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of Health Risks from Chemicals and The Dutch Expert
Committee on Occupational Standards

126. 1,2,3-Benzotriazole

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Preface

An agreement has been signed by the Dutch Expert Committee on Occupational Standards (DECOS) of the Health Council of the Netherlands and the Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals (NEG). The purpose of the agreement is to write joint scientific criteria documents which could be used by the national regulatory authorities in both the Netherlands and in the Nordic Countries.

The document on health effects of 1,2,3-benzotriazole was written by H. Stouten, A.A.J.J.L Rutten, I.A. van de Gevel, and F. De Vrijer from the Toxicology Division of TNO Nutrition and Food Research, Zeist, the Netherlands and has been reviewed by DECOS as well as by NEG.

The document has been adapted to the different formats used by DECOS and NEG.

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Abbreviations

bw	body weight
h	hour
CI	confidence interval
LC ₅₀	lethal concentration for 50% of the exposed animals
LC ₁₀	lowest lethal concentration
LD ₅₀	lethal dose for 50% of the exposed animals
LD ₁₀	lowest lethal dose
LOAEL	lowest observed adverse effect level
NOAEL	no observed adverse effect level
ppm	parts per million (v/v)10 ⁻⁶ , cm ³ /m ³
UV	ultraviolet

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1. Introduction

Benzotriazole is an odourless, white to tannish crystalline powder. It is sparingly soluble in water and soluble in a number of organic solvents. It has a low vapour pressure, and it is therefore likely to occur as dust at the workplace. When finely divided and mixed with air, dust explosions may occur.

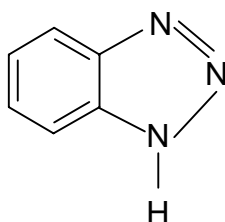
Benzotriazole is an industrial compound primarily used as a corrosion inhibitor, as a plastic stabiliser, and as a chemical intermediate.

In the present report, the consequences of occupational exposure to 1,2,3-benzotriazole (further referred to as benzotriazole) are discussed.

2. Identity, properties and monitoring

2.1 Identity

2.1.1 Structure



2.1.2 Chemical names and synonyms/registry numbers

Name	benzotriazole
CAS registry number	95-14-7
CAS index name	1 <i>H</i> -benzotriazole
Synonyms	1,2,3-benzotriazole; benzisotriazole; benztriazole; 1,2-amino-azophenylene; azimidobenzene; aziminobenzene; benzene azimide; 2,3-diazaindole; 1,2,3-triaza-1 <i>H</i> -indene; 1,2,3-triazaindene; benzene azimide; 2,3- diazaindole
EINECS No.	202-394-1
RTECS No.	DM1225000

2.2 Physical and chemical properties (5, 12, 18, 27)

Molecular formula	C ₆ H ₅ N ₃
Molecular weight	119.14
Boiling point (101.3 kPa)	350°C
Melting point (101.3 kPa)	99°C
Relative density (water = 1)	1.4
Vapour density (air = 1)	4.1
Vapour pressure (20°C; 101.3 kPa)	<0.01 kPa
Relative density of saturated vapour/air mixture (air = 1; 20°C)	1.00
Auto-ignition temperature	400°C
Explosive limits (% , in air)	2.4-?
Solubility in water (25°C)	2.0 g/100 ml
Solubility in organic solvents	soluble in alcohol, benzene, toluene, chloroform, dimethylformamide
Partition coefficient (Log P octanol/water)	1.44 (experimental value)
Physical form	white to tan, crystalline powder; needles when crystallised from benzene
Odour	odourless

Benzotriazole exists in two tautomeric forms. The first tautomer has the formula *1H*-benzotriazole. The second tautomer has the formula *2H*-benzotriazole and is also referred to as pseudo-azimidobenzene or 2,1,3-benzotriazole. The *1H*-tautomer represents the more stable and essentially exclusive molecular structure (12).

Dust explosions can occur when finely divided powder is mixed with air. It can explode when heated and during vacuum distillation. When heated or combusted, benzotriazole decomposes into toxic vapours (nitrogen dioxide). It is very stable toward acids and alkalis, and toward oxidation and reduction.

2.3 Validated analytical methods

2.3.1 Environmental monitoring

No method for monitoring benzotriazole in air is available. However, since benzotriazole will hardly vaporise at room temperature, it will be present as dust, and general dust measurement methods should be applicable. The benzotriazole content in the dust may be assessed by analytical-chemical methods after extraction with a suitable solvent. In view of the specificity and the height of the detection limit, UV-spectrophotometry, high performance liquid chromatography, or gas chromatography-mass spectrometry may be most appropriate (see section 2.3.2).

2.3.2 Biological monitoring

No method for the determination of benzotriazole in biological samples was found.

Gas chromatography-mass spectrometry, high performance liquid chromatography, and spectrophotometric methods for the determination of benzotriazole in aqueous solutions have been published (12, 19).

No validated method for biological monitoring of workers exposed to benzotriazole was found.

3. Sources

3.1 Natural occurrence

No data available.

3.2 Man-made sources

3.2.1 Production

Benzotriazole is produced by reaction of *o*-phenylenediamine with nitrous acid in the presence of glacial acetic acid or by reaction of hydrochloric acid or nitrous acid with *o*-phenylenediamine. The production of benzotriazole in the US was mentioned to be probably higher than approximately 7 and 4.5 tonnes in 1977 and 1979, respectively (27).

3.2.2 Uses

Benzotriazole is used as a corrosion inhibitor, as a plastic stabiliser, and as a chemical intermediate for dyes, pharmaceuticals, and fungicides. Derivates of benzotriazole are used as UV absorbers and as restrainers in photographic emulsions (27).

Benzotriazole is used in metalworking and art restoration as an anticorrosive, and in the construction industry as a tarnish remover and a protective coating of metal. It functions as a corrosion inhibitor in water cooling systems such as automobile radiators and boilers, and in dry cleaning equipment. It is included in some formulations of automatic dishwasher detergents to prevent tarnishing of metal pots and silverware, and to inhibit the corrosion of metal machine parts. Benzotriazole is used in synthetic greases, lubricants, and hydraulic fluids to prevent the oxidation of these materials, which is catalysed by metal ions. In the electronics industry, it is used to treat packing materials for copper electronic parts, and to extend the life of polymers that are used as insulators for copper wire. Furthermore, benzotriazole is used in electrolytic processing, where the stripping of metals from copper cathodes is eased by pre-treatment of the cathode with benzotriazole. In photographic processing, benzotriazole acts as an anti-fogging agent in silver-halide emulsions, restraining the developer and preventing the blackening or fogging of the image due to overdevelopment (25).

Coolant lubricants and corrosion inhibiting fluids may contain up to 0.05% benzotriazole (17, 24).

4. Exposure

4.1 General population

No data available.

4.2 Working population

No data available.

5. Toxicokinetics

There is very little information found with respect to the toxicokinetics of benzotriazole.

5.1 Absorption

Based on its molecular weight (119.14) and its partition coefficient (log P octanol/water), dermal absorption might be expected.

Because benzotriazole is a weak base ($pK_b=1.6$) as well as a weak acid ($pK_a=8.57$) (1), a low degree of ionisation of benzotriazole can be predicted at the physiological pHs of stomach, intestine, and blood, suggesting that it would easily pass the respective membranes. It is, however, noticed that other factors than passive diffusion play a role in passing membranes.

5.2 Distribution

No information available.

5.3 Biotransformation

Benzotriazole was incubated for one hour with a microsome suspension obtained from phenobarbital-induced rat livers at a final concentration in the incubation solution of 0.2 mg/ml. During this one-hour incubation period, the overall metabolism of benzotriazole was relatively low (<5% of the amount added to incubation mixture), and the 5-hydroxy metabolite of benzotriazole was formed 4-5 times more than 4-hydroxybenzotriazole (1.6 vs. 0.32% of the amount added) (22).

5.4 Excretion

No information available.

5.5 Biological monitoring

No information available.

5.6 Summary

No information was found on distribution, excretion, and biological monitoring. Based on physico-chemical data (molecular weight, partition coefficient), dermal absorption might be expected. *In vitro*, using a rat liver microsomal suspension and an incubation period of one hour, benzotriazole is metabolised to 4- and 5-hydroxybenzotriazole at a low rate.

6. Effects

6.1 Observations in man

6.1.1 Irritation and sensitisation

Four cases of contact dermatitis among workers exposed to benzotriazole-containing industrial oils or greases showed positive reactions (varying from weakly to strongly positive) in a patch test using 2% benzotriazole in petrolatum (14). However, since there were no data on negative controls and an immunological reaction was not ascertained, it is not possible to assess whether the effects were the consequence of irritation or sensitisation.

Forty out of 286 workers from ten Dutch metalworking factories had contact dermatitis of the hands and/or forearms. Benzotriazole was not listed among the compounds that induced contact allergy in eight out of these 40 workers upon patch-testing (4).

In conclusion, based on the available data on humans, an irritating and/or sensitising potential of benzotriazole cannot be excluded.

6.1.2 Toxicity due to experimental or occupational exposure

No data available.

6.2 Animal experiments

6.2.1 Irritation and sensitisation

Eye

Referring to unpublished industrial reports from the mid 1970s, it was stated that 100 mg of benzotriazole instilled into one eye of rabbits (n=6) produced severe irritation effects, amongst others complete corneal opacity and severe chemosis (swelling of lids) in four animals. Immediate washing with water greatly reduced the irritation (2, 12).

In an unpublished study, benzotriazole (granules; "Preventol CI-8"; purity (from (20)): 99.83%) was slightly irritating to the eyes of female albino rabbits (New Zealand White; n=3). The study was performed to corresponding OECD and EU guidelines. When 100 µl of the test substance was instilled into the

rabbit's eye, left there for 24 h before washing out, the following Draize scores were obtained (28):

Scores ^a observed after:	1 h	24 h	48 h	72 h	7 days
cornea					
opacity	1,1,1	1,1,1	1,0,1	0,0,0	0,0,0
iris	1,1,0	1,0,0	1,0,0	0,0,0	0,0,0
conjunctivae					
redness	1,1,1	1,1,2	2,1,2	1,0,1	0,0,0
chemosis	2,2,3	1,2,1	1,0,0	0,0,0	0,0,0
discharge	2,2,2	2,2,0	1,0,0	0,0,0	0,0,0

^a Figures are irritation scores per animal. Grades for scoring range from 0-2 for iris, 0-3 for conjunctival redness and from 0-4 for corneal opacity and conjunctival chemosis and discharge.

Skin

In an unpublished study, benzotriazole (granules; "Preventol CI-8"; purity (from (20)): 99.83%) was not irritating to the skin of female albino rabbits (New Zealand White; n=3). The study was performed to corresponding OECD and EU guidelines. No erythema or oedema - all scores were 0 - was observed when 500 mg test material (vehiculum: water) was applied under semi-occlusive conditions for 4 h to the intact clipped skin (observation times: 1, 24, 48, and 72 h and 7 and 14 days) (28).

Benzotriazole and commercial benzotriazole (purity: unknown) were only minimally irritating (primary irritation index: 1.0, and 0.7, respectively; maximum score: 8.0) when applied to the abraded and intact, clipped skin (under 24-h occlusion) of New Zealand white rabbits (n=3/sex) at a concentration of 50% in polyethylene glycol (PEG 400) and saline (70:30) (6, 9).

Referring to unpublished industrial reports from the mid 1970s, it was mentioned that benzotriazole was mildly irritating to guinea pig skin at a concentration of 50% in ethanol. Furthermore, in a separate experiment, no irritation was reported after 24 and 72 h (no other evaluation time points mentioned) when about 80 mg/cm² (of the pure material) was administered to the intact or abraded skin of rabbits (n=6/group) and covered for 24 h. However, there were signs of irritation, consisting of a well defined but transient erythema observed at experimental day 2, in three out of five rabbits following 24-h covered contact with 2000 mg/kg bw applied to abraded skin (observation period: 14 days) (2, 12).

Sensitisation

In an unpublished study, benzotriazole ("Benzotriazol Granulat"/"Preventol CI-8"; purity: 99.83%) was not a skin sensitiser when tested in male guinea pigs (Winkelmann DHPW; n=20; controls: n=10) using the Magnusson-Kligman maximisation test. The study was performed according to corresponding OECD and EU guidelines (good laboratory practice statements were included). A positive control group treated with formaldehyde was included to demonstrate the sensitivity of the test. Following an intradermal and topical (one week later) induction of a 5 and 25% solution in propylene glycol, respectively, an epidermal

challenge application of a 12% solution, three weeks later, caused a positive reaction in 1/20 animals. This positive response was, however, very weak. It was observed only 24 h after challenge and not confirmed by the 48-h observation. In the control group, a weak positive response was found in 1/10 animals at the 24-h observation (20).

In guinea pigs, the optimisation test showed negative results with purified and commercial (purity: unknown) benzotriazole after intradermal as well as epidermal challenge application. In the maximisation test, negative results were obtained with purified benzotriazole (exact composition not known), but an epidermal challenge application of 30% of commercial benzotriazole caused slight erythemas in 3/20 animals (controls: 0/19) (24). In two other, not published studies (industrial reports from the mid 1970s), it was reported not to be a skin sensitiser when tested in guinea pigs (no experimental details presented) (2).

Conclusion

From data with benzotriazole of known purity, it is concluded that, in experimental animals the compound is slightly irritating to the eyes and, at most, slightly irritating to the skin. In addition, benzotriazole is apparently not a skin sensitiser.

6.2.2 Acute toxicity

When rats (male; n=10/group) were exposed during 3 h to benzotriazole aerosols of 780, 1460, 2030, 2230, and 2710 mg/m³ (no data on particle size, particle size distribution humidity, etc), mortality was 10%, 20%, 50%, and 100%, respectively. Almost all animals died during the exposure period, usually during the first half-hours, and showed severe accumulation of white frothy liquid in the trachea and haemorrhages in the lungs. There was no pulmonary oedema. In the surviving animals, the only sign of intoxication observed was deep abdominal breathing and open mouth gasping in the animals exposed to the two highest concentrations. From these mortality data, an LC₅₀ of 1910 mg/m³ (95% CI: 1590-2290 mg/m³) was presented (see 2, 12). [In view of the experimental data, an LC₅₀ of 2153 mg/m³ (95% CI: 1908-2402 mg/m³) seems more reasonable.]

Dermal LD₅₀ values were reported to be greater than 1000 and 2000 mg/kg bw in rats (26) and rabbits (2), respectively. Only the rabbit study was described in more detail. Apart from skin irritation (see also section 6.2.1), no other toxic effects were reported. However, histological examinations were not included, and the absence of a control group prevented the evaluation of body weight gain data (12).

Oral LD₅₀ values ranged from 500 to 965 mg/kg/bw in rats, whereas in mice oral LD₅₀s of 615 (26), 831 (17) and greater than 4500 mg/kg bw (2) have been mentioned. In guinea pigs, it was estimated to 500 mg/kg bw (26). No description of toxic effects was given. In a range-finding toxicity test preceding an unpublished micronucleus test (see also section 6.2.5), single oral (gavage) doses of 500, 750, 850, and 1000 mg/kg bw of benzotriazole (granules; "Preventol CI8-100"; purity: 99.83%) to male and female NMRI mice caused mortality in the two higher dose groups (850 mg/kg: 2/5; 1000 mg/kg: 3/5). Symptoms observed included apathy, reduced motility, unkempt coat, lateral position, abdominal

position, cramp, convulsion, and rapid breathing. Data relating incidence/severity and dose levels were not given (21).

Intraperitoneal LD₅₀ values ranged from 500-900 and 500-1000 mg/kg bw in rats and mice, respectively. In mice, effects on the central nervous system (convulsions; flaccid paralysis) were seen at doses of approximately 250 mg/kg bw. Following single intravenous injections to mice, an LD₅₀ was found to be 238 mg/kg bw, while effects on the central nervous system (reduced reflexes; paralysis) were observed at a dose of 55 mg/kg bw. In mice, intravenous and intraperitoneal doses caused death by respiratory arrest (2).

In conclusion, based on the acute lethal toxicity data (inhalation LC rat*: 2153 mg/m³; oral LD₅₀ rat: 500-965 mg/kg) and using EC-classification criteria, benzotriazole is considered harmful following inhalation and oral exposure. Oral and intraperitoneal LD₅₀s in rats and mice were of similar order of magnitude (500-1000 mg/kg bw).

6.2.3 Short-term toxicity (up to 90 days)

In a pilot experiment prior to a carcinogenicity study, groups of male (n=5) and female (n=5) rats (Fischer 344) and mice (B6C3F1) were fed benzotriazole at concentrations of 300, 1000, 3000, 10 000, and 30 000 ppm (rats: approximately 13-1325 mg/kg bw/d; mice: approximately 37-3710 mg/kg bw/d)** in the diet for eight weeks. This experiment was performed in order to estimate the maximum tolerated dose. It was not reported which end points were investigated, and only statements on survival and body weight were presented. In rats, body weight decreases*** were not higher than 12% at each dose ranging from 300 to 10 000 ppm, while a sharp decrease of 34-40% in body weight was observed at 30 000 ppm. All animals survived. In mice, a small effect on body weight (i.e., a decrease of approximately 5%) was observed at a concentration of 30 000 ppm only (25). However, considering the low number of animals used and the limited scope (range finding for a 2-year carcinogenicity study) and reporting (only statements on survival and body weight), no conclusions regarding a NOAEL can be drawn from this study.

Undefined toxic effects on the peripheral blood system, liver, and kidney were observed in rats (sex not known) after oral administration of 2.4, 12, and 60 mg/kg bw/day for 30 days. A daily oral dose of 0.6 mg/kg bw for six months induced toxic effects (not specified), while 0.06 mg/kg bw/day did not (original paper in Russian). No further details were reported (17), and therefore this study is not suitable for evaluation of the health hazard.

The available short-term toxicity studies suffer from various limitations that exclude them from being used for health hazard assessment purposes.

* This value was calculated by DECOS from the experimental data presented in (2) and (12) (see section 6.2.2).

** To convert oral doses from ppm or mg/kg diet into mg/kg bw the following default values (or their averages) are used throughout this report: rat male bw 500 g, daily food intake 20 g; female bw: 350 g, daily food intake 17.5 g; mouse male bw 30 g, daily food intake 3.6 g; female bw 25 g, daily food intake 3.25 g.

*** Probably decreased body weight gain was meant.

6.2.4 Long-term toxicity and carcinogenicity

In a carcinogenicity study, male and female rats (Fischer 344; n=50/sex/group) were fed time-weighted average doses of 6700 and 12 100 ppm (i.e., approximately 295 and 535 mg/kg bw/day; dosage adjusted during the experimental period) for 78 weeks. This was followed by an observation period of 27 weeks. Animals were observed twice daily for signs of toxicity, clinical observations were recorded every month, and body weights were recorded every two weeks for the first twelve weeks and every month thereafter. The pathological evaluation consisted of gross and microscopical examinations of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. Data on both neoplastic and nonneoplastic lesions were presented. No data on organ weights were given. Consistently lower mean body weights were found in the exposed groups when compared with controls (only growth curves presented). In male rats, survival rates were not affected by treatment, while in dosed females survival was slightly higher than in controls. No compound-related clinical signs were seen. At post-mortem examinations, nonneoplastic lesions observed were: inflammation of the prostate and the uterus and changes in the liver ("clear-cell changes", "basophilic cytoplasm change", "eosinophilic cytoplasm change").

With respect to neoplastic lesions, benign liver tumours (nodules), not present in either the control or low-dose male rats, were seen in five out of the 45 (11%) high-dose males, a statistically significant increase. However, since incidences of 10-11% had been seen in two out of thirteen groups of untreated rats of the same strain at the same laboratory (in previous experiments), the investigators concluded that the tumours "cannot be clearly associated with administration of the test chemical". There were brain tumours in three out of the 44 (7%) low-dose males and in one out of the 50 (2%) high-dose females, but neither in male nor in female controls. Previous results, from the same laboratory, showed a low incidence of brain tumours in untreated rats of the same strain (none in 250 males and one in 249 females studied). The investigators concluded that the tumours were "suggestive of, but not considered as sufficient evidence of, carcinogenicity". The low-dose females had a significantly higher level of benign uterus tumours compared with the controls (10/45 or 22% vs. 2/48 or 4%). However, since the 16% incidence in the high-dose group did not attain statistical significance, the investigators concluded that these tumours "cannot be associated with administration of the chemical" (25). In addition, incidences of 12-15% had been seen in untreated groups of the same strain of rat in other laboratories (2). Benign thyroid tumours were seen in four out of the 43 (9%) low-dose female rats while malignant thyroid tumours occurred in one out of the 43 (2%) low-dose and three out of the 50 (6%) high-dose females. These tumours were not present in the female controls (25). Previous results, in other laboratories (again with the same strain), have shown the incidence of these types of benign and malignant thyroid tumours in untreated female groups to be 4-5% and 1-4%, respectively (2). The above results, therefore, may indicate a weak carcinogenic action at this site, although the investigators do not discuss these findings. There was no statistically significant increase in thyroid tumour incidence in the male rats (25).

A similar carcinogenicity study was performed in mice (B6C3F1; n=50/sex/group) according to a similar protocol. When fed average time-weighted doses of 11 700 and 23 500 ppm (i.e., ~1455 and ~2925 mg/kg bw/day; dosage adjusted during the experimental period) for 104 weeks followed by an observation period of two weeks, mean body weights were dosis-relatedly decreased (only growth curves presented). As in rats, survival rates were affected in dosed females (i.e., higher) only, and there were no clinical signs. Treatment-induced nonneoplastic changes were found in the bone marrow (myelofibrosis) and mesenteric lymph nodes (haemorrhages). No convincing evidence of carcinogenicity was seen. However, a higher number of lung tumours were found in the treated females; incidences in the controls, low-dose, and high-dose animals being 0, 9, and 3 out of 49, respectively (0%, 18%, 6%, respectively). In previous control groups of females kept at the laboratory, the incidences of these tumours varied from 0 to 7%, with a mean of 4%, which led the investigators to describe the benzotriazole findings as only suggesting a possible carcinogenic effect (2, 25).

Death and decreased growth were noted when mice, already suffering from mammary tumours, were given eleven to fifteen injections (unspecified route) of 50-150 mg/kg bw/day (2). Subcutaneous administration of 100 mg/kg bw/day to rats, for 46 weeks, caused liver damage (2). No details were reported and therefore, this study is not suitable for the evaluation of the health risk.

When 315 male and female mice were administered oral doses of 100 mg/kg bw weekly for 46 weeks (by stomach tube), an incidence of leukaemia of 13.7% was reported. This incidence was significantly higher than that of 3.4% which was observed in a group of untreated mice. However, in a control group, where the solvent (type unspecified) used for the above dosing was administered alone, 11.1% developed leukaemia (2).

In a tumour promotion study, rats were fed benzotriazole at about 250 mg/kg bw/day for eight weeks, along with a known liver carcinogen. Benzotriazole had no effect on the incidence of liver tumours (2).

From the carcinogenicity studies with rats and mice there is inconclusive evidence that benzotriazole is carcinogenic. Although higher incidences of - mostly benign - tumours in some organs were observed in treated than in concurrent control animals, these tumours had mostly higher incidences in the low-dose than in the high-dose group, and occurred at fairly high rates in historical controls. No NOAEL could be assessed since effects were observed in rats (neoplastic effects: brain tumours in males, thyroid tumours in females; nonneoplastic effects: decreased body weight gain, histological changes in liver cells, inflammation of prostate and uterus) and mice (neoplastic effects: lung tumours in females; nonneoplastic effects: decreased body weight gain, bone marrow myelofibrosis, haemorrhages in mesenteric lymph nodes) at the lowest doses tested. This study resulted in LOAELs of 295 and 1455 mg/kg bw/day in rats and mice, respectively.

6.2.5 Genotoxicity

A summary of *in vitro* genotoxicity studies is presented in Table 1.

Benzotriazole was found mutagenic in one *Salmonella typhimurium* strain (strain TA 1535) in the presence of a metabolic activation system (7, 8, 16, 30), while for this strain both positive (7, 8) and negative (16, 30) results were found in the absence of a metabolic activation system. In another study, a positive response was obtained in strain TA1535 in the presence of hamster liver S9 preparations only (mouse and rat liver S9 gave negative results) (15). When commercial benzotriazole (purity: unknown) was tested, positive results were obtained in strains TA98, TA1537, and TA1538 as well (10, 11). In *E coli* (strain WP2 *uvrA*), mutagenicity was observed both in the presence and in the absence of a metabolic activation system (16).

As to *in vitro* mammalian cell systems, benzotriazole ("Benzotriazol Granulat"/"Preventol CI-8"; purity: 99.83%; vehicle: DMSO) was tested in the HGPRT forward mutation assay in Chinese hamster ovary (CHO) cells. Without adding a metabolic activating system, negative results were obtained in two independent trials at dose ranges of 400-1000 µg/ml (5 duplicate doses/trial). In the presence of an induced rat-liver-derived S9 mix, a slight statistically significant increase in mutation frequency was observed at one of the mid-dose levels in one of the trials (dose range: 200-1000 µg/ml; 5 duplicate doses/trial). However, this increase was within historical control levels, not dose related, and not found in the other trial (3). In view of the survival rates (approximately 72% at 1000 µg/ml) in the nonactivating test, higher levels could have been tested. However, from the results presented for the dose range tested a positive result is unlikely to occur at higher doses than tested. Thus, (in accordance with (3)) benzotriazole is judged negative in this assay.

In validating an *in vitro* transformation assay, which was developed to detect mutagens/carcinogens by measuring the acquisition of attachment independence (recognised as being characteristic of transformed cells), benzotriazole was positive (29).

In vivo, benzotriazole was investigated for its potential to induce clastogenic effects with the mouse bone marrow micronucleus test. In this unpublished study, performed according to relevant OECD guidelines, there was no increase in the incidence of micronuclei in polychromatic erythrocytes obtained from mice (NMRI; n=5/sex/sacrifice) 24, 48, and 72 hours after administration of a single oral dose (gavage; vehicle: PEG 400) of 800 mg/kg bw of benzotriazole (granules; "Preventol CI8-100"; purity: 99.83%). Treatment did not affect the polychromatic/normochromatic erythrocyte ratio. The level, selected from a preceding range-finding test, was clearly toxic as was shown by compound-related symptoms such as apathy, reduced motility, abdominal position, cramp, convulsion, and rapid and feeble breathing and mortality (in 1/40) (see also section 6.2.2) (21).

Table 1. In vitro studies of genotoxic effects of benzotriazole

Organism/target cells	Endpoint	Concentration	Metabolic activation	Res-ponse	Ref.
TA98, TA100, TA1537, TA1538	Gene mutation	33-3333 µl/plate	no	neg	15
TA1535			yes ^a	neg	
TA98, TA100, TA1537, TA1538	Gene mutation	0.3-10 000 µl/plate	no	neg	16
TA1535			yes ^b	neg	
TA97, TA98, TA100	Gene mutation	33-1666 µg/plate	no	neg	30
TA1535			yes ^c	neg	
TA98, TA100, TA1537, TA1538	Gene mutation	444-2250 µg/0.1 ml; test repeated with 500-8000 µg/0.1 ml	no	neg	7
TA1535			yes ^b	neg	
TA98, TA100, TA1537, TA1538	Gene mutation	25-2025 µg/0.1 ml; test repeated with 444-2250 µg/0.1 ml	no	neg	8
TA1535			yes ^c	neg	
TA100	Gene mutation	25-2025 µg ^e /0.1 ml; test repeated with 50-4050 µg ^e /0.1 ml	no	neg	10
TA98, TA1537, TA1538 ^d			yes ^c	neg	
TA100	Gene mutation	250-4000 µg ^e /0.1ml	no	neg	11
TA98, TA1535, TA1537, TA1538			yes ^b	neg	
<i>E. Coli</i> WP2 <i>uvrA</i>	Gene mutation	0.3-10 000 µg/plate	no	pos	16
			yes ^a	pos	
<i>E. Coli</i> PQ37	SOS induction (DNA damage)	Up to 100 mM or up to solubility limit ^f	no	neg	23
			yes	neg	
Chinese hamster ovary cells	HGPRT forward mutation	50-1000 mg/ml	no	neg	3
			yes ^c	neg	

^a From rat, mouse, and hamster liver (non-induced and Arochlor-induced).

^b From hamster liver (Arochlor-induced).

^c From rat liver (Arochlor-induced).

^d TA1538 used in repeated test only.

^e Commercial benzotriazole tested (purity: unknown).

^f A great number of compounds were tested at 3-5 different concentrations at half-log intervals at a maximum level of 100 mM or the limit of solubility. Specific concentrations per compounds were not given.

In conclusion, *in vitro*, benzotriazole was mutagenic in bacterial cell systems (*Salmonella typhimurium*, *E. coli*), but not in mammalian cells (Chinese hamster ovary cells). An indication test for DNA damage (SOS chromotest in *E. coli*) was negative as well. *In vivo*, benzotriazole did not induce micronuclei in the bone marrow of orally treated mice. Benzotriazole induced cell transformation.

6.2.6 Reproduction toxicity

In the German documentation, two Russian studies are mentioned in which the effects of pre-mating exposure of female rats on developmental parameters were examined. Exposure might have induced changes in hormonal balance (increased cycles). Data on developmental parameters (foetal and pup mortality, anomalies) were inconsistent and could not be properly evaluated because they were inadequate as well (17).

In conclusion, no valid data on the reproduction toxicity of benzotriazole were found.

6.3 Summary

Contact dermatitis in metalworkers was observed after skin exposure to benzotriazole.

In experimental animals, pure benzotriazole was a slight eye irritant and at most a slight skin irritant; benzotriazole is not a skin sensitiser. Based on acute lethal toxicity data and using EC-classification criteria, benzotriazole should be classified as harmful following inhalation and oral exposure.

No valid repeated-dose short-term toxicity studies were available. From long-term carcinogenicity studies, there is inconclusive evidence that benzotriazole is carcinogenic in rats and mice, since these tumours had mostly higher incidences in the low-dose than in the high-dose group, and occurred at fairly high rates in historical controls. A NOAEL could not be established since effects were observed in rats (neoplastic effects: brain tumours in males, thyroid tumours in females; nonneoplastic effects: decreased body weight gain, histological changes in the liver, inflammation of prostate and uterus) and mice (neoplastic effects: lung tumours in females; nonneoplastic effects: decreased body weight gain, bone marrow myelofibrosis, haemorrhages in mesenteric lymph nodes) at the lowest dose tested. The LOAELs were set at 295 and 1455 mg/kg bw/d in rats and mice, respectively.

In vitro, benzotriazole is mutagenic in *Salmonella typhimurium* TA 1535 and in *E. coli*, but not in Chinese hamster ovary cells. The SOS chromotest in *E. coli*, an indicator test for DNA damage was negative. *In vivo*, benzotriazole was negative in an oral mouse bone marrow micronucleus assay.

There were no valid data on reproduction toxicity.

7. Existing guidelines, standards and evaluations

7.1 General population

No guidelines for the general population were found.

7.2 Working population

No occupational exposure limits/standards for benzotriazole were established or recommended in the Netherlands, the Nordic countries, the UK, or by the American Conference of Governmental Industrial Hygienists (ACGIH). In Germany, benzotriazole was listed among those compounds for which no limit could be established (13).

In 1988, Germany concluded that occupational exposure to benzotriazole could be irritating to the eyes, but not to the skin. Sensitisation may occur. Cytostatic effects were induced at such high dose levels that these effects are concluded not to occur at workplace exposure conditions. Since (reversible) central nervous system effects following single oral or inhalation exposure were induced at relatively high levels and since the dose-response curve might be steep, acute toxic effects are not expected from using/handling benzotriazole-containing products (namely, coolant lubricants which contain 0.05% benzotriazole). Toxic effects following long-term exposure could not be evaluated conclusively, since there were no data available from (semi)chronic inhalation studies or from reproduction toxicity studies. On the other hand, significant toxic effects (decreased body weight gain) were induced at only relatively high dietary levels. Carcinogenic effects might occur at high doses, although animal experiments were inconclusive. Benzotriazole would be a weak carcinogen only (17).

8. Hazard assessment

8.1 Assessment of health hazard

Based on experimental animal data with benzotriazole of unknown purity and limited human data, it is concluded that benzotriazole is slightly irritating to the eyes and, at most, slightly irritating to the skin. Benzotriazole is considered not to be a skin sensitiser.

Based on acute lethal toxicity data and using EC-classification criteria, benzotriazole should be classified as harmful following inhalation and oral exposure.

No data from valid repeated-dose short-term toxicity or developmental toxicity studies were found.

Benzotriazole was mutagenic in *in vitro* bacterial cells, but not in mammalian cell systems. The bacterial test indicator for DNA damage was negative. *In vivo*, benzotriazole did not induce micronuclei in the bone marrow of orally treated mice.

In oral carcinogenicity studies in rats and mice, higher incidences of - mostly benign - tumours in some organs were observed in treated than in concurrent

control animals. These tumours had mostly higher incidences in low-dose than in high-dose groups, and occurred at fairly high rates in historical controls. The results of these studies are inconclusive with respect to carcinogenic potential of benzotriazole. No NOAEL can be assessed from these studies since several effects were observed in rats (neoplastic effects: brain tumours in males, thyroid tumours in females; nonneoplastic effects: decreased body weight gain, histological changes in the liver, inflammation of prostate and uterus) and mice (neoplastic effects: lung tumours in females; nonneoplastic effects: decreased body weight gain, bone marrow myelofibrosis, haemorrhages in mesenteric lymph nodes) at the lowest dose tested. The LOAELs were set at 295 and 1455 mg/kg bw/day in rats and mice, respectively.

In view of the (inconclusive) evidence on the carcinogenic potential of benzotriazole in rodents and the mutagenic effects of benzotriazole in bacterial systems along with the absence of mutagenic and genotoxic effects in mammalian cells and in mouse bone marrow *in vivo*, the data base is considered inconclusive regarding carcinogenicity of this chemical. Thus, benzotriazole should be considered as a suspected human carcinogen.

Benzotriazole appeared to be a slight eye irritant in experimental animals. This may indicate that eye and/or respiratory tract irritation cannot be excluded to occur in workers exposed to benzotriazole. Since no eye irritation studies in humans were available, and repeated-dose inhalation toxicity studies in experimental animals were absent, it is not possible to derive any LOAEL or NOAEL for these effects.

8.2 Groups at extra risk

No specific groups at extra risk are identified in the literature.

9. Recommendations for research

- Genotoxicity: a gene mutation test and a chromosome aberration assay using eukaryotic cells and depending on the results an appropriate *in vivo* test.
- Reproduction toxicity studies.
- Subchronic and chronic inhalation toxicity studies in rats.
- A human volunteer respiratory and dermal irritation test.

10. Summary

H. Stouten, A.A.J.J.L Rutten, I.A. van de Gevel, and F. De Vrijer.
The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and The Dutch Expert Committee on Occupational Standards.
126. 1,2,3-Benzotriazole. *Arbete och Hälsa* 2000;24:1-20. National Institute for Working Life, Solna.

Benzotriazole is an odourless, white to tannish crystalline powder. It is sparingly soluble in water and soluble in a number of organic solvents. It has a low vapour pressure, and it is therefore likely to occur as dust at the workplace. When finely divided and mixed with air, dust explosions may occur. Benzotriazole is an industrial compound primarily used as a corrosion inhibitor, as a plastic stabiliser, and as a chemical intermediate. There is very little information found with respect to the toxicokinetics of benzotriazole.

Based on experimental animal data with benzotriazole of unknown purity and limited human data, it is concluded that benzotriazole is slightly irritating to the eyes and, at most, slightly irritating to the skin. Benzotriazole is considered not to be a skin sensitiser.

In the carcinogenicity studies, higher incidences of - mostly benign - tumours in some organs were observed in treated than in concurrent control animals. These tumours had mostly higher incidences in the low-dose than in the high-dose group, and occurred at fairly high rates in historical controls. The LOAELs were set at 295 and 1455 mg/kg bw/day in rats and mice, respectively.

In view of the inconclusive evidence for carcinogenic potential of benzotriazole in rodents and the mutagenic effects of benzotriazole in bacterial systems along with the absence of mutagenic and genotoxic effects *in vitro* in mammalian cells and *in vivo* in experimental animals, the data base is too poor to justify a conclusion regarding genotoxicity and carcinogenicity of this chemical.

Keywords: benzotriazole, irritation, occupational exposure limit, risk assessment, toxicology

11. Summary in Swedish

H. Stouten, A.A.J.J.L Rutten, I.A. van de Gevel, and F. De Vrijer.
The Nordic Expert Group for Criteria Documentation of Health Risks from Chemicals and The Dutch Expert Committee on Occupational Standards.
126. 1,2,3-Benzotriazole. *Arbete och Hälsa* 2000;24:1-20. National Institute for Working Life, Solna.

Bensotriazol är ett luktlöst, vitt till ljusbrunt kristallint pulver. Det är svårlösligt i vatten men lösligt i många organiska lösningsmedel. Ämnet har lågt ångtryck och förekommer därför troligen som damm i arbetsmiljön. När det finfördelas och blandas med luft finns risk för dammexplosioner. Bensotriazol är en industrikemikalie som framför allt används för att förhindra korrosion, som stabiliserare i plaster och som kemisk intermediär. Informationen om upptag och omsättning av bensotriazol är mycket sparsam.

Baserat på djurförsök med bensotriazol av okänd renhet och på begränsade humandata får bensotriazol anses vara svagt irriterande på ögon, och som mest, något irriterande på hud. Bensotriazol anses inte vara hudsensibiliserande.

I cancerstudier observerades en högre incidens av - huvudsakligen godartade - tumörer i vissa organ hos de behandlade djuren jämfört med de jämsides löpande kontrolldjuren. Tumörerna hade vanligen en högre incidens i lågdos- än i högdosgruppen, och förekom med relativt hög frekvens hos de historiska kontrollerna. LOAEL-värdena var 295 och 1455 mg/kg kroppsvikt/dag hos råttor respektive mus.

På grund av icke-konklusiva data vad gäller bensotriazols carcinogena potential hos gnagare samt ämnets mutagena effekt i bakteriesystem i kombination med frånvaron av mutagena och genotoxiska effekter *in vitro* i däggdjursceller och *in vivo* hos försöksdjur kan inga slutsatser dras vad gäller bensotriazols genotoxicitet och carcinogenicitet.

Nyckelord: bensotriazol, hygieniskt gränsvärde, irritation, riskbedömning, toxikologi

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13. Data bases used in search for literature

For the preparation of this document, literature has been retrieved from online data bases such as Medline, Toxline and CA (last update online search: May 1998). HSDB and RTECS, data bases available from CD-ROM, were consulted as well (26, 27).

Conclusions are based on scientific publications prior to March 2000.

Before finalising the document, an additional literature search was performed in Medline (May 1998 - August 2000) and Toxline (May 1998 - April 2000). The results of this search were no reason for adjustment of the recommendations.

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