermediate and in the manufacture of numerous chemical products, including acrylic acid and methionine. It is directly used as a biocide, specifically, as an herbicide in recirculating water systems. Acrolein is formed during combustion of fuels, wood, and plastics, and is present in cigarette smoke and vapours from electronic cigarettes. In kitchens, high-temperature roasting and deep-fat frying produce measurable amounts of acrolein in the air. Acrolein is also formed during fermentation and is found in various alcoholic beverages. Acrolein is routinely measured in studies monitoring outdoor air pollution, and it has been identified in various combustion emissions in numerous reports. Firefighters are also exposed to acrolein. Occupational and environmental exposure guidelines exist for acrolein.

The urinary metabolite *N*-acetyl-*S*-(3-hydroxypropyl)-L-cysteine (3-hydroxypropylmer - capturic acid, HPMA) has been applied to estimate exposure, and a reference value for workplace substances is available. However, it is often challenging to differentiate endogenous from exogenous exposure due to the various external sources including air pollution, exposure to secondhand smoke, and consumption of fried and fermented foods.

5.2 Cancer in humans

One occupational cohort study, two hospital-based case–control studies, and three nested case–control studies in occupational or population-based cohorts were available, with little consistency in the cancer sites evaluated.

The study in an occupational cohort, one case–control study on urothelial cancer in patients with chronic kidney disease, and one nested case– control study on lymphohaematopoietic cancer in an occupational cohort were uninformative due to small numbers, poor external exposure assessment, and flaws in design. The other case– control study detected higher levels of acrolein– DNA adducts in buccal swabs of patients with oral cancer compared with healthy controls, but the study did not find an association between adduct levels and external exposures, including tobacco smoking or betel chewing. Finally, two nested case–control studies in a population-based cohort studied several biomarkers (including metabolites of acrolein) in relation to lung cancer among current smokers and non-smokers respectively, without demonstrating a direct etiological involvement of acrolein. In summary, all studies were judged to be uninformative and did not provide evidence on a causal relationship between acrolein exposure and cancer in humans. The studies were either of poor quality regarding design or exposure assessment, or they were of a mechanistic nature.

5.3 Cancer in experimental animals

Exposure to acrolein caused an increase in the incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two species.

In an inhalation study in B6D2F$_1$/Crlj mice, female mice exposed to acrolein showed a significant positive trend in the incidence of malignant lymphoma.

In an inhalation study in F344/DuCrlCrlj rats, there was a significant positive trend in the incidence of rhabdomyoma of the nasal cavity and of squamous cell carcinoma or rhabdomyoma (combined) of the nasal cavity in females exposed to acrolein. There was also a significant increase in the incidence of squamous cell carcinoma or rhabdomyoma (combined) of the nasal cavity. Rhabdomyoma of the nasal cavity and squamous cell carcinoma of the nasal cavity are very rare tumours in the strain of rats used in the study.

5.4 Mechanistic evidence

The available data on absorption and distribution of acrolein in humans are scarce. Acrolein is absorbed after inhalation or oral exposure. A slow absorption rate from air was observed in experiments with human skin in vitro. In humans, the delivery of acrolein to the lower respiratory tract can be higher than in rats; in rats, which are obligate nasal breathers, a significant portion of acrolein (up to 98%) is absorbed in the upper respiratory tract. Acrolein is a reactive electrophile that reacts spontaneously with cellular glutathione as well as with nucleophilic sites in proteins and DNA. It is efficiently metabolized by three detoxification pathways: (i) conjugation with glutathione leading eventually to HPMA and *N*-acetyl-*S*-(carboxyethyl)-L-cysteine (2-carboxyethylmercapturic acid, CEMA), which are excreted in urine; (ii) reduction by aldo-keto reductases to allyl alcohol; and (iii) oxidation by aldehyde dehydrogenases to acrylic acid, which is further converted to 3-hydropropionic acid and thereby enters physiological catabolism. Metabolic activation by cytochrome P450s (CYPs) to glycidaldehyde is a minor metabolic pathway leading to 2-carboxy-2-hydroxyethylmercapturic acid through glutathione conjugation. Acrolein is excreted in urine, exhaled air, and faeces. Excretion half-time in humans is approximately 9 hours as measured by urinary HPMA levels. Small amounts of acrolein from both endogenous and exogenous sources have been detected in

exhaled air. In rats, 26–31% of both intravenous and oral doses were exhaled as carbon dioxide.

There is consistent and coherent evidence that acrolein exhibits key characteristics of carcinogens. Acrolein is a strongly electrophilic α,β-unsaturated aldehyde (enal) that readily reacts with DNA bases and proteins forming DNA and protein adducts in vivo and in vitro. Among these adducts, the most widely studied are the cyclic deoxyguanosine adducts, which are formed as a pair of α and γ regioisomers, α- and γ-hydroxy-1,N^2-propano-2′-deoxyguanosine (α- and γ-OH-PdG). γ-OH-PdG has been consis tently detected in humans in various samples (including from lung, liver, brain, urothelial mucosa, and saliva), as well as in experimental animals, with detected levels dependent on species, tissue types, exposure, and physiological conditions. Elevated levels of acrolein-derived adducts are found in tobacco smokers, or under chronic inflammatory conditions, such as non-alcoholic fatty liver disease. This indicates their formation by acrolein from tobacco smoke; their presence in tissues of non-smokers is indicative of acrolein formation by endogenous processes, including lipid peroxidation. In acrolein-treated human lung cells, acrolein–DNA adducts were preferentially formed at lung cancer *TP53* mutational hotspots, and acrolein preferentially adducted guanines at cytosine methylation CpG sites. Acroleinderived DNA adducts have been detected in the liver of untreated rodents as well as in various tissues of rodents exposed to cigarette smoke, automobile exhaust, or a high-fat diet. Acroleinderived DNA adducts have also been detected in dogs exposed to cyclophosphamide, and in cockerels exposed to acrolein.

Acrolein is genotoxic. No data in humans in vivo were available. In several studies in human primary cells, acrolein consistently induced DNA strand breaks and DNA–protein crosslinks. In cultured human cell lines, acrolein consistently induced DNA strand breaks, mutations, and micronucleus formation, and was suggestive of inducing DNA–protein crosslinks. A limited number of in vivo studies of genotoxic end-points were available and were largely negative; however, across many in vitro experimental systems acrolein was found to consistently induce DNA strand breaks, DNA–protein crosslinks, mutations, and sister-chromatid exchanges. In *Salmonella* strains tested without metabolic activation, acrolein induced both base-pair substitution and frameshift mutations. The mutagenicity of acrolein has also been demonstrated in experiments with plasmid DNA.

Acrolein alters DNA repair or causes genomic instability. No data in humans in vivo were available. Multiple studies in human cells have demonstrated that acrolein directly inhibits proteins in three major DNA-repair pathways. Acrolein induced concentration-dependent inhibition of nucleotide excision repair, base excision repair and mismatch repair in primary human lung fibroblasts and bronchial epithelial cells, as well as in cultured human lung and urothelial cells. Acrolein inhibited the DNA repair enzyme O^6-methylguanine–DNA methyltransferase in human bronchial fibroblasts. It also inhibited excision repair due to the accumulation of DNA single-strand breaks in normal skin fibroblasts.

Acrolein induces oxidative stress. No in vivo human data were available. In vitro studies using multiple human- and rodent-derived cells showed that acrolein induces biochemical changes consistent with depletion of glutathione and increased generation of ROS and protein carbonyls, indicative of oxidative stress. Multiple studies in rodents have likewise shown that acrolein administration via multiple routes of exposure including inhalation, oral, and intraperitoneal injection resulted in decreased

tissue glutathione concentrations, and increased lipid peroxidation and protein carbonyl production. A statistically significant increase in levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) has been reported in rodent lung DNA after acrolein inhalation.

Acrolein is immunosuppressive. No data in humans in vivo were available. In studies in vitro with human immune cells, acrolein exposure can impair cytokine release and result in cytotoxicity. Human alveolar macrophages exposed to acrolein have reduced ability to clear *Mycobacterium tuberculosis* infection. Multiple studies in rodents have demonstrated that acrolein inhalation alters bacterial-induced mortality, bactericidal activity, or innate immune function. Mouse splenic cells exposed to acrolein exhibited decreased T- and B-cell proliferation.

Acrolein induces chronic inflammation. No data were available in humans, but acrolein exposure can produce chronic inflammation in rodents. Shorter (i.e. acute to subchronic) rodent studies showed that acrolein administration via multiple routes, including oral and inhalation, produces inflammation at the site of entry.

Additionally, acrolein alters cell proliferation, cell death, or nutrient supply. No in vivo data were available in humans. In vitro studies using multiple human and rodent cell types showed that acrolein inhibited tumour suppressor genes and activated proto-oncogenes either by directly binding and modulating the protein or by disrupting signalling cascades that promote cell proliferation. Hyperplasia, metaplasia, and dysplasia were seen in the respiratory system of rodents exposed chronically or acutely by inhalation. After chronic exposure, rodents treated with acrolein by oral gavage developed forestomach epithelial hyperplasia.

There is suggestive evidence that acrolein induces epigenetic alterations via DNA methylation and histone modification. One study using mouse tissues and cells treated with acrolein reported alteration of global DNA methylation and accumulation of DNA damage because of silencing of DNA repair genes. This result was consistent with findings in mouse tissues and cells. DNA methylase was inhibited in two different rat strains. In vitro studies using human- and rodent-derived cells suggest that acrolein compromises chromatin assembly through inhibition of acetylation of the N-terminal tails of cytosolic histones.

Acrolein was essentially without effects in the assay battery of the Toxicity Testing in the 21st Century (Tox21) and Toxicity Forecaster (ToxCast) research programmes.

6. Evaluation and Rationale

6.1 Cancer in humans

There is *inadequate evidence* in humans regarding the carcinogenicity of acrolein.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of acrolein.

6.3 Mechanistic evidence

There is *strong evidence* that acrolein exhibits multiple key characteristics of carcinogens, primarily from studies with human primary cells and studies in experimental systems, supported by studies in humans for DNA adducts.

6.4 Overall evaluation

Acrolein is *probably carcinogenic to humans (Group 2A)*.

6.5 Rationale

The *Group 2A* evaluation for acrolein is based on *sufficient evidence* of cancer in experimental animals and *strong* mechanistic evidence. The *sufficient evidence* of carcinogenicity in experimental animals is based on an increased incidence of either malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two species. There is *strong* evidence that acrolein exhibits multiple key characteristics of carcinogens; acrolein is electrophilic; it is genotoxic; it alters DNA repair or causes genomic instability; it induces oxidative stress; it is immunosuppressive; it induces chronic inflammation; and it alters cell proliferation, cell death, or nutrient supply. The supporting data that acrolein exhibits these key characteristics comes primarily from studies with human primary cells and studies in experimental systems, and is supported by studies in humans for DNA adducts.

The evidence regarding cancer in humans is *inadequate*. The few available studies related to acrolein exposure and human cancer were inconsistent in the cancer sites evaluated, and most studies were small. All had poor assessment of external exposure to acrolein or could not distinguish the effects of acrolein exposure from other constituents of cigarette smoking.

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Crotonaldehyde / But-2-enal

MAK Value Documentation

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the germ cell mutagenicity and carcinogenicity of crotonaldehyde [4170-30-3, 123-73-9]. Crotonaldehyde is a highly reactive mutagenic and cytotoxic compound without metabolic activation.
An oral carcinogenicity study in male rats, with the liver as the only organ examined, provides at most only an indication of a carcinogenic potential. Crotonaldehyde remains assigned to Carcinogen Category 3B because of the still limited database.
Despite some methodical deficiencies, new in vivo studies with positive results for bone marrow and spermatocyte chromosomal aberrations as well as dominant lethal mutations in mice lead to a reclassification in Category 3A for Germ Cell Mutagens.

Keywords

crotonaldehyde; beta-methylacrolein; crotonic aldehyde; 2-butenal; but-2-enal; mechanism of action; genotoxicity; carcinogenicity; germ cell mutagenicity; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

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Crotonaldehyde

[4170-30-3, 123-73-9]

Supplement 2018

MAK value	**–**
Peak limitation	**–**
Absorption through the skin (1981)	**H**
Sensitization	**–**
Carcinogenicity (1981)	**Category 3B**
Prenatal toxicity	**–**
Germ cell mutagenicity (2017)	**Category 3A**
BAT value	**–**
1 ml/m³ (ppm) ≙ 2.908 mg/m³	**1 mg/m³ ≙ 0.344 ml/m³ (ppm)**

Since 1981, crotonaldehyde has been classified in Carcinogen Category 3B and designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts) (see documentation "Crotonaldehyde" 2007).

The supplement published in 2007 (supplement "2-Butenal" 2007, available in German only) assessed germ cell mutagenicity and the sensitizing effects of crotonaldehyde. As a result, the substance was classified in category 3B for germ cell mutagens. A new study of the genotoxic effects in vivo (Jha et al. 2007) has made it necessary to re-assess classification of the substance in one of the categories for germ cell mutagens. As part of this re-assessment, the data for the carcinogenic effects have also been updated.

Crotonaldehyde was once used in the manufacture of *sec*-butyl alcohol; today, it has been replaced by other technical steps during its synthesis. The substance is used in further applications, such as in the preparation of vulcanization accelerators for rubber, in leather tanning, as a denaturant of ethyl alcohol, as a warning agent (odoriferous substance) in fuel gases and in the detection of leaks in pipes. Today, crotonaldehyde is used mainly as an intermediate in the synthesis of sorbic acid and crotonic acid. Crotonaldehyde is formed during the incomplete combustion and pyrolysis of organic substances, in particular during the combustion of gases in petrol and diesel-powered engines, wood combustion and tobacco smoking. Crotonaldehyde is also produced endogenously and occurs naturally in many plants, foods and beverages (SCOEL 2013).

Mechanism of Action

Genotoxicity

Crotonaldehyde reacts with cellular macromolecules. Protein adducts and DNA–histone crosslinks were induced in vitro. Cyclic 1,N^2-propanodeoxyguanosine adducts were detected in various tissues of mice and rats both in vitro and in vivo; these are formed also endogenously in animals and humans (supplement "2-Butenal" 2007, available in German only). In humans, these adducts were detected more frequently in the lungs than in the liver, and were not detected in blood (SCOEL 2013).

In addition, N^2-(3-hydroxybutylidene)deoxyguanosine and N^2-[2-(2-hydroxypropyl)-6-methyl-1,3-dioxan-4-yl]deoxyguanosine ("N^2-paraldol-dG") were detected in vitro (supplement "2-Butenal" 2007, available in German only).

Crotonaldehyde protein adducts have been found in the brains of patients with Alzheimer's disease (SCOEL 2013). 1,N^2-propanodeoxyguanosine adducts inhibit DNA synthesis and are mutagenic after incorporation into DNA vectors and after transfection into human xeroderma pigmentosum cells (Stein et al. 2006). In addition, 1,N^2-propanodeoxyguanosine adducts can form DNA crosslinks (Kozekov et al. 2003; Liu et al. 2006).

Cytotoxicity

The gene expression profile and cytotoxicity of human bronchial epithelial cells were examined by microassay after 2 or 6-hour exposure to crotonaldehyde concentrations of 40 or 80 μM. The results revealed a gene expression profile for various processes that suggested cytotoxicity and tissue injury, including inflammatory responses, exogenous metabolism, cell cycle, heat shock and antioxidant responses (SCOEL 2013).

Another study with human bronchial epithelial cells performed by the same research group found that exposure to crotonaldehyde concentrations of 10 to 120 μM led to a decrease in intracellular glutathione levels and an increase in reactive oxygen species, both of which were dependent on the concentration. Crotonaldehyde induced apoptosis and, at higher concentrations, necrosis. Other studies suggested that crotonaldehyde-induced apoptosis was activated by a caspase-dependent signalling pathway (SCOEL 2013).

Animal Experiments and in vitro Studies

Genotoxicity

In vitro

Since the 2007 supplement was published (supplement "2-Butenal" 2007, available in German only), a new in vitro study has become available.

In mouse lymphoma cells, crotonaldehyde caused an increase in mutations at concentrations of 25 μM and above; however, these were primarily small cell colonies (Demir et al. 2011).

In vivo

Since the 2007 supplement was published (supplement "2-Butenal" 2007, available in German only), three new investigations in mice have become available: two studies examined chromosomal aberrations in bone marrow cells or spermatocytes, another carried out a dominant lethal test in mice (Jha et al. 2007).

In Swiss albino mice given single intraperitoneal injections of crotonaldehyde of 0, 8, 16 or 32 µl/kg body weight (6.8, 13.6 or 27.2 mg/kg body weight), a dose-dependent increase in the percentage of aberrant metaphases in bone marrow cells was recorded. However, the mitotic index in the bone marrow was significantly reduced after 6-hour treatment at the low crotonaldehyde dose of 8 µl/kg body weight and above and after 12 or 24-hour treatment at 16 µl/kg body weight and above, and the number of agglutinated and pulverised chromosomes in the metaphase cells increased in a dose-dependent manner. There was a dose-related increase in the number of chromosomal aberrations in spermatocytes at 16 µl/kg body weight and above (Jha et al. 2007). The incidence of chromosomal aberrations in bone marrow cells and in spermatocytes was significantly increased only when accompanied by a reduced mitotic index in the bone marrow, which shows that genotoxic effects occur only concomitantly with cytotoxic effects.

In a dominant lethal test, male Swiss albino mice were given intraperitoneal injections of crotonaldehyde of 0, 8, 16 or 32 µl/kg body weight (6.8, 13.6 or 27.2 mg/kg body weight) for 5 days; the animals were then mated with untreated female mice. The treatment led to a significant decrease in the fertility indices, the total number of implantations and the number of implantations per female. There was a dose-related increase in the number of dead implantations per animal and the percentage of dominant lethal mutations (Jha et al. 2007). The study is flawed by poor methodology; it does not provide any data regarding the time of cervical opening, early or late post-implantation losses, or resorptions, and describes the effects observed in the untreated female animals rather than in the treated male animals. In addition, the analysis of the dominant lethal test was inadequate. Whether the effects observed in the dominant lethal test were genotoxic or cytotoxic cannot be determined because suitable parameters were not assessed (Ehling and Neuhäuser-Klaus 1993).

Carcinogenicity

Long-term studies

Groups of 23 to 27 male rats were exposed for 113 weeks via the drinking water to crotonaldehyde concentrations of 0, 0.6 or 6.0 mM (equivalent to 42 or 421 mg/l, or about 2.1 or 21 mg/kg body weight and day (conversion factor 0.05, long-term, according to EFSA 2012)); at the end of the exposure, only the liver was histopathologically examined. Survival was not affected in any group. At concentrations of 0, 42 or 421 mg/l, the incidence of hepatocellular carcinomas was 0/23, 2/27 or 0/23, that of neoplastic nodules was 0/23, 9/27 or 1/23 and that of liver cell foci was 1/23, 23/27 or 13/23, respectively (Chung et al. 1986). As only male rats and only a few animals per dose group were investigated and the incidences of preneoplastic and neoplastic findings were not dose-related, the findings from this study indicate at

best that crotonaldehyde has the potential to act as a liver carcinogen. As no other organs were examined, the findings on the carcinogenic potential of crotonaldehyde are, overall, of limited relevance.

Manifesto (carcinogenicity, germ cell mutagenicity)

Carcinogenicity. As described in the documentation published in 1981 (documentation "Crotonaldehyde" 2007), crotonaldehyde is a highly reactive compound and causes mutagenic and cytotoxic effects, which manifest themselves even without metabolic activation. In the meantime, a new study has been published on the carcinogenic effect of crotonaldehyde on the liver of male rats (Chung et al. 1986). However, the findings from this study merely indicate that crotonaldehyde has the potential to act as a liver carcinogen because only male animals were investigated and the incidences of preneoplastic and neoplastic findings were not dose-related. As there is little data available and no definite conclusions on the carcinogenic risk may be drawn from this data, crotonaldehyde remains classified in Carcinogen Category 3B.

Germ cell mutagenicity. In the 2007 supplement (supplement "2-Butenal" 2007, available in German only), crotonaldehyde was classified in category 3B for germ cell mutagens. In vitro studies demonstrated the genotoxic potential of crotonaldehyde. In vivo, X-chromosomal recessive lethal mutations and reciprocal translocations were detected in tests on germ cells of Drosophila melanogaster after the injection of crotonaldehyde. Mutations in TA100 occurred in the host mediated assay. Following administration by gavage and dermal application, covalent DNA binding was observed in the liver, lungs, kidneys and epidermis of mice and rats. Two micronucleus tests yielded negative results in mice after daily administration by gavage for up to 2 days or 13 weeks. There is no evidence of cytotoxicity in bone marrow cells or the peripheral blood.

Chromosomal aberrations in bone marrow cells or spermatocytes were detected after intraperitoneal injection of the substance only if the mitotic index was reduced, thus at cytotoxic doses. Dominant lethal mutations occurred in mice after intraperitoneal injection (Jha et al 2007). However, the study is flawed by poor methodology (see Section "Genotoxicity in vivo"), so that no distinction can be made between genotoxic and cytotoxic effects.

The accessibility of the germ cells was documented in the 2007 supplement (supplement "2-Butenal" 2007, available in German only). This was based on the following data: evidence of DNA adducts in the liver, lungs and kidneys after administration by gavage, studies with methodological shortcomings that investigated germ cells and found degenerative damage to the nucleus in the stages of spermatogenesis after exposure via the drinking water, and evidence of chromosomal aberrations, dominant lethal mutations and sperm head anomalies after intraperitoneal injection.

In view of the flawed methodology of the study of Jha et al. (2007) and the fact that it is not possible to determine with any certainty whether the effects observed in the chromosomal aberration test and in the dominant lethal test after intraperitoneal

injection are genotoxic or cytotoxic, and because of the negative results yielded by the micronucleus tests after administration by gavage, crotonaldehyde has not been classified in category 2 for germ cell mutagens, but moved from category 3B to category 3A for germ cell mutagens.

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completed March 22, 2017

Crotonaldehyde: Human health tier II assessment

05 February 2016

Chemicals in this assessment

Chemical Name in the Inventory	CAS Number
2-Butenal, (E)-	123-73-9
2-Butenal	4170-30-3

Preface

This assessment was carried out by staff of the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) using the Inventory Multi-tiered Assessment and Prioritisation (IMAP) framework.

The IMAP framework addresses the human health and environmental impacts of previously unassessed industrial chemicals listed on the Australian Inventory of Chemical Substances (the Inventory).

The framework was developed with significant input from stakeholders and provides a more rapid, flexible and transparent approach for the assessment of chemicals listed on the Inventory.

Stage One of the implementation of this framework, which lasted four years from 1 July 2012, examined 3000 chemicals meeting characteristics identified by stakeholders as needing priority assessment. This included chemicals for which NICNAS already held exposure information, chemicals identified as a concern or for which regulatory action had been taken overseas, and chemicals detected in international studies analysing chemicals present in babies' umbilical cord blood.

Stage Two of IMAP began in July 2016. We are continuing to assess chemicals on the Inventory, including chemicals identified as a concern for which action has been taken overseas and chemicals that can be rapidly identified and assessed by using Stage One information. We are also continuing to publish information for chemicals on the Inventory that pose a low risk to human health or the environment or both. This work provides efficiencies and enables us to identify higher risk chemicals requiring assessment.

The IMAP framework is a science and risk-based model designed to align the assessment effort with the human health and environmental impacts of chemicals. It has three tiers of assessment, with the assessment effort increasing with each tier. The Tier I assessment is a high throughput approach using tabulated electronic data. The Tier II assessment is an evaluation of risk on a substance-by-substance or chemical category-by-category basis. Tier III assessments are conducted to address specific concerns that could not be resolved during the Tier II assessment.

These assessments are carried out by staff employed by the Australian Government Department of Health and the Australian Government Department of the Environment and Energy. The human health and environment risk assessments are conducted and published separately, using information available at the time, and may be undertaken at different tiers.

This chemical or group of chemicals are being assessed at Tier II because the Tier I assessment indicated that it needed further investigation.

For more detail on this program please visit:**www.nicnas.gov.au**

Disclaimer

ACRONYMS & ABBREVIATIONS

Grouping Rationale

The chemical, 2-butenal (CAS No. 4170-30-3), is an a,β-unsaturated aldehyde that is commercially available as a mixture of two geometric isomers. Both the commercial product and the predominant trans isomer (> 95%), E-2-butenal (CAS No. 123-73-9), are listed separately on the Australian Inventory of Chemical Substances (AICS). The minor cis isomer, Z-2-butenal (CAS No. 15798-64-8) is not listed on the AICS. The (E)-isomer and the isomeric mixture are considered together in this group assessment.

As most of the available toxicology data relate to either commercial 2-butenal (CAS No. 4170-30-3) or on the purified trans isomer (CAS No. 123-73-9), these compounds have been grouped together and data can be read across from available sources on either compound. There are limited data available for Z-2-butenal (CAS No. 15798-64-8), although this chemical is expected to have a similar toxicological profile to that of the commercial product and the trans isomer, as the toxicity appears to occur by reactive mechanisms. The isomeric mixture (95% trans) is therefore expected to have an almost identical profile to the pure trans isomer.

For the purposes of this assessment, "the chemical" refers to the isomeric mixture 2-butenal (CAS No. 4170-30-3) unless stated otherwise. Where necessary, the trans isomer is specified separately.

Import, Manufacture and Use

Australian

No specific Australian use, import, or manufacturing information has been identified.

International

The following international uses have been identified through:

- the European Union (EU) Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) dossiers;
- Galleria Chemica;
- the Substances and Preparations in Nordic countries (SPIN) database;
- the European Commission Cosmetic Ingredients and Substances (CosIng) database;
- the United States (US) Personal Care Products Council International Nomenclature of Cosmetic Ingredients (INCI) Dictionary;
- the OECD High Production Volume chemical program (OECD HPV);
- the US Environmental Protection Agency's Aggregated Computer Toxicology Resource (ACToR);
- the US National Library of Medicine's Hazardous Substances Data Bank (HSDB); and
- various international assessments (IARC, 1995; ATSDR).

The chemical has reported cosmetic use as a fragrance additive (CosIng, INCI). However, due to its highly pungent, suffocating odour it is expected that only very low concentrations are used.

The chemical has reported commercial uses, including:

- in rubber accelerators as an antioxidant and a rubber strengthener;
- in leather tanning;
- as a warning agent in fuel gases;
- as a stabiliser for tetraethyl lead;
- as an alcohol denaturant;
- in the preparation of surface active agents;
- in the preparation of construction materials such as fillers; and
- in the purification of mineral and lubricating oils.

The chemical has reported site-limited uses, including:

- as an intermediate in the manufacture of chemicals such as sorbic acid (which is a food preservative);
- in the manufacture of polymers such as resins and polyvinyl actetals;
- as a solvent and short-stopper for polyvinyl chloride manufacture;
- in the preparation of adhesives; and
- in the manufacture of tear gas.

The chemical has reported non-industrial use, including:

- in flavouring agents; and
- in the preparation of pesticidal compounds, fertilisers and chemotherapeutic agents.

Restrictions

Australian

No known restrictions have been identified.

International

The chemicals (CAS No. 4170-30-3 and 123-73-9) are listed on the following (Galleria Chemica):

Cosmetic:

- ASEAN Cosmetic Directive Annex II Part 1: List of substances which must not form part of the composition of cosmetic products;
- Chile List of substances which must not form part of the composition of cosmetic products;
- China List of Banned substances for use in Cosmetics;
- EU Cosmetics Regulation 1223/2009 Annex II—List of substances prohibited in cosmetic products; and
- New Zealand Cosmetic Products Group Standard—Schedule 4: Components cosmetic products must not contain.

Other:

- Council of Europe Resolution AP (92) 2 on control of aids to polymerisation for plastic materials and articles - Limits for finished articles; and
- EU, Commission Regulation (EC) No 552/2009 of 22 June 2009 amending Regulation (EC) No 1907/2006 of the European Parliament and of the Council on REACH as regards Annex XVII - "The chemical cannot be used as a substance or as mixtures in aerosol dispensers where these aerosol dispensers are intended for supply to the general public for entertainment and decorative purposes".

Chemical warfare:

The chemical can be a precursor in the manufacture of chemical weapons (tear gas) but also has legitimate large scale industrial use and is currently listed on the US Department of Homeland Security (DHS) - Chemical Facility Anti-Terrorism Standards (CFATS) - Chemicals of Interest.

Existing Worker Health and Safety Controls

Hazard Classification

The chemicals (CAS No. 4170-30-3 and 123-73-9) are classified as hazardous, with the following risk phrases for human health in Hazardous Substances Information System (HSIS) (Safe Work Australia):

- Mut. Cat 3; R68 (mutagenicity)
- T+; R26 (acute toxicity)
- T; R24/25 (acute toxicity)
- Xn; R48/22 (repeated dose toxicity)
- Xi; R37/38-41 (irritation)

Exposure Standards

Australian

The chemical (CAS No. 4170-30-3) has an exposure standard of 5.7 mg/m^3 (2 ppm) time weighted average (TWA) (HSIS). No short-term exposure limits (STEL) are available.

International

The following exposure standards are identified for the chemicals (CAS No. 4170-30-3 and 123-73-9) (Galleria Chemica):

An exposure limit of 0.5–6 mg/m^3 (0.34–2 ppm) time weighted average (TWA) and 0.87–18 mg/m^3 (0.3–6 ppm) short-term exposure limit (STEL)/MAK/occupational exposure limit (OEL) are listed in different countries such as Canada (Yukon), Denmark, France, Germany, Greece, Iceland, Indonesia, Ireland, Mexico, Norway, Poland, Singapore, South Africa, Spain, Taiwan, the United Arab Emirates (UAE) and the United States (US).

Health Hazard Information

The chemical 2-butenal occurs naturally at low concentrations (up to 0.7 ppm) in many plants, foods and beverages. It is also formed endogenously in humans and animals via lipid peroxidation (which is increased by cigarette smoking), and has been detected in human milk. The chemical is also present at low levels in the ambient atmosphere, as it is a component of products generated from combustion processes such as wood smoke, tobacco smoke and engine exhaust (CICAD, 2008).

The chemical has a highly reactive carbonyl functionality, and an activated carbon-carbon double bond that can undergo Michael-type reactions with amino groups on proteins and nucleosides to form stable, protein-bound 2-butenal and DNA adducts. These adducts have been found in almost every tissue investigated in rats and mice (skin, liver, lung, kidney, brain, intestinal epithelial cells and leukocytes), with or without exogenous administration of the chemical (CICAD, 2008).

Toxicokinetics

Based on the available animal studies, it has been established that 2-butenal can enter the body by the oral, dermal and inhalation routes (SCOEL, 2013; MAK, 2012; IARC, 1995). In particular, the chemical is readily absorbed through the skin (see **Acute Toxicity - Dermal**).

After oral exposure of rats to ^{14}C-labelled 2-butenal in doses of 0.7–35 mg/kg bw, over 90 % of the substance was absorbed and rapidly metabolised— 60–78 % of the radioactivity was excreted in urine and breath within 12 hours of dosing and after 72 hours this increased to 82–86 %. Approximately 7 % was eliminated in faeces. In another study, following intravenous injection, 40 % of the dose was eliminated within 6 hours in urine, 33 % in exhaled air (as CO_2) and < 1 % in faeces (REACH; MAK, 2012).

Most aldehydes are mainly metabolised in the liver by oxidation to the corresponding carboxylic acids, and further degraded through fatty acid metabolism. However, it has been found that 2-butenal is not easily oxidised by aldehyde dehydrogenase, and instead reacts strongly with cellular thiol groups in proteins, and in particular glutathione, depletion of which may cause cell death via oxidative stress. This is thought to be the major detoxification pathway of the chemical at low concentrations, and metabolites of this process (3-hydroxymethyl-1-propylmercapturic acid and 2-carboxyl-1-methylethylmercapturic acid) were identified in urine following subcutaneous injection of the chemical in rats (REACH).

Acute Toxicity

Oral

The chemicals are classified as hazardous with the risk phrase ‘Toxic if swallowed’ (T; R25) in HSIS (Safe Work Australia).

Based on a limited number of test results, the chemical has high acute oral toxicity in rats and mice. The median lethal dose (LD50) is 174–300 mg/kg bw in rats and 104–240 mg/kg bw in mice (CICAD, 2008; SCOEL, 2013; MAK, 2012).

In an acute oral toxicity fixed dose study (conducted similarly to the Organisation for Economic Cooperation and Development (OECD) Test Guideline (TG) 420), male and female Sprague Dawley (SD) rats (5 animals/group) were administered the chemical by gavage at doses of 64.5, 107.5, 180, 300 and 500 mg/kg bw and observed for 14 days. Within 24 hours post-treatment, there were 27 out of 50 mortalities, including all animals in the 300 and 500 mg/kg bw groups and 7/10 deaths in the 180 mg/kg bw group. Observed sublethal effects for the surviving animals included lethargy, salivation, changes in motor activity and lacrimation. The LD50 was determined to be 174 mg/kg bw (REACH).

Dermal

The chemicals are classified as hazardous with the risk phrase ‘Toxic in contact with skin’ (T; R24) in HSIS (Safe Work Australia). The available data (rabbit: LD50 128–380 mg/kg bw; guinea pig: 26 mg/kg bw) support this classification (CICAD, 2008; NIOSH, 1979). Reported signs of toxicity include local effects such as necrosis, oedema, erythema and congestion of capillaries, as well as damage to internal organs (REACH). The low LD50 values in two different animal species indicate that the chemical readily penetrates the skin and may induce systemic toxicity.

Inhalation

The chemicals are classified as hazardous with the risk phrase ‘Very toxic by inhalation’ (T+; R26) in HSIS (Safe Work Australia). The available data (median lethal concentration for 4 hours (LC50) 69–120 ppm, equivalent to 0.19–0.34 mg/litre/4h) support this classification (SCOEL, 2013; REACH).

Reported signs of toxicity include irritation and neurotoxicity. Examination of the deceased animals revealed haemorrhagic rhinitis, proliferative lesions in the bronchioles, pulmonary congestion and pulmonary oedema as well as haemorrhages of the lung, liver, heart and kidneys (SCOEL, 2013).

Corrosion / Irritation

Respiratory Irritation

The chemicals are classified as hazardous with the risk phrase 'Irritating to respiratory system' (Xi; R37) in HSIS (Safe Work Australia). The available human data (see **Observation in Humans**) support this classification.

In a non-guideline study, sensory irritation was quantified by measuring respiratory rate depression upon exposure of B6C3F1 mice to the chemical. The animals were sealed in an airtight vessel and exposed to 5 different concentrations for 10 minutes. The dose resulting in a 50 % decrease in respiratory rate (RD50) was determined to be 4.88 ppm. Little or no recovery was reported (REACH).

The chemicals 2-butenal and acrolein (which are the most abundant a,β-unsaturated aldehydes in cigarette smoke) were also demonstrated to elicit neurogenic inflammatory responses in the airways of guinea pigs exposed to the individual chemicals and cigarette smoke extract itself (Andre et al., 2008).

Skin Irritation

The chemicals are classified as hazardous with the risk phrase 'Irritating to skin' (Xi; R38) in HSIS (Safe Work Australia). Several available study reports suggest that the chemicals may be corrosive. However, these old studies contained methodological deficiencies and were not conducted according to OECD test guidelines. An EU harmonised classification

concluded that the chemical was a skin irritant after consideration of the available data. In the absence of further reliable information, amendment of the existing classification is not warranted.

In a non-guideline study, 0.5 mL of undiluted 2-butenal was applied to the abraded and non-abraded skin of rabbits under occlusive conditions. The test substance was allowed to remain on the skin for 4 hours, then signs of irritation or corrosivity were recorded at 4, 24 and 72 hours after exposure and scored on a graded scale of 0–4. The chemical was classified as corrosive to rabbit skin, with maximum scoring attained. No description of the severity and type of skin effects are reported (REACH).

In another non-guideline study, undiluted chemical on intact rabbit skin for 15 minutes produced severe erythema and oedema after 5–9 hours. Hyperaemia appeared immediately after the skin came into contact with the chemical. After 2–3 days desquamation began, the skin became covered with serous crusts and regions of ulceration were seen. Symptoms on the exposed areas persisted for 12–15 days, then gradually healed towards the end of the observation period (2 months). After 15–17 days, partial detachment of necrotised regions of the ear or complete detachment of its distal portion were observed (REACH). The study results indicated that the chemical was corrosive to rabbit skin.

Eye Irritation

The chemicals are classified as hazardous with the risk phrase 'Risk of serious damage to eyes' (Xi; R41) in HSIS (Safe Work Australia). The available data support this classification.

In an eye irritation study, the chemical was found to cause serious damage to rabbit eyes with volumes of 0.001–0.5 mL of undiluted 2-butenal applied to the cornea. After 24 hours, the observed eye irritation was described as being equal to that of acetic anhydride, which is corrosive. No reversibility data were reported (REACH).

Observation in humans

Humans exposed to the chemical have reported cases of skin, eye and respiratory irritation (CICAD, 2008). The vapour of 2-butenal is so highly irritating to the eyes that people are unable to remain in the presence of dangerous concentrations—at 45 ppm the odour is extremely obnoxious and there is considerable eye discomfort.

In eight instances of industrial corneal injury from 2-butenal, healing is reported to have been complete in 48 hours, although the severity of exposure was not specified (REACH).

In a poorly reported irritation study carried out on volunteers, the chemical was administered to human skin for 10 minutes. Details of the dose (concentration, volume, test conditions) were not provided. Hyperaemia, elevation of skin temperature by 5–7 °C compared to intact regions, localised infiltrate and a sense of pain and burning appeared from the very first seconds of exposure. After 4 days, surface erosion formed with subsequent epithelialisation of the skin under the scab by the end of the month. The chemical was concluded to be corrosive in this study (REACH).

Sensitisation

Skin Sensitisation

Limited data are available.

The chemical was not demonstrated to be sensitising in a dose-dependent contact hypersensitivity test in female B6C3F1 mice. The concentrations of 2-butenal ranged from 0.3 % to 3.0 % in a solution of acetone in olive oil (4:1) for sensitisation and 10 % for the challenge. The mice received 20 µL of the chemical directly on prepared skin for 5 consecutive days. The chemical 2,4-Dinitrofluorobenzene (0.5 % dose) was used as a positive control (REACH; NTP, 1989).

Observation in humans

There are no valid human data available. Subjects in several studies reacted positively to solutions of 2-butenal in patch tests, although methodological deficiencies prevent a classification from being made using this information (REACH; IARC, 1995; MAK, 2012; CICAD, 2008).

A mixture of 7.5 % 2-butenal and 4 % sodium lauryl sulphate was a primary irritant, but was not sensitising in a patch test with 33 subjects (SCOEL, 2013).

Repeated Dose Toxicity

Oral

The chemicals are classified as hazardous with the risk phrase 'Danger of serious damage to health by prolonged exposure if swallowed' (Xn; R48/22) in HSIS (Safe Work Australia). While the data are limited, the available data support this classification.

In a 14-day repeated dose oral toxicity study, groups of male and female SD albino rats were administered the chemical in feed at doses of 0, 22, 44, 88 and 175 mg/kg bw/day. No mortality was observed during the study and no evidence of treatment-related toxicity was observed in any of the parameters examined (REACH).

In a 90-day study, rats and mice (10 animals/sex/group) were gavaged with the chemical in doses of 0, 2.5, 5, 10, 20 and 40 mg/kg bw/day for 5 days/week for 13 weeks (REACH; SCOEL, 2013). There were dose-related increases in mortality and in inflammation of the nasal cavity in rats (but not in mice) at doses of 5 mg/kg bw/day and above, with a no observable adverse effect level (NOAEL) of 2.5 mg/kg bw/day established. Lesions of the forestomach were produced in rats at doses of 10 mg/kg bw/day and above (dose-related) and in mice of the highest dose group. However, these data were only presented in a journal abstract and no other details were provided.

In a chronic study, 23–27 male rats were exposed for 113 weeks to the chemical in the drinking water at concentrations of 0, 0.6 and 6 mmol/L (equivalent to 0, 7.3 and 53.9 mg/kg bw/day). The higher dose resulted in reduced body weight gain, while survival was not affected. Nearly half of the high-dose animals had moderate to severe non-neoplastic liver lesions (fatty metamorphosis, focal necrosis, fibrosis and cholestasis) and all the remaining animals (high and low dose) developed liver cell foci (Chung et al, 1986; SCOEL, 2013).

Dermal

Reliable animal studies on the effects of repeated dermal exposure were not available (SCOEL, 2013).

Inhalation

Reliable animal studies are not available (SCOEL, 2013; CICAD, 2008).

In a non-guideline study, rats were continuously exposed to 1.2 mg/m^3 of 2-butenal for 3 months. Changes in motor activity and blood haemoglobin levels were observed. However, as no pathology or histology studies were undertaken, the data were insufficient to judge the applicability of these results (REACH).

Genotoxicity

The chemicals are classified as Category 3 mutagens with the risk phrase ‘Possible risk of irreversible effects’ (Xn; R68) in HSIS (Safe Work Australia). The available data support an amendment to this classification (refer to **Recommendation** section).

Previous international reports regarding the chemical have concluded that there is a concern for mutagenicity based on the weight of evidence from a range of in vitro and in vivo experiments (IARC, 1995; MAK, 2012; SCOEL, 2013). Indication that the chemical can induce mutations in germ cells was demonstrated by positive results in a sex-linked recessive lethal test in *Drosophila melanogaster*, as well as a positive mouse sperm abnormality test. However, the mouse sperm study did not report

any positive or negative controls to validate the results. Recently, a reliable dominant lethal study via the intraperitoneal (i.p.) route demonstrated that systemically-available 2-butenal could enter germ cells and induce mutations (REACH). The additional data provided by this study provide sufficient evidence for upgrading the hazard classification of these chemicals to Category 2 mutagens, with the risk phrase 'May cause heritable genetic damage' (T; R46) in HSIS (Safe Work Australia).

In vitro studies

The chemical 2-butenal has been found to bind to DNA and induce DNA-protein cross-links in vitro via Michael addition. In a non-guideline study, DNA adducts were observed in calf thymus DNA treated with 1.0 mM solution of the chemical, either directly or with metabolic activation. The adducts that formed were identified as cyclic 1,N^2-propanodeoxyguanosine (REACH). Adducts were also formed in CHO cells (REACH). 'Both the 1- and N^2 positions of guanine are involved in base-pairing, hence the presence of the cyclic adduct may lead to mutations' (IARC, 1995).

In an Ames test conducted similarly to OECD TG 471, 2-butenal was tested at 0.05–0.4 µL per plate for point mutations against *Salmonella typhimurium* strains TA 98, 100, 1535, 1537 and 1538 with or without S9 metabolic activation. The chemical had no mutagenic activity in any of the strains tested using the plate incorporation method. However, when a preincubation method was employed, it was mutagenic in *S. typhimurium* strain TA 100 with and without metabolic activation (REACH; IARC, 1995).

In another Ames test, 2-butenal was tested in *S. typhimurium* strains TA 102 and 104 with and without metabolic activation at concentrations of 0.075–1.4 µmol per plate. Using the preincubation method, the chemical was positive for mutagenicity in TA 104 without metabolic activation and negative in TA 102 (REACH; IARC, 1995).

In a non-guideline intrasanguineous mouse host-mediated assay, 2-butenal was administered orally (gavage) to CD-1 mice (0.009–0.094 mg/kg bw) during simultaneous intravenous injection of *S. typhimurium* TA 100. The chemical was found to be mutagenic, with a three-fold increase in revertants of TA 100 recovered from mouse blood compared to the control, at a dose of 0.032 mg/kg bw (REACH; CICAD, 2008; MAK, 2012).

In a sister chromatid exchange assay in mammalian cells conducted similarly to OECD TG 479, 2-butenal was tested in Chinese hamster ovary (CHO) cells. The results were positive from 0.5 µg/mL and above without activation (dose range tested: 0.16–1.6 µg/mL), and positive from 1.6 µg/mL with S9 metabolic activation (dose range tested: 1.6–160 µg/mL) (REACH). Positive results were also observed in other sister chromatid exchange studies carried out on human blood lymphocytes and lymphoblastoid Namalva cells (REACH).

In a mammalian chromosome aberration assay conducted similarly to OECD TG 473, 2-butenal was tested in CHO cells with positive results from 1.6 µg/mL onwards without metabolic activation (dose range tested: 0.5–5 µg/mL) and positive at the highest dose tested (16 µg/mL) with S9 metabolic activation (dose range tested: 1.6–16 µg/mL) (REACH). In another chromosome aberration study in human blood lymphocytes and lymphoblastoid Namalva cells (dose range tested: 5–250 µM), increased micronuclei were observed from 200 µM and above for lymphocytes, and from 100 µM and above for Namalva cells (REACH).

In a SOS-Chromotest, DNA repair functions were induced in *Escherichia coli* PQ37 using ethanol as a solvent instead of dimethyl sulfoxide (DMSO). A weak SOS result was obtained using the *S. typhimurium* strain TA1535/pSK1002 without metabolic activation (IARC, 1995; SCOEL, 2013; CICAD, 2008).

The chemical 2-butenal has been tested for mutagenic activity in several other in vitro assays, including DNA damage and repair assays in mammalian and bacterial cells. Positive results were obtained in primary rat epithelial cells (stomach and colon). However, in a test conducted similarly to OECD TG 482, no unscheduled DNA synthesis was observed in a single DNA repair test in rat hepatocytes (REACH).

In vivo studies

In a study conducted similarly to OECD TG 475, chromosomal aberrations were observed in mouse bone marrow cells after 12 hours when the animals were administered a single dose of the chemical (8, 16, 32, or 200 µL/kg bw) by i.p. injection (REACH).

In a non-guideline study, 2-butenal was found to covalently bind to DNA and form cyclic DNA adducts in the dermis of Sencar mouse skin after topical application of the chemical (total dose 1.4 mmol, 98 mg) five times per week for three weeks (IARC, 1995; MAK, 2012). No background adducts were found in the skin of untreated mice. Systemic availability of the chemical was demonstrated by increased numbers of DNA adducts in the liver, lung and kidneys of rats after administration of 2-butenal at high doses via gavage (IARC, 1995; MAK, 2012).

In a study conducted similarly to OECD TG 477, sex-linked recessive lethal mutations and reciprocal translocations were induced in *D. melanogaster* injected with a single dose of 2-butenal at 3500 ppm (IARC, 1995; REACH). In another study, 2-butenal (4000 ppm) was administered to *D. melanogaster* via oral feeding, although the chemical was not found to be mutagenic after three days.

In a study conducted similarly to OECD TG 483, 2-butenal induced chromosomal damage in the spermatogonia of mice after oral administration in drinking-water or by i.p. injection. Special meiotic anomalies, such as degenerated cell nuclei, multispindle cells, polyploids and sperm anomalies were observed. However, no positive and negative controls were reported, rendering this study inadequate for the evaluation of germ cell mutagenicity (IARC, 1995; MAK, 2012; REACH). In another study conducted similarly to OECD TG 478, dominant lethal frequencies increased with dose (8, 16 or 32 µL/kg bw) in a mouse study following i.p. administration (REACH).

Carcinogenicity

Limited data are available. The available data do not warrant hazard classification.

The International Agency for Research on Cancer (IARC) has classified the chemical as 'Not classifiable as to its carcinogenicity to humans' (Group 3) (IARC, 1995) based on inadequate evidence for carcinogenicity in humans and animals.

In a single, non-guideline study, the trans isomer (E-2-butenal, CAS No. 123-73-9) was administered to male Fischer 344 (F344) rats (23–27 animals/group) in drinking water at 0, 0.6 or 6.0 mM (equivalent to 0, 7.3 and 53.9 mg/kg bw/day) for 113 weeks (Chung et al., 1986). There were statistically significant increases in the incidence of hepatocellular neoplasms (including neoplastic nodules and hepatocellular carcinomas) in the low dose group. The incidences were 0/23, 9/27 and 1/23 in the control, low- and high-dose groups, respectively. The incidences of hepatocellular carcinomas alone were 0/23, 2/27 and 0/23, respectively. The incidences of enzyme-altered liver foci, which are considered precursors of neoplasms, were 1/23, 23/27 and 13/23 in the control, low- and high-dose groups, respectively. The increased incidences in both the low- and high-dose groups were statistically significant relative to controls. The lower incidence of neoplastic and preneoplastic lesions at the higher dose compared with the higher dose was not explained. However, the study was only carried out on a single sex and only using two doses. In addition, the incidence of tumours did not appear to be dose-related (IARC; Chung et al., 1986).

The systemic availability and genotoxicity of 2-butenal in vitro and in vivo (see **Genotoxicity**) suggest that this chemical can play a role in human carcinogenesis. However, the limited information is not sufficient to warrant hazard classification.

Reproductive and Developmental Toxicity

In a one-generation reproductive toxicity study, no reproductive effects were seen at the doses tested. The available information does not meet the criteria for hazard classification in regards to reproductive toxicity.

In a one-generation reproductive toxicity study carried out similarly to OECD TG 415, male and female F344 rats were treated with the chemical (0, 2.5, 5 and 10 mg/kg bw/day) by gavage daily until sacrifice. Males were dosed for 61 days prior to breeding, and females were dosed 31 days prior to breeding. There were no notable clinical observations with regards to gonadal function, mating behaviour or fertility in either male or female rats. A NOAEL of 10 mg/kg bw/day for both sexes was established for reproductive effects (REACH).

In another study, a single i.p. injection of 2-butenal (0, 8, 16 or 32 µL/kg bw, corresponding to 0, 6.8, 13.7 and 27.2 µg/kg bw) was administered to male Swiss albino mice. A statistically significant increase in the percentage of abnormal sperm heads was recorded at 16 and 32 µL/kg bw at 3 weeks, and at only the highest dose at 5 weeks. However, there were methodological deficiencies in this study, and the route of exposure is not appropriate for humans (REACH).

Other Health Effects

Neurotoxicity

Limited data are available.

Evidence of 2-butenal-protein adducts has been found in the human brain. Using a specific antibody against these adducts, it was shown that the number of protein-bound 2-butenal-immunoreactive cells in the grey matter was larger in patients with Alzheimer's disease than in controls. It was suggested that increased oxidative stress and 2-butenal formation in glial cells is implicated in the pathogenesis of Alzheimer's disease (Kawaguchi-Niida, 2006).

Risk Characterisation

Critical Health Effects

The critical health effects for risk characterisation include systemic long-term effects (mutagenicity) and systemic acute effects (acute toxicity from oral, dermal and inhalation exposure). The chemicals can also cause harmful effects following repeated exposure if swallowed, serious damage to eyes and irritation to the skin and respiratory system.

Public Risk Characterisation

The chemicals in this group have reported use overseas as fragrance additives. However, due to their highly pungent, suffocating odour, it is expected that only very low concentrations are used. Given the main uses identified for the chemicals are commercial and site-limited uses, it is unlikely that the public will be exposed at levels that warrant concern. Hence, the public risk from these chemicals is not considered to be unreasonable.

Occupational Risk Characterisation

During product formulation, dermal, ocular and inhalation exposure may occur, particularly where manual or open processes are used. These could include transfer and blending activities, quality control analysis, and cleaning and maintaining equipment. Worker exposure to the chemicals at lower concentrations could also occur while using formulated products containing the chemicals. The level and route of exposure will vary depending on the method of application and work practices employed. Oral exposure is also possible but can be prevented by good hygiene practices.

Given the critical systemic long-term, systemic acute and local health effects, the chemicals could pose an unreasonable risk to workers unless adequate control measures to minimise dermal, ocular and inhalation exposure are implemented. The chemicals should be appropriately classified and labelled to ensure that a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) has adequate information to determine the appropriate controls.

The data available support an amendment to the hazard classification in the HSIS (Safe Work Australia) (refer to **Recommendation** section).

NICNAS Recommendation

Assessment of these chemicals is considered to be sufficient, provided that the recommended amendment to the classification is adopted, and labelling and all other requirements are met under workplace health and safety and poisons legislation as adopted by the relevant state or territory.

Regulatory Control

Work Health and Safety

The chemicals are recommended for classification and labelling under the current approved criteria and adopted GHS as below. This assessment does not consider classification of physical and environmental hazards.

Hazard	Approved Criteria (HSIS)[a]	GHS Classification (HCIS)[b]
Acute Toxicity	Toxic if swallowed (T; R25)* Toxic in contact with skin (T; R24)* Very toxic by inhalation (T+; R26)*	Toxic if swallowed - Cat. 3 (H301) Toxic in contact with skin - Cat. 3 (H311) Fatal if inhaled - Cat. 1 (H330)
Irritation / Corrosivity	Risk of serious eye damage (Xi; R41)* Irritating to skin (Xi; R38)* Irritating to respiratory system (Xi; R37)*	Causes serious eye damage - Cat. 1 (H318) Causes skin irritation - Cat. 2 (H315) May cause respiratory irritation - Specific target organ tox, single exp Cat. 3 (H335)
Repeat Dose Toxicity	Harmful: danger of serious damage to health by prolonged exposure if swallowed (Xn; R48/22)*	May cause damage to organs through prolonged or repeated exposure - Cat. 2 (H373)
Genotoxicity	Muta. Cat 2 - May cause heritable genetic damage (T; R46)	May cause genetic defects - Cat. 1B (H340)

[a] Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)].

[b] Globally Harmonized System of Classification and Labelling of Chemicals (GHS) United Nations, 2009. Third Edition.

[*] Existing Hazard Classification. No change recommended to this classification

Advice for industry

Control measures

Control measures to minimise the risk from oral, dermal, ocular and inhalation exposure to the chemicals should be implemented in accordance with the hierarchy of controls. Approaches to minimise risk include substitution, isolation and engineering controls. Measures required to eliminate, or minimise risk arising from storing, handling and using a hazardous chemical depend on the physical form and the manner in which the chemicals are used. Examples of control measures that could minimise the risk include, but are not limited to:

- using closed systems or isolating operations;
- using local exhaust ventilation to prevent the chemicals from entering the breathing zone of any worker;
- health monitoring for any worker who is at risk of exposure to the chemicals, if valid techniques are available to monitor the effect on the worker's health;
- air monitoring to ensure control measures in place are working effectively and continue to do so;
- minimising manual processes and work tasks through automating processes;
- work procedures that minimise splashes and spills;
- regularly cleaning equipment and work areas; and
- using protective equipment that is designed, constructed, and operated to ensure that the worker does not come into contact with the chemicals.

Guidance on managing risks from hazardous chemicals are provided in the *Managing risks of hazardous chemicals in the workplace—Code of practice* available on the Safe Work Australia website.

Personal protective equipment should not solely be relied upon to control risk and should only be used when all other reasonably practicable control measures do not eliminate or sufficiently minimise risk. Guidance in selecting personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

Obligations under workplace health and safety legislation

Information in this report should be taken into account to help meet obligations under workplace health and safety legislation as adopted by the relevant state or territory. This includes, but is not limited to:

- ensuring that hazardous chemicals are correctly classified and labelled;
- ensuring that (material) safety data sheets ((M)SDS) containing accurate information about the hazards (relating to both health hazards and physicochemical (physical) hazards) of the chemical are prepared; and
- managing risks arising from storing, handling and using a hazardous chemical.

Your work health and safety regulator should be contacted for information on the work health and safety laws in your jurisdiction.

Information on how to prepare an (M)SDS and how to label containers of hazardous chemicals are provided in relevant codes of practice such as the *Preparation of safety data sheets for hazardous chemicals—Code of practice* and *Labelling of workplace hazardous chemicals—Code of practice*, respectively. These codes of practice are available from the Safe Work Australia website.

A review of the physical hazards of these chemicals have not been undertaken as part of this assessment.

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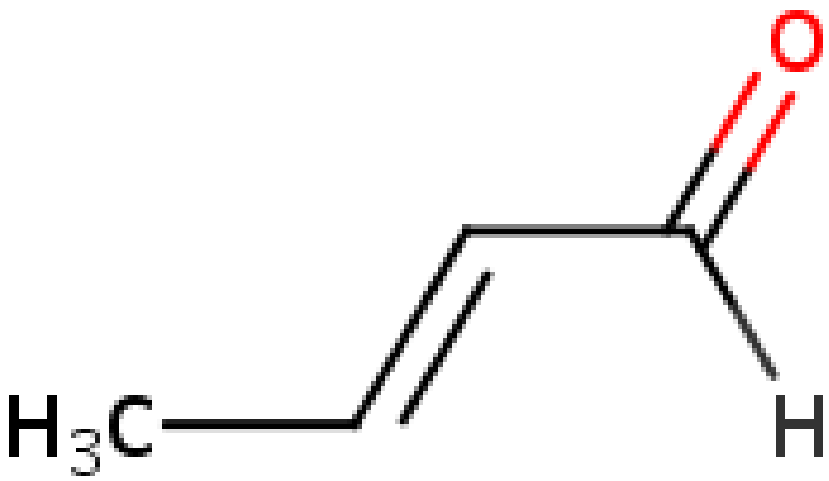
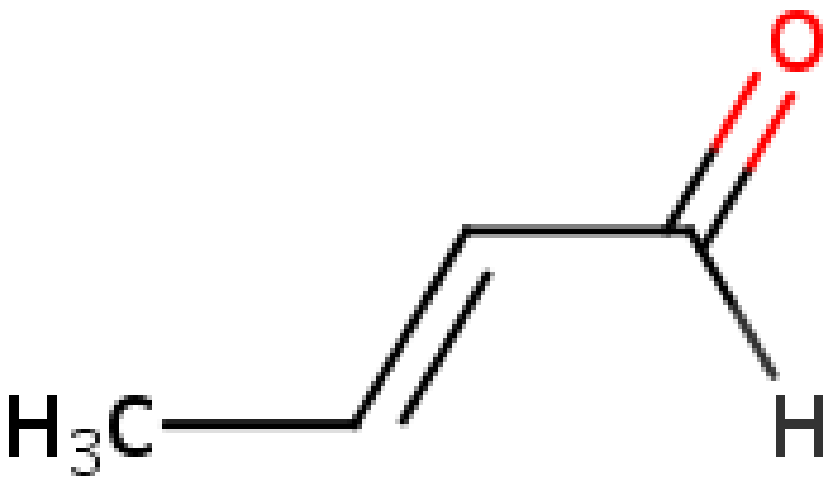
Last Update 05 February 2016

Chemical Identities

Chemical Name in the Inventory and Synonyms	**2-Butenal, (E)-** crotonaldehyde, (E)- trans-2-butenal trans-crotonaldehyde
CAS Number	123-73-9
Structural Formula	

	
Molecular Formula	C4H6O
Molecular Weight	70.09

Chemical Name in the Inventory and Synonyms	**2-Butenal** crotonaldehyde 2-butenaldehyde crotonal crotylaldehyde propylene aldehyde
CAS Number	4170-30-3
Structural Formula	

	
Molecular Formula	C4H6O
Molecular Weight	70.09

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Recommendation from the Scientific Committee on Occupational Exposure Limits for 2-Butenal

SCOEL/SUM/180
March 2013

Employment, Social Affairs and Inclusion

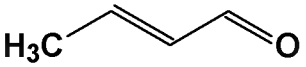
Table of Contents

Recommendation from the Scientific Committee on Occupational Exposure Limits for 2-Butenal

8-hour TWA:	-
STEL (15-min):	-
Additional classification:	Skin notation

This evaluation is based on ACGIH (2001), BUA (1993), DFG (2005), ECB (2000), IARC (1995), AEGL (2007) and IPCS (2008) and the references cited in these reviews as well as additional references from database searches (the final search performed in February 2013). As most of the available studies were performed with commercial 2-butenal, which consists of about 95 % *trans*-2-butenal and 5 % *cis*-2-butenal, the recommendation applies to both the pure *trans* isomer and the mixture of isomers.

1. Substance identification, physico-chemical properties

Chemical name:	2-Butenal
Synonyms:	But-2-enal; 2-butenaldehyde; crotonaldehyde; crotonic aldehyde; ß-methylacrolein, ß-methylacrolein; 1-formylpropene
Molecular formula:	C_4H_6O
Structural formula:	
EC No.:	224-030-0 (mixed isomers) 204-647-1 (*trans* isomer)
CAS No.:	4170-30-3 (mixed isomers) 123-73-9 (*trans* isomer) 15798-64-8 (*cis* isomer)
Annex I Index No.:	605-009-00-9
Molecular weight:	70.09 g/mol
Conversion factors: (20 °C, 101.3kPa)	1 ppm = 2.92 mg/m^3 1 mg/m^3 = 0.343 ppm

EU classification:

Flam. Liq. 2	H225	Highly flammable liquid and vapour
Muta. 2	H341	Suspected of causing genetic defects
Acute Tox. 2	H330	Fatal if inhaled
Acute Tox. 3	H311	Toxic in contact with skin
Acute Tox. 3	H301	Toxic if swallowed
STOT RE 2	H373	May cause damage to organs through prolonged or repeated exposure
STOT SE 3	H335	May cause respiratory irritation
Skin Irrit. 2	H315	Causes skin irritation
Eye Dam. 1	H318	Causes serious eye damage
Aquatic Acute 1	H400	Very toxic to aquatic life

2-Butenal is a colourless liquid with a pungent, suffocating odour. It is an α,β-unsaturated aldehyde and consequently a very reactive compound. The boiling point of the substance is 101–105 °C, and the vapour pressure is 25–43 hPa at 20 °C. The water solubility of 2-butenal is 150–181 g/l at 20 °C and the calculated log P_{OW} is 0.63. The substance has a flash point of 12.8 °C (open cup) and a density of 0.850–0.856 g/cm^3 (ACGIH 2001, ECB 2000, IARC 1995, IPCS 2008).

2. Occurrence/use and occupational exposure

In the past, 2-butenal has been used mainly in the manufacture of 2-butanol, but this process has been mostly replaced by other technical syntheses. 2-Butenal has also been used in the preparation of rubber accelerators, in leather tanning, as a denaturant of ethyl alcohol, as a warning agent in fuel gases and to detect leaks in pipes. Currently, the most extensive use of 2-butenal is as an intermediate in the synthesis of sorbic acid and crotonic acid. 2-Butenal is formed during incomplete combustion and pyrolysis of organic substances, in particular during combustion of fuels in gasoline- and diesel-powered engines, wood combustion, and tobacco smoking. 2-Butenal is produced endogenously and occurs naturally in many plants, foods and beverages (Eder and Budiawan 2001, IARC 1995, IPCS 2008). Low amounts of 2-butenal has been reported, along with a variety of other aldehydes, in settled dust from indoor residences (around 1 µg/g dust) (Nilsson *et al* 2005).

3. Health significance

3.1. Toxicokinetics

2-Butenal is formed endogenously during lipid peroxidation and forms protein and DNA adducts in animals and humans (IPCS 2008).

3.1.1. Human data

2-Butenal-protein adducts have been found in the brains of patients with Alzheimer's disease (Kawaguchi-Niida *et al* 2006) and in human skin (Hirao and Takahashi 2005). 2-Butenal–DNA adducts have been detected in human liver (Nath & Chung 1994), leukocytes and mammary glands (Nath *et al* 1996), and in oral tissues (Chung *et al* 1999). 2-Butenal has been detected in human milk (AEGL 2007).

3.1.2. Animal data

No data concerning the inhalation or dermal route were available. However, low dermal LD_{50} values indicate significant skin absorption (Section 3.2.2). After oral exposure of rats to carbon-14 labelled 2-butenal in doses of 0.7–35 mg/kg, over 90 % of the substance was absorbed and rapidly metabolised; 60–78 % of the radioactivity was excreted in urine and breath within 12 hours of dosing, and after 72 hours, this increased to 82–86 %. Approximately 7 % was eliminated via faeces (ECB 2000, AEGL 2007). Following intravenous injection, 40 % of the dose was eliminated within 6 hours in urine, 33 % in exhaled air (as CO_2) and < 1 % in faeces. The metabolites were not identified, the urine contained traces only of 2-butenal and 2-butenoic acid (ECB 2000, DFG 2005).

2-Butenal is suspected to be metabolised mainly in the liver by oxidation to 2-butenoic acid, which is further degraded in the fatty acid metabolism. 2-Butenal reacts *in vitro* with cellular thiol groups in proteins and glutathione. After subcutaneous injection in rats, 3-hydroxy-methyl-propylmercapturic acid (6–15 % of the administered dose of 53 mg/kg) and small amounts of 2-carboxyl-1-methyl-propylmercapturic acid were the metabolites identified in the urine (ECB 2000, DFG 2005).

DNA and protein adducts have been found endogenously and after exogenous administration of 2-butenal in almost all investigated tissues (skin, liver, lung, kidney, intestinal epithelial cells) from rats and mice (Nath & Chung 1994, Eder *et al* 1996, 1999, Nath *et al* 1996).

3.1.3. Biological monitoring

There were no data available.

3.2. Acute toxicity

3.2.1. Human data

No reports on acute intoxications were available. The strong odourous and irritative properties of 2-butenal may limit exposure to higher concentrations, thereby avoiding other toxic effects (Henschler 1981).

3.2.2. Animal data

The inhalation LC_{50} (4 hours) in rats was 69–100 ppm. Acute inhalation of high concentrations produced signs of irritation and neurotoxicity. Deceased animals revealed haemorrhagic rhinitis, proliferative lesions in the bronchioles, pulmonary congestion and pulmonary oedema as well as haemorrhages of the lung, liver, heart and kidneys (BUA 1993, Rinehart 1967).

The oral LD_{50} values were 206–300 mg/kg in rats and about 100 mg/kg in mice. The dermal LD_{50} was 128–170 mg/kg in rabbits and 25 mg/kg in guinea pigs (BUA 1993, ECB 2000).

3.2.3. In vitro data

The gene expression profile and cytotoxicity of normal human bronchial epithelial cells was examined after exposure to 2-butenal at 40 or 80 µM for 3 or 6 hours using microarrays technology. The gene expression analyses revealed that several biological processes representing cytotoxicity and tissue injury were dysregulated, including inflammatory responses, exogenous metabolism, cell cycle, heat shock responses and antioxidant responses (Liu *et al* 2010a).

Another study with human bronchial epithelial cells performed by the same group showed that 2-butenal at 10–120 µM caused decreases of intracellular reduced glutathione levels and increases of reactive oxygen species in a dose-dependent manner. 2-Butenal induced cell death by apoptosis, which gradually transitioned to necrosis at higher concentrations. Additional studies suggested that the 2-butenal-induced apoptosis was activated in a caspase-dependent way (Liu *et al* 2010b).

3.3. Irritation and corrosivity

3.3.1. Human data

The odour threshold (detection) of 2-butenal is in the range of 0.035–0.2 ppm. Human studies on odour and irritation are summarised in Table 1.

Sim and Pattle (1957) exposed 12 volunteers to 4.1 ppm 2-butenal. After 30 sec of exposure, lacrimation appeared, but the eye irritation did not increase with increasing exposure duration. At 15 min exposure duration, the substance was highly irritating to all exposed mucosal surfaces, especially those of the nose and upper respiratory tract. The activity levels of the test subjects were not provided and there was co-exposure to cigarette smoke. In a study by Rinehart (1967), cited by AEGL (2007), inhalation exposure of 2–3 volunteers to 45 ppm was very disagreeable within less than 30 sec and caused conjunctival irritation. Exposure to a concentration of 15 ppm for up to 30 sec was detectable (strong odour), but not irritating to the eyes. Fannick (1982) studied the effects in workers exposed to a mean of 0.56 ppm (range < 0.35–1.1 ppm) 2-butenal for < 8 hours and reported occasional minor eye irritation. The workers

Table 1. Human data on odourous and irritative properties of 2-butenal (adapted from AEGL 2007).

Exposure level (ppm)	Exposure duration	Effects	References
0.035–0.2 0.037–1.05 0.12	Undefined (few seconds)	Odour threshold. Secondary sources, descriptions of most original studies unavailable.	Verschueren 1996, Ruth 1986, Amoore and Hautala 1983
0.038	Undefined (few seconds)	Subjects exposed multiple times. Roughly half of them detected odour at this level.	Tepikina *et al* 1997
0.17	1 min	Odour detection and/or irritation, exposure via mask, undefined analytical method.	Trofimov 1962
0.56	< 8 hours	Occasional eye irritation, concentration up to 1.1 ppm, co-exposure to other chemicals.	Fannick 1982
4.1	15 min	Marked respiratory irritation, lacrimation after 30 sec, co-exposure to cigarette smoke.	Sim and Pattle 1957
3.5–14	Undefined	Irritation sufficient to wake a sleeping person.	Fieldner *et al* 1954
3.8	10 sec	"Irritating within 10 sec"; no further details.	
7.3	Undefined (seconds?)	Very sharp odour and strong irritation to the eye and nose; no experimental details.	Dalla *et al* 1939
8 14 (nose) 19 (eyes)	Undefined (few seconds)	Irritation threshold; methods used to determine or define "irritation" not given.	Ruth 1986, Amoore and Hautala 1983
15	<30 sec	Lab workers "sniffed" 2-butenal. Odour strong but not intolerable; no eye discomfort.	Rinehart 1967
45–50		Odour strong, pungent, and dis-agreeable. Burning eye sensation but no lacrimation.	

were exposed to other chemicals (e.g. acetic acid and acetaldehyde, but 2-butenal was likely the most irritant among these chemicals (AEGL 2007). Trofimov (1962) reported a threshold for mucosal irritation in humans of 0.17 ppm. In this experiment, volunteers inhaled 2-butenal vapour through a mask for 1 min; it was not specified how the vapour was generated or how the concentrations were measured. Factors taken into account were odour detection and irritation of the eyes and mucous membranes of the nose and trachea; it was not specified on which of these endpoints the estimated irritation threshold was actually based (AEGL 2007). Amoore and Hautala (1983) reported irritation thresholds of 14 ppm and 19 ppm for nose and eyes, respectively. The irritation threshold was 8 ppm in a study by Ruth 1986, cited by AEGL 2007.

A mixture of 7.5 % 2-butenal and 4 % sodium lauryl sulphate was a primary irritant in an aluminium patch test in 19 of the 33 test persons (Coenraads *et al* 1975). Dermal exposure to 0.12 % 2-butenal in plant oil (24 hours) was irritating to the human skin (Bainova and Madzhunov 1984).

There are 8 case reports of corneal injury due to exposure to unknown amounts of liquid 2-butenal. Healing was complete within 48 hours (ACGIH 2001).

3.3.2. Animal data

Skin
Dermal exposure of rabbit skin to 2-butenal produced irritation and inflammation (ECB 2000).

Eyes
2-Butenal was highly irritating to the rabbit eye, causing severe damage (ECB 2000).

Respiratory tract
The RD_{50} values (concentrations causing a 50 % depression of the respiratory rate due to sensory irritation of the respiratory tract) in Swiss Webster and B6C3F1 mice were 3.5 ppm and 4.9 ppm, respectively. The RD_{50} in F-344 rats was 23.2 ppm (Steinhagen and Barrow 1984, Schaper 1993). Trofimov (1962) reported a threshold for mucosal irritation in rabbits and cats of 17 ppm and 3.1 ppm, respectively.

André *et al* (2008) found that aqueous extracts of cigarette smoke (CSE), 2-butenal, and acrolein all mobilised Ca^{2+} in cultured guinea pig jugular ganglia neurons and promoted contraction of isolated guinea pig bronchi in a similar fashion. The responses were abolished by a TRPA1-selective antagonist and by the aldehyde scavenger glutathione but not by the TRPV1 antagonist capsazepine or by ROS scavengers. Treatment with CSE or aldehydes increased Ca^{2+} influx in TRPA1-transfected cells, but not in control HEK293 cells, and promoted neuropeptide release from isolated guinea pig airway tissue. The effect of CSE and aldehydes on Ca^{2+} influx in dorsal root ganglion neurons was abolished in TRPA1-deficient mice. The results indicate the aldehydes as the main causative agents in cigarette smoke that cause neurogenic inflammation via TRPA1 stimulation.

3.4. Sensitisation

3.4.1. Human data

One case of allergic dermatitis is known. This person was occupationally exposed to dimethoxane, which hydrolyses to 2-butenal. A patch test revealed a positive reaction 72 hours following dermal exposure to a 1 % solution of 2-butenal in water or olive oil. Exposure to a 0.1 % solution did not provoke a reaction (Shmunes and Kempton 1980).

A mixture of 7.5 % 2-butenal and 4 % sodium lauryl sulphate was a primary irritant, but was not sensitising in a patch test with 33 subjects (Coenraads *et al* 1975).

3.4.2. Animal data

A study regarding the sensitising properties of 2-butenal by NTP is completed (NTP 2012). According to other authors (BUA 1993, ECB 2000; without further details), the result of this study is "not sensitising".

3.5. Repeated dose toxicity

3.5.1. Human data

Human data on the effects of repeated exposure were not available.

3.5.2. Animal data

Inhalation

Valid animal studies on the effects of repeated inhalation exposure were not available. There is a poorly reported study by Voronin *et al* (1982), indicating alterations of motor activity and blood haemoglobin content of rats and mice continuously exposed to concentrations of 1.2 mg/m^3 (0.4 ppm) and above for 3 months.

Oral

Rats and mice (10 animals per sex and group) were gavaged with 2-butenal in doses of 0, 2.5, 5, 10, 20 and 40 mg/kg/day on 5 days/week for 13 weeks (Wolfe *et al* 1987). There was a dose-related increase in mortality and inflammation of the nasal cavity in rats (but not in mice) at doses of 5 mg/kg/day and above (NOAEL 2.5 mg/kg/day). Lesions of the forestomach were produced in rats at doses of 10 mg/kg/day and above (dose-related) and in mice of the highest dose group. These data are only presented as an abstract.

Chung *et al* (1986) exposed 23–27 male rats for 113 weeks to 2-butenal in the drinking water at concentrations of 0, 0.6 and 6 mmol/l (42 and 421 mg/l). The higher dose produced reduced body weight gain, while survival was not affected. Nearly half of the high-dose animals had moderate to severe non-neoplastic liver lesions (fatty metamorphosis, focal necrosis, fibrosis and cholestasis) and all the remaining animals (high and low dose) developed liver cell foci (see Section 3.7.2).

Dermal

Valid animal studies on the effects of repeated dermal exposure were not available.

3.6. Genotoxicity

3.6.1. In vitro

2-Butenal induced forward and reverse mutations in bacteria (*Salmonella typhimurium* strains TA100, TA104, BA9) with and without metabolic activation, but only when a preincubation method or the liquid suspension technique was used. Plate incubation protocols yielded negative results.

There was no mutagenic response in the SOS chromotest in *Escherichia coli* PQ37 and PQ243 (DFG 2005, IARC 1995). However, when ethanol was used as solvent instead of DMSO, 2-butenal was clearly positive (PQ37). A weak SOS response was seen in *S. typhimurium* TA1535/pSK1002 without metabolic activation (IPCS 2008, AEGL 2007).

Exposure of primary human lymphocytes or Namalva (Burkitt's lymphoma) cells resulted in increases of sister chromatid exchanges, chromosomal aberrations and micronuclei (Dittberner *et al* 1990).

In Chinese hamster ovary (CHO) hamster cells *in vitro*, the substance produced sister chromatid exchanges and chromosomal aberrations with or without metabolic transformation (Galloway *et al* 1987), but no gene mutations in a HPRT test (Foiles *et al* 1990).

Incubation of rat colon mucosa cells with 2-butenal resulted in DNA damage in the comet assay (Gölzer *et al* 1996). 2-Butenal did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes (Williams *et al* 1989).

The substance bounds covalently to DNA of *E. coli* HB101pUC13, to calf thymus DNA and to DNA of CHO cells or human fibroblasts *in vitro*, forming cyclic adducts with deoxyguanosine (DFG 2005, ECB 2000). 1,N^2-Propano-deoxyguanosine adducts (which are produced as the main adducts also after *in vivo* exposure of animals) caused mutations in mammalian cells with a yield of about 5 %, when they were incorporated in DNA plasmids and transfected into COS-7 monkey kidney cells (Fernandes *et al* 2005). These adducts also inhibited DNA synthesis and were mutagenic after incorporation into DNA vectors and transfection into human xeroderma pigmentosum cells (Stein *et al* 2006). In addition, 1,N^2-propano-deoxyguanosine adducts were capable of forming DNA crosslinks (Kozekov *et al* 2003, Liu *et al* 2006) or DNA-protein crosslinks *in vitro* (Kurtz and Lloyd 2003). Hecht *et al* (2001a,b) and Wang *et al* (2001) described the formation of several other minor adducts to deoxyguanosine after reaction of 2-butenal with calf thymus DNA.

Using the mouse lymphoma cells, Demir *et al* (2011) found that 2-butenal induced increased mutant frequencies at concentrations of 50 µM in the first experiment and 25 µM in the second.

3.6.2. In vivo – Human data

Zhang *et al* (2006) isolated adducts of 2-butenal with deoxyguanosine (1,N^2-propano-deoxyguanosine) from DNA of humans (not occupationally exposed to 2-butenal). These adducts were more frequently detected in lung DNA than in liver DNA and were not detectable in DNA from blood.

Nath *et al* (1998) found higher levels (5.5- to 8-fold) of 2-butenal-DNA adducts in gingival tissue DNA from smokers compared to non-smokers (not occupationally exposed to 2-butenal).

3.6.3. In vivo – Animal data

A host mediated assay in CD1 mice with a single oral exposure of the animals to 8–80 mg/kg 2-butenal and simultaneous injection of *S. typhimurium* TA100 yielded a positive finding (Jagannath 1980). Oral exposure of mice (doses of 0.8–80 mg/kg, administered twice) did not induce chromosomal damage in the bone marrow micronuclei test (Mayer *et al* 1980). Oral exposure (1 month in drinking water at concentrations of 200 mg/l) or a single intraperitoneal injection (30 mg/kg) produced chromosomal damage in all stages of spermatogenesis and special meiotic anomalies in mice (Auerbach *et al* 1977, Moutschen-Dahmen *et al* 1975). Abnormal sperm heads, indicative of genotoxicity, were observed by Jha and Kumar (2006) in mice after a single intraperitoneal injection. The effect reached statistical significance 1 and 3 weeks after exposure at doses over 16 µl/kg and 5 weeks after exposure at the highest dose of 32 µl/kg.

A single oral high dose of 200 or 300 mg/kg 2-butenal caused an increase in DNA adducts in rat liver cells (about 3 adducts/10^8 nucleotides of cyclic 1,N^2-propane-deoxyguanosine adducts, 20 hours after exposure). Lower amounts of adducts were detected in lung, kidney and large intestine. Repeated gavage to rats in doses of 1 and 10 mg/kg/day (30 applications within 6 weeks) produced a dose-dependent increase in these DNA adducts in liver cells (2.1 and 6.3 adducts /10^8 nucleotides 20 hours after the last exposure). The adducts persisted partially and declined within 15 days to about 20 % of the level detected 20 hours post-exposure (Eder *et al* 1996, 1999, Eder and Budiawan 2001). The same kind of adducts were also detected in DNA of the skin of mice treated dermally with 2-butenal at doses of 300 mg/kg (IARC 1995).

The genotoxicity of 2-butenal was evaluated by employing bone marrow and spermatocyte chromosomal aberration and dominant lethal mutation assays in Swiss albino mice. Single intraperioneal doses of 2-butenal (8, 16 and 32 µl/kg bw) in olive oil caused dose-dependent increases in percentage aberrant metaphases in bone marrow cells. At the same doses, a dose-dependent increase in chromosomal aberrations was also seen in spermatocytes from male mice given the same doses. A lethal mutation study was performed with males given the same doses as above once daily for 5 days and then mated with untreated females. The treatment resulted in significant decreases in fertility indices, total number of implants and number of live implants per female, and increased number of dead implants per female. The percentage dominant lethal mutations increased with the dose (Jha *et al* 2007).

3.7. Carcinogenicity

3.7.1. Human data

A study by Bittersohl (1974) reported 9 malignant tumours (2 squamous cell carcinomas of the oral cavity, one adenocarcinoma of the stomach, one adenocarcinoma of the caecum and 5 squamous cell tumours of the lung) among 150 workers exposed to concentrations of 1–7 mg/m^3 (0.3–2.4 mg/m^3) 2-butenal for 20 years. All cases were smokers. There was also exposure to acetaldehyde, butyraldehyde and higher aldehydes, to *n*-butanol and higher alcohols and possibly also to butadiene.

3.7.2. Animal data

Chung *et al* (1986) exposed 23–27 male rats for 113 weeks to 2-butenal via the drinking water in concentrations of 0, 42 and 421 mg/l. Survival was not affected in any group. The incidence of hepatocellular carcinomas was 0/23, 2/27 and 0/23, and neoplastic nodules in the liver were found in 0/23, 9/27 (significant increase) and 1/23 in the control, lower and higher dose group, respectively. Liver cell foci (according to the authors precursors of hepatocellular neoplasms) were found in 1/23 controls, in 23/27 at the low dose and in 13/23 at the high dose. The increase in exposed groups was significantly different from controls but not dose-related. Ten of the high-dose animals had moderate to severe non-neoplastic liver lesions, but none of these animals developed preneoplastic lesions or tumours. The remaining 13 animals were found to have the liver cell foci without further liver lesions. The authors considered these foci as preneoplastic, however, the observed foci were mainly of the eosinophilic type. Basophilic hepatocellular foci are generally considered to be putative preneoplastic, whereas foci of the eosinophilic type are not.

When neonatal B6C3F1 mice were injected intraperitoneally with total doses of 1.5 or 3 µmol (105 or 210 mg, split on days 8 and 15), there was no significant increase in liver tumours at 12–15 months of age (von Tungeln *et al* 2002). The authors suggested that this assay is not sensitive enough to detect carcinogens that induce an increase in endogenous DNA adduct formation through lipid peroxidation or oxidative stress.

In its evaluation of 2-butenal, IARC concluded that the available data were too limited to form the basis for an evaluation of the carcinogenicity to humans. The increased incidences of hepatic neoplastic nodules and altered liver-cell foci seen in the male rat drinking water study were not dose-related. The overall evaluation was Group 3, i.e. not classifiable as to its carcinogenicity to humans (IARC 1995).

3.8. Reproductive toxicity

3.8.1. Human data

Human data on reproductive or developmental effects were not available.

3.8.2. Animal data

Fertility

Oral exposure (one month in drinking water at concentrations of 200 mg/l or a single intraperitoneal injection (1 mg/animal, about 30 mg/kg) produced chromosomal damage in all stages of spermatogenesis and meiotic anomalies in mice (Moutschen-Dahmen *et al* 1975, Auerbach *et al* 1977, see Section 3.6.3). The study had neither positive nor negative controls but suggests that 2-butenal reaches the germ cells (IPCS 2008).

A dose-related increase in abnormal sperm heads was reported in mice treated with single intraperitoneal doses of 8, 16 and 32 µl/kg 2-butenal (6.8, 13.6 and 27.2 mg/kg). The effect reached statistical significance at doses of ≥ 16 µl/kg 1 and 3 weeks after exposure and at the highest dose of 32 µl/kg 5 weeks after exposure (Jha and Kumar 2006).

Developmental toxicity

Animal studies on developmental effects were not available.

4. Recommendation

Irritation

2-Butenal is a highly reactive and strong irritant. The RD_{50} values in mice are 3.5–4.9 ppm, depending on the strain (Steinhagen und Barrow 1984). Scattered human data indicate that 2-butenal is similarly irritating to humans. Thus irritation has been reported after acute exposures (seconds to minutes) at between 0.17 and 15 ppm (Table 1).

Systemic effects

2-Butenal is endogenously formed by lipid peroxidation. No adequate inhalation studies were available to assess the systemic toxicity. The NOAELs of subchronic and chronic animal studies with oral exposure are 2.5 and 5.9 mg/kg/day, respectively. Hepatotoxicity and inflammation of the respiratory tract were observed at higher doses (Wolfe *et al* 1987, Chung *et al* 1986).

Genotoxicity and carcinogenicity

2-Butenal is mutagenic *in vitro* and *in vivo*. 2-Butenal produces cyclic 1,N^2-propane-deoxyguanosine and other minor deoxyguanosine adducts with DNA *in vitro* and *in vivo*. The recent study by Jha and Khumar (2006) indicates that 2-butenal reaches germ cells *in vivo*.

Data concerning carcinogenic effects are limited. The human data of Bittersohl (1974) are not useful due to the smoking status of the workers and co-exposure to other chemicals. A slight increase in liver tumours was shown in the long-term rat study by Chung *et al* (1986), but without a clear dose-response relationship (hepatocellular carcinomas in the low-dose but not in the high-dose group). In view of the genotoxic properties, a possible carcinogenic potency of 2-butenal in humans cannot be dismissed. However, the limited human and animal data are too meagre to draw definite conclusions.

Overall assessment

In conclusion, no health-based OEL can be established at the present state of knowledge.

A "skin" notation is proposed because of low dermal LD_{50} values in rabbits and guinea pigs, similar to or even lower than the oral LD_{50} values in rats and mice.

Only one case of allergic contact dermatitis to 2-butenal in humans is known. A controlled study with 33 subjects revealed no sensitisation and animal studies show negative results. Therefore, there is little concern for sensitisation by 2-butenal.

No data on biological monitoring were available.

The present Recommendation was adopted by SCOEL on 20 March 2013.

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