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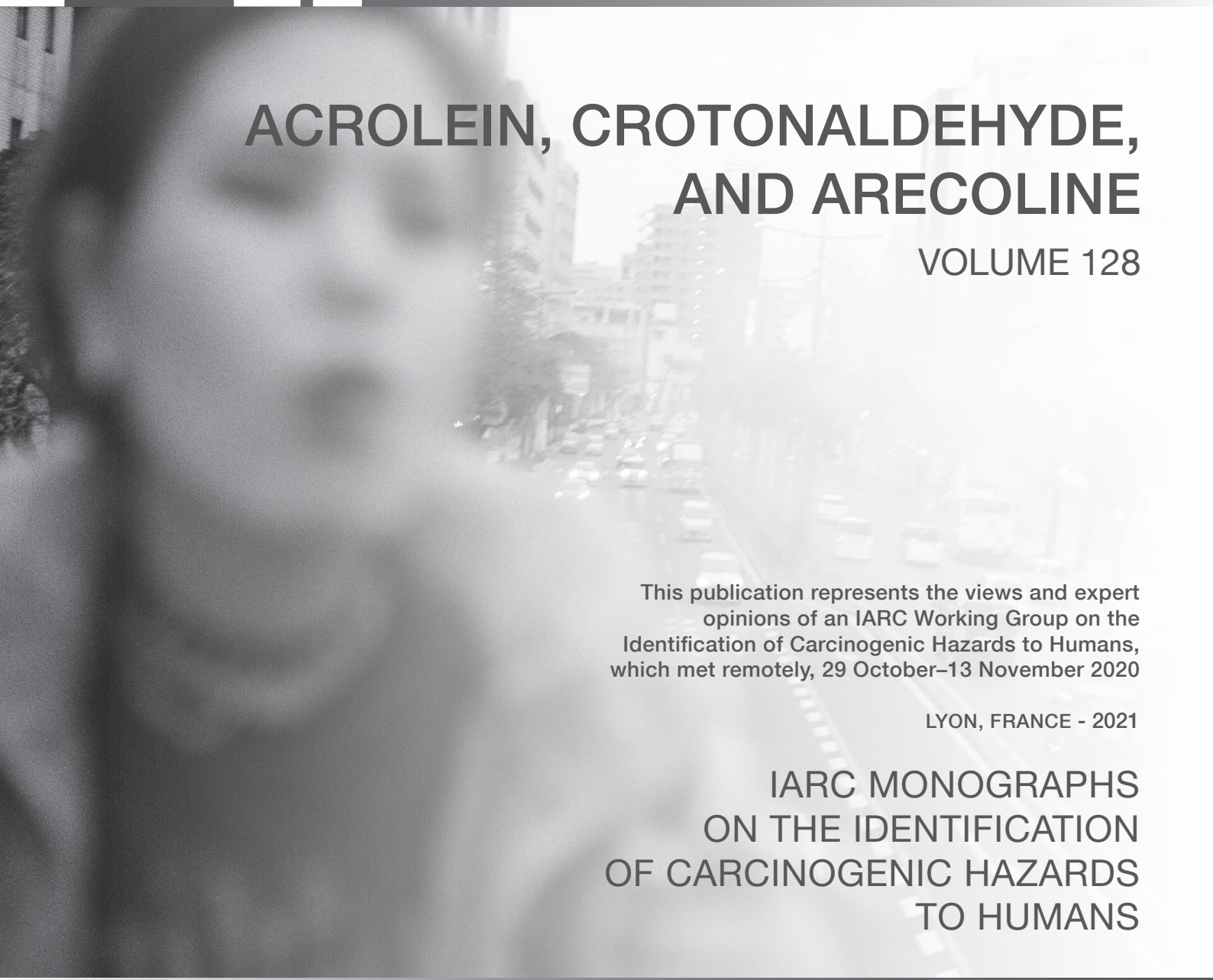

# ACROLEIN, CROTONALDEHYDE, AND ARECOLINE

VOLUME 128

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IARC MONOGRAPHS ON THE IDENTIFICATION OF CARCINOGENIC HAZARDS TO HUMANS

International Agency for Research on Cancer

World Health Organization

# CROTONALDEHYDE

## 1. Exposure Characterization

### 1.1 Identification of the agent

#### *1.1.1 Nomenclature*

*Chem. Abstr. Serv. Reg. No.*: 4170-30-3 (*E*/*Z*); 15798-64-8 (*Z*); 123-73-9 (*E*)

*Chem. Abstr. Serv. name*: 2-butenal (*E*/*Z*); (*Z*)-2-butenal; (*E*)-2-butenal

*EC/List No.*: 224-030-0; 204-647-1 (*E*)

*IUPAC name*: but-2-enal (*E*/*Z*); (*Z*)-but-2-enal; (*E*)-but-2-enal

*ICSC No.*: 0241 (ILO, 2018)

*RTECS No.*: GP9499000 (NIOSH, 2019)

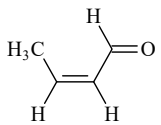
*DSSTox substance ID*: DTXSID8024864 (US EPA, 2020a)

*Common name*: crotonaldehyde

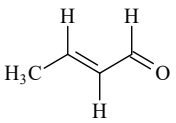
*Synonyms*: 2-butenaldehyde; crotonal; crotonic aldehyde; crotylaldehyde; 1-formylpropene; propylene aldehyde; methylpropenal; 3-methylacrolein; β-methylacrolein; BDQ; Topanel; butenal; topanelca; 2-butenal; bu-2-tenal; NCI-C56279; Topanel CA (ChemicalBook, 2019; NCBI, 2020).

#### *1.1.2 Structural and molecular formulae, and relative molecular mass*

*Structural formulae:*




*Z- (cis-)* isomer




*E- (trans-)* isomer

*Molecular formula*: $C_4H_6O$

*Relative molecular mass*: 70.09

#### *1.1.3 Chemical and physical properties*

*Description*: pure crotonaldehyde is a colourless liquid with a suffocating odour, which degrades when exposed to light and air and turns pale yellow as it oxidizes to a peroxide and then to crotonic acid. It can polymerize in the presence of small amounts of mineral acids. If heated with alkali chemicals, it will also polymerize, condense, or resinify (Celanese Corporation, 2011; NCBI, 2020). The information below pertains to mixtures of the *trans-* (*E-*) and *cis-* (*Z-*) isomers of crotonaldehyde, unless stated otherwise.

*Boiling point*: 104–105 °C (Lide, 1993)

*Melting point*: –74 °C (Lide, 1993)

*Relative density*: 0.8495 at 20 °C/4 °C (Lide, 1993)

*Solubility*: soluble in water (150 g/L at 20 °C), acetone, benzene, diethyl ether, and ethanol; miscible with gasoline, kerosene, solvent naphtha, and toluene (Eastman Chemical Co., 1991; Lide, 1993; Larrañaga et al., 2016)

*Vapour pressure*: 32 mm Hg [4.3 kPa] at 20 °C; relative vapour density, 2.4 (air = 1) (Budavari, 1989; Eastman Chemical Co., 1994)

*Flash point*: 13 °C (closed cup) (ILO, 2018)

*Stability*: readily dimerizes when pure; slowly oxidizes to crotonic acid (Budavari, 1989); polymerizes to become inflammable and explosive (Eastman Chemical Co., 1994)

*Reactivity*: lower explosive limit, 2.15% at 24 °C; reacts violently with bases, strong oxidizing agents, and polymerization initiators (Eastman Chemical Co., 1994)

*Octanol/water partition coefficient (*P*)*: log $K_{ow}$, 0.63 (United States National Library of Medicine, 1994)

*Odour perception threshold*: 0.035–0.2 ppm [0.10–0.57 mg/m$^3$] (European Commission, 2013)

*Conversion factor*: 1 ppm = 2.87 mg/m$^3$ (IARC, 1995).

### *1.1.4 Technical products and impurities*

Commercial crotonaldehyde is stabilized with 0.1–0.2% BHT (butylated hydroxytoluene, 2,6-di-*tert*-butyl-4-methylphenol) and is available at purities of 90–99%. One common commercial product consists of > 95% *trans*- (*E*-) and < 5% *cis*- (*Z*-) isomer and contains 0.1–0.2% BHT and 1% water as stabilizers (Sigma-Aldrich, 2020a); another commercial product that is > 99% *trans*- (*E*-) isomer is stabilized with 0.1–0.2% BHT and 1% water (Sigma-Aldrich, 2020b). A typical specification for crotonaldehyde is as follows: minimal purity, 90%; acidity (as crotonic acid), 0.15% maximum; water content, 8.5% maximum; aldol, 0.1% maximum; butyraldehyde, 0.02% maximum; low boiling-point compounds (including acetaldehyde, see IARC, 1987; and butyraldehyde), 0.20% maximum; butyl alcohol, 0.15% maximum; and high-boiling-point compounds, 1.0% maximum (Blau et al., 1987; Eastman Chemical Co., 1993; Spectrum Chemical MFG Corp., 1994).

## 1.2 Production and use

### *1.2.1 Production process*

Crotonaldehyde is usually produced by the aldolization reaction of acetaldehyde, catalysed by one of various basic catalysts, e.g. alkali metal or alkaline earth metal catalysts, ammonium salts, zeolites, molecular sieves and claylike materials, followed by dehydration of the acetaldol and distillation (Blumenstein et al., 2015).

### *1.2.2 Production volume*

Crotonaldehyde is a High Production Volume chemical according to the Organization for Economic Co-operation and Development (OECD) (OECD, 2020) and the United States Environmental Protection Agency (US EPA) (US EPA, 2020a). Less than 500 tonnes were used in the USA in 1977 (Baxter, 1979). In 1986, 1990, 1994, and 1998, between 10 and 50 million pounds [4500–22 700 tonnes] were produced annually in the USA. However, production fell to between 1 and 10 million pounds [450–4500 tonnes] in 2002 (NCBI, 2020). In 2012, 2013, and 2014 only two companies reported the use of crotonaldehyde to the US EPA, and each reported producing less than a million pounds [less than 450 tonnes] in each of those years (US EPA, 2020a).

In 2020, there were two major producers of crotonaldehyde in the USA, one in Germany, and another in western Europe (Market Watch, 2020;

NCBI, 2020; US EPA, 2020a). The major producer and user of crotonaldehyde is currently China (ResearchMoz, 2020), although production and use is growing in India. The global crotonaldehyde market was valued at US $244 million in 2019.

Crotonaldehyde was on the Pollutant Release and Transfer Register (PRTR) of Canada with a threshold use of 10 000 kg/year (no facilities reported), until it was removed in 2018 (Government of Canada, 2019). It remains on the National Pollutant Release Inventory (NPRI) of Japan with a threshold use of 1000 kg/year (three facilities), and the USA Toxics Release Inventory (TRI) with a threshold manufacturing or processing use of 11 340 kg/year or other use of 4536 kg/year (seven facilities) at the time of reporting (OECD, 2014).

### *1.2.3 Uses*

Crotonaldehyde is a reactive chemical, with an aldehyde functional group that is conjugated to the olefinic double bond, and is a reducing agent. These characteristics make crotonaldehyde particularly versatile and useful for synthesizing other chemicals for diverse industries. The main use of crotonaldehyde in the past was in the manufacture of *n*-butanol (Baxter, 1979). In 1964, 88% of crotonaldehyde was used for the synthesis of *n*-butanol, 10% for *n*-butyraldehyde, and 2% for crotonic acid and sorbic acid (NCBI, 2020). Its predominant use today is as an intermediate in organic chemical synthesis and in the production of sorbic acid and intermediates such as crotonic acid (Blau et al., 1987), crotyl alcohol, *n*-butanal, as well as *n*-butanol (Celanese Corporation, 2011; Blumenstein et al., 2015). The primary industries that use crotonaldehyde as an intermediate are pharmaceuticals, rubber, chemicals, leather, food, and agriculture (Coherent Market Insights, 2020).

Crotonaldehyde is used in the synthesis of sorbic acid, a food preservative, and vitamin E (Blumenstein et al., 2015). Crotonaldehyde reacts with urea to form crotonylidene ureas, which are slow-release fertilizers, and is also used to make pesticides (Celanese Corporation, 2011).

Crotonaldehyde is an intermediate in the synthesis of chemicals including quinaldines, thiophenes, pyridines, and 3-methoxybutanol, which is a speciality solvent used in lacquers and varnishes to control viscosity, drying behaviour, and gloss. Crotonaldehyde can also be used to control polymerization. Other products include pharmaceuticals, resins, paints and coatings, dyestuffs, rubbers, adhesives, and chemicals used to tan leather (Blumenstein et al., 2015).

The *E*-isomer of crotonaldehyde is listed by the US EPA among the chemicals associated with hydraulic fracturing (US EPA, 2020b). Owing to its pungent odour and strong lacrimating properties, crotonaldehyde is also used as a warning agent in fuel gases and for locating breaks and leaks in pipes (Budavari, 1989) as well as in the purification of lubricating oils (NCBI, 2020). It can be used as a solvent for vegetable and mineral oils, fats, waxes, resins, and polyvinyl chloride (Celanese Corporation, 2011; NCBI, 2020).

## 1.3 Methods of detection and quantification

Methods for the detection and quantification of crotonaldehyde have evolved steadily since the agent was last evaluated by the *IARC Monographs* programme in Volume 63 (IARC, 1995). Techniques are now available to measure crotonaldehyde in air, water, foodstuffs, and biological specimens. Other methodologies estimate human exposure via metabolites, and both protein and DNA adducts. Table 1.1 summarizes these methods by sample matrix and indicates sample requirements and sensitivity parameters. These techniques are sufficiently sensitive to measure concentrations reliably in ambient air, water, food, and in human

**Table 1.1 Representative methods for the detection and quantification of crotonaldehyde, its metabolites, and its DNA adducts**

| Sample matrix | Sample collection/preparation | Assay procedure | Limit of detection (unless otherwise stated) | Reference |
|---|---|---|---|---|
| *Crotonaldehyde* | | | | |
| Air | DNPH-impregnated XAD and glass fibre filters; sampling rate, 30 mL/min; exposure time, 8 h | GC-FID and GC-ECD after sampling with immediate derivatization | FID, 2–20 µg/m³<br>ECD, 0.2–4 µg/m³ | Otson et al. (1993) |
| Urban outdoor and indoor air | DNPH-silica sorbent tubes and US EPA Method TO-5 DNPH solution in midget impinger; sampling rate, ~0.5 L/min; sampling time, 2–3 h; maximum sample volume, 80 L | HPLC-UV after derivatization with DNPH | 1.59 µg/m³ (US EPA Method TO-5)<br>1.02 µg/m³ (cartridge) | Williams et al. (1996) |
| Air | DNPH-impregnated silica gel, sampling rate 300 mL/min, sampling time, 1 h | Electrochromatography | 0.26 mg/L [0.26 g/m³] | Fung & Long (2001) |
| Air | Passive button sampler; silica gel impregnated with DNPH, sampling time, 7 days | HPLC-UV – RP C18 method after DNPH derivatization | Calibration curve range, 0.05–10 ppm [0.14–29 mg/m³] | Liu et al. (2001) |
| Air in cigarette smoking chamber | DNPH-coated silica gel, flow rate, 200 mL/min; thermal desorption tubes, flow rate, 60 mL/min; sampling time, 4 h | HPLC-DAD-UV | LOQ, 0.62 µg/m³ | Liu et al. (2017) |
| Air | DNPH-coated $C_{18}$ cartridges; flow rate, 0.55–0.77 L/min; sampling times, 4, 5, 8, and 11 h; sampling volumes, 0.14–0.37 m³ | HPLC-UV-visible detector | 26 ng/sample; LOQ in ambient air, 0.06 ppb [0.17 µg/m³] | Grosjean et al. (1996) |
| Air | Tedlar bags and carbox tubes (thermally desorbed); flow rate, 100 mL/min; sampling time, 2 min | GC/MS | 0.079 ng/sample; 0.02 ppb [0.080 µg/m³] | Ahn et al. (2014) |
| Air vapours and particulate | DNPH-coated diffusion cell and DNPH-coated filter in line; flow rate, 1.0 L/min; sampling time, 60 min | GC-TSD<br>GC-MS<br>GC-ECD | 1.03 ng/mL [1.03 g/m³]<br>0.53 ng/mL [0.53 g/m³]<br>0.006 ng/mL [0.006 g/m³] | Dugheri et al. (2019) |
| Automobile exhaust | Two impingers connected in series; flow rate, 0.5 L/min | GC-ECD | LOQ, 0.15 µg in 2 mL of absorption solution | Nishikawa et al. (1987) |
| Water | PFBOA derivatization/hexane extraction | GC-ECD<br>GC-MS-SIM | 1.2 µg/L<br>11.2 µg/L | Glaze et al. (1989) |
| Food products | Oil sample, 5 g; headspace<br>PFPH derivatization/solvent extraction<br>DNPH derivatization/solvent extraction | Isotopic dilution/GC-MS<br>Isotopic dilution/GC-MS<br>Isotopic dilution/HPLC-MS/MS | 3 µg/kg; LOQ, 9 µg/kg<br>2 µg/kg; LOQ, 6 µg/kg<br>1.5 µg/kg; LOQ, 4.5 µg/kg | Granvogl (2014) |
| Food products: fried clams | Lipid/oil sample, 1 g; solvent extraction of food product DNPH derivatization | HPLC-MS/MS | 75 nM; LOQ, 300 nM | Liu et al. (2020) |
| Food products: oils | Oil sample: 10 mg; DNPH derivatization/SPE | HPLC-MS/MS | 2.5 ng/mL; LOQ, 8 ng/mL | Suh et al. (2017) |

**Table 1.1 (continued)**

| Sample matrix | Sample collection/preparation | Assay procedure | Limit of detection (unless otherwise stated) | Reference |
|---|---|---|---|---|
| Food products: liquors | Beverage sample: 4.0 mL; DNPH derivatization | HPLC-UV-visible PDA | 10–50 µg/L | Nascimento et al. (1997) |
| Human serum | Serum separation: 3000 rpm for 10 min; sample volume, 250 µL; acidification with 330 µL of 0.1 M HCl/SPME/ headspace | GC-MS | 0.147 µg/L; LOQ, 0.147 µg/L | Silva et al. (2018) |
| Human serum | Sample volume, 100 µL; fluorescent derivatization | HPLC-PO-CL | ~4–6 nmol/injection | Ali et al. (2014) |
| *Metabolites* | | | | |
| Human urine: metabolite HMPMA | Sample volume in assay 50 µL urine, diluted 1:10 with buffer (50 µL undiluted urine + 25 µL working mixed internal standard + 425 µL 15 mM ammonium acetate, pH 6.8) | UPLC-ESI-MS/MS | 2.00 ng/mL | Alwis et al. (2012) |
| Human urine: metabolite HMPMA | Sample volume, 400 µL; 96-well plate/SPE | HPLC-MS/MS | 0.82 ng/mL | Carmella et al. (2013) |
| *DNA adducts* | | | | |
| Human oral tissue: DNA adduct CdG | Gingival tissue or buccal mucosa samples, 50–300 mg each; DNA extraction; $^{32}$P-postlabelling | HPLC | LLR, 0.026 µmol/mol CdG | Nath et al. (1998) |
| Human saliva: DNA adduct CdG | Sample volume, 3 mL; DNA sample, 25 µg; SPE | HPLC-NSI-MS/MS | LOQ, 0.5 pg | Chen & Lin (2011) |

CdG, crotonaldehyde-derived 1,$N^2$-propanodeoxyguanosine; DAD, diode array detector; DNPH, 2,4-dinitrophenylhydrazine; ECD, electron-capture detector; ESI, electrospray ionization; FID, flame-ionization detector; GC, gas chromatography; h, hour; HCl, hydrochloric acid; HPLC, high-performance liquid chromatography; HMPMA, *N*-acetyl-*S*-(3-hydroxy-1-methylpropyl)-L-cysteine; ICP, inductively coupled plasma; LLR, lowest level recorded; LOQ, limit of quantification; MS, mass spectrometry; min, minute; NSI, nanospray ionization; PDA, photodiode array detector; PFBOA, O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine; PFPH, pentafluorophenylhydrazine; PO-CL, peroxyoxalate chemiluminescence; ppb, parts per billion; ppm, parts per million; RP, reversed phase; SIM, selected ion monitoring; SPE, solid-phase extraction; SPME, solid-phase microextraction; TSD, thermionic specific detector; UPLC, ultra high-performance liquid chromatography; UV, ultraviolet.

biological specimens, and can distinguish background levels from higher exposures (e.g. to combustion products, crotonaldehyde-containing foodstuffs, or in occupational settings). However, sensitivity varies across methods, and not all methods – including National Institute for Occupational Safety and Health (NIOSH) method 3516 (NIOSH, 1994) – have sufficient sensitivity to measure air levels in occupational settings, i.e. at the current American Conference of Governmental Industrial Hygienists (ACGIH) recommended a threshold limit value (TLV) of 0.3 ppm [0.86 mg/m$^3$] (ACGIH, 2020).

### 1.3.1 Air

Air sampling for the measurement of crotonaldehyde concentrations was most often done by drawing air through impingers or midget bubblers (NIOSH, 1994; Zervas et al., 2002; Zhang et al., 2019a). A significant advance has been the quantitative chemical trapping of crotonaldehyde for the analysis of the corresponding hydrazone by high-performance liquid chromatography (HPLC) (Zhang & Smith, 1999) or gas chromatography (GC) (Otson et al., 1993). 2,4-Dinitrophenylhydrazine (DNPH) can be used in the impinger collection fluid (Zervas et al., 2002) or dried upon glass-fibre filters (OSHA, 1990), passive samplers (Otson et al., 1993), silica gel (Zhang & Smith, 1999; Fung & Long, 2001; Mitova et al., 2016), or octadecane sampling cartridges (Grosjean et al., 1996). Prepared DNPH tubes are available commercially (Williams et al., 1996; Liu et al., 2017). Ahn et al. (2014) used both polyester aluminium film sampling bags and sorbent tubes packed with carbon black to collect the air above fried fish to measure crotonaldehyde levels. Air from the bag was directly analysed by gas chromatography-mass spectrometry (GC-MS) while the crotonaldehyde on sorbent tubes was thermally desorbed before injection. Various forms of GC-MS and liquid chromatography-mass spectrometry (LC-MS) have been employed to detect crotonaldehyde from air samples collected as described above.

### 1.3.2 Water

Methods for the analysis of crotonaldehyde in water have involved derivatization of samples with pentafluorobenzyl hydroxylamine before hexane extraction and injection into GC equipped with a $^{63}$Ni electron-capture detector (ECD) or GC-MS (Glaze et al., 1989). Wang et al. (2009) used DNPH derivatization before HPLC but did not detect crotonaldehyde in any rainwater samples tested (other aldehydes were detected). Baños & Silva (2009) evaluated six solid-phase extraction systems for the analysis of aldehydes in water. They described a continuous DNPH derivatization and pre-concentration step before analysis with LC-MS/MS. However, they found no crotonaldehyde in several samples of swimming pool water in which other aldehydes were detected.

### 1.3.3 Soil

No data were available to the Working Group.

### 1.3.4 Food, beverages, and consumer products

Methods for the analysis of crotonaldehyde in food are similar to those used for air, with the exception that crotonaldehyde must either first be extracted from the food matrix, or the headspace above the matrix must be sampled. Granvogl (2014) reported on three different methods involving isotopic dilution that gave good agreement and similar limits of detection and quantification in heat-processed fats and oils, and fried food. In the first method, headspace was sampled directly into the GC-MS. The second method involved derivatization with pentafluorophenylhydrazine, followed by extraction and injection into the GC-MS.

The third method involved derivatization with DNPH, followed by extraction and injection into a HLPC-MS system. Because of ease of application, the first method was used for the analysis of food products. The latter two methods were more sensitive than the first method but involved pre-analytical steps.

### 1.3.5 Biological specimens

Several methods are available for the direct analysis of crotonaldehyde in saliva, urine, and serum, as well as for the analysis of crotonaldehyde metabolites or DNA and protein adducts (Table 1.1). The urinary biomarker *N*-acetyl-*S*-(3-hydroxy-1-methylpropyl)-L-cysteine (HMPMA; 3-hydroxy-1-methylpropylmercapturic acid) was commonly analysed. *N*-Acetyl-*S*-(3-carboxy-1-methylpropyl)-L-cysteine (CMEMA; 2-carboxy-1-methylethylmercapturic acid) has also been analysed. Both can be detected using LC-MS/MS methods.

No data on a validated biomarker for crotonaldehyde were available to the Working Group.

## 1.4 Occurrence and exposure

### 1.4.1 Environmental and natural occurrence

Crotonaldehyde occurs naturally in a ubiquitous fashion. It is produced endogenously by plants and animals (including humans) as part of lipid peroxidation and metabolism (WHO; IPCS; IOMC, 2008). Crotonaldehyde has been measured in gases emitted by volcanoes (Graedel et al., 1986), but has also been detected as a biogenic emission from pine trees (0.19 μg/m$^3$) and deciduous forests (0.49 μg/m$^3$) (Ciccioli et al., 1993). Scotter et al. (2005) measured levels of crotonaldehyde in the headspace of fungal cultures. Detectable crotonaldehyde levels (mean, 0.106 μg/m$^3$; standard deviation, SD, 0.005 μg/m$^3$) were found in the air of a room when people were exercising, but not when they were resting (< 0.0636 μg/m$^3$) (Mitova et al., 2020).

Crotonaldehyde is found in burned and unburned tobacco (Bagchi et al., 2018), as a combustion product of burning wood and plastic, cooking fires (3.8–91.6 mg/kg fuel), and automobile exhaust (0.07–1.35 ppm [0.20–3.87 mg/m$^3$], depending on engine size and operating conditions) and diesel exhaust (15–27 mg/kWh energy produced, depending on fuel type and operating conditions) (Nishikawa et al., 1987; Zhang & Smith, 1999; Song et al., 2010).

An overview of exposure measurements of crotonaldehyde in outdoor air, indoor air and dust, and water is provided in Table 1.2.

### 1.4.2 Exposure in the general population

Important sources of exposure to crotonaldehyde in the general population include tobacco and tobacco-related products, indoor and outdoor air, food, and beverages. Table 1.3 presents concentrations of crotonaldehyde in cigarettes, engine emissions, and other sources. Table 1.4 presents data on levels of crotonaldehyde biomarkers in humans. including from studies of known exposures (e.g. to tobacco products) and from studies in which the exposure source was not characterized (e.g. in children). Studies on DNA adducts in humans (e.g. Chen & Lin, 2009) are further addressed in Section 4.2.1. Table 1.5 presents crotonaldehyde concentrations measured in food and beverages.

#### (a) Tobacco products and tobacco-related products

See Table 1.3 and Table 1.4.

Cigarette smoke is a major source of exposure to crotonaldehyde (Counts et al., 2004; Carmella et al., 2009). The amount of crotonaldehyde measured per cigarette varies widely depending on the source of the tobacco and the sampling protocol (Hammond & O'Connor, 2008; see Table 1.3). The mean concentration of

**Table 1.2 Concentrations of crotonaldehyde in outdoor air, indoor air and dust, and water**

| Sample | Concentration (μg/m³) mean ± SD unless specified otherwise | Country or region | Reference |
|---|---|---|---|
| Volatile emissions from Chinese arborvitae *Thuja orientalis* | Identified but not quantified | USSR | Isidorov et al. (1985) |
| Air of forest areas of northern and southern Europe and a remote site in the Himalaya region | 0.19<br>0.49<br>3.32<br>1.41<br>2.19<br>0.24 | Storkow (Germany)<br>Castel Porziano (Italy)<br>K2-A (Nepal)<br>K2-B (Nepal)<br>K2-C1 (Nepal)<br>K2-C2 (Nepal) | Ciccioli et al. (1993) |
| Ambient air | Annual average, 13.1 (range, 0.8–151.2) | Eastern Himalaya, India | Sarkar et al. (2017) |
| Ambient air | Range, 0.009–0.112 | Northern California, USA | Seaman et al. (2006) |
| Ambient air | 0.30 ± 0.10 ppb [0.86 ± 0.29] | Los Angeles, USA | Grosjean et al. (1996) |
| Ambient air, downtown | 0.05 ppb [0.14] | Porto Alegre, Brazil | Grosjean et al. (1999) |
| Air outside urban residences:<br>Spring<br>Summer<br>Fall<br>Winter | <br>0.20 ± 0.07<br>0.51 ± 0.17<br>0.32 ± 0.19<br>0.44 ± 0.36 | New Jersey, USA | Liu et al. (2006) |
| Air adjacent to a six-lane level roadway | 2.17–3.71% of total aldehydes excluding acetone | Raleigh, NC, USA | Zweidinger et al. (1988) |
| Ambient air at the Oakland–San Francisco Bay Bridge toll plaza (occupational exposure) | Morning: 0.147 ± 0.004<br>Afternoon: 0.093 ± 0.002 | San Francisco, USA | Destaillats et al. (2002) |
| Polluted air | 442 ± 22.2 ppb [1.29 ± 0.064] | Osaka, Japan | Kuwata et al. (1979) |
| Urban roadside sites | <br>3.5 ± 2.6<br>1.4 ± 0.4<br>1.6 ± 0.8<br>ND | London, UK<br>Ealing, residential<br>Ealing, commercial<br>Wood Green, residential<br>Wood Green, commercial | Williams et al. (1996) |
| Air samples in rooms with:<br>no people<br>3 persons (morning)<br>3 persons, no prior air purge (afternoon)<br>3 persons, prior air purge (afternoon) | <br>< 0.0636<br>< 0.0636<br>< 0.0636<br>< 0.0636 | Neuchatel, Switzerland | Mitova et al. (2020) |
| Indoor air samples from 234 homes<br>Personal exposure concentrations | 0.7<br>1.3 | Elizabeth, New Jersey, Houston, Texas, and Los Angeles County, California, USA | Liu et al. (2007) |

## Table 1.2 (continued)

| Sample | Concentration (μg/m³) mean ± SD unless specified otherwise | Country or region | Reference |
|---|---|---|---|
| Indoor dust from 389 children's bedrooms | Quantified in 80%<br>0.9 μg/g (range, 0.01–10 μg/kg) | Värmland, Sweden | Nilsson et al. (2005) |
| Hospital indoor and outdoor air | 0.16 (range, ND–0.37) | Guangzhou, China | Lü et al. (2010) |
| Indoor air risk-assessment demonstration for analytical laboratories | 0.00835 | Kanpur, India | Dhada et al. (2016) |
| Indoor air subjected to heated tobacco products<br>Indoor air subjected to cigarettes | Median, < 0.182<br>Median, 2.04 | Neuchatel, Switzerland | Mitova et al. (2016) |
| Train carriage air | Range, 2.6–3.6 | Hangzhou, China | Lu & Zhu (2007) |
| Air in a closed room (27 m³) during burning of 5 kg of polypropylene | 1.1 ppm [3200] | Borehamwood, Herts, England | Woolley (1982) |
| Volatile emissions from burning wood (cedar, red oak, and green ash) in a fireplace | [0.043 g/kg]<br>(range, ND–0.116 g/kg) | Warren, Michigan, USA | Lipari et al. (1984) |
| Colours and chemicals production plant (occupational exposure) | General area: ND–3200<br>Personal samples: 1900–2100 | East Hanover, NJ, USA | NIOSH (1982) |
| Diesel-fuelled automobile exhaust | 0.01 ppm [290] | Warren, Michigan, USA | Lipari & Swarin (1982) |
| Automobile exhaust gas at different engine speeds | 0.51 ppm<br>[1475; range, 200–3870] | Gifu, Japan | Nishikawa et al. (1987) |
| Vapours emitted from polyurethane foam | Crotonaldehyde identified | Ottawa, Canada | Krzymien (1989) |
| Exhaust from a compressed natural gas heavy-duty engine<br>Exhaust from a diesel engine | 0.12 mg/kWh<br>0.42 mg/kWh | Naples, Italy | Gambino et al. (1993) |
| Emissions from polyethylene resin samples in a 30 000 m³ applications area during extrusion operations | Area: < 0.02 to < 0.05 ppm [< 60 to < 140]<br>Personal: < 0.03 to < 0.05 ppm [< 90 to < 140] | Calgary, Canada | Tikuisis et al. (1995) |
| Emissions from a two-stroke chain saw engine using ethanol and ethanol-blended gasoline | 0.012–0.063 g/kWh | Umeå, Sweden | Magnusson et al. (2002) |
| Industrial emission sources from 77 companies | 8.66 ± 27.7 ppb [24.9 ± 79.5] | An-San and Si-Hung city, Republic of Korea | Kim et al. (2008) |
| Polyester-manufacturing plant, wastewater | 5.64 mg/L, estimated | Brazil | Caffaro-Filho et al. (2010) |
| Ship diesel engine emissions:<br>standard diesel fuel<br>heavy fuel oil | <br>18 ± 4 μg/MJ<br>43 ± 13 μg/MJ | Rostock, Germany | Reda et al. (2014) |
| Air in the process chimney of a waste-treatment plant | 8 ± 3 (range, 3–14) | Barcelona, Spain | Gallego et al. (2016) |

ND, not detected; ppb, parts per billion; ppm, parts per million; SD, standard deviation.

## Table 1.3 Concentrations of crotonaldehyde in cigarettes, engine emissions, and other sources

| Source | No. of samples | Concentration | | Country or region | Reference |
|---|---|---|---|---|---|
| | | Average concentration (unless otherwise stated) | Range (unless otherwise stated) | | |
| *Cigarettes and related exposures* | | | | | |
| Breathing-zone concentrations of cigarette smoke in garages (occupational exposure): | | | | USA | Zhang et al. (2003) |
| smokers in garages | 22 | 0.96± 0.94 mg/m$^3$ | NR | | |
| non-smokers in garages | 31 | 0.53± 0.79 mg/m$^3$ | | | |
| smokers control | 11 | 0.29± 0.48 mg/m$^3$ | | | |
| non-smokers control | 22 | 0.25± 0.34 mg/m$^3$ | | | |
| Mainstream cigarette smoke (ISO machine-smoking regimen) | 5 studies<br>9 samples | 13.9 µg/cig<br>9.8 µg/cig | 95% CI, 11.1–16.6<br>95% CI, 6.1–13.5 | Richmond, USA | Counts et al. (2004) |
| Mainstream smoke of "light" cigarettes (modified ISO machine-smoking regimen) | 7 | 33% reduction | 26–47% reduction | Canada | Gendreau & Vitaro (2005) |
| Smokeless tobacco: | | | | Indianapolis, Dallas, Austin, Minneapolis, USA, and Sweden | Stepanov et al. (2008) |
| traditional products | 5 | 2.98 µg/g dry weight | 0.98–6.35 µg/g dry weight | | |
| new products | 12 | 9.12 µg/g dry weight | 0.55–19.4 µg/g dry weight | | |
| Mainstream cigarette smoke: | NR | Total, 12.5 ± 1.8 µg/cig | NR | Kentucky, USA | Eschner et al. (2011) |
| ISO machine-smoking regimen | | Vapour phase, 8 ± 1 µg/cig | | | |
| "Canadian Intense" machine-smoking protocol | | Vapour phase, 49 ± 7 µg/cig | | | |
| Mainstream cigarette smoke ("Canadian Intense" machine-smoking protocol) | 95 | 55.4 µg/cig ($n$ = 61) | 35.4–75.1 µg/cig ($n$ = 95) | USA | Bodnar et al. (2012) |
| Mainstream cigarette smoke: | | | | Bayreuth, Germany (cigarette brands sold worldwide) | Eldridge et al. (2015) |
| ISO machine-smoking regimen | 39 | 4.8–12.1 µg/cig[a] | SD, 0.7–1.5 | | |
| "Canadian Intense" machine-smoking protocol | 40 | 37.9–47.1 µg/cig[a] | SD, 3.5–4.3 | | |
| Mainstream smoke of cigarettes (ISO machine-smoking regimen) | 148 | 1.9–20.5 µg/cig [a] | NR | 19 different laboratories | ISO (2018) |
| Mainstream smoke of: | | | | Greece and USA | Farsalinos et al. (2018) |
| regular heated tobacco product | 5 | 1.4–3.0 µg/stick[b] | SD, 0.4–0.7 | | |
| menthol heated tobacco product | 5 | 1.9–3.3 µg/stick[b] | SD, 0.2–0.9 | | |
| e-cigarettes | 5 | ND µg/12 puffs | ND | | |
| regular cigarettes | 5 | 40.5–65.7 µg/cig[b] | SD, 8.6–14.6 | | |

**Table 1.3 (continued)**

| Source | No. of samples | Concentration | | Country or region | Reference |
|---|---|---|---|---|---|
| | | Average concentration (unless otherwise stated) | Range (unless otherwise stated) | | |
| Mainstream smoke of: | | | | Various locations | Cai et al. (2019) |
| experimental cigarettes | 48 | 19.79 µg/cig | NR | | |
| cigarettes from Chinese market | 163 | 42–67 µg/cig | NR | | |
| Heated tobacco products: | | | | France | Cancelada et al. (2019) |
| mainstream smoke | 9 | 4.9–5.2 µg/heatstick[c] | SD, 0.5–0.6 | | |
| sidestream smoke | 9 | 0.3–0.4 µg/heatstick[c] | SD, 0.2 | | |
| Tobacco heatstick | 32 | < 3.0 µg/stick | NR | Sigmaringen, Germany | Mallock et al. (2018) |
| E-cigarette refill solutions | 45 | [0.19 µg/mL] | ND–0.75 µg/mL[c] | Republic of Korea | Lee et al. (2020) |
| | 30 | [0.26 µg/mL] | ND–1.35 µg/mL[c] | USA | |
| | 15 | ND | ND | Japan | |
| *Engine exhaust* | | | | | |
| Exhaust from a one-cylinder diesel research engine | 3 | 0.04 [0.11] ± 0.088 [± 0.25] ppm [mg/m$^3$] | NR | Waukesha, Wisconsin | Creech et al. (1982) |
| Jet engine exhaust | 7 | 0.009 [0.03] ppm [mg/m$^3$] | 0–0.051 [0–0.15] ppm [mg/m$^3$] | Tokyo, Japan | Miyamoto (1986) |
| *Other sources* | | | | | |
| Barbecue charcoal combustion | 4 | 42.5 [122] ppb [µg/m$^3$] | 11.5–121 [33.0–347] ppb [µg/m$^3$] | Republic of Korea, Indonesia, China, Malaysia | Kabir et al. (2010) |
| Steel protective paints (polyvinyl butyral) | 1 | 6 mg/m$^3$ | | Turku, Finland | Henriks-Eckerman et al. (1990) |
| *Aspergillus flavus* | 5 | Very low, < 75 cps | | Christchurch, New Zealand | Scotter et al. (2005) |
| *Aspergillus fumigatus* | 5 | Very low, < 75 cps | | | |
| *Candida albicans* | 5 | NR | Low, 0 to < 300 cps | | |
| *Cryptococcus neoformans* | 3 | NR | Low, 0 to < 1000 cps | | |
| *Fusarium solani* | 3 | NR | Low, 0 to < 1000 cps | | |
| *Mucor racemosus* | 3 | NR | Low/moderate, < 75 to < 300 cps | | |

**Table 1.3 (continued)**

| Source | No. of samples | Concentration | | Country or region | Reference |
|---|---|---|---|---|---|
| | | Average concentration (unless otherwise stated) | Range (unless otherwise stated) | | |
| Scented candles: | | Burning (before lighting): | | Republic of Korea, USA, and China | Ahn et al. (2015) |
| clean cotton | 2 | 0.15 (0.15) [0.43 (0.43)] ppb[μg/m$^3$] | | | |
| floral | 2 | 0.15 (4.72) [0.43 (13.5)] ppb[μg/m$^3$] | | | |
| kiwi melon | 2 | 0.15 (0.15) [0.43 (0.43)] ppb[μg/m$^3$] | | | |
| strawberry | 2 | 109 (1.2) [313 (3.44)] | | | |
| vanilla | 2 | 0.15 (0.15) [0.43 (0.43)] ppb[μg/m$^3$] | | | |
| plain | 2 | 8.54 (2.2) [24.5 (6.31)] ppb[μg/m$^3$] | | | |

cig, cigarette; cps, counts per second; e-cigarette, electronic cigarette; ND, not detected; NR, not reported; ppb, parts per billion; ppm, parts per million; SD, standard deviation.

[a] Range of means.

[b] Range of means of three different puffing regimens.

[c] Range of means of different products.

## Table 1.4 Levels of crotonaldehyde biomarkers in humans

| Sample and source | Biomarker | No. of samples | Average concentration (unless otherwise stated) | Range (unless otherwise stated) | Country or region | Reference |
|---|---|---|---|---|---|---|
| Urine after smoking:<br>cellulose acetate filter-tipped<br>charcoal filter-tipped cigarettes | HMPMA | <br>20<br>19 | <br>6220 µg/24 h<br>5152µg/24 h | <br>SD, 3063<br>SD, 2517 | Munich, Germany | Scherer et al. (2006) |
| Urine of:<br>e-cigarette smokers<br>conventional cigarette smokers<br>non-smokers (stopped) | HMPMA | <br>60<br>20<br>20 | <br>750 µg/24 h<br>2320 µg/24 h<br>299 µg/24 h | <br>SD, 466<br>SD, 1405<br>SD, 166 | Richmond, USA | Frost-Pineda et al. (2008) |
| Urine of smokers, 3–56 days after stopping smoking | HMPMA | 17 | 242–331 nmol/24 h | SD, 83–153 | Minneapolis, USA | Carmella et al. (2009) |
| Urine of Chinese non-smoking women who regularly cook at home | HMPMA | 54 | 1158 pmol/mg creatinine | NR | Singapore | Hecht et al. (2010) |
| Urine of smokers who:<br>developed lung cancer<br>did not develop lung cancer | HMPMA | <br>343<br>392 | <br>GM, 7915 pmol/mg creatinine<br>GM, 5749 pmol/mg creatinine | <br>95% CI, 6906–9071<br>95% CI, 5022–6581 | Shanghai, China | Yuan et al. (2012) |
| Urine from cigarette smokers | HMPMA | 2613 | 3302 pmol/mL | SD, 3341 | Minnesota, south California and Hawaii, USA | Carmella et al. (2013) |
| Urine of non-smokers, who:<br>developed lung cancer<br>did not develop lung cancer | HMPMA | <br>80<br>82 | <br>GM, 1750 pmol/mg creatinine<br>GM, 1714 pmol/mg creatinine | <br>95% CI, 1425–2150<br>95% CI, 1384–2123 | Shanghai, China | Yuan et al. (2014) |
| Urine of never-smoking Chinese women who regularly cook at home:<br>≤ ×1/week<br>≥ ×7/week | HMPMA | <br>90<br>95 | <br>GM, 894 pmol/mg creatinine<br>GM, 1167 pmol/mg creatinine | <br>IQR, 749–1067<br>IQR, 1022–1332 | Singapore | Hecht et al. (2015) |
| Urine of adults aged ≥ 20 yr:<br>male non-smokers<br>male smokers<br>female non-smokers<br>female smokers | HMPMA | <br>1244 (all men)<br>1084 (all women) | <br>GM, 485 ng/mL<br>GM, 848 ng/mL<br>GM, 488 ng/mL<br>GM, 1162 ng/mL | <br>95% CI, 436–540<br>95% CI, 706–1017<br>95% CI, 433–549<br>95% CI, 993–1360 | USA | Jain (2015b) |

**Table 1.4 (continued)**

| Sample and source | Biomarker | No. of samples | Average concentration (unless otherwise stated) | Range (unless otherwise stated) | Country or region | Reference |
|---|---|---|---|---|---|---|
| Urine of cigarette smokers: | HMPMA | | | | Minnesota, southern California and Hawaii, USA | Park et al. (2015) |
| African American | | 361 | Median, 2948 pmol/mL | IQR, 1418–5194 | | |
| Native Hawaiian | | 329 | Median, 2766 pmol/mL | IQR, 1473–4493 | | |
| White | | 440 | Median, 2535 pmol/mL | IQR, 1423–4492 | | |
| Latino | | 452 | Median, 1986 pmol/mL | IQR, 1079–3602 | | |
| Japanese American | | 702 | Median, 2134 pmol/mL | IQR, 1037–3507 | | |
| Urine of pregnant women with no smoke exposure or some smoke exposure | HMPMA | 362 + 93 | Median, 342 ng/mL | NR–17 700 ng/mL | New York, North Carolina, Utah, California, Pennsylvania, Wisconsin | Boyle et al. (2016) |
| Urine of cigarette smokers who switched to e-cigarettes: | HMPMA | 20 | | | Silesia, Poland | Goniewicz et al. (2017) |
| after 1 week | | | 632 µg/g creatinine | IQR, 312–856 | | |
| after 2 weeks | | | 616 µg/g creatinine | IQR, 331–706 | | |
| Urine of: | HMPMA | | | | USA | Bagchi et al. (2018) |
| users of combusted tobacco | | 867 | Median, 1.63 mg/g creatinine | IQR, 0.68–3.29 | | |
| non-users | | 3825 | Median, 0.313 mg/g creatinine | IQR, 0.23–0.45 | | |
| Urine of adolescents: | HMPMA | | | | San Francisco, USA | Rubinstein et al. (2018) |
| e-cigarette smokers | | 67 | Median, 149 ng/mg creatinine | 0–793 | | |
| e-cigarette & tobacco smokers | | 16 | Median, 185 ng/mg creatinine | 110–438 | | |
| non-smokers | | 20 | Median, 100 ng/mg creatinine | 0–522 | | |
| Urine after consumption of broccoli-sprout beverages | HMPMA | 48 | Median, 0.481–0.486 nmol/mg creatinine | IQR, 0.319–0.721 (SFR)<br>IQR, 0.312–0.904 (GRR) | Qidong, China | Kensler et al. (2012) |
| Urine after consumption of: | HMPMA | | | | Qidong, China | Egner et al. (2014) |
| broccoli-sprout beverages | | 137 | GM, 1312 pmol/mg creatinine | IQR, 829–1790 | | |
| placebo | | 130 | GM, 1510 pmol/mg creatinine | IQR, 880–1959 | | |
| Salivary DNA | CdG | 27 | 7.5 adducts/$10^8$ nucleotides | SD, 12 | Ming-Hsiung, Taiwan, China | Chen & Lin (2011) |
| Urine of children aged 6–11 yr: | HMPMA | | | | USA | Jain (2015a) |
| males | | 203 | GM, 338 ng/mL | 95% CI, 298–382 | | |
| females | | 214 | GM, 311 ng/mL | 95% CI, 276–351 | | |

CI, confidence interval; e-cigarette, electronic cigarette; GRR, glucoraphanin-rich; h, hour; HMPMA, *N*-acetyl-*S*-(3-hydroxy-1-methylpropyl)-L-cysteine; IQR, interquartile range; GM, geometric mean; NR, not reported; SD, standard deviation; SFR, sulforaphane-rich.

## Table 1.5 Concentrations of crotonaldehyde in food and beverages

| Item | No. of samples | Concentration | | Country of study or purchase | Reference |
|---|---|---|---|---|---|
| | | Average concentration | Range | | |
| Rice seeds | 12 | ~10–20 ng/g | ~2–5 ng/g | Nanchang, China | Shenzao et al. (2018) |
| Carrot roots | > 8 | 0.04–0.1 mg/kg | NR | Piikkiö, Finland | Linko et al. (1978) |
| Apples, guavas, grapes, strawberries, tomatoes | NR | < 0.01 ppm [mg/kg] | NR | NR | Feron et al. (1991) |
| Cabbage, carrots, celery leaves, cauliflower, Brussels sprouts | | 0.02–0.1 ppm [mg/kg] | NR | | |
| Bread, cheese, milk, meat, fish, beer | | 0–0.04 ppm [mg/kg] | NR | | |
| Wine | | 0–0.7 [mg/L] | NR | | |
| Heavily salted cod | 14 | 1.02 µM/kg | NR | Canada | Yurkowski & Bordeleau (1965) |
| Whole-grain soft wheat | 1 | Detected but not quantified | | USA | McWilliams & Mackey (1969) |
| Heated beef fat | 1 | Detected but not quantified | | Tokyo, Japan | Yamato et al. (1970) |
| Bottled beer | NR | 17 µg/L | NR | Takasaki, Japan | Hashimoto & Eshima (1977) |
| Beer | 3 | 1.33 ppb [µg/L] | 0.77–1.82 ppb [µg/L] | London, UK | Greenhoff & Wheeler (1981) |
| Scotch whisky (brand x) | 4 | 0.03 ± 0.01 ppm [mg/L] | NR | Oxford, OH, USA | Miller & Danielson (1988) |
| Scotch whisky (brand y) | 4 | 0.21 ± 0.02 ppm [mg/L] | NR | | |
| Kentucky bourbon | 3 | 0.04± 0.002 ppm [mg/L] | NR | | |
| Vodka | 3 | < 0.02 (SD, NR) | NR | | |
| Alcoholic beverages | NR | Detected but not quantified | | Baltimore, USA | Theruvathu et al. (2005) |
| Mothers' milk | 12 | Identified in 1 sample | | Bridgeville, PA; Bayonne, NJ; Jersey City, NJ; and Baton Rouge, LA, USA | Pellizzari et al. (1982) |
| Soymilk, ultra-high pressure homogenized | 2 | Detected but not quantified | | Barcelona, Spain | Poliseli-Scopel et al. (2013) |
| Fish oil | 10 | 1.0–21.7 (range of averages) µg/g | SD, 0.1–1.0 | Lake Alfred, FL, USA | Suh et al. (2017) |
| Olive oil, extra virgin | 3 | 0.067 ± 0.006 mg/kg | NR | Cordoba, Spain | Garrido-Delgado et al. (2011) |
| Olive oils, extra virgin | 251 | Detected but not quantified | | Italy | Melucci et al. (2016) |

**Table 1.5 (continued)**

| Item | No. of samples | Concentration | | Country of study or purchase | Reference |
|---|---|---|---|---|---|
| | | Average concentration | Range | | |
| Volatile components of raw and stir-fried fruits | 7 | Detected but not quantified | | Chengu, China | Zhong et al. (2015) |
| Canola oil (180 °C) | 2 | 1.0–1.7 mg/h per L oil | 0.1–0.2[a] | Alicante, Spain | Fullana et al. (2004) |
| Canola oil (240 °C) | 2 | 1.2–2.5 mg/h per L oil | 0.1–0.6[a] | | |
| Extra virgin olive oil (180 °C) | 2 | 0.9–2.8 mg/h per L oil | 0.1–0.4[a] | | |
| Extra virgin olive oil (240 °C) | 2 | 2.9–5.1 mg/h per L oil | 0.1–0.3[a] | | |
| Olive oil (180 °C) | 2 | 0.9–1.9 mg/h per L oil | 0.1–0.2[a] | | |
| Olive oil (240 °C) | 2 | 0.8 mg/h per L oil | 0.1[a] | | |
| Frying process, clam | 18 | 1.44–2.20 µg/g | SD, 0.02–0.11 | Dalian, Liaoning, China | Liu et al. (2020) |
| Cooking oil fumes of soybean oil, sunflower oil, rapeseed oil, and palm oil when cooking potatoes and pork loin | 8 | Quantified together with other aldehydes | | Taiwan, China | Peng et al. (2017) |
| *Coffea arabica* flowers | 3 | Detected but not quantified | | Bucaramanga, Colombia | Stashenko et al. (2013) |

NR, not reported; ppb, parts per billion; ppm, parts per million; SD, standard deviation.

[a] Standard error of the mean.

crotonaldehyde in cigarettes from the Chinese market was 42–67 µg/cigarette (Cai et al., 2019). Zhang et al. (2019a) analysed the gas phase of mainstream smoke from 16 different brands of Chinese flue-cured cigarettes and reported an average crotonaldehyde concentration of 13.4 µg/cigarette under the International Organization for Standardization ISO 3308 machine-smoking regimen (35 mL puff volume, 2 second puff duration, 60 second puff interval). Similar concentrations were reported by Ding et al. (2016) and Sampson et al. (2014) when using the ISO regimen, whereas using the "Canadian Intense" protocol (55 mL puff volume, 2 second puff duration, 30 second puff interval) gave values that were 2–5 times higher; however, levels as high as 228 µg/cigarette have been reported previously. Brands originating in the USA appear to contain higher levels of crotonaldehyde, with Ding et al. (2016) reporting average levels of 25–72 [mean, 48] µg/cigarette in 10 USA brands under the "Canadian Intense" protocol. [The Working Group noted that the "Canadian Intense" method may provide higher values that correspond better to human exposure during smoking.] Several research groups have reported lower levels of crotonaldehyde in mainstream smoke when electronic cigarettes were machine-smoked (Farsalinos et al., 2018; Mallock et al., 2018).

Among smokers in the National Health and Nutrition Examination Survey (NHANES) study conducted by the United States Centers for Disease Control and Prevention (CDC), there was an increase in HMPMA concentration with increasing number of cigarettes smoked. Approximately 20% of participants were smokers (Bagchi et al., 2018). The urinary HMPMA concentrations in smokers were about 5 times higher than in non-smokers (1.63 versus 0.313 mg/g creatinine). HMPMA was detected in 99.9% of all urine samples (Bagchi et al., 2018). Median concentrations were 419 and 369 µg/L for the 2011–12 and 2013–14 sampling periods, respectively, while the 95th percentiles were 3700 and 3040 µg/L, respectively (NHANES, 2019). [The Working Group noted that the latter values are likely to include smokers and/or persons with significant occupational exposure.] The lowest concentrations were reported for non-Hispanic Black people (median, 253 µg/g creatinine for non-users and 1070 µg/g creatinine for users of exclusively smoked tobacco products; interquartile range, 195–356 and 489–1870 µg/g creatinine, respectively). These data indicate widespread crotonaldehyde exposure within the population and confirm that tobacco smoke is a major source of exposure (Bagchi et al., 2018; see Table 1.4).

Alwis et al. (2012) analysed urinary HMPMA concentrations in 1203 non-smokers and 347 smokers. They found the average (± SD) concentrations to be 429 µg/L (± 478 µg/L) in non-smokers and 1992 µg/L (± 2009 µg/L) in smokers, a highly significant difference. Carmella et al. (2009) studied HMPMA concentrations in 17 people who quit smoking. They found that concentrations were reduced by 80% when re-sampling occurred on the next return visit after 3 days (allowing an estimate of the maximum possible half-life of 36 hours for HMPMA) and then remained at approximately this level for the next 56 days of follow-up. Scherer et al. (2006) conducted a study of HMPMA comparing regular-filter cigarettes to those with a charcoal filter. HMPMA concentrations in week 1 were lower in smokers using cigarettes with charcoal filters than in smokers using cigarettes with regular filters. However, the difference disappeared when the groups crossed over after 1 week, although the glutathione-depleting activity of smoke passed through the charcoal filters was significantly less than of smoke passed through regular filters.

Park et al. (2015) studied HMPMA in more than 2200 smokers of five ethnicities. They found a significant difference between the ethnic groups, with native Hawaiians having the highest geometric mean concentrations of HMPMA and Latinos the lowest at 2759 and 2210 pmol/mL

urine, respectively. These data strongly suggest an ethnic influence on exposure effect.

Pluym et al. (2015) measured both HMPMA and CMEMA concentrations in three groups: non-smokers, light smokers (≤ 10 cigarettes/day) and heavier smokers (> 10 cigarettes/day). They reported a robust concentration–response relationship for HMPMA but not for CMEMA. Median concentrations in non-smokers were 18.9 (range, 9.7–64.4) µg/g creatinine for HMPMA, and 201 (range, 104–756) µg/g creatinine for CMEMA. These values were 95.9 (range, 55–268) µg/g creatinine and 226 (range, 125–408) µg/g creatinine in light smokers, and 121.7 (range, 57–220) µg/g creatinine and 226 (range, 121–299) µg/g creatinine in heavier smokers. In addition, there was only a weak correlation between HMPMA and CMEMA concentrations, and no correlation between CMEMA and cotinine concentrations.

### *(b) Indoor air*

Indoor cooking can be a source of airborne exposure to crotonaldehyde. Zhang & Smith (1999) studied the emissions from 22 different methods of cooking in China and found that crotonaldehyde production ranged from not detected to 92 mg/kg fuel for wood used in a brick stove with a flue. Relatively large amounts (up to 88 mg/kg fuel; mean, 60 mg/kg fuel) were produced when liquefied petroleum gas was used as fuel while coal and coal briquette fuels produced the lowest levels. Consistent with these data, Weinstein et al. (2020) reported a non-significant 4% reduction in urinary HMPMA concentrations in women in Guatemala when wood-burning stoves were replaced by liquefied petroleum gas-powered stoves (from 193 µg/g creatinine with wood-burning stoves to 186 µg/g creatinine with liquefied petroleum gas). Mitova et al. (2020) found a mean concentration of 2.06 µg/m³ (SD, ± 0.01 µg/m³) in Switzerland where people warmed a cheese dish on an electric hotplate. Ahn et al. (2014) reported that crotonaldehyde concentrations ranged from 4.96 to 51.7 ppb [14.2 to 148 µg/m³] when mackerel were pan-fried using butane as a fuel in the Republic of Korea. Hecht et al. (2015) compared HMPMA concentrations in non-smoking women in Singapore who cooked once per week or less frequently with a wok (including boiling, stir frying, and deep frying) with those who cooked between 2 and 6 times per week and with those who cooked 7 times per week or more frequently. They reported a highly significant trend with increasing wok use, with the groups at either extreme (< 1 meal/week versus > 7 meals/week) having a geometric mean of 894 (95% confidence interval, CI, 749–1067) pmol/mg creatinine versus 1167 (95% CI, 1022–1332) pmol/mg creatinine). There was also an effect of the oil used to cook, with rapeseed oil (829 pmol/mg creatinine) and sunflower oil (1329 pmol/mg creatinine) being the extremes.

Ochs et al. (2016) reported that varnishing a door during apartment renovation was the source of an increase in crotonaldehyde concentrations that peaked at 80 µg/m³ but dissipated rapidly thereafter.

Lu & Zhu (2007) measured crotonaldehyde concentrations aboard six carriages in different trains during the 2004 Spring Festival in China when tens of millions of people used the train system; they reported concentrations of between 2.6 and 3.6 µg/m³.

### *(c) Outdoor air pollution*

Grosjean et al. (1996) reported that concentrations of crotonaldehyde in outdoor air in Los Angeles, California, USA, peaked at about 0.5 ppb [1.4 µg/m³] with an average concentration of 0.3 ppb [0.86 µg/m³]. Concentrations seemed to increase with traffic, consistent with reports of crotonaldehyde in the exhaust of gasoline and diesel engines (Nishikawa et al., 1987; Zervas et al., 2002; Song et al., 2010). Similarly, Dugheri et al. (2019) reported that crotonaldehyde concentrations in four roads with heavy traffic in Florence,

Italy, were 0.8–1.3 μg/m³ (mean, 1.0 μg/m³), while in a low-traffic area, the mean concentration was 0.2 μg/m³. The bulk of the crotonaldehyde was found in the vapour phase.

*(d) Food and beverages*

See Table 1.4 and Table 1.5.

Crotonaldehyde is present in many foodstuffs, including vegetables (Brussels sprouts, cabbages, carrots, cauliflower, celery leaves; at concentrations of 0.02–0.1 ppm [mg/kg]), fruits (apples, grapes, guavas, tomatoes and strawberries; at > 0.01 ppm [mg/kg]), dairy products and meats (milk, bread, cheese, meat, clams and fish), beer, and wine (at 0–0.07 ppm [mg/kg, mg/L]) (Feron et al., 1991; Liu et al., 2020). Whisky and vodka contain from < 0.02 to 0.21 ppm [mg/L] (Miller & Danielson, 1988). Fruit intake was significantly associated with increased urinary HMPMA levels in the NHANES survey (Bagchi et al., 2018).

Recent data indicated that heated cooking oil is a significant source of exposure to crotonaldehyde in food. In a study conducted in Germany, Granvogl (2014) reported that while cooking oils differ intrinsically due to composition, the amount of crotonaldehyde in each oil increases significantly with temperature (100–180 or 220 °C) and heating time. Concentrations of crotonaldehyde in the oils ranged from below 9 μg/kg in unheated oils to 34 mg/kg [34 000 μg/kg] for linseed oil heated to 180 °C for 24 hours. Foods cooked in these oils also contained crotonaldehyde, albeit at lower concentrations. Both potato chips and doughnuts cooked in rapeseed oil contained twice as much crotonaldehyde as those cooked in olive oil (24.8 and 18.2 μg/kg, and 12.6 and < 9 μg/kg, respectively). Liu et al. (2020) measured crotonaldehyde concentrations in clams before and during deep frying in China. They found that the concentration of crotonaldehyde increased with both oil temperature and cooking time, from 0.04 μg/g for fresh clams to 1.46 μg/g for clams fried at 180 °C for 15 minutes. Crotonaldehyde concentrations in pre-marinated control clams also increased over 15 minutes when they were fried at 160 °C.

*(e) Exposures in infants and children*

See Table 1.4.

Regarding infants, El-Metwally et al. (2018) compared urinary concentrations of HMPMA in newborns in cribs versus those born pre-term and placed in incubators (median ages, 16 and 11 days, respectively). Median concentrations did not differ (394 μg/L versus 376 μg/L, respectively), suggesting that there were relatively high crotonaldehyde exposures in neonatal intensive care units (compared with children aged 6–11 years, see above). Boyle et al. (2016) studied 488 pregnant women and reported a 50th percentile HMPMA value of 342 μg/L which was virtually identical to the value of 352 μg/L reported by NHANES for girls and women aged 6–11 years, 12–19 years, and ≥ 20 years (NHANES, 2019). Boyle et al. reported that the highest value measured was 17 700 μg/L (5% of their sample were tobacco smokers).

### *1.4.3 Occupational exposure*

One of the largest current commercial uses of crotonaldehyde is in the production of sorbic acid as a food preservative (E200), and crotonic acid (European Commission, 2013). However, no data could be found on workers' exposure during this process. A survey conducted by NIOSH (1983) suggested that fewer than 400 workers (metal-plating machine operators in the transportation equipment industry, and separating, filtering, and clarifying machine operators in the chemicals and allied products industry) had potential exposure to crotonaldehyde in the USA, but no measurements were made. Since that time, it has become appreciated that far more workers are exposed to crotonaldehyde via exposure to pyrolysis products. Therefore, studies have been

performed in cooks, coke-oven workers, and traffic officers, at toll booths, and particularly on firefighters, but have focused on biological monitoring rather than air concentrations.

The *IARC Monographs* programme in its previous evaluation of crotonaldehyde (Volume 63; IARC, 1995) noted that a variety of measurements for the compound were made in 24 Finnish businesses and that all measurements were below the Finnish standard at the time of 6 mg/m³. Linnainmaa et al. (1990) found concentrations of 0.23 mg/m³ near a doughnut-frying station in a Finnish bakery. In a chemical plant in the USA, area samples ranged from not detected to 3.2 mg/m³, with two personal samples of 1.9 and 2.1 mg/m³ (NIOSH, 1982). Crotonaldehyde was detected at concentrations of 1–7 mg/m³ in a plant producing aldehydes in Germany. More recently, Zhang et al. (2003) measured exposure to crotonaldehyde via inhalation in parking-garage workers ($n$ = 53) and controls ($n$ = 33) and reported that smoking parking-garage workers had a mean crotonaldehyde air concentration of 0.96 µg/m³ (SD, ± 0.94 µg/m³) and non-smoking parking-garage workers' mean concentrations were 0.53 µg/m³ (± 0.79 µg/m³). Smoking controls were exposed to crotonaldehyde at 0.29 µg/m³ (± 0.48 µg/m³), and non-smoking controls at 0.25 µg/m³ (± 0.32 µg/m³). Destaillats et al. (2002) measured concentrations at toll booths in San Francisco, USA, and reported concentrations (mean ± SD) of 0.061 ± 0.012 µg/m³ and 0.093 ± 0.002 µg/m³ in the afternoon and 0.147 ± 0.004 µg/m³ in the morning.

For firefighters, Dills et al. (2008) reported crotonaldehyde concentrations as high as 4.3 mg/m³ in the overhaul smoke of a demonstration fire (wood with polyvinyl chloride), when water was used to knock down the fire. In another demonstration-fire study (household materials), Jones et al. (2016) reported concentrations as high as 0.07 ppm [0.2 mg/m³] during the overhaul phase (smouldering) of the exercise. In a third demonstration study, Kirk & Logan (2015) measured concentrations between 1 and 11 µg/m³ off-gassing from a structural firefighting ensemble for 24 hours after four hostile attack evolutions (resin-bonded wood panels).

Frigerio et al. (2020) measured urinary CMEMA and HMPMA concentrations in coke-oven workers, but there was no statistical difference between concentrations in workers and controls, the latter being slightly higher.

## 1.5 Regulations and guidelines

### *1.5.1 Exposure limits and guidelines*

#### *(a) Occupational exposure limits*

Occupational exposure regulations and guidelines for various countries and states are given in Table 1.6. Crotonaldehyde is a potent irritant of the skin, eyes, and mucous membranes throughout the respiratory tract. The ACGIH TLV of 0.86 mg/m³ for crotonaldehyde is based on analogy with formaldehyde as an irritant. The TLV is a ceiling level, i.e. a level that should never be exceeded. Crotonaldehyde is also given a "skin" notation by ACGIH indicating that there are data suggesting that the liquid is well-absorbed through the skin (ACGIH, 2020). Although the TLVs are established to provide professional guidance for practicing industrial hygienists, they have been adopted by many governmental regulatory agencies. The TLV for crotonaldehyde was last updated by the ACGIH in 1998 with the ceiling value being adopted. As can be seen from Table 1.6, values established before 2000 are significantly higher than those promulgated after 2000, the sole exception being United States Occupational Safety and Health Administration (OSHA) and NIOSH. Furthermore, the pre-2000 limits are time-weighted averages as opposed to the ceilings that should never be exceeded for many values set after 2000.

**Table 1.6 Occupational exposure limits for crotonaldehyde[a] in various countries**

| Country or agency | Year | Concentration (mg/m³) | Interpretation | Notation, category |
|---|---|---|---|---|
| Australia | 2018 | 5.7 | TWA | |
| Austria | 2011 | 1 | MAK | |
| | | 4 | STEL | |
| Argentina[b] | 2019 | 0.86 | Ceiling | Skin A3 Carcinogen |
| Belgium | 2009 | 0.87 | STEL | |
| Bulgaria[b] | 2019 | 0.86 | Ceiling | Skin A3 Carcinogen |
| Canada – Alberta | 2001 | 5.8 | TWA | |
| Canada – Ontario | 2020 | 0.86 | Ceiling | Skin |
| Canada – Quebec | 2020 | 5.7 | TWA | Skin A3 Carcinogen |
| China | 2019 | 12 | MAC | |
| Columbia[b] | 2019 | 0.86 | Ceiling | |
| Denmark | 1999 | 6 | TWA | Skin |
| European Union – SCOEL | 2013 | | | Skin |
| Finland | 2000 | 0.29 | TWA | Skin |
| | | 0.87 | STEL | |
| France | 2016 | 6 | VLEP | |
| Germany – MAK | 2006 | | | Skin 3B Carcinogen |
| Ireland | 2007 | 6 | TWA | |
| | | 18 | STEL | |
| Jordan[b] | 2019 | 0.86 | Ceiling | Skin A3 Carcinogen |
| New Zealand[b] | 2019 | 0.86 | Ceiling | Skin A3 Carcinogen |
| Norway | 2013 | 6 | TWA | Skin |
| Philippines | 1993 | 6 | TWA | |
| Poland | 2018 | 1 | TWA | Skin |
| | 2018 | 2 | STEL | |
| Portugal | 2004 | 0.86 | Ceiling | |
| Republic of Korea[b] | 2019 | 0.86 | Ceiling | Skin A3 Carcinogen |
| Romania | 2018 | 25 | STEL | |
| Singapore[b] | 2014 | 5.7 | PEL (long-term) | Skin A3 Carcinogen |
| Spain | 2019 | 0.87 | STEL | Skin |
| Switzerland | 2005 | 1 | MAK-W | Skin |
| United Kingdom | 1993 | 6 | LTEL | |
| | | 18 | STEL | |
| USA –ACGIH TLV[c] | 2019 | 0.86 | Ceiling | |
| USA – OSHA PEL | 2019 | 6 | TWA | Skin A3 Carcinogen |
| USA – NIOSH REL | 2019 | 6 | TWA | |
| USA – Connecticut | 2011 | 0.12 | Ambient air | |
| USA – Nevada | 2011 | 0.143 | Ambient air | |
| USA – North Dakota | 2011 | 0.18 | Ambient air | |

**Table 1.6 (continued)**

| Country or agency | Year | Concentration (mg/m³) | Interpretation | Notation, category |
|---|---|---|---|---|
| USA – Virginia | 2011 | 0.10 | Ambient air | |
| Viet Nam[b] | 2019 | 0.86 | Ceiling | Skin A3 Carcinogen |

ACGIH, American Conference of Governmental Industrial Hygienists; LTEL, long-term exposure limit (8 hours); MAC, maximum allowable concentration (ceiling value); MAK, MAK-W, Maximale Arbeitsplatz-Konzentration (maximum workplace concentration), in the workplace air which generally does not have known adverse effects on the health of the employee nor cause unreasonable annoyance even when the person is repeatedly exposed for 8 hours daily assuming on average a 40-hour working week; PEL, permissible exposure limit; PEL (long-term), permissible exposure level over an 8-hour working day and a 40-hour working week; REL, recommended exposure limit; SCOEL, Scientific Committee on Occupational Exposure Limits; STEL, short-term exposure limit, based on a 15 minute average; TLV, threshold limit value, the level to which a worker may be repeatedly exposed, day after day, over a working lifetime without adverse health effects; TWA, 8-hour time-weighted average; VLEP, *Valeur limite d'exposition professionnelle* (8-hour occupational exposure limit value).

[a] Includes *trans*- (*E*-), *cis*- (*Z*-), and a mixture of both.

[b] Use ACGIH TLVs as local regulations.

[c] Based on analogy with formaldehyde.

From ACGIH (2020); Pohanish (2012); European Commission (2013); Finland Ministry of Social Affairs and Health (2018); Ontario Ministry of Labour, Training and Skills Development (2020).

### *(b) Environmental exposure limits*

Crotonaldehyde has not been widely regulated in the environment. As with acrolein and other reactive aldehydes, occupational guidelines for acute exposures (100–300 ppb) [0.29–0.86 mg/m³] are approximately 10 to 100 times the environmental guidelines for acute exposures (1–5 ppb [2.9–14 µg/m³] or for subacute exposures).

In 2008 the National Advisory Committee for Acute Exposure Guideline Levels (AEGLs) for Hazardous Substances of the United States National Academy of Sciences evaluated crotonaldehyde exposure concentrations and times that could be classified as nondisabling (AEGL-1), disabling (AEGL-2), and lethal (AEGL-3) (National Research Council, 2007). These are presented in Table 1.7. Note that AEGL-3, which is lethal, is reached after 10 minutes exposure to crotonaldehyde at 44 000 ppb (44 ppm) [130 mg/m³], whereas exposure for any duration of time from 10 minutes to 8 hours to 190 ppb [0.55 mg/m³] leads to slight eye irritation and discomfort.

## *1.5.2 Reference values for biological monitoring of exposure*

There are currently no regulations or guidelines for measuring levels of crotonaldehyde metabolites or other biomarkers in biological samples. While there have been important studies involving metabolites in smokers, there are very few data related to metabolite concentrations, air concentrations, or effect markers (e.g. DNA adducts and metabolites), which are the parameters needed to provide guidance relevant for occupational exposure. In addition, there remain other data gaps that prevent development of such a biological exposure index. This includes data on metabolite elimination half-life, which is needed to recommend the timing of sample collection (ACGIH, 2020). One alternative for guidance is using "population" reference values based on the 95th percentile levels in the general population (ACGIH, 2020). [The Working Group noted that there appeared to be ample data to establish a population value for crotonaldehyde.]

**Table 1.7 Summary of acute exposure guideline levels for crotonaldehyde**

| Classification | 10 minutes | 30 minutes | 1 hour | 4 hours | 8 hours | End-point | Reference |
|---|---|---|---|---|---|---|---|
| Classification AEGL-1a (nondisabling) | 0.19 ppm (0.55 mg/m³) | 0.19 ppm (0.55 mg/m³) | 0.19 ppm (0.55 mg/m³) | 0.19 ppm (0.55 mg/m³) | 0.19 ppm (0.55 mg/m³) | Mild eye irritation in humans | NIOSH (1982) |
| AEGL-2 (disabling) | 27 ppm (77 mg/m³) | 8.9 ppm (26 mg/m³) | 4.4 ppm (13 mg/m³) | 1.1 ppm (3.2 mg/m³) | 0.56 ppm (1.6 mg/m³) | Impaired pulmonary function, NOAEL for bronchiole lesions | Rinehart (1967) |
| AEGL-3 (lethal) | 44 ppm (130 mg/m³) | 27 ppm (77 mg/m³) | 14 ppm (40 mg/m³) | 2.6 ppm (7.4 mg/m³) | 1.5 ppm (4.3 mg/m³) | Lethality NOEL | Rinehart (1967) |

AEGL, acute exposure guideline levels; NOAEL, no-observed-adverse-effect level; NOEL, no-observed-effect level; ppm, parts per million.
From National Research Council (2007).

## 1.6 Quality of exposure assessment in key epidemiological studies

Table S1.6 and Table S1.7 (Annex 2, Supplementary material for crotonaldehyde, Section 1, Exposure Characterization, web only; available from: https://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 crotonaldehyde. Only four studies of human cancer were identified: one occupational cohort and three nested case–control studies, two of lung cancer and one of colorectal cancer. The occupational cohort study assigned exposure based on expert evaluation of company records on the use of chemicals and on employment. The two case–control studies on lung cancer were nested within the same general population cohort and assessed exposure by measuring urinary metabolites (HMPMA). The case–control study on colorectal cancer applied an untargeted adductomics approach. The majority of the mechanistic studies in humans can be considered demonstration studies, as noted below.

### *1.6.1 Quality of exposure assessment in key cancer epidemiology studies*

Bittersohl (1975) investigated cancer frequency in an aldehyde factory and assigned exposure to crotonaldehyde based on employment records. Quantitative crotonaldehyde measurements, available for some departments, were not used to quantify exposure intensity or cumulative exposure. Workers were likely to be exposed simultaneously to other chemical agents (e.g. acrolein, see the first monograph in the present volume). The two nested case–control studies of lung cancer (Yuan et al., 2012, 2014) assessed exposure by measuring a urinary metabolite of crotonaldehyde, HMPMA. A single void urine sample was collected from each participant at baseline, and these urine samples were analysed for cases and controls to determine the concentration of HMPMA. Information on smoking was available from a questionnaire, and smokers were studied separately from non-smokers.

The nested case–control study on colorectal cancer applied an untargeted approach to measure Cys34 adducts of albumin to crotonaldehyde in human serum. Serum samples were collected at time of recruitment to the cohort. Information on body mass index and lifestyle factors such as smoking, alcohol drinking, and

meat consumption was collected by questionnaire. The formation of crotonaldehyde adducts was not related to external exposures, such as smoking, and was instead attributed to endogenous production after oxidation of membrane lipids by reactive oxygen species.

### *1.6.2 Quality of exposure assessment in mechanistic studies in humans*

As noted above, the majority of the mechanistic studies can be considered demonstration studies, simply reporting that it is possible to use the technique described to detect the particular biomarker in human samples (Nath & Chung, 1994; Nath et al., 1996, 1998; Zhang et al., 2006; Chen & Lin, 2009, 2011; Garcia et al., 2013; Alamil et al., 2020). Most studies were early validations and are not used to assess carefully the relationship between external exposure and mechanistic end-points. For compounds like crotonaldehyde that have widespread environmental sources, are produced endogenously, and are also present in basic foods and beverages, careful documentation of food, tobacco, and alcohol consumption, and significant exposure to automotive exhaust is required to determine contributions from different exposure sources. This was lacking in several studies. If samples are collected from cases (Grigoryan et al., 2019), the potential exists that the disease itself could cause differences in DNA adduct levels and/or that exposure for the cases may have changed between the time the case was identified and the time that the sample collected. In all these studies, only a single exposure marker was reported at a single point in time, making it difficult or impossible to assess exposure sources and duration, since the marker is then used as an outcome.

## 2. Cancer in Humans

### 2.1 Descriptions of individual studies

See Table 2.1.

One cohort study and three nested case–control studies in cohorts have been published on the relationship between cancer and exposure to crotonaldehyde.

#### *Cohort studies*

Bittersohl (1975) recorded cancer cases in a small cohort of 220 workers in an aldehyde factory in the former German Democratic Republic who were diagnosed between 1967 and 1972. Workers who left the factory for whatever reason were not included. Measurements in some factory departments showed values of crotonaldehyde of 1–7 mg/m$^3$. Four different cancer types were observed in nine men (5 cases of squamous cell lung carcinoma, 2 cases of squamous cell carcinoma of the oral cavity, 1 case of adenocarcinoma of the stomach, and 1 case of adenocarcinoma of the colon). Two cases in women (one leukaemia and one cancer of the ovary) were excluded from analysis due to short duration of exposure to aldehydes. There was no formal comparison group; a comparison was made with incidence rates in the general population of the former German Democratic Republic (source not reported). [The Working Group noted that the study design was weak. Not all those ever employed in the factory were included, only those currently employed (possible selection bias), and a small number of cases (9 cases) at four different sites were recorded. Exposure was based on measurements in some unspecified departments and there were multiple undifferentiated exposures experienced by workers. The exposure–disease association was not quantified because comparison rates for the general population were not provided.]

## Table 2.1 Epidemiological studies of cancer in humans exposed to crotonaldehyde

| Reference, location, enrolment/ follow-up period, study-design | Population size, description, exposure assessment method | Cancer type | Exposure category or level | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments |
|---|---|---|---|---|---|---|---|
| Bittersohl (1975)<br>Former German Democratic Republic<br>1967–1972<br>Cohort | 220 workers employed in an aldehyde factory operating since 1936; included men presently in the factory<br>Exposure assessment method: quantitative measurements; workers exposed to multiple aldehyde derivatives containing traces of crotonaldehyde; exposure was assumed based on employment within the aldehyde factory, with measurable airborne levels of crotonaldehyde (1–7 mg/m$^3$) | Lung (squamous cell carcinoma) | Men, NR | 5 | NR | None | *Exposure assessment critique*: Poorly defined exposure. No attempt to assess exposure (semi-) quantitatively by measurements of duration. No separate exposure assessment for different chemical agents present in the factory, hence it is not possible to separate the effect of different chemical agents.<br>*Strengths*: cancers among workers in the factory were recorded.<br>*Limitations*: small sample size; selection bias, as only presently employed workers were included; relationship with the exposure could not be established; calculation of RR was not possible. |
| | | 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, enrolment/ follow-up period, study-design | Population size, description, exposure assessment method | Cancer type | Exposure category or level | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments |
|---|---|---|---|---|---|---|---|
| Yuan et al. (2012) Shanghai, China Enrolment, 1986–1989; follow-up through 2006 Nested case–control | Cases: 343 cases of incident lung cancer and deaths; current smokers at enrolment, identified through annual in-person interviews and reviewed through Shanghai Cancer Registry and Shanghai Municipal Vital Statistics Office; cohort of 18 244 men aged 45–64 yr at baseline<br>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 mo) and neighbourhood of residence at enrolment<br>Exposure assessment method: exposure to crotonaldehyde was determined based on measurement of its urinary metabolite HMPMA; 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 HMPMA (pmol/mg creatinine) (OR): | | | 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*: No external exposure assessment. All study subjects were smokers. Smoking history was included as confounder in the analyses. Urine samples were collected at baseline, so clearly preceded the health outcome; however, only one urine sample was collected. Cancer risk was evaluated at increasing metabolite levels.<br>*Other comments*: urinary levels of HMPMA were statistically significantly associated with increased risk of lung cancer; however, after adjustment for cotinine, a biomarker of nicotine, there was no longer an association.<br>*Strengths*: active follow-up with annual in-person interviews; 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 verified by urinary cotinine.<br>*Limitations*: intraindividual variation in exposure not captured; 35% of cases were not histologically confirmed. |
| | | | First quartile | 47 | 1 | | |
| | | | Second quartile | 74 | 1.34 (0.83–2.17) | | |
| | | | Third quartile | 94 | 1.58 (0.98–2.56) | | |
| | | | Fourth quartile | 128 | 1.95 (1.22–3.12) | | |
| | | | Trend-test *P* value, 0.004 | | | | |
| | | Lung, incidence | Quartile of urinary HMPMA (pmol/mg creatinine) (OR) | | | Age at diagnosis and place of residence, smoking intensity and duration, duration of urine samples storage before laboratory analysis, urinary total NNAL and PheT | |
| | | | First quartile | 47 | 1 | | |
| | | | Second quartile | 74 | 1.19 (0.73–1.95) | | |
| | | | Third quartile | 94 | 1.33 (0.81–2.18) | | |
| | | | Fourth quartile | 128 | 1.58 (0.96–2.57) | | |
| | | | Trend-test *P* value, 0.058 | | | | |
| | | Lung, incidence | Quartile of urinary HMPMA (pmol/mg creatinine) (OR): | | | Age at diagnosis and place of residence, smoking duration and intensity, duration of urine samples storage before laboratory analysis, total urine cotinine | |
| | | | First quartile | 47 | 1 | | |
| | | | Second quartile | 74 | 0.90 (0.53–1.52) | | |
| | | | Third quartile | 94 | 0.95 (0.56–1.62) | | |
| | | | Fourth quartile | 128 | 0.97 (0.56–1.66) | | |
| | | | Trend-test *P* value, 0.956 | | | | |

## Table 2.1 (continued)

| Reference, location, enrolment/ follow-up period, study-design | Population size, description, exposure assessment method | Cancer type | Exposure category or level | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments |
|---|---|---|---|---|---|---|---|
| Yuan et al. (2014)<br>China, Shanghai enrolment, 1986–1989/ follow-up through 2008<br>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), aged 45–64 yr at enrolment and resided in one of four small geographically defined communities in Shanghai, China<br>Controls: 83 participants in the Shanghai Cohort study without cancer, non-smokers and alive at the time of cancer diagnosis of the case; matched on age at enrolment (± 2 yr), year and month of urine sample collection (± 1 mo), and neighbourhood of residence at recruitment.<br>Exposure assessment method: in-person questionnaire (for smoking status); exposure to crotonaldehyde was determined based on measurement of its urinary metabolite HMPMA; urine samples were collected at baseline survey of the cohort in which the case–control study was nested. | Lung, incidence | Quartile of HMPMA (OR):<br>First quartile<br>Second quartile<br>Third quartile<br>Fourth quartile<br>Trend-test *P* value, 0.99 | 24<br>17<br>19<br>20 | 1<br>0.75 (0.31–1.83)<br>0.8 (0.33–1.97)<br>1 (0.41–2.41) | Age at baseline, neighbourhood of residence at enrolment, years of sample storage and urinary cotinine level | *Exposure assessment critique*: Internal exposure assessment only. No information on external exposure. Only never-smokers were included. Urine samples were collected at baseline, so clearly preceded the health outcome; however, only one urine sample at baseline was collected (intraindividual variations).<br>*Strengths*: active follow-up with annual in-person interviews; long follow-up (22 yr); losses to follow-up low (5.4%); self-reported smoking status was confirmed by urinary cotinine levels.<br>*Limitations*: no external exposure assessment; relatively small sample size; 26% of cases not histologically confirmed. |

**Table 2.1 (continued)**

| Reference, location, enrolment/ follow-up period, study-design | Population size, description, exposure assessment method | Cancer type | Exposure category or level | Exposed cases or deaths | Risk estimate (95% CI) | Covariates controlled | Comments |
|---|---|---|---|---|---|---|---|
| Grigoryan et al. (2019) Turin, Italy, EPIC study Enrolment, 1993 through 1997/follow-up, ≤ 14 yr Nested case–control | Cases: 57 men and women aged 36–65 yr at enrolment Controls: 72 men and women aged 36–64 yr at enrolment; included 47 case–control pairs matched on age, sex, and enrolment year and season Exposure assessment method: no data on external exposure; study of Cys34 adducts in serum samples, including for crotonaldehyde by untargeted adductomics. questionnaire for data on diet, BMI, and lifestyle factors | Colon and rectum, incidence | Seven Cys34 adducts to albumin were statistically significantly associated with colorectal cancer. One of the five adducts found to be more abundant in cases than in controls was identified as a crotonaldehyde adduct and clustered with the *s*-methanethiol adduct. | | | Age, sex | *Exposure assessment critique*: No external exposure assessment, such as on smoking status. Serum samples collected at baseline, before disease occurrence. Crotonaldehyde may have been produced endogenously.<br>*Strengths*: cancer of the colon or rectum confirmed by colonoscopy and biopsy; data in various lifestyle factors collected by questionnaire.<br>*Limitations*: small sample size. |

BMI, body mass index; CI, confidence interval; HMPMA, *N*-acetyl-*S*-(3-hydroxy-1-methylpropyl)-L-cysteine; mo, month; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NR, not reported; OR, odds ratio; PheT, r-1,t-2,3,c-4-tetrahydroxy1,2,3,4-tetrahydrophenanthrene; RR, relative risk; yr, year.

Yuan et al. (2012) and Yuan et al. (2014) published results from two nested case–control studies from the Shanghai Cohort Study, which included 18 244 men residing in one of four small communities in Shanghai and aged 45–65 years at enrolment (1986–1989). The methodology of the two nested case–control studies was very similar. Besides in-person interviews, a spot urine sample was taken from each participant at baseline and stored until laboratory analysis. Lung cancer incidence and mortality data were obtained from annual in-person interviews of all surviving participants, the local cancer registry, and the vital statistics office. Exposure to crotonaldehyde was represented by its urine metabolite HMPMA at enrolment. [The Working Group noted that both studies used a nested case–control design, with a long follow-up and few losses to follow-up. As a measure of exposure, tobacco-specific biomarkers were determined in urine samples. Urine biomarkers were based on single urine samples at enrolment, and smoking status was also collected at enrolment. The rate of histopathological confirmation of lung cancer was moderate, at 65% and 74% of cases for each study respectively. Otherwise, the classification was based on clinical diagnosis.]

In the first study (Yuan et al., 2012), the cohort was followed for 20 years through 2006; loss during follow-up was 4.6%. The aim of the study was to examine the relationship between some volatile carcinogens and toxicants from tobacco smoke and lung cancer development in smokers. A total of 706 cases of lung cancer were identified, of which 574 were in current smokers at baseline. For each case in a smoker, one control was selected, also a smoker, who was alive and free of cancer at the time of cancer diagnosis and matched on age at enrolment, date of urine sample collection, and neighbourhood of residence. After excluding cases and controls whose urine samples were depleted and had missing values for one or more mercapturic acid metabolites, 343 lung cancer cases and 392 controls were included in the analysis (all current smokers at baseline). Urine samples were analysed for mercapturic acids, including a metabolite of crotonaldehyde (HMPMA), as well as for metabolites of polycyclic aromatic hydrocarbons (*r*-1,t-2,3,c-4-tetrahydroxy1,2,3,4-tetrahydrophenanthrene; PheT), tobacco-specific nitrosamines (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL), and nicotine (total cotinine). Smoking duration was 34.4 years for cases and 30.8 years for controls. Lung cancer cases had significantly higher concentrations of HMPMA than did controls ($P < 0.001$), and HMPMA concentration was positively associated with the daily number of cigarettes smoked and duration of smoking ($P < 0.001$). Comparing the highest with the lowest quartiles of HMPMA concentration, risk of lung cancer was almost 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 (PheT) and tobacco-specific nitrosamines (NNAL) and/or cotinine, no association was found between HMPMA concentration and lung cancer. [The Working Group noted that there were multiple correlated exposures as measured by biomarkers. The strengths of the study included a relatively large sample size, a sufficiently long follow-up (20 years), few losses to follow-up, that the urinary biomarker HMPMA was collected before disease occurrence, and that smoking status was verified by total cotinine. The nearly two-fold increase in risk of lung cancer associated with the highest quartile of HMPMA concentration when adjusting only for intensity and duration of smoking disappeared with further adjustment for other smoking biomarkers such as cotinine. HMPMA is likely to be a biomarker of smoking. Overall, the study was not informative regarding the carcinogenicity of crotonaldehyde.]

In the second study (Yuan et al., 2014), a similar design as in the paper published in

2012 was applied. Male never-smokers at baseline were included to examine the relationship between environmental exposure to air pollutants, including secondhand smoke, and lung cancer. The follow-up was extended through 2008 (22 years). Loss to follow-up was 5.4%. A total of 80 cases of lung cancer and 82 controls (all never-smokers at baseline) were included in the analysis, after excluding cases with urinary cotinine concentrations above 18 ng/mL (indicating that they may have been smokers) and missing values for cotinine and mercapturic acids. The same biomarkers as in the previous paper were measured, including HMPMA for crotonaldehyde; PheT; 3-OH-Phe (3-hydroxyphenanthrene) and total OH-Phe (total hydroxyphenanthrenes, the sum of 1-, 2-, 3- and 4-OH-Phe) for polycyclic aromatic hydrocarbons; and cotinine for nicotine. Urinary concentrations of HMPMA were similar in both cases and controls. After adjustment for matching factors and urinary cotinine concentration, HMPMA was not associated with elevated risk of lung cancer (fourth quartile versus first quartile OR, 1.00; 95% CI, 0.41–2.41). [The Working Group noted that only internal exposure to crotonaldehyde was assessed. In addition, urinary cotinine represents a short-term biomarker of passive smoking and therefore there may not have been full adjustment for long-term secondhand smoke exposure.]

Grigoryan et al. (2019) published results of a nested case–control study on cancer of the colon or rectum within the cohort study European Prospective Investigation into Cancer and Nutrition, in Italy (EPIC-Italy), with participants recruited from 1993 through 1997. Serum samples were obtained at baseline to detect Cys34 adducts of albumin, as an exposure marker. Cases of colorectal cancer were confirmed by colonoscopy and biopsy. Healthy controls were selected from the cohort, and matched on age, sex, and enrolment year and season. Data on different lifestyle factors were obtained by questionnaire at baseline. After excluding gelled serum samples and samples from two subjects with a high percentage of missing adducts, 57 cases and 72 controls were included in analyses (including 47 matched case–control pairs). Seven Cys34 adducts were associated in a statistically significant manner with colorectal cancer. Five adducts were found to be more abundant in the cases than in controls. One of these was identified as a crotonaldehyde adduct and clustered with the *s*-methanethiol adduct. These adduct findings may have resulted from the infiltration of gut microbes into the intestinal mucosa and subsequent inflammatory response. [The Working Group noted the small sample size and the lack of information regarding external exposure to crotonaldehyde as limitations of this study.]

## 2.2 Evidence synthesis for cancer in humans

Epidemiological evidence available on crotonaldehyde in relation to cancer in humans comprised one occupational cohort study (Bittersohl, 1975) and three nested case–control studies in population-based cohorts (Yuan et al., 2012, 2014; Grigoryan et al., 2019). Regarding cancer sites evaluated across these studies, three of the four studies examined lung cancer (Bittersohl, 1975; Yuan et al., 2012, 2014), while the occupational cohort study (Bittersohl, 1975) also reported on cancers of the oral cavity, stomach, and colon. One nested case–control study (Grigoryan et al., 2019) reported on cancers of the colon or rectum.

### *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. One study considered external occupational exposure to crotonaldehyde (Bittersohl, 1975), but provided no quantitative exposure assessment, and

therefore no exposure–response analyses could be carried out. In addition, study participants were simultaneously exposed to multiple, undifferentiated chemical agents, and the potential associations between individual chemicals and cancer risk could not be evaluated.

The two other studies (Yuan et al., 2012, 2014) considered crotonaldehyde exposure in two nested case–control studies of smokers and non-smokers, respectively, as determined by urinary metabolites. These studies did not consider external exposure to crotonaldehyde explicitly. Although information on smoking was available and may have been an important source of crotonaldehyde exposure, these studies adjusted for smoking through restriction or statistical adjustment.

#### *2.2.2 Cancers of the lung 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 study among current smokers, and one among never-smokers at baseline). Analyses conducted among the smokers (Yuan et al., 2012) revealed a two-fold risk of lung cancer for the highest compared with the lowest quartile of the crotonaldehyde biomarker HMPMA adjusted for intensity and duration of smoking. Further adjustment for markers of smoking (NNAL, PheT, cotinine) diminished the association between crotonaldehyde and lung cancer, which suggested that crotonaldehyde represents a biomarker of smoking. The study examined the relationship between some volatile carcinogens and toxicants from tobacco smoke and lung cancer development in smokers and was considered uninformative regarding the carcinogenicity of crotonaldehyde as such.

One cohort study (Bittersohl, 1975) among workers currently employed in an aldehyde factory and exposed to multiple chemicals, including aldehydes, reported four different types of cancer among nine male workers (lung, oral cavity, stomach, colon). The study was considered uninformative due to the poorly defined external exposure, small number of cases, and flaws in study design.

One nested case–control study (Grigoryan et al., 2019) found an association between cancers of the colon or rectum and an albumin adduct of crotonaldehyde, interpreted as the effect of an inflammatory response to the gut microbiota infiltrating the colon mucosa.

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

## 3. Cancer in Experimental Animals

In a previous evaluation, the *IARC Monographs* programme concluded that there was *inadequate evidence* in experimental animals for the carcinogenicity of crotonaldehyde (IARC, 1995).

Studies on the carcinogenicity of crotonaldehyde 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 Crj:$BDF_1$ [$B6D2F_1$/Crlj] mice (age, 6 weeks) were treated by inhalation with crotonaldehyde (purity, > 99.9%; CAS No., 123-73-9) by whole-body exposure for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2001a, b, c). The

## Table 3.1 Studies of carcinogenicity with crotonaldehyde in experimental animals

| Study design<br>Species, strain (sex)<br>Age at start<br>Duration<br>Reference | Route<br>Purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals | Results | Significance | Comments |
|---|---|---|---|---|
| Full carcinogenicity<br>Mouse, B6D2F$_1$/Crlj (M)<br>6 wk<br>104 wk<br>JBRC (2001a) | Inhalation (whole-body)<br>> 99.9%<br>Clean air<br>0, 3, 6, 12 ppm<br>6 h/day, 5 days/wk<br>50, 50, 50, 50<br>33, 30, 38, 43 | *All sites*: no significant increase in the incidence of tumours | | Principal strengths: multiple-dose study; use of males and females; study complied with GLP.<br>Other comments: the incidence of hyperplasia of the respiratory tract 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<br>Mouse, B6D2F$_1$/Crlj (F)<br>6 wk<br>104 wk<br>JBRC (2001a) | Inhalation (whole-body)<br>> 99.9%<br>Clean air<br>0, 3, 6, 12 ppm<br>6 h/day, 5 days/wk<br>50, 50, 50, 50<br>30, 25, 30, 34 | *All sites*: no significant increase in the incidence of tumours | | Principal strengths: multiple-dose study; use of males and females; study complied with GLP.<br>Other comments: the incidence of hyperplasia of the respiratory tract 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<br>Mouse, B6C3F$_1$ (M)<br>Neonatal (age 8 days)<br>12 mo<br>Von Tungeln et al. (2002) | Intraperitoneal injection<br>NR<br>DMSO<br>0, 3000 nmol<br>Injections with 1/3 and 2/3 of the total dose in 30 µL DMSO at age 8 and 15 days, respectively<br>24, 24<br>24, 24 | *Liver*<br>Adenoma<br>Incidence: 0/24, 4/24<br>Carcinoma<br>Incidence: 0/24, 1/24<br>Adenoma or carcinoma (combined)<br>Incidence: 0/24, 4/24<br>Multiplicity: 0, 1.3 | <br><br>NS<br><br>NS<br><br>NS<br>NR | Principal strengths: use of males and females.<br>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<br>Mouse, B6C3F$_1$ (F)<br>Neonatal (age 8 days)<br>12 mo<br>Von Tungeln et al. (2002) | Intraperitoneal injection<br>NR<br>DMSO<br>0, 3000 nmol<br>Injections with 1/3 and 2/3 of the total dose in 30 µL DMSO at age 8 and 15 days, respectively<br>24, 24<br>23, 24 | *Liver*<br>Adenoma<br>Incidence: 0/23, 0/24<br>Carcinoma<br>Incidence: 0/23, 0/24<br>Adenoma or carcinoma (combined)<br>Incidence: 0/23, 0/24<br>Multiplicity: 0, 0 | <br><br>NA<br><br>NA<br><br>NA<br>NA | Principal strengths: use of males and females.<br>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<br>Species, strain (sex)<br>Age at start<br>Duration<br>Reference | Route<br>Purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals | Results | Significance | Comments |
|---|---|---|---|---|
| Full carcinogenicity<br>Mouse, B6C3F$_1$ (M)<br>Neonatal (age 8 days)<br>15 mo<br>Von Tungeln et al. (2002) | Intraperitoneal injection<br>NR<br>DMSO<br>0, 1500 nmol<br>Injections with 1/3 and 2/3 of the total dose in 30 µL DMSO at age 8 and 15 days, respectively<br>24, 24<br>24, 23 | *Liver*<br>Adenoma<br>Incidence: 4/24, 4/23<br>Carcinoma<br>Incidence: 0/24, 0/23<br>Adenoma or carcinoma (combined)<br>Incidence: 4/24, 4/23<br>Multiplicity: 1.0, 1.3 | <br><br>NS<br><br>NA<br><br>NS<br>NR | Principal strengths: use of males and females.<br>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<br>Mouse, B6C3F$_1$ (F)<br>Neonatal (age 8 days)<br>15 mo<br>Von Tungeln et al. (2002) | Intraperitoneal injection<br>NR<br>DMSO<br>0, 1500 nmol<br>Injections with 1/3 and 2/3 of the total dose in 30 µL DMSO at age 8 and 15 days, respectively<br>24, 24<br>24, 24 | *Liver*<br>Adenoma<br>Incidence: 0/24, 0/24<br>Carcinoma<br>Incidence: 0/24, 0/24<br>Adenoma or carcinoma (combined)<br>Incidence: 0/24, 0/24<br>Multiplicity: 0, 0 | <br><br>NA<br><br>NA<br><br>NA<br>NA | Principal strengths: use of males and females.<br>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<br>Rat, F344/DuCrj (M)<br>6 wk<br>104 wk<br>JBRC (2001d) | Inhalation (whole-body)<br>> 99.9%<br>Clean air<br>0, 3, 6, 12 ppm<br>6 h/day, 5 days/wk<br>50, 50, 50, 50<br>39, 39, 45, 38 | *Nasal cavity*<br>Adenoma<br>Incidence: 0/50, 1/50 (2%), 1/50 (2%), 2/50 (4%)<br>Rhabdomyosarcoma<br>Incidence: 0/50, 0/50, 0/50, 1/50 | <br><br>NS<br><br>NS | Principal strengths: multiple-dose study; use of males and females; study complied with GLP.<br>Other comments: historical control data in F344 male rats: nasal cavity adenoma, 1/1199 (0.08%); nasal cavity rhabdomyosarcoma, 0/1199; the incidence of hyperplasia of the respiratory tract 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<br>Rat, F344/DuCrj (F)<br>6 wk<br>104 wk<br>JBRC (2001d) | Inhalation (whole-body)<br>> 99.9%<br>Clean air<br>0, 3, 6, 12 ppm<br>6 h/day, 5 days/wk<br>50, 50, 50, 50<br>39, 38, 40, 40 | *Nasal cavity:* adenoma<br>Incidence: 0/50, 0/50, 0/50, 1/50 | NS | Principal strengths: multiple-dose study; use of males and females; study complied with GLP.<br>Other comments: historical control data for nasal cavity adenoma in F344 female rats, 0/1097; the incidence of hyperplasia of the respiratory tract 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)**

| Study design<br>Species, strain (sex)<br>Age at start<br>Duration<br>Reference | Route<br>Purity<br>Vehicle<br>Dose(s)<br>No. of animals at start<br>No. of surviving animals | Results | Significance | Comments |
|---|---|---|---|---|
| Full carcinogenicity<br>Rat, F344 (M)<br>6 wk<br>113 wk<br>Chung et al. (1986a) | Oral administration (drinking-water)<br>> 99%<br>Distilled water<br>0, 0.6, 6.0 mmol/L in drinking-water<br>23, 27, 23<br>16 (at 110 wk), 17 (at 110 wk), 13 (at 110 wk) | *Liver*<br>Hepatocellular adenoma<br>Incidence: 0/23, 9/27*, 1/23<br>Hepatocellular carcinoma<br>Incidence: 0/23, 2/27, 0/23<br>Hepatocellular adenoma or carcinoma (combined)<br>Incidence: 0/23, 9/27*, 1/23<br>*Urinary bladder:* transitional cell papilloma<br>Incidence: 0/23, 2/27, 0/23 | *[$P = 0.0022$, two-tailed Fisher exact test]<br>[NS]<br>*[$P = 0.0022$, two-tailed Fisher exact test]<br>[NS] | Principal strengths: long-term study (> 2 yr).<br>Principal limitations: small number of rats per group; use of males only, increased mortality and lower body weight observed at the highest dose; rationale for doses not given. |

DMSO, dimethyl sulfoxide; F, female; GLP, Good Laboratory Practice; h, hour; M, male; mo, month; NA, not applicable; NR, not reported; NS, not significant; ppm, parts per million; wk, week; yr, year.

concentration in the exposure chamber was set to 0 (clean air, control), 3, 6 or 12 ppm for males and females. The mean ± SD values monitored every 15 minutes for the groups at 3, 6, and 12 ppm were 3.0 ± 0.0, 6.0 ± 0.0, and 12.0 ± 0.1 ppm, respectively. Survival in males and females was not affected by exposure. Survival in the groups at 0, 3, 6, and 12 ppm, respectively, was: 33/50, 30/50, 38/50, and 43/50 in males, and 30/50, 25/50, 30/50, and 34/50 in females. Male mice at 6 and 12 ppm showed a significant decrease in body-weight gain compared with the control value from week 7 to week 78, and from the first week to the end of exposure, respectively. The relative final body weight in males at 3, 6, and 12 ppm was 101%, 90%, and 66%, respectively, of the value for controls. There was a significant decrease in body-weight gain in female mice at 12 ppm from the first week to the end of the exposure when compared with the control value. The relative final body weight in females at 3, 6, and 12 ppm was 103%, 101%, and 79%, respectively, of the value for controls. All mice underwent complete necropsy, and all organs and tissues were examined microscopically. In all groups of treated male and female mice, there was no significant increase in the incidence of any tumours.

Regarding non-neoplastic lesions in the respiratory tract (see also Section 4 of this monograph), a significant increase in the incidence and/or severity of necrosis, atrophy, cuboidal change, and squamous cell metaplasia in the respiratory epithelium; atrophy and respiratory metaplasia in the olfactory epithelium; exudate; oedema of lamina propria; and hyperplasia and respiratory metaplasia of the nasal glands was observed in the nasal cavity in mice at 12 ppm. The incidence of cuboidal change in the respiratory epithelium was also significantly increased in male mice at 6 ppm. A significant increase in the incidence and/or severity of necrosis, atrophy, inflammation, hyperplasia, cuboidal change, and squamous cell metaplasia in the respiratory epithelium; atrophy and respiratory metaplasia in the olfactory epithelium; exudate; and respiratory metaplasia of the glands was observed in the nasal cavity of female mice at 12 ppm. The incidence of cuboidal change in the respiratory epithelium was also significantly increased in female mice at 6 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 used males and females.]

### *3.1.2 Intraperitoneal injection*

In the first experiment in a study of carcinogenicity focused on the induction of liver and lung tumours in mice (Von Tungeln et al., 2002), groups of 24 male and 24 female B6C3F$_1$ mice (age, 8 days) were given crotonaldehyde [purity, not reported; assumed to be predominantly *trans*-2-butenal] by intraperitoneal injection in 30 µL of dimethyl sulfoxide (DMSO) at a dose of 3000 nmol, with one third of the total dose [1000 nmol] given at age 8 days and two thirds [2000 nmol] at age 15 days. Control groups of 24 males and 24 females were given 30 µL of DMSO by intraperitoneal injection. There was no significant effect on survival. Mice were killed at age 12 months and underwent complete necropsy. The livers, lungs, and all gross lesions of all mice were examined microscopically. In treated males, a non-statistically significant increase in the incidence of liver adenoma (controls, 0/24, controls; treated, 4/24), and liver adenoma or carcinoma (combined) (controls, 0/24, controls; treated, 4/24) was observed. No liver tumours were observed in treated or 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 crotonaldehyde at a dose of 1500 nmol by intraperitoneal injection in 30 µL of DMSO, with one

third [500 nmol] of the total dose given at age 8 days and two thirds [1000 nmol] at age 15 days. Control groups of 24 males and 24 females were given 30 μL of DMSO by intraperitoneal injection. There was no significant effect on survival. Mice were killed at age 15 months. No statistically significant differences in the incidence of liver adenoma or liver carcinoma were observed in treated male animals compared with controls. No liver tumours were observed in treated or control females. [The Working Group noted that the principal strength of the study was the use of males and females. The principal limitations were the use of a single dose, that justification for the dose used was not provided, only data regarding liver tumours were reported, and no data on body weight were reported.]

## 3.2 Rat

### *3.2.1 Inhalation*

In a study that complied with GLP, groups of 50 male and 50 female F344/DuCrj rats (age, 6 weeks) were treated by inhalation with crotonaldehyde (purity, > 99.9%; CAS No., 123-73-9) by whole-body exposure for 6 hours per day, 5 days per week, for 104 weeks (JBRC, 2001d, e, f). The concentration in the exposure chamber was set to 0 (clean air, control), 3, 6, or 12 ppm for males and females. The mean ± SD values monitored every 15 minutes for the groups at 3, 6, and 12 ppm were 3.0 ± 0.0, 6.0 ± 0.0, and 12.0 ± 0.1 ppm, respectively. Survival in males and females was not affected by exposure. Survival in the groups at 0, 3, 6, and 12 ppm was 39/50, 39/50, 45/50, and 38/50 in males, respectively; and 39/50, 38/50, 40/50, and 40/50 in females, respectively. Male rats at 12 ppm showed a significant decrease in body-weight gain compared with the value for controls throughout the exposure period. The relative final body weight in males at 3, 6, and 12 ppm was 99%, 96%, and 91% of the value for controls, respectively. Female rats at 12 ppm showed a significant decrease in body-weight gain from week 2 to the end of exposure compared with the value for controls. The relative final body weight in females at 3, 6, and 12 ppm was 100%, 99%, and 91% of the value for controls, 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. The incidence of nasal cavity adenoma was 0/50 (control), 1/50 (2%, 3 ppm), 1/50 (2%, 6 ppm) and 2/50 (4%, 12 ppm). [The Working Group noted an apparent dose–response relationship, although it was not statistically significant.] Although it was not statistically significant, the value for males at 12 ppm (4%) was in excess of the incidence in historical controls (1/1199, 0.08%). One (1/50, 2%) rhabdomyosarcoma of the nasal cavity was observed in a male rat at 12 ppm; this tumour was not observed in 1199 male historical controls. [The Working Group considered that the adenomas of the nasal cavity were exposure-related, and that the rhabdomyosarcoma of the nasal cavity may have been exposure-related.]

In treated female rats, there was no significant increase in the incidence of any tumours. One (1/50, 2%) adenoma of the nasal cavity, never reported in historical controls (incidence, 0/1097), was observed in a female rat at 12 ppm. [The Working Group considered that this rare adenoma of the nasal cavity may have been be related to exposure.]

Regarding non-neoplastic lesions in the respiratory tract (see also Section 4 of this monograph), a significant increase in the incidence and/or severity of: inflammation (at ≥ 3 ppm), hyperplasia (at ≥ 6 ppm), squamous cell metaplasia (at ≥ 3 ppm), and squamous cell hyperplasia (at 12 ppm) in the respiratory epithelium; atrophy (at 12 ppm) and respiratory metaplasia (at ≥ 3 ppm) in the olfactory epithelium; and inflammation with foreign body (at ≥ 6 ppm)

was observed in the nasal cavity of treated males. A significant increase in the incidence and/or severity of: inflammation (at ≥ 6 ppm), hyperplasia (at ≥ 6 ppm), and squamous cell metaplasia (at ≥ 3 ppm) in the respiratory epithelium; atrophy (at 12 ppm) and respiratory metaplasia (at 12 ppm) in the olfactory epithelium; and inflammation with foreign body (at 12 ppm) was observed in the nasal cavity of treated females. [The Working Group considered that the 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 used males and females.]

### *3.2.2 Oral administration (drinking-water)*

Groups of 23–27 male Fischer 344 rats (age, 6 weeks) were given drinking-water containing crotonaldehyde (purity, > 99%; *trans*-2-butenal) at a dose of 0 (control, distilled water only), 0.6, or 6.0 mmol/L for 113 weeks (Chung et al., 1986a). In rats at the highest dose of crotonaldehyde, increased mortality (survival at 110 weeks: controls, 16/23; 0.6 mmol/L, 17/27; and 6.0 mmol/L, 13/23) and lower body weight [no statistics provided, but approximately –12% read from graph] were observed. Gross lesions and representative samples from all major organs [not further specified] were taken for microscopic examination. In treated rats, a significant increase in the incidence of liver neoplastic nodules [hepatocellular adenoma] was observed at the lower dose compared with controls, with an incidence of 0/23 (control), 9/27 [$P = 0.0022$], and 1/23, respectively. In treated rats, a non-statistically significant increase in the incidence of hepatocellular carcinoma was also observed at the lower dose, with an incidence of 0/23 (control), 2/27, and 0/23, respectively. Overall, there was a significant increase in the incidence of hepatocellular adenoma or carcinoma (combined) at the lower dose, with an incidence of 0/23 (control), 9/27 [$P = 0.0022$; Fisher exact test], and 1/23, respectively. The unusual dose–response relationship was attributed to extensive hepatotoxicity (fatty metamorphosis, focal liver necrosis, fibrosis, cholestasis, and mononuclear cell infiltration) in the group at the higher dose. [The increased mortality and lower body weight of the rats at the higher dose might at least in part explain the lack of a dose–response relationship for the induction of hepatocellular adenoma and hepatocellular carcinoma. The small number of animals used might at least in part explain why the increase in the incidence of hepatocellular carcinoma was not statistically significant.] In treated rats, a non-statistically significant increase in the incidence of urinary bladder transitional cell papilloma was also observed at the lower dose, with an incidence of 0/23 (control), 2/27, and 0/23, respectively. Regarding pre-neoplastic lesions, a significant increase in the incidence of altered liver foci was observed at the lower and higher doses. [The Working Group noted that the principal strength of the study was that it was a long-term study (> 2 years). The principal limitations were the small number of animals per group, the use of males only, that the rationale for the doses used was not provided, and that increased mortality and lower body weight were observed at the higher dose.]

## 3.3 Evidence synthesis for cancer in experimental animals

The carcinogenicity of crotonaldehyde has been assessed in one GLP study in male and female mice and one GLP study in male and female rats treated by inhalation with whole-body exposure. The other available studies included two studies in newborn male and female mice treated by intraperitoneal administration, and one study in male rats treated by oral administration (in the drinking-water).

The GLP inhalation study with crotonaldehyde in F344/DuCrj rats reported a low incidence of nasal cavity adenoma and a single nasal cavity rhabdomyosarcoma in exposed male rats. The incidence of nasal cavity adenoma had an apparent dose-related positive trend, and the nasal cavity rhabdomyosarcoma was observed at the highest dose. A single nasal cavity adenoma was also reported in females at the highest dose. Both nasal cavity adenoma and nasal cavity rhabdomyosarcoma are very rare in the rat strain used in the study (JBRC, 2001d, e, f).

The GLP inhalation study in B6D2F$_1$/Crlj mice did not report a significant increase in the incidence of any tumours in male or female mice exposed to crotonaldehyde (JBRC, 2001a, b, c).

Crotonaldehyde administered in the drinking-water of male Fischer 344 rats caused a significant increase in the incidence of hepatocellular adenoma and of hepatocellular adenoma or carcinoma (combined) at the lowest but not the highest dose tested. The lack of a dose–response relationship was attributed to extensive hepatotoxicity at the highest dose of crotonaldehyde (Chung et al., 1986a).

Treating neonatal B6C3F$_1$ mice by intraperitoneal injection did not result in an increased incidence of tumours (Von Tungeln et al., 2002).

# 4. Mechanistic Evidence

## 4.1 Absorption, distribution, metabolism, and excretion

The information below pertains to mixtures of the *trans*- (*E*-) and *cis*- (*Z*-) isomers of crotonaldehyde, unless stated otherwise.

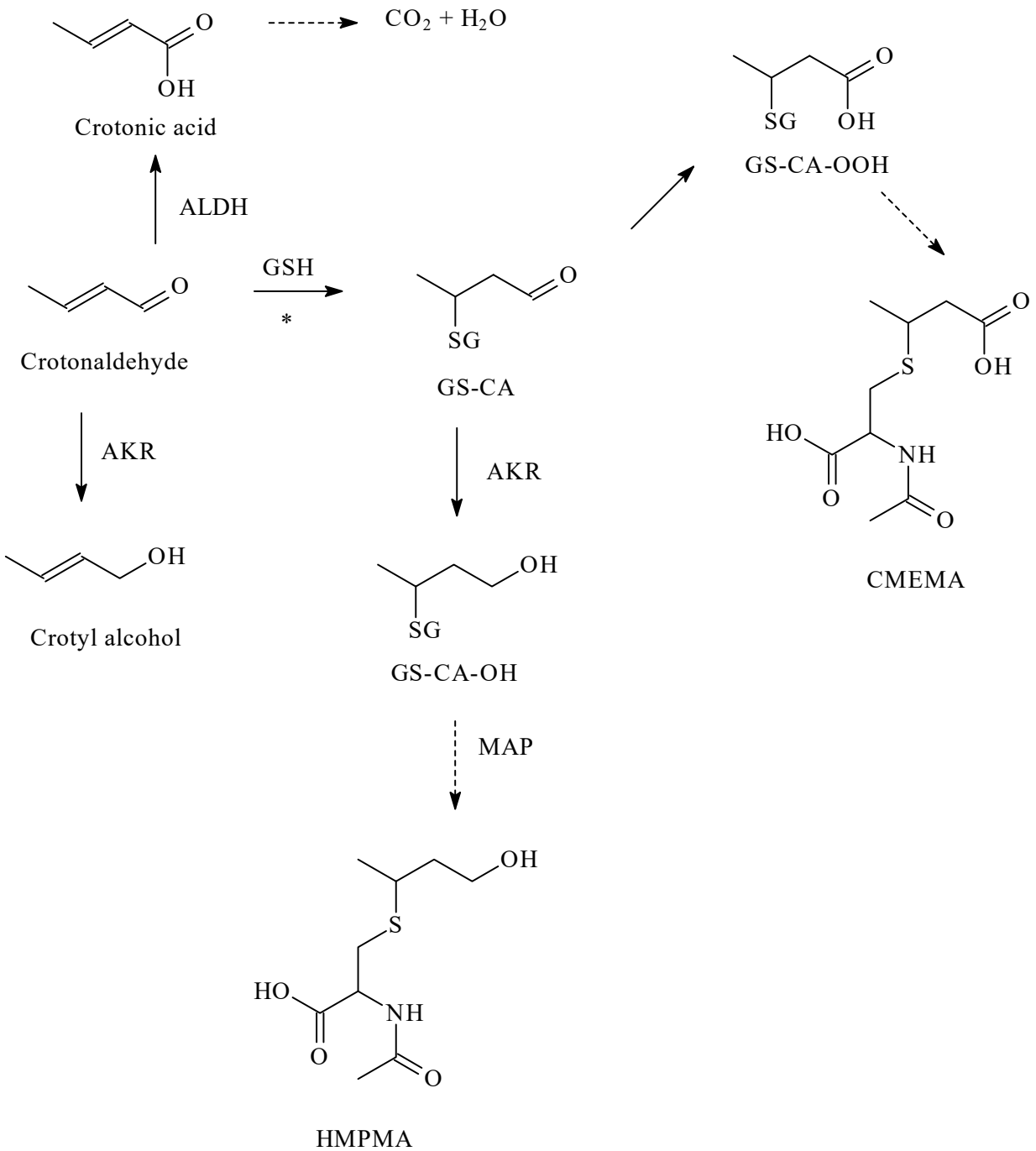
### *4.1.1 Humans*

#### *(a) Exposed humans*

Sparse information was available to the Working Group on the absorption and distribution of crotonaldehyde in humans.

The most extensive data on the fate of crotonaldehyde in humans are related to the detection and quantification of urinary crotonaldehyde-specific mercapturates (Fig. 4.1). Numerous studies have reported the use of sensitive analytical methods, primarily based on LC-MS/MS, to assess urinary biomarkers of human exposure to mixtures of volatile organic compounds, including crotonaldehyde (Scherer et al., 2006, 2007; Carmella et al., 2009; Hecht et al., 2010; Alwis et al., 2012; Carmella et al., 2013; Zhang et al., 2014; Hecht et al., 2015; Pluym et al., 2015; Frigerio et al., 2019). These studies have consistently demonstrated the ubiquitous presence of HMPMA in human urine, at statistically significant higher concentrations (3- to 7-fold) in smokers than in non-smokers. Smoking cessation or switching to cigarettes with lower crotonaldehyde delivery resulted in significant reductions in urinary HMPMA concentrations (Scherer et al., 2006, 2007). When measured up to 56 days after smoking cessation, urinary HMPMA concentrations rapidly decreased, from a baseline value of 1965 ± 1001 (mean ± SD) to 265 ± 113 nmol/24 hours after 3 days, and remained approximately constant thereafter (Carmella et al., 2009). Some of these studies (Scherer et al., 2007; Pluym et al., 2015; Frigerio et al., 2019) also reported the detection and quantification of a second crotonaldehyde-derived mercapturate, CMEMA, in human urine. In contrast to rats (see Section 4.1.2), in which CMEMA was found to be a minor urinary metabolite, urinary concentrations of CMEMA in humans were comparable to, or even higher than, those of HMPMA. However, whereas HMPMA concentrations were significantly correlated with smoking status, this was not the case

**Fig. 4.1 Major pathways of crotonaldehyde metabolism**




ALDH, aldehyde dehydrogenase; AKR, aldo-keto reductase; CMEMA, *N*-acetyl-*S*-(3-carboxy-1-methylpropyl)-L-cysteine; GS–CA, glutathione–crotonaldehyde adduct; GS–CA-OH, reduced glutathione–crotonaldehyde adduct; GS–CA-OOH, oxidized glutathione–crotonaldehyde adduct; GSH, glutathione; HMPMA, *N*-acetyl-*S*-(3-hydroxy-1-methylpropyl)-L-cysteine; MAP, mercapturic acid pathway; *, with and/or without catalysis by glutathione *S*-transferase.
Compiled by the Working Group.

for CMEMA (Scherer et al., 2007; Pluym et al., 2015). Conversely, concentrations of CMEMA (but not HMPMA) were significantly higher in non-smoking gasoline-station attendants than in unexposed workers (Frigerio et al., 2019). [The Working Group noted that the reasons for these discrepancies are not clear. Both HMPMA and CMEMA may also be formed from exposure to crotonaldehyde present in food and ambient air, or formed endogenously. Elevated concentrations of CMEMA might reflect exposure to crotonic acid or crotonates in humans.]

A genome-wide association study conducted in samples from more than 2200 smokers from five ethnic groups reported a significant association between urinary HMPMA concentration and a variant on chromosome 12 near the *TBX3* gene, which is involved in encoding transcription factors, but the implications of this association with regard to crotonaldehyde metabolism and excretion were not clear (Park et al., 2015). Moreover, no association was detected with chromosome 11, which contains the glutathione *S*-transferase pi *1 (GSTP1)* gene. [These observations suggest that glutathione conjugation with crotonaldehyde, ultimately leading to formation of HMPMA, is mainly a non-enzyme-catalysed process in humans.]

#### *(b) Human cells in vitro*

Although crotonaldehyde reacts rapidly with glutathione in vitro (see Section 4.1.2), some degree of enzyme-catalysed conjugation has been demonstrated in vitro with several allelic variants of human GSTP1-1, with catalytic efficiencies ($k_{cat}/K_m$) in the range of 12–17 $mM^{-1}$ $s^{-1}$ (Pal et al., 2000). Consistent with glutathione conjugation, human polymorphonuclear leukocytes had a dose-related decrease in surface and soluble sulfhydryl (SH) groups after treatment with crotonaldehyde in vitro (Witz et al., 1987).

In studies with purified recombinant aldo-keto reductase family 1 B10 (AKRB10), which is expressed in the human colon and small intestine, the enzyme was demonstrated to catalyse the reduction of crotonaldehyde to crotyl alcohol at 0.9 μM, with $K_m$ = 86.7 ± 14.3 μM and $V_{max}$ = 2647.5 ± 132.2 nmol/mg protein per min, and also the carbonyl reduction of the glutathione–crotonaldehyde conjugate at 0.5 μM, with $K_m$ = 245.7 ± 21.2 μM and $V_{max}$ = 1900.7 ± 90.9 nmol/mg protein per min (Yan et al., 2007; Zhong et al., 2009). *AKRB10* downregulation enhanced the susceptibility of colorectal cancer HCT-8 cells to crotonaldehyde, resulting in rapid cell death (Yan et al., 2007). In a subsequent study, catalytic efficiency for the reduction of crotonaldehyde was 400 times lower for purified recombinant aldo-keto reductase family 1 B1 (AKRB1) (ubiquitously expressed in humans) than for AKRB10. Although AKRB1 appeared to have higher specificity than AKRB10 for glutathione-conjugated carbonyls, data for the glutathione–crotonaldehyde conjugate were not presented (Shen et al., 2011).

### *4.1.2 Experimental systems*

#### *(a) Non-human mammals in vivo*

The available data on the absorption, distribution, metabolism, and excretion of crotonaldehyde in experimental animals are few. Nonetheless, protein and DNA adducts of crotonaldehyde have been detected in multiple tissues from rats and mice (see Section 4.2.1), demonstrating that crotonaldehyde undergoes systemic distribution.

In a study with groups of three or four adult male Fischer 344 rats given a single dose of [$^{14}$C]-crotonaldehyde (radiochemical purity, > 96%) at 2.6–2.9 mg/kg body weight (bw) in aqueous ethanol by intravenous injection, approximately 31% of the administered radiolabel was excreted as [$^{14}$C]-labelled carbon dioxide in the expired air and 37% in the urine within 6 hours after dosing. At the same time-point, the excretion of other volatiles in the breath accounted for approximately 1%

of the total radiolabel, whereas the amount of radiolabel associated with blood and selected tissues (skin, muscle, adipose tissue, and liver) accounted for 10% of the total dose administered. At 72 hours after dosing, the elimination of crotonaldehyde as [$^{14}$C]-labelled carbon dioxide had increased to approximately 41%, and urinary metabolites accounted for 48% of the administered radiolabel, with negligible (< 0.5%) faecal elimination and low accumulation of [$^{14}$C] (< 5% radioactivity) detected in the analysed tissues. The parent crotonaldehyde accounted for > 1% of the urinary excretion of [$^{14}$C] and its oxidation product, crotonic acid, for < 2%. The elimination of [$^{14}$C] in the breath and urine appeared to be biphasic, with similar half-lives of approximately 2 hours for the initial phase and 13 hours for the second phase estimated for both routes (NTP, 1985).

In a concomitant study, adult male Fischer 344 rats were given [$^{14}$C]-labelled crotonaldehyde by gavage as a single dose at 0.7, 3, or 35 mg/kg bw. Absorption from the gastrointestinal tract occurred readily. By 12 hours after dosing, elimination in exhaled air and urine combined accounted for 78% and 60% of the administered radiolabel at the lowest and highest dose, respectively. By 72 hours, 44–49% of the administered dose was excreted in the breath as [$^{14}$C]-labelled carbon dioxide, 38–39% in the urine, and 6–7% in the faeces, indicating that the absorption of [$^{14}$C]-labelled crotonaldehyde from oral doses was > 93%. Elimination of [$^{14}$C] from the tissues and blood was biphasic; there was an initially rapid elimination stage, with half-lives of approximately 1 hour or less, followed by a much slower elimination of the last 10% of the dose, with terminal half-lives of 2.5 days or longer (NTP, 1985).

In an earlier study, groups of male albino and black hooded rats were given a single subcutaneous injection of crotonaldehyde at 0.75 mmol/kg bw [approximately 53 mg/kg bw] in olive oil. Two mercapturate metabolites were identified in urine collected in the 24 hours after dosing. The major metabolites, which accounted for 6–15% of the administered dose, was characterized as HMPMA by hydrolytic conversion to *S*-(3-hydroxy-1-methylpropyl)-L-cysteine and comparison with a synthetic standard of the latter. The minor urinary metabolite, which was detected occasionally but not quantified, was characterized as CMEMA (Gray & Barnsley, 1971). HMPMA was also detected in the urine of adult male C57BL6/J mice after whole-body exposure to mainstream cigarette smoke (equivalent to 12 cigarettes over 6 hours) but not in the urine of mice exposed to electronic cigarette aerosols (Conklin et al., 2018) or smokeless tobacco extracts in tap water (Malovichko et al., 2019).

The structures of the urinary mercapturates are indicative of Michael-type addition of glutathione to the α,β-unsaturated carbonyl of crotonaldehyde, followed by either reduction or oxidation of the aldehyde group and subsequent catabolism (Fig. 4.1). When given by intraperitoneal injection at a dose of 2 mmol/kg bw [140 mg/kg bw] to male Wistar rats, crotonaldehyde did cause an early decrease in the hepatic glutathione concentrations, as measured 3 hours after dosing. However, the approximate liver glutathione content in rats treated with crotonaldehyde at 0.75 mmol/kg bw was comparable to that of control rats when measured 12 hours after dosing (Oguro et al., 1990).

### *(b) Non-human mammalian cells in vitro*

Upon in vitro incubation with stomach content homogenate from an untreated rat, at an amount equivalent to a dose of 1.8 mg/kg bw, [$^{14}$C]-labelled crotonaldehyde remained essentially intact after 2 hours, with 94% of the radioalabel being recovered as the parent compound (assessed by HPLC) and approximately 5% found to be bound to particulate matter (NTP, 1985). In contrast, incubation of [$^{14}$C]-labelled crotonaldehyde (approximately 7.33 µg/g) with rat plasma at

37 °C demonstrated that the compound was not stable under these conditions. After 5 minutes, only 42% of the radiolabelled parent compound remained intact, and this had decreased to 15% after 30 minutes. The initial degradation of crotonaldehyde subsequently became much slower, with 8% of the parent compound still present after 20 hours. The reaction products were not identified (NTP, 1985).

Crotonaldehyde reacts readily with SH groups in vitro. It undergoes spontaneous reaction with glutathione, although some degree of enzyme catalysis has also been documented after incubation with rat liver preparations (Boyland & Chasseaud, 1967; Gray & Barnsley, 1971) or with purified glutathione *S*-transferases (Stenberg et al., 1992; Eisenbrand et al., 1995). In an additional study, rat pulmonary alveolar macrophages exhibited a dose-related decrease in surface and soluble SH groups after treatment with crotonaldehyde in vitro (Witz et al., 1987).

Upon incubation of adult rat lung alveolar cells with crotonaldehyde at 100, 200, or 500 µM for 20 minutes, the effective concentration ($EC_{50}$) for 50% intracellular glutathione depletion was estimated to be 130 µM. At the crotonaldehyde concentrations used in the study, the rate of glutathione depletion was characteristic of a non-enzymatic second-order reaction for adduct formation (Meacher & Menzel, 1999). [The Working Group noted that the data from this study indicated a key role for molecular reactivity in the process.]

While the reaction between crotonaldehyde and glutathione in buffer solution yields the expected 1,4-addition product (glutathione–crotonaldehyde adduct; GS–CA, Fig. 4.1), this species is only detected at very low levels in cell media. In contrast, a glutathione–crotonaldehyde adduct resulting from subsequent reduction of the aldehyde carbonyl (GS–CA-OH, Fig. 4.1) was clearly identified after a 30-minute incubation of B16-BL6 mouse melanoma cells with crotonaldehyde at 10 µM (Horiyama et al., 2016). The same crotonaldehyde-specific adduct was readily detected ($t \leq 1$ minute) in sheep erythrocytes exposed to cigarette smoke extract (Horiyama et al., 2018), indicating that the initially formed glutathione–crotonaldehyde adduct is a substrate for mammalian intracellular carbonyl reductases.

Several studies have also addressed the oxidative metabolism of crotonaldehyde to crotonic acid in rat hepatocytes and rat liver mitochondrial, cytosolic, and microsomal fractions. Crotonaldehyde was consistently found to be both a poor substrate for the liver aldehyde dehydrogenases (ALDHs), with a $K_m$ of 515 µM calculated for the microsomal ALDH, and was a potent inhibitor of the high-affinity mitochondrial and cytosolic ALDH isoforms (Cederbaum & Dicker, 1982; Dicker & Cederbaum, 1984; Mitchell & Petersen, 1993).

## 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 crotonaldehyde is electrophilic or can be metabolically activated to an electrophile; is genotoxic; induces oxidative stress; induces chronic inflammation; or is immunosuppressive. Insufficient data were available for the evaluation of other key characteristics of carcinogens.

### *4.2.1 Is electrophilic or can be metabolically activated to an electrophile*

#### *(a) Human*

##### *(i) Exposed humans*

See Table 4.1.

Crotonaldehyde forms α-methyl-γ-hydroxy-1,$N^2$-propano-2′-deoxyguanosine adducts in DNA, of which there are two identified diastereoisomeric forms – 8*R*,6*R* and 8*S*,6*S* (see Fig. 4.2

## Table 4.1 Crotonaldehyde-derived DNA adducts in exposed humans

| Biosample | Location, setting | No. of subjects | Adduct frequency (analytical method) Response (significance) | Comments | Reference |
|---|---|---|---|---|---|
| Liver | Autopsy samples from Columbia University (NY), USA | 2 M, 3 F | 0.13–1.0 adducts/$10^6$ G ($^{32}$P-postlabelling) | | Nath & Chung (1994) |
| Peripheral blood | Healthy volunteers | 2 M (1 smoker), 2 F (1 smoker) | 0.003–0.025 µmol/mol G ($^{32}$P-postlabelling) No difference between smokers and non-smokers | Smoking | Nath et al. (1996) |
| Mammary tissue | Breast-reduction surgery samples from Anderson Cancer Center, Houston (TX), USA | 3 F | 0.004–0.077 µmol/mol G ($^{32}$P-postlabelling) | | |
| Gingival tissue | Samples from surgery at a periodontal clinic at New York University Dental Center (NY), USA | 11 smokers (4 M, 7 F); 12 non-smokers (8 M, 4 F) | Adduct levels significantly higher in smokers ($P = 0.003$) ($^{32}$P-postlabelling) CdG1 adduct: 0.53 ± 0.44 µmol/mol G in smokers; 0.06 ± 0.07 µmol/mol G in non-smokers ($P = 0.0015$) CdG2 adduct: 1.72 ± 1.26 µmol/mol G in smokers; 0.31 ± 0.40 µmol/mol G in non-smokers ($P = 0.0014$) | Smoking | Nath et al. (1998) |
| Liver | Surgical samples obtained from the Cancer Center Tissue Procurement Facility, University of Minnesota, USA | 23 | 4/23 positive 6S,8S adduct: 6.70 ± 2.92 fmol/µmol dG (mass spectrometry) 6R,8R adduct: 7.87 ± 4.47 fmol/µmol dG | Smoking status of donors unknown. | Zhang et al. (2006) |
| Lung | Surgical samples obtained from the Cancer Center Tissue Procurement Facility, University of Minnesota, USA | 45 | 16/45 positive 6S,8S adduct: 7.19 ± 4.14 fmol/µmol dG (mass spectrometry) 6R,8R adduct: 12.8 ± 7.6 fmol/µmol dG | Samples were from self-reported smokers (but not clear whether past or present). | |
| Peripheral blood | 9 buffy-coat samples from the University of Minnesota Transdisciplinary Tobacco Use Research Center, and 2 from Mid-South Regional Blood Center, Memphis (TN), USA | 11 | 0/11 positive LOQ, 4 fmol/µmol dG (mass spectrometry) | Smoking status not reported. | |
| Peripheral blood | Healthy volunteers, Taiwan, China | 9 | 6.2 ± 3.8 adducts/$10^8$ nucl (mass spectrometry) | | Chen & Lin (2009) |
| Placenta | Commercial DNA sample | 1 | 26 adducts/$10^8$ nucl | | |

**Table 4.1 (continued)**

| Biosample | Location, setting | No. of subjects | Adduct frequency (analytical method) Response (significance) | Comments | Reference |
|---|---|---|---|---|---|
| Saliva | Healthy volunteers, Taiwan, China | 27 | 22/27 individuals positive<br>Mean, 7.5 ± 12 (range, 0–48.5) adducts/$10^8$ nucl (mass spectrometry) | Smoking status not reported. Study included simultaneous detection of other adducts potentially derived from products of lipid peroxidation. | Chen & Lin (2011) |
| Urinary samples | Urban (São Paulo City) and rural (São João da Boa Vista) dwellers, Brazil | 47 urban, 35 rural | Urban: median 20.8 (range, ND–330.0) fmol/mg creatinine (mass spectrometry)<br>Rural: median, 7.9 (range, 2.6–53.1) fmol/mg creatinine ($P < 0.05$) | Publication is a short communication, lacking details on study subjects or sources of exposure. | Garcia et al. (2013) |
| Urinary samples | China | 13 | 6S,8S adduct: 1.01 ± 0.85 nmol/mol creatinine<br>6R8R adduct: 0.89 ± 0.67 nmol/mol creatinine | | Zhang et al. (2016a) |
| Lung | Lung Tissue Research Consortium of the National Heart Lung and Blood Institute (NIH), USA | 41 lung cancer patients (smokers); 13 non-lung cancer patients (non-smokers) | Significantly higher levels of CdG in smokers than non-smokers (immunoassay and $^{32}$P-postlabelling) | *P* value not reported; adduct levels shown graphically (range, 0 to ~40 adducts/$10^7$ dG). | Weng et al. (2018) |
| Buccal cells | | 33 smokers; 17 non-smokers | PdG adducts (derived from acrolein and crotonaldehyde combined) significantly higher in smokers ($P < 0.0001$) | Adduct levels shown graphically (range, 0 to ~2.5 adducts/$10^5$ dG). | |
| Sputum | | 22 smokers; 8 non-smokers | PdG adducts (derived from acrolein and crotonaldehyde combined) significantly higher in smokers ($P < 0.0193$) | Immunoassay method only was used. Adduct levels shown graphically (range, 0 to ~2.5 adducts/$10^5$ dG). | |
| Peripheral blood | | 1 smoker, 1 non-smoker | Smoker: 28.3 adducts/$10^7$ nucl (mass spectrometry)<br>Non-smoker: 3.5 adducts/$10^7$ nucl | | Alamil et al. (2020) |

CdG, crotonaldehyde-derived l,$N^2$-propano-deoxyguanosine; dG, deoxyguanosine; F, female; G, guanine; LOQ, limit of quantification; M, male; nucl, nucleotides, ND, not detected; PdG, cyclic 1,$N^2$-propano-deoxyguanosine.

**Fig. 4.2 Diastereoisomeric adducts, 8*R*,6*R* and 8*S*,6*S***


CdG 1
(6*S*, 8*S*-isomer)

CdG 2
(6*R*, 8*R*-isomer)

CdG, crotonaldehyde-derived 1,$N^2$-propano-deoxyguanosine.
Adapted from Nath et al. (1996).

and Section 4.2.1.b). These crotonaldehyde adducts were detected in normal human liver at levels ranging from 0.13 to 1.0 adducts/$10^6$ deoxyguanosine (Nath & Chung, 1994). In subsequent studies, these adducts were detected in other normal tissues, including in peripheral blood and mammary tissue (Nath et al., 1996); in oral (gingival) tissue (Nath et al., 1998); in liver, lung, and blood cells (Zhang et al., 2006); in placenta, blood cells, and saliva (Chen & Lin, 2009, 2011); in urine samples (Garcia et al., 2013; Zhang et al., 2016a); and in peripheral blood (Alamil et al., 2020). [The Working Group noted that different methods were used in these studies, which may account for differences in levels detected.]

In studies comparing smokers and non-smokers, adduct levels were significantly elevated in smokers, indicating their formation by crotonaldehyde from tobacco smoke; the presence of adducts in tissues of non-smokers is widely interpreted as being indicative of formation from endogenous sources such as lipid peroxidation (Nath et al., 1996). In a comparison of residents in two areas of Brazil, adduct levels in urine samples were significantly higher in the urban population than in rural residents (Garcia et al., 2013). This was attributed to differences in levels of air pollution as the source of exposure to crotonaldehyde.

In a study from the EPIC-Italy colon cancer cohort, Cys34 adducts of crotonaldehyde in serum albumin were more abundant in cases than in controls, suggesting an inflammatory response involving the generation of crotonaldehyde via lipid peroxidation (Grigoryan et al., 2019). Interestingly, this adduct, along with several other adducts that can result from lipid peroxidation, was also present at significantly higher concentrations in the serum albumin of workers exposed to benzene than in unexposed controls (Grigoryan et al., 2018).

In a study on various smoking-related DNA adducts in different human tissues, crotonaldehyde-derived 1,$N^2$-propano-2′-deoxyguanosine adducts were the most common adducts detected in buccal cells from smokers and in normal lung tissue from lung cancer patients who were smokers, but not in lung tissues of non-smokers (Weng et al., 2018).

*(ii) Human cells in vitro*

See Table 4.2.

Several studies have demonstrated the formation of crotonaldehyde-derived DNA adducts or DNA damage in human cells treated in vitro with crotonaldehyde. Adducts characteristic of

## Table 4.2 Genetic and related effects of crotonaldehyde in human cells in vitro

| End-point | Tissue, cell line | Results[a] | | Concentration (LEC or HIC) | Comments | Reference |
|---|---|---|---|---|---|---|
| | | Without metabolic activation | With metabolic activation | | | |
| DNA adducts ($^{32}$P-postlabelling) | Human xeroderma pigmentosum fibroblasts (GM 5509) | + | NT | 1 μM | | Wilson et al. (1991) |
| DNA adducts ($^{32}$P-postlabelling) | Human primary normal bronchial fibroblasts | + | NT | 100 μM | Only one concentration tested. | Wilson et al. (1991) |
| DNA adducts ($^{32}$P-postlabelling) | Human skin fibroblasts from a cystic fibrosis patient (GM 4539) | + | NT | 100 μM | Only one concentration tested. | Wilson et al. (1991) |
| DNA adducts (mass spectrometry) | MRC5 fibroblast cell line | + | NT | 1 μM | 6S,8S and 6R,8R adducts were detected in untreated cells; levels were enhanced by crotonaldehyde treatment across the range 1–100 μM. | Zhang et al. (2016b) |
| DNA interstrand crosslinks (comet assay, thermal denaturation, circular dichroism) | Lymphocytes | + | NT | 50 mM | High concentration tested. Lack of positive control. | Ul Islam et al. (2014) |
| DNA interstrand crosslinks (dynamic light scattering) | Placental DNA | + | NT | 50 mM | Not a standard genotoxicity assay. High concentration tested. Lack of positive control. | Ul Islam et al. (2016) |

GM, geometric mean; HIC, highest ineffective concentration; LEC, lowest effective concentration, NT, not tested.

[a] +, positive.

1,$N^2$-propano-2′-deoxyguanosine were detected by $^{32}$P-postlabelling in the DNA of human xeroderma pigmentosum (XP) fibroblasts treated with crotonaldehyde at 1–100 μM (Wilson et al., 1991). The same range of crotonaldehyde concentrations increased the levels of these adducts in MRC5 cells above the levels already present in untreated cells (Zhang et al., 2016b).

DNA interstrand crosslinks were detected in human lymphocytes and placental DNA treated with crotonaldehyde at 50 mM (Ul Islam et al., 2014, 2016).

Treatment of human HepG2 liver cells with the carcinogen aflatoxin $B_1$ resulted in the formation of aflatoxin–DNA adducts, and also crotonaldehyde-derived DNA adducts (at a 30-fold higher level) induced by lipid peroxide generation of crotonaldehyde (Weng et al., 2017). Both types of adducts were preferentially formed at codon 249 of the *TP53* gene, a hotspot for mutation in hepatocellular carcinoma associated with aflatoxin exposure.

### *(b) Experimental systems*

#### *(i) DNA and protein binding in chemical reactions*

Crotonaldehyde is a bifunctional α,β-unsaturated aldehyde (enal) that can form cyclic adducts in DNA, DNA interstrand crosslinks, and DNA–protein crosslinks.

Michael addition of the $N^2$-amino group of deoxyguanosine and of deoxyguanosine residues in DNA, to C3 of crotonaldehyde, followed by ring closure between N1 of deoxyguanosine and C1 of crotonaldehyde forms α-methyl-γ-hydoxy-1,$N^2$-propano-2′-deoxyguanosine adducts, frequently referred to as crotonaldehyde-derived 1,$N^2$-propano-2′-guanosine adducts (Eder et al., 1982; Chung & Hecht, 1983; Chung et al., 1984; Chung et al., 1986b). These are guanine positions that are involved in base pairing in DNA. Chirality at the methyl-bearing carbon atom in the 1,$N^2$-propano ring results in a pair of diastereoisomeric adducts, 8*R*,6*R* and 8*S*,6*S* (see Fig. 4.2).

Monoclonal antibodies specific for the 8*R*,6*R* and 8*S*,6*S* stereoisomers have been produced (Foiles et al., 1987). Methods for detecting crotonaldehyde derived DNA adducts using $^{32}$P-postlabelling analysis (Chung et al., 1989, Foiles et al., 1990, Nath et al., 1994, Pan et al., 2006) and mass spectrometry (Doerge et al., 1998, Zhang et al., 2006, Chen & Lin, 2009, Garcia et al., 2013, Zhang et al., 2016b, Alamil et al., 2020) have also been reported.

Reaction of deoxyguanosine with an excess of crotonaldehyde at 80 °C gave rise not only to 1,$N^2$-propano adducts but also to *N7*,C8 cyclic adducts and 1,$N^2$,7,8 bicyclic adducts (Eder & Hoffman, 1992). Reaction of crotonaldehyde with deoxyadenosine produces 1,$N^6$-propano-2′-deoxyadenosine adducts equivalent to the deoxyguanosine adducts (Chen & Chung, 1994).

Crotonaldehyde is a metabolite of *N*-nitrosopyrrolidine (NPYR), a carcinogenic environmental nitrosamine. α-Acetoxy-NPYR, a synthetic stable precursor to the proposed proximate carcinogen α-hydroxy-NPYR, reacts with DNA to form crotonaldehyde-derived 1,$N^2$-propano-2′-deoxyguanosine and cyclic *N7*,C8 guanine adducts (Wang et al., 1989, 1998).

Crotonaldehyde-derived 1,$N^2$-propano-2′-deoxyguanosine may also be generated by endogenous processes. Their formation by ω-3 polyunsaturated fatty acids, including docosahexaenoic acid, linoleic acid, and eicosapentaenoic acid (Pan & Chung, 2002), suggests a possible source, as products of lipid peroxidation, of adducts detected in human and animal tissues not knowingly exposed to crotonaldehyde.

The ability of crotonaldehyde to form interstrand crosslinks in DNA depends on the stereochemistry at the C6 position of the 1,$N^2$-propano-2′-deoxyguanosine adduct. It requires a 5′-CpG-3′ (cytosine-phosphate-guanine) sequence where the orientation of the aldehyde within the minor groove favours reaction of

the 6*R* configuration relative to the 6*S* (Kozekov et al., 2003; Stone et al., 2008; Minko et al., 2009). Molecular modelling studies predict less disruption of the duplex structure, and greater thermodynamic stability for the crosslink formed by the *R* adduct (Cho et al., 2006a, b, 2007).

Histones, which are rich in basic amino acids such as arginine and lysine, accelerate the reaction of crotonaldehyde with deoxyguanosine and DNA under physiological conditions (Sako et al., 2003; Inagaki et al., 2004). Crotonaldehyde reacts with lysine and histidine in bovine serum albumin (Ichihashi et al., 2001) and can also form DNA–protein crosslinks (Kuykendall & Bogdanffy, 1992). Crotonaldehyde-derived 1,$N^2$-propano-2′-deoxyguanosine adducts crosslink to peptides via Schiff base linkage (Kurtz & Lloyd, 2003).

Several studies have indicated that crotonaldehyde, and crotonaldehyde-derived DNA adducts, can arise from acetaldehyde, a metabolite of alcohol, under physiological conditions or at biologically relevant concentrations of acetaldehyde (Stornetta, et al., 2018), indicating that alcohol exposure is confounding when performing studies of crotonaldehyde–DNA binding. Micromolar concentrations of acetaldehyde in the presence of spermidine led to formation of α-methyl-γ-hydroxy-1,$N^2$-propano-2′-deoxyguanosine adducts in DNA (Theruvathu et al., 2005). Crotonaldehyde can be produced in aqueous solutions of acetaldehyde by aldol condensation. Enzymatic or neutral hydrolysis of DNA in the presence of crotonaldehyde produces paraldol, the dimer of 3-hydroxy-butanal (aldol) that, when it reacts with DNA, generates a class of adducts described by Wang et al. (2000). Base treatment of acetaldehyde results in the formation of its trimer, aldoxane, which is in equilibrium with crotonaldehyde in solution. This too can lead to the formation of adducts in DNA (Wang et al., 2001), although it is not known whether aldoxane or paraldol are produced from acetaldehyde in vivo.

*(ii) DNA adducts in experimental systems*

See Table 4.3 and Table 4.4.

After treatment of Fischer 344 rats by gavage with a single dose of crotonaldehyde (200 mg/kg bw), 2.9 adducts/$10^8$ nucleotides were detected in the liver; treatment with repeated doses (1 mg/kg bw, five times per week for 6 weeks) resulted in a similar level of adduct formation (2.0 adducts/$10^8$ nucleotides) (Eder et al., 1999; Budiawan et al., 2000). No adducts were detected in the livers of untreated rats in these studies (limit of detection, 3 adducts/$10^9$ nucleotides), in contrast to studies by other investigators who reported the presence of adducts in the livers of both untreated and treated mice and rats (Chung et al., 1989; Nath & Chung, 1994; Nath et al., 1996; Pan et al., 2006). [The Working Group noted that in one of these studies treatment of rats with *N*-nitrosopyrrolidine (NPYR) also gave rise to crotonaldehyde-derived 1,$N^2$-propano-2′-deoxyguanosine adducts in liver (Chung et al., 1989).] DNA adducts have also been detected in mouse skin after topical treatment with crotonaldehyde (Chung et al., 1989) and in multiple tissues (lung, kidney, colonic mucosa, prostate, mammary tissue, brain, and leukocytes) of untreated rats and also in the skin of untreated mice (Nath et al., 1996).

Tissues of mice exposed to mainstream tobacco smoke (5 days per week for 12 weeks) were analysed for multiple DNA adducts, including those derived from benzo[*a*]pyrene, 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK), acrolein, and crotonaldehyde (Weng et al., 2018). Adducts derived from crotonaldehyde were detected in the lung and urinary bladder, but not in the heart and liver.

Male Wistar rats were exposed via inhalation to exhaust from either ultra-low sulfur diesel (ULSD) or biodiesel containing 30% rapeseed methyl ester in ULSD. No significant increases in the frequency of lung crotonaldehyde–DNA adducts were observed in either treatment group

## Table 4.3 Detection of crotonaldehyde-derived DNA adducts in non-human mammals in vivo

| Species, strain (sex) | Tissue | Exposure | Results (LED or HID) | Comments[a] | Reference |
|---|---|---|---|---|---|
| Rat, F344 (F) | Liver, lung, kidney, colon | Gavage; 200 mg/kg single dose; 1 mg/kg, 5×/wk for 6 wk | +<br>200 mg/kg single dose; 30 mg/kg (total) multiple dose.<br>No adducts detected in livers of untreated animals; LOD, 3 adducts/$10^9$ nucl | Purity, NR. | Eder et al. (1999); Budiawan et al. (2000) |
| Mouse, A/J (M)<br>Rat, F344 (M) | Liver<br>Liver | None (untreated aimals)<br>None | + | Mice, 4.<br>Rats, 4. | Nath & Chung (1994) |
| Mouse, A/J (F) | Skin | None (untreated animals) | + | Mice, 5 | Nath et al. (1996) |
| Rat, F344 (M, F) | Lung, kidney, colonic mucosa, prostate, mammary tissue, brain, and leukocytes | None (untreated animals) | + | Up to 6 rats studied. | Nath et al. (1996) |
| Mouse, Sencar (F) | Skin | Topical, 6.7 mg 5×/wk for 3 wk | +<br>100 mg | | Chung et al. (1989) |
| Rat, F344 (M) | Liver | None (untreated animals) | + | | |
| Rat, F344 (M) | Liver | 6 mM NPYR in drinking-water for 14 days | + | | |
| Rat, Long Evans | Liver | None (untreated animals) | +<br>LOD, 9 adducts/$10^9$ nucl | One rat analysed. | Pan et al. (2006) |
| Mouse, FVBN (M) | Lung | Tobacco smoke, ~75 mg/$m^3$ for 12 wk | + | | Weng et al. (2018) |

F, female; HID, highest ineffective dose; LED, lowest effective dose; LOD, limit of detection; M, male; nucl, nucleotides; NPYR, *N*-nitrosopyrrolidine; NR, not reported; wk, week.

[a] +, positive.

**Table 4.4 Detection of crotonaldehyde-derived adducts with oligonucleotides and DNA**

| Test system (species, strain) | Results[a] | Concentration (LEC or HIC) | Comments | Reference |
|---|---|---|---|---|
| | Without metabolic activation | | | |
| Calf thymus DNA (acellular) | + | 21 mg/mL [300 mM] | | Chung et al. (1984) |
| T/C 25-mer (acellular) | + | 1.25 M | Only one concentration tested. | Borys-Brzywczy et al. (2005) |
| Nucleosides and 5′-mononucleotides (acellular) | + | 70 mg/mL [1 M] | | Eder & Hoffman (1992) |
| Deoxycytidine (acellular) | + | 292 mM | Only one concentration tested. | Borys-Brzywczy et al. (2005) |
| Deoxythymidine (acellular) | – | 292 mM | Only one concentration tested. | Borys-Brzywczy et al. (2005) |

HIC, highest effective concentration; LEC, lowest effective concentration; NT, not tested.
[a] +, positive; –, negative.

when compared with rats exposed to filtered air (Douki et al., 2018).

It has also been reported that crotonaldehyde forms 1,$N^2$-propano-2′-deoxyguanosine adducts, as detected by $^{32}$P-postlabelling analysis in Chinese hamster ovary cells (Foiles et al., 1990).

In acellular studies, crotonaldehyde induced the formation of DNA adducts in calf thymus DNA (Chung et al., 1984; Kailasam & Rogers, 2007), as well as in oligonucleosides and mononucleotides (Eder & Hoffman, 1992; Borys-Brzywczy et al., 2005; see Table 4.4).

### *4.2.2 Is genotoxic*

[The information below pertains to mixtures of the *trans*- (*E*-) and *cis*- (*Z*-) isomers of crotonaldehyde, unless stated otherwise.]

*(a) Humans*

*(i) Exposed humans*

No data were available to the Working Group.

*(ii) Human cells in vitro*

See Table 4.5.

Crotonaldehyde-induced DNA single-strand breaks were observed in human lymphoblastoid (Namalwa) cells (Eisenbrand et al., 1995). Dittberner et al. (1995) obtained a positive result for sister-chromatid exchange, structural chromosomal aberration, and micronucleus formation in both human primary lymphocytes and Namalwa cells treated with crotonaldehyde. However, a negative result was obtained for centromere-positive micronuclei, as detected by fluorescence in situ hybridization (FISH), in both cell lines. [The Working Group noted that this indicates a clastogenic effect.] Additionally, the lymphocytes were only examined for the number of aneuploid metaphases; no significant increase was found (Dittberner et al., 1995).

In three experiments, plasmids containing the *supF* gene were reacted with crotonaldehyde and then transfected into various human cell types to allow for repair and replication; the *supF* mutant frequency was subsequently assessed in *Escherichia coli* and found to be significantly increased in a dose-dependent manner in all cases (Czerny et al., 1998; Kawanishi et al., 1998; Weng et al., 2017). In one study in which the exposed plasmid was transfected into human hepatocellular carcinoma cells (HepG2), crotonaldehyde induced G→C transversions (41%), G→T transversions (37%), deletions (16%), and G→A

## Table 4.5 Genetic and related effects of crotonaldehyde in human cells in vitro

| End-point | Tissue, cell line | Results[a] | | Concentration (LEC or HIC) | Comments | Reference |
|---|---|---|---|---|---|---|
| | | Without metabolic activation | With metabolic activation | | | |
| DNA single-strand breaks (alkaline elution) | Lymphoblastoid Namalwa cells | + | NT | 0.2 mM | | Eisenbrand et al. (1995) |
| Chromosomal aberrations – structural; sister-chromatid exchanges | Primary lymphocytes | + | NT | 10 µM | | Dittberner et al. (1995) |
| Chromosomal aberrations – structural | Lymphoblastoid Namalwa cells | + | NT | 100 µM | | Dittberner et al. (1995) |
| Chromosomal aberrations – numerical | Primary lymphocytes | – | NT | 250 µM | | Dittberner et al. (1995) |
| Micronucleus formation | Lymphoblastoid Namalwa cells, primary lymphocytes | + | NT | 40 µM | | Dittberner et al. (1995) |
| Micronuclei – centromere positive | Lymphoblastoid Namalwa cells, primary lymphocytes | – | NT | 150 µM | | Dittberner et al. (1995) |
| Sister-chromatid exchange | Lymphoblastoid Namalwa cells | + | NT | 20 µM | | Dittberner et al. (1995) |
| Plasmid pZ189 (exposed acellularly); transfected into transformed human normal lymphoblasts (GM0621) | Forward mutation (*supF*) | + | NT | 10 mM | Plasmids exposed to crotonaldehyde then transfected into human cells to allow for repair and replication. | Czerny et al. (1998) |
| Plasmid pMY189 (exposed acellularly); transfected into transformed normal human fibroblasts (W138-VA13) | Forward mutation (*supF*) | + | NT | 1.2 M | Plasmids exposed to crotonaldehyde then transfected into human cells to allow for repair and replication. | Kawanishi et al. (1998) |
| Plasmid pSP189 (exposed acellularly); transfected into human hepatocellular carcinoma cells (HepG2) | Forward mutation (*supF*) | + | NT | 5 mM | Plasmids exposed to crotonaldehyde then transfected into human cells to allow for repair and replication. | Weng et al. (2017) |

HIC, highest ineffective concentration; LEC, lowest effective concentration; NT, not tested.

[a] +, positive; –, negative.

transitions (6%) (Weng et al., 2017). In another *supF* shuttle-vector study using normal human fibroblasts (W138-VA13), 85% of the crotonaldehyde-induced mutations were base substitutions (single substitutions, 47%; tandem or multiple substitutions, 38%), 14% were deletions, and 1% were insertions; of the base substitutions, they found that G→T transversions predominated (50%), followed by G→A transitions (23%), and G→C transversions (13%) (Kawanishi et al., 1998). In a study in which the exposed plasmid was transfected into transformed human normal lymphoblasts (GM0621), crotonaldehyde induced primarily deletions (46%), as well as base-pair substitutions (39%), insertions (12%), and inversions (3%); two hot spot deletions were identified, which represented 62% of all deletions (Czerny et al., 1998).

In another study, a DNA vector containing either the 8*R*,6*R* or 8*S*,6*S* adducts was introduced into human xeroderma pigmentosum A (XPA) cells; both adduct isomers were found to inhibit DNA synthesis, with the 8*S*,6*S* adduct being more mutagenic than the 8*R*,6*R* isomer (10% versus 5%, respectively). Additionally, for the 8*S*,6*S* adduct, G→T transversions were the most common, followed by G→C transversions, and G→A transitions, whereas with the 8*R*,6*R* isomer, G→T and G→A were induced at almost the same frequency, followed by G→C (Stein et al., 2006).

### *(b) Experimental systems*

#### *(i) Non-human mammals in vivo*

See Table 4.6.

Chromosomal aberrations in the bone marrow were observed in male and female Swiss albino mice exposed to crotonaldehyde as a single intraperitoneal injection, with a significant response seen at sampling times of 6, 12, and 24 hours (Jha et al., 2007). Chromosomal aberrations were observed in spermatozoa analysed 24 hours after exposure to crotonaldehyde (Jha et al., 2007). A significant increase in abnormal sperm head morphology (an end-point used as an indicator of mammalian germ cell mutagens) was observed in male Swiss albino mice in samples obtained 1, 3, and 5 weeks after a single intraperitoneal dose of crotonaldehyde (Jha & Kumar, 2006). Male Swiss albino mice exposed by intraperitoneal injection to crotonaldehyde once daily for 5 days were mated with untreated females during the post-exposure periods in weeks 1, 2, 3, 4, or 5. An increase in the number of dominant lethal mutations (DLMs) and the number of dead implants per female was reported (Jha et al., 2007). From mating week 1, a significant increase in DLMs was induced by the highest dose; for mating weeks 2 and 3, DLMs were induced by all three doses; for mating week 4, DLMs were induced by the highest dose, and from mating week 5, there was a small but non-significant dose-related increase in DLMs (Jha et al., 2007). [The Working Group noted that the examination of these end-points after different post-exposure mating schedules allows for analysis of the sensitivity of the male germ cells at different developmental stages, and that these results indicated that male mouse germ cells appear to be most sensitive to the mutagenic effects of crotonaldehyde when exposed during the repair-proficient spermatid and late spermatocyte stages.]

#### *(ii) Non-human mammalian cells in vitro*

See Table 4.7.

In crotonaldehyde-treated primary rat hepatocytes, a significant increase in the frequency of DNA single-strand breaks was observed at 1 mM, as assessed by the alkaline elution assay (Eisenbrand et al., 1995). Crotonaldehyde treatment of primary rat colon and stomach mucosa cells induced DNA damage at 0.4 mM, as assessed by the alkaline comet assay (Gölzer et al., 1996). Higher doses (up to 71.3 mM) failed to elicit a significant increase in the amount of DNA in the comet tail in primary rat hepatocytes when assessed by the comet assay; however, condensed comet heads characteristic of DNA cross links

## Table 4.6 Genetic and related effects of crotonaldehyde in non-human mammals in vivo

| End-point | Species, strain, (sex) | Tissue | Results[a] | Dose (LED or HID) | Route, duration, dosing regimen | Comments | Reference |
|---|---|---|---|---|---|---|---|
| Chromosomal aberrations | Mouse, Swiss albino, (M, F) | Bone marrow | + | 8 µL/kg bw [7 mg/kg bw] | Intraperitoneal, 1×; 6 h sampling time | | Jha et al. (2007) |
| Chromosomal aberrations | Mouse, Swiss albino, (M, F) | Bone marrow | + | 8 µL/kg bw [7 mg/kg bw] | Intraperitoneal, 1×; 12 h sampling time | | Jha et al. (2007) |
| Chromosomal aberrations | Mouse, Swiss albino, (M, F) | Bone marrow | + | 8 µL/kg bw [7 mg/kg bw] | Intraperitoneal, 1×; 24 h sampling time | | Jha et al. (2007) |
| Chromosomal aberrations | Mouse, Swiss albino, (M) | Spermatozoa | + | 16 µL/kg bw [14 mg/kg bw] | Intraperitoneal, 1×; 24 h sampling time | | Jha et al. (2007) |
| Sperm head morphology | Mouse, Swiss albino (M) | Spermatozoa | + | 16 µL/kg bw [14 mg/kg bw] | Intraperitoneal 1×, 1-wk sampling time | Treated cells were spermatozoa. | Jha & Kumar (2006) |
| Sperm head morphology | Mouse, Swiss albino (M) | Spermatozoa | + | 16 µL/kg bw [14 mg/kg bw] | Intraperitoneal 1×, 3-wk sampling time | Treated cells were spermatids (repair-proficient). | Jha & Kumar (2006) |
| Sperm head morphology | Mouse, Swiss albino (M) | Spermatozoa | + | 27 mg/kg bw | Intraperitoneal 1×, 5-wk sampling time | Treated cells were preleptotene spermatocytes. | Jha & Kumar (2006) |
| Dominant lethal | Mouse, Swiss albino, (M) | Embryos in non-exposed pregnant females | + | 27 mg/kg bw | Intraperitoneal, 1×/day for 5 days, mating schedule 1–7 days | Treated cells were spermatids (repair-deficient due to highly condensed chromatin). | Jha et al. (2007) |
| Dominant lethal | Mouse, Swiss albino, (M) | Embryos in non-exposed pregnant females | + | 7 mg/kg bw | Intraperitoneal, 1×/day for 5 days, mating schedule 8–14 days | Treated cells were spermatids (repair-proficient). | Jha et al. (2007) |
| Dominant lethal | Mouse, Swiss albino, (M) | Embryos in non-exposed pregnant females | + | 7 mg/kg bw | Intraperitoneal, 1×/day for 5 days, mating schedule 15–21 days | Treated cells were spermatocytes. | Jha et al. (2007) |
| Dominant lethal | Mouse, Swiss albino (M) | Embryos in non-exposed pregnant females | + | 27 mg/kg bw | Intraperitoneal, 1×/day for 5 days, mating schedule 22–28 days | Treated cells were preleptotene spermatocytes and spermatocytes. | Jha et al. (2007) |

**Table 4.6 (continued)**

| End-point | Species, strain, (sex) | Tissue | Results[a] | Dose (LED or HID) | Route, duration, dosing regimen | Comments | Reference |
|---|---|---|---|---|---|---|---|
| Dominant lethal | Mouse, Swiss albino (M) | Embryos in non-exposed pregnant females | – | 27 mg/kg bw | Intraperitoneal, 1×/day for 5 days, mating schedule 29–35 days | Treated cells were spermatogonia. | Jha et al. (2007) |

bw, body weight; F, female; h, hour; HID, highest ineffective dose; LED, lowest effective dose; M, male; wk, week.
[a] +, positive; –, negative.

## Table 4.7 Genetic and related effects of crotonaldehyde 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 single-strand breaks (alkaline elution assay) | Wistar rat, primary hepatocytes | + | NT | 1 mM | | Eisenbrand et al. (1995) |
| DNA damage (comet, alkaline) | Sprague-Dawley rat, primary colon mucosa cells | + | NT | 0.4 mM | Comets were classified into three size classes depending on tail length. | Gölzer et al. (1996) |
| DNA damage (comet, alkaline) | Sprague-Dawley rat, primary colon mucosa cells | + | NT | 0.4 mM | Comets were classified into three size classes depending on tail length. | Gölzer et al. (1996) |
| DNA damage (comet, alkaline) | Wistar rat, primary hepatocytes | – | NT | 71.3 mM | 94% of cells had a central condensed spot characteristic of DNA and/or protein crosslinks. High concentrations used. | Kuchenmeister et al. (1998) |
| DNA and/or protein cross-links (comet, alkaline) | Wistar rat, primary hepatocytes | + | NT | 28.5 mM | | Kuchenmeister et al. (1998) |
| Unscheduled DNA synthesis | Rat, primary rat hepatocytes | – | NT | 125 µM | | Williams et al. (1989) |
| Gene mutation (*Tk*) | Mouse, lymphoma L5178Y/*Tk*$^{+/-}$ -3.7.2C cells | + | NT | 25 µM | | Demir et al. (2011) |
| Gene mutation (*Hgprt*) | Chinese hamster, fibroblasts, V79-4 | + | NT | 10 µM | Only tested concentration. | Li et al. (2012) |
| Gene mutation (*Hgprt*) | Chinese hamster, fibroblasts, V79-4, expressing human AKR7A2 | + | NT | 10 µM | Only tested concentration. | Li et al. (2012) |

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

[a] +, positive; –, negative.

were observed at 28.5 mM (Kuchenmeister et al., 1998). [The Working Group noted that similar comet responses for other cross-linking chemicals have been reported elsewhere (Pfuhler & Wolf, 1996; Merk & Speit, 1999). The Working Group also noted the high concentrations used.] A negative response was obtained for unscheduled DNA synthesis in crotonaldehyde-treated primary rat hepatocytes (Williams et al., 1989). Crotonaldehyde treatment resulted in a significant increase in the frequency of mutations of the *Tk* gene in mouse lymphoma cells (L5178Y) (Demir et al., 2011), and the *Hgprt* gene in standard Chinese hamster fibroblasts (V79-4), as well as in V79-4 cells expressing the human aldo-keto reductase enzyme AKR7A2 (Li et al., 2012).

Using shuttle vectors containing either adduct isomer, the 8*R*,6*R* and 8*S*,6*S* crotonaldehyde-derived 1,$N^2$-propano-2′-guanosine adducts were found to be mutagenic in African green monkey kidney (COS-7) cells, with similar percentage mutagenicity observed for both isomers (i.e. 4.7% and 6.2%, respectively) (Fernandes et al., 2005).

*(iii) Non-mammalian experimental systems*

See Table 4.8.

In *Drosophila melanogaster*, a negative result was obtained for sex-linked recessive lethal mutation when crotonaldehyde was administered in the feed, but the result was positive for both sex-linked recessive lethal mutations and heritable translocations when crotonaldehyde was administered by injection (Woodruff et al., 1985). A positive response was observed in the somatic mutation and recombination test (SMART) wing spot mutation assay with crotonaldehyde in the feed (Demir et al., 2013).

Crotonaldehyde has been evaluated in several *Salmonella typhimurium* strains that are sensitive to base-pair substitutions (i.e. strains TA1535, TA100, and TA104) and frameshift mutations (i.e. strains TA1537, TA1538, and TA98). However, no strains specific to the detection of cross-linking agents (e.g. TA102) were employed. In some cases, tests were only carried out without metabolic activation (–S9); with metabolic activation (+S9), the number of revertants was lowered. In the base-pair substitution strains, crotonaldehyde gave negative results with and without metabolic activation in several plate-incorporation assays with strain TA1535 (Lijinsky & Andrews, 1980; Neudecker et al., 1981; Haworth et al., 1983) and TA100 (Lijinsky & Andrews, 1980; Neudecker et al., 1981); however, in strain TA100 two positive results with and without metabolic activation were also observed (Haworth et al., 1983; Neudecker et al., 1989). When the more sensitive preincubation version of the assay was employed, a negative result was still obtained in strain TA1535 (Grúz et al., 2018). However, in strain TA100 the result was positive with and (when tested) without metabolic activation in five of the six preincubation assays (Lijinsky & Andrews, 1980; Neudecker et al., 1981, 1989; Cooper et al., 1987; Eder et al., 1992; Grúz et al., 2018). A positive response was obtained in strain TA104 without metabolic activation (Marnett et al., 1985). Crotonaldehyde gave negative results with and without metabolic activation in the frameshift strains TA1537, TA1538, and TA98 (Lijinsky & Andrews, 1980; Neudecker et al., 1981, 1989; Haworth et al., 1983; Eder et al., 1992; Grúz et al., 2018). Positive results without metabolic activation were observed in several YG test strains engineered with different polymerases (Grúz et al., 2018). A weak positive response for SOS induction was observed in strain TA1535 (Benamira & Marnett, 1992), and two negatives and a weak positive result were obtained in the SOS chromotest in *Escherichia coli* when DMSO was used as the solvent (Eder et al., 1992, 1993; Eder & Deininger, 2002); however, when ethanol was used as the solvent in two additional assays, robust positive responses were observed (Eder et al., 1993; Eder & Deininger, 2002). Negative results were obtained for both forward and reverse mutation in *Salmonella typhimurium* BA9 when the plate-incorporation version was

## Table 4.8 Genetic and related effects of crotonaldehyde 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* | Sex-linked recessive lethal mutation | – | NA | 4000 ppm [57 mM] (feed) | | Woodruff et al. (1985) |
| *Drosophila melanogaster* | Sex-linked recessive lethal mutation | + | NA | 3500 ppm [50 mM] (injection) | | Woodruff et al. (1985) |
| *Drosophila melanogaster* | Heritable translocation | + | NA | 3500 ppm [50 mM] (injection) | | Woodruff et al. (1985) |
| *Drosophila melanogaster* | SMART wing spot mutation | + | NA | 25 mM (feed) | Small spots only, mwh/flr3 only. | Demir et al. (2013) |
| *Salmonella typhimurium* TA1535 pSK 1002 | SOS (*umu*) induction assay, DNA damage | (+) | NT | 21 µg/mL | | Benamira & Marnett (1992) |
| *Salmonella typhimurium* TA1535 | Reverse mutation | – | – | 167 µg/plate | Purity, 83%. | Haworth et al. (1983) |
| *Salmonella typhimurium* TA1535 | Reverse mutation | – | – | 1000 µg/plate | | Lijinsky & Andrews (1980) |
| *Salmonella typhimurium* TA1535 | Reverse mutation | – | – | NR | | Neudecker et al. (1981) |
| *Salmonella typhimurium* TA1535, preincubation assay | Reverse mutation | – | NT | 3 µg/plate | | Grúz et al. (2018) |
| *Salmonella typhimurium* TA100 | Reverse mutation | + | + | 21 µg/mL | | Neudecker et al. (1989) |
| *Salmonella typhimurium* TA100 | Reverse mutation | + | + | 100 µg/plate | Purity, 83%. | Haworth et al. (1983) |
| *Salmonella typhimurium* TA100 | Reverse mutation | – | – | 1000 µg/plate | | Lijinsky & Andrews (1980) |

**Table 4.8 (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 | – | – | NR | | Neudecker et al. (1981) |
| *Salmonella typhimurium* TA100, preincubation assay | Reverse mutation | + | + | 10 µg/plate | | Lijinsky & Andrews (1980) |
| *Salmonella typhimurium* TA100, preincubation assay | Reverse mutation | – | NT | 0.54 mM [37.8 µg/mL] | Purity, 85%. | Cooper et al. (1987) |
| *Salmonella typhimurium* TA100, preincubation assay | Reverse mutation | + | NT | 70 µg/plate | | Neudecker et al. (1989) |
| *Salmonella typhimurium* TA100, preincubation assay | Reverse mutation | + | NT | 2 µg/plate | | Grúz et al. (2018) |
| *Salmonella typhimurium* TA100, preincubation assay | Reverse mutation | + | + | NR | | Eder et al. (1992) |
| *Salmonella typhimurium* TA100, liquid suspension | Reverse mutation | + | + | 0.025 µl/mL [21 µg/mL] (-S9), 0.075 µl/mL [64 µg/mL] (+S9) | Modified pre-incubation assay was performed suspended in either 0.1 M phosphate buffer or nutrient broth. Both were positive, but a more sensitive result was obtained with phosphate buffer. | Neudecker et al. (1981) |
| *Salmonella typhimurium* TA104 | Reverse mutation | + | NT | 20 µg/plate | | Marnett et al. (1985) |
| *Salmonella typhimurium* TA1538 | Reverse mutation | – | – | 1000 µg/plate | | Lijinsky & Andrews (1980) |

**Table 4.8 (continued)**

| Test system (species, strain) | End-point | Results[a] | | Concentration (LEC or HIC) | Comments | Reference |
|---|---|---|---|---|---|---|
| | | Without metabolic activation | With metabolic activation | | | |
| *Salmonella typhimurium* TA1538 | Reverse mutation | – | – | NR | | Neudecker et al. (1981) |
| *Salmonella typhimurium* TA98 | Reverse mutation | – | – | 1000 µg/plate | | Lijinsky & Andrews (1980) |
| *Salmonella typhimurium* TA98 | Reverse mutation | – | – | 167 µg/plate | Purity, 83%. | Haworth et al. (1983) |
| *Salmonella typhimurium* TA98 | Reverse mutation | – | – | NR | | Neudecker et al. (1981) |
| *Salmonella typhimurium* TA1537 | Reverse mutation | – | – | 167 µg/plate | Purity, 83%. | Haworth et al. (1983) |
| *Salmonella typhimurium* TA1537 | Reverse mutation | – | – | 500 µg/plate | | Lijinsky & Andrews (1980) |
| *Salmonella typhimurium* TA1537 | Reverse mutation | – | – | NR | | Neudecker et al. (1981) |
| *Salmonella typhimurium* YG6248, preincubation assay | Reverse mutation | + | NT | 2 µg/plate | | Grúz et al. (2018) |
| *Salmonella typhimurium* YG5197, YG9060 preincubation assay | Reverse mutation | + | NT | 1 µg/plate | | Grúz et al. (2018) |
| *Salmonella typhimurium* YG9028, YG6251, YG9135 preincubation assay | Reverse mutation | + | NT | 2 µg/plate | | Grúz et al. (2018) |
| *Salmonella typhimurium* YG5196, preincubation assay | Reverse mutation | – | NT | 3 µg/plate | | Grúz et al. (2018) |

**Table 4.8 (continued)**

| Test system (species, strain) | End-point | Results[a] | | Concentration (LEC or HIC) | Comments | Reference |
|---|---|---|---|---|---|---|
| | | Without metabolic activation | With metabolic activation | | | |
| *Salmonella typhimurium* BA9 | Forward mutation | – | NT | 1836 nM | | Ruiz-Rubio et al. (1984) |
| *Salmonella typhimurium* BA9 | Reverse mutation | – | NT | 1836 nM | | Ruiz-Rubio et al. (1984) |
| *Salmonella typhimurium* BA9, preincubation assay | Forward mutation | + | NT | 612 nmol/plate [43 µg/plate] | | Ruiz-Rubio et al. (1984) |
| *Salmonella typhimurium* BA9, preincubation assay | Reverse mutation | + | NT | 612 nmol/plate [43 µg/plate] | | Ruiz-Rubio et al. (1984) |
| *Escherichia coli* PQ37, SOS chromotest | DNA damage | – | NT | NR | | Eder et al. (1992) |
| *Escherichia coli* PQ37, SOS chromotest | DNA damage | – | NT | NR | | Eder et al. (1993) |
| *Escherichia coli* PQ37, SOS chromotest | DNA damage | + | NT | NR | Ethanol used as solvent in place of DMSO. | Eder et al. (1993) |
| *Escherichia coli* PQ37, SOS chromotest | DNA damage | (+) | NT | NR | Weak positive when tested with DMSO (no SOSIP; $I_{max}$, < 1.5). | Eder & Deininger (2002) |
| *Escherichia coli* PQ37, SOS chromotest | DNA damage | + | NT | NR | Positive when ethanol used as solvent. | Eder & Deininger (2002) |
| Calf thymus DNA (acellular) | DNA damage (fluorescence screening for changes in DNA melting and annealing behaviour) | + | NT | 100 mM | | Kailasam & Rogers (2007) |

DMSO, dimethyl sulfoxide; flr, flare; HIC, highest effective concentration; $I_{max}$, maximal concentration for induction; LEC, lowest effective concentration; mwh, multiple wing hairs; NA, not applicable; NR, not reported; NT, not tested; ppm, parts per million; SMART, somatic mutation and recombination test; SOSIP, SOS-inducing potency.

[a] +, positive; –, negative; (+), positive in a study of limited quality.

carried out, but robust increases in forward and reverse mutation were observed with the more sensitive preincubation version (Ruiz-Rubio et al., 1984).

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 crotonaldehyde in an acellular study (Kailasam & Rogers, 2007).

### 4.2.3 Alters DNA repair

A single study on the ability of crotonaldehyde to alter DNA repair was available. Using the host cell reactivation assay, crotonaldehyde was found to inhibit both nucleotide excision repair and base excision repair capacity in human hepatocellular carcinoma cells (HepG2) (Weng et al., 2017). In a subsequent experiment, nucleotide excision repair was instantaneously inhibited when crotonaldehyde was added to cell lysates, indicating that crotonaldehyde reacts with and inhibits proteins that are critical for nucleotide excision repair (Weng et al., 2017).

### 4.2.4 Induces oxidative stress

#### (a) Humans

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

In vitro studies in human vein endothelial cells demonstrated that crotonaldehyde (50 μM; 1 hour) increases the formation of reactive oxygen species (Ryu et al., 2013). Crotonaldehyde treatment also increased gene expression and protein levels of haem oxygenase 1 in a dose-dependent manner, consistent with a cellular response to oxidative stress (Lee et al., 2011). In human bronchial epithelial cells, crotonaldehyde decreased concentrations of intracellular glutathione (at up to 10 μM) and increased the formation of reactive oxygen species (at 40 μM) (Liu et al., 2010).

#### (b) Experimental systems

See Table 4.9.

In rats, depletion of hepatic glutathione (a marker of oxidative stress) occurs after acute intraperitoneal administration of crotonaldehyde (Cooper et al., 1992). In male Wistar rats, subchronic oral administration of crotonaldehyde increased production of proinflammatory cytokines and elevated serum malondialdehyde concentrations, indicative of increased lipid peroxidation (Zhang et al., 2019b). In another study in male Wistar rats, subchronic (up to 120 days) oral exposure to crotonaldehyde decreased serum glutathione peroxidase and superoxide dismutase activity and elevated malondialdehyde concentration (Li et al., 2020).

In vitro studies have shown that crotonaldehyde exposure can inhibit glutathione *S*-transferase activity, resulting in depletion of intracellular glutathione (van Iersel et al., 1996). Crotonaldehyde exposure for 4 hours decreased intracellular glutathione concentration (at 25 μM) and increased reactive oxygen species formation (at ≥ 25 μM) in a rat alveolar macrophage cell line (Yang et al., 2013a).

### 4.2.5 Induces chronic inflammation

#### (a) Humans

No data were available to the Working Group.

#### (b) Experimental systems

See Table 4.9.

In a study of subchronic toxicity (120 days) in rats treated by gavage, crotonaldehyde was associated with myocardial necrosis, cardiac fibrosis, renal tubular epithelial cell oedema, and renal lymphocyte infiltration, suggestive of an inflammatory response (Zhang et al., 2019b). In a study of chronic toxicity in male rats treated by inhalation, crotonaldehyde was associated with a dose-dependent increase in the incidence and severity of inflammation in the nasal respiratory

**Table 4.9 Effects of crotonaldehyde on markers of oxidative stress or chronic inflammation in non-human mammals in vivo**

| End-point | Species, strain (sex) | Tissue | Results[a] | Dose (LED or HID) | Route, duration, dosing regimen | Reference |
|---|---|---|---|---|---|---|
| *Oxidative stress markers* | | | | | | |
| GSH | Rat, F344 (M) | Liver | ↓ | 450 µmol/kg [31.5 mg/kg] | Intraperitoneal, 1× | Cooper et al. (1992) |
| MDA | Rat, Wistar (M) | Serum | ↑ | 8.5 mg/kg per day | Oral (gavage), 120 days | Zhang et al. (2019b) |
| GPx, SOD | Rat, Wistar (M) | Serum | ↓ | 8.5 mg/kg per day | Oral (gavage), 120 days | Zhang et al. (2019b) |
| GPx, MDA, SOD | Rat, Wistar (M) | Lung | ↓ | 4.5 mg/kg per day | Oral (gavage), 120 days | Li et al. (2020) |
| *Inflammation markers* | | | | | | |
| Inflammatory cell infiltration, oedema, or inflammatory markers | Rat, Wistar (M) | Heart<br>Kidney | ↑<br>↑ | 4.5 mg/kg per day | Oral (gavage), 120 days | Zhang et al. (2019b) |
| Respiratory epithelial inflammation | Rat, F344 (M, F) | Nasal cavity | ↑ | 3 ppm (M), 6 ppm (F) | Inhalation, 6 h/day, 5 days/wk, 104 wk | JBRC (2001e) |
| Respiratory epithelial inflammation | Mouse, Crj:BDF1 (M,F) | Nasal cavity | ↑ | 12 ppm (F only; no effect in M) | Inhalation, 6 h/day, 5 days/wk, 104 wk | JBRC (2001b) |
| Inflammatory cell infiltration, macrophage phagocytic ability and number, shifts in T lymphocyte populations | Rat, Wistar (M) | Lung (BALF) | ↑ | 4 µL/kg [3.4 mg/kg] | Intratracheal instillation, 1× | Wang et al. (2018) |
| Inflammatory cell infiltration | Rat, Wistar (M) | Lung | ↑ | 4.5 mg/kg per day | Oral (gavage), 120 days | Li et al. (2020) |

BALF, bronchoalveolar lavage fluid; F, female; GPx, glutathione peroxidase; GSH, glutathione; h, hour; HID, highest ineffective dose; LED, lowest effective dose; M, male; MDA, malondialdehyde; ppm, parts per million; SOD, superoxide dismutase; wk, week.
[a] ↑, increase; ↓, decrease.

epithelium (JBRC, 2001b). In female mice and rats exposed to crotonaldehyde by inhalation in a study of chronic toxicity, inflammation was seen at 12 and 6 ppm, respectively (JBRC, 2001e, b); see also Section 3. [The Working Group noted that changes in cell proliferation in response to crotonaldehyde exposure has not been evaluated in experimental systems.] Intratracheal instillation of crotonaldehyde resulted in inflammatory cell infiltration, shift in the number of CD4+ and CD8+ T lymphocytes, decreased numbers of mononuclear phagocytes in bronchoalveolar lavage fluid in male Wistar rats (Wang et al., 2018). In a study of subchronic toxicity (up to 120 days) in male Wistar rats, oral exposure to crotonaldehyde (at 4.5 mg/kg bw) resulted in increased inflammatory cell infiltration, as well as increased lung concentrations of TNFα, interleukin 6 (IL6), and IL1β (Li et al., 2020).

### 4.2.6 Other key characteristics

#### (a) Is immunosuppressive

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

In cultured human monocytic U937 cells differentiated along the macrophagic line, crotonaldehyde increased the release of IL8 and TNFα (Facchinetti et al., 2007). In cultured human macrophages, human lung fibroblasts, and small

airway epithelial cells, crotonaldehyde increased the release of IL8, and this response was mediated via p38 MAPK- and ERK1/2-dependent pathways (Moretto et al., 2009).

Shifts in T-lymphocyte populations, decreased numbers of mononuclear phagocytes in bronchoalveolar lavage fluid, and decreased lung macrophage function were reported in male Wistar rats after intratracheal instillation of crotonaldehyde in the study of Wang et al. (2018) referenced above (see Table 4.9). Crotonaldehyde was found to suppress phagocytic function in cultured rat alveolar macrophages and was associated with a dose-dependent decrease in cell viability (Yang et al., 2013b).

*(b) Modulates receptor-mediated effects*

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

Crotonaldehyde activated peroxisome proliferator-activated receptor PPARγ and PPARβ/δ luciferase reporter activity in a dose-dependent manner in cultured TSA201 cells derived from human embryonic kidney cells (HEK293) (Matsushita et al., 2019). Crotonaldehyde enhanced thyroid hormone action by modulating thyroid hormone binding to thyroid hormone receptors (TR) resulting in upregulation of gene transcription in cultured human embryonal kidney (TSA 201) cells (Hayashi et al., 2018). In TSA201 cells transfected with the ligand-binding domain of TRα1 or TRβ1 coupled to a luciferase reporter system, it was demonstrated that, in the presence of thyroid hormone, crotonaldehyde induced TRα1-mediated transcription activity while not affecting TRβ1 (Hayashi et al., 2018).

*(c) Multiple characteristics*

Transcript profiling has been performed in a human monocytic leukaemia THP-1 cell line exposed to crotonaldehyde (Yang et al., 2014). In this system, 342 or 663 genes were statistically significantly differentially expressed after either a 6- or 12-hour exposure, respectively, to crotonaldehyde at 80 µM (Yang et al., 2014). Crotonaldehyde affected the expression of genes related to oxidative stress, including several involved in glutathione metabolism. Haeme oxygenase 1 (*HO-1*) was also upregulated after crotonaldehyde exposure (Yang et al., 2014). Other pathways dysregulated by crotonaldehyde exposure included those involved in apoptosis and regulating cellular responses to DNA damage (Yang et al., 2014).

Liu et al. (2010) evaluated transcript profiles in human bronchial epithelial cells exposed to crotonaldehyde. Multiple inflammatory responsive genes (e.g. *XCL1, CXCL2, CXCL3, CCL2, CSF1, CSF2, NFKBIA, NFKBIZ*) were downregulated by crotonaldehyde, whereas fewer genes (*CMTM, PAG1,* and *PTX3)* were upregulated. Some genes involved in cytokine production and inflammation (*IL6, IL8*) were downregulated, whereas *HOX-1* was upregulated after treatment with crotonaldehyde (Liu et al., 2010).

## 4.3 Other relevant evidence

### 4.3.1 Humans

No data were available to the Working Group.

### 4.3.2 Experimental systems

See Table 4.9.

Two-year studies have been performed in F344/DuCrj rats and Crj:BDF1 mice treated with crotonaldehyde by inhalation with whole-body exposure (JBRC, 2001b, e). Rats and mice (in groups of 50 per species, sex, and dose group) were exposed at 0, 3, 6, or 12 ppm (6 hours per day, 5 days per week for 104 weeks). In male and female rats, chronic inhalation of crotonaldehyde was associated with a dose-dependent increase in the incidence and severity of inflammation and squamous cell hyperplasia and metaplasia in the nasal respiratory epithelium, and necrosis and atrophy in the olfactory epithelium (JBRC,

2001e). In male and female mice, chronic inhalation of crotonaldehyde of 12 ppm was associated with an increased incidence of squamous cell metaplasia of the nasal respiratory epithelium (JBRC, 2001b). Evidence of inflammation in the nasal respiratory epithelium was only seen in female mice at 12 ppm. Atrophy and metaplasia of the olfactory epithelium was seen in male and female mice at 12 ppm (JBRC, 2001b).

## 5. Summary of Data Reported

### 5.1 Exposure characterization

Crotonaldehyde is a High Production Volume chemical that is produced by the aldolization reaction of acetaldehyde. It is a reactive chemical and is widely used for synthesizing other chemicals, including the food preservative sorbic acid and vitamin E (two major products), but also for the production of intermediates such as crotonic acid, crotyl alcohol, *n*-butanal, and *n*-butanol in different industries such as pharmaceuticals, rubber, chemicals, leather, and food and agriculture.

Crotonaldehyde occurs naturally in a ubiquitous fashion. It is produced endogenously by plants and animals including humans as part of lipid peroxidation and metabolism. It is found in many foods and beverages.

Tobacco smoke is a major exposure source in the general population, followed by gasoline and diesel engine exhaust, indoor cooking on wood-burning stoves, heating by coal and coal briquette fuels, and heated cooking oil. The urinary metabolites *N*-acetyl-*S*-(3-hydroxy-1-methylpropyl)-L-cysteine (HMPMA) and *N*-acetyl-*S*-(3-carboxy-1-methylpropyl)-L-cysteine (CMEMA) have been studied as markers to assess exposure, but no accepted reference values are available for these metabolites.

Occupational exposure to crotonaldehyde occurs through its application in industry and wherever organic material is burned; however, no data were found on workers' exposure during these processes. Air concentrations of crotonaldehyde were reported in studies among workers in a plant producing aldehydes, garage workers, workers in toll booths, firefighters, as well as coke-oven workers.

Occupational exposure reference values exist for crotonaldehyde and acute environmental exposure values are also available.

### 5.2 Cancer in humans

One occupational cohort study and three nested case–control studies in population-based cohorts were available. The study in an occupational cohort was uninformative due to small numbers, poor external exposure assessment and flaws in design. Two nested case–control studies in a population-based cohort studied several biomarkers (including metabolites of crotonaldehyde) in relation to lung cancer among current smokers and non-smokers respectively, without demonstrating an etiological association with crotonaldehyde exposure. The third nested case–control study reported on colorectal cancers in relation to crotonaldehyde adducts. In summary, all studies were judged to be uninformative in terms of providing evidence on a causal relationship between crotonaldehyde 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 crotonaldehyde caused an increase in the incidence of an appropriate combination of benign and malignant neoplasms in a single sex and species in one study, and an increase in the incidence of a very rare benign neoplasm in a second study.

In the first study, there was a significant increase in the incidence of hepatocellular adenoma and of hepatocellular adenoma or carcinoma (combined) in male Fischer 344 rats given drinking-water containing crotonaldehyde.

In the second study, there was a low incidence of nasal cavity adenoma in male F344/DuCrj rats exposed to crotonaldehyde by inhalation. Nasal cavity adenoma is a very rare tumour in the rat strain used in this study.

## 5.4 Mechanistic evidence

The available data on the absorption and distribution of crotonaldehyde in humans are scarce. Nonetheless, increased concentrations of crotonaldehyde metabolites in the urine of tobacco smokers are consistent with absorption. Crotonaldehyde is efficiently conjugated with glutathione, ultimately yielding HMPMA and CMEMA as urinary metabolites in humans and in rats. Other metabolic pathways are reduction to crotyl alcohol, catalysed by aldo-keto reductases, and oxidation to crotonic acid, catalysed by aldehyde dehydrogenases. In rats treated intraperitoneally or by oral gavage the primary routes of elimination are through the urine (as mercapturates) and the breath (as exhaled carbon dioxide).

There is consistent and coherent evidence that crotonaldehyde exhibits multiple key characteristics of carcinogens. Crotonaldehyde is an electrophilic bifunctional α,β-unsaturated aldehyde (enal) that can form cyclic adducts in DNA, DNA interstrand crosslinks and DNA–protein crosslinks. It forms DNA adducts in vivo and in vitro. The identified adducts formed in vivo are two diastereoisomeric forms of α-methyl-γ-hydroxy-1,$N^2$-propano-2′-deoxyguanosine. These crotonaldehyde adducts have been detected in normal human liver and in other normal tissues including peripheral blood, mammary tissue, oral (gingival) tissue, liver, and placenta, and in saliva and urine. In studies in which smokers and non-smokers were compared, adduct levels were significantly elevated in tobacco smokers, indicating their formation by crotonaldehyde in tobacco smoke; their presence in tissues of non-smokers which may be indicative of crotonaldehyde formation by endogenous processes, including lipid peroxidation, or from other external sources. In human cells treated in vitro with the agent, several studies have demonstrated the formation of crotonaldehyde-derived DNA adducts. In rats treated with crotonaldehyde by gavage, DNA adducts were detected in the liver. In some studies, but not all, the presence of crotonaldehyde-derived adducts has been reported in various tissues, including the liver, of untreated rodents. In mice chronically exposed to mainstream tobacco smoke, DNA adducts derived from crotonaldehyde were detected in the lung and bladder, but not in the heart and liver. Crotonaldehyde and crotonaldehyde-derived DNA adducts can also be formed in the presence of biologically relevant concentrations of acetaldehyde, a metabolite of ethanol, under physiological conditions. Crotonaldehyde is also a metabolite of *N*-nitrosopyrrolidine, a carcinogenic environmental nitrosamine.

Crotonaldehyde is genotoxic. No data in exposed humans were available to the Working Group. In human primary cells and human cell lines, crotonaldehyde was clastogenic. In Swiss albino mice, crotonaldehyde induced dominant lethality in embryos, and induced chromosomal aberrations in bone marrow and spermatozoa. In cultured rodent cells, crotonaldehyde induced DNA damage and gene mutations at *Tk* and *Hprt* loci. Crotonaldehyde induced mutations in *Drosophila melanogaster*, and induced base-pair substitution mutations in the absence of metabolic activation in *Salmonella typhimurium*. Crotonaldehyde induced *supF* mutations in exposed plasmids.

Crotonaldehyde induces oxidative stress. No data in exposed humans were available. In vitro exposure of human endothelial cells or bronchial

epithelial cells to crotonaldehyde resulted in increased production of reactive oxygen species. Crotonaldehyde also decreased intracellular glutathione concentration in human bronchial epithelial cells. Depletion of hepatic glutathione occurs in rats after acute intraperitoneal administration of crotonaldehyde. Subchronic oral administration of crotonaldehyde to rats increased proinflammatory cytokine concentrations and elevated serum malondialdehyde concentration, indicating increased lipid peroxidation. Subchronic oral administration of crotonaldehyde to rats increased lung malondialdehyde concentration. In vitro studies in rodent cells showed that crotonaldehyde inhibits glutathione *S*-transferase activity, depletes intracellular glutathione concentrations, and increases the formation of reactive oxygen species.

Crotonaldehyde induces chronic inflammation, with mild increases in inflammation in the nasal respiratory epithelium reported in rats and mice in studies of chronic toxicity. In studies of subchronic toxicity in rodents, crotonaldehyde showed either renal lymphocyte infiltration after oral exposure or a dose-dependent increase in the incidence and severity of inflammation in the nasal respiratory epithelium after inhalation.

Few data were available regarding other key characteristics of carcinogens. Regarding whether crotonaldehyde is immunosuppressive, crotonaldehyde exposure altered cytokine release in human cells in vitro. Shifts in T-lymphocyte populations, decreased numbers of mononuclear phagocytes in bronchoalveolar lavage fluid, and decreased lung macrophage function have been observed in rats after intratracheal instillation of crotonaldehyde. Crotonaldehyde also suppressed phagocytic function in cultured rat alveolar macrophages.

# 6. Evaluation and Rationale

## 6.1 Cancer in humans

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

## 6.2 Cancer in experimental animals

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

## 6.3 Mechanistic evidence

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

## 6.4 Overall evaluation

Crotonaldehyde is *possibly carcinogenic to humans (Group 2B).*

## 6.5 Rationale

The *Group 2B* evaluation for crotonaldehyde is based on *strong* mechanistic evidence. There is *strong evidence* in human primary cells that crotonaldehyde exhibits key characteristics of carcinogens; crotonaldehyde is electrophilic and genotoxic. It also induces oxidative stress and induces chronic inflammation in experimental systems. In addition, there is supporting evidence from studies in humans for DNA adducts.

There is also *limited evidence* for cancer in experimental animals, based on an increase in the incidence of an appropriate combination of benign and malignant neoplasms in a single sex and species in one study, and an increase in the incidence of a very rare benign neoplasm in a

second study. The evidence regarding cancer in humans is *inadequate*. The few available studies were small, and/or had major design limitations, and/or could not distinguish the effects of crotonaldehyde exposure from other constituents of cigarette smoking.

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