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

*SCOEL/SUM/180*
*March 2013*

Employment, Social Affairs and Inclusion

# Table of Contents

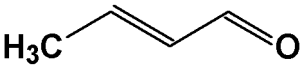
1. Substance identification, physico-chemical properties ..... 3
2. Occurrence/use and occupational exposure ..... 4
3. Health significance ..... 4
3.1. Toxicokinetics ..... 4
3.1.1. Human data ..... 4
3.1.2. Animal data ..... 4
3.1.3. Biological monitoring ..... 5
3.2. Acute toxicity ..... 5
3.2.1. Human data ..... 5
3.2.2. Animal data ..... 5
3.2.3. In vitro data ..... 5
3.3. Irritation and corrosivity ..... 5
3.3.1. Human data ..... 5
3.3.2. Animal data ..... 7
3.4. Sensitisation ..... 7
3.4.1. Human data ..... 7
3.4.2. Animal data ..... 7
3.5. Repeated dose toxicity ..... 8
3.5.1. Human data ..... 8
3.5.2. Animal data ..... 8
3.6. Genotoxicity ..... 8
3.6.1. In vitro ..... 8
3.6.2. In vivo – Human data ..... 9
3.6.3. In vivo – Animal data ..... 9
3.7. Carcinogenicity ..... 10
3.7.1. Human data ..... 10
3.7.2. Animal data ..... 10
3.8. Reproductive toxicity ..... 11
3.8.1. Human data ..... 11
3.8.2. Animal data ..... 11
4. Recommendation ..... 11

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

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

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

## 1. Substance identification, physico-chemical properties

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

*EU classification:*

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

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

# 2. Occurrence/use and occupational exposure

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

# 3. Health significance

## 3.1. Toxicokinetics

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

### 3.1.1. Human data

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

### 3.1.2. Animal data

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

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

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

### 3.1.3. Biological monitoring

There were no data available.

## 3.2. Acute toxicity

### 3.2.1. Human data

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

### 3.2.2. Animal data

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

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

### 3.2.3. In vitro data

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

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

## 3.3. Irritation and corrosivity

### 3.3.1. Human data

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

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

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

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

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

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

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

#### 3.3.2. Animal data

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

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

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

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

## 3.4. Sensitisation

### 3.4.1. Human data

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

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

### 3.4.2. Animal data

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

## 3.5. Repeated dose toxicity

### 3.5.1. Human data

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

### 3.5.2. Animal data

*Inhalation*

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

*Oral*

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

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

*Dermal*

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

## 3.6. Genotoxicity

### 3.6.1. In vitro

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

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

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

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

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

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

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

### 3.6.2. In vivo – Human data

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

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

### 3.6.3. In vivo – Animal data

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

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

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

## 3.7. Carcinogenicity

### 3.7.1. Human data

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

### 3.7.2. Animal data

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

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

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

## 3.8. Reproductive toxicity

### 3.8.1. Human data

Human data on reproductive or developmental effects were not available.

### 3.8.2. Animal data

*Fertility*

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

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

*Developmental toxicity*

Animal studies on developmental effects were not available.

## 4. Recommendation

*Irritation*

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

*Systemic effects*

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

*Genotoxicity and carcinogenicity*

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

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

*Overall assessment*

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

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

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

No data on biological monitoring were available.

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

# 5. References

ACGIH, American Conference of Governmental Industrial Hygienists (2001). Crotonaldehyde. In: ACGIH, American Conference of Governmental Industrial Hygienists, Threshold limit values for chemical substances and physical agents and biological exposure indices, 2004, Cincinnati, OH.

AEGL (2007). Committee on Acute Exposure Guideline Levels, Committee on Toxicology, National Research Council. Acute exposure guideline levels for selected airborne chemicals: Volume 6. Crotonaldehyde trans and cis + trans. ISBN: 0-309-11214-1, 318 pages. http://www.nap.edu/catalog/12018.html.

AIHA, American Industrial Hygiene Association (1997). Odor thresholds for chemicals with established occupational health standards. American Industrial Hygiene Association, Fairfax, VA.

Alarie Y, Nielsen GD, Schaper MM (2001). Animal bioassays for evaluation of indoor air quality. Chapter 23. In: Spengler JD, McCarthy JF, Samet JM. Indoor air quality handbook. McGraw-Hill, 23.1-23.49.

Auerbach C, Moutschen-Dahmen M, Moutschen J (1977). Genetic and cytogenetical effects of formaldehyde and related compounds. Mutat Res 39:317-362, cited in BUA 1993 and ECB 2000.

Bainova A, Madzhunov N (1984). Quantitative determination of the irritating effect on human skin of butanol, octanol, acetaldehyde and crotonaldehyde [in Bulgarian]. Probl Khig 9:66-72, cited in BUA 1993.

Bittersohl G (1974). Epidemiologische Untersuchungen über Krebserkrankungen bei Arbeiten mit Aldol und aliphatischen Aldehyden. Arch Geschwulstforsch 43:172-176, cited in BUA 1993 and ECB 2000.

BUA, Beratergremium für umweltrelevante Altstoffe (1993). Crotonaldehyd (2-Butenal), BUA-Stoffbericht 98. S. Hirzel Verlag, Stuttgart.

Chung FL, Tanaka T, Hecht SS (1986). Induction of liver tumors in F344 rats by crotonaldehyde. Cancer Res 46:1285-1289.

Chung FL, Zhang L, Ocando JE, Nath RG (1999). Role of 1,N2-propanodeoxyguanosine adducts as endogenous DNA lesions in rodents and humans. IARC Sci Publ 150:45-54.

Coenraads PJ, Bleumink E, Nater JP (1975). Susceptibility to primary irritants. Age dependence and relation to contact allergic reactions. Contact Dermatitis 1:377-381, cited in BUA 1993 and ECB 2000.

Demir A, Kayaa B, Soriano C, Creus A, Marcos R (2011). Genotoxic analysis of four lipid-peroxidation products in the mouse lymphoma assay. Mutat Res 726(2):98-103.

DFG, Deutsche Forschungsgemeinschaft (2005). Toxikologische Beurteilung α,β-ungesättigter aliphatischer Aldehyde in Lebensmitteln. In: Lebensmittel und Gesundheit II/Food and Health II, Wiley-VCH, Weinheim, Germany.

Dittberner U, Eisenbrand G, Zankl H (1990). Cytogenetic effects of the α,β-unsaturated aldehydes crotonaldehyde and E-2-hexene-1-al. Mutagenesis 5:618, cited in BUA 1993.

ECB, European Chemicals Bureau (2000). IUCLID, International Uniform Chemical Information Database. Edition II. EUR 19559 EN, European Commission.

Eder E, Budiawan (2001). Cancer risk assessment for the environmental mutagen and carcinogen crotonaldehyde on the basis of TD(50) and comparison with 1,N(2)-propanodeoxyguanosine adduct levels. Cancer Epidemiol Biomarkers Prev 10:883-888.

Eder E, Budiawan, Schuler D (1996). Crotonaldehyde: a carcinogenic and mutagenic air, water and food pollutant. Cent Eur J Public Health 4 (Suppl.): 21-22.

Eder E, Schuler D, Budiawan (1999). Cancer risk assessment for crotonaldehyde and 2-hexenal: an approach. In: Singer B, Bartsch H. IARC Scientific Publication No. 150. Exocyclic DNA adducts in mutagenesis and carcinogenesis. IARC, International Agency for Research on Cancer, Lyon, 219-232.

Fannick N (1982). Health hazard evaluation report, No. HETA-81-102-1244, Sandoz Colors and Chemicals, East Hanover, NJ, US National Institute for Occupational Safety and Health. Hazard Evaluations and Technical Assistance Branch, Cincinnati, OH, cited in AEGL 2007.

Fernandes PH, Kanuri M, Nechev LV, Harris TM, Lloyd RS (2005). Mammalian cell mutagenesis of the DNA adducts of vinyl chloride and crotonaldehyde. Environ Mol Mutagen 45:455-459.

Foiles P, Akerkar A, Miglietta I, Chung FL (1990). Formation of cyclic deoxyguanosine adducts in Chinese hamster ovary cells by acrolein and crotonaldehyde. Carcinogenesis 11:2059-2061, cited in DFG 2005.

Galloway SM, Armstrong MJ, Reuben C, Colman S, Brown B, Cannon C, Bloom AD, Nakamura F, Ahmed M, Duk S, Rimpo J, Margolin BH, Resnick MA, Anderson B, Zeiger E (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. Environ Mol Mutagen 10 (Suppl. 10):1-175.

Gölzer P, Janzowski C, Pool-Zobel BL, Eisenbrand G (1996). (E)-2-hexenal-induced DNA damage and formation of cyclic 1,N2-(1,3-propano)-2'-deoxyguanosine adducts in mammalian cells. Chem Res Toxicol 9:1207-1213, cited in DFG 2002.

Hecht SS, McIntee EJ, Cheng G, Shi Y, Villalta PW, Wang M (2001a). New aspects of DNA adduct formation by the carcinogens crotonaldehyde and acetaldehyde. Adv Exp Med Biol 500:63-71.

Hecht SS, McIntee EJ, Wang M (2001b). New DNA adducts of crotonaldehyde and acetaldehyde. Toxicology 166:31-36.

Henschler D (1981). Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeits-medizinische Begründungen von MAK-Werten, Loseblattsammlung, 8. Lfg. DFG, Deutsche Forschungsgemeinschaft, VCH Verlag Weinheim.

IARC, International Agency for Research on Cancer (1995). IARC Monographs on the evaluation of carcinogenic risks to humans. Vol. 63. Dry cleaning, some chlorinated solvents and other industrial chemicals. WHO, World Health Organization, Geneva.

IPCS, International Programme on Chemical Safety (2008). Concise International Chemical Assessment Document 74. 2-Butenal. ISBN 978 92 4 153074 3, http://www.inchem.org/documents/cicads/cicads/cicad74.pdf.

Jagannath DR (1980). Intra sanguineous mouse host-mediated assay crotonaldehyde. Unpublished report (LDI Project No. 20998), Litton Biometics, Inc., Kensington, MD, (on behalf of Gewerbetoxikologie, Hoechst AG, Frankfurt/Main; Report No. 06/81), 1-17, cited in BUA 1993.

Jha AM, Kumar M (2006). In vivo evaluation of induction of abnormal sperm morphology in mice by an unsaturated aldehyde crotonaldehyde. Mutat Res 603:159-163.

Jha AM, Singh AC, Sinha U, Kumar M (2007). Genotoxicity of crotonaldehyde in the bone marrow and germ cells of laboratory mice. Mutat Res 632:69–77.

Kawaguchi-Niida M, Shibata N, Morikawa S, Uchida K, Yamamoto T. Sawada T, Kobayashi M (2006). Crotonaldehyde accumulates in glial cells of Alzheimer's disease brain. Acta Neuropathologica 111:422-429.

Kozekov ID, Nechev LV, Moseley MS, Harris CM, Rizzo CJ, Stone MP, Harris TM (2003). DNA interchain cross-links formed by acrolein and crotonaldehyde. J Am Chem Soc 125:50–61.

Kurtz AJ, Lloyd RS (2003). 1,N2-deoxyguanosine adducts of acrolein, crotonaldehyde, and trans-4-hydroxynonenal cross-link to peptides via Schiff base linkage. J Biol Chem 278:5970-5976.

Liu XY, Yang ZH, Pan XJ, Zhu MX, Xie JP (2010a). Gene expression profile and cytotoxicity of human bronchial epithelial cells exposed to crotonaldehyde. Toxicol Lett 197:113-122.

Liu XY, Yang ZH, Pan XJ, Zhu MX, Xie JP (2010b). Crotonaldehyde induces oxidative stress and caspase-dependent apoptosis in human bronchial epithelial cells. Toxicol Lett 195:90-98.

Mayer D, Weigand W, Kramer M (1980). Bericht über die Prüfung von Crotonaldehyd auf mutagene Wirkung im Mikronukleus-test an NMRI-Mäusen nach oraler Verabreichung. Unpublished report No. 53/80 of Pharma Forschung Toxikologie, Hoechst AG, Frankfurt/Main, 1-11, cited in BUA 1993.

Moutschen-Dahmen J, Moutschen-Dahmen M, Degraeve N, Houbrechts N, Colizzi A (1975). Genetical hazards of aldehydes from mouse experiments. Mutat Res 29:205.

Nath RG, Ocando JE, Guttenplan JB, Chung F (1998). 1,N2-propanodeoxyguanosine adducts: Potential new biomarkers of smoking-induced DNA damage in human oral tissue. Cancer Res 58:581-584.

Nath RG, Chung F (1994). Detection of exocyclic 1,N2-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans (acrolein/crotonaldehyde). Proc Natl Acad Sci USA 91:7491-7495.

Nath RG, Ocando JE, Chung F (1996). Detection of 1,N2-propanodeoxyguanosine adducts as potential endogenous DNA lesions in rodent and human tissues Cancer Res 56:452-456.

Nilsson A, Lagesson V, Bornehag CG, Sundell J, Tagesson C (2005). Quantitative determination of volatile organic compounds in indoor dust using gas chromatography-UV spectrometry. Environ Int 31:1141-1148.

NTP, National Toxicology Program (2012). Crotonaldehyde.
http://ntp.niehs.nih.gov/?objectid=BCAC7858-123F-7908-7BEEB291F013C986.

Rinehart WE (1967). The effect on rats of single exposures to crotonaldehyde vapour. Am Ind Hyg Assoc J 28:561-566.

Schaper M (1993). Development of a database for sensory irritants and its use in establishing occupational exposure limits. Am Ind Hyg Assoc J 54:488-544.

Shmunes E, Kempton RJ (1980). Allergic contact dermatitis to dimethoxane in a spin fish. Contact Dermatitis 6:421-424, cited in BUA 1993 and ECB 2000.

Sim VM, Pattle RE (1957). Effect of possible smog irritants on human subjects. J Am Med Assoc 165:1908-1913.

Stein S, Lao Y, Yang IY, Hecht SS, Moriya M (2006). Genotoxicity of acetaldehyde- and crotonaldehyde-induced 1,N2-propanodeoxyguanosine DNA adducts in human cells. Mutat Res 608:1-7.

Steinhagen WH, Barrow CS (1984). Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. Toxicol Appl Pharmacol 72: 495-503.

Tepikina LA, Skvortsova EL, Shipulina ZV, Kartashova AV, Mol'kov IUN, Sizova NN. 1997. Substantiation of MAC for crotonaldehyde in environmental air [in Russian]. Gig Sanit 3:3-5, cited in AEGL 2007.

Trofimov LV (1962). Comparative toxic action of crotonaldehyde and butyraldehyde (in Russian, English abstract). Gig Tr Prof Zabol 6:34-40.

von Tungeln LS, Yi P, Bucci TJ, Samokyszyn VM, Chou MW, Kadlubar FF, Fu PP (2002). Tumorigenicity of chloral hydrate, trichloroacetic acid, trichloroethanol, malondialdehyde, 4-hydroxy-2-nonenal, crotonaldehyde, and acrolein in the B6C3F(1) neonatal mouse. Cancer Lett 185:13-19.

Voronin VA, Bel'gova IN, Voronka LA, Grigor'ev ON, Zhdanov VA, Nezhentsev MV, Antelava NA, Gusel' VA, Rotleder AM (1982). Korrektur der Toxizitätsbefunde über Crotonaldehyd (CA; Technische Vorschriften TU 6-09-3667-74). Gig Tr Prof Zabol 26:54-55, cited in BUA 1993 and ECB 2000.

Wang M, McIntee EJ, Cheng G, Shi Y, Villalta PW, Hecht SS (2001). A Schiff base is a major DNA adduct of crotonaldehyde. Chem Res Toxicol 14:423-430.

Williams GM, Mori H, McQueen CA (1989). Structure-activity relationships in the rat hepatocyte DNA-repair test for 300 chemicals. Mutat Res 221:263-286, cited in IARC 1995 and ECB 2000.

Wolfe GW, Rodwin M, French JE, Parker GA (1987). Thirteen week subchronic toxicity study of crotonaldehyde (CA) in F344 rats and B6C3F1 mice. Abstr. No. 835. Toxicologist 7:20.

Zhang S, Villalta PW, Wang M, Hecht SS (2006). Analysis of crotonaldehyde- and acetaldehyde-derived 1,N2-propanodeoxyguanosine adducts in DNA from human tissues using liquid chromatography electrospray ionization tandem mass spectrometry. Chem Res Toxicol 19:1386-1392.