Danish Ministry of the Environment
Environmental Protection Agency

Benzotriazole and Tolyltriazole

Evaluation of health hazards and proposal of health based quality criteria for soil and drinking water

Environmental Project No. 1526, 2013

Title:

Benzotriazole and Tolyltriazole. Evaluation of health hazards and proposal of health based quality criteria for soil and drinking water

Author:

Vibe Beltoft
Elsa Nielsen
Ole Ladefoged
Division of Toxicology and Risk Assessment
National Food Institute, Technical University of Denmark

Published by:

The Danish Environmental Protection Agency
Strandgade 29
1401 Copenhagen K
Denmark
www.mst.dk/english

Year:

Authored in 2006
Published in 2013

ISBN no.

978-87-93026-81-0

Content

Preface

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to Benzotriazole and Tolyltriazole and a proposal of health based quality criteria for soil and drinking water. This resulted in 2006 in the present report, which was prepared by Vibe Beltoft, Elsa Nielsen and Ole Ladefoged, Department of Toxicology and Risk Assessment, Danish Institute for Food and Veterinary Research, i.e. the present Division of Toxicology and Risk Assessment, National Food Institute, Technical University of Denmark.

The report has been subjected to review and discussion and has been endorsed by a steering committee consisting of representatives from the following Danish authorities:

The Danish Nature Agency,
The Danish Ministry of Food, Agriculture and Fisheries (The Faculty of Agricultural Sciences),
The Danish Veterinary and Food Administration,
The National Board of Health, Denmark,
Danish Regions (former Amternes Videncenter for Jordforurening)
The Danish Environmental Protection Agency.

The Danish Environmental Protection Agency
Copenhagen, December 2013.

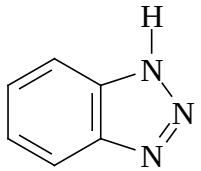
1 General description

This document considers the toxicity of 1) benzotriazole and 2) tolyltriazole. The term tolyltriazole (CAS 29385-43-1) is generally used for the commercial mixture composed of approximately equal amounts of 4- and 5-methylbenzotriazole with small quantities of the 6- and 7- methyl isomers (TNO BIBRA 1998).

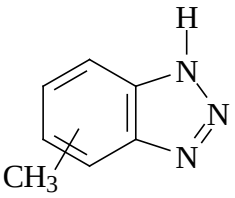
1.1 Identity

Molecular formula: 1) $C_6H_5N_3$
2) $C_7H_7N_3$

Structural formula: 1)



2)



Molecular weight: 1) 119.13
2) 133.17

CAS-no.: 1) 95-14-7
2) 29385-43-1

Synonyms: 1) 1,2-Aminozophenylene
Azimidobenzene
Aziminobenzene
Benzene azimide
1H-Benzotriazole
1,2,3-Benzotrazole
Benztriazole
Benzisotriazole

2) Methylbenzotriazole
Methyl-1H-Benzotriazole
Tolyltriazol

1.2 Physical / chemical properties

Description:	1) Benzotriazole occurs as an odourless, white to light tan, crystalline powder. 2) Tolyltriazole occurs as tan to light brown granules with a characteristic odour.
Purity:	-
Melting point:	1) 98.5°C 2) 76-87°C
Boiling point:	1) 204°C (at 15 mm Hg), 159°C (at 2 mm Hg) 2) 210-212°C (at 12 mm Hg), 160°C (at 2 mm Hg)
Density:	1) - 2) 1.24 g/ml
Vapour pressure:	1) - 2) 0.03 mmHg (Pa) (at 20°C)
Concentration of saturated vapours:	1) - 2) 39 ppm (220 mg/m^3) (calculated) (20°C/760 mmHg)
Vapour density:	1) 4.1 2) 4.6 (air = 1)
Flash point:	1) - 2) 182.2°C
Flammable limits:	-
Autoignition temp.:	-
Solubility:	1) Water: 1-5 g/l (at 23.7°C). Soluble in alcohol, benzene, toluene, chloroform, and dimethylformamide. 2) Water: <0.1 g/l (at 18°C). Alcohol: ≥100 g/l (at 21°C), acetone: ≥100 g/l (at 21°C). Soluble in methanol, isopropanol, ethylene glycol, toluene and methyl ethyl ketone.
References:	NTP (1991a), NTP (1991b), Merck Index (1996), HSDB (1998), TNO BIBRA (1998), Chemfinder (1999).

1.3 Production and use

Benzotriazole is used as a component of aircraft de-icing fluid, pickling inhibitor in boiler scale removal, restrainer, developer and antifogging agent in photographic emulsions, corrosion inhibitor for copper, chemical intermediate for dyes, in pharmaceuticals, and as fungicide. (HSDB 1998).

Tolyltriazole is used as inhibitor of corrosion of copper and copper alloys, in antioxidants, and photographic developers (NTP 1991b).

In Denmark, benzotriazole and tolyltriazole are reported to be used in small amounts (0.1-0.2 %) in de-icing fluids, e.g. propylene glycol (MST 1999). They are also used as a corrosion inhibitor in antifreeze chemicals containing glycol (MST 2000).

1.4 Environmental occurrence

In Denmark, benzotriazole and tolyltriazole have been detected in drainage water from de-icing platforms in Kastrup Airport and in the ground water besides the platforms. Around 30 µg/l of benzotriazole and around 160 to 180 µg/l of tolyltriazole were measured in the ground water besides the platforms. (MST 1999).

No further data considering environmental occurrence have been found. However, under laboratory conditions, neither barley nor tomatoes were effected by the exposure to benzotriazole (barley roots were dipped or the intact tomatoplants were sprayed). Also under laboratory conditions, different strains of fish were exposed for one day to 5000 or 10000 µg/l benzotriazole in an aquatic environment. Effects on behaviour were reported for fish exposed to 5000 µg/l, and mortality was observed among the fishes exposed to 10000 µg/l (no further data). (Ecotoxicology database as of September the 12th 2000).

1.5 Environmental fate

No data were found.

1.6 Human exposure

No data were found.

2 Toxicokinetics

2.1 Absorption, distribution

No data were found.

2.2 Elimination

Benzotriazole was metabolised by rat liver microsomes *in vitro* to 4-hydroxy-benzotriazole and 5-hydroxy-benzotriazole (Hoffmann and Pooth 1982 - quoted from Patty 1994).

2.3 Mode of action

No data were found.

3 Human toxicity

3.1 Single dose toxicity

No data were found.

3.2 Irritation

No data were found.

3.3 Sensitisation

Four cases have been described, where patch tests revealed allergic type reaction to products containing benzotriazole (Ducombs et al. 1980).
In one case, a male metal worker presented with contact dermatitis of the hands and forearms. Patch tests revealed allergic type reactions to lubricating oil used by the worker. Benzotriazole was isolated from this oil. Re-testing with benzotriazole gave a strong positive reaction and histology of the skin showed an eczematous reaction.
In another case of a worker with eczema of the hands, face and neck patch tests were positive to benzotriazole as well as to other components of industrial greases to which the worker was in daily contact.
Two other subjects with contact dermatitis had weakly positive tests with benzotriazole.

Benzotriazole (1% in petrolatum) has been included in special patch test series for car mechanics and metal workers with contact dermatitis. None of the 145 patients reacted to benzotriazole in 48-hour covered patch tests. (De Boer et al. 1989, Meding et al. 1994).

3.4 Repeated dose toxicity

No data were found.

3.5 Toxicityto reproduction

No data were found.

3.6 Mutagenic and genotoxic effects

No data were found.

3.7 Carcinogenic effects

No data were found.

4 Animal toxicity

4.1 Single dose toxicity

4.1.1 Inhalation

4.1.1.1 Benzotriazole

Groups of 10 male rats were exposed to benzotriazole at concentration levels of 780, 1460, 2030, 2230, and 2710 mg/m^3 for 3 hours. Mortality was observed at all concentration levels with the LC_{50}-value being 1910 mg/m^3 (95% confidence limits = 1590 to 2290 mg/m^3). Clinical observations included deep abdominal breathing with open mouth gasping. Gross necropsy revealed a severe accumulation of white frothy fluid in the trachea and a moderate to severe incidence of dark red haemorrhagic areas in the lungs (number of animals with symptoms were not stated). (Sherwin-Williams 1976 - quoted from BIBRA 1995; EPA/OTS 1989 - quoted from TOXLINE pre 1982 - 1990).

4.1.1.2 Tolyltriazole

Bleeding of the respiratory tract, probably due to local irritation, and effects on the liver and kidneys was seen in rats exposed to tolyltriazole (aerosol) at 1730 mg/m^3 for one hour. The LC_{50}-value was reported to be > 1730 mg/m^3. (Sherwin-Williams 1976b - quoted from TNO BIBRA 1998).

Respiratory irritation, indicated by a decrease in breathing rate, was observed in mice exposed to tolyltriazole at 95 to 323 mg/m^3 for 3 hours (Detwiler-Okabayashi and Schaper 1996 - quoted from TNO BIBRA 1998).

4.1.2 Oral intake

4.1.2.1 Benzotriazole

The reported LD_{50}-values for benzotriazole in rats are in the range from 500 to 965 mg/kg b.w. In mice, the LD_{50}-values reported are in the range from 615 to 831 mg/kg b.w., however, a single study reported an LD_{50}-value of > 4500 mg/kg b.w. (strain not stated). In the guinea pig, an LD_{50}-value of 500 mg/kg b.w. benzotriazole has been reported. (Anon. 1953, Geigy Co. 1964, Sherwin-Williams Co. 1976, NCI 1978 - all quoted from BIBRA 1995). Death following the administration of the fatal doses occurred within two days (Sherwin-Williams Co. 1976 - quoted from BIBRA 1995). The acute toxicity studies suggested that benzotriazole acts on the central nervous system (no further data available) (Sherwin-Williams Co. 1976 - quoted from BIBRA 1995).

In rats receiving benzotriazole by gavage (130, 200, 300, 450, 670, or 2250 mg/kg), the clinical observations included initial weight loss, temporary prostration and lethargy. Gross necropsy findings were not reported. Mortality was observed at the two highest dose levels (number of deaths were not stated). (EPA/OTS 1989 - quoted from TOXLINE pre 1982 - 1990).

Groups of 5 rats were given benzotriazole, by gavage at dose levels of 46.4, 100, 215 mg/kg. No mortalities were observed at any dose level. Clinical observations included depressed righting and placement reflexes among animals at all three dose levels.
In other groups of 5 rats, benzotriazole was given by gavage at 464, 1000 or 2150 mg/kg. Mortality was observed within 14 days of dosing (4 rats each in the 1000 and 2150 mg/kg) with the LD_{50}-value being 909 mg/kg (95% confidence limits = 546 to 1510 mg/kg). At the high dose level, observations included depressed righting and placement reflexes, absence of pain, shallow respiration, followed by death. Gross necropsy revealed paled extremities, slight to moderate congestion (of blood or mucus? - was not stated in the abstract) of lungs, kidneys and adrenals, and slight irritation of the small intestines. (EPA/OTS 1989 - quoted from TOXLINE pre 1982- 1990).

4.1.2.2 Tolyltriazole

The LD_{50}-values reported for tolyltriazole were in the range of 675 to 3400 mg/kg b.w. in rats; however, it is not clear whether the compound tested was the commercial tolyltriazole or the specific isomer 5-methylbenzotriazole. An LD_{50}-value for 5-methylbenzotriazole in mice was reported to be 800 mg/kg b.w. (Sherwin-Williams 1972c, Olin Mathieson 1968, Olin Corporation 1973, Eastman Kodak 1975 - all quoted from TNO BIBRA 1998).

Administration by gavage of 1 to 100 mg/kg b.w./day of 5-methylbenzotriazole for 2 weeks did not produce any overt signs of toxicity in rats (10 animals/sex/group). Blood analysis and tissue examination of the major organs revealed no adverse effects. (Komsta et al. 1989 - quoted from TNO BIBRA 1998).

In rats given 500 mg/kg b.w. and above of tolytriazole by gavage caused central nervous system effects. Effects on lungs, stomach and liver were seen at doses of 1000 mg/kg b.w. and above. Death occurred at higher doses, generally 2000 mg/kg b.w. and above (no further details given, it is not clear whether the test material was tolyltriazole or 5-methylbenzotriazole). (Olin Mathieson 1968 - quoted from TNO BIBRA 1998).

4.1.3 Dermal contact

4.1.3.1 Benzotriazole

Five female rabbits received a single application of 2000 mg/kg to the clipped skin for 24 hours. No mortality was observed. Gross necropsy findings were not reported. (Sherwin-Williams Co. 1976 - quoted from BIBRA 1995; EPA/OTS 1989 - quoted from TOXLINE pre 1982- 1990).

4.1.3.2 Tolyltriazole

The LD_{50}-value reported for tolyltriazole (24 hours, covered contact) for rabbits was > 2000 mg/kg b.w. The LD_{50}-value for 5-methylbenzotriazole (24 hours, covered contact) for guinea-pigs was reported to be > 1000 mg/kg b.w. (TNO BIBRA 1998).

4.2 Irritation

4.2.1 Skin irritation

4.2.1.1 Benzotriazole

Signs of skin irritation were seen in three out of five rabbits following a 24-hour covered contact with 2000 mg/kg b.w. to abraded skin **[enheden (mg/kg b.w.) er korrekt citeret]**. No irritation was reported after 72 hours when about 80 mg/cm^2 was applied to the intact or abraded skin of 12 rabbits and covered for 24 hours. (Sherwin-Williams Co. 1976 - quoted from BIBRA 1995).

Benzotriazole was mildly irritating to guinea pigs at a concentration of 50% in ethanol (McAlack 1974 - quoted from BIBRA 1995).

4.2.1.2 Tolyltriazole

Covered 24-hour application of neat tolyltriazole to abraded rabbit skin caused slight skin redness in three of five animals; however, in a similar test, no irritation was noted in a further six animals. No redness or swelling was evident following similar exposure of intact skin (5-6 rabbits/ group) (Sherwin-Williams 1972a, 1972b - quoted from TNO BIBRA 1998).

Covered 24-hour contact with a 40% aqueous paste caused slight swelling of abraded skin and redness of the intact and abraded skin of four rabbits (it is not clear whether tolyltriazole or 5-methylbenzotriazole was applied in this study (Olin Mathieson 1968 - quoted from TNO BIBRA 1998).

An industrial grade (no further details given) of neat 5-methylbenzotriazole was irritating to rabbits given a 24-hour covered patch (Olin corp. 1973 - quoted from TNO BIBRA 1998).

In guinea pigs, slight skin irritation was observed with 24-hour covered exposure to neat 5-methylbenzotriazole (Eastman Kodak 1975 - quoted from TNO BIBRA 1998).

No skin reactions were seen when ten guinea pigs were given a 24-hour covered patch test with 10% tolyltriazole in petrolatum (Ciba Geigy 1982a - quoted from TNO BIBRA 1998).

4.2.2 Eye irritation

4.2.2.1 Benzotriazole

Benzotriazole (100 mg of dry powder) was a severe irritant in rabbits producing complete corneal opacity in four out of six rabbits (McAlack 1974, Sherwin-Williams 1976 - quoted from BIBRA 1995).

4.2.2.2 Tolyltriazole

Tolyltriazole was an eye irritant in rabbits following instillation of 100 mg neat material or 0.1 ml of a 35% solution in isopropanol (approximately 35 mg) (Sherwin-Williams 1977 - quoted from TNO BIBRA 1998). Administration of 10

mg tolyltriazole caused mild to moderate eye irritation in six rabbits. (Olin Mathieson 1968 - quoted from TNO BIBRA 1998).

4.3 Sensitisation

4.3.1.1 Benzotriazole

Group of ten male and ten female guinea pigs were given several intra-dermal injections of 0.1% of benzotriazole in saline together with an adjuvant. This was followed 2 weeks later by a challenge intra-dermal injection using the same concentration and, after a further 1 week, a 24-48- hour covered dermal application using a 30% solution in petroleum. No skin reactions indicative of sensitisation were found.

In another study, a 1% solution of benzotriazole in saline were given intra-dermally for several times to guinea pigs (10 animals/sex/group) followed 1 week later by a single 48-hour covered skin application of 30% in petrolatum. After a 2-week rest period, a challenge (24/48-hour) covered skin application was made with the same concentration. A slight redness to the challenge application was seen in three animals. No skin reactions indicative of sensitisation were seen when a purified preparation was similarly tested.
(Maurer and Meier 1984 - quoted from BIBRA 1995).

4.3.1.2 Tolyltriazole

Tolyltriazole failed to induce skin sensitisation in twenty guinea pigs (ten of each sex) when given as multiple subcutaneous injections of a diluted solution over 3-week period and followed by a challenge firstly on week 5 by another intradermal injection, and then during week 7 by covered 24-hour skin contact with a 10% solution in petrolatum. (Giba Geigy 1982a - quoted from TNO BIBRA 1998).

4.4 Repeated dose toxicity

4.4.1 Inhalation

No data were found.

4.4.2 Oral intake

4.4.2.1 Benzotriazole

Subchronic feeding studies were conducted with F344 rats and B1C3F1 mice (5 animals/sex/group) to estimate the maximum tolerated doses of benzotriazole (NCI 1978).
Benzotriazole was administered in the diet 7 days/week for 8 weeks at doses of 0, 300, 1000, 3000, 10.000, or 30.000 ppm (in rats equivalent to 0, 15, 50, 150, 500, or 1500 mg/kg b.w./day; in mice equivalent to 0, 45, 150, 450, 1500, or 4500 mg/kg b.w./day).
In rats, the mean body weight depressions were no greater than 12% at dose levels ranging from 300 to 10.000 ppm when compared to the control group; at 30.000 ppm, the body weight depression was 40 and 34% for males and females, respectively.

In mice, only a slight body weight depression of approximately 5% was seen in both sexes at 30.000 ppm.

Fischer 344 rats (50 animals/sex/group) were fed benzotriazole at dietary levels of 0, 6700 or 12100 ppm (time-weighted average dose) (equivalent to around 0, 335, or 605 mg/kg b.w./day) for 78 weeks and then observed for 27 weeks. (NCI 1978).
Survival of animals in dosed and control groups was at least 60%. Decreased growth and cellular effects on several tissues, particularly in the liver, kidney, prostate, and uterus, were reported at both dose levels but in a non-dose dependent manner:
Different cytoplasmic changes in the liver cells (clear cell, eosinophilic, and basophilic alterations) were observed in both dose groups (low-dose male rats: from 4/46 (9%) to 13/46 (28%); high-dose male rats: from 6/45 (13%) to 11/45 (24%); low-dose female rats: from 3/48 (6%) to 28/48 (58%); and high-dose female rats: from 4/50 (8%) to 37/50 (74%)).
In the kidney, nephrosis was seen only in the dosed groups (low-dose male rats: 40/45 (89%); high dose male rats; 36/45 (78%); low-dose female rats: 16/48 (33%); high dose female rats: 17/50 (34%)). Nephropathy was observed in 73% of male control rats, and in 37% of female control rats; none were observed in the exposed animals.
In the prostate, inflammation was seen in 49% of low-dose and in 27% of high-dose male rats.
In uterus, inflammation was observed in 24% of the female rats in both low- and high-dose groups. Acute inflammation was seen in the ovary at 9% in the low-dose and 4% in the high-dose groups.
Other effects observed in female rats were bronchiostasis in the lungs (10%) and inflammation in the pancreas (8%); both effects were observed in the low-dose group only.
In this study, the LOAEL was 335 mg/kg b.w./day.

B6C3F1 mice (50 animals/sex/group) were administered benzotriazole at dietary levels of 0, 11700, or 23500 ppm (equivalent to 0, 1755, or 3525 mg/kg b.w./day) for 104 weeks and then observed for 2 weeks. On arrival at the laboratory, the mice showed evidence of intestinal parasites and were therefore administered piperazine adipate in the drinking water (3.0 g/l) for two three-day periods, with a three-day interval between the two periods. Before the study was initiated, the mice were quarantined for two weeks. (NCI 1978).
At the end of the study, survival of animals in dosed and control groups was at least 60%.
Decreased growth and damage to the bone marrow, lymph nodes and to some other organs were observed at both dose levels, but not in a dose dependent manner.
Damage to the bone marrow were observed in female mice (in 21/47 (45%) in the low-dose group, and in 13/48 (27%) in the high-dose group), but not in male mice.
Damage to the lymph nodes (necrosis) were observed in the dosed male mice (low-dose 13/35 (37%); high-dose 13/43 (30%)). Haemorrhage was observed in lymph nodes in dosed female mice in 9/42 (21%) in the low-dose group, and in 4/44 (9%) in the high-dose group.
In the kidneys, nephrosis was observed in low-dose male mice (21/43 (49%)) and in female mice (low-dose: 25/48 (52%); high-dose: 3/50 (6%)).
In the spleen, hyperplasia was observed in low-dose male (3/43 (7%)) and female mice (5/47 (11%)) and erythropoiesis in low-dose male mice (3/43 (8%)).
In the lungs, haemorrhage was observed in high-dose male mice (5/46 (11%)), and hyperplasia was observed in low-dose male mice (4/43 (9%)). In female mice, inflammation of the lungs (3/49 (6%)) was observed in both dose groups.
In this study, the LOAEL was 1755 mg/kg b.w./day.

Other observations reported was parasitism in the colon (no further explanation) in both rats and mice. It was observed in male rats in the low- (5%) and in the high-dose group (14%) and in female rats in the high-dose group (4%). In mice, parasitism in the colon was reported in low- (10%) and high-dose male mice (5%).

4.4.2.2 Tolyltriazole

Fifteen male rats were exposed to 0.5% 5-methylbenzotriazole, about 375 mg/kg b.w./day, in the diet for eight weeks and observed for another 8 weeks; no deaths were reported (no further details are given) (Clayton and Abbott 1958 - quoted from TNO BIBRA 1998).

4.4.3 Dermal contact

No data were found.

4.5 Toxicityto reproduction

No data were found.

4.6 Mutagenic and genotoxic effects

4.6.1.1 Benzotriazole

Benzotriazole was positive in *S. typhimurium* (in the presence but not in the absence of a liver metabolic activation system) and in *E. coli* mutagenicity assays (both in the presence and absence of a liver metabolic activation system) (Dunkel et al. 1985 - quoted from Patty 1994).

Zeiger et al. (1987) reported that benzotriazole was weakly positive in the *Salmonella*/microsome test both in the absence and presence of liver S-9.

No evidence of DNA damage in the SOS chromotest using *E. Coli* was seen in the presence and absence of a liver metabolic activation system (von der Hude et al. 1988).

Benzotriazole was reported to induce chromosome aberrations and sister chromatid exchanges (no further details were given) (NIEHS 1999).

No *in vivo* studies were found.

4.6.1.2 Tolyltriazole

In Ames tests, tolyltriazole was mutagenic or weakly mutagenic to *Salmonella typhimurium* in the presence, but not in the absence, of a liver metabolic activation fraction (Ciba Geigy 1982b, Crowley and Margard 1978, Zeiger et al. 1988 - all quoted from TNO BIBRA 1998).
5-Methylbenzotriazole gave no convincing evidence of a mutagenic effect when tested similarly at up to 1 mg/plate (Blakey et al. 1994 - quoted from TNO BIBRA 1998 and from MEDLINE 1993-1994).

5-Methylbenzotriazole did not induce chromosome aberrations in hamster cells, with or without the addition of a metabolic activation fraction derived from rat liver (Blakey et al. 1994).

Tolyltriazole did not cause the transformation of mouse cells and did not damage the DNA of human lung cells. Metabolic activation systems were not used in these studies. (Crowley and Margard 1978 - quoted from TNO BIBRA 1998).

No *in vivo* studies were found.

4.7 Carcinogenic effects

4.7.1.1 Benzotriazole

Fischer 344 rats (50 animals/sex/group) were fed benzotriazole at dietary levels of 6700 or 12100 ppm (time-weighted average dose) (equivalent to around 335 or 605 mg/kg b.w./day) for 78 weeks and then observed for 27 weeks.
Survival of animals in dosed and control groups was at least 60%.
In male rats, neoplastic nodules of the liver occurred at a statistically significant incidence (P = 0.024) in the high-dose group when compared with the control group (controls 0/48, low-dose 0/46, high-dose 5/45 (11%)). According to the authors, these tumours cannot be clearly associated with administration of the test chemical because the incidence in the high-dose group is no higher than has been observed generally in control groups at the same laboratory (0 to 11%).
Brain tumours occurred in three low-dose male rats (one oligodendroglioma, two gliomas), in one high-dose female rat (glioma), and in none of the controls. According to the authors, the occurrence of this rare tumour in dosed animals is suggestive of, but not considered as sufficient evidence of, carcinogenicity.
In female rats, the incidence of endometrial stromal polyps in the low-dose group was significantly higher (P = 0.010) than that in the corresponding controls (controls 2/48, low-dose 10/45, high-dose 8/49). According to the authors, these tumours cannot be associated with administration of the test chemical because the incidence in the high-dose group was not significant, and because when the incidences of endometrial stromal polyps and endometrial stromal sarcomas were combined, they were not significant in either the low- or high-dose groups.
A none-dose related increase in the incidence of C-cell adenomas and carcinomas of the thyroid was reported. The incidence in the control rats was 0/43, in the low-dose group 5/43, and in the high-dose group 3/50.
Benign thyroid tumours were seen in low-dose female rats (4/43 (9%)) while malignant thyroid tumours occurred in low-dose (1/43 (2%)) and high-dose (3/50 (6%)) female rats; there was no statistically significant increase in thyroid tumour incidence in the male rats. According to the authors, previous results in other laboratories have shown the incidence of these types of benign and malignant thyroid tumours in untreated females to be 4-5% and 1-4%, respectively.
(NTP 1999; NCI 1978).

B6C3F1 mice (50 animals/sex/group) were administered benzotriazole at dietary levels of 11700 or 23500 ppm (equivalent to 1755 or 3525 mg/kg b.w./day) for 104 weeks and then observed for 2 weeks. Survival of animals in dosed and control groups was at least 60%. In female mice, alveolar/bronchiolar carcinomas occurred at a statistically significant incidence (P = 0.001) in the low-dose group when compared with the control group (controls 0/49, low-dose 9/49 (18%), high-dose (3/49 (6%)). According to the authors, the occurrence of this tumour in female mice cannot be clearly related to the administration of the test chemical because the incidence in the high-dose group was not significant, and the data did not show a

dose-related trend; furthermore, the incidence of these tumours in control female mice at the same laboratory has varied from 0 to 7% with a mean of 4%. In male mice, no tumours occurred in dosed groups at incidences that were significantly higher than those in controls. (NTP 1999; NCI 1978).

4.7.1.2 Tolyltriazole

The incidence of liver tumours produced by a known carcinogen was not altered by concomitant exposure of 15 rats to dietary levels of 0.5% tolyltriazole (equal to about 375 mg/kg b.w./day) for 8 weeks, when examined at week 16. (Clayton & Abbott 1958 - quoted from TNO BIBRA 1998).

5 Regulations

5.1 Ambient air

-

5.2 Drinking water

-

5.3 Soil

-

5.4 Occupational Exposure Limits

-

5.5 Classification

-

5.6 IARC

-

5.7 US-EPA

-

6 Summary and evaluation

6.1 Description

Benzotriazole is an odourless white to light tan crystalline powder. Tolyltriazole is tan to light brown granules with a characteristic odour.

6.2 Environment

In Denmark, benzotriazole and tolyltriazole have been detected in the ground water besides de-icing platforms in Kastrup Airport at around 30 µg/l or 160 to 180 µg/l, respectively.

6.3 Human exposure

No data were found.

6.4 Toxicokinetics

In an *in vitro* metabolism study, benzotriazole was metabolised to 4- and 5-hydroxy-benzotriazole. No other data have been found.

6.5 Human toxicity

Patch tests have revealed a weakly positive (two cases) to positive (two cases) response for allergic reaction to benzotriazole for metal workers with either contact dermatitis (three cases) or eczema (one case).
However, in special patch test series for car mechanics and metal workers with contact dermatitis, none of the 145 patients tested reacted to benzotriazole.

6.6 Animal toxicity

6.6.1 Single dose toxicity

For benzotriazole, most the oral LD_{50}-values reported are in the range of 500 to 1000 mg/kg b.w. (rats, mice and guinea pigs). For tolyltriazole, the LD_{50}-values reported range from 675 to 3400 mg/kg b.w. (rats and mice). Clinical observations suggest that benzotriazole (dose levels from around 50 mg/kg b.w.) and tolyltriazole (dose levels from 500 mg/kg b.w.) act on the central nervous system. Following dermal contact, the LD_{50}-values reported for rabbits are above 2000 mg/kg b.w. for both triazoles.

6.6.2 Irritation

For benzotriazole, skin irritation has been noted in rabbits at a high dose level (2000 mg/kg b.w.) when applicated to abraded skin; no skin irritation was seen in rabbits following application (80 mg/cm^2) to abraded or intact skin. Benzotriazole (dry powder) was a severe eye irritant when instilled into rabbit eyes.

For tolyltriazole, the data on skin irritation are equivocal as both positive and negative results have been reported for rabbits following application of the test material to abraded or intact skin. Tolyltriazole (dry powder or in solution) was reported to be an eye irritant in rabbits.

6.6.3 Sensitisation

Benzotriazole did not show skin sensitisation in guinea pigs (2 studies).

Tolyltriazole did not induce skin sensitization in guinea pigs (1 study).

6.6.4 Repeated dose toxicity

In Fischer 344 rats, decreased growth and cellular effects in particularly the liver, prostate and uterus were reported following administration of benzotriazole at dietary levels corresponding to about 335 or 605 mg/kg b.w./day for 78 weeks. In B6C3F1 mice, decreased growth and damage to the bone marrow and lymph nodes, and to some other organs were observed following dietary administration of benzotriazole corresponding to about 1755 or 3525 mg/kg b.w./day for 104 weeks.

No deaths were reported in 15 rats orally exposed to 5-methyltriazole at about 375 mg/kg b.w./day for eight weeks.

6.6.5 Toxicity to reproduction

No data were found.

6.6.6 Mutagenic and genotoxic effects

Benzotriazole has shown positive results in *S. typhimurium* and in *E. coli* mutagenicity assays; in the SOS chromotest using *E. Coli* no evidence of DNA damage was found. Benzotriazole has been reported to induce chromosome aberrations and sister chromatid exchanges.

Tolyltriazole was reported to be mutagenic in *S. typhimurium* in the presence but not in the absence of a metabolic activation system, whereas 5-methyltriazole did not show evidence of a mutagenic effect when tested similarly. 5-Methyltriazole did not induce chromosome aberrations in hamster cells. Tolyltriazole did not induce cell transformation in mouse cells and did not damage the DNA of human lung cells.

No *in vivo* data have been found for either benzotriazole or tolyltriazole.

6.6.7 Carcinogenic effects

In Fischer 344 rats, an increased incidence of liver, brain, uterus and thyroid tumours were observed in treated animals fed benzotriazole in the diet (about 335 or 605 mg/kg b.w./day for 78 weeks and observed for a further 27 weeks) when compared to the control animals. In treated female B6C3F1 mice (about 1755 or 3525 mg/kg b.w./day of benzotriazole in the diet for 104 weeks), a higher incidence of lung tumours were observed when compared to untreated females.

6.7 Evaluation

The data on human health effects of benzotriazole and tolyltriazole (triazoles) are very limited.

Different results concerning patch tests have been reported: Four cases reported that patch tests revealed a positive or weakly positive response for allergic type reaction to benzotriazole with contact dermatitis. However, in special patch test series for workers with contact dermatitis, none of the 145 patients tested reacted to benzotriazole.
This is supported by the animal data which have shown that benzotriazole and tolyltriazole did not induce skin sensitisation in guinea pigs (3 studies).
Based on these data, benzotriazole is considered to have only a very weak potential for skin sensation, if any.

Both benzotriazole and tolyltriazole have showed moderate acute toxocity as most of the oral LD_{50}-values reported for rodents are in the range of 500 to 3400 mg/kg b.w.

In a 78-week study of rats and a 2-year study of mice, decreased growth and effects on various organs and tissues were reported following dietary administration of benzotriazole at 335 or 605 mg/kg b.w./day (rats) and 1755 or 3525 mg/kg b.w./day (mice).
In rats, effects on the liver (clear cell, eosinophilic, and basophilic alternations), on the kidney (nephrosis), and inflammation (in the prostate in males, and in the uterus and ovary in the females) were observed at both dose levels in both sexes, but not in a dose dependent manner. Parasitism was seen in both male and female rats.
In mice, damage to the bone marrow (female mice), lymph nodes (necrosis in both male and female mice), kidneys (nephrosis in male mice), lungs (haemorrhage and hyperplasia in male mice, inflammation in both sexes), and spleen (hyperplasia in both sexes) were observed, but not in a dose-dependent manner.
Based on these studies, a LOAEL of approximately 335 mg/kg b.w./day can be established for rats and approximately 1755 mg/kg b.w./day for mice for various effects observed. However, as both rats and mice were reported to have parasitism, it cannot be excluded that this might possibly have an influence on the animals.

In the 78-week study in rats, adenomas and carcinomas of the liver occurred at a statistically significant incidence in the high-dose group (5/45) when compared with the control group (0/48). The figure for the high dose group is rather high and this should be taken into consideration, when evaluating the evidence of carcinogenicity. Another observation which points towards a carcinogenic effect of benzotriazole was the occurrence of rare brain tumours in three low-dose male rats (one oligodendroglioma and two gliomas) and in one high-dose female (glioma) with no brain tumours observed in the control group. Furthermore, an increased incidence of tumours in the uterus and thyroid has also been observed in the rats.

Low-dose female mice showed a statistical significant increase in lung-tumours; also the high-dosed females showed an increased incidence of lung-tumours compared to control animals, although not statistically significant.
The occurrence of tumours in both rats and mice is suggestive of a possible carcinogenic effect of benzotriazole in both species.

Equivocal results have been reported in mutagenicity and genotoxicity tests of benzotriazole and tolyltriazole *in vitro*; no *in vivo* data have been found.
For benzotriazole, positive results have been obtained in *S. typhimurium* and in *E. coli* both in the presence and absence of metabolic activation. No evidence of DNA damage was found in the SOS chromotest using *E. coli*. In cultured mammalian cells, benzotriazole has been reported to induce chromosome aberrations and sister chromatid exchanges.
Based on the fact that no *in vivo* data were found and that there are only few available *in vitro* data for benzotriazole, a clear conclusion whether benzotriazole is a genotoxic substance cannot be drawn.
Tolyltriazole was reported to be mutagenic in *S. typhimurium* in the presence but not in the absence of metabolic activation, whereas 5-methylbenzotriazole was not mutagenic in *S. typhimurium*. Tolyltriazole was negative in a cell transformation assay (mouse cells) and did not induce DNA damage in human lung cells. 5-methylbenzotriazole did not induce chromosome aberrations in hamster cells with and without metabolic activation.
As for benzotriazole, no *in vivo* data were found and no clear conclusion whether tolyltriazol is genotoxic can be made from the few *in vitro* data reported here.

No long-term studies of tolyltriazole have been found; however, based on the structure-activity relationship between benzotriazole and tolyltriazole (methylbenzotriazole), it is considered that the same type of effects will be observed following administration of tolyltriazole as have been observed for benzotriazole.

6.7.1 Critical effect and NOAEL

The available data do not allow a clear conclusion on which effect(s) to be considered as the critical one(s). A number of effects have been observed in various organs and tissues in rats and mice when benzotriazole was administered at dietary concentrations from 6700 ppm (approximately 335 mg/kg b.w./day) to rats and from 11700 ppm (approximately 1755 mg/kg b.w./day) to mice.

For the purpose of establishing health based quality critera in soil and drinking water, the lowest dose level of approximately 335 mg/kg b.w./day in the 78-week rat study (dietary administration of benzotriazole) is considered to be a LOAEL for the effects which have been observed in various organs and tissues.

7 TDI and quality criteria

7.1 TDI

For the estimation of a TDI, the lowest dose level of approximately 335 mg/kg b.w./day in the 78-week rat study of benzotriazole is considered to be a LOAEL for the effects which have been observed in various organs and tissues following dietary administration of benzotriazole.

$$TDI = \frac{LOAEL}{UF_I * UF_{II} * UF_{III}} = \frac{335 \text{ mg/kg b.w./day}}{10 * 10 * 500} = 0.0067 \text{ mg/kg b.w./day}$$

The uncertainty factor UF_I accounting for interspecies variability is set to 10 assuming that humans are more sensitive than animals. The UF_{II} accounting for intraspecies variability is set to 10 reflecting the range in biological sensitivity within the human population. The UF_{III} is set to 500 because of using a LOAEL in stead of a NOAEL and because it cannot be clearly evaluated whether benzotriazole and tolyltriazole have genotoxic and carcinogenic properties.

7.2 Allocation

The general population can be exposed to benzotriazole and tolyltriazole from other sources such as components in antifreeze chemicals. Therefore, only 10% is allocated to ingestion of soil and 10% to drinking water.

7.3 Quality criterion in soil

The quality criterion in soil QC_{soil} is calculated based on the TDI of 0.0067 mg/kg b.w. per day and assuming a daily ingestion of 0.2 g soil for a child weighing 10 kg (w_{child}):

$$QC_{soil} = \frac{TDI * X * w_{child}}{ingestion_{soil}} = \frac{0.0067 \text{ mg/kg day} * 0.1 * 10 \text{ kg}}{0.0002 \text{ kg/day}}$$

$$= 33.5 \text{ mg/kg soil}$$

7.3.1 Quality criteria in soil

30 mg/kg soil.

7.4 Quality criterion in drinking water

The quality criterion in drinking water QC_{dw} is calculated based on the TDI of 6.7 µg/kg b.w. per day and assuming a daily ingestion of 2 litres of drinking water for an adult weighing 70 kg (w_{adult}):

$$QC_{dw} = \frac{TDI * Y * w_{adult}}{ingestion_{dw}} = \frac{6.7\ \mu g/kg\ day * 0.1 * 70\ kg}{2\ l/day}$$

$$= 23.5\ \mu g/l$$

7.4.1 Quality criterion in drinking water

20 µg/l.

8 References

BIBRA (1995). Toxicity profile. Benzotriazole. 2nd ed. BIBRA international. Great Britain.

Blakey DH, Maus KL, Bell R, Bayley J, Douglas GR, and Nestmann ER (1994). Mutagenic activity of 3 industrial chemicals in a battery of in vitro and in vivo tests. Mutation Research **320**, 273-283.

Chemfinder (1999). Benzotriazole or tolyltriazole. In chemfinder data base. (http://www.chemfinder.com).

De Boer EM, Van Ketel WG, and Bruynzeel DP (1989). Dermatoses in metal workers. (II) Allergic contact dermatitis. Contact Dermatitis **20**, 280-286.

Ducombs G, Tamisier JM, and Texier L (1980). Contact dermatitis to 1-H benzotriazole. Contact dermatitis **6**(3), 224-225.

Ecotoxicology database as of September the 12th 2000. ECETOX: Ecotoxicology database from U.S. EPA. Office of Research and development. National Health and Environmental Effects Research Laboratory. Mid-Continent Ecology Division.

HSDB (1998). Benzotriazole. In: Hazardous Substances Data Base.

IARC (1990). Pathology of tumours in laboratory animals. Volume 1 - tumours of the rat. Eds.: Turusov V and Mohr U. 2nd edition. IARC Scientific Publications. No 99. Lyon.

Meding B, Barregård L, and Marcus K (1994). Hand eczema in car mechanics. Contact Dermatitis **30**, 129-134.

MEDLINE 1993-1994.

Merck Index (1996). 1H-Benzotriazole. 12th. ed., Rahway, New Jersey, Merck & Co., Inc., 186.

MST (1999). Personal communication from Susanne Rasmussen.

MST (2000). Personal communication from Janne Forslund.

NCI (1978). Bioassay of 1H-Benzotriazole for possible carcinogenicity CAS No. 95-14-7. U.S. National Cancer Institut, Bethesda, Md. U.S. Department of Commerce. National Technical Information Service. PB-285 202.

NIEHS (1999). 1,2,3-Benzotriazole. In: National Institute of Environmental Health Sciences (http://ntp-server.niehs.nih.gov/htdocs /Results_status/ResstatB/10453-G.html).

NTP (1999). Bioassay of 1H-Benzotriazole for possible carcinogenicity (CAS No. 95-14-7). Abstract. (http://ntp-server.niehs.nih.gov/htdocs/LT-studies/TR088.html).

NTP (1991a). Benzotriazole. In: Chemical Health and Safety Data, National Toxicological Program (http://ntp-server.niehs.nih.gov/htdocs/ CHEM_H&S/NTP_Chem9/Radian95-14-7.html).

NTP (1991b). Tolyltriazole. In: Chemical Health and Safety Data, National Toxicological Program (http://ntp-server.niehs.nih.gov/htdocs/ CHEM_H&S/NTP_Chem2/Radian29385-43-1.html).

Patty: Patty's industrial hygiene and toxicology (1994) 4th ed.. Edited by Clayton GD and Clayton FE. Vol. II part E, 3346-3347.

TNO BIBRA (1998). Toxicity Profile, Tolyltriazole. 3rd ed. TNO BIBRA International Ltd. Great Britain.

TOXLINE pre 1982-1999.

Von der Hude W, Behm C, Gürtler R, and Basler A (1988). Evaluation of the SOS chromotest. Mutation Research **203**, 81-94.

Zeiger E, Anderson B, Haworth S, Timothy L, Mortelmans K, Speck W (1987). Salmonella Mutagenicity tests: III. Results from the testing of 255 chemicals. Environ Mutagen **9** (Suppl. 9) 1-110.

Benzotriazole and Tolyltriazole

The Danish Environmental Protection Agency has requested an evaluation of health hazards by exposure to to benzotriazole and tolyltriazole. This resulted in 2006 in the present report which includes estimation of quality criteria in soil and in drinking water for the mentioned substances.

Danish Ministry of the Environment
Environmental Protection Agency

Strandgade 29
1401 Copenhagen K, Denmark
Tel.: (+45) 72 54 40 00

www.mst.dk