The MAK-Collection for Occupational Health and Safety

GMS PUBLISSO ZB MED-Publikationsportal Lebenswissenschaften

Benzotriazole

MAK Value Documentation, supplement – Translation of the German version from 2019

A. Hartwig[1,*] MAK Commission[2,*]

1 *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

2 *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

* *E-Mail: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Keywords:

benzotriazole, glioma, oligodendroglioma, carcinogenicity, genotoxicity, reproductive toxicity, skin absorption, irritation, sensitization, hazardous substance

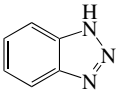
Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated benzotriazole [95-14-7], considering all toxicological endpoints. Available publications and unpublished study reports are described in detail. A genotoxic potential is not found in bacterial or mammalian cell systems in vitro and benzotriazole does not induce micronuclei in the bone marrow of mice. In oral carcinogenicity studies in rats and mice, benzotriazole causes tumours in various organs with a low incidence of three gliomas and one oligodendroglioma in the brain of rats. These rarely occur in control animals and are therefore considered to be substance-induced. For this reason, benzotriazole is suspected of being carcinogenic and is classified in Carcinogen Category 3 B. A NOAEL (no observed adverse effect level) of 12.5 mg/kg body weight and day is obtained for bleeding of mucous membranes at nose and mouth and salivation at 5 mg/kg body weight and day from a subchronic oral toxicity study in rats. As an inhalation study has not been performed, but benzotriazole is irritating to the eye and is therefore expected to be irritating to the respiratory tract, a maximum concentration at the workplace (MAK value) cannot be derived. In a reproductive toxicity study with exposure of rats to benzotriazole, at 300 mg/kg body weight and day the body weight of pups is reduced during lactation. Teratogenicity was not examined. Skin contact is expected to contribute significantly to systemic toxicity. Therefore, benzotriazole is designated with an “H”. Limited data show no sensitization.

Citation Note:
Hartwig A, MAK Commission. Benzotriazole. MAK Value Documentation, supplement – Translation of the German version from 2019. MAK Collect Occup Health Saf. 2020 Dec;5(4):Doc078.
DOI: 10.34865/mb9514kske5_4ad

Manuscript completed:
21 Mar 2018

Publication date:
21 Dec 2020

MAK value	–
Peak limitation	–
Absorption through the skin (2018)	**H**
Sensitization	–
Carcinogenicity (2018)	**Category 3 B**
Prenatal toxicity	–
Germ cell mutagenicity	–
BAT value	–
Synonyms	aziminobenzene 1,2,3-benzotriazole 2,3-diazaindole
Chemical name (IUPAC)	1*H*-benzotriazole
CAS number	95-14-7
Structural formula	
Molecular formula	$C_6H_5N_3$
Molar mass	119.14 g/mol
Melting point	100 °C (ECHA 2017)
Boiling point at 1013.25 hPa	204 °C (ECHA 2017)
Vapour pressure at 25 °C	0.0689 hPa (calculated; ECHA 2017)
log K_{OW}	1.34 (ECHA 2017)
Solubility in water	20 g/l (ECHA 2017)
1 ml/m³ (ppm) ≙ 4.944 mg/m³	**1 mg/m³ ≙ 0.202 ml/m³ (ppm)**
Stability	thermally stable, stable in the presence of electromagnetic radiation in the UV range (US EPA 1977)
Production	reaction of *o*-phenylenediamine with nitrous acid (NCBI 2020)
Purity	in studies, the purity is given as > 99% (Bayer AG 1988; Connect Chemicals GmbH 2012 c)
Impurities	no data
Uses	anticorrosive agent in metal-working fluids, detergent (NCBI 2020)

Note: The substance can occur simultaneously as vapour and aerosol.

Documentation is available for benzotriazole from 1988 (translated in Henschler 1991). As a result of new data this supplement has been drawn up. Benzotriazole is contained in metal-working fluids in concentrations up to a maximum of 0.5% (Hartwig 2014, available in German only).

1 Toxic Effects and Mode of Action

Benzotriazole is irritating to the eyes of rabbits. In long-term oral studies with rats and mice, benzotriazole produced tumours in several organs; particularly the occurrence of three gliomas and one oligodendroglioma in the brain of rats suggests that the substance has carcinogenic effects. The acute toxicity of benzotriazole in the animals used can be attributed mainly to effects on the central nervous system (CNS). After subchronic administration of benzotriazole by gavage, occasional bleeding of mucous membranes and salivation were observed at 50 mg/kg body weight and day and above. The studies of reproductive toxicity revealed reduced body weights in the offspring on postnatal days 0 and 4 at the dose level of 300 mg/kg body weight and day.

2 Mechanism of Action

2.1 Neurotoxicity

Effects on the nervous system are found in a number of species after the administration of single oral and intravenous doses of benzotriazole. In mice, intravenous injection causes progressive paralysis up to respiratory arrest (see Section 5.1.4). This does not involve an effect on the peripheral nervous system, as the conduction velocity of peripheral nerves and transmission of the signal to the muscle end-plate are not affected by benzotriazole. The central nervous paralysis is explained by the depression of polysynaptic reflex arcs at the spinal cord and brain stem levels (Domino et al. 1952).

2.2 Carcinogenicity

The paralysing effects of benzotriazole can be attributed to CNS effects (see above); the accessibility of the brain at concentrations such as those used in the carcinogenicity study (see Section 5.7) appears in principle to be possible.

In male and female F344 rats, an increase in gliomas or oligodendrogliomas compared with the incidence in controls was observed, but this was not statistically significant (see Section 5.7).

In order to identify the pathogenesis of the development of brain tumours in rodents more exactly, studies with transgenic animal models (p53 heterozygous mice) were carried out. It was found that the loss of normal p53 gene expression may represent an early event in rodent brain carcinogenesis. According to the authors, these findings are all the more important as loss or mutation of the p53 gene is common in glial tumours in man (Sills et al. 1999). However, there are no studies that investigate whether benzotriazole is able to modulate the tumour suppressor p53.

In addition, a relationship between the formation of brain tumours and reactive oxygen species (ROS) has been suggested (Rinaldi et al. 2016). As benzotriazole has not been found to have genotoxic potential, and there is thus no evidence for the generation of ROS by benzotriazole, this mechanism does not seem to be of prime importance.

The statistically significant increase in incidence of alveolar/bronchiolar carcinomas found in the carcinogenicity study with B6C3F1 mice is not relevant to humans due to the high incidence of adenomas and carcinomas in the lungs of mice occurring spontaneously and induced by chemicals and the high doses used. The higher sensitivity of mice for lung tumours is due to the pulmonary adenoma susceptibility 1 (Pas1) locus (Dassano et al. 2014; Malkinson 1989).

It is questionable whether the tumours of the thyroid gland, uterus and liver that occurred in the F344 rat are substance-related and they cannot be assigned to a specific mechanism (see Section 5.7).

3 Toxicokinetics and Metabolism

There is no new information available regarding the toxicokinetics and metabolism of benzotriazole.

The absorption, distribution and elimination of benzotriazole have not been investigated in either animals or humans. In vitro, 4-hydroxybenzotriazole and 5-hydroxybenzotriazole are produced as metabolites of benzotriazole (Henschler 1991).

Experimental studies of the dermal absorption of benzotriazole are not available. According to the models of Fiserova-Bergerova et al. (1990), Guy and Potts (1993) and Wilschut et al. (1995), fluxes of 0.67, 0.06 and 0.07 mg/cm^2 and hour, respectively, are calculated for a saturated aqueous solution (exposed area 2000 cm^2, exposure duration one hour) and therefore the possible absorption of 1340 mg, 120 mg and 140 mg benzotriazole. In metal-working fluids, the maximum concentration of benzotriazole used is 0.5% (5 g/l). The corresponding calculated dermal absorption under standard conditions is 335 mg, 30 mg and 35 mg, respectively.

4 Effects in Humans

Only studies of allergenic effects are available.

Allergenic effects

Two cases of contact dermatitis in metal workers have been described. The workers were exposed to lubricants containing benzotriazole and produced strong reactions in the patch tests (3+) or a positive reaction (no other details) to a 2% preparation of benzotriazole in petrolatum, which the authors had isolated from the lubricant oil used. Two other workers with contact dermatitis produced an erythematous or weakly positive (1+) reaction to the preparation (Ducombs et al. 1980; Henschler 1991).

In other studies in workers exposed to metal-working fluids, no positive reactions to benzotriazole (tested in 1% petrolatum) were obtained in 52 workers with contact dermatitis from a total collective of 74 (Angelini and Meneghini 1977; Henschler 1991). Furthermore, no reactions occurred in 40 tested persons with contact dermatitis from a collective totaling 286 metal workers in 10 factories (de Boer et al. 1989) and no reactions were observed in 199 (Geier et al. 2004), 110 (Geier et al. 2006) and 182 tested persons (Gruvberger et al. 2003). Also after testing with a 1% aqueous preparation of the sodium salt of the methyl derivative of benzotriazole (methyl-1*H*-benzotriazole, sodium salt, CAS No. 29385-43-1), no positive reactions were produced in 125 tested persons (Geier et al. 2003).

In 105 tested car mechanics (Meding et al. 1994) and in two collectives of 230 (Holden and Gawkrodger 2005) and 731 (Katugampola et al. 2005) tested patients with dermatitis on the hand or foot, no or only one positive reaction to benzotriazole (Katugampola et al. 2005) was found. In a review covering the years 1985 to 1997, the results of the recorded patch tests with antibacterial ingredients conducted at the university clinic of Leuven included two positive reactions in 8521 tested persons (Goossens et al. 1998).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

In the documentation of 1988 (Henschler 1991), a 3-hour LC_{50} of 1900 mg benzotriazole/m^3 was reported in rats; accumulation in the trachea of a white frothy fluid and haemorrhages in the lungs (no pulmonary oedema) were observed. After 2 to 3 days, the animals were normal again (no other details).

For the structurally related methyl-1*H*-benzotriazole, an RD_{50} of 205 mg/m^3 was determined in mice for pulmonary irritation (Hartwig 2011, available in German only).

5.1.2 Oral administration

In the documentation of 1988 (Henschler 1991), oral LD_{50} values of between 550 and 965 mg/kg body weight for rats, 831 mg/kg body weight for mice and 500 mg/kg body weight for guinea pigs were reported. The first effect observed in the animals was the loss of the righting reflex in the hind limbs.

In a recent study carried out according to OECD Test Guideline 423, all 3 female Sprague Dawley rats died within 3 hours after receiving a benzotriazole dose of 2000 mg/kg body weight. The signs of intoxication observed were reduced spontaneous activity, reduced muscle tone, reduced righting and Preyer's reflexes, mydriasis, lacrimation, delayed breathing and in some cases drooping of the eyelids. In the gross-pathological examination, a reduction in the size of the forestomach and the associated tissue with white discoloration and black spots was found. None of the 6 female Sprague Dawley rats died at 300 mg/kg body weight. During the initial hours following administration of the substance, the same clinical findings in a weakened form were found, from which all animals recovered completely within 24 hours. There were no substance-related gross-pathological findings. The LD_{50} was therefore above 300 and below 2000 mg/kg body weight (Connect Chemicals GmbH 2012 b).

5.1.3 Dermal application

The dermal LD_{50} values were > 2000 mg benzotriazole/kg body weight for rabbits and > 1000 mg/kg body weight for guinea pigs (Eastman Kodak Company 1983; Polaroid Corporation 1965; Sherwin-Williams Company 1972 a).

5.1.4 Intravenous and intraperitoneal injection

Studies of the effects of benzotriazole after intravenous injection in mice yielded a mean paralysing dose of 55 mg/kg body weight. The loss of the righting reflex started in the hind limbs. The paralysis was reversible after administration of non-lethal doses. In mice, the intravenous LD_{50} was 238 mg/kg body weight, at which respiratory arrest occurred (Domino et al. 1952; Henschler 1991), the intraperitoneal LD_{50} was 500 mg/kg body weight after administration in oil and 1000 mg/kg body weight after administration without oil (Henschler 1991).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

There are no studies available.

5.2.2 Oral administration

In a dose range finding study, groups of 5 male and 5 female Fischer 344 rats and B6C3F1 mice were given benzotriazole with the diet for 8 weeks. The concentrations were 0, 300, 1000, 3000, 10 000 and 30 000 mg/kg diet (corre-

sponding to doses of 0, about 27, 90, 270, 900, 2700 mg/kg body weight and day in rats or 0, about 60, 200, 600, 2000, 6000 mg/kg body weight and day in mice, using conversion factors of 0.09 for rats and 0.2 for mice in accordance with EFSA (2012)). In rats, the body weight gain was decreased (up to 12% at doses between 27 and 900 and 34% to 40% at 2700 mg benzotriazole/kg body weight and day). In B6C3F1 mice, there was no decrease in body weight gain until the dose of 6000 mg benzotriazole/kg body weight and day; at this dose there was a decrease of up to 5% (Henschler 1991).

In a study according to OECD Test Guideline 421, 12 male and 12 female Wistar rats were given gavage doses of benzotriazole of 0, 12.5, 50 or 200 mg/kg body weight and day, the males for 39 to 50 days, the females for 46 to 56 days (purity > 99%). Females showing no signs of having mated were subjected to a second mating cycle and were then exposed for between 67 and 70 days. In some animals of all treatment groups, respiratory sounds occurred occasionally after application. One female in the high dose group and one control died as a result of gavage errors. From day 5 onwards, individual animals treated with the high and the medium dose started wiping their mouth through the cage bedding immediately after application. This was observed with increasing incidence throughout the treatment period and in nearly all animals of these dose groups towards the end of the in-life phase. Pronounced salivation was observed particularly in the animals of the high dose group on day 15 of administration and beyond; this was found to a reduced extent also in the animals of the middle dose group. Occasional bleeding at nose and mouth was observed in individual animals of the high and the medium dose groups. There was no statistically significant difference in body weights, body weight gains and food consumption between the exposed animals and those not exposed. In the females, the water consumption in the high and low dose groups was statistically significantly increased compared with that in the controls, in the middle dose group it was statistically significantly reduced, while in the males it was not affected. Swollen and reddened axillary and mesenteric lymph nodes were found in animals of all groups. In addition, effects in the digestive tract such as a reddened jejunum and patches in the duodenum were found, with a higher incidence in the females than in the males. As the effects on the lymph nodes and in the digestive tract were found also in the controls, the authors attributed these findings to the solvent selected and regarded them as not substance-related. The histopathological examination did not reveal any substance-related findings in either the parent or young animals (Connect Chemicals GmbH 2012 c). If the occasional bleeding of the mucous membranes and the salivation found in some animals at 50 mg/kg body weight and day and above are regarded as substance-related effects, the NOAEL (no observed adverse effect level) is then 12.5 mg/kg body weight and day.

In a screening study according to OECD Test Guideline 422, 12 female and 12 male Crl:CD rats per group were given gavage doses of 0, 30, 100 or 300 mg/kg body weight and day. The males were treated for 42 days, the females for about 54 days (14 days prior to mating, 14-day mating period, 22-day pregnancy, 4 days lactation). As a substance-related effect, a statistically significant reduction in body weights were observed in the male and female offspring of the high dose group on day 1 and on day 4 of lactation (mean value ± standard deviation on lactation day 4: ♀ control group: 10.4 ± 0.7 g; ♀ high dose group: 9.3 ± 0.8 g; ♂ control group: 10.8 ± 0.7 g; ♂ high dose group: 9.5 ± 0.7 g). All other end points were without substance-related findings. The NOAEL for the parents was 300 mg/kg body weight and day, the highest dose tested (ECHA 2017). As the original study report is not available, the study cannot be used for this evaluation. The study description is no longer available on the website of ECHA.

5.2.3 Dermal application

There are no data available.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

In the documentation of 1988 (Henschler 1991), benzotriazole was evaluated as not irritating to the intact skin of rabbits. The studies available since the documentation of 1988 are given below. They confirm the classification as "not irritating".

In a study according to OECD Test Guideline 404, 500 mg of benzotriazole as a paste with water was applied over 6 cm^2 rabbit skin and covered semi-occlusively. The covering was removed after 4 hours, the exposed skin area was washed and the skin was examined for irritation after 1, 24, 48 and 72 hours and 7, 14 and 21 days following application using the Draize evaluation criteria. Erythema, eschar formation or oedema were not observed at any time (Bayer AG 1987 a). Minimal irritation was found on the intact and the scarified skin of 3 male and 3 female rabbits after the application for 24 hours under occlusive conditions of 50% benzotriazole (100% pure) as well as of 50% benzotriazole (purity not specified) dissolved in 70% polyethylene glycol (PEG 400) and 30% saline solution (Ciba-Geigy Limited 1982 e, f).

Benzotriazole (0.5 g) was applied to the shaved intact and the scarified dorsal skin of 6 female New Zealand White rabbits and left for 24 hours. Evaluation of the irritation was carried out immediately after the end of the application as well as after 72 hours. Benzotriazole was not irritating to the rabbit skin (Sherwin-Williams Company 1972 b).

In an earlier study, 1 g benzotriazole, homogenized with distilled water, was applied to the skin (4 cm^2) of 4 rabbits and covered occlusively for 3 days. Evaluation of the skin was carried out after 3, 11 and 26 days. No effects could be found on the skin of these animals (Polaroid Corporation 1965).

5.3.2 Eyes

In the documentation of 1988 (Henschler 1991), benzotriazole was classified as irritating to the eyes of rabbits. The studies available since the 1988 documentation confirm this evaluation; they are described below.

The eye irritation caused by benzotriazole was investigated in 3 New Zealand White rabbits according to OECD Test Guideline 405. After the instillation of 100 µl test substance, the eyelids were held closed for about one second. The treated eye was rinsed with physiological saline after 24 hours. The second, untreated eye, served as the control. The irritation was investigated after 1, 24, 48 and 72 hours as well as after 7 and 14 days following administration of the substance and assessed using the Draize criteria. The irritation scores for the cornea, iris and conjunctivae are given in Table 1. All forms of irritation had subsided 7 or 14 days after the instillation of the substance. Benzotriazole was irritating to the eyes of rabbits (Bayer AG 1987 a).

Tab. 1 Eye irritation scores (24, 48 and 72 hours) in rabbits after the instillation of benzotriazole (Bayer AG 1987 a)

	Animal 1	Animal 2	Animal 3
Corneal opacity (max. 4)	0.6	0.3	0.6
Iris (max. 2)	0.6	0	0
Conjunctivae			
redness (max. 3)	1.3	0.6	1.6
chemosis (max. 4)	0.6	0.6	0.3
discharge (max. 3)	1	0.6	0

max: maximum irritation score

The instillation of 100 mg benzotriazole into one eye of 6 albino rabbits produced corneal opacity, iritis, conjunctival inflammation and bleaching of the conjunctiva 24, 48 and 72 hours after application (Sherwin-Williams Company 1969).

The eye irritation caused by 100 mg benzotriazole, suspended in 0.2 ml distilled water, was investigated in 4 New Zealand White rabbits. Five minutes after instillation, the eyes were rinsed with physiological saline and the irritation of the cornea was evaluated using the Draize method. Irritation of the cornea was observed in all animals, which was still clearly visible after 26 days. In all 4 animals, the corneal irritation score was 3 (maximum irritation score 4) after 24 hours, and between 2 and 4 after 7 and 28 days (Polaroid Corporation 1965). The irritating effect of benzotriazole on the eyes has been confirmed in two other studies (Eastman Kodak Company 1983; Sherwin-Williams Company 1974).

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

In a maximization test with groups of 20 Pirbright White guinea pigs, purified benzotriazole did not produce reactions in any of the animals, while commercially available benzotriazole produced reactions in 3 of 20 animals. Intradermal induction was carried out with 1% preparations of the test substances in physiological saline, and topical induction and challenge with 30% preparations of the test substance in petrolatum (Maurer and Meier 1984). There is no information given as to whether pretreatment of the animals with sodium lauryl sulfate was carried out before topical induction.

In another maximization test, groups of 20 Dunkin Hartley guinea pigs were exposed to benzotriazole with a purity of 99.83%; intradermal induction treatment was carried out with 5% of the test substance in propylene glycol. For the topical induction and challenge treatment, 25% and 12% preparations of benzotriazole in propylene glycol were used, respectively. At the challenge, a reaction was observed in one of the 20 treated animals and in one of the 10 controls after 24 hours, but no longer after 48 hours (ECHA 2017).

An optimization test carried out in groups of 20 Pirbright White guinea pigs with purified and commercially available benzotriazole yielded negative results for both test substances in all treated animals and in the 20 controls after intradermal induction with preparations of 0.1% test substance in physiological saline and topical challenge with 30% preparations in petrolatum. The intradermal challenge with 0.1% preparations in physiological saline produced reactions in 5 of 20 animals with the commercially available substance and reactions in 3 of 20 animals with the purified substance. In the vehicle control group, reactions occurred in 2 of 20 animals (Maurer and Meier 1984).

In an earlier study with repeated application of 200 mg/kg body weight within 30 days, no reaction was obtained in guinea pigs (Henschler 1991).

5.4.2 Sensitizing effects on the airways

No data are available.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In the study carried out according to OECD Test Guideline 421 described in Section 5.2.2, Wistar rats were given daily benzotriazole (purity > 99%) doses of 0, 12.5, 50 or 200 mg/kg body weight by gavage, the males for between 39 and 50 days, and the females for between 46 and 56 days. Females showing no signs of having mated were subjected to a second mating cycle and then exposed for between 67 and 70 days. No treatment-related findings were observed in the reproductive organs of either males or females, in the recorded fertility parameters or in the histopathological examinations of the parent animals (Connect Chemicals GmbH 2012 c). If the haemorrhage of the mucous membranes and the salivation that occasionally occurred in some of the animals at and above 50 mg/kg

body weight and day are regarded as substance-related effects, the NOAEL for the parent animals is 12.5 mg/kg body weight and day; the NOAEL for fertility is 200 mg/kg body weight and day, the highest dose tested.

In the study described in Section 5.2.2 carried out according to OECD Test Guideline 422, 12 female and 12 male Crl:CD rats per group were given benzotriazole doses of 0, 30, 100 and 300 mg/kg body weight and day by gavage. The males were dosed for 42 days and the females for about 54 days (14 days before mating, 14-day mating period, 22 days pregnancy, 4 days lactation). There were no treatment-related findings for the end points regarding fertility. The NOAEL for fertility and systemic parental toxicity was therefore the highest dose tested of 300 mg/kg body weight and day (ECHA 2017). As the original study report is not available, the study cannot be used for this evaluation. The study description is no longer available on the website of ECHA.

5.5.2 Developmental toxicity

In the study described in Sections 5.2.2 and 5.5.1 carried out according to OECD Test Guideline 421, female Wistar rats were given daily benzotriazole doses of 0, 12.5, 50 or 200 mg/kg body weight (purity > 99%) by gavage for between 39 and 70 days up to postnatal day 4 of the offspring. No substance-related changes were found in the developmental parameters. In the high dose group, the weights of the newborn rats were not statistically significantly reduced compared with those of the controls. No substance-related findings in the parents or pups were observed in the histopathological examination (Connect Chemicals GmbH 2012 c). If the occasionally occurring haemorrhage in the mucous membranes and the salivation in some of the animals at 50 mg/kg body weight and day and above are regarded as substance-related effects in the dams, the maternal NOAEL is 12.5 mg/kg body weight and day. As no skeletal and visceral examinations were carried out in the offspring, a NOAEL for development toxicity cannot be established.

In the study described in Sections 5.2.2 and 5.5.1 carried out according to OECD Test Guideline 422, 12 female and 12 male Crl:CD rats per group received gavage doses of benzotriazole of 0, 30, 100 and 300 mg/kg body weight and day. The males were dosed for 42 days and the females for about 54 days (14 days before mating, 14-day mating period, 22 days pregnancy, 4 days lactation). A statistically significant reduction in body weights were found in the male and female offspring (mean value ± standard deviation on lactation day 4: ♀ control group: 10.4 ± 0.7 g; ♀ high dose group: 9.3 ± 0.8 g; ♂ control group: 10.8 ± 0.7 g; ♂ high dose group: 9.5 ± 0.7 g) in the high dose group on postnatal days 0 and 4. For all other end points there were no treatment-related findings. Skeletal and visceral examinations of the offspring were not carried out, so that no statements can be made regarding teratogenicity. The parental NOAEL was the highest dose tested of 300 mg/kg body weight and day (ECHA 2017). As the original study report is not available, the study cannot be used for this evaluation. The study description is no longer available on the website of ECHA.

5.6 Genotoxicity

5.6.1 In vitro

All available studies of gene mutations in vitro are listed in Table 2. Mutagenicity was found in the bacterial test systems using Salmonella typhimurium (S. typhimurium) and Escherichia coli (E. coli). Among the cited studies with S. typhimurium, in two studies in which positive results were found in the strains TA98, TA1535, TA1537 and TA1538 only a substance designation (TK 10'637) was given, without verification that benzotriazole was involved and without any specification of purity (Ciba-Geigy Limited 1982 c, d). The validity of these studies is therefore questionable. In all other studies with S. typhimurium identified as yielding positive results, a mutagenic effect was found in the strain TA1535 only. Strain TA1535, like the strain TA100, contains a hisG46 mutation. These two strains differ in that the plasmid pKM101 was introduced into strain TA100. A positive result in strain TA1535 is easier to detect, as the background incidence of mutations is lower, which means that fewer mutations per plate are necessary for strain TA1535 (8–20) compared with for strain TA100 (90–160) (Prival and Zeiger 1998). Substances that induce

a positive result only in strain TA1535, but not in strain TA100, are at best weakly mutagenic, which could not be verified in the case of benzotriazole in a more recent study. In S. typhimurium strains TA97a, TA98, TA100, TA102 and TA1535, benzotriazole (purity 99.87%) was not mutagenic in the presence and absence of a metabolic activation system in a series of investigations carried out according to OECD Test Guideline 471 at concentrations of 50 to 5003 µg/plate. No signs of cytotoxicity occurred, the negative and positive controls yielded the expected results. Likewise, in a second series of investigations carried out according to OECD Test Guideline 471 in the same Salmonella strains with preincubation and benzotriazole concentrations of 313 to 5002 µg/plate (purity 99.87%), neither mutagenicity nor cytotoxicity occurred in the presence and absence of a metabolic activation system. The negative and positive controls produced the expected results (Connect Chemicals GmbH 2012 a).

Tab. 2 Gene mutation tests with benzotriazole in vitro

Test system	Concentration	Cytotoxicity	Effective concentration	Result		Remarks	References
				–m. a.	+m. a.		
S. typhimurium TA98, TA100, TA1537, TA1538	1.0–1000 µg/plate	no data	–	–	–	purity > 99%	Dunkel and Simmon 1980
TA1535	1.0–1000 µg/plate	no data	1000 µg/plate (+m. a.)	–	+		
S. typhimurium TA98, TA100, TA1538	0.3–10 000 µg/plate	at 3333 µg/plate and above	–	–	–	purity > 99%	Dunkel et al. 1985
TA1535	0.3–10 000 µg/plate	at 10 000 µg/plate and above	10 000 µg/plate (–m. a.) 333, 1000, 3333, 10 000 µg/plate (+m. a.)	+	+		
E. coli WP2 uvrA	0.3–10 000 µg/plate	at 10 000 µg/plate and above	333, 1000, 3333 µg/plate (–m. a.) 33, 100, 333, 1000, 3333 µg/plate (+m. a.)	+	+		
S. typhimurium TA97, TA98, TA100	33–1666 µg/plate	at 1666 µg/plate and above	–	–	–	purity 99%	Zeiger et al. 1987
TA1535	33–1666 µg/plate	at 1666 µg/plate and above	333, 1000, 1666 µg/plate	–	+		
S. typhimurium TA98, TA100, TA1535, TA1537	25–2025 and 444–2250 µg/plate	no data	–	–	–	purity about 100%	Ciba-Geigy Limited 1982 a
TA1535	25–2025 and 444–2250 µg/plate	no data	2025 and 667, 1500 µg/plate (–m. a.) 2025 and 1500 µg/plate (+m. a.)	+	+		
S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	444–2250 and 500–8000 µg/plate	no data	–	–	–	purity about 100%	Ciba-Geigy Limited 1982 b
TA1535	444–2250 and 500–8000 µg/plate	no data	1000, 1500 µg/plate (–m. a.) 444, 667, 1000, 1500, 2250 and 2000 µg/plate (+m. a.)	+	+		

Tab. 2 *(continued)*

Test system	Concentration	Cytotoxicity	Effective concentration	Result		Remarks	References
				–m. a.	+m. a.		
S. typhimurium TA100	25–2025 and 50–4050 µg/plate	at 4050 µg/plate and above	–	–	–	purity unknown, study questionably valid	Ciba-Geigy Limited 1982 c
TA98	25–2025 and 50–4050 µg/plate	at 4050 µg/plate and above	75, 225, 675, 2025 and 50, 150, 450, 1350 µg/plate (–m. a.) 2025 and 1350, 4050 µg/plate (+m. a.)	+	+		
TA1535	25–2025 and 50–4050 µg/plate	at 4050 µg/plate and above	2025 and 450, 1350 µg/plate (–m. a.) 675, 2025 and 1350 µg/plate (+m. a.)	+	+		
TA1537	25–2025 and 50–4050 µg/plate	at 4050 µg/plate and above	75, 225, 675, 2025 and 50, 150, 450, 1350 µg/plate (–m. a.) 675, 2025 and 150, 1350, 4050 µg/plate (+m. a.)	+	+		
TA1538	25–2025 and 50–4050 µg/plate	at 4050 µg/plate and above	50, 150, 450, 1350 µg/plate (–m. a.) 150, 450, 1350, 4050 µg/plate (+m. a.)	+	+		
S. typhimurium TA100	250–4000 µg/plate	no data	–	–	–	purity unknown, study questionably valid	Ciba-Geigy Limited 1982 d
TA98	250–4000 µg/plate	no data	250, 500, 1000, 2000, 4000 µg/plate (–m. a.) 250, 500, 1000, 2000, 4000 µg/plate (+m. a.)	+	+		
TA1535	250–4000 µg/plate	at 4000 µg/plate and above	250, 500, 1000, 2000, 4000 µg/plate (–m. a.) 500, 1000, 2000 µg/plate (+m. a.)	+	+		
TA1537	250–4000 µg/plate	no data	250, 500, 1000, 2000, 4000 µg/plate (–m. a.) 250, 500, 1000, 2000, 4000 µg/plate (+m. a.)	+	+		
TA1538	250–4000 µg/plate	no data	250, 500, 1000, 2000, 4000 µg/plate (–m. a.) 250, 500, 1000, 2000, 4000 µg/plate (+m. a.)	+	+		
S. typhimurium TA97a, TA98, TA100, TA102, TA1535	50–5003 and 313–5002 µg/plate	not detected	–	–	–	purity 99.87%, according to OECD Test Guideline 471	Connect Chemicals GmbH 2012 a
HPRT locus in CHO cells	50–1000 µg/ml	no data	–	–	–	according to OECD Test Guideline 476	Bayer AG 1987 b

CHO: Chinese hamster ovary cells; +m. a.: with metabolic activation; –m. a.: without metabolic activation

The results of mutagenicity studies in mammalian cells (HPRT locus in Chinese hamster ovary cells) were negative (Bayer AG 1987 b).

5.6.2 In vivo

In a dose-finding study for a micronucleus test in erythroblasts from the bone marrow of male and female NMRI mice, single benzotriazole doses of 0, 500, 750, 850 or 1000 mg/kg body weight (purity 99.83%) were administered. Three of 5 animals in the high dose group and 2 of 5 animals in the 850 mg/kg group died. The following clinical signs were found: apathy, reduced motility, abdominal position, convulsions and rapid breathing. For the main study, a dose of 800 mg benzotriazole/kg body weight in polyethylene glycol 400 (PEG 400) was selected. After single gavage doses, benzotriazole was not clastogenic after 24, 48 and 72 hours. Over a period of 6 hours after administration, most animals were found to have the same clinical signs that were observed in the dose-finding study. One of the 40 animals treated with benzotriazole died after 24 hours. The ratio between polychromatic and normochromatic erythrocytes was unchanged. The animals given 20 mg cyclophosphamide/kg body weight as a positive control or only PEG 400 as a negative control yielded the expected results (Bayer AG 1988).

Conclusions

In earlier studies, mutagenic effects were sometimes described in bacterial test systems (S. typhimurium, E. coli) for benzotriazole. Since the purity of the substance was not specified in some of these studies with S. typhimurium, their validity is questionable. In all other studies with S. typhimurium that yielded positive results, mutagenic effects were found only in strain TA1535, but not in strain TA100; this indicates weak mutagenicity. On the other hand, in a valid new study in accordance with OECD Test Guideline 471, which included also the S. typhimurium strains TA1535 and TA100, benzotriazole was not found to be mutagenic. In addition, there was no increase in HPRT mutations in mammalian cells (Chinese hamster ovary cells). All in all, the Commission regards benzotriazole as not mutagenic in vitro.

In an in vivo study in erythrocytes from the bone marrow of mice, benzotriazole was not clastogenic after a single oral dose of 800 mg/kg body weight.

In summary, there is no evidence from the available studies that benzotriazole has genotoxic potential.

5.7 Carcinogenicity

In the documentation of 1988 (Henschler 1991), a cell transformation test, a very concisely described carcinogenicity study published in Russian, and a second carcinogenicity study by the NCI dating from 1978 are cited. New data for the end point carcinogenicity are not available, however, the results of the NCI study are re-evaluated: In this study, male and female rats (Fischer 344, 50 animals/group) were given 6700 or 12 100 mg benzotriazole/kg diet (doses of about 335 or 605 mg/kg body weight and day, conversion factor 0.05 according to EFSA (2012)) on 7 days per week, for 78 weeks. The animals were observed for 104 to 106 weeks. Male and female mice (B6C3F1, 50 animals/group) were fed with 11 700 or 23 500 mg benzotriazole/kg diet (doses of about 1755 or 3525 mg/kg body weight and day, conversion factor 0.15 according to EFSA (2012)) on 7 days per week, for 104 weeks. The animals were observed for 106 to 109 weeks. The results are given in Tables 3 and 4.

Tab. 3 Studies of the carcinogenicity of benzotriazole in the B6C3F1 mouse (NCI 1978)

Author:		NCI 1978		
Substance:		benzotriazole		
Species:		**mouse**, B6C3F1, 50 ♂, 50 ♀ per group		
Administration route:		oral (with diet)		
Dose:		0, 1755, 3525 mg/kg body weight and day		
Duration:		104 weeks, 7 days/week		
		dose (mg/kg body weight and day)		
		0	1755	3525
Lungs:				
alveolar/bronchiolar carcinomas	♂	2/39 (5%)	5/43 (12%)	5/46 (11%)
	♀	0/49	9/49 (18%)*a)	3/49 (6%)
alveolar/bronchiolar adenomas	♂	2/39 (5%)	2/43 (5%)	0/46
	♀	0/49	1/49 (2%)	1/49 (2%)
alveolar/bronchiolar carcinomas or adenomas	♂	4/39 (10%)	7/43 (17%)	4/49 (8%)
	♀	0/49	10/49 (20%)*	5/46 (11%)

*p = 0.001; a) historical controls from the same laboratory: 0–7%, with a mean value of 4%

Tab. 4 Studies of the carcinogenicity of benzotriazole in the F344 rat (NCI 1978)

Author:		NCI 1978		
Substance:		benzotriazole		
Species:		**rat**, Fischer 344, 50 ♂, 50 ♀ per group		
Administration route:		oral (with diet)		
Dose:		0, 335, 605 mg/kg body weight and day		
Duration:		104 weeks, 7 days/week		
		dose (mg/kg body weight and day)		
		0	335	605
Liver:				
neoplastic nodules	♂	0/48	0/46	5/45 (11%)*a)
	♀	0/50	0/48	2/50 (4%)
Brain:				
gliomas	♂	0/46	2/44 (5%)	0/46
	♀	0/50	0/47	1/50 (2%)
oligodendrogliomas	♂	0/46	1/44 (2%)	0/46
	♀	0/50	0/47	0/50

Tab. 4 *(continued)*

Uterus:				
endometrial stromal polyps	♀	2/48 (4%)	10/45 (22%)**	8/50 (16%)
endometrial stromal sarcomas	♀	2/48 (4%)	0/45	2/50 (4%)
Thyroid gland:				
follicular carcinomas	♂	0/43	0/40	1/44 (2%)
	♀	0/43	0/43	1/50 (2%)
C-cell adenomas	♂	3/43 (7%)	0/40	1/44 (2%)
	♀	0/43	4/43 (9%)	0/50
C-cell carcinomas	♂	2/43 (5%)	1/40 (3%)	1/44 (2%)
	♀	0/43	1/43 (2%)	3/50 (6%)

*p = 0.024; **p = 0.010

[a)] historical controls from the same laboratory: 0%–11%, with 2/13 historical control groups with incidences of 10%–11%

A statistically significant increase in alveolar/bronchiolar carcinomas in female mice with an incidence of 18% (9/49 animals) was found only in the low dose group of 1755 mg/kg body weight and day. When the incidence of alveolar/bronchiolar adenomas and carcinomas are combined, a statistically significant increase was again found only in low dose females.

In male rats, the 11% increase in the incidence of neoplastic nodules in the liver (5/45 animals compared with 0/48 controls) was statistically significant at 605 mg/kg body weight. In the uterus of the female rats, the increase in the incidence of endometrial stromal polyps was statistically significant with an incidence of 22% (10/45 animals compared with 2/48 controls) in the low dose group of 335 mg/kg body weight and day. The incidences of endometrial stromal sarcomas and also the combined incidences of endometrial stromal polyps and sarcomas were not statistically significantly different from those in the controls. In the females, slight increases in thyroid gland C-cell adenomas (9%, 4/43 animals) in the low dose group and C-cell carcinomas in the low dose (2%, 1/43 animals) and high dose group (6%, 3/50 animals) were without statistical significance. In the brain, there were two gliomas (2/44) in male rats of the low dose group and one in a female rat (1/50) of the high dose group, as well as one oligodendroglioma in male rats (1/44) of the low dose group. A more precise classification of the gliomas or the oligodendroglioma was not given. There were no brain tumours in the female and male rats of the control group (Henschler 1991; NCI 1978).

Due to the high spontaneous incidence of tumours in the strain used (up to 7% of all animals in historical controls) and the very high doses administered, the increase in alveolar/bronchiolar carcinomas in the female mice of the low dose group does not represent clear evidence of the carcinogenicity of benzotriazole. Also the neoplastic nodules found in the liver of male rats are, with an incidence of 11%, at the level of historical control data and can therefore not be clearly attributed to benzotriazole. In the uterus of rats, the increase in the incidence of endometrial stromal polyps, but not that of sarcomas, was statistically significant. The low incidences of C-cell adenomas and C-cell carcinomas in the thyroid gland of treated female rats were not statistically significant, for which reason they are to be regarded as questionably substance-related. All in all, the neoplasms in the liver, uterus and thyroid gland of the rats and in the lungs of the mice do not represent clear evidence of a carcinogenic effect of benzotriazole, and may be a highly variable chance finding.

However, the occurrence of brain tumours is to be regarded as critical. In a publication by Sills et al. (1999), 500 substances were compared that had been investigated as regards their carcinogenicity in the National Toxicology Program. Only in the case of 10 substances were neoplastic effects in the brain found, and the incidence of gliomas and oligodendrogliomas in male and female Fischer 344 rats was between 0% and 2%. Although the incidence of brain tumours after the administration of benzotriazole was low and without statistical significance and occurred mainly

in the low dose group, this finding needs to be given special attention due to the rare occurrence of neoplasms in the brain.

Taking all the data into account, there were neoplasms in different organs of two species and in both sexes; particularly in the case of the brain tumours a substance-specific effect cannot be excluded. Benzotriazole is thus a suspected carcinogen at high doses.

6 Manifesto (MAK value/classification)

The critical effects of benzotriazole are the occurrences of three gliomas and one oligodendroglioma in the brain of rats in a long-term oral study, which are possibly substance-related even if not statistically significant, and the irritation in the rabbit eye.

MAK value and peak limitation. There are no data in humans available that are suitable for the derivation of a MAK value.

A gavage study carried out according to OECD Test Guideline 421 in Wistar rats yielded a NOAEL of 12.5 mg benzotriazole/kg body weight if the occasional haemorrhage of the mucous membranes and the salivation occurring in some animals at and above 50 mg/kg body weight and day are regarded as substance-related effects. This NOAEL is far below the dose of 335 mg/kg body weight and day, at which an increase in brain tumours that was not statistically significant was found in rats, though not in mice. The following toxicokinetic data are taken into consideration for the extrapolation of the NOAEL of 12.5 mg benzotriazole/kg body weight and day to a concentration in workplace air: a possible increase of the effects with time (1 : 4 because the exposure duration was between subacute and subchronic), the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7 : 5), the species-specific correction value for the rat (1 : 4), the assumed oral absorption (100%), the body weight (70 kg) and respiratory volume (10 m^3) of the person, the assumed 100% absorption by inhalation and the extrapolation of the data from an animal experiment to humans (1 : 2). The concentration calculated from this is 3.8 mg/m^3. In view of the irritant effects of benzotriazole in the rabbit eye, it is, however, unclear whether this concentration would still lead to irritation. The structurally related methyl-1*H*-benzotriazole has an RD_{50} of 205 mg/m^3 for pulmonary irritation. From this, a threshold value of 3.4 mg/m^3 would be calculated. However, this procedure was not used to derive the MAK value for methyl-1*H*-benzotriazole, as there are too many uncertainties (Hartwig 2011). Accordingly, the data for methyl-1*H*-benzotriazole cannot be used to derive a MAK value for benzotriazole. A longer-term inhalation study is not available, therefore, the irritation potential of benzotriazole after inhalation cannot be assessed and a MAK value cannot be derived. Peak limitation is thus not applicable.

Prenatal toxicity. The available data for reproductive toxicity revealed reduced body weights in the offspring of rats on postnatal days 0 and 4 after oral administration of 300 mg benzotriazole/kg body weight and day (ECHA 2017). Teratogenic effects were not investigated. As no MAK value has been established for benzotriazole, the substance has not been assigned to a Pregnancy Risk Group.

Carcinogenicity. In a carcinogenicity study (NCI 1978) with high doses, alveolar/bronchiolar carcinomas and adenomas were found in mice and rats developed neoplastic nodules in the liver, endometrial stromal polyps and sarcomas in the uterus, C-cell adenomas and carcinomas in the thyroid gland, as well as two gliomas in males (2/44), one in females (1/50), and one oligodendroglioma in males (1/44). The neoplasms in the brain are particularly critical effects. The historical control data for these tumours indicate a very low spontaneous incidence of 0% to 2% in the strain of rat used (Sills et al. 1999). Taking all data into account, a suspected carcinogenicity of benzotriazole at high doses cannot be excluded, especially as findings were obtained in a number of organs and in both sexes of two species. In particular, the sporadic occurrence of brain tumours may be a substance-specific effect. Therefore, benzotriazole is classified in Carcinogen Category 3 B.

Germ cell mutagenicity. Earlier studies described mutagenic effects of benzotriazole in bacterial test systems (Salmonella typhimurium, Escherichia coli) which have not been confirmed in recent studies. In addition, no in-

crease in HPRT mutations in mammalian cells (Chinese hamster ovary cells) was observed. Benzotriazole is therefore regarded as not mutagenic in vitro. In vivo, no clastogenic effects in the form of micronuclei were found in erythrocytes from the bone marrow of mice. Studies with germ cells are not available. A germ cell mutagenic potential is not suspected, and benzotriazole is, therefore, not classified in one of the categories for germ cell mutagens.

Absorption through the skin. Model calculations of dermal absorption yielded values of 120 to 1340 mg for a saturated aqueous solution or 30 to 335 mg for 0.5% benzotriazole as used in a metal-working fluid preparation. The systemic NOAEL for rats in a study according to OECD Test Guideline 421 with daily oral administration was 12.5 mg/kg body weight. In humans, this dose corresponds to a concentration of 3.8 mg/m^3 at the workplace (see above) and, assuming 100% absorption by inhalation and a respiratory volume of 10 m^3, to a systemically tolerable amount of 38 mg. The calculated dermal absorption is considerably above this value both for saturated solutions and for metal-working fluid preparations, so that benzotriazole is designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. Only very few questionably reliable clinical findings in humans are available for skin sensitization caused by benzotriazole. Contact sensitization cannot be deduced from experimental studies with animals. Findings on respiratory sensitization are not available. Benzotriazole is therefore not designated with "Sh" (for substances which cause sensitization of the skin) or "Sa" (for substances which cause sensitization of the airways).

References

Angelini G, Meneghini CL (1977) Dermatitis in engineers due to synthetic coolants. Contact Dermatitis 3: 219–220. DOI: 10.1111/j.1600-0536.1977.tb03662.x

Bayer AG (1987 a) Benzotriazol. Untersuchungen zum Reiz-/Ätzpotential an der Haut und am Auge (Kaninchen). Bayer AG, Fachbereich Toxikologie, Report No. 15529, Bayer AG, Wuppertal, unpublished

Bayer AG (1987 b) Benzotriazole Granulat in the CHO HPRT forward mutation assay. Hazleton Biotechnologies, Veenendaal, Study number: E-9553-0-435, Bayer AG, Wuppertal, unpublished

Bayer AG (1988) Micronucleus test on the mouse to evaluate for clastogenic effects. Bayer AG, Fachbereich Toxikologie, Study number: T8024075, Bayer AG, Wuppertal, unpublished

de Boer EM, van Ketel WG, Bruynzeel DP (1989) Dermatoses in metal workers. Contact Dermatitis 20: 280–286. DOI: 10.1111/j.1600-0536.1989.tb03146.x

Ciba-Geigy Limited (1982 a) Salmonella/mammalian-microsome mutagenicity test with TK 11'237. NTIS/OTS0538210. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0538210.xhtml, accessed 29 May 2017

Ciba-Geigy Limited (1982 b) Salmonella/mammalian-microsome mutagenicity test with TK 11'237. NTIS/OTS0538209. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0538209.xhtml, accessed 29 May 2017

Ciba-Geigy Limited (1982 c) Salmonella/mammalian-microsome mutagenicity test with TK 10'637. NTIS/OTS0538213. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0538213.xhtml, accessed 29 May 2017

Ciba-Geigy Limited (1982 d) Salmonella/mammalian-microsome mutagenicity test with TK 10'637. NTIS/OTS0538214. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0538214.xhtml, accessed 29 May 2017

Ciba-Geigy Limited (1982 e) Report on skin irritation in the rabbit after single application of TK 11'237. NTIS/OTS0538206. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0538206.xhtml, accessed 29 May 2017

Ciba-Geigy Limited (1982 f) Report on skin irritation in the rabbit after single application of TK 10'367. NTIS/OTS0538211. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0538211.xhtml, accessed 29 May 2017

Connect Chemicals GmbH (2012 a) Determination of the mutagenic potential of 1,2,3-benzotriazole-REACH 01 with bacterial reverse mutation test following OECD 471 and EU B.13/14. LAUS GmbH, Kirrweiler, Study number: 12020701G803, Connect Chemicals GmbH, Ratingen, unpublished

Connect Chemicals GmbH (2012 b) 1,2,3-Benzotriazole-REACH 01. Evaluation of acute oral toxicity in rats – acute toxic class method. Phycher Bio Development, Cestas, Study number: TAO423-PH-12/0094, Connect Chemicals GmbH, Ratingen, unpublished

Connect Chemicals GmbH (2012 c) Toxicity study of 1,2,3-benzotriazole-REACH 01 for a reproduction/developmental toxicity screening test (OECD 421). Vivo Science GmbH, Study report L09-019, Connect Chemicals GmbH, Ratingen, unpublished

Dassano A, Colombo F, Trincucci G, Frullanti E, Galvan A, Pettinicchio A, De Cecco L, Borrego A, Martinez Ibanez OC, Dragani TA, Manenti G (2014) Mouse pulmonary adenoma susceptibility 1 locus is an expression QTL modulating Kras-4d. PLoS Genet 10: e1004307. DOI: 10.1371/journal.pgen.1004307

Ducombs G, Tamisier JM, Texier L (1980) Contact dermatitis to 1-H benzotriazole. Contact Dermatitis 6: 224–225. DOI: 10.1111/j.1600-0536.1980.tb05612.x

Dunkel VC, Simmon VF (1980) Mutagenic activity of chemicals previously tested for carcinogenicity in the National Cancer Institute bioassay program. In: Montesano R, Bartsch H, Tomatis L (eds) Molecular and cellular aspects of carcinogen screening tests, IARC Scientific Publications No 27. IARC, Lyon, 283–302

Dunkel VC, Zeiger E, Brusick D, McCoy E, McGregor D, Mortelmans K, Rosenkranz HS, Simmon VF (1985) Reproducibility of microbial mutagenicity assays: II. Testing of carcinogens and noncarcinogens in Salmonella typhimurium and Escherichia coli. Environ Mutagen 7 Suppl 5: 1–248. DOI: 10.1002/em.2860070902

Domino EF, Unna KR, Kerwin J (1952) Pharmacological properties of benzazoles. I. Relationship between structure and paralyzing action. J Pharmacol Exp Ther 105: 486–497

Eastman Kodak Company (1983) Basic toxicity of benzotriazole. NTIS/OTS0516745, EPA/OTS Doc ID 86-890000208. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0516745.xhtml, accessed 18 May 2017

ECHA (European Chemicals Agency) (2017) Information on registered substances. Dataset on benzotriazole (CAS Number 95-14-7), joint submission, first publication 25 Jun 2013, last modification 14 Aug 2017. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14234, accessed 26 Oct 2017

EFSA (European Food Safety Authority) (2012) Guidance on selected default values to be used by the EFSA Scientific Committee, Scientific Panels and Units in the absence of actual measured data. EFSA J 10: 2579. DOI: 10.2903/j.efsa.2012.2579

Fiserova-Bergerova V, Pierce JT, Droz PO (1990) Dermal absorption potential of industrial chemicals: criteria for skin notation. Am J Ind Med 17: 617–635. DOI: 10.1002/ajim.4700170507

Geier J, Lessmann H, Frosch PJ, Pirker C, Koch P, Aschoff R, Richter G, Becker D, Eckert C, Uter W, Schnuch A, Fuchs T (2003) Patch testing with components of water-based metalworking fluids. Contact Dermatitis 49: 85–90. DOI: 10.1111/j.0105-1873.2003.00187.x

Geier J, Lessmann H, Dickel H, Frosch PJ, Koch P, Becker D, Jappe U, Aberer W, Schnuch A, Uter W (2004) Patch test results with the metalworking fluid series of the German Contact Dermatitis Research Group (DKG). Contact Dermatitis 51: 118–130. DOI: 10.1111/j.0105-1873.2004.00416.x

Geier J, Lessmann H, Becker D, Bruze M, Frosch PJ, Fuchs T, Jappe U, Koch P, Pföhler C, Skudlik C (2006) Patch testing with components of water-based metalworking fluids: results of a multicentre study with a second series. Contact Dermatitis 55: 322–329. DOI: 10.1111/j.1600-0536.2006.00993.x

Goossens A, Claes L, Drieghe J, Put E (1998) Antimicrobials: preservatives, antiseptics and disinfectants. Contact Dermatitis 39: 133–134. DOI: 10.1111/j.1600-0536.1998.tb05864.x

Gruvberger B, Isaksson M, Frick M, Pontén ANN, Bruze M (2003) Occupational dermatoses in a metalworking plant. Contact Dermatitis 48: 80–86. DOI: 10.1034/j.1600-0536.2003.480205.x

Guy RH, Potts RO (1993) Penetration of industrial chemicals across the skin: a predictive model. Am J Ind Med 23: 711–719. DOI: 10.1002/ajim.4700230505

Hartwig A (ed) (2011) Methyl-1H-benzotriazol. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten, 50. Lieferung, Wiley-VCH, Weinheim. Also available from DOI: 10.1002/3527600418.mb2938543kskd0050

Hartwig A (ed) (2014) Komponenten von Kühlschmierstoffen, Hydraulikflüssigkeiten und anderen Schmierstoffen. In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werten, 56. Lieferung, Wiley-VCH, Weinheim. Also available from DOI: 10.1002/3527600418.mb0215khsd0056

Henschler D (ed) (1991) 1H-Benzotriazole. MAK Value Documentation, 1988. In: Occupational Toxicants, vol 2. VCH, Weinheim, 231–237. Also available from DOI: 10.1002/3527600418.mb9514kske0002

Holden CR, Gawkrodger DJ (2005) 10 years' experience of patch testing with a shoe series in 230 patients: which allergens are important? Contact Dermatitis 53: 37–39. DOI: 10.1111/j.0105-1873.2005.00631.x

Katugampola RP, Statham BN, English JSC, Wilkinson MM, Foulds IS, Green CM, Ormerod AD, Stone NM, Horne HL, Chowdhury MM (2005) A multicenter review of the footwear allergens tested in the UK. Contact Dermatitis 53: 133–135. DOI: 10.1111/j.0105-1873.2005.00662.x

Malkinson AM (1989) The genetic basis of susceptibility to lung tumours in mice. Toxicology 54: 241–271. DOI: 10.1016/0300-483x(89)90062-0

Maurer T, Meier F (1984) Sensitization potential of benzotriazole. Contact Dermatitis 10: 163–165. DOI: 10.1111/j.1600-0536.1984.tb00024.x

Meding B, Barregård L, Marcus K (1994) Hand eczema in car mechanics. Contact Dermatitis 30: 129–134. DOI: 10.1111/j.1600-0536.1994.tb00692.x

NCI (National Cancer Institute) (1978) Bioassay of 1H-benzotriazole for possible carcinogenicity, CAS No 95-14-7. TR 88. NCI, Bethesda, MD. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr088.pdf, accessed 17 May 2017

NCBI (National Center for Biotechnology Information) (2020) PubChem Database. 1,2,3-Benzotriazole, Source = HSDB. https://pubchem.ncbi.nlm.nih.gov/source/hsdb/4143, accessed 05 May 2020

Polaroid Corporation (1965) Letter from the Polaroid Corporation regarding the enclosed results of the acute toxicity, corneal and dermal irritation studies on 1H-benzotriazole with attachments. NTIS/OTS0520182, EPA/OTS Doc ID 86-890001039. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0520182.xhtml, accessed 18 May 2017

Prival M, Zeiger E (1998) Chemicals mutagenic in Salmonella typhimurium strain TA1535 but not in TA100. Mutat Res 412: 251–260. DOI: 10.1016/s1383-5718(97)00196-4

Rinaldi M, Caffo M, Minutoli L, Marini H, Abbritti RV, Squadrito F, Trichilo V, Valenti A, Barresi V, Altavilla D, Passalacqua M, Caruso G (2016) ROS and brain gliomas: an overview of potential and innovative therapeutic strategies. Int J Mol Sci 17: 984. DOI: 10.3390/ijms17060984

Sherwin-Williams Company (1969) Acute dermal toxicity and eye irritation studies on sample Bt-d, sample #SD-7413 in rabbits. NTIS/OTS0520633, EPA/OTS Doc ID 86-890000594. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0520633.xhtml, accessed 18 May 2017

Sherwin-Williams Company (1972 a) Acute dermal toxicity investigation in rabbits of Cobratec 99 (BT) sample #6023. NTIS/OTS0520634, EPA/OTS Doc ID 86-890000595. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0520634.xhtml, accessed 31 May 2017

Sherwin-Williams Company (1972 b) Primary skin irritation study on the rabbits of Cobratec 99 (BT) sample #6023. NTIS/OTS0520635, EPA/OTS Doc ID 86-890000596. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0520635.xhtml, accessed 31 May 2017

Sherwin-Williams Company (1974) Eye irritation study in the rabbit of benzotriazole (Cobratec99) sample #1088. NTIS/OTS0520636, EPA/OTS Doc ID 86-890000597. NTIS, Alexandria, VA. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0520636.xhtml, accessed 31 May 2017

Sills RC, Hailey JR, Neal J, Boorman GA, Haseman JK, Melnick RL (1999) Examination of low-incidence brain tumour responses in F344 rats following chemical exposures in National Toxicology Program carcinogenicity studies. Toxicol Pathol 27: 589–599. DOI: 10.1177/019262339902700513

US EPA (US Environmental Protection Agency) (1977) Investigation of selected potential environmental contaminants: Benzotriazoles. US EPA, Washington, DC

Wilschut A, ten Berge WF, Robinson PJ, McKone TE (1995) Estimating skin permeation. The validation of five mathematical skin permeation models. Chemosphere 30: 1275–1296. DOI: 10.1016/0045-6535(95)00023-2

Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K, Speck W (1987) Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals. Environ Mutagen 9 Suppl 9: 1–109. DOI: 10.1002/em.2860090602