1,2,3-Benzotriazole

Health-based recommended occupational exposure limit

Aan de Minister van Volksgezondheid, Welzijn en Sport

Onderwerp	:	toezending advies over 1,2,3-benzotriazol.
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Mevrouw de Minister,

Bij brief van 3 december 1993, nr. DGV/BMO-U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid om gezondheidskundige advieswaarden af te leiden ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen.

Per 1 januari 1994 heeft mijn voorganger daartoe een commissie ingesteld die de werkzaamheden voortzet van de Werkgroep van Deskundigen (WGD). De WGD was een door genoemde Minister ingestelde adviescommissie.

Hierbij stuur ik u ter kennisname - gehoord de Beraadsgroep Gezondheid en Omgeving - een publicatie van de Commissie WGD over 1,2,3-benzotriazol.
Deze publicatie heb ik heden aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid aangeboden.

Hoogachtend,
w.g.
prof. dr JJ Sixma

1,2,3-Benzotriazole

Health-based recommended occupational exposure limit

Dutch expert committee on occupational standards,
a Committee of the Health Council of the Netherlands

to:

the Minister and State Secretary of Social Affairs and Employment

No. 2000/14OSH, The Hague, 22 November 2000

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues...” (Section 21, Health Act).

The Health Council receives most requests for advice from the ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature Prereservation & Fisheries. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

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Samenvatting en advieswaarde

1 Vraagstelling

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid leidt de Commissie WGD van de Gezondheidsraad gezondheidskundige advieswaarden af voor beroepsmatige blootstelling aan toxische stoffen in de lucht. Die afleiding is de eerste fase van een drietrapsprocedure die moet leiden tot wettelijke grenswaarden.

In het voorliggende rapport bespreekt de commissie de gevolgen van blootstelling aan 1,2,3-benzotriazol (in het vervolg aangeduid met benzotriazol) in de lucht op de werkplek. De conclusies van de commissie zijn gebaseerd op wetenschappelijke publicaties die vóór maart 2000 zijn verschenen.

2 Vóórkomen; fysische en chemische eigenschappen

Benzotriazol is een reukloos, wit tot lichtbruin kristallijn poeder. Het lost matig op in water en goed in een aantal organische oplosmiddelen. Benzotriazol heeft een lage dampspanning en zal daarom op de werkplek vooral als stof voorkomen. Stofexplosies zijn mogelijk, indien het in fijn verdeelde vorm gemengd wordt met lucht.

Benzotriazol wordt gebruikt in de industrie als middel om corrosie tegen te gaan, als stabilisator voor plastics en als intermediair bij de synthese van andere chemische verbindingen.

3 Monitoring

Gezien de lage dampspanning van benzotriazol is het waarschijnlijk, dat deze verbinding op de werkplek alleen geadsorbeerd aan stof voorkomt. Algemene methoden voor het meten van inhaleerbaar stof zouden dan geschikt kunnen zijn voor het meten van benzotriazol. De fractie benzotriazol in het stof kan, na extractie met een geschikt oplosmiddel, analytisch chemisch worden bepaald. Gezien de specificiteit en de hoogte van de detectiegrens, lijken UV-spectrofotometrie of analyse met HPLC of GC-MS het meest geschikt.

4 Grenswaarden

Er is geen grenswaarde voor benzotriazol vastgesteld of aanbevolen in Nederland, de Noord-Europese landen, het Verenigd Koninkrijk en de Verenigde Staten. In Duitsland is benzotriazol geplaatst op een lijst met stoffen waarvoor geen grenswaarde kon worden afgeleid.

5 Kinetiek

De commissie heeft geen gegevens gevonden over de kinetiek van benzotriazol.

Op basis van physisch chemische gegevens, mag verwacht worden, dat benzotriazol via de huid wordt opgenomen.

Incubatie met rattenlevermicrosomen gedurende 1 uur resulteerde in een langzame omzetting in 4- en 5-hydroxybenzotriazol.

6 Effecten

Benzotriazol veroorzaakte contactdermatitis bij metaalbewerkers na blootstelling van de huid.

In onderzoek met dieren bleek benzotriazol in ernstige mate irriterend te zijn voor de ogen en, hooguit, in lichte mate voor de huid; een zwak sensibiliserende potentie kan niet worden uitgesloten. Op basis van sterfte na eenmalige blootstelling (inhalatoire LC_{50} rat*: 2153 mg/m^3; orale LD_{50} rat: 500-965 mg/kg lichaamsgewicht) en bijbehorende EG-richtlijnen zou benzotriazol geclassificeerd moeten worden als een stof die schadelijk is bij inademing en bij opname door de mond.

* Deze waarde is berekend door de commissie op basis van dierexperimentele gegevens.

Afgezien van orale carcinogeniteitsproeven met ratten en muizen heeft de commissie geen deugdelijke studies gevonden naar effecten van herhaalde blootstelling (inbegrepen reproductie-studies).

In de carcinogeniteitsstudies werden hogere incidenties van — meestal goedaardige — tumoren gevonden bij behandelde dieren dan bij controles. Deze incidenties waren meestal hoger bij de lage dan bij de hoge doseringsgroep en de achtergrondincidenties bij de controledieren waren hoog. De commissie kon geen NOAEL vaststellen, aangezien bij de laagste dosering nog effecten werden gezien. De LOAELs werden vastgesteld op 295 en 1455 mg/kg lichaamsgewicht per dag bij respectievelijk ratten en muizen.

In *in vitro* onderzoek was benzotriazol mutageen in *S. typhimurium* en in *E. coli*, maar niet in CHO-cellen ('Chinese hamster ovary' cellen). Een test waarin DNA-schade *in vitro* werd bepaald was ook negatief. Na orale blootstelling van muizen aan benzotriazol was de in vivo (beenmerg) micronucleus test eveneens negatief.

7 Evaluatie

Op basis van humane en/of dierexperimentele gegevens concludeert de commissie, dat sensibilisering en oogirritatie als gevolg van beroepsmatige blootstelling aan benzotriazol niet kunnen worden uitgesloten.

Op basis van de carcinogeniteitsstudies met ratten en muizen concludeert de commissie, dat er onvoldoende bewijs is voor de carcinogeniteit van benzotriazol. Hoewel in enkele organen hogere incidenties van — meestal goedaardige — tumoren werden aangetroffen bij behandelde dieren dan bij de controles, waren deze incidenties meestal hoger bij de lage dan bij de hoge doseringsgroep en waren de achtergrondincidenties bij historische controledieren hoog.

Omdat er onvoldoende bewijs is voor carcinogene potentie van benzotriazol bij knaagdieren engezien de mutagene effecten van benzotriazol in bacteriële systemen maar het ontbreken van *in vitro* genotoxiciteitstesten met zoogdiercellen en *in vivo* genotoxiciteitsonderzoek bij proefdieren, is de commissie van mening, dat de gegevens te mager zijn om een conclusie te rechtvaardigen met betrekking tot de carcinogeniteit en mutageniteit van benzotriazol. De gegevens zijn onvoldoende om benzotriazol te classificeren als een stof die als kankerverwekkend voor de mens beschouwd moet worden, maar geven wel reden tot bezorgdheid. 1,2,3-Benzotriazol wordt dan ook geclassificeerd als verdacht carcinogeen.

Wanneer de commissie er van uit zou gaan, dat benzotriazol geen carcinogene eigenschappen bezit, dan zouden de beschikbare orale gegevens gebruikt kunnen worden om een gezondheidskundige advieswaarde af te leiden. De "lowest-observed-adverse-effect level" van 295 mg/kg lg/d uit de chronische rattenstudie zou als uitgangspunt gebruikt kunnen worden. Het zal duidelijk zijn, dat extrapolatie vanuit deze orale dosis die bij

proefdieren nog effect veroorzaakt, met toepassing van de gebruikelijke overwegingen en aannames, zal leiden tot een gezondheidskundige advieswaarde die aanzienlijk hoger is dan de maximaal aanvaarde concentraties die in Nederland zijn vastgesteld voor respirabel en inhaleerbaar hinderlijk stof, namelijk respectievelijk 5 en 10 mg/m^3. Omdat benzotriazol bij kamertemperatuur op de werkplek voor zal komen als stof, zouden deze waarden van toepassing zijn.

Omdat het echter niet uitgesloten kan worden, dat benzotriazol een genotoxisch carcinogeen is, achtte de commissie het wenselijk om het kankerrisico te berekenen dat samenhangt met een beroepsmatige blootstelling aan 10 mg/m^3. Op basis van een "worst case" benadering (zie Annex) zou beroepsmatige blootstelling aan 10 mg/m^3 gedurende 40 jaar gepaard gaan met een extra kans op overlijden als gevolg van kanker van 5 per 10.000.

Voorts is gebleken, dat benzotriazol ernstige oogirritatie veroorzaakt bij proefdieren. Dit zou kunnen betekenen, dat irritatie van de ogen en/of de luchtwegen of zelfs ernstigere effecten op deze orgaansystemen van werkers blootgesteld aan benzotriazolconcentraties van 5 mg/m^3 als respirabele en van 10 mg/m^3 als inhaleerbare deeltjes kunnen optreden, of in ieder geval niet uitgesloten kunnen worden. Omdat er geen humane gegevens betreffende oogirritatie beschikbaar zijn en toxiciteitsstudies waarin proefdieren herhaaldelijk inhalatoir zijn blootgesteld, ontbreken, concludeert de commissie, dat het niet mogelijk is om op basis van de beschikbare gegevens een gezondheidskundige advieswaarde af te leiden.

8 Gezondheidskundige advieswaarde

De Commissie WGD van de Gezondheidsraad concludeert, dat de beschikbare toxicologische gegevens over benzotriazol onvoldoende zijn voor het afleiden van een gezondheidskundige advieswaarde.

De commissie classificeert 1,2,3-benzotriazol als verdacht carcinogeen.

Executive summary

1 Scope

At the request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands recommends health-based occupational exposure limits for the concentration of toxic substances in air at the workplace. These recommendations are made by the Council's Dutch Expert Committee on Occupational Standards. They constitute the first step in a three-step procedure that leads to legally-binding limit values.

In the present report, the committee discusses the consequences of occupational exposure to 1,2,3-benzotriazole (further referred to as benzotriazole) and, if appropriate, recommends a health-based occupational exposure limit. The committee's conclusions are based on scientific publications prior to March 2000.

2 Occurrence, physical and chemical properties

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 stabilizer, and as a chemical intermediate.

3 Monitoring

In view of the low vapour pressure at room temperature, it is likely that benzotriazole will only occur as dust at the workplace. A general method for measuring inhalable dust should than be suitable for measuring benzotriazole. 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 or a HPLC or GC-MS method may be most appropriate.

4 Limit values

No occupational exposure limits/standards for benzotriazole have been established or recommended in the Netherlands, the Nordic countries, the UK, and the USA (ACGIH). In Germany, benzotriazole was listed among the compounds for which no limit could be established.

5 Kinetics

The committee did not find data on the toxicokinetics of benzotriazole. Based on physico-chemical data, dermal absorption might be expected. In an *in vitro* experiment using rat liver microsomes and a one-hour incubation period, it was metabolized to 4- and 5-hydroxybenzotriazole at a low rate.

6 Effects

In metal workers, contact dermatitis was observed after skin exposure to benzotriazole.

In experimental animals, benzotriazole appeared to be a severe eye irritant and, at most, a slight skin irritant; Benzotriazole is not a skin sensitizer. Based on acute letal toxicity data (inhalation LC_{50} rat*: 2153 mg/m^3; oral LD_{50} rat: 500-965 mg/kg bw) and using EC-classification criteria, benzotriazole should be classified as harmful following inhalation and oral exposure.

Apart from oral carcinogenicity studies in rats and mice, the committee did not find data from valid repeated-dose toxicity (including developmental toxicity) studies.

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

* Calculated by the committee.

at fairly high rates in historical controls. The committee could not assess a NOAEL since several effects were observed at the lowest dose tested. The LOAELs were set at 295 and 1455 mg/kg bw/day in rats and mice, respectively.

Benzotriazole is, *in vitro*, mutagenic in *S. 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.

7 Hazard assessment

Based on the human and/or experimental animal data, the committee concludes that eye irritation following occupational exposure to benzotriazole cannot be excluded.

From the carcinogenicity studies with rats and mice, the committee concludes that 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.

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 committee considers the data base too poor to justify a conclusion regarding genotoxicity and carcinogenicity of this chemical. Clearly, the data base is inadequate to classify benzotriazole as a probable carcinogen to humans, but it may raise concern for humans. Therefore, 1,2,3-benzotriazole is classified as a suspect carcinogen.

Assuming — for the time being — benzotriazole does not possess carcinogenic properties, one might use the available oral data to derive an occupational exposure limit. The LOAEL of 295 mg/kg bw/d from the chronic rat study could be used as a starting point. It will be clear that extrapolation from this LOAEL applying the usual considerations and assumptions will lead to a limit value much higher than the maximum accepted concentrations set in the Netherlands for respirable and inhalable nuisance dust, viz, 5 and 10 mg/m^3, respectively. Since benzotriazole at room temperature will be present at the workplace as dust, these values could then be applied as occupational exposure limits for benzotriazole.

Since, however, it cannot be excluded that benzotriazole is a genotoxic carcinogen, the committee deemed it desirable to calculate the cancer risk associated with an occupational exposure level of 10 mg/m^3. Using a “worst-case” approach (see Annex), occupational exposure to 10 mg/m^3 for 40 years should be associated with an excess cancer mortality risk of 5 per 10.000.

Furthermore, benzotriazole appeared to be a slight eye irritant in experimental animals. This may indicate that eye and/or respiratory tract irritation can not be excluded to occur in workers exposed to benzotriazole concentrations of 5 mg/m^3 for respirable particles and to 10 mg/m^3 for inhalable ones. Since no eye irritation studies in humans were available and repeated-dose inhalation toxicity studies in experimental animals were absent, the committee concludes that it is not justifiable to derive a health-based occupational exposure limit from the available data.

8 Health-based recommended occupational exposure limit

The Dutch Expert Committee on Occupational Standards considered the data base on benzotriazole too poor to justify recommendation of a health-based occupational exposure limit.

The committee classifies 1,2,3-benzotriazole as a suspected human carcinogen.

Chapter 1

Scope

1.1 Background

In the Netherlands, occupational exposure limits for chemical substances are set using a three-step procedure. In the first step, a scientific evaluation of the data on the toxicity of the substance is made by the Dutch Expert Committee on Occupational Standards (DECOS), a committee of the Health Council of the Netherlands, on request of the Minister of Social Affairs and Employment (Annex A). This evaluation should lead to a health-based recommended exposure limit for the concentration of the substance in air. Such an exposure limit cannot be derived, if sufficient data are not available or if the toxic action cannot be evaluated using a threshold model. In the latter case, an exposure-response relationship is recommended for use in regulatory standard setting.

In the next phase of the three-step procedure, the Social and Economic Council advises the Minister on feasibility of using the health based value as a regulatory Occupational Exposure Limit (OEL), or recommends a different OEL. In the final step of the procedure, the Minister of Social Affairs and Employment sets the official Occupational Exposure Limit.

1.2 Committee and method of work

This document is a co-production of DECOS and the Nordic Expert Group for Documentation of Occupational Exposure Limits (NEG). It is a result of an agreement between both groups to prepare jointly criteria documents which can be used by the

regulatory authorities in the Netherlands and in the Nordic countries. The draft document has been prepared 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 was first reviewed by DECOS and thereafter by NEG.

In 1999, the President of the Health Council released a draft of the report for public review. The individuals and organizations that commented on the draft are listed in Annex C. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Data

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 (NIO00, NLM00).

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

Chapter **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*H*-benzotriazole
Synonyms 2,3-diazaindole	:	1,2,3-benzotriazole; benzisotriazole; benztriazole; 1,2-amino-azophenylene; azimidobenzene; aziminobenzene; benzene azimide; 2,3-diazaindole; 1,2,3-triaza-1*H*-indene; 1,2,3-triazaindene; benzene azimide; 2,3-diazaindole
EINECS No	:	202-394-1
RTECS No	:	DM1225000

2.2 Physical and chemical properties (Che99, Dav77, Han95, NLM00)

Molecular formula	:	$C_6H_5N_3$
Molecular weight	:	119.14
Boiling point (101.3 kPa)	:	350 ºC
Melting point (101.3 kPa)	:	99 ºC
Relative density (water=1)	:	1.4
Vapour density (air=1)	:	4.1
Vapour pressure (20 ºC; 101.3 kPa)	:	< 0.01 kPa
Relative density of saturated vapour/air mixture (air=1; 20 ºC)	:	1.00
Auto-ignition temperature	:	400 ºC
Explosive limits (%, in air)	:	2.4-?
Solubility in water (25 ºC)	:	2.0 g/100 ml
Solubility in organic solvents	:	soluble in alcohol, benzene, toluene, chloroform, dimethylformamide
Log Poctanol/water	:	1.44 (experimental value)
Physical form	:	white to tan, crystalline powder; needles when crystallized from benzene
Odour	:	odourless

Benzotriazole exists in two tautomeric forms. The first tautomer has the formula 1*H*-benzotriazole. The second tautomer has the formula 2*H*-benzotriazole and is also referred to as pseudo-azimidobenzene or 2,1,3-benzotriazole. The 1*H*-tautomer represents the more stable and essentially exclusive molecular structure (Dav77).

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 alkalies, and toward oxidation and reduction.

2.3 Validated analytical methods

2.3.1 Environmental monitoring

No method for monitoring benzotriazole in air was available. However, since benzotriazole will hardly vaporize at room temperature, it will be present as dust, and general dust

measurement methods should be applicable (for overview: see Bol95). 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 or a HPLC or GC-MS method 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.

GC-MS, HPLC, and spectrophotometric methods for the determination of benzotriazole in aqueous solutions have been published (Dav77, Haw81).

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

Chapter 3

Sources

3.1 Natural occurrence

No data available.

3.2 Man-made sources

No data available.

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 (NLM00).

3.2.2 Uses

Benzotriazole is used as a corrosion inhibitor, as a plastic stabilizer, and as a chemical intermediate for dyes, pharmaceuticals, and fungicides. Derivates of benzotriazole are used as UV absorbers and as restrainers in photographic emulsions (NLM00).

Benzotriazole is used in metal working and in 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 dishwater 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 catalyzed 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 pretreatment of the cathode with benzotriazole. In photographic processing, benzotriazole acts as an antifogging agent in silver-halide emulsions, restraining the developer and preventing the blackening or fogging of the image due to overdevelopment (NCI78).

Coolant lubricants and corrosion inhibiting fluids may contain up to 0.05% benzotriazole (Gre99, Mau84).

Chapter 4

Exposure

4.1 General population

No data available.

4.2 Working population

No data available.

Chapter 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=1.6) as well as a weak acid (pKa = 8.57) (Alb48), a low degree of ionization 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) (Hof82).

5.4 Excretion

No information available.

5.5 Biological monitoring

No information available.

5.6 Summary

The committee did not find information 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 metabolized to 4- and 5-hydroxybenzotriazole at a low rate.

Chapter 6

Effects

6.1 Observations in man

6.1.1 Irritation and sensitization

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 (Duc80). However, since there were no data on negative controls and an immunological reaction was not ascertained, the committee can not assess whether the effects were the consequence of either irritation or sensitization.

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 (Boe89).

Conclusion

Based on the available data on humans, an irritating and/or sensitizing 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 sensitization

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, amongst others complete corneal opacity and severe chemosis (swelling of lids) in four animals. Immediate washing with water greatly reduced the irritation (BIB95, Dav77).

In an unpublished study, benzotriazole (granules; "Preventol CI-8"; purity (from Hei88): 99.83%) was slightly irritating to the eyes of female albino rabbits (HC:NZW; 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 hours before washing out, the following Draize scores were obtained (Ruf87):

scores[a] observed after:	1 hour	24 hours	48 hours	72 hours	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 conjuctival chemosis and discharge.

Skin

In an unpublished study, benzotriazole (granules; "Preventol CI-8"; purity (from Hei88): 99.83%) was not irritating to the skin of female albino rabbits (HC:NZW; 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 four hours to the intact clipped skin (observation times: 1, 24, 48, and 72 hours and 7 and 14 days) (Ruf87).

Benzotriazole and commercial benzotriazole (purity: unknown) were only minimally irritating (primary irritation index: 1.0, 0.7, respectively; maximum score: 8.0) when applied to the abraded and intact, clipped skin (under 24-hours occlusion) of New Zealand

white rabbits (n=3/sex) at a concentration of 50% in polyethylene glycol (PEG 400) and saline (70:30) (Cib93a, Cib93d).

Referring to unpublished industrial reports from the mid 1970s, it was mentioned that benzotriazole was mildly irritating to guinea-pigs at a concentration of 50% in ethanol. Furthermore, in a separate experiment, no irritation was reported after 24 and 72 hours (no other evaluation time points mentioned) when about 80 mg/cm^2 (of the pure material) was administered to the intact or abraded skin of rabbits (n=6/group) and covered for 24 hours. 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-hour covered contact with 2000 mg/kg bw applied to abraded skin (observation period: 14 days) (Dav77, BIB95).

Sensitization

In an unpublished study, benzotriazole ("Benzotriazol Granulat"/"Preventol CI-8"; purity: 99.83%) was not a skin sensitizer when tested in male guinea pigs (Winkelmann DHPW; n=20; controls: n=10) using the Magnusson-Kligman maximization test. The study was performed according to corresponding OECD and EU guidelines (GLP 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 hours after challenge and not confirmed by the 48-hour observation. In the control group, a weak positive response was found 1/10 animals at the 24-hour observation (Hei88).

In guinea pigs, the optimization test showed negative results with purified and commercial (purity: unknown) benzotriazole after intradermal as well as epidermal challenge application. In the maximization 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) (Mau84). In two other, not published studies (industrial reports from the mid 1970s), it was reported not to be a skin sensitizer when tested in guinea-pigs (no experimental details presented) (BIB95).

Conclusion

From data with benzotriazole of known purity, the committee concludes that, in experimental animals, the compound is slightly irritating to the eyes and, at most, slightly irri-

tating to the skin. In addition, the committee is of the opinion that benzotriazole is not a skin sensitizer.

6.2.2 *Acute toxicity*

When rats (male; n=10/group) were inhalatory exposed during three hours to benzotriazole aerosols of 780, 1460, 2030, 2230, and 2710 mg/m^3 (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 LC_{50} of 1910 mg/m^3 (95% CI: 1590-2290 mg/m^3) was presented (see BIB95, Dav77). [The committee is of the opinion that in view of the experimental data, this LC_{50} of 1910 mg/m^3 is rather unlikely. From the same data the committee concluded that an LC_{50} of 2153 mg/m^3 (95% CI: 1908-2402 mg/m^3) seems more reasonable.]

Dermal LD_{50} values were reported to be greater than 1000 and 2000 mg/kg bw in rats (NIO00) and rabbits (BIB95), 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 (Dav77).

Oral LD_{50} values ranged from 500 to 965 mg/kg/bw in rats, whereas in mice oral LD_{50}s of 615 (NIO00), 831 (Gre99) and greater than 4500 mg/kg bw (BIB95) have been mentioned. In guinea pigs, it was estimated at 500 mg/kg bw (NIO98). 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 groups of, a total of, five mice (Bor:NMRI; both males and females included) 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 (Her88).

Ip LD_{50} 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 iv injections to mice, a LD_{50} 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, iv and ip doses caused death by respiratory arrest (BIB95).

Conclusion

Based on the acute lethal toxicity data (inhalation LC_{50} rat*: 2153 mg/m^3; oral LD_{50} rat: 500-965 mg/kg) and using EC-classification criteria, the committee concludes that benzotriazole is harmful following inhalation and oral exposure. Oral and ip LD_{50}s 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 (5) and female (5) rats (Fischer 344) and mice (B6C3F1) were fed benzotriazole at concentrations of 300, 1000, 3000, 10,000, and 30,000 ppm (rats: approx 13-1325 mg/kg bw/d; mice: approx 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 approx 5%) was observed at a concentration of 30,000 ppm only (NCI78). However, considering the low number of animals used and the limited scope (range-finding for a 2-y 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 (sexe 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/d did not (original paper in Russian). No further details were reported (Gre99), and therefore this study is not suitable for evaluation of the health hazard.

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* This value was calculated by the committee from the experimental data presented in BIB95 and Dav77 (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: 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

Conclusion

The available short-term toxicity studies suffered from various limitations excluding them from being used for health hazard assessment purposes.

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., approx 295 and 535 mg/kg bw/d; 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" (NCI78). In addition, incidences of 12-15% had been seen in untreated groups of the same strain

of rat in other laboratories (BIB95). 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 (NCI78). 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 (BIB95). 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 (NCI78).

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/d; 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 (BIB95, NCI78).

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 (BIB95). Sc administration of 100 mg/kg bw/day to rats, for 46 weeks, caused liver damage (BIB95). 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 (BIB95).

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 (BIB95).

Conclusion

From the carcinogenicity studies with rats and mice, the committee concludes that 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. The committee could not assess a NOAEL 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 *S. typhimurium* strain (strain TA 1535) in the presence of a metabolic activation system (Cib93b, Cib93c, Dun85, Zei87), while for this strain both positive (Cib93b, Cib93c) and negative (Dun85, Zei87) 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 S-9 preparations only (mouse and rat liver S9 gave negative results) (Dun80). When commercial benzotriazole (purity: unknown) was tested, positive results were obtained in strains TA98, TA1537, and TA1538 as well (Cib93e, Cib93f). In *E coli* (strainWP2 *uvrA*), mutagenicity was observed both in the presence and in the absence of a metabolic activation system (Dun85).

organism/ target cells	endpoint	concentration	metabolic activation	response	ref.
S. typhimurium					
TA98, TA100, TA1537, TA1538	gene mutation	33-3333 µl/plate	-	negative	Dun80
			+[a]	negative	
TA1535			-	negative	
			+[b]	positive	
TA98, TA100, TA1537, TA1538	gene mutation	0.3-10,000 µl/plate	-	negative	Dun85
			+[a]	negative	
TA1535			-	negative	
			+[a]	positive	
TA97, TA98, TA100	gene mutation	33-1666 µg/plate	-	negative	Zei87
			+[c]	negative	
TA1535			-	negative	
			+[c]	positive	
TA98, TA100, TA1537, TA1538	gene mutation	444-2250 µg/0.1 ml;	-	negative	Cib93b
		test repeated with	+[b]	negative	
TA1535		500-8000 µg/0.1 ml	-	positive	
			+[b]	positive	
TA98, TA100, TA1537, TA1538	gene mutation	25-2025 µg/0.1 ml;	-	negative	Cib93c
		test repeated with	+[c]	negative	
TA1535		444-2250 µg/0.1 ml	-	positive	
			+[c]	positive	
TA100	gene mutation	25-2025 µg[e]/ 0.1 ml;	-	negative	Cib93e
		test repeated with	+[c]	negative	
TA98, TA1537, TA1538[d]		50-4050 µg[e]/0.1 ml	-	positive	
			+[c]	positive	
TA100,	gene mutation	250-4000 µg[e]/0.1 ml	-	negative	Cib93f
			+[b]	negative	
TA98, TA1535, TA1537, TA1538			-	positive	
			+[b]	positive	
E. Coli	Gene mutation	0.3-10,000 µg/plate	-	positive	Dun85
WP2 *uv*ra			+[a]	positive	
E. Coli	SOS induction (DNA da-	up to 100 mM or up to solu-	-	negative	
PQ37	mage)	bility limit[f]	+	negative	
Chinese hamster ovary cells	HGPRT forward	50-1000 mg/ml	-	negative	
	mutation		+[c]	negative	

nr = not reported.

[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.

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 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 (Boe87). In view of the survival rates (ca. 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, the committee concludes that a positive result is unlikely to occur at higher doses than tested. The committee concludes (in accordance with Boe87) that benzotriazole is 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 (recognized as being characteristic of transformed cells), benzotriazole was positive (Tra81).

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 (Bor: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 §6.2.2) (Her88).

Conclusion

In vitro, benzotriazole was mutagenic in bacterial cell systems (*S. 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 DFG documentation, two Russian studies are mentioned in which the effects of premating exposure of female rats on developmental parameters were examined. Exposure might have induced changes in hormonal balance (increased cycles). Data on developmental parameters (fetal and pup mortality, anomalies) were inconsistent and could not be properly evaluated because they were inadequate as well (Gre99).

Conclusion

The committee did not find valid data on the reproduction toxicity of benzotriazole.

6.3 Summary

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

In experimental animals, pure benzotriazole was a severe eye irritant and at most a slight skin irritant; benzotriazole is not a skin sensitizer. 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 in 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 ratsand mice, respectively.

In vitro, benzotriazole is mutagenic in *S. 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 the reproduction toxicity.

Chapter 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, and by the ACGIH (USA). In Germany, benzotriazole was listed among those compounds for which no limit could be established (DFG99).

In 1988, DFG concluded that occupational exposure to benzotriazole could be irritating to the eyes, but not to the skin. Sensitization 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) CNS 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 (Gre99).

Chapter 8

Hazard assessment

8.1 Assessment of health hazard

Based on experimental animal data with benzotriazole of unknown purity and limited human data, the committee concludes that benzotriazole is slightly irritating to the eyes and, at most, slightly irritating to the skin. In addition, benzotriazole is not a skin sensitizer.

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

The committee did not find data from valid repeated-dose short-term toxicity or developmental toxicity studies.

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 committee considers the results of these studies to be inconclusive with respect to carcinogenic potential of benzotriazole. The committee could not assess a NOAEL in 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 committee considers the data base inconclusive regarding carcinogenicity of this chemical. Clearly, the data base is inadequate to classify benzotriazole as 'known to be carcinogenic to humans' or as 'should be regarded as carcinogenic to humans', but it may raise concern for humans. Therefore, the committee classifies 1,2,3-benzotriazole as a suspected human carcinogen (comparable with EU class 3B).

Assuming — for the time being — that benzotriazole does not possess carcinogenic properties, one might use the available oral data to derive an occupational exposure limit. The LOAEL of 295 mg/kg bw/d from the chronic rat study could be used as a starting point. It will be clear that extrapolation from this LOAEL will lead to a limit value* much higher than the maximum accepted concentrations set in the Netherlands for respirable and inhalable nuisance dust, viz, 5 and 10 mg/m^3, respectively. Since benzotriazole at room temperature will be present at the workplace as a dust, these values could then be applied as occupational exposure limits for benzotriazole.

Since, however, it cannot be excluded that benzotriazole is a genotoxic carcinogen, it was deemed desirable to calculate the cancer risk associated with an occupational exposure level of 10 mg/m^3. Using a "worst-case" approach (see Annex), occupational exposure to 10 mg/m^3 for 40 years should be associated with an excess cancer mortality risk of 5 per 10.000.

Furthermore, benzotriazole appeared to be a slight eye irritant in experimental animals. This may indicate that eye and/or respiratory tract irritation can not be excluded to occur in workers exposed to benzotriazole concentrations of 5 mg/m^3 for respirable particles and to 10 mg/m^3 for inhalable ones. Since no eye irritation studies in humans were available, and repeated-dose inhalation toxicity studies in experimental animals were absent, the committee concludes that it is not justifiable to derive a health-based occupational exposure limit from the available data.

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* The following considerations should be taken into account: intra- and interspecies variation, differences between experimental conditions and the exposure pattern of the worker, type of critical effect, dose-response-curve, the absence of a NOAEL, the confidence of the data base. The resulting oral dose is converted to an inhalation concentration (HBR-OEL) assuming a respiratory volume of 10 m^3 for an eight-hour working day and a worker body weight of 70 kg.

8.2 Groups at extra risk

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

8.3 Health-based recommended occupational exposure limit

The Dutch Expert Committee for Occupational Standards considers the data base on benzotriazole too poor to justify recommendation of a health-based occupational exposure limit.

The committee classifies 1,2,3-benzotriazole as a suspected human carcinogen (comparable with EU class 3(B)).

Chapter **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.

The Hague, 22 November 2000,
for the committee

dr ASAM van der Burght,
scientific secretary

Prof. dr GJ Mulder,
chairman

References

Alb48 Albert A, Goldacre R, Phillips J. The strenght of heterocyclic bases. J Chem Soc 1948; 2240-9.

BIB95 BIBRA Toxicology International. Toxicity profile of benzotriazole. Carshalton (Surrey), UK: BIBRA Toxicology International, 1995.

Boe8 Den Boer WC. Mutagenicity test on Benzotriazol Granulat in the CHO HGPRT forward mutation assay. Veenendaal, the Netherlands: Hazleton Biotechnologies Veenendaal Laboratory, 1987; HBC study no E-95553-0-435 (study submitted to Bayer AG, Institut für Toxikologie, Wuppertal, Germany) (unpublished data submitted to THE COMMITTEE by Bayer AG).

Boe89 Boer de EM, van Ketel WG, Bruynzeel DP. Dermatoses in metal workers. 2. Allergic contact dermatitis. Contact Dermatitis 1989; 20: 280-6.

Che99 Chemiekaarten: gegevens voor het veilig werken met chemicaliën/ [ed by] samenwerkingsverband-chemiekaarten (TNO Arbeid, VNCI) 15 th ed. The Hague, The Netherlands. Ten HAgen & Stam, 1999:133.

Cib93a Ciba-Geigy Corp. Report on skin irritation in the rabbit after single application of TK11’237 with cover letter dated 072893. Springfield VA, USA: National Technical Information Service (NTIS), 1993; order no NTIS/OTS0538206.

Cib93b Ciba-Geigy Corp. Salmonella/mammalian-microsome mutagenicity test with TK 11’237 with cover letter dated 072893. Springfield VA, USA: National Technical Information Service (NTIS), 1993; order no NTIS/OTS0538209.

Cib93c Ciba-Geigy Corp. Salmonella/mammalian-microsome mutagenicity test with TK 11’237 with cover letter dated 072893. Springfield VA, USA: National Technical Information Service (NTIS), 1993; order no NTIS/OTS0538210.

Cib93d Ciba-Geigy Corp. Report on skin irritation in the rabbit after single application of TK10'637 with cover letter dated 072893. Springfield VA, USA: National Technical Information Service (NTIS), 1993; order no NTIS/OTS0538211.

Cib93e Ciba-Geigy Corp. Salmonella/mammalian-microsome mutagenicity test with TK 10'637 with cover letter dated 072893. Springfield VA, USA: National Technical Information Service (NTIS), 1993; order no NTIS/OTS0538213.

Cib93f Ciba-Geigy Corp. Salmonella/mammalian-microsome mutagenicity test with TK 10'637 with cover letter dated 072893. Springfield VA, USA: National Technical Information Service (NTIS), 1993; order no NTIS/OTS0538214.

Dav77 Davis LN, Santodonato J, Howard PH, *et al*. Investigation of selected potential environmental contaminants: benzotriazoles. Springfield VA, USA: National Technical Information Service (NTIS), 1977; rep no PB266366.

DFG99 Deutsche Forschungsgemeinschaft (DFG): Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe. MAK- und BAT-Werte-Liste 1999. Maximale Arbeitsplatzkonzentrationen und biologische Arbeitsstofftoleranzwerte. Weinheim, FRG: Wiley-VCH Verlag GmbH, 1999: 28 (Mitteilung 35).

Duc80 Ducombs G, Tamisier JM, Texier L. Contact dermatitis to 1-H benzotriazole. Contact Dermatitis 1980; 6: 224-5.

Dun80 Dunkel VC, Simmon VF. Mutagenic acitivity of chemicals previously tested for carcinogenicity in the national cancer institute bioassay program. IARC Sci Publ 1980; 27: 283-302.

Dun85 Dunkel VC, Zeiger E, Brusick, *et al*. Reproducibility of microbial mutagenicity assays. 2. Testing of carcinogens and noncarcinogens in Salmonella typhimurium and E. coli. Environ Mutagen 1985; 7 (suppl 5): 1-248.

Gal85 Galloway SM, Bloom AD, Resnick M, *et al*. Development of a standard protocol for in vitro cytogenetic testing with chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. Environ Mutagen 1985; 7: 1-51.

Gol91 Gold SL, Slone TH, Manley NB, *et al*. Target organs in chronic bioassays of 533 chemical carcinogens. Environ Health Perspect 1991; 93: 233-46.

Gre99 Greim H, ed. Benzotriazol. In: Gesundheidsschädliche Arbeitsstoffe. Toxikologisch-arbeitsmedizinische Begründungen von MAK-Werte (Maximale Arbeitsplatz-Konzentrationen). 1st-29th ed. Weinheim, FRG: Wiley-VCH Verlag GmbH, 1999.

Han95 Hansch C, Leo A, Hoekman D. Exploring QSARs. Hydrophobic, electronic and steric constants; Washington DC, USA: Amer Chem Soc, 1995; 18.

Haw81 Hawn GG, Diehl PA, Talley CP. High-performance liquid-chromatographic determination of aromatic triazole corrosion inhibitors in aqueous solutions. J Chromatogr Sci 1981; 19: 567-9.

Hea95 Health Council of the Netherlands: Dutch Expert Committee on Occupational Standards (DECOS). Calculating cancer risk. Rijswijk, the Netherlands: Health Council of the Netherlands, 1995; pub no 1995/06WGD.

Hei88 Heimann KG. Benzotriazol Granulat (Preventol CI-8). Untersuchungen auf hautsensibilisierende Wirkung bei Meerschweinchen (Maximierungstest). Wuppertal, Germany: Bayer AG, Fachbereich Toxikologie, 1988; rep no 16482 (unpublished data submitted to THE COMMITTEE by Bayer AG).

Her88 Herbold BA. Preventol CI8-100. Micronucleus test on the mouse to evaluate for clastogenic effects. Wuppertal, Germany: Bayer AG, Fachbereich Toxikologie, 1988; rep no 16737 (unpublished data submitted to THE COMMITTEE by Bayer AG).

Hof82 Hoffman H, Pooth R. Biotransformation von 1H-Benzotriazol und N-1-Alkylbenzotriazolen in vitro. Arch Pharm 1982; 315: 422-8.

Hud88 von der Hude W, Behm C, Gürtler R, *et al*. Evaluation of the SOS chromotest. Mutat Res 1988; 203: 81-94.

Huf91 Huff J, Cirvello, Haseman J, *et al*. Chemicals associated with site-specific neoplasia in 1394 long-term carcinogenesis experiments in laboratory rodents. Environ Health Perspect 1991; 93: 247-70.

Mau84 Maurer T, Meier F. Sensitization potential of benzotriazole. Contact Dermatitis 1984; 10 : 163-5.

NCI78 National Cancer Institute (NCI). Bioassay of 1H-Benzotriazole for possible carcinogenicity; CAS No. 95-14-7. SpringfieldVA, USA: National Technical Information Service (NTIS), 1978; order no PB285202 (NTP Techn Rep Ser No. 88).

NIO00 US National Institute of Occupational Health (NIOSH). 1H-Benzotriazole In: Registry of Toxic Effects of Chemical Substances (RTECS)[CD-ROM], issue April 2000. SilverPlatter International, 1998 (last update benzotriazole file: April 1999).

NLM00 US National Library of Medicine (NLM). 1,2,3-Benzotriazole in: Hazardous Substances Databank (HSDB) [CD-ROM], issue March 2000. SilverPlatter International, 2000 (last update benzotriazole file: Februari 2000).

Ros90 Rosenkranz HS, Ennever FK, Klopman G. Relationship between carcinogenicity in rodents and the induction of sister chromatid exchanges and chromosomal aberrrations in Chinese hamster ovary cells. Mutagenesis 1990; 5: 559-71.

Ruf87 Ruf J. Untersuchungen zum Reiz-/Ätzpotential an Haut und Auge (Kaninchen) nach OECD-Richtlinie No. 404 und 405. Wuppertal, Germany: Bayer AG, Fachbereich Toxikologie, 1987; rep no 15529 (unpublished data submitted to THE COMMITTEE by Bayer AG).

Tra81 Traul KA, Takayama K, Kachevsky V, *et al*. A rapid in vitro assay for carcinogenicity of chemical substances in mammalian cells utilizing an attachment-independence endpoint. 2 - Assay validation. J Appl Toxicol 1981; 1: 190-5.

Zei87a Zeiger E. Carcinogenicity of mutagens: predictive capability of the Salmonella mutagenesis assay for rodent carcinogenicity. Cancer Res 1987; 47: 1287-96.

Zei87b Zeiger E, Anderson B, Haworth S, *et al*. Salmonella mutagenicity tests. 3. Results from the testing of 255 chemicals. Environ. Mutagen 1987; 9 (suppl. 9): 1-110.

Annexes

Chapter **A**

Request for advice

In a letter dated October 11, 1993, ref DGA/G/TOS/93/07732A, to, the State Secretary of Welfare, Health and Cultural Affairs, the Minister of Social Affairs and Employment wrote:

Some time ago a policy proposal has been formulated, as part of the simplification of the governmental advisory structure, to improve the integration of the development of recommendations for health based occupation standards and the development of comparable standards for the general population. A consequence of this policy proposal is the initiative to transfer the activities of the Dutch Expert Committee on Occupational Standards (DECOS) to the Health Council. DECOS has been established by ministerial decree of 2 June 1976. Its primary task is to recommend health based occupational exposure limits as the first step in the process of establishing Maximal Accepted Concentrations (MAC-values) for substances at the work place.

In an addendum, the Minister detailed his request to the Health Council as follows:

The Health Council should advice the Minister of Social Affairs and Employment on the hygienic aspects of his policy to protect workers against exposure to chemicals. Primarily, the Council should report on health based recommended exposure limits as a basis for (regulatory) exposure limits for air quality at the work place. This implies:

- A scientific evaluation of all relevant data on the health effects of exposure to substances using a criteria-document that will be made available to the Health Council as part of a specific request for advice. If possible this evaluation should lead to a health based recommended exposure limit, or, in

the case of genotoxic carcinogens, a 'exposure versus tumour incidence range' and a calculated concentration in air corresponding with reference tumour incidences of 10^{-4} and 10^{-6} per year.

- The evaluation of documents review the basis of occupational exposure limits that have been recently established in other countries.
- Recommending classifications for substances as part of the occupational hygiene policy of the government. In any case this regards the list of carcinogenic substances, for which the classification criteria of the Directive of the European Communities of 27 June 1967 (67/548/EEG) are used.
- Reporting on other subjects that will be specified at a later date.

In his letter of 14 December 1993, ref U 6102/WP/MK/459, to the Minister of Social Affairs and Employment the President of the Health Council agreed to establish DECOS as a Committee of the Health Council. The membership of the Committee is given in annex B.

Chapter **B**

The committee

- GJ Mulder, *chairman*
  professor of toxicology; Leiden University, Leiden
- RB Beems
  toxicologic pathologist; National Institute of Public Health and the Environment, Bilthoven
- PJ Borm
  toxicologist; Heinrich Heine Universität Düsseldorf (Germany)
- JJAM Brokamp, *advisor*
  Social and Economic Council, The Hague
- VJ Feron,
  professor of toxicology; TNO Nutrition and Food Research Institute, Zeist
- DJJ Heederik
  epidemiologist; Agricultural University, Wageningen
- LCMP Hontelez, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- G de Jong
  occupational physician; Shell International Petroleum Maatschappij, The Hague
- J Molier-Bloot
  occupational physician; BMD Akers bv, Amsterdam
- IM Rietjens
  professor in biochemical toxicology; Wageningen University, Wageningen

- H Roelfzema, *advisor*
  Ministry of Health, Welfare and Sport, Den Haag
- T Smid
  occupational hygienist; KLM Health Safety & Environment, Schiphol and professor of working conditions, Free University, Amsterdam
- GMH Swaen
  epidemiologist; Maastricht University, Maastricht
- HG Verschuuren
  toxicologist; DOW Europe, Horgen (Switzerland)
- F de Wit
  occupational physician; Labour Inspectorate, Arnhem
- CA Bouwman, *scientific secretary*
  Health Council of the Netherlands, Den Haag
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, Den Haag

The first draft of the present advisory report was prepared by H Stouten, MSc, from the Department of Occupational Toxicology of the TNO Nutrition and Food Research, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: J Toet.
Lay-out: J van Kan.

Chapter **C**

Comments on the public draft

A draft of this report was released in 1999 for public review. The following persons or organisation commented on the draft document:

- A Aalto
  Occupational Safety and Health division, Tampere, Finland
- HJ Weideli
  CIBA Specialty Chemicals, Basel, Switzerland
- E Bomhard and G Stropp
  Bayer AG, Wuppertal, Germany

Chapter **D**

Calculation of the cancer risk of benzotriazole

In the female rats of the low dose group, a statistical significant increase in the incidence of thyroid tumours (C-cell adenomas and carcinomas in 5/43; p=0.028) was found (NCI78).

The incidence of tumour bearing animals per mg/kg bw/day (lifespan conditions, assuming a linear dose-response relationship), I_{dose}, is calculated as follows:

$$I^*_{dose} = \frac{I_e - I_c}{C \times (X_{po}/L) \times (X_{pe}/L) \times \text{exposure hours per day}/24 \times \text{exposure days per week}/7}$$

$$= \frac{15/43 - 0/43}{(295\ \text{mg}/kg/d) \times (78 \times 7^d/1000^d) \times (105 \times 7^d/1000^d) \times 24/24 \times 7/7}$$

$$= 9.8 \times 10^{-4}\ [\text{mg/kg/d}]^{-1}$$

Assuming the average worker lives 75 years, is exposed 8 h/d, 5 d/w, 48 w/y, for 40 years, and inhales 10 m^3 per 8-hour working day and using the estimated incidence of 9.8 x

* I_{dose}= the carcinogenic activity attributable to the exposure to the substance per unit daily dose under lifespan conditions, assuming a linear dose response relationship, usually expressed per mg/m^3 or per mg/kg bw/day.
I_e and I_c = incidence of tumour bearing animals or tumours in exposed and control animals, respectively,
X_{po} = exposure period, X_{pe} = experimental period
L = standard lifespan for the animals in question (L rat is assumed to be 1000 days)

10^{-4} as a starting point, the additional lifetime cancer risk per mg/m^3 under occupational conditions (HBC-OCRV) amounts to:

$9.8 \times 10^{-4} \times 40/75 \times 5/7 \times 48/52 \times 10/70 = 5 \times 10^{-5}$

From this, the additional lifetime cancer risk for 40 years of occupational exposure to 10 mg/m^3 amounts to $10 \times (5 \times 10^{-5}) = 5 \times 10^{-4}$

Chapter **E**

Abbreviations

bp	boiling point
EC_{50}	concentration at which a described effect is found in 50% of the exposed animals or at which the effect is decreased up to 50% of the control value
HBR-OEL	health based recommended occupational exposure limit
h	hour
IC_{50}	concentration at wAbbreviationshich inhibition of a certain function is found up to 50% of the control value
LC_{50}	lethal concentration for 50% of the exposed animals
LC_{lo}	lowest lethal concentration
LD_{50}	lethal dose for 50% of the exposed animals
LD_{lo}	lowest lethal dose
LOAEL	lowest observed adverse effect level
MAC	maximaal aanvaarde concentratie (maximal accepted concentration)
MAEL	minimal adverse effect level
MAK	Maximale Arbeitsplatz Konzentration
MOAEL	minimal observed adverse effect level
MTD	maximum tolerated dose
NAEL	no adverse effect level
NEL	no effect level
NOAEL	no observed adverse effect level
OEL	occupational exposure limit
PEL	permissible exposure limit
ppb	parts per billion $(v/v)10^{-9}$
ppm	parts per million $(v/v)10^{-6}$
RD_{50}	concentration at which a 50% decrease of respiratory rate is observed
REL	recommended exposure limit
STEL	short term exposure limit

tgg	tijd gewogen gemiddelde
TLV	threshold limit value
TWA	time weighted average
V_{max}	maximal reaction velocity of an enzyme

Organisations

ACGIH	American Conference of Governmental Industrial Hygienists
CEC	Commission of the European Communities
DECOS	Dutch Expert Committee on Occupational Standards
DFG	Deutsche Forschungsgemeinschaft
EPA	Environmental Protection Agency (USA)
FDA	Food and Drug Administration (USA)
HSE	Health and Safety Executive (UK)
IARC	International Agency for Research on Cancer (WHO)
INRS	Institut National de Recherche et de Sécurité (France)
NIOSH	National Institute for Occupational Safety and Health (USA)
NTP	National Toxicology Programme (USA)
OECD	Organisation for Economic Cooperation and Development
OSHA	Occupational Safety and Health Association (USA)
RTECS	Registry of Toxic Effects of Chemical Substances
SER	Social and Economic Council (Sociaal-Economische Raad NL)
WATCH	Working Group on the Assessment of Toxic Chemicals (UK)
WHO	World Health Organisation

Toxicological terms

bid	*bis in diem* (twice per day)
bw	body weight
CARA	chronic non-specific respiratory diseases
CHD	coronary heart disease
CNS	central nervous system
ECG	electrocardiogram
EEG	electro encephalogram
FCA	Freunds Complete Adjuvans
FEV	forced expiratory volume
FSH	follicle stimulating hormone
GD	gestation day(s)
GPMT	guinea pig maximisation test
GSH	glutathione
HLiA	hamster liver activated
IHD	ischaemic heart disease
im	intramuscular
ip	intraperitoneal
ipl	intrapleural
it	intratracheal
iv	intravenous
LH	lutheinising hormone
MAC	minimal alveolar concentration
MFO	mixed function oxidase

NA	not activated
PNS	peripheral nervous system
po	*per os* (= oral)
RBC	red blood cells
RLiA	rat liver activated
SCE	sister chromatid exchange
sc	subcutaneous
UDS	unscheduled DNA-synthesis

Statistical terms

GM	geometric mean
OR	Odds Ratio
RR	relative risk
SD	standard deviation
SEM	standard error of mean
SMR	standard mortality ratio

Analytical methods

AAS	atomic absorption spectroscopy
BEEL	biological equivalent exposure limit
BEI	biological exposure index
BEM	biological effect monitoring
BM	biological monitoring
ECD	electron capture detector
EM	environmental monitoring
FID	flame ionisation detector
GC	gas chromatography
GLC	gas liquid chromatography
GSC	gas solid chromatography
HPLC	high performance liquid chromatography
IR	infrared
MS	mass spectrometry
NMR	nuclear magnetic resonance
PAS	personal air sampling
TLC	thin layer chromatography
UV	ultraviolet