

The MAK Collection for Occupational Health and Safety

Crotonaldehyde

MAK Value Documentation, addendum – Translation of the German version from 2018

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Crotonaldehyde / But-2-enal

MAK Value Documentation

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the germ cell mutagenicity and carcinogenicity of crotonaldehyde [4170-30-3, 123-73-9].

Crotonaldehyde is a highly reactive mutagenic and cytotoxic compound without metabolic activation.

An oral carcinogenicity study in male rats, with the liver as the only organ examined, provides at most only an indication of a carcinogenic potential. Crotonaldehyde remains assigned to Carcinogen Category 3B because of the still limited database.

Despite some methodical deficiencies, new *in vivo* studies with positive results for bone marrow and spermatocyte chromosomal aberrations as well as dominant lethal mutations in mice lead to a reclassification in Category 3A for Germ Cell Mutagens.

Keywords

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

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Crotonaldehyde

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

Supplement 2018

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

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

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

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

Mechanism of Action

Genotoxicity

Crotonaldehyde reacts with cellular macromolecules. Protein adducts and DNA–histone crosslinks were induced in vitro. Cyclic 1,N²-propanodeoxyguanosine adducts were detected in various tissues of mice and rats both in vitro and in vivo; these are formed also endogenously in animals and humans (supplement “2-Butenal” 2007, available in German only). In humans, these adducts were detected more frequently in the lungs than in the liver, and were not detected in blood (SCOEL 2013).

In addition, N²-(3-hydroxybutylidene)deoxyguanosine and N²-[2-(2-hydroxypropyl)-6-methyl-1,3-dioxan-4-yl]deoxyguanosine (“N²-paraldol-dG”) were detected in vitro (supplement “2-Butenal” 2007, available in German only).

Crotonaldehyde protein adducts have been found in the brains of patients with Alzheimer’s disease (SCOEL 2013). 1,N²-propanodeoxyguanosine adducts inhibit DNA synthesis and are mutagenic after incorporation into DNA vectors and after transfection into human xeroderma pigmentosum cells (Stein et al. 2006). In addition, 1,N²-propanodeoxyguanosine adducts can form DNA crosslinks (Kozekov et al. 2003; Liu et al. 2006).

Cytotoxicity

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

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

Animal Experiments and in vitro Studies

Genotoxicity

In vitro

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

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

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In vivo

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

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

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

Carcinogenicity

Long-term studies

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

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

Manifesto (carcinogenicity, germ cell mutagenicity)

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

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

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

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

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

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

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