Crotonaldehyde: Human health tier II assessment

05 February 2016

Chemicals in this assessment

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

Preface

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

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

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

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

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

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

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

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

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

Disclaimer

ACRONYMS & ABBREVIATIONS

Grouping Rationale

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

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

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

Import, Manufacture and Use

Australian

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

International

The following international uses have been identified through:

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

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

The chemical has reported commercial uses, including:

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

The chemical has reported site-limited uses, including:

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

The chemical has reported non-industrial use, including:

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

Restrictions

Australian

No known restrictions have been identified.

International

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

Cosmetic:

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

Other:

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

Chemical warfare:

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

Existing Worker Health and Safety Controls

Hazard Classification

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

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

Exposure Standards

Australian

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

International

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

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

Health Hazard Information

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

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

Toxicokinetics

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

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

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

Acute Toxicity

Oral

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

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

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

Dermal

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

Inhalation

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

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

Corrosion / Irritation

Respiratory Irritation

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

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

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

Skin Irritation

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

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

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

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

Eye Irritation

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

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

Observation in humans

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

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

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

Sensitisation

Skin Sensitisation

Limited data are available.

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

Observation in humans

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

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

Repeated Dose Toxicity

Oral

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

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

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

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

Dermal

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

Inhalation

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

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

Genotoxicity

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

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

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

In vitro studies

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

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

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

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

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

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

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

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

In vivo studies

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

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

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

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

Carcinogenicity

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

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

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

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

Reproductive and Developmental Toxicity

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

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

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

Other Health Effects

Neurotoxicity

Limited data are available.

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

Risk Characterisation

Critical Health Effects

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

Public Risk Characterisation

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

Occupational Risk Characterisation

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

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

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

NICNAS Recommendation

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

Regulatory Control

Work Health and Safety

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

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

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

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

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

Advice for industry

Control measures

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

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

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

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

Obligations under workplace health and safety legislation

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

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

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

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

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

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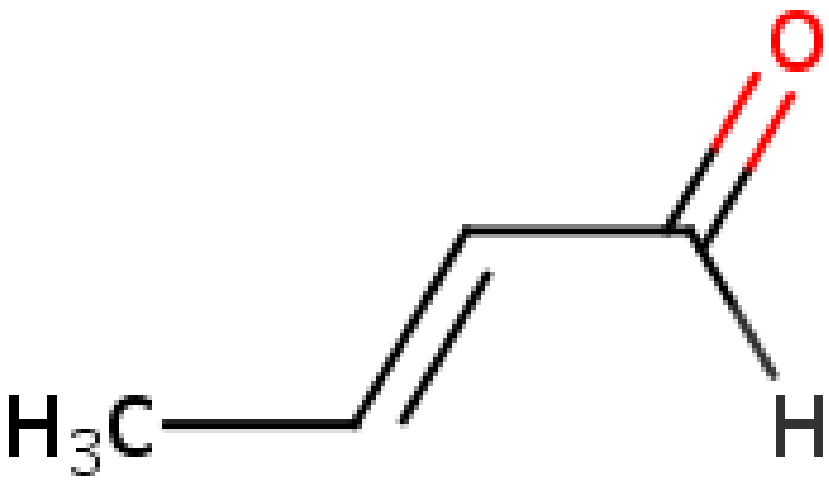
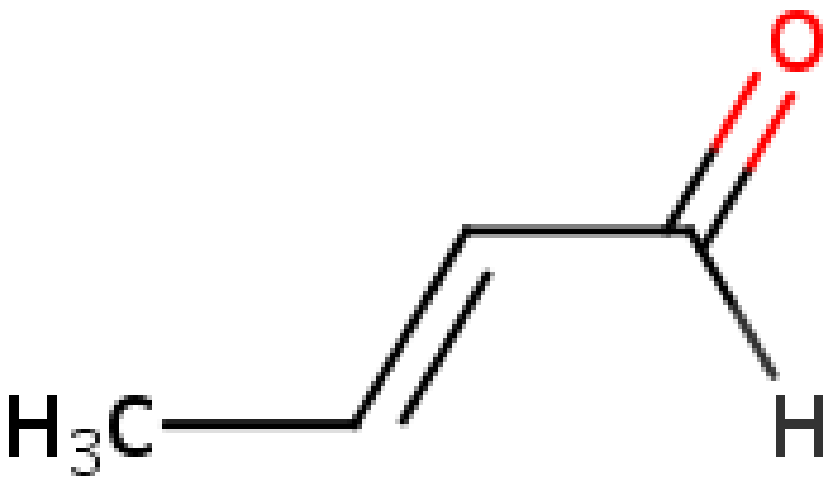
Chemical Identities

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

	
Molecular Formula	C4H6O
Molecular Weight	70.09

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

	
Molecular Formula	C4H6O
Molecular Weight	70.09

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