FOREWORD INTRODUCTION

N-TERT-BUTYLBENZOTHIAZOLE-2-SULPHENAMIDE

CAS N°: 95-31-8

SIDS Initial Assessment Report

For

SIAM 16

Paris, May 27-30, 2003

1. Chemical Name: N-tert-butylbenzothiazole-2-sulphenamide

2. CAS Number: 95-31-8

3. Sponsor Country: Japan

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4. Shared Partnership with: Ouchi-Shinko Chemical Industrial Co., Ltd. and Sanshin Chemical Industry Co., Ltd. The industry contact points are:

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5. Roles/Responsibilities of the Partners: The industry consortium collected new data, prepared the updated IUCLID and drafted versions of the SIAR and SIAP.

- Name of industry sponsor /consortium
- Process used

6. Sponsorship History This substance is sponsored by Japan under the ICCA Initiative and is submitted for first discussion at SIAM 16.

- How was the chemical or category brought into the OECD HPV Chemicals Programme ?

7. Review Process Prior to the SIAM: Japanese government peer-reviewed the documents and audited selected studies.

8. Quality check process: Japanese government peer-review committee performed spot checks on randomly selected endpoints and compared original

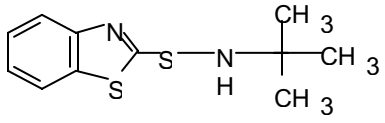
studies with data in the SIDS dossier.

9. Date of Submission: February 21, 2003

10. Date of last Update:

11. Comments:

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	95-31-8
Chemical Name	N-tert-butylbenzothiazole-2-sulphenamide
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

There is no information on toxicokinetics. Based on the available data, it can be concluded that the chemical or its hydrolysis products can be absorbed by oral dermal or inhalation routes. Toxicity may be via the hydrolysis products. Hepatic metabolism of the parent material or the hydrolysis products is likely. Excretion is most likely via the kidney.

The oral LD50 (rat) was greater than 2000 mg/kg [OECD TG 423]. The dermal LD50 (rabbit) was greater than 7940 mg/kg. The substance shows signs of mild irritation in the skin and eyes of rabbits but is not considered to be a skin or eye irritant. A hydrolysis product, mercaptobenzothiazole, is a strong skin sensitiser. This is further supported by animal testing (Buehler assay) and human patch tests with the parent chemical reported in the secondary literature.

In a combined oral repeat dose reproductive/developmental toxicity screening test [OECD TG 422] rats were dosed by gavage at 40, 200 and 1000 mg/kg bw/day. Toxicologically significant effects were identified in liver (hepatocyte hypertrophy), kidneys (vacuolar degeneration) and erythrocytes (haemolytic anaemia).The LOAEL was 40 mg/kg/day for males, based on anaemia (female data unavailable). In a 90 day oral toxicity study, which cannot be validated, females showed increased liver and kidney weight at 1000 mg/kg/day together with increased cholesterol and urine specific gravity. Bodyweight was reduced in males at 300 mg/kg/day and 1000 mg/kg/day. The NOAEL was 100 mg/kg/day. A 28 day repeated exposure inhalation study (5 days per week exposure) has been conducted but cannot be validated. The highest exposure level, 0.084 mg/L, produced a decrease in bodyweight and effects to liver and lymph nodes. The NOAEL was 0.029 mg/L.

Based on these studies the LOAEL for repeated dose toxicity (oral) is considered to be 40 mg/kg/day for males, based on anaemia (female data unavailable). The NOAEL for repeated dose toxicity (inhalation) is 0.029 mg/L (non-validated data).

The chemical was not mutagenic in bacteria [OECD TG 471 and 472] and in several *in vitro* mammalian gene mutation assays. Positive responses, however, were seen in several mouse lymphoma assays in the presence of exogenous metabolic activation. It also induced chromosomal aberrations in mammalian cells *in vitro* in the presence of an exogenous metabolic activation system [OECD TG 473]. Because the chemical was non-mutagenic in bacteria and mammalian cells and clastogenic in mammalian cells, the positive response in the mouse lymphoma assays seemed to be derived from chromosomal aberrations. The chemical was negative in the mouse micronucleus assay [OECD TG 474] tested up to 2000 mg/kg. Accordingly this chemical was clastogenic *in vitro* but not *in vivo*.

For the above mentioned reproduction/developmental toxicity screening test [OECD TG 422], the chemical was

given for 42 days in males and from 14 days before pregnancy to day 3 of lactation in females. No adverse effects were observed in terms of fertility, delivery and nursing in parent animals. The viability and body weight of offspring were unaffected and no malformations were detected. Both male and female reproductive tissues were well examined and no abnormalities were observed. Changes in fertility index were observed at 40 and 1000 mg/kg bw/day, but not at 200 mg/kg bw/day.

In a developmental toxicity study reported in the secondary literature, female rats were dosed with the chemical at up to 500 mg/kg bw/day between days 6-15 of gestation. No effects were seen, either in females or offspring, at any dose level and under the conditions of the test the chemical was considered to have no effect on reproduction. No further details of the study could be obtained. The NOAEL for developmental toxicity was 500 mg/kg bw/day.

Environment

The chemical has a log Pow of 3.9 at room temperature, a vapour pressure of less than 0.0000021 hPa at 25°C and a water solubility of 0.345 mg/L at 20°C. Fugacity model Mackay level III calculations suggest that the majority of the chemical would distribute to soil if released to the air or soil compartments and to water if released to the water compartment. The chemical is not ionised at environmental pHs ($pKa_{(1)}$ 1.75, $pKa_{(2)}$ –3.43, conjugate acid). The chemical is not readily biodegradable (0%) but it does hydrolyse in less than 1 day at pH 9 or less (t1/2 = 1.7h at pH 4, 1.8h at pH 7 and 21.5h at pH 9). The identified hydrolysis products are mercaptobenzothiazole, di(benzothiazoyl-2)disulfide, t-butylamine, and benzothiazole. These hydrolysis products have been tested and shown to have a low potential for bioaccumulation. Based on these findings, the chemical is judged to have low potential for bioaccumulation also. Indirect photo-oxidation by hydroxy radicals is predicted to occur with a half-life estimated at 2.8 hours. The substance does not inhibit the action of wastewater treatment microorganisms EC50 (3h) > 10000 mg/L. The hydrolysis products, such as mercaptobenzothiazole, di(benzothiazoyl-2)disulfide and benzothiazole, are non-volatile and not readily biodegradable in the environment.

In an acute fish toxicity study (OECD TG 203, *Oryzias latipes*, 96h) a LC50 = 0.345 mg/L (limit of water solubility) was reported. In Daphnia magna, an acute toxicity value of 48h EC50 = 0.345 mg/L (limit of water solubility) was reported. The results in algae (OECD TG 201) were an E_rC50 = 0.071 mg/L, a $NOEC_b$ (0 to 72 h, biomass) = 0.011 mg/L and a $NOEC_r$ (24 to 72 h, growth rate) = 0.023 mg/L (measured concentrations). In a prolonged fish toxicity study (OECD TG 204, *Oryzias latipes*) a 14d LC50 = 0.345 mg/L (limit of water solubility) and a 14d NOEC = 0.15 mg/L were presented. The chronic toxicity to *Daphnia magna* (OECD TG 211) was a NOEC (14d, reproduction) = 0.042 mg/L (measured) and NOEC (21d, parental) = 0.068 mg/L (measured).

Some of the degradation products of N-tert-butylbenzothiazole-2-sulfenamide, and especially mercapto-benzothiazole, show similar levels of aquatic toxicity compared to the parent compound.

Exposure

The production volume of the chemical in 2000 is 16,000 – 20,000 t/y worldwide, with production in Japan being 4,000 – 4,500 t/y. The chemical is an accelerator for the vulcanization of rubber. Due to the chemical transformation processes involved in vulcanization, the finished rubber products only contain small amounts of the chemical in addition to substances produced from the synthesis, such as benzothiazole, 2-mercaptbenzothiazole and 2-mercaptobenzothiazole disulfide. The substance and its degradation products may be released to the environment during the use of rubber products, such as tyres.

Occupational hygiene measurements suggest that occupational exposure is 0.64 mg/m^3. Personal Protective equipment (dust masks, goggles, protective clothing, gloves) is worn during operations. Consumer exposure is minimal.

RECOMMENDATION

The chemical is a candidate for further work.

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

The chemical possesses properties indicating a hazard for the environment. Also some of the degradation products show similar hazards to the environment. The substance and its degradation products are present in many rubber products and a release to the environment is possible. An exposure assessment, and if necessary a risk assessment for the environment of the chemical and its degradation products should be performed. The currently on-going assessment of di(benzothiazoyl-2)disulfide (CAS No 120-78-5) and of N-cyclohexylbenzothiazole-2-sulfenamide (CAS No 95-33-0) should be taken into account.

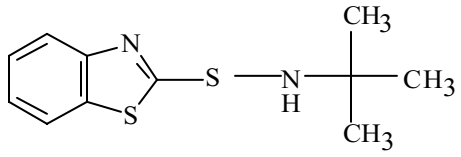
The chemical also possesses properties indicating a hazard for human health (sensitisation and anaemia). An exposure assessment and, if necessary, a risk assessment for human health should be performed.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 95-31-8
IUPAC Name: N-tert-butylbenzothiazole-2-sulphenamide
Molecular Formula: $C_{11}H_{14}N_2S_2$
Structural Formula:



Molecular Weight: 238.39
Synonyms: Benzothiazolesulfenamide, N-(1,1-dimethylethyl)-
Benzothiazolyl-2-tert-butylsulfenamide
Benzothiazyl-2-tert-butylsulfenamid
Cure-rite BBTS
Delac NS
N-(1,1-dimethylethyl)-2-benzothiazolesulfenamide
N-tert,-butyl-2-benzothiazolsulfenamide
N-tert,butyl-2-benzothazyl sulphonamide
Accel TBS-R
Nocceler NS-P
Pennac Tbbs
Perkacit NS
Sanceler NS-G
Santocure NS
Santocure NS vulcanization accelerator
TBBS
TBBS/EGC
Tertiary-butylbenzothiazolesulfenamide
Vanax NS
Vulkacit NZ

1.2 Purity/Impurities/Additives

Purity: 97 % w/w

Impurities included: Dibenzothiazyldisulfide, CAS No. 120-78-5 (ca. 0.8%)

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value
Physical state Melting point	 105 °C
Boiling point	Unmeasurable (degrades at 207 °C)
Relative density	ca. 1.28 – 1.29 g/cm3 at 25 °C
Vapour pressure	< 0.0000021 hPa at 25°C
Water solubility	Water 0.345 mg/L at 20 °C
Partition coefficient n-octanol/water (log value)	Log Pow = 3.9 at room temperature
Henry's law constant	
Hydrolysis	T½ = 1.71 hours at 25 °C, pH 4 T½ = 1.8 hours at 25 °C, pH 7 T½ = 21.5 hours at 25 °C, pH 9
pKa	$pKa_{(1)}$ 1.75, $pKa_{(2)}$ –3.43 (prediction, conjugate acid)

2 GENERAL INFORMATION ON EXPOSURE

- Production of the chemical during 2000 is 16,000 – 20,000 t/y worldwide, with production in Japan 4,000 – 4,500 t/y. The chemical is an organic solid.
- The chemical is used as an accelerator in the vulcanisation of rubber.
- The main source of pollution is emission in the place of use.
- The chemical and/or related substances produced during synthesis may be released from the final products, for example tyres, during use.

2.1 Environmental Exposure and Fate

Fugacity Model Mackay level III (Harada, 2003) calculations suggest that the majority of the chemical would distribute to soil if released to the air or soil compartments and to water if released to the water compartment.

Table 1. Environmental distribution of this substance using Fugacity Model Mackay Level III under three scenarios

Release	Distribution (%)			
	Air	Water	Soil	Sediment
100% to air	0.3	0.0	99.7	0.0
100% to water	0.0	73.6	3.2	23.2
100% to soil	0.0	0.0	100.0	0.0

The chemical is not ionised at environmental pHs ($pKa_{(1)}$ 1.75, $pKa_{(2)}$ –3.43, conjugate acid) (Cuthbert, 2003).

Indirect photo-oxidation by hydroxy radicals is predicted to occur with a half-life estimated at 2.8 hours (calculated using AOPWIN rate constant).

The chemical is not readily biodegradable (0%, OECD TG 301C) (ECB, 2000; ACC 2001)

The substance undergoes rapid abiotic degradation by hydrolysis at pH less than or equal to 7. The identified hydrolysis products are mercaptobenzothiazole, di(benzothiazoyl-2)disulfide, t-butylamine and benzothiazole, all of which have been tested for bioaccumulation and have shown low bioaccumulation characteristics (BCF < 8, OECD TG 305) (METI, 2000)

2.1.1 Environmental Fate of Degradation Products

Di(benzothiazoyl-2)disulfide (MBTS, CAS No 120-78-5) (see corresponding SIAR and BUA, 1994)

Degradation

No degradation has been found in different tests performed for inherent biological degradation. A solution of MBTS in river water proved to be unstable; the half life was estimated to be ca. 9 days. Mercaptobenzothiazol (MBT) was found as transformation product, other products were not identified. It is not known whether decomposition is caused by hydrolysis, or other reactions. Decomposition of MBTS is strongly dependant on the matrix, it proceeds more rapidly in distilled water and organic solvents than in river water (BLfW, 1993).

Photolytic degradation was found in water solution. However, a reaction constant under environmental conditions cannot be derived. A half life of 1.9 hours is calculated for the photochemical-oxidative degradation in atmosphere by hydroxyl radicals. This route of degradation is however of no environmental relevance due to the physical chemical properties of MBT.

Distribution

The water solubility of MBTS is dependent on the pH, values of 88 mg/l (pH 5) and 49 mg/l (pH 9) were measured (SRI, 1980). With a Henry constant of 9.9.10-5 Pa.m3/mol, MBTS is to be classified as non volatile from aqueous solution. Regarding soil adsorption, the log Pow of 4.5 indicates a high geoaccumulation potential. The stability of MBTS in soil is not known. Using the fugacity model Mackay Level I on the distribution in compartments the following values are obtained: water 16.9%, soil 42.9%, sediment 40.1%.

Accumulation

The measured log Pow value of 4.5 indicates a high bioaccumulation potential. However, in tests carried out in carps *(Cyprinus carpio)* BCF values of up to 51 were found. This substance can therefore be classified to have a low bioaccumulation potential.

Mercaptobenzothiazole (MBT, CAS No 149-30-4)

Degradation

In several investigations on biological degradation performed independently, degradation was not observed even after adaptation. Following adaptation of activated sludge to benzothiazole-2-sulfonic acid a low degradation of 10-35 % of MBT was observed. Within another test, a degradation of 70-95 % was achieved after long-term adaptation to a mixture from MBT and benzothiazole (BUA, 1991). As these tests allowed very special adaptations, MBT must be considered as not biodegradable under aerobic environmental conditions.

MBT can methylate biologically to 2-methylthiobenzothiazole. This reaction was shown in a sediment test (Brownlee, 1992) and during the treatment of tannery waste waters (Reemtsma, 1994). From the data available, safe conclusions on the degradation of MBT in municipal sewage treatment plants cannot be made.

Under environment-relevant conditions MBT is stable to hydrolysis. Benzothiazole, 2-benzo-thiazolone and other no further identified benzothiazole derivatives were found as photolysis products in an aqueous solution. In summer, the half life in humic acid water amounts to 3.4 hours and to 29 hours in winter (secondary conditions: clear water close to the surface). By taking into account light deviation due to water dullness and individual absorption of the river water, significantly greater environmental half lives are to be assumed (Brownlee, 1992).

A half life of < 1 d was estimated for the photochemical-oxidative degradation in the atmosphere by hydroxyl radicals (BUA, 1991). This way of degradation is of no environmental relevance due to the physical chemical properties of MBT (see below).

Distribution

On the basis of a water solubility of 0.117 g/l (20oC, pH 7) and a vapour pressure of 2.98.10-6 Pa the Henry-constant is calculated to be 4.2.10-6 Pa.m3.mol-1 (BUA, 1991). MBT is thus to be considered as not volatile from hydrous solution. In combination with metals such as Al, Cu and Zn, MBT forms poorly soluble complexes (Bosch, 1985). Koc values between 326 and 1829 have been measured for different soil types and between 2130 and 3560 for sediments. An exact analysis of the two investigations available does however show that MBT adsorbs to a relatively small

extent onto the organic fraction, and more onto clay minerals. Mobility in soil is medium to low (BUA, 1991).

Accumulation

The measured log Pow of 2.42 does not indicate a bioaccumulation potential. This statement is confirmed by the measured BCF values of < 8 (BUA, 1991).

Benzothiazole (CAS No 95-16-9)

Benzothiazole exhibits a water solubility of 3 g/l (24oC) and a logPow of 1.99 (Brownlee, 1992). For benzothiazole, bioconcentration factors of up to 350 were experimentally determined. In leeches *(Hirudinea)* concentrations of up to 1,400 µg/l were measured (Metcalfe, 1988). In fish liver benzothiazole was qualitatively detected (Spies, 1987). The substance is mobile in soil and not subject to degradation (Demirjian, 1984, 1987).

An aqueous solution of benzothiazole exposed to sunlight for 4 months proved to be stable (Spies, 1987). In a creek, a half life of ca. 3 h was determined by concentration measurements which, according to the authors, might be caused by volatilization and biodegradation in biofilm (Brownlee, 1992). In another investigation, most of the emitted benzothiazole was however found several miles away from the point where it had been discharged (Jungclaus, 1976).

2.2 Human Exposure

2.2.1 Occupational Exposure

Generally products are manufactured in closed systems in Japan. Gloves and protective clothing are worn.

Exposure data was obtained using personal monitoring (JISHA, 2003). In terms of an 8 hour time weighted average, the most exposed group of workers were loaders filling bags with the substance. In one factory where filling was performed indoors for 24 h/d, arithmetic mean exposure levels were 0.64 mg/m^3 (8 h TWA assuming loaders performed 8 hour shifts). In another factory filling was performed outdoors for 7 h/d and, as the arithmetic mean measured exposure level was 0.33 mg/m^3 for a 7 hour day.

Risk reduction measures (the wearing of gloves, mask and protective clothing) means that the actual human exposure (dermal) is essentially zero.

2.2.2 Consumer Exposure

Consumer exposure to the substance and/or products derived from the substance during the vulcanisation process may occur during use of the finished products, for example tyres.

This exposure is likely to be negligible.

2.2.3 Indirect Exposure Via The Environment

- Exposure via this route is unlikely. The chemical is not readily biodegradable, but it is predicted not to be bioaccumulative based on the bioaccumulation potential of the hydrolysis products.
- Environmental exposure from the manufacturing plant:

The substance is manufactured at two sites in Japan. Wastewater from the manufacturing process is treated by activated sludge, chemical oxidation and activated carbon before discharge to municipal

drains. Exhaust gas from the manufacturing process is either treated with acid or alkali or incinerated to remove any chemical before release. The liquid waste resulting from chemical treatment is processed through the wastewater treatment plant as described above. Waste chemical from spillage or cleaning is disposed of via landfill. It is estimated that approximately 0.12% of manufactured material is disposed of in this manner.

- Environmental exposure from consumer use:

Due to the chemical transformation processes involved in vulcanization, the finished rubber products only contain small amounts of the chemical in addition to substances produced from the synthesis, such as benzothiazole, 2-mercaptbenzothiazole and 2-mercaptobenzothiazole disulfide (Sullivan, 1992). The substance and its degradation products may be released to the environment during the use of rubber products, such as tyres. There may also be release from disposal/recycling of products containing the substance and its degradants.

2.3 Conclusions

Production of the chemical during 2000 is 16,000 – 20,000 t/y worldwide, with production in Japan 4,000 – 4,500 t/y. The chemical is an accelerator for the vulcanization of rubber. The manufacture of rubber products using TBBS as an accelerator is conducted in closed systems in Japan, and therefore worker exposure is minimal. However some dry manipulation may be undertaken under local exhaust ventilation when loading hoppers, etc.

In terms of an 8 hour time weighted average, the most exposed group of workers were loaders filling bags with the substance. In the worst case, filling was performed indoors and exposure levels were 0.64 mg/m^3 (8 h TWA, assuming loaders performed 8 hour shifts).

Due to the chemical transformation processes involved in vulcanization, the finished rubber products only contain small amounts of the chemical in addition to substances produced from the synthesis, such as benzothiazole, 2-mercaptbenzothiazole and 2-mercaptobenzothiazole disulfide (Sullivan, 1992). The substance and its degradation products may be released to the environment during the use of rubber products, such as tires.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

No information is available on toxicokinetics. Based on the available data it can be concluded that the chemical may be absorbed from the gastro-intestinal route as well as by dermal or inhalation exposure. It is likely that the test material will undergo hydrolysis either following initial exposure or following systemic exposure. The *in vivo* study results suggest that it is the hydrolysis products that are responsible for the toxicity. Further hepatic metabolism of the parent chemical or the hydrolysis products takes place. The principal route of excretion is the kidney (Wood, 2003).

3.1.2 Acute Toxicity

Inhalation

There is no information available on acute inhalation toxicity.

Dermal

There are two acute dermal toxicity studies. In the most reliable of these (which could not be validated) the chemical was applied directly to the intact skin of rabbits (1 male/1 female) at a dose of 7940 mg/kg and covered with a semi-occlusive dressing for 24 hours. There were no deaths or signs of systemic toxicity. The viscera of the animals appeared normal at necropsy. The LD50 was > 7940 mg/kg.

The data are summarised in Table 2.

Table 2. Acute toxicity in experimental animals

Route	Species (sex)	Value	Reference
Oral	Rat (female)	LD_{50} > 2000 mg/kg	(Highton, 2002)
Dermal	Rabbit (male/female)	LD_{50} > 7940 mg/kg	(ECB, 2000; JACT, 1990)

There were no clinical signs of toxicity during the acute oral or acute dermal study.

Oral

There are four acute oral toxicity studies. Of these, only one is considered to be reliable. In this study [OECD TG 423] female rats were dosed by gavage at 2000 mg/kg. There were no deaths or signs of systemic toxicity. All animals, except one, showed expected gains in body weight over the study period and no abnormalities were noted at necropsy. The LD_{50} was > 2000 mg/kg.

Conclusion

Oral LD_{50} (rat) was greater than 2000 mg/kg [OECD TG 423]. Dermal LD_{50} (rabbit) was greater than 7940 mg/kg. There is no information on acute inhalation toxicity.

3.1.3 Irritation

Skin Irritation

Skin irritation was investigated in several studies (ECD, 2000), which could not be validated. In variations of the standard Draize test, an average of 6 rabbits were exposed for 24 hours to the undiluted substance. Some slight irritation was noted in the studies but the chemical is not considered to be a skin irritant.

Eye Irritation

Eye irritation was investigated using the Draize test or modifications of the test, in several studies (ECB, 2000), which could not be validated. On average, 6 rabbits were exposed to the chemical for an unspecified time. There were signs of mild irritation but the chemical is not considered to be an eye irritant.

Conclusion

The substance shows signs of mild irritation in the skin and eyes of rabbits but is not considered to be a skin or eye irritant.

3.1.4 Sensitisation

Studies in Animals

The chemical was a sensitiser in a guinea pig Buehler test (ECB, 2000), which could not be validated due to lack of details, at a dose level of 25% in ethanol.

Studies in Humans

Skin

The substance was reported as a dermatological sensitiser in human patch tests, producing sensitisation rates of 9/55 and 13/45 (ECB, 2000). In a third study, the substance was applied to subjects previously sensitised to mercaptobenzothiazole, a hydrolysis product of the substance (Fossereau, 1983). A sensitisation rate of 13/14 was reported. It is possible that sensitisation was due to the hydrolysis product (mercaptobenzothiazole) rather than the parent substance, as hydrolysis occurs within 24 hours.

Conclusion

A hydrolysis product, mercaptobenzothiazole, is a strong skin sensitiser. This is further supported by animal testing (Buehler assay) and human patch tests on the parent chemical reported in the secondary literature.

3.1.5 Repeated Dose Toxicity

There are five repeat dose studies, one of which is valid. All of the studies were performed over a 28-day or longer period.

Table 3. Repeated dose toxicity studies

Species	Dose	NOEL(NOAEL)	Principal toxic effect	Reference
Rat (Sprague-Dawley)	0, 40, 200, 1000 mg/kg b.w./day by gavage for 38 to 42 days	Male: LOAEL 40 mg/kg b.w./day	Kidney, liver effects. Haematology and spleen effects in males and females	(MHW, 1997a)
Rat (Sprague-Dawley)	0, 10, 50, 300, 1000, 3000 mg/kg b.w./day by oral feed for 28 days	Male/female: 1000 mg/kg b.w./day	Decrease in b.w. and food consumption	(ECB, 2000)
Rat (Sprague-Dawley)	0, 100, 300, 1000, 3000 mg/kg b.w./day by gavage for 30 days	Male: 100 mg/kg b.w./day; Female: LOAEL 100 mg/kg b.w./day	Heart, kidney, liver effects	(ECB, 2000)
Rat (Sprague-Dawley)	0, 100, 300, 1000 mg/kg b.w./day by gavage for 90 days	Male/female: 100 mg/kg b.w./day;	Decrease in b.w. and organ weights	(ECB, 2000)
Rat (Sprague-Dawley)	0, 0.0024, 0.029, 0.084 mg/L, 6 hours/day by inhalation for 28 days	Male/female: 0.029 mg/L	Liver, lymph node effects, increased ASAT	(ECB, 2000)

In the combined repeat dose reproductive/developmental toxicity screening study [OECD TG 422] 13 animals of each sex were dosed once daily by gavage. Males were dosed for 42 days and females for 38 days (from 14 days prior to pregnancy to day 3 of lactation). In both males and females temporary salivation after each administration was observed in the 200 mg/kg and higher dose groups. Body weight gain was suppressed in males in the 1000 mg/kg group and slightly suppressed in pregnant females at the same dose. On pathological examination, various changes in the kidneys, such as increase in eosinophilic bodies and vacuolar degeneration in proximal tubules plus an increase in relative weight were observed in males and females in the 200 mg/kg b.w./day and 1000 mg/kg b.w./day group. Liver effects such as hypertrophy of hepatocytes, and increases in relative weight were also noted in both males and females of the 200 and 1000 mg/kg b.w./day groups. Males of the 200 and 1000 mg/kg b.w./day groups showed changes in haematology, such as haemoglobin and haemocrit decrease, induced haemolytic anaemia, as well as spleen related changes, such as increased haemosiderin deposits. Anaemia was also evident in females. The number of male rats with eosinophilic bodies in the kidney was increased in all compound-treated groups. LOAEL was 40 mg/kg b.w./day for males, based on anaemia (female data unavailable).

In the 28-day oral feed study, which could not be validated, groups of 5 animals of each sex at each dose level were dosed once daily. There were no effects noted at any dose level except at the highest dose of 3000 mg/kg/day. Animals in the highest dose group showed decreases in body weight and food consumption. The NOAEL was 1000 mg/kg b.w./day.

In the 30-day study, which could not be validated, 5 animals of each sex were dosed by oral gavage, once daily. All females and one male rat in the highest dose level group of 3000 mg/kg b.w./day died on Day 6. All other high dose group males were sacrificed on Day 6 in a moribund condition. Females dosed at 300 and 1000 mg/kg b.w./day showed a decrease in heart weight and an increase in kidney and liver weights. Females dosed at 100 mg/kg b.w./day and males dosed at 300 and 1000 mg/kg b.w./day were observed to have decreased body weight. The NOAEL was 100 mg/kg b.w./day for males. This dose represented the LOAEL for females.

In the 90-day study, which could not be validated, 5 animals of each sex were dosed by oral gavage, once daily. Males in the 300 and 1000 mg/kg b.w./day showed decreased body weights. Females in the highest dose group showed increases in liver and kidney weight, increased cholesterol in serum and increased specific gravity of urine. Hence, the NOAEL was 100 mg/kg b.w./day.

In the 28-day inhalation study, which could not be validated, animals were dosed for 6 hours per day, 5 days a week. No animals died during the test. The animals in the highest dose group showed a decrease in body weight. Histopathology of the highest dose group also showed effects to the liver and lymph nodes. An increase in aspartate aminotransferase was observed at both the high and mid dose level. The NOAEL was 0.029 mg/L, 6h/day, equivalent to 29 mg/m^3, 6 h/day or 22 mg/m^3, 8 h/day. The report was only available from the secondary literature and no details were given on histopathology at the 0.029 mg/L dose.

Conclusion

Taking into account the valid study only, the LOAEL for males (oral, rat) is 40 mg/kg bw/day (female data unavailable). The NOAEL for repeated dose toxicity (inhalation, rat) is 0.029 mg/L (6 h/day) (29 mg/m^3).

3.1.6 Mutagenicity

In vitro Studies

Table 4. Genotoxicity studies.

Type of test	Test system	Dose	Result	Reference
Bacterial test (reverse mutation)	*S. typhimurium* TA 98, TA100, TA 1535, TA 1537. *E. coli* WP2uvrA	Up to 7 doses between 1.56 to 5000 µg/plate	Negative, with and without metabolic activation	(MHW, 1997b)
In vitro chromosome aberration assay	CHL/IU	-S9: (continuous exp.) 0.015, 0.03, 0.06 mg/mL +/- S9: (6h exp.) 0.05, 0.1, 0.2 mg/mL	Positive with metabolic activation and negative without metabolic activation	(MHW, 1997c)

The chemical has been tested for reverse mutation in Salmonella typhimurium and Escherichia coli with and without exogenous metabolic activation by standard Japanese test methods in full compliance with OECD TG 471 and 472 (MHW, 1997b). Cytotoxicity was observed from 25 µg/plate (T1537) without S9 and from 1000 µg/plate (TA1537) with S9. Positive controls [2-(2-furyl)-3-(5-nitro-2-furyl)-acrylamide, sodium azide, 9-aminoacridine (all –S9) and 2-aminiantracene (+S9)] gave the expected response. The tests were negative, both in the presence and absence of a metabolising system.

An *in vitro* chromosome aberration study in CHL cells was conducted in accordance with Japanese guidelines similar to OECD TG 473 (MHW, 1997c). The highest dose level was cytotoxic. Positive controls [mitomycin C, Cyclophosphamide] gave the expected response (chromosomal aberrations). A small number of polyploid cells were induced in mid and high test concentration groups, with and without metabolic activation. However, the induction of polyploid cells was judged not to be biologically significant since the incidence was low. The substance was weakly clastogenic with metabolic activation under the conditions of the test.

An *in vitro* mammalian gene mutation study in BALB/3T3 cells, which cannot be validated, at dose levels of up to 35 µg/ml with and without a metabolic activation system showed cytotoxicity at the highest dose level tested (ECB, 2000). The substance did not show any indication of mutagenicity under the conditions of the test.

Several mouse lymphoma assays using the L5187Y cell line, which cannot be validated, have been conducted to various protocols (ECB, 2000). Doses up to 100 µg/ml both in the absence and

presence of a metabolic activation system have been tested. All studies have shown a positive result with the metabolising system and a negative result without. There are insufficient details in the available secondary literature to determine whether the positive response was due to chromosomal aberrations or point mutations. Based on the negative results seen in the Ames tests and the in vitro mammalian gene mutation studies, it is likely that the chemical caused chromosomal aberrations in the mouse lymphoma assays.

In vivo Studies

In a mouse micronucleus assay [OECD TG 474] (Durwood, 2002) animals were dosed via i.p. injection with 500, 1000 and 2000 mg/kg bw of chemical. There were no statistically significant increases in the frequency of micronucleated PCEs in any of the dose groups when compared to the concurrent vehicle control.

Conclusion

The chemical was not mutagenic in bacteria [OECD TG 471 and 472] or in several *in vitro* mammalian gene mutation assays. It did induce chromosomal aberrations in mammalian cells *in vitro* with metabolic activation [OECD TG 473]. Positive responses were seen in mouse lymphoma cells with metabolic activation and it is assumed based on the other *in vitro* results that these were chromosomal aberrations. The chemical was determined not to be genotoxic in an *in vivo* mouse micronucleus assay [OECD TG 474].

3.1.7 Carcinogenicity

There is no information available.

3.1.8 Toxicity for Reproduction and Development

A combined repeat dose with reproduction/developmental toxicity screening test [OECD TG 422] was performed (MHW, 1997a). 13 animals of each sex were dosed once daily by gavage (0, 40, 200, 1000 mg/kg b.w./day). Males were dosed for 42 days and females for 38 days (from 14 days prior to pregnancy to day 3 of lactation). Test chemical administration had no influence on mating ability, duration of oestrus or duration of pregnancy and parturition. Changes in fertility index were observed at 40 and 1000 mg/kg bw/day, but not at 200 mg/kg bw/day. Body weight of offspring was not affected by the test chemical and no abnormalities were seen on external examination at birth. Both male and female reproductive tissues were well examined and no abnormalities were observed.

In a developmental toxicity study conducted to GLP, reported in the secondary literature, female rats were dosed daily by gavage (0, 50, 150, 500 mg/kg b.w./day) on days 6 – 15 of gestation (ACC, 2001). No effects of this chemical were observed in females or offspring at any dose level and, under the conditions of the test, the chemical was considered to have no effect on reproduction. No further details of the study could be obtained. The NOAEL for both maternal toxicity and developmental toxicity was 500 mg/kg b.w./day.

Conclusion

In a reproduction/developmental toxicity screening test [OECD 422] males were dosed for 42 days and females for 38 days (from 14 days prior to pregnancy to day 3 of lactation). No adverse effects were observed in terms of copulation, delivery and nursing of parents. Changes in fertility index were observed at 40 and 1000 mg/kg bw/day, but not at 200 mg/kg bw/day. The viability and body weight of offspring were unaffected and no malformations were detected. Both male and female reproductive tissues were well examined and no abnormalities were observed.

The NOAEL for maternal toxicity and developmental toxicity were both 500 mg/kg b.w./day in a developmental toxicity study conducted to GLP when female rats were dosed with the chemical between days 6 – 15 of gestation. No effects were observed, either in the females or offspring, at any dose level and under the conditions of the test the substance was considered to have no effect on reproduction. No further details of the study could be obtained.

3.2 Initial Assessment for Human Health

There is no information on toxicokinetics. Based on the available data it can be concluded that the chemical may be absorbed from the gastro-intestinal route as well as by dermal or inhalation exposure. It is likely that the test material will undergo hydrolysis either following initial exposure or following systemic exposure. The *in vivo* study results suggest that it is the hydrolysis products that are responsible for the toxicity. Further hepatic metabolism of the parent chemical or the hydrolysis products takes place. The principal route of excretion is the kidney. Kidney and liver were identified as the principal target organs for toxicity. Haemolytic anaemia was noted in male and female rats.

Oral LD_{50} (rat) was greater than 2000 mg/kg [OECD TG 423]. Dermal LD_{50} (rabbit) was greater than 7940 mg/kg. There is no information on acute inhalation toxicity. The substance shows signs of mild irritation in the skin and eyes of rabbits but is not considered to be a skin or eye irritant. A hydrolysis product, mercaptobenzothiazole, is a strong skin sensitiser. This is further supported by animal testing (Buehler assay) and human patch tests on the parent chemical reported in the secondary literature.

Based on the results of a valid repeat dose study, the LOAEL for repeat dose toxicity (oral) is considered to be 40 mg/kg bw/day for males, based on anaemia (female data unavailable). The NOAEL for repeated dose toxicity (inhalation) is 0.029 mg/L (6 h/day) (29 mg/m^3).

The chemical was not mutagenic in bacteria [OECD TG 471 and 472] or in several *in vitro* mammalian gene mutation assays. It did induce chromosomal aberrations in mammalian cells *in vitro* with metabolic activation [OECD TG 473]. Positive responses were seen in mouse lymphoma cells with metabolic activation and it is assumed based on the other *in vitro* results that these were chromosomal aberrations. The chemical was determined not to be genotoxic in an *in vivo* mouse micronucleus assay [OECD TG 474].

In a reproduction/developmental toxicity screening test [OECD 422] males were dosed for 42 days and females for 38 days (from 14 days prior to pregnancy to day 3 of lactation). No adverse effects were observed in terms of copulation, delivery and nursing of parents. Changes in fertility index were observed at 40 and 1000 mg/kg bw/day, but not at 200 mg/kg bw/day. The viability and body weight of offspring were unaffected and no malformations were detected. Both male and female reproductive tissues were well examined and no abnormalities were observed.

The NOAEL for maternal toxicity and developmental toxicity were both 500 mg/kg b.w./day in a developmental toxicity study conducted to GLP when female rats were dosed with the chemical between days 6 – 15 of gestation. No effects were observed, either in the females or offspring, at any dose level and under the conditions of the test the substance was considered to have no effect on reproduction. No further details of the study could be obtained.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute and Chronic Toxicity Test Results

In the following table the results from acute and chronic tests with aquatic organisms are presented.

Table 5: Acute and chronic studies in aquatic organisms

Organism	Test duration	Result (mg/L)	Reference
Fish			
Oryzias latipes	96 hours (ss)	LC_{50} (96 h) = 0.345 mg/L (limit of water solubility)	(MOE, 1996a)
Oryzias latipes	14 days (ft)	LC_{50} (14 days) = 0.345 mg/L (limit of water solubility)	(MOE, 1996b)
	14 days (ft)	NOEC (14 days) = 0.15 mg/L	(MOE, 1996b)
Invertebrates			
Water Flea (*Daphnia magna*)	48 hours (s)	EC_{50} = 0.345 mg/L (limit of water solubility)	(MOE, 1996c)
	14 days (ss)	NOEC (reproduction) = 0.042 mg/L (measured)	(MOE, 1996d)
	21 days (ss)	EC_{50} (reproduction) > 0.16 mg/L (measured) NOEC(reproduction) > 0.16 mg/L (measured) NOEC (parental) = 0.068 mg/L (measured)	
Aquatic Plants			
Green algae *Selenastrum capricornutum*	72 hours (s)	E_bC_{50} (0 - 72 h) = 0.037 mg/L (measured) $NOEC_b$ (0 - 72 h) = 0.011 mg/L (measured) E_rC_{50} (0 -72 h) = 0.071 mg/L (measured) $NOEC_r$ (24-72 h) = 0.023 mg/L (measured)	(MOE, 1996e)

(s): Static conditions
(ss): Semi-static conditions
(ft) Flow through

There are five acute fish toxicity studies, one of which is considered reliable. In this study [OECD TG 203] *Oryzias latipes* were exposed under semi-static conditions to the chemical at nominal concentrations of 0, 0.25, 0.50, 1.00, 2.00 and 4.00 mg/L for 96 hours, using DMF/HCO-30 as the vehicle. Abnormal swimming was observed at test concentrations over 0.518 mg/L (set-up concentration 1.00 mg/L). No abnormal symptoms were seen in the control groups during the exposure period. Toxicity to fish was exhibited at dose levels above the level of water solubility. LC_{50} (96 hour) = 0.345 mg/L (limit of water solubility). In a number of other studies (ECB, 2000), which could not be validated because they were taken from the secondary literature, LC_{50} values were again reported to be above the limit of water solubility.

In a reliable prolonged fish toxicity study [OECD TG 204], *Oryzias latipes* were exposed under flow through conditions to the chemical at concentrations of 0, 0.049, 0.150, 0.440 and 1.30 mg/L for 14 days, using DMF/HCO-30 as the vehicle. Abnormal swimming was observed at test concentrations over 0.44 mg/L. No abnormal symptoms were seen in the control group during the exposure period. Decrease in food intake was seen at test concentrations higher than 0.440 mg/L. No significant differences were seen in body weight or body length in test groups compared to controls. NOEC (14 days) = 0.15 mg/L, LC_{50} (14 days) = 0.345 mg/L (limit of water solubility).

In an acute aquatic invertebrate toxicity study [OECD TG 202], *Daphnia magna* were exposed under semi-static conditions to the chemical at concentrations of 0, 0.50, 0.75, 1.10, 1.70 and 2.50 mg/L for 48 hours, using DMF/HCO-50 as the vehicle. The EC_{50} was 0.345 mg/L (limit of water solubility).

In a chronic toxicity study [OECD TG 202], *Daphnia magna* were exposed under semi-static conditions to the chemical at concentrations of 0, 0.02, 0.04, 0.08, 0.15 and 0.30 mg/L. It was considered that the chemical might affect the larva of daphnia by exposure of 0.068 mg/L or more, although recovery was observed at 21 days after the start of exposure. EC_{50} (reproduction) > 0.16 mg/L (measured), NOEC (14d, reproduction) = 0.042 mg/L (measured) and NOEC (parental) = 0.068 mg/L (measured).

In a reliable study [OECD TG 201], *Selenastrum capricornutum* were exposed under static conditions to the chemical at nominal concentrations of 0, 0.004, 0.008, 0.016, 0.032, 0.063, 0.13 and 0.25 mg/L. The substance was toxic to algae under the conditions of the test. E_rC_{50} (0 -72 h) = 0.071 mg/L, $NOEC_r$ (24-72 h) = 0.023 mg/L (measured concentrations).

Aquatic Toxicity of Degradation Products

(See draft SIAR for di(benzothiazoyl-2)disulfide, CAS Nr 120-78-5)

di(benzothiazoyl-2)disulfide (MBTS)

The effect concentrations given are only nominal values. Owing to the instability of MBTS in aqueous solution it may be assumed that the effects observed are mainly attributed to MBT (BUA, 1994).

Algae
Selenastrum capricornutum EC50 = 0.6 mg/l (96 h)
NOEC < 0.3 mg/l (96 h)
(Effect: inhibition of chlorophyll-a-synthesis)

Scenedesmus subspicatus at 40 mg/l after 72 h no inhibition of biomass

Invertebrates
Daphnia magna EC50 = 82 mg/l (48 h)
(Effect: immobilization)

Fish
Salmo gairdneri LC50 = 66 mg/l (96 h)
Lepomis macrochirus LC50 = 82 mg/l (96 h)
Oryzias latipes LC50 = 19 mg/l (48 h)

Mercaptobenzothiazole (MBT)
MBT and its salts are not considered separately. It is however indicated when NaMBT instead of MBT was used in an investigation.

Protozoa
Tetrahymena pyriformis EC50 = 10 mg/l (24 h) (BUA, 1991)
(Effect: biomass)

Algae
Selenastrum capricornutum EC50 = 0.25 mg/l (96 h)
EC50 = 0.3 mg/l (96 h) NaMBT
NOEC = 0.1 mg/l (96 h)
(Nominal concentrations, effect: biomass) (BUA, 1991)

Invertebrates

Daphnia magna	LC50 = 4.1 mg/l (48 h) LC0 = 1.8 mg/l (48 h) LC50 = 19 mg/l (48 h) NaMBT LC0 = 10 mg/l (48 h) NaMBT NOEC = 0.24 mg/l (21 d) mortality NOEC = 0.22 mg/l (21 d) reprod. rate	(BUA, 1991)

Vertebrates

Oncorhynchus mykiss (= Salmo gairdneri)	LC50 = 0.73 mg/l (96 h) LC50 = 1.8 mg/l (96 h) NaMBT NOEC = 0.041 mg/l (89 d) length of larvae	
(Measured concentrations)	(BUA, 1991)	

Benzothiazole

Fish

Oryzias latipes	LC50 = 110 mg/l (48 h)	

Protozoa

Tetrahymena pyriformis (Effect: biomass)	EC50 = 160 mg/l (24 h)	(Yoshioka, 1986)

Based on the above data, some of the degradation products of N-tert-butylbenzothiazole-2-sulfenamide, and especially mercaptobenzothiazole, show similar levels of aquatic toxicity to the parent compound.

Conclusion

Reliable acute toxicity data are available for one species of fish (*Oryzias latipes*, LC_{50} = 0.345 mg/L, i.e. limit of water solubility). In *Daphnia magna*, an acute toxicity value of 48h EC_{50} = 0.345 mg/L (limit of water solubility) was reported. The results in algae were E_rC_{50} = 0.071 and $NOEC_r$ (24 to 72 h) = 0.023 mg/L (measured concentrations). In a prolonged fish toxicity study (*Oryzias latipes*) the results were 14d LC50 = 1.02 mg/L and 14d NOEC = 0.15 mg/L. The chronic toxicity data for *Daphnia magna* were EC_{50} > 0.16 mg/L (measured), NOEC = 0.042 mg/L (14d, reproduction, measured) and NOEC = 0.068 (21d, parental, measured). The results for algal toxicity and chronic NOEC values in fish and Daphnia have been derived and are less than the water solubility. Some of the degradation products of N-tert-butylbenzothiazole-2-sulfenamide, and

especially mercaptobenzothiazole, show similar levels of aquatic toxicity to the parent compound.

4.2 Terrestrial Effects

There is no available information.

4.3 Other Environmental Effects

The chemical is not harmful to activated sewage sludge, EC_{50} (3 h) > 10000 mg/L [ISO 8192] (ECB, 2000). There is no other available information.

4.4 Initial Assessment for the Environment

The chemical has a log Pow of 3.9 at room temperature, a vapour pressure of less than 0.0000021 hPa at 25°C and a water solubility of 0.345 mg/L at 20°C. Fugacity model Mackay level III

calculations suggest that the majority of the chemical would distribute to soil if released to the air or soil compartments and to water if released to the water compartment. The chemical is not ionised at environmental pHs ($pKa_{(1)}$ 1.75, $pKa_{(2)}$ – 3.43, conjugate acid).

The substance is not readily biodegradable (0%) but it does hydrolyse in less than 1 day at pH 9 or less. The identified hydrolysis products are mercaptobenzothiazole, di(benzothiazoyl -2)disulfide, t-butylamine and benzothiazole. The substance can be predicted not to bioaccumulate based on results of bioaccumulation tests on the hydrolysis products. Indirect photo-oxidation by hydroxy radicals is predicted to occur with a half-life estimated at 2.8 hours. The substance does not inhibit the action of wastewater treatment microorganisms EC_{50} (3h) > 10000 mg/L. The hydrolysis products, such as mercaptobenzothiazole, di(benzothiazoyl-2)disulfide and benzothiazole, are non-volatile and not readily biodegradable in the environment.

Reliable acute toxicity data are available for one species of fish (*Oryzias latipes*, LC_{50} = 0.345 mg/L, i.e. limit of water solubility). In a prolonged fish toxicity study (*Oryzias latipes*, 14 d) the results were LC50 = 1.02 mg/L and NOEC = 0.15 mg/L. In Daphnia magna, an acute toxicity value of 48h EC_{50} = 0.345 mg/L (limit of water solubility) was reported. The chronic toxicity data for Daphnia magna were EC_{50} > 0.16 mg/L (measured), NOEC = 0.042 mg/L (14d, reproduction, measured) and NOEC = 0.068 mg/L (21d, parental, measured). The results in algae were E_rC_{50} = 0.071 mg/L and $NOEC_r$ (24 to 72 h) = 0.023 mg/L (measured concentrations).

The predicted no effect concentration (PNEC) of 0.00023 mg/L is estimated from the lowest chronic value ($NOEC_r$ (24 to 72 h) of 0.023 mg/L, Algae), by applying an assessment factor of 100, since the algae was the most sensitive species.

Some of the degradation products of N-tert-butylbenzothiazole-2-sulfenamide, and especially mercaptobenzothiazole, show similar levels of aquatic toxicity to the parent compound.

5 RECOMMENDATIONS

The chemical is a candidate for further work.

The chemical possesses properties indicating a hazard for the environment. Also some of the degradation products show similar hazards to the environment. The substance and its degradation products are present in many rubber products and a release to the environment is possible. An exposure assessment, and if necessary a risk assessment for the environment of the chemical and its degradation products should be performed. The currently on-going assessment of di(benzothiazoyl-2)disulfide (CAS No 120-78-5) and of N-cyclohexylbenzothiazole-2-sulfenamide (CAS No 95-33-0) should be taken into account.

The chemical also possesses properties indicating a hazard for human health (sensitisation and anaemia). An exposure assessment and, if necessary, a risk assessment for human health should be performed.

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I U C L I D Data Set

Existing Chemical	:	ID: 95-31-8
Memo	:	ICCA HPV 2003
CAS No.	:	95-31-8
EC No.	:	202-409-1
EINECS Name	:	N-tert-butylbenzothiazole-2-sulphenamide
Molecular Formula	:	C11 H14 N2 S2

Producer related part

Company	:	Safepharm Laboratories
Creation date	:	26.03.2001

Substance related part

Company	:	Safepharm Laboratories
Creation date	:	26.03.2001
Status	:	
Memo	:	N-tert-butylbenzothiazole-2-sulphenamide: ICCA 2003
Printing date	:	20.02.2003
Revision date	:	
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Number of pages	:	2
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	cooperating company
Name	:	Ouchi-Shinko Chemical Industrial Co. Ltd.
Contact person	:	Mr Iwasaki
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Homepage	:	

08.02.2002

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Cedex	:	
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Homepage	:	

08.02.2002

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	
Smiles Code	:	N(c(c(S1)ccc2)c2)=C1SNC(C)(C)C
Molecular formula	:	C11H14N2S2
Molecular weight	:	238.39
Petrol class	:	

08.02.2002

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type : typical for marketed substance
Substance type : organic
Physical status : solid
Purity : ca. 97 % w/w
Colour :
Odour :

Reliability :
26.03.2001

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

Accel TBS-R

13.01.2003

Benzothiazolesulfenamide, N-(1,1-dimethylethyl)-

26.03.2001

Benzothiazolyl-2-tert-butylsulfenamide

26.03.2001

Benzothiazyl-2-tert-butylsulfenamid

26.03.2001

Cure-rite BBTS

26.03.2001

Delac NS

26.03.2001

N-(1,1-dimethylethyl)-2-benzothiazolesulfenamide

26.03.2001

N-tert-butyl-2-benzothiazolesulfenamide

25.11.2002

N-tert-butyl-2-benzothiazolsulfenamide

25.11.2002

N-tert-butyl-2-benzothiazyl sulphenamide

25.11.2002

Nocceler NS-P

07.01.2003

Pennac Tbbs

26.03.2001

Perkacit NS

26.03.2001

Sanceler NS-G

05.07.2002

Santocure NS

26.03.2001

Santocure NS vulcanization accelerator

26.03.2001

TBBS

26.03.2001

TBBS/EGC

26.03.2001

Tertiary-butylbenzothiazolesulfenamide

26.03.2001

Vanax NS

26.03.2001

Vulkacit NZ

26.03.2001

1.3 IMPURITIES

Purity	:	typical for marketed substance
CAS-No	:	120-78-5
EC-No	:	
EINECS-Name	:	Dibenzothiazyldisulfide
Molecular formula	:	
Value	:	ca. .8 % w/w

13.01.2003

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Remark : Production of the chemical worldwide is 16,000 - 20,000 t/y. Production in Japan is 4,000 - 4,500 t/y.

13.12.2002

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : use
Category : Vulcanizing accelerator agents

26.03.2001

Type of use : industrial
Category : Chemical industry: used in synthesis

26.03.2001

Type of use : type
Category : Use resulting in inclusion into or onto matrix

26.03.2001

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

Type : EINECS
Additional information :

26.03.2001

Type : TSCA
Additional information : EPA TSCA Test submission (TSCATS) data base, December 1999

26.03.2001

Type : DSL
Additional information : Canadian Inventory

18.12.2002

Type : AICS
Additional information : Australian Inventory

18.12.2002

Type : ECL
Additional information : Korean Inventory of Chemicals

18.12.2002

Type : ENCS
Additional information : Japanese Inventory

18.12.2002

Type : PICCS
Additional information : Philippine Inventory

18.12.2002

Type : CHINA
Additional information : Inventory of Existing Chemical Substances in China

18.12.2002
Type : DENMARK

Additional information : The Danish Product Register
13.05.2003

Type : SWEDEN
Additional information : The Swedish Products Register

13.05.2003

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

Source of exposure : Human: exposure by production
Exposure to the : Substance
Method : Sampling for the chemical was performed during the time workers were exposed. Samples were taken from the air around the worker's mouth. Workers performing extremely short tasks repeated the task several times for sampling. Samples were taken on both sides of workeers to take into account air current effects.

Method of sampling and analysis:
Sample air was taken at 2L/min using sampling tubes (glass fiber + XAD-7 (270/140 mg).

HPLC:	Agilent 1100 series
Column:	TSK gel ODS-80TsQA (5µm, 2.0 mm x 25 cm)
Mobile phase:	Acetonitrile/water 65/35 v/v%
Column temperature:	40°C
Flow rate:	0.2mL/min
Detector wavelength:	225 nm
Injection amount:	5.0 µL

Year : 2003
Result : The concentration of the chemical in the air ranged from 0.04– 14.99 mg/m^3. The higher concentrations were associated with cleaning of the manufacturing equipment

Table 1: Concentration in the air of N-t-butylbenzothizole-2-sulphenamide for each task

Factory	Task	Frequency of the Task	Working time	Workers	Range		Arithmetical mean		Geometrical mean	
					minimum (mg/m^3)	maximum (mg/m^3)	Mean (mg/m^3)	S.D.	Mean (mg/m^3)	S.D.
A	Sampling	Once a day	5 min each	2	0.75	3.61	2.17	-	1.62	-
	Checking	2-3times/day	2-5min each	2	<0.21	0.28	0.25	-	0.24	-
	Filling	-	2-3 h/day	2	0.48	0.79	0.64	-	0.62	-
	Inspection	-	40min/day	4	<0.05	<0.11	0.08	0.035	0.07	1.577
	Cleaning		3 min/day	4	3.49	14.99	8.47	5.327	7.18	1.967
	Shipping	-	60/day	2	<0.09	<0.09	0.09	-	0.09	-
B	Sampling	10 times/day	1 min each	2	<0.54	<0.54	0.54	-	0.54	-
	Cleaning I	-	80 min/day	2	<0.03	0.59	0.36	-	0.28	-
	Cleaning	-	3.5 - 4.5	4	0.11	1.20	0.49	0.513	0.30	3.225
	Filling	-	7 h/day	4	0.04	0.75	0.33	0.299	0.22	3.400
	Inspection	-	26 min/day	2	<0.07	<0.07	0.07	-	0.07	-
	Collecting	8	10 min each	2	0.45	4.18	2.32	-	1.37	-

Table 2: Measurements taken at Factory A

Measure-ment No.	Task	Places measured	Spots measured	Local venti-lation	Temp/ humidity (°C) (%)	Air flow (m/s)	Time measured (mins)	Period sampled (L/min x min)	Amount sampled (L)	Concnt. in the air (mg/m^3)	Note
A1	Sampling	Indoor	Left side of worker's mouth	Yes	8.0 75	0.07 - 0.32	9:12	2 x 2	4	0.75	The number of times of sampling of the product is one time
A2	Sampling	Indoor	Right side of worker's mouth	Yes			9:12	2 x 2	4	3.61	The number of times of sampling of the product is one time
A3	Checking	Indoor	Left side of worker's mouth	No		0.0 -0.16	9:19	2 x 5	10	<0.21	Checking of pellet maker
A4	Checking	Indoor	Right side of worker's mouth	No			9:19	2 x 5	10	0.28	Checking of pellet maker
A5	Filling	Indoor	Left side of worker's mouth	Yes	10.0 65	0.05 -0.18	9:37	2 x 17	34	0.48	Packing in bags (20kg)
A6	Filling	Indoor	Right side of worker's mouth	Yes			9:37	2 x 17	34	0.79	Packing in bags (20kg)
A7	Inspection	Indoor	Right side of worker's mouth	No	24.5 33	0.0 -0.02	10:02	2 x 22	44	<0.05	Weighting, melting, sieving
A8	Inspection	Indoor	Left side of worker's mouth	No			10:02	2 x 22	44	<0.05	Weighting, melting, sieving
A9	Inspection	Indoor	Left side of worker's mouth	No			10:12	2 x 10	20	<0.11	Weighting, melting, sieving
A10	Inspection	Indoor	Right side of worker's mouth	No			10:12	2 x 10	20	<0.11	Weighting, melting, sieving
A11	Cleaning	Indoor	Right side of worker's mouth	Yes	12.0 68	0.06 -0.4	13:13	2 x 4	8	14.99	Cleaning of drier
A12	Cleaning	Indoor	Left side of worker's mouth	Yes			13:13	2 x 4	8	4.81	Cleaning of drier
A13	Cleaning	Indoor	Right side of worker's mouth	Yes			13:21	2 x 5	10	3.49	Cleaning of measure
A14	Cleaning	Indoor	Left side of worker's mouth	Yes			13:21	2 x 5	10	10.57	Cleaning of measure

A15	Shipping	Outdoor	Shipping place	No	10.5	51	0.5-1.7	14:06	2 x 12	24	<0.09	Near side of loading to a lorry
A16	Shipping	Outdoor	Shipping place	No				14:06	2 x 12	24	<0.09	Near side of loading to a lorry

Table 3: Measurements taken at Factory B

Measure-ment No.	Task	Places measured	Spots measured	Local venti-lation	Temp/ humidity (C)	(%)	Air flow (m/s)	Time measured (mins)	Period sampled (l/min x min)	Amount sampled (l)	Concnt. in the air (mg/m^3)	Note
B1	Cleaning I	Indoor	Left side of worker's mouth	No	13.0	45	0.08	9:10	2 x 8	16	<0.13	Cleaning of pellet maker
B2	Cleaning I	Indoor	Right side of worker's mouth	No				9:10	2 x 8	16	0.59	Cleaning of pellet maker
B3	Filling	Indoor	Right side of worker's mouth	Yes	8.0	57	0.23	9:32	2 x 30	60	0.04	Packing in bags
B4	Filling	Indoor	Left side of worker's mouth	Yes				9:32	2 x 30	60	0.75	Packing in bags
B5	Sampling	Indoor	Right side of worker's mouth	No	8.0	57	0.23	10:07	2 x 2	4	<0.54	
B6	Sampling	Indoor	Left side of worker's mouth	No				10:07	2 x 2	4	<0.54	
B7	Cleaning II	Indoor	Right side of worker's mouth	Yes	15.5	41	0.10	10:32	2 x 16	32	0.12	Scraping off filter cloths of centrifuge
B8	Cleaning II	Indoor	Left side of worker's mouth	Yes				10:32	2 x 16	32	0.11	Scraping off filter cloths of centrifuge
B9	Cleaning II	Indoor	Right side of worker's mouth	Yes	14.5	48	0.09	10:59	2 x 2	4	1.20	Scraping off inside of hoppers
B10	Cleaning II	Indoor	Left side of worker's mouth	Yes				10:59	2 x 2	4	<0.54	Scraping off inside of hoppers
B11	Collecting	Indoor	Left side of worker's	Yes	11.5	52	0.18	11:25	2 x 11	22	0.45	Collecting at container bag

			mouth								
B12	Collecting	Indoor	Right side of worker's mouth	Yes			11:25	2 x 11	22	4.18	Collecting at container bag
B13	Inspection	Indoor	Right side of worker's mouth	No	22.0	31	0.25	13:07	2 x 16	32	<0.07	Preparation of samples for inspection
B14	Inspection	Indoor	Left side of worker's mouth	No				13:07	2 x 16	32	<0.07	Preparation of samples for inspection
B15	Filling	Indoor	Near worker A's mouth	Yes	12.5	64	0.35	14:02	2 x 4	8	<0.27	Filling into container
B16	Filling	Indoor	Near worker B's mouth	Yes				14:02	2 x 4	8	<0.27	Filling into container

10.02.2003 (26)

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

Type of search : Internal and External
Chapters covered : 2, 3, 4, 5
Date of search : 23.4.2003
Remark : Aquire (1992 - 2002)
Biodegradation Data (BIODEG) (1992 - 2002)
Biodegradation Bibliographic References (BIOLOG)(1992 - 2002)
Biological Abstracts - BIOSIS (1969 - present)
CA Search (1967 – present)
ECOTOX database
EMBASE (1974 - present)
EMBSINFO (1977 - present)
Enviroline (1970 - present)
Environmental Bibliography (1974 - present)
Gene-Tox (1992 - 2002)
HSELINE (1977 - present)
IRIS database
Medline (1966 - present)
National Technical Information Service (NTIS)(1964 - present)
NIOSH (1973 - present)
PASCAL (1984 - present)
TERRETOX (1992 – 2002)
TSCATS (1977 - present)
Toxfile (1965 - present)

Search terms:

CAS No. 95-31-8
TBBS

N-tert-butylbenzothiazole-2-sulphenamide
Ecotoxicology
Toxicology

25.11.2002

1.13 REVIEWS

Memo : RTECS

Remark : CHEMICAL IDENTIFICATION:
Chemical name
Molecular formula
Synonyms/trade names
Other reference numbers/names
TOXICITY:
Acute oral toxicity
Acute intraperitoneal toxicity
Acute intravenous toxicity
Acute dermal toxicity
MUTAGENICITY:
Mouse lymphoma assay
Morphological transformation in the mouse
OCCUPATIONAL EXPOSURE DATA

26.03.2001

Memo : IUCLID

Remark : Non-confidential dataset from Year 2000 CD-ROM edition.
Merged datasets submitted under Council Regulation (EEC) No. 793/93.

Submitting Companies
Tradenames/synonyms
Physico-chemical properties
Toxicity
Ecotoxicity

References to reports

26.03.2001

2.1 MELTING POINT

Value	:	105 °C
Decomposition	:	yes, at 207 °C
Sublimation	:	no
Method	:	other: DSC method
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	Conditions of measurement: Standard test: a -alumina Atmosphere: in nitrogen Temperature range: room temperature - 450°C Temperature gradient: 10°C/min Range: DSC +/- 16 mcal/mol, TG 20 mg Speed of recording chart: 5 mm/min
Reliability	:	(2) valid with restrictions In-house data generated by Ouchi Shinko Chemical Company
Flag	:	Critical study for SIDS endpoint
15.04.2002		

Value	:	ca. 103 - 109 °C
Decomposition	:	no, at °C
Sublimation	:	no
Method	:	other: no data
Year	:	
GLP	:	no data
Test substance	:	
Reliability	:	(4) not assignable Secondary literature
26.11.2002		(1) (6)

2.2 BOILING POINT

Decomposition	:	yes
Method	:	Ebullioscopic method
Year	:	1996
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The boiling point was unmeasurable due to decomposition of the chemical (turned to black).
Reliability	:	(1) valid without restriction Study conducted to standard test method under GLP
Flag	:	Critical study for SIDS endpoint
18.02.2003		(27)

Value	:	358.3 °C
Remark	:	Figure calculated by EPI Modelling Program v3.04
Reliability	:	(4) not assignable Secondary literature

25.11.2002

2.3 DENSITY

Type : density
Value : ca. 1.28 - 1.29 g/cm³ at 25 °C
Method : other: no data
Year :
GLP :
Test substance :

Reliability : (4) not assignable
Secondary literature
26.11.2002 (6)

Type :
Value : = 1.29 g/cm³ at 25 °C
Method : other: no data
Year :
GLP :
Test substance :

Reliability : (4) not assignable
Secondary literature
26.11.2002 (1)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : < .0000021 hPa at 25 °C
Decomposition :
Method : OECD Guide-line 104 "Vapour Pressure Curve"
Year : 2002
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Reliability : (1) valid without restriction
Study conducted to standard test method under GLP
Flag : Critical study for SIDS endpoint
25.11.2002 (19)

Value : ca. .000000611 - .00000137 hPa at 25 °C
Decomposition :
Method : other (measured): no data
Year :
GLP :
Test substance :

Reliability : (4) not assignable
Secondary literature
26.11.2002 (1) (6)

Value : = .0000547 hPa at 25 °C

Decomposition	:	
Method	:	other (calculated): no data
Year	:	
GLP	:	
Test substance	:	
Reliability	:	(4) not assignable Secondary literature

26.11.2002 (6)

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	3.9 at room temperature
pH value	:	
Method	:	OECD Guide-line 117 "Partition Coefficient (n-octanol/water), HPLC Method"
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	pH: Testing was carried out without pH correction to the mobile phase and at approximately neutral pH since the test material has no mode of dissociation within the pH range of the test.
Reliability	:	(1) valid without restriction Study conducted to standard test method under GLP
Flag	:	Critical study for SIDS endpoint

25.11.2002 (17)

Partition coefficient	:	octanol-water
Log pow	:	ca. 4.38 - 4.67 at 22 °C
pH value	:	-
Method	:	other (measured): no information available
Year	:	1991
GLP	:	yes
Test substance	:	
Reliability	:	(4) not assignable Only secondary literature available (IUCLID data set)

25.11.2002 (1) (6)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	.345 mg/l at 20 °C
pH value	:	6.6 - 7.2
concentration	:	.345 mg/l at 20 °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	slightly soluble (0.1 - 100 mg/L)
Stable	:	yes
Deg. product	:	no
Method	:	OECD Guide-line 105
Year	:	2002
GLP	:	yes

Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	A peak was evident in the sample chromatograms around the dead time of the HPLC column. This was assumed to be a water soluble impurity and not degradation of the parent material since the peak was evident to approximately the same extent in all samples'. Additionally, no gradual decrease in concentration was evident in the samples measured at different times, which would have been indicative of degradation. The glass beads used in the test are coated with an excess of test material, so that if degradation did occur, the test material would be replaced with freshly dissolved material. Thus it is believed that the solubility measured in this study is close to the actual solubility of the test material. Nevertheless, it is indeed possible that there are also dissolved hydrolysis products, which presumably were not detected under the chromatographic conditions used to determine the parent substance.
Reliability	:	(1) valid without restriction Study conducted to standard test method under GLP
Flag	:	Critical study for SIDS endpoint
25.11.2002		(17)

Solubility in	:	Water
Value	:	< 1 mg/l at 20 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	of very low solubility
Stable	:	
Deg. product	:	
Method	:	other: no information available
Year	:	
GLP	:	
Test substance	:	

Reliability	:	(4) not assignable Only secondary literature available (IUCLID data set)
25.11.2002		(1) (6)

Solubility in	:	Water
Value	:	300 mg/l at 24.5 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other:
Year	:	
GLP	:	no data
Test substance	:	no data

Method	:	100 mL of water placed in a flask and the test material added with stirring until saturated. After stirring for 1 hour, the solution was filtered. 20 mL of filtrate was taken using a pipette and evaporated to dryness using a rotary evaporator and the remaining sample dried in a desiccator for 3 hours then weighed. The result is the average of 3 replicates.

Remark	:	The method used leads to an overestimation of the solubility of the chemical. Due to the rapid hydrolysis of the chemical, the residue obtained after evaporating the filtered solution to dryness will be composed of the the chemical itself as well as various hydrolysis products.
Reliability	:	(3) invalid Manufacturer data

26.11.2002

Solubility in	:	Organic Solvents
Value	:	at °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other:
Year	:	
GLP	:	no data
Test substance	:	no data

Method	:	100 mL of solvent placed in a flask and the test material added with stirring until saturated. After stirring for 1 hour, the solution was filtered. 20 mL of filtrate was taken using a pipette and evaporated to dryness using a rotary evaporator and the remaining sample dried in a desiccator for 3 hours then weighed. The result is the average of 3 replicates.
Result	:	SOLUBILITIES: in g/100ml Methanol - 5.92 at 16.5°C Ethanol - 4.3 at 22°C Acetone - 5.5 at 27.5°C Chloroform - 25.01 at 17.6°C Benzene - 11.43 at 28°C Toluene - 8.34 at 24.3° n-Hexane - 1.03 at 24°C
Reliability	:	(3) invalid Manufacturer data

26.11.2002

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

Result	:	not explosive

Method : other
Year :
GLP :
Test substance :

Method : Predication based on structure
16.04.2002

2.11 OXIDIZING PROPERTIES

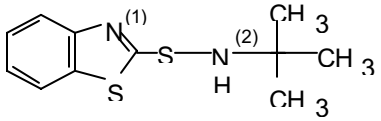
Result : no oxidizing properties
Method : other
Year :
GLP :
Test substance :

Method : Prediction based on structure
16.04.2002

2.12 DISSOCIATION CONSTANT

Acid-base constant : pKa(1) = 1.75, pKa(2) = -3.43
Method : other: Calculation using ACD/I-Lab Web service (ACD/pKa 6.0)
Year : 2002
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result :



$pKa_{(1)}$ = 1.75 (conjugate acid)
$pKa_{(2)}$ = -3.43 (conjugate acid)

Reliability : (4) not assignable
Prediction using ACD/pKa 6.0

10.12.2002

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

INDIRECT PHOTOLYSIS

Halflife t1/2	:	= 2.8 hour(s)
Degradation	:	% after
Quantum yield	:	
Rate constant	:	45.63 x 10^{-12} cm^3/molecule-sec
Method	:	Calculated using AOP Program, version 1.90. EPIWIN modeling program. Meylan, W. and Howard, P. (1999) Syracuse Research Corporation. Environmental Science center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510
Test condition	:	Concentration of hydroxyl radicals in the air = 1.5xE6 molecules/cm3
Reliability	:	(2) valid with restrictions Model reliable for most chemicals, but has not been validated. No details of inputs used.

12.12.2002

Type	:	other: dilute phosphate buffer
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	11.1 mg/l at 20 °C

DIRECT PHOTOLYSIS

Halflife t1/2	:	.1 - .2 day(s)
Degradation	:	100 % after
Quantum yield	:	0

INDIRECT PHOTOLYSIS

Sensitizer	:	other: dissolved organic matter
Conc. of sensitizer	:	10 mg/l
Rate constant	:	ca. 3.1 cm^3/(molecule*sec)
Degradation	:	98 % after
Deg. product	:	
Method	:	other (measured): test conditions undocumented
Year	:	1992
GLP	:	no data
Test substance	:	other TS
Remark	:	for direct - ph 7, rate constant 8.1-8.7; for indirect-initial conc. = 1.9 mg/L, temp = 1-10 deg. C, total solar radiation = 36.3E/m2, pH=7, t1/2=0.28-0.44 day, quantum yield=0.0013; similar results obtained when natural water was used - quantum yield = 0.0015 and 100% reduction; products formed with and without sensitizer and in natural water - benzothiazole (28-47%), 2-hydroxybenzothiazole (4-15%) and unidentified product.
Test substance	:	MErcaptobenzothiazole

14.05.2003 (6)

Type	:	other: ethanol
Light source	:	other: Hanovia mercury lamp-uv irradiation
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	860 mg/l at °C
Deg. product	:	
Method	:	other (measured): Parkanyi, C. et al protocol; see test conditions
Year	:	1985
GLP	:	no data
Test substance	:	other TS

Remark	:	Final product was benzothiazole sulfate with solutions of methanol, ethanol or acetonitrile; When dry benzene or toluene was the reaction medium, bis-(2-benzothiazoyl) disulfide was formed that could then be degraded to benzothiazole; oxygen is necessary for this reaction to take ppace and water is needed fo r the last step.
Test condition	:	ommersion-well type; water cooled Ace Glass photochemical reactor; air saturated 96% ethanol; irradiated 22 hours; 450 watts.
Test substance	:	Mercaptobenzothiazole
14.05.2003		(6)

Type	:	other: filter paper
Light source	:	other: germicidal lamp
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Deg. product	:	
Method	:	other (measured): Mitchell, E.C. protocol; see test conditions
Year	:	1961
GLP	:	no data
Test substance	:	other TS

Remark	:	classified as 'little or no degradation'
Test condition	:	10 mg quantities of a pesticide chemical are spotted on filter paper and the spot is exposed to a germicidal light (30 Watt)
Test substance	:	Mercaptobenzothiazole
14.05.2003		(6)

Type	:	water
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	.459 mg/l at 36 °C
DIRECT PHOTOLYSIS		
Halflife t1/2	:	31.1 minute(s)
Degradation	:	86 % after 90 minute(s)
Quantum yield	:	
INDIRECT PHOTOLYSIS		
Sensitizer	:	water with additives
Conc. of sensitizer	:	
Rate constant	:	$cm^3/(molecule*sec)$
Degradation	:	% after
Deg. product	:	
Method	:	other (measured): Federal Register 53(173) page 34522-34530
Year	:	1989
GLP	:	yes
Test substance	:	other TS

Test condition	:	Indirect photolysis measurement was with humic acid. Half life estimated to be 27.4 minutes.
Test substance	:	Mercaptobenzothiazole
14.05.2003		(6)

Type	:	water
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	1.1 mg/l at °C
DIRECT PHOTOLYSIS		
Halflife t1/2	:	3.7 hour(s)

Degradation : % after
Quantum yield :
Deg. product :
Method : other (measured): test conditions undocumented
Year : 1980
GLP : yes
Test substance : other TS

Remark : Four photodegradation by-products were observed
Test substance : Mercaptobenzothiazole
14.05.2003 (6)

Type : air
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight
Deg. product :
Method : other (calculated): acc. to Atkinson: SRC-AOP for Microsoft Windows
Year :
GLP :
Test substance :

Remark : Sensitizer: OH
Conc. of sensitizer: 0.5E6 OH/cm3
Rate constant: 7.0E-12 cm3/molecule-sec
Half life time: 4.584 days
Test substance : Benzothiazole
14.05.2003 (6)

3.1.2 STABILITY IN WATER

Type : abiotic
t1/2 pH4 : = 1.71 hour(s) at 25°C
t1/2 pH7 : = 1.80 hour(s) at 25°C
t1/2 pH9 : = 21.5 hour(s) at 25°C
Degradation :
Deg. product : yes
Method : OECD Guideline 111
Year : 1996
GLP : yes
Test substance : as prescribed by 1.1 - 1.4
Deg. products : Not identified in this study
Result :

	Rate Constant ($hour^{-1}$)	Half life at 25°C (hour)
pH 4	4.05×10^{-1}	1.71
pH 7	3.84×10^{-1}	1.80
pH 9	3.23×10^{-2}	21.5

Test condition : Concentration of test material: Approx 1mg/L
Temperature and pH of test solution:

pH 4	20 +/- 1°C,	30 +/- 1°C
pH 7	30 +/- 1°C,	40 +/- 1°C
pH 9	30 +/- 1°C,	40 +/- 1°C

Reliability : (1) valid without restriction

Guideline study conducted to GLP

04.12.2002 (25)

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	= 6.1 hour(s) at °C
t1/2 pH9	:	at °C
Degradation	:	= 100 % after 24 hour(s) at pH 7 and °C
Deg. product	:	yes
Method	:	other: ABC Laboratories method
Year	:	1984
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Deg. products	:	149-30-4 205-736-8 benzothiazole-2-thiol 75-64-9 200-888-1 tert-butylamine
Method	:	METHOD FOLLOWED: No details on method given Estimation of half-life.
Result	:	Report indicates that the substance hydrolyzed completely. Mercaptobenzothiazole and t-butylamine were identified as hydrolysis products.
Test condition	:	TEST TYPE: Test medium: Deionised water Test system: Deionised water filtered through a 0.45µm filter and adjusted to pH 7.00 +/- 0.05 using 0.1M NaOH/0.1M KH2PO4 buffer system. Stock solutions were prepared in acetone and then aliquots transferred to the water system.
Test substance	:	Name: Santocure NS Supplier: Monsanto Polymer Products Company Lot No.: NC06-107 Purity: 97%
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	= 10.4 - 13.2 hour(s) at °C
t1/2 pH9	:	= 40.5 hour(s) at °C
t1/2 pH 5	:	= 5.1 hour(s) at °C
Deg. product	:	yes
Method	:	other: ABC Laboratories Method
Year	:	1984
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Deg. products	:	149-30-4 205-736-8 benzothiazole-2-thiol
Method	:	METHOD FOLLOWED: No details on method given
Result	:	Neither sunlight nor added minerals from the well water impacted hydrolysis significantly. This study confirms the results obtained in a previous study that the substance has a half-life of less than 24 hours in water.
Test condition	:	TEST TYPE: Test medium: Deionised and environmental water Test system: Deionised and environmental water (well water)

were filtered through a 0.45µm filter. The pH was adjusted to one of 3 pH's. At pH 5 a potassium hydrogen phthalate buffer was used, at pH 7 a potassium dihydrogen phosphate buffer was used, whilst at pH 9 a sodium borate buffer was used. Hydrolysis in sunlight and in the dark and for samples prepared in buffered well water and in buffered deionised water were used to evaluate the impact of sunlight and other minerals from the hydrolysis rate.

DURATION: Unknown

Test substance : Name: Santocure NS
Supplier: Monsanto Polymer Products Company
Lot No.: NC06-107
Purity: 97%

Reliability : (4) not assignable
Secondary literature data

04.12.2002 (6)

Type : abiotic
t1/2 pH4 : at °C
t1/2 pH7 : at °C
t1/2 pH9 : at °C
Deg. product :
Method : other: Reflux
Year : 1981
GLP : no data
Test substance : other TS
Deg. products : 149-30-4 205-736-8 benzothiazole-2-thiol

Method : METHOD FOLLOWED:

Laboratory purified TBBS was refluxed (80°C) in a 90/10 isopropanol:water mixture (3% w/w. TBBS).

ANALYTICAL METHODS:

Sulfenamide assay: Based on the titration of amines liberated by the reduction of sulfenamides, published by Lichty et al (1963).

Free amine content:
Titration with strong acid
Alcohol insolubles;
Quantitative filtration, drying and weighing.

Result : The substance undergoes an autocatalytic reaction. As the hydrolysis proceeds the substance becomes more basic and greater amounts of the free amine are released

Test condition : TEST TYPE:
Test medium; isopropanol/water
Test system; Straight chemical hydrolysis

DURATION: ca. 7 hours

Test substance : Source: Commercially manufactured chemical (Monsanto), purified in the laboratory by recrystallization.

Purity: Not specified.

Conclusion : Degradation of the substance occurs by hydrolysis of the sulfur-nitrogen bond. Di-mercaptobenzothiaziole is the principle component formed as degradation occurs. The degradation products and impurities act as catalysts and speed up the hydrolysis reaction.

The substance is therefore unstable in storage.

Reliability : (4) not assignable
Non-standard test method

04.12.2002 (8)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

Type of measurement : background concentration
Media : other: Water and sediment
Concentration : Below limit of detection
Method : No details of the test method are available
Remark : In 1998 13 locations in Japan were sampled for water and 12 for sediment and no substance was detected in any of the locations:

In water			In sediment		
Samples detected/ Total samples	Sites detected/ Total sites	Limit of detection	Samples detected/ Total samples	Sites detected/ Total sites	Limit of detection
0/39	0/13	0.1 ppb	0/36	0/12	0.0047 ppm

Reliability (4) not assignable
Secondary literature

17.01.2003 (23)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III

Table 1: Summary of emissions for release to separate compartments

	1000 kg/h emission to these compartments separately		
	Air	**Water**	**Soil**
In air	0.3	0.0	0.0
In water	0	73.6	0.0
In soil	99.7	3.2	100.0
In sediment	0.0	23.2	0.0

Method : other: Fugacity Model Level III
Year : 2002

Test condition : Inputs:

Molecular weight 238.39
Melting point 105°C
Vapour pressure 0.00021 Pa
Water solubility 0.345 g/m3
Log Kow 3.9

Temperature 25°C
T1/2 (air) 8.4 h (estimate)
T1/2 (water) 1.8 h (measured)
T1/2 (soil) 240000 h (model default)
T1/2 (sediment) 720000 h (model default)

Reliability : (1) valid without restriction
Reliable model

13.12.2002

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge
Concentration : 100 mg/l related to test substance
related to
Contact time :
Degradation : = 0 (±) % after 28 day(s)
Result : Under the test conditions, no biodegradation was observed. However, the chemical did hydrolyse to produce mercaptobenzothiazole, di(benzothiazoyl-2)disulfide, t-butylamine, 2-sulfo(sulfino)benzothiazole and benzothiazole as the degradation products.

Degradation rates of aniline after 7 days and 14 days obtained from BOD were 63% and 74%, respectively, thereby confirming the validity of the test conditions.

	Degradation after 28 days (%)			
	Expt 1	Expt 2	Expt 3	Average
Degradation by BOD	0	0	0	0
Degradation by HPLC	80	81	100	87

Deg. product :
Method : OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year : 1995
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Reliability : (1) valid without restriction
Guideline study conducted to GLP

04.12.2002 (24)

Type : aerobic
Inoculum : activated sludge, adapted
Concentration : 29.4 mg/l related to Test substance
related to
Contact time :

Degradation	:	(±) % after
Result	:	other: 63.5% ThCO2 evolved after 32 days
Deg. product	:	
Method	:	other
Year	:	1975
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Test condition	:	TEST TYPE: Test medium: Activated sludge Test system: 60 ml of acclimated bacterial seed is mixed with 440 ml of minimal salts media in fluted 2 litre flask. (Appl. Microbiol. 30:922 (1975))
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Elimination	:	
Method	:	other: Japanese MITI Reports
Year	:	2001
GLP	:	no data
Test substance	:	other TS
Method	:	METHOD FOLLOWED: MITI Bioaccumulation. Studies conducted in accordance with generally accepted principles
Result	:	N-tert-butylbenzothiazole-2-sulphenamide (TBBS) hydrolyses in water to produce 2-mercaptobenzothiazole, di(benzothiazolyl-2)disulfide, tert-butylamine and benzothiazole.

These hydrolysis products have been tested and shown to have low potential for bioaccumulation. Based on these findings, TBBS is judged to have low potential for bioaccumulation also.

Bioconcentration factors:
2-mercaptobenzothiazole:
Level 1 area: < 0.8 (nominal concentration = 100 µg/L)
Level 2 area: < 8 (nominal concentration = 10 µg/L)
Di(benzothiazolyl-2)disulfide:
Level 1 area: 1.0 – 7.2 (nominal concentration = 0.2 µg/L)
Level 2 area: <1.4 - 51 (nominal concentration = 0.02 µg/L)
Benzothiazole:
Level 1 area: 2.1 – 5.1 (nominal concentration = 200 µg/L)
Level 2 area: < 4.1 – 7.5 (nominal concentration = 20 µg/L)
tert-butylamine:
Level 1 area: <0.36 - 1.3 (nominal concentration = 1 mg/L)
Level 2 area: <3.8 - 16 (nominal concentration = 0.1 mg/L)

Test condition	:	TEST TYPE: Test medium; 2-mercaptobenzothiazole: Hardened caster oil (HCO 20) and sodium hydroxide Di(benzothiazolyl-2)disulfide: HCO-20 and DMSO Test system: Standard Test duration: 6 weeks
Test substance	:	SOURCE: Test substances supplied by various manufacturers submitting MITI biodegradation studies. PURITY: 2-Mercaptobenzothiazole: 98.4% Di(benzothiazolyl-2)disulfide: >= 98% Benzothiazole: >= 99% N-(t-Butyl)-2-benzothiazolyl sulfenamide: ca. 98% tert-butylamine: 99.7% ANY OTHER INFORMATION: No batch specific information given.
		Solubility of degradants: 2-mercaptobenzothiazole: 118 mg/L Di(benzothiazolyl-2)disulfide: < 10 mg/L Benzothiazole: 4.3 g/L tert-butylamine: 100 g/L
Conclusion	:	The substance is considered not to bioaccumulate based upon the characteristics of the hydrolysis products.
Reliability	:	(4) not assignable Secondary literature review from MITI summary reports on bioaccumulation.

04.12.2002 (2)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	flow through
Species	:	Oryzias latipes (Fish, fresh water)
Exposure period	:	14 day(s)
Unit	:	mg/l
LC0	:	= .15
LC50	:	= 1.02
LC100	:	= 1.3
LC50 (7 days)	:	= 1.3
LOEL (14 days)	:	= .44
Limit test	:	
Analytical monitoring	:	yes
Method	:	OECD Guide-line 204 "Fish, Prolonged Toxicity Test: 14-day Study"
Year	:	1996
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method :

METHOD FOLLOWED: OECD Guideline 204

DEVIATIONS FROM GUIDELINE: No

STATISTICAL METHODS:
Body weight and body length: Multiple comparison of Dunnet, two sided test, alpha = 0.05

ANALYTICAL METHOD:
HPLC:
Column: Inertsil ODS-2, 5µm, 4.6x150 mm (GL Science Inc.)
Mobile phase: 80% acetonitrile/20% water
Flow rate: 1.0 mL/min
uv detector: 225 nm
Sample size: 50 µL

Test condition :

TEST ORGANISMS:
Size: 1.72-2.48 cm
Weight: 0.072-0.222 g
Loading: 10 fish per dose level

DILUTION WATER:
Dechlorinated tap water supplied by Yokohama City

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle/solvent: DMF and HC0-30
Concentration of vehicle/solvent: 96 mg/L

STABILITY OF THE TEST CHEMICAL SOLUTIONS:
The ratios of the concentration measured to the set-up concentration were within +/- 20% in all the test doses. All test concentrations were therefore based on nominal test substance concentrations.

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Concentrations: Control, vehicle control, 0.049, 0.150, 0.440, and 1.30 mg/L
Dosing rate: Continuous
Renewal of test solution: 6 times/day

Exposure vessel type: 5.0 L glass beaker
Number of replicates, fish per replicate: 1 test vessel per concentration, 10 fish per vessel.
Test temperature: 24 +/- 2 °C
Dissolved oxygen: 5.5 - 8.2 mg/L
pH: 7.4 - 7.9
Intensity of irradiation: Room light
Photoperiod; 16 hours

TEST PARAMETER: Mortality

MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured at 0, 7 and 14 day time periods.

Result : 0.440 mg/L > LC50 >1.30 mg/L (95% confidence interval)

OBSERVATIONS:
Abnormal swimming was observed at test concentrations over 0.44 mg/L. No abnormal symptoms were seen in the control group during the exposure period.

Decrease in food intake was seen in at test concentrations higher than 0.440 mg/L. No significant differences were seen in body weight or body length in test groups compared to the controls.

CONTROL:
Number of adverse effects: None

Table 1: Measured concentrations of test substance during 14-day exposure

Nominal Concentration mg/L	Measured Concentration, mg/L (Percent of Nominal)			Mean Measured Concentration mg/L
	0 Day	7 Day	14 Day	
Control	<0.004	<0.004	<0.004	-
Solvent Control	<0.004	<0.004	<0.004	-
0.049	0.043 (88)	0.045 (92)	0.045 (92)	0.044 (91)
0.150	0.122 (81)	0.131 (87)	0.127 (85)	0.127 (84)
0.440	0.444 (11)	0.364 (83)	0.474 (108)	0.427 (97)
1.30	1.21 (93)	1.42 (109)	1.51 (116)	1.38 (106)

Table 2: Mortality of Medaka (Oryzias latipes)

Nominal Concentration mg/L	Mean Measured Concentration mg/L	Cumulative mortality (Percent Mortality)					
		2 days	5 Days	7 Days	9 Days	12 Days	14 Days
Control	-	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	-	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.049	0.044	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.150	0.127	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.440	0.427	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.30	1.38	1 (10)	5 (50)	5 (50	6 (60)	6 (60)	7 (70)

Table 3: Symptoms of toxicity observed in Medaka (Oryzias latipes)

Nominal Concentration mg/L	Mean Measured Concentration mg/L	Symptoms					
		2 days	5 Days	7 Days	9 Days	12 Days	14 Days
Control	-	N	N	N	N	N	N
Solvent Control	-	N	N	N	N	N	N
0.049	0.044	N	N	N	N	N	N
0.150	0.127	N	N	N	N	N	N
0.440	0.427	LA	LA	LA	LA	LA	LA AS-2
1.30	1.38	LA AS-9	LA AS-5	LA AS-5	LA AS-4	LA AS-4	LA AS-3

N: No toxicological symptom was observed
LA: loss of appetite
AS: abnormal swimming

Conclusion : The substance shows toxicity to fish at the limit of water solubility and above.
Reliability : (1) valid without restriction
Guideline study conducted to GLP
04.12.2002 (14)

Type : semistatic
Species : Oryzias latipes (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC0 : = .518
LC50 : = 1.38
LC100 : = 3.99
Limit test :
Analytical monitoring : yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1996
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : METHOD FOLLOWED: OECD guideline 203

		DEVIATIONS FROM GUIDELINE: No STATISTICAL METHODS: Binomial method use to calculate LC50 METHOD OF CALCULATION: Test substance concentration calculated using geometric means ANALYTICAL METHODS: HPLC: Column: Inertsil ODS-2, 5µm, 4.6x150 mm (GL Science Inc.) Mobile phase: 80% acetonitrile/20% water Flow rate: 1.0 mL/min uv detector: 225 nm Sample size: 50 µL
Test condition	:	TEST ORGANISMS: Size: 1.59-2.30 cm Weight: 0.059-0.217 g Loading: 10 fish per dose level DILUTION WATER: Source: Dechlorinated tap water supplied by Yokohama City STOCK AND TEST SOLUTION AND THEIR PREPARATION: Vehicle/solvent: DMF and HC0-30 Concentration of vehicle/solvent: 96 mg/L STABILITY OF THE TEST CHEMICAL SOLUTIONS: The ratios of the concentration measured to the set-up concentration exceeded +/- 20%, hence values were based on the geometric mean of the measured concentrations. REFERENCE SUBSTANCE: None TEST SYSTEM: Concentrations: Control, vehicle control, 0.250, 0.500, 1.00, 2.00, 4.00 mg/L Renewal of test solution: Total amount of test water was replaced every 24 hours Exposure vessel type: 5.0L glass beaker Number of replicates, fish per replicate: 1 test vessel per concentration, 10 fish per vessel. Test temperature: 24 +/- 1 °C Dissolved oxygen: 5.0 - 8.3 mg/L pH: 7.2 - 7.9 Intensity of irradiation: Room light Photoperiod; 16 hours light/8 hours darkness TEST PARAMETER: Mortality MONITORING OF TEST SUBSTANCE CONCENTRATION: Test concentrations measured at 0, 24 hour time periods.
Result	:	0.518 mg/L < LC50 (96 hr) < 3.99 mg/L (95% confidence interval) OBSERVATIONS: Abnormal swimming was observed at test concentrations over 0.518 mg/L (set-up concentration 1.00 mg/L). No abnormal symptoms were seen in the control groups during the exposure period. CONTROL:

Number of adverse effects: None

Table 4: Measured concentrations of test substance during a 96-hour exposure

Nominal Concentration mg/L	Measured Concentration, mg/L (Percent of Nominal)		Mean Measured Concentration mg/L
	0 Hr (new)	24 Hr (old)	
Control	<0.004	<0.004	-
Solvent Control	<0.004	<0.004	-
0.250	0.223 (89)	0.139 (56)	0.176 (71)
0.500	0.465 (93)	0.240 (48)	0.334 (67)
1.00	0.909 (91)	0.295 (30)	0.518 (52)
2.00	2.03 (102)	0.351 (18)	0.844 (43)
4.00	3.99 (100)	**	3.99 (100)

new: freshly prepared test solutions

old: test solutions after 24 hours exposure period

**: No measurement made because all fish were dead at this observation time.

Table 5: Mortality of Medaka (Oryzias latipes)

Nominal Concentration mg/L	Mean[a] Measured Concentration mg/L	Cumulative mortality (Percent Mortality)			
		24 Hours	48 Hours	72 Hours	96 Hours
Control	-	0 (0)	0 (0)	0 (0)	0 (0)
Solvent Control	-	0 (0)	0 (0)	0 (0)	0 (0)
0.250	0.176	0 (0)	0 (0)	0 (0)	0 (0)
0.500	0.334	1 (10)	1 (10)	1 (10)	1 (10)
1.00	0.518	0 (0)	0 (0)	0 (0)	0 (0)
2.00	0.844	0 (0)	1 (10)	2 (20)	2 (20)
4.00	3.99	10 (100)	10 (100)	10 (100)	10 (100)

a: geometric mean

Table 6: Symptoms of toxicity observed in Medaka (Oryzias latipes)

Nominal Concentration mg/L	Mean Measured Concentration mg/L	Symptoms			
		24 Hours	48 Hours	72 Hours	96 Hours
Control	-	N	N	N	N
Solvent Control	-	N	N	N	N
0.250	0.176	N	N	N	N
0.500	0.334	N	N	N	N
1.00	0.518	AS-10	AS-10	AS-10	AS-10
2.00	0.844	AS-10	AS-9	AS-8	AS-8
4.00	3.99	**	**	**	**

N: No toxicological symptom was observed

**: All fish were dead at this observation time

AS: abnormal swimming

Conclusion : The substance is toxic to fish under the conditions of this study. Toxicity is seen at the limit of water solubility and above.
Reliability : (1) valid without restriction
Guideline study conducted to GLP

04.12.2002 (16)

Type : flow through
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 14 day(s)
Unit : mg/l
LC50 : > .3
Limit test :
Analytical monitoring : yes
Method : other
Year : 1979
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : Toxicity was greater than the water solubility of the substance (0.3 mg/l).
Test condition : TEST SPECIES:
Weight/length: mean weight = 0.43 g; mean length = 38 mm

TEST SYSTEM:
Solvent: DMF (0.33 mg/l)
Renewal of test solution: continuous flow diluter; rate = 2 ml/l.
Conclusion : The substance is toxic to fish under the conditions of the test.
Reliability : (4) not assignable
Seconday literature

04.12.2002 (1) (6)

Type : static
Species : Brachydanio rerio (Fish, fresh water)
Exposure period : 96 hour(s)

Unit	:	mg/l
LC50	:	> .5
Limit test	:	
Analytical monitoring	:	yes
Method	:	Directive 84/449/EEC, C.1 "Acute toxicity for fish"
Year	:	1993
GLP	:	yes
Test substance	:	other TS
Result	:	No mortality found beneath the detection limit of the analytical method (0.5 mg/L).
Test substance	:	PURITY: 99%
Conclusion	:	The substance is not harmful to fish under the conditions of this test.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (1) (6)

Type	:	static
Species	:	Lepomis macrochirus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	1.2
Limit test	:	
Analytical monitoring	:	no
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1984
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	LC50 (96 hr) = 1.2 mg/l (C.I. = 0.98 - 1.6 mg/l) LC50 (24 hr) = 3.6 mg/l LC50 (48 hr) = 1.5 mg/l
Test condition	:	TEST SPECIES: Weight/length: mean length = 3.8 cm TEST SYSTEM: Solvent: acetone Temperature = 22°C
Conclusion	:	Under the conditions of this test the substance was found not to be harmful at the level of water solubility but toxic to fish above this level.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (1) (6)

Type	:	static
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	1.6
Limit test	:	
Analytical monitoring	:	no
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1984
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	LC50 (96 hr) = 1.6 mg/l (C.I. = 1.3 - 1.9 mg/l)

LC50 (24 hr) = 1.6 mg/l
LC50 (48 hr) = 1.6 mg/l

Test condition : TEST SPECIES:
Weight/length: mean length = 3.7 cm

TEST SYSTEM:
Solvent: acetone
Temperature = 12°C

Conclusion : Under the conditions of this test the substance is not harmful to fish at the level of water solubility, but it is toxic above this level.

Reliability : (4) not assignable
Secondary literature

04.12.2002 (1) (6)

Type : static
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : 5.6
LC50 : 21
Limit test :
Analytical monitoring : no
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1984
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Result : LC50 (96 hr) = 21 mg/l (C.I. = 6 - 27 mg/l)
NOEC (96hr) = 5.6 mg/l
LC50 (24 hr) = 24 mg/l
LC50 (48 hr) = 21 mg/l

Test condition : TEST SYSTEM:
Solvent: acetone
Temperature = 22°C

Conclusion : Under the conditions of this test the substance is not harmful at the level of water solubility but is harmful to fish above this level.

Reliability : (4) not assignable
Secondary literature

04.12.2002 (1) (6)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : semistatic
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : = .948
EC50 : = 1.31
EC100 : = 1.69
Analytical monitoring : yes
Method : OECD Guide-line 202
Year : 1996
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : METHOD FOLLOWED:

OECD Guideline 202.

DEVIATIONS FORM GUIDELINE: No

STATISTICAL METHODS:
EC50 calculated using moving averages

METHOD OF CALCULATION:
Test concentrations calculated using geometric means

ANALYTICAL METHOD:
HPLC:
Column: Inertsil ODS-2, 5µm, 4.6x150 mm (GL Science Inc.)
Mobile phase: 80% acetonitrile/20% water
Flow rate: 1.0 mL/min
uv detector: 225 nm
Sample size: 50 µL

Result : 1.20 < EC50 (48 hr) < 1.44 mg/L (95% confidence interval)

EXPOSED:

Effect data (immobilisation);
EC50 (24 hr) = 1.45 mg/l (C.I. = 1.28 - 1.75 mg/l)
NOEC (24 hr) = 0.458 mg/l
EC100 (24 hr) > 1.69 mg/l

CONTROL:
Number of adverse effects: 0

Test condition : TEST ORGANISMS:
Loading: 20 organisms per test concentration

DILUTION WATER:
Dechlorinated tap water supplied by Yokohama City.

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle, solvent: HCO-50 and DMF
Concentration of vehicle/solvent: 100 mg/l

STABILITY OF THE TEST CHEMICAL SOLUTIONS:
The concentration of test material measured at preparation and after 24 hours were greater than +/- 20% of the set-up values, therefore, the measured values (geometric average) were used.

REFERENCE SUBSTANCE: none

TEST SYSTEM:
Concentrations: Control, vehicle control, 0.50, 0.750, 1.10, 1.70 and 2.50 mg/L
Renewal of test solution: Total amount of test solution was replaced at 24 hours
Exposure vessel type: 100 mL glass beaker
Number of replicates, individuals per replicate: 4 replicates per test concentration, 5 individuals per replicate.
Test temperature: 20.4 +/- 0.3°C
Dissolved oxygen: 7.8 - 8.1 mg/L.
pH: 7.9 -8.1
Intensity of irradiation:
Photoperiod: 16 hours light/8 hours darkness

DURATION OF TEST: 48 hours

TEST PARAMETER: immobilisation

Table 1: Measured concentrations of the test substance under semi-static test conditions during a 48-hour daphnia magna immobilization test

Nominal Concentration mg/L	Measured Concentration, mg/L				Geometric Mean during 24 hours mg/L
	0 Hour new	Percent of Nominal	24 Hour old	Percent of Nominal	
Control	< 0.004	-	< 0.004	-	-
Solvent Control	< 0.004	-	< 0.004	-	-
0.500	0.549	110	0.382	76	0.458
0.750	0.855	114	0.549	73	0.685
1.10	1.25	114	0.719	65	0.948
1.70	1.91	112	0.908	53	1.32
2.50	2.84	114	1.00	40	1.69

new: freshly prepared test solutions

old: test solutions after 24 hours exposure period

Table 2: The numbers of immobile Daphnia magna exposed to the test substance under semi-static conditions

Nominal Concentration mg/L	Measured Concentration mg/L	Cumulative Numbers of Immobilized Daphnia (Percent Immobility)	
		24 Hour	48 Hour
Control	-	0 (0)	0 (0)
Solvent Control	-	0 (0)	0 (0)
0.500	0.458	1 (5)	0 (0)
0.750	0.685	5 (25)	1 (5)
1.10	0.948	6 (30)	0 (0)
1.70	1.32	3 (15)	7 (35)
2.50	1.69	18 (90)	20 (100)

Conclusion : The substance is toxic to Daphnia under the conditions of the test. Toxicity was observed to be greater than the limit of water solubility of the substance.

Reliability : (1) valid without restriction
Guideline study conducted to GLP

04.12.2002 (12)

Type :

Species : Daphnia magna (Crustacea)

Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	> .3
EC50	:	> .3
Analytical monitoring	:	no
Method	:	OECD Guide-line 202
Year	:	1984
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	LC50 (24 hr) > 100 mg/l LC50 (48 hr)> 100 mg/l NOEC (48 hr) = 100 mg/l
Test condition	:	TEST SYSTEM: Solvent: acetone
Conclusion	:	The toxicity of the substance is greater than the water solubility level.
Reliability	:	(4) not assignable Guideline study (OECD 202)

04.12.2002 (1) (6)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	growth rate
Exposure period	:	72 hour(s)
Unit	:	mg/l
NOEC	:	= .023 (measured)
EC50	:	= .071 (measured)
Limit test	:	
Analytical monitoring	:	yes
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	1996
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	METHOD FOLLOWED: OECD Guideline No. 201. DEVIATIONS FROM GUIDELINE: No STATISTICAL METHODS: The EC50 values and associated 95% confidence limits were determined by least squares linear regression analysis of the logarithm of nominal test concentration against the inhibition rates based on the solvent control. The NOEC values were determined by analysis of variance (ANOVA) and Williams' t-test, subsequent to Bartlett's test for homogeneity of variances. Statistical analyses were performed using Yukms Statlight #4 software (Yukms Corp, Tokyo). ANALYTICAL METHOD: HPLC: Column: Inertsil ODS-2, 5µm, 4.6x150 mm (GL Science Inc.) Mobile phase: 80% acetonitrile/20% water Flow rate: 1.0 mL/min uv detector: 225 nm Sample size: 100 µL (working curve: 50 µL)
Result	:	EXPOSED:

Effect data/Element values:

E_bC_{50} (0-72h) = 0.037 mg/L (C.I. 0.033 – 0.042 mg/L) (measured)
$NOEC_b$ (0-72h) = 0.011 mg/L (measured)
E_rC_{50} (24-72h) = 0.071 mg/L (measured)*
$NOEC_r$ (24-72h) = 0.023 mg/L (measured)

* It was not possible to calculate 95% confidence limits for the ErC50 value as the data generated did not fit the models available for the calculation of confidence limits.

Cell density data:
Growth curves: Cell concentrations after 72 hours in the control and vehicle control groups were increased by 178 and 205 times respectively, in average showing normal growth under the test conditions. In the test groups growth was 208 times at the 0.004 mg/l dose level, 204 times at the 0.008 mg/l dose level, 191 times at the 0.016 mg/l dose level, 152 times at the 0.032 mg/l dose level, 96 times at the 0.063 mg/l dose level, 30 times at the 0.13 mg/l dose level and 10 times at the 0.25 mg/l dose level.

CONTROL:
Number/percentage showing adverse effects:
Nature of adverse effects:

Test condition : TEST ORGANISMS:
Initial cell concentration: 10000 cells/mL

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle, solvent: HCO-50 and DMF
Concentration of vehicle/solvent: 100 mg/L

STABILITY OF THE TEST CHEMICAL SOLUTIONS:
The concentration of the test material at the end of the exposure period was between 19 and 90% of the nominal value at the start of study. Geometric mean measured values were used for calculation of the growth inhibition concentration.

REFERENCE SUBSTANCE: None

TEST SYSTEM:
Test type: Static, Shaking culture (100 rpm)
Concentrations: Control, vehicle control, 0.004, 0.008, 0.016, 0.032, 0.063, 0.13, and 0.25 mg/L (nominal)
Renewal of test solution: No
Exposure vessel type: 300 mL glass Erlenmeyer flask fitted with a breathable silicone plug.
Number of replicates: 3 replicates per test concentration.
Test temperature: 23.5 +/- 0.7°C
pH: Initial = 7.8, Final 7.8 - 9.7 (increased pH seen at dose levels of =< 0.032 mg/l)
Adjustment of pH: None
Intensity of irradiation: 4000 - 5000 lux
Photoperiod: continuous light

TEST PARAMETER: Growth inhibition

Table 1: Measured concentrations during a 72-hour exposure

Nominal Concentration mg/L	Measured Concentration, mg/L				
	0 Hour	Percent of Nominal	72 Hour	Percent of Nominal	Geometric Mean
Control	< 0.0005	-	< 0.0005	-	
Solvent Control	< 0.0005	-	< 0.0005	-	
0.0040	0.0047	118	0.0036	90	0.0041
0.0080	0.0089	111	0.0056	70	0.007
0.0160	0.0148	93	0.0078	49	0.011
0.0320	0.0317	99	0.0162	51	0.023
0.0630	0.0634	101	0.0240	38	0.039
0.130	0.128	98	0.0379	29	0.069
0.250	0.250	100	0.0468	19	0.11

Table 2: Cell Density of Selenastrum capricornutum

Nominal Concn (Measured Concn)* mg/L	Vessel No.	Cell Density (cells/mL)			
		0	24	48	72
Control	1	10000	63500	462400	1635300
	2	10000	62800	442200	1913300
	3	10000	59400	361800	1784300
	Average	10000	61900	422100	1777600
	SD	0	2200	53200	139100
Solvent control	1	10000	67300	477100	2394300
	2	10000	63100	459400	1878300
	3	10000	58100	408500	1882300
	Average	10000	62800	448300	2051600
	SD	0	4600	35600	296800
0.0040 (0.0041)	1	10000	64600	484300	2327300
	2	10000	61700	426600	2283300
	3	10000	58100	388300	1615300
	Average	10000	61500	433100	2075300
	SD	0	3300	48300	399000
0.0080 (0.007)	1	10000	66300	428300	2185300
	2	10000	61600	387200	1889300
	3	10000	59500	392100	2058300
	Average	10000	62500	402500	2044300
	SD	0	3500	22400	148500

0.0160	1	10000	59300	418300	2314300
(0.011)	2	10000	55700	349900	1694300
	3	10000	54400	346700	1732300
	Average	10000	56500	371600	1913600
	SD	0	2500	40400	347500
0.0320	1	10000	60400	318100	1744300
(0.023)	2	10000	54600	282200	1430300
	3	10000	47200	282800	1397300
	Average	10000	54100	294400	1524000
	SD	0	6600	20600	191500
0.0630	1	10000	55600	285700	1380300
(0.039)	2	10000	51900	216200	798300
	3	10000	43000	202300	687000
	Average	10000	50200	234700	955200
	SD	0	6500	44700	372300
0.130	1	10000	48500	128800	307100
(0.069)	2	10000	47800	135200	397400
	3	10000	48600	129400	198400
	Average	10000	48300	131100	301000
	SD	0	400	3500	99600
0.250	1	10000	37600	68300	100800
(0.11)	2	10000	38800	74600	102700
	3	10000	36300	69400	105100
	Average	10000	37600	70800	102900
	SD	0	1300	3400	2200

*1 values in parentheses are the geometric mean measured concentration

Table 3: Growth Inhibition of Selenastrum capricornutum

Nominal Conc. (Measured Conc.) [*1]		Area[*2]	Inhibition (%)[*5]	Rate[*3]	Inhibition (%)[*5]	Rate[*4]	Inhibition (%)[*5]
mg/L	No.	A(0-72h)	IA(0-72h)	μ(24-48h)	Im(24-48h)	μ(24-72h)	Im(24-72h)
Control	1	31645000		0.0827		0.0677	-
	2	34480000		0.0813		0.0712	-
	3	30920000		0.0753		0.0709	-
	Average	32348000	-	0.0798	-	0.0699	-
	SD	1881000		0.0039		0.0019	
Solvent control	1	41197000		0.0816		0.0744	
	2	34480000		0.0827		0.0707	
	3	33186000		0.0813		0.0725	
	Average	36288000	-	0.0819	-	0.0725	-
	SD	4301000		0.0007		0.0019	
0.0040 (0.0041)	1	40501000		0.0839		0.0747	
	2	38519000		0.0806		0.0752	
	3	29497000		0.0792		0.0693	
	Average	36172000	0.3	0.0812	0.9	0.0731	-0.8
	SD	5865000		0.0024		0.0033	
0.0080 (0.007)	1	37494000		0.0777		0.0728	
	2	32843000		0.0766		0.0713	
	3	34938000		0.0786		0.0738	
	Average	35092000	3.3	0.0776	5.3	0.0726	-0.1
	SD	2329000		0.0010		0.0013	
0.0160 (0.011)	1	38634000		0.0814		0.0763	
	2	29466000		0.0766		0.0711	
	3	29814000		0.0772		0.0721	
	Average	32638000	10.1	0.0784	4.3	0.0732	-1.0
	SD	5196000		0.0026		0.0028	
0.0320 (0.023)	1	29416000		0.0692		0.0701	
	2	24647000		0.0684		0.0680	
	3	24088000		0.0746		0.0706	
	Average	26050000	28.2**	0.0707	13.7**	0.0696	4.0
	SD	2928000		0.0034		0.0014	
0.0630 (0.039)	1	24155000		0.0682		0.0669	
	2	15414000		0.0595		0.0569	
	3	13531000		0.0645		0.0577	
	Average	1770000	51.2**	0.0641	21.7**	0.0605	16.6**
	SD	5669000		0.0044		0.0056	
0.130 (0.069)	1	7340000		0.0407		0.0385	
	2	8561000		0.0433		0.0441	
	3	6053000		0.0408		0.0293	

	Average	7318000	79.8**	0.0416	49.2**	0.0373	48.6**
	SD	1254000		0.0015		0.0075	
0.250	1	3151000		0.0249		0.0205	
(0.11)	2	3354000		0.0272		0.0203	
	3	3198000		0.0270		0.0221	
	Average	3234000	91.1**	0.0264	67.8**	0.0210	71.0**
	SD	106000		0.0013		0.0010	

*1 values in parentheses are the geometric mean measured concentration

*2 Area under the growth curves (0-72h)

*3 Growth rates (24-48h)

*4 Growth rates (24-72h)

*5 Values are the inhibition rates based on the solvent control

** Indicates a significant difference (p=0.10) from the solvent control

Conclusion : The substance is toxic to algae under the conditions of the test.
Reliability : (1) valid without restriction
Guideline study conducted to GLP
04.12.2002 (13)

Species : Selenastrum capricornutum (Algae)
Endpoint : Biomass
Exposure period : 96 hour(s)
Unit : mg/l
EC50 : > .3
Limit test :
Analytical monitoring : No
Method : other: US EPA (1971) Algal Assay Procedure: Bottle Test EPA 1972-795-146/1
Year : 1971
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : 1 < EC50 (96 hr) 15 mg/L (95 % confidence)
In vivo chorophyll results:
EC50 (24 hr) > 10 mg/L
EC50 (48 hr) = 6 mg/L
EC 50 (72 and 96 hr) = 4 mg/L
Test condition : TEST SYSTEM:
Solvent: acetone
Temperature = 24°C
Intensity of irradiation = 4000 lux
Initial cell concentration: 10,000 cells/mL
Conclusion : The substance is not harmful to algae below the limit of water solubility but it is toxic above this level.
Reliability : (4) not assignable
Secondary literature
04.12.2002 (1) (6)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type : Aquatic
Species : activated sludge

Exposure period	:	3 hour(s)
Unit	:	mg/l
EC50	:	> 10000
Analytical monitoring	:	No
Method	:	ISO 8192 "Test for inhibition of oxygen consumption by activated sludge"
Year	:	1990
GLP	:	Yes
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (1) (6)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species	:	Daphnia magna (Crustacea)
Endpoint	:	reproduction rate
Exposure period	:	21 day(s)
Unit	:	mg/l
NOEC (21d, reproduction)	:	> .16 (measured)
NOEC (14d, reproduction)	:	0.042 (measured)
LCEC	:	> .16 (measured)
EC50	:	> .16 (measured)
LC50 (parental)	:	> .16 (measured)
NOEC (parental)	:	0.068 (measured)
Analytical monitoring	:	yes
Method	:	OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test"
Year	:	1996
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method :

METHOD FOLLOWED:
OECD Guideline No. 202.

DEVIATIONS FROM GUIDELINE: No

STATISTICAL METHODS:
Dunnett multiple comparison procedure (two sided test) used to compare cumulative numbers of juveniles produced per adult.

METHOD OF CALCULATION:
Time weighted average used to calculate test material concentration.

ANALYTICAL METHOD:
HPLC:
Column: Inertsil ODS-2, 5µm, 4.6x150 mm (GL Science Inc.)
Mobile phase: 80% acetonitrile/20% water
Flow rate: 1.0 mL/min
uv detector: 225 nm
Sample size: 50 µL

Result :

EXPOSED:

Number of mortalities and death rate: Death rates of parent Daphnia in the control and vehicle control were 15% and 7.5% respectively by the end of the exposure period. These values fulfilled the standard of less than 20% which is the requirement of the study.

In the test groups (based on measured concntrations) the death rates were as follows:
0.012 mg/l = 5%
0.022 mg/l = 2.5%
0.042 mg/l = 5%
0.068 mg/l = 5%
0.16 mg/l = 35%

Day of first birth: In the control and vehicle control the first day of birth was 7 days after the start of exposure and fulfilled the standard level within 9 days which is the requirement for the study. In the test groups the day of first birth was 7 days for the 0.012, 0.022, 0.042 and 0.068 mg/l dose levels (based on measured test concentrations) and 10 days for the 0.16 dose level.

Mean value of accumulated offspring (based on measured concentrations):
Control group = 82
Vehicle control = 96
0.012 mg/l = 103
0.022 mg/l = 105
0.042 mg/l = 108
0.068 mg/l = 87
0.16 mg/l = 88

Lowest observed effect concentration (LOECr) on accumulated number of born offspring per one parent Daphnia > 0.16 mg/l (measured)

Size and condition of parent: There were no observable differences in the size of parent Daphnia between the control, the vehicle control and the 0.012 and 0.022 mg/l test groups. In comparison to the control group the parent Daphia was small on day 9 in the 0.042 mg/l group, on days 7-15 in the 0.068 mg/l group and on days 7-17 in the 0.16 mg/l group.

Dormant eggs: There were no dormant eggs in any of the groups throughout the study.

Numbers of offspring: Numbers of offspring in the 0.068 and 0.16 mg/l dose groups were inclined to decrease compared with those in the control and vehicle control groups until 14 days after exposure. However, numbers of offspring increased after 14 days in comparison with the other test groups. The numbers exceeded those in the control group and recovered to the level of those in the vehicle control group.

CONTROL:

Number/percentage showing adverse effects: 0

Test condition : DILUTION WATER SOURCE:
Dechlorinated tap water supplied by Yokoyama City

STOCK AND TEST SOLUTION AND THEIR PREPARATION:
Vehicle, solvent: HCO-50 and DMF

Concentration of vehicle/solvent: 30 mg/l

STABILITY OF THE TEST CHEMICAL SOLUTIONS:
The concentration of test material in the test solution at the time of preparation of the test solution was 0.019 - 0.304 mg.l (set-up value 0.02 - 0.3 mg/l) and the concentration before the exchange of solution was 0.004 - 0.021 mg/l. In addition the ratios of test material concentration measured to the set-up value in each concentration range were 88 - 135% at the time of preparation and 5 - 25% before the exchange.
The concentration of test material measured at preparation and before exchange during the exposure period were +/- 20% of the set-up values, therefore, the measured (time-weighted average) were used.

REFERENCE SUBSTANCE: one

TEST SYSTEM:
Test type: Semi-static
Concentrations: Control, vehicle control, 0.020, 0.040, 0.080, 0.15 and 0.3 mg/l
Renewal of test solution: Every two days
Exposure vessel type: 1 L glass beaker
Number of replicates, individuals per replicate: 4 replicates per test concentration, 10 individuals per replicate.
Test temperature: 19.8 - 20.3°C
Dissolved oxygen: 6.7 - 8.5 mg/L.
pH: 7.3 -8.1
Photoperiod: 16 hours light/ 8 hours dark

DURATION OF TEST: 21 days

ENDPOINTS ASSESSED: Mortality (LC50), inhibition of reproduction (ErC50), No Observed Effect Concentration, Lowest Observed Effect Concentration, Number of offspring, Date of first birth, Mean number of offspring, size and condition of parents, number of dormant eggs.

TEST PARAMETER: Effects on parents and first generation offspring within 21 days of exposure.

SAMPLING: Every two days.

Table 1: Measured concentrations of test substance during a 21-day exposure of Daphnia magna under the semi-static test conditions

Nominal Concentration (mg/L)	Measured Concentration (mg/L) (Percent of Nominal)						Time-Weighted Mean during 21-days (mg/L)
	0 day new	1 day old	2 day old	14 day new	15 day old	16 day old	
Control	<0.001 (-)	<0.001 (-)	<0.001 (-)	<0.001 (-)	<0.001 (-)	<0.001 (-)	-
Solvent Control	<0.001 (-)	<0.001 (-)	<0.001 (-)	<0.001 (-)	<0.001 (-)	<0.001 (-)	-
0.020	0.027 (135)	0.015 (75)	0.005 (25)	0.019 (95)	0.009 (45)	0.004 (20)	0.012
0.040	0.045	0.026	0.008	0.040	0.018	0.009	0.022

	(113)	(65)	(20)	(100)	(45)	(23)	
0.080	0.085 (106)	0.042 (53)	0.014 (18)	0.075 (94)	0.038 (48)	0.015 (19)	0.042
0.150	0.157 (105)	0.066 (35)	0.018 (7)	0.138 (92)	0.061 (41)	0.017 (11)	0.068
0.300	0.304	0.105	0.021	0.263	0.097	0.014	0.160

new: freshly prepared test solution

old: test solutions 1 day or 2 days after preparation

Table 2: Cumulative Numbers of Dead Parental Daphnia

Nominal conc.(mg/L)	Days																					
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
cont.	0	0	1	1	1	1	1	1	1	1	1	1	1	1	2	3	3	4	5	5	6	6
sol. cont.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2	3	3	3	3	3
0.020	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2
0.040	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1
0.080	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	2	2	2
0.150	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	2	2
0.300	0	0	1	1	1	1	11	11	11	11	11	11	11	11	11	12	12	12	13	13	14	14

Table 3: Mortality (%) of Parental Daphnia

Nominal conc. (mg/L)	Measured conc. (mg/L)	Days					
		1	2	4	7	14	21
cont.	-	0	3	3	3	5	15
sol. cont.	-	0	0	0	0	5	8
0.020	0.012	0	0	0	0	0	5
0.040	0.022	0	0	0	0	0	3
0.080	0.042	0	0	0	0	0	5
0.150	0.068	0	0	0	0	0	5
0.300	0.160	0	3	3	28	28	35

Table 4: Time (day) to First Brood Production

Vessel No.	Nominal concentration (mg/L)						
	Control	Solvent Control	0.020	0.040	0.080	0.150	0.300
1	7	7	7	7	7	7	7
2	7	7	7	7	7	10	10
3	7	7	7	7	7	7	10
4	7	7	7	7	7	10	7
Mean	7.00	7.00	7.00	7.00	7.00	8.50	8.50

Table 5: Mean cumulative Numbers of Juveniles Produced per Adult (ΣF1/P)

Nominal Conc. (mg/L)	Measured Conc. (mg/L)	Days										
		0	6	7	8	10	12	14	16	18	20	21
cont.	-	0.0	0.0	5.4	6.1	23.2	23.2	48.9	77.8	78.5	81.6	81.7
sol. cont.	-	0.0	0.0	2.4	5.0	17.5	21.0	49.0	73.9	77.2	95.9	95.9
0.020	0.012	0.0	0.0	1.6	1.8	16.5	17.4	48.7	82.8	83.1	103.1	103.1
0.040	0.022	0.0	0.0	1.4	1.6	14.5	18.3	48.4	81.5	85.1	105.2	105.2
0.080	0.042	0.0	0.0	0.6	1.0	17.6	18.6	50.6	90.7	91.7	107.5	107.5
0.150	0.068	0.0	0.0	0.2	0.2	6.4	7.6	27.2	54.9	57.0	87.2	87.2
0.300	0.160	0.0	0.0	0.2	0.2	11.2	12.4	29.7	57.9	640	88.4	88.4

Table 6: Mean cumulative numbers of juveniles produced per adult in control and test vessels after 21 days

Vessel No.	Nominal Concentration, mg/L (Measured Concentration, mg/L)						
	Control	Sol. Cont.	0.020 (0.012)	0.040 (0.022)	0.080 (0.042)	0.150 (0.068)	0.300 (0.160)
1	53.6	115.0	101.4	106.0	99.5	98.6	84.9
2	91.0	96.5	104.0	109.1	109.9	79.8	94.9
3	95.7	83.1	102.7	96.7	113.5	91.3	68.1
4	86.3	89.0	104.2	108.8	107.1	78.9	105.6
Mean	81.7	95.9	103.1	105.2	107.5	87.2	88.4
S.D.	19.1	13.9	1.3	5.8	5.9	9.5	15.9
Inhibition rate (%)		-	-7.5	-9.6	-12.1	9.1	7.8

No significant differences from solvent control (α=0.05, 0.01)

Table 7: Mean cumulative numbers of juveniles produced per adult in control and test vessels after 14 days

Vessel No.	Nominal Concentration, mg/L (Measured Concentration, mg/L)						
	Control	Sol. Cont.	0.020 (0.012)	0.040 (0.022)	0.080 (0.042)	0.150 (0.068)	0.300 (0.160)
1	34.7	59.2	43.5	46.6	41.2	37.1	30.3
2	50.9	44.8	54.1	55.0	56.1	21.9	32.5
3	54.8	44.4	49.1	45.1	60.6	27.5	21.5
4	55.1	47.5	47.9	47.0	44.6	22.4	34.6
Mean	48.9	49.0	48.7	48.4	50.6	27.2	39.7
S.D.	9.6	7.0	4.4	4.5	9.2	7.1	5.8
Inhibition rate (%)			0.7	1.1	-3.4	44.4	39.3
Significant difference						**	**

** Indicates a significant difference (α=0.01) from the solvent control

Conclusion : It was considered that the test material might affect the larva of Daphnia by exposure of 0.068 mg/l or more after 14 days, although recovery in reproduction was observed and there is no significant difference between the 0.160 mg/L and control at 21 days of exposure (end of the test period).

Reliability : (1) valid without restriction
Guideline study (OECD 202) conducted to GLP

04.12.2002 (15)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

Remark : **AQUATIC TOXICITY OF DEGRADATION PRODUCTS**

(See draft SIAR for di(benzothiazoyl-2)disulfide, CAS Nr 120-78-5)

di(benzothiazoyl-2)disulfide (MBTS)
The effect concentrations given are only nominal values. Owing to the instability of MBTS in aqueous solution it may be assumed that the effects observed are mainly attributed to MBT (BUA, 1994).

Algae
Selenastrum capricornutum EC_{50} = 0.6 mg/l (96 h)
NOEC < 0.3 mg/l (96 h)
(Effect: inhibition of chlorophyll-a-synthesis)
Scenedesmus subspicatus at 40 mg/l after 72 h no inhibition of biomass

Invertebrates
Daphnia magna EC_{50} = 82 mg/l (48 h)
(Effect: immobilization)

Fish
Salmo gairdneri LC_{50} = 66 mg/l (96 h)
Lepomis macrochirus LC_{50} = 82 mg/l (96 h)
Oryzias latipes LC_{50} = 19 mg/l (48 h)

Mercaptobenzothiazole (MBT)
MBT and its salts are not considered separately. It is however indicated when NaMBT instead of MBT was used in an investigation.

Protozoa
Tetrahymena pyriformis EC_{50} = 10 mg/l (24 h) (BUA, 1991)
(Effect: biomass)

Algae
Selenastrum capricornutum EC_{50} = 0.25 mg/l (96 h)
EC_{50} = 0.3 mg/l (96 h) NaMBT
NOEC = 0.1 mg/l (96 h)
(Nominal concentrations, effect: biomass) (BUA, 1991)

Invertebrates
Daphnia magna LC_{50} = 4.1 mg/l (48 h)
LC_0 = 1.8 mg/l (48 h)
LC_{50} = 19 mg/l (48 h) NaMBT
LC_0 = 10 mg/l (48 h) NaMBT
NOEC = 0.24 mg/l (21 d) mortality
NOEC = 0.22 mg/l (21 d) reprod. rate (BUA, 1991)

Vertebrates
Oncorhynchus mykiss (= Salmo gairdneri)

LC_{50} = 0.73 mg/l (96 h)
LC_{50} = 1.8 mg/l (96 h) NaMBT
NOEC = 0.041 mg/l (89 d) length of larvae
(Measured concentrations) (BUA, 1991)

Benzothiazole

Fish
Oryzias latipes LC_{50} = 110 mg/l (48 h)

Protozoa
Tetrahymena pyriformis EC_{50} = 160 mg/l (24 h)
(Yoshioka, 1986)
(Effect: biomass)

Based on the above data, some of the degradation products of N-tert-butylbenzothiazole-2-sulfenamide, and especially mercaptobenzothiazole, show similar levels of aquatic toxicity to the parent compound.

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

Remark : The test material is a solid. No details of particle size distribution have been given, but a repeat-dose toxicity study using the inhalation route indicates some systemic toxicity. The vapour pressure value for the test material is low, therefore exposure via inhalation of volatile products is low. The test material is expected to hydrolyse readily at pH values below 9. This suggests that systemic exposure to degradants can be expected, particularly following oral ingestion. The log oil/water partition coefficient value is moderate, which suggests that test material passage across biological membranes is possible.

Absorption

The results of the acute and repeat dose oral toxicity studies in the rat suggest that the test material is absorbed from the gastro-intestinal tract. Systemic effects are observed, particularly with cumulative exposure. Because of the rapid hydrolysis of the test material, it is likely that toxicity is a result of exposure to degradants. The test material has a low water solubility which may restrict absorption of the parent molecule but hydrolysis may enhance water solubility. The moderate log oil/water partition coefficient of the parent molecule will allow passage across the biological membranes of the gastro-intestinal tract. The results of the acute dermal toxicity studies in the rabbit show that the test material is not more toxic by this route. The results of human patch tests and a sensitisation study in the guinea pig show that the test material (or a product of hydrolysis, mercaptobenzothiazole) is absorbed through the skin. The results of a repeat dose study in the rat by inhalation exposure shows that the test material (or a hydrolysis product) can be absorbed by inhalation.

Distribution

The results of the repeat dose oral and inhalation studies in the rat suggest some systemic distribution. Following oral ingestion it is likely that the test material (or hydrolysis products) is distributed via the portal circulation system. The positive sensitisation response suggests that the hydrolysis products may bind to circulatory proteins. The moderate log oil/water partition coefficient value suggests that the test material could potentially accumulate in body fat. Because the test material hydrolyses, it is likely to result in products with a lower partition coefficient value.

Metabolism

The widespread distribution of hydrolases throughout tissues such as the gastro-intestinal tract, and the tendency of the test material to undergo hydrolysis suggest that initial metabolism of the material will be widespread and non-specific. The results of the repeat dose oral and inhalation studies in the rat do show microscopic changes in the liver. This may be indicative of further metabolism of the hydrolysis products. The results of *in vitro* mammalian cell genotoxicity studies show that a positive genotoxic effect is seen, but only in the presence of S9 metabolising system. This indicates that metabolism of the parent test material or a hydrolysis product is required to produce a positive response. The results of separate reproduction/developmental toxicity studies in the rat with the test material and an analogue (either N-cyclohexylbenzothiazole-2-sulfenamide or N,N-dicyclohexylbenzothiazole-2-sulfenamide) show differences in developmental toxicity. If, as expected, the analogues undergo hydrolysis/metabolism as does the test material, it may be suggested that the profile of the metabolites may be significant in the toxicity of the

product.
Excretion

The results of some of the repeat dose oral toxicity studies show changes in the kidneys of rats. This suggests that urinary excretion is a significant route for removal of test material.

05.12.2003

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : > 2000 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : female
Number of animals : 6
Vehicle : peanut oil
Doses : 2000 mg/kg
Method : OECD Guide-line 423
Year : 2002
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Result : Mortality: There were no deaths

Clinical Observations: There were no signs of systemic toxicity

Bodyweight: All animals, except one, showed expected gains in bodyweight over the study period

Necropsy: No abnormalities were noted at necropsy.

Table 1: Individual Bodyweights and Weekly Bodyweight Changes

Dose Level mg/kg	Animal Number and Sex	Bodyweight (g) at Day			Bodyweight Gain (g) During Week	
		0	7	14	1	2
2000	1-0 Female	236	256	275	20	19
	1-1 Female	232	259	259	27	0
	1-2 Female	234	261	276	27	15
	2-0 Female	189	224	243	35	19
	2-1 Female	189	219	240	30	21
	2-2 Female	207	248	280	41	32

Conclusion : The substance is not harmful by acute oral exposure
Reliability : (1) valid without restriction
Study conducted to standard test method under GLP

04.12.2002 (5)

Type : LD50
Value : > 6310 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : male/female

Number of animals	:	20
Vehicle	:	other: 25.0% suspension in corn oil
Doses	:	6310, 7949 mg/kg
Method	:	other: Younger Laboratory method
Year	:	1973
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Clinical signs of toxicity: Reduced appetite and activity (lasting 3 to 5 days in survivors), increasing weakness, collapse and death. Necropsy: Autopsy of decedents showed lung congestion, liver discolouration, and acute gastrointestinal inflammation. Viscera of surviving animals appeared normal at sacrifice.
Test condition	:	Mode of administration: gavage Number of animals: 5/dose (M/F)
Conclusion	:	The substance is not classified as harmful via oral exposure.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (1) (6) (7)

Type	:	LD50
Value	:	= 6850 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	male/female
Number of animals	:	5
Vehicle	:	other: 10% w/w suspension in water
Doses	:	6850 mg/kg
Method	:	other: no information available
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Mortality observed.
Test condition	:	Number of animals: 2 Male, 3 Female
Conclusion	:	Under the conditions of the test the substance was not classified as harmful by oral exposure. The test is not considered to be valid as too few animals were tested to corroborate the test result.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

Type	:	LD0
Value	:	> 10000 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	male
Number of animals	:	1
Vehicle	:	other: 25% w/w suspension in water
Doses	:	10000 mg/kg
Method	:	other: screening test, no further information available
Year	:	

GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	No mortality observed
Conclusion	:	Under the conditions of the screening test the substance is not considered to be harmful be oral exposure. The screening test is not a valid test for acute oral toxicity.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 7940 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	male/female
Number of animals	:	2
Vehicle	:	other: 40% suspension in corn oil
Doses	:	7940 mg/kg
Method	:	other: Younger Laboratories method
Year	:	1973
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Mortality: none Clinical signs: none Necropsy: viscera appeared normal at sacrifice
Test condition	:	Mode of administration: Applied directly to clipped, intact skin using semi-occlusive dresssings. Exposure time: 24 hours
Conclusion	:	Under the conditions of the test the substance is not considered to be harmful by dermal exposure.
Reliability	:	(4) not assignable Only secondary literature available

04.12.2002 (6) (7)

Type	:	LD0
Value	:	> 6000 mg/kg bw
Species	:	rabbit
Strain	:	
Sex	:	male/female
Number of animals	:	2
Vehicle	:	
Doses	:	6000 mg/kg
Method	:	other: no information available
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Mortality: none

Conclusion	:	Under the conditions of the study the substance is not considered to be harmful by dermal exposure.
Reliability	:	(4) not assignable Only secondary literature available (IUCLID data set)

04.12.2002 (6)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	LD50
Value	:	= 5000 - 7000 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	other: no information available
Year	:	
GLP	:	no data
Test substance	:	no data
Reliability	:	(4) not assignable Secondary literature source. Review paper on hygiene maintenance for food products

04.12.2002 (21)

Type	:	LD50
Value	:	= 180 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.v.
Exposure time	:	
Method	:	other: no data
Year	:	
GLP	:	no d ata
Test substance	:	other TS
Reliability	:	(4) not assignable Secondary literature source: RTECS review

04.12.2002 (18)

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	.5 g
Exposure	:	Semiocclusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	other: moistened with saline
PDII	:	.1
Result	:	not irritating
Classification	:	not irritating
Method	:	Draize Test

Year	:	1982
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Score: 0.1/8.0
Conclusion	:	The substance is not classified under the conditions of the test.
Reliability	:	(4) not assignable Secondary literature.

04.12.2002 (7)

Species	:	rabbit
Concentration	:	
Exposure	:	
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	water
PDII	:	0
Result	:	not irritating
Classification	:	not irritating
Method	:	other: Younger Laboratories method
Year	:	1973
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	EU mean erythema score: 0.0 EU mean oedema score: 0.0 PII: 0/8
Test condition	:	Mode of administration: as per FHSA Observation times: 24, 48, 72, 168 hours Skin: intact
Conclusion	:	Under the conditions of the test the substance is considered not to be irritating to the skin of rabbits.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

Species	:	rabbit
Concentration	:	
Exposure	:	
Exposure time	:	24 hour(s)
Number of animals	:	3
Vehicle	:	
PDII	:	1
Result	:	slightly irritating
Classification	:	not irritating
Method	:	other: modified Draize Test
Year	:	1944
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	PII: 1.0/8
Test condition	:	Number of animals: 1 Male, 2 Females Healing time: 2 hours
Conclusion	:	Under the conditions of the test the substance is not considered to be an irritant to the skin of rabbits.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

Species	:	rabbit
Concentration	:	
Exposure	:	
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	1.5
Result	:	slightly irritating
Classification	:	irritating
Method	:	other: modified Draize
Year	:	1944
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	PII: 1.5/8 Healing time: Irritation still observed after 72 hours
Test condition	:	Animals: 6 Males Skin: intact
Conclusion	:	The substance is considered to be irritating to the skin of rabbits under the conditions of this test due to the persistance of signs of irritation after the 72 hour observation.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	100 other: mg
Exposure time	:	unspecified
Comment	:	no data
Number of animals	:	6
Vehicle	:	
Result	:	slightly irritating
Classification	:	not irritating
Method	:	other
Year	:	1973
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Score: 2.5/110.0
Conclusion	:	The substance is not classified as irritating
Reliability	:	(4) not assignable Secondary literature.

04.12.2002 (7)

Species	:	rabbit
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	6
Vehicle	:	
Result	:	slightly irritating
Classification	:	not irritating

Method	:	Draize Test
Year	:	1944
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	EU mean erythema score: 2.53 EU mean chemosis score: 0 EU mean corneal score: 0 EU mean iris score: 0 Draize score: 2.5/110
Conclusion	:	Under the conditions of this test the substance is considered not to be irritating to the eyes of rabbits.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

Species	:	rabbit
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	3
Vehicle	:	
Result	:	slightly irritating
Classification	:	not irritating
Method	:	other: modified Draize method
Year	:	1944
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Draize score: 2.4/110
Test condition	:	Animals: 2 Male, 1 Female Healing time: 72 hours
Conclusion	:	Under the conditions of the test the substance is considered not to be an irritant to the eyes of rabbits.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

Species	:	rabbit
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	6
Vehicle	:	
Result	:	slightly irritating
Classification	:	not irritating
Method	:	Draize Test
Year	:	1944
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Draize score: 2.5/110
Test condition	:	Animals: 6 Male Healing time: 48 hours
Conclusion	:	Under the conditions of this test the substance is considered not to be an irritant to the eyes of rabbits.
Reliability	:	(4) not assignable

Secondary literature

04.12.2002 (6)

5.3 SENSITIZATION

Type	:	Patch-Test
Species	:	human
Number of animals	:	55
Vehicle	:	
Result	:	sensitizing
Classification	:	sensitizing
Method	:	other: no information available
Year	:	1969
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Irritation: Not a primary irritant. Sensitisation: 9/55 sensitisation rate
Test condition	:	Subjects: 18 Male, 37 Female Exposure type: occluded Exposure period: 24 hours Readministration: alternate days Length of test: 14 days
Conclusion	:	Under the conditions of the test, the substance is considered to be a sensitiser.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

Type	:	Patch-Test
Species	:	human
Number of animals	:	14
Vehicle	:	petrolatum
Result	:	sensitizing
Classification	:	sensitizing
Method	:	other: no information available
Year	:	1983
GLP	:	no data
Test substance	:	other TS
Result	:	Sensitisation rate: 13/14
Test condition	:	Application: 1% of the substance in petrolatum Subjects: 14 subjects previously sensitised to MBT (hydrolysis product). Analysis: The substance was not analysed for traces of MBT.
Test substance	:	SOURCE: Commercial grade material supplied by Monsanto. PURITY: Not specified.
Conclusion	:	Under the conditions of the test, the substance can be considered to be a sensitiser. The sensitisation reaction being a cross sensitisation from the substance MBT which occurs as a hydrolysis product in the substance. The validity of the study should be considered as total hydrolysis of the substance to MBT and related products occurs within 24 hours. The substance was not analysed for MBT, therefore, sensitisation could have been through exposure to MBT not the substance.

Reliability : (4) not assignable
Secondary literature
04.12.2002 (4)

Type : Patch-Test
Species : human
Number of animals : 45
Vehicle : petrolatum
Result : sensitizing
Classification : sensitizing
Method : other: product investigations method
Year : 1982
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Result : 13/45 sensitisation rate
Test condition : Application: 60% preparation in petrolatum
Conclusion : Under the conditions of the test the substance was considered to be a sensitiser.
Reliability : (4) not assignable
Secondary literature
04.12.2002 (6)

Type : Buehler Test
Species : guinea pig
Number of animals :
Vehicle : other: ethanol
Result : sensitizing
Classification : sensitizing
Method : other: Pharmakon Laboratory method
Year : 1980
GLP : yes
Test substance : other TS

Test condition : Substance applied as a 25% preparation in ethanol.
Test substance : The substance tested came from three different locations.
Conclusion : Under the conditions of the test the substance is considered to be a sensitiser in the guinea pig.

The validity of the study should be considered as no sensitisation rate has been specified.
Reliability : (4) not assignable
Secondary literature
04.12.2002 (6)

Type : other: comedogenicity assay
Species : rabbit
Number of animals :
Vehicle : other: chloroform
Result : not sensitizing
Classification : not sensitizing
Method : other: Pharmakon Laboratory method
Year : 1982
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Test condition : Application:
Sample #1: 0.01, 0.1 or 10% in chloroform
Sample #2: 1, 10 or 100% in chloroform

Sample #3: 0.01, 0.1 or 1% in chloroform

Conclusion : Under the conditions of this test the substance is considered not to be sensitising in rabbits.

The validity of the study should be considered based on the evidence of the guinea-pig and human studies.

Reliability : (4) not assignable
Secondary literature

04.12.2002 (6)

5.4 REPEATED DOSE TOXICITY

Type	:	
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	
Frequency of treatm.	:	daily
Post exposure period	:	
Doses	:	0, 40, 200 and 1000 mg/kg
Control group	:	yes, concurrent vehicle
LOAEL	:	= 40 mg/kg bw (males)
Method	:	OECD combined study TG422
Year	:	
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Result : LOAEL:
Males 40 mg/kg bw

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
Clinical signs: In both males and females, temporary salivation after each administration was observed in the 200 mg/kg or more groups.
Bodyweight gain: In males bodyweight gain was suppressed in the 1000 mg/kg group. In females a slight suppression of bodyweight gain was observed in the 1000 mg/kg group during pregnancy.
Food consumption: In males food consumption was suppressed in the 1000 mg/kg group and in females of that group food consumption was decreased prior to mating.
Clinical chemistry/pathology/histopathology:
Males: At autopsy after 42 days treatment, the number of males with eosinophilic bodies in the kidney was increased in all treated groups, and increases in the degree of this change and the absolute and relative kidney weights were noted in the 1000 mg/kg group. Although slight hypertrophy of hepatocytes in the central zone was observed in the 200 mg/kg or more groups, increase in the relative liver weight related to the administration was observed only in the 1000 mg/kg group. Decrease in fatty change of hepatocytes in the periportal zone was also observed in the livers of the 1000 mg/kg group. In the 200 mg/kg or more groups, slight increase in the total bilirubin concentration and dose-dependent increase in hemosiderin deposits in the spleen were observed. In addition, hemoglobin and hematocrit value were slightly decreased and hemolytic anemia was induced in the 1000 mg/kg group.
Females: At autopsy on postpartum day 4, slight vacuolar degeneration in proximal tubules of the kidney was observed in the 200 mg/kg or more groups, as well as slight increase in the relative kidney weight. Slight

hypertrophy of hepatocytes in the central zone in the 200 mg/kg or more groups and relative liver weights in the 1000 mg/kg group were found. Deposits of brown pigment in the spleen in the 1000 mg/kg group tended to increase. No haematological findings were reported for females, but based on other findings it is deduced that anemia also occurs in females.

Test condition : TEST ORGANISMS:
Number of animals: 104 (13 males/13 females per group)

ADMINISTRATION/EXPOSURE:
Males 42 days
Females from 14 days prior to mating to day 3 of lactation

ORGANS EXAMINED AT NECROPSY:
Liver, kidneys, spleen, thymus, testes, epididymides, heart, adrenal gland and ovary.

VEHICLE: 5% Gum arabic

Test substance : PURITY: 96.4%

Remark : The study does not report information on the following tissues/organs as required according to the guidelines: brain, spinal cord, large and small intestine, stomach, thyroid, trachea and lungs, uterus urinary bladder, lymph nodes, peripheral nerve, and bone marrow.

Table 1: Body weights of male rats treated orally with N-tert-butyl-2-benzothiazolesulfenamide in the combined repeat dose and reproductive/developmental toxicity screening test

Days of administration	Dose (mg/kg)			
	0	40	200	1000
1 (Init wt.)	281.4 ± 8.6	281.8 ± 9.4	281.9 ± 9.9	281.9 ± 9.0
8	327.1 ± 15.8	333.7 ± 16.4	336.2 ± 17.2	317.3 ± 17.8
15	372.7 ± 18.2	376.4 ± 22.0	385.1 ± 21.1	357.0 ± 24.2
22	401.3 ± 23.7	410.3 ± 27.7	414.9 ± 22.0	385.6 ± 30.2
29	430.6 ± 27.5	440.8 ± 33.0	442.5 ± 24.5	411.5 ± 36.1
36	458.6 ± 32.8	469.2 ± 36.0	471.1 ± 27.3	431.9 ± 40.6
42	479.4 ± 37.7	488.2 ± 37.7	487.2 ± 31.3	445.8 ± 44.5

Values are expressed as Mean ± S.D. in grams – 13 animals used in each group throughout the study

Table 2: Body weights of female rats treated orally with N-tert-butyl-2-benzothiazolesulfenamide in the combined repeat dose and reproductive/developmental toxicity screening test

Days of administration (pre-mating period)	Dose (mg/kg)			
	0	40	200	1000
1 (Init. wt.)	190.2 ± 7.2 (13)	189.5 ± 6.4 (13)	189.7 ± 6.8 (13)	190.0 ± 5.8 (13)
8	211.2 ± 8.2 (13)	208.2 ± 10.0 (13)	211.4 ± 8.1 (13)	207.3 ± 7.6 (13)
15	229.8 ± 11.8 (13)	225.9 ± 11.7 (13)	229.5 ± 10.9 (13)	222.5 ± 8.6 (13)
Days of pregnancy				
0	234.2 ± 13.5 (10)	236.9 ± 21.0 (7)	239.0 ± 11.5 (13)	233.5 ± 16.4 (9)
7	272.2 ± 17.1 (10)	273.8 ± 29.3 (7)	276.0 ± 16.2 (13)	258.9 ± 19.0 (9)
14	308.1 ± 18.1 (10)	311.3 ± 34.9 (7)	312.5 ± 17.7 (13)	290.4 ± 22.6 (9)
20	374.1 ± 23.4 (10)	380.4 ± 41.4 (7)	385.1 ± 26.1 (13)	343.0 ± 40.4 (9)
Days of lactation				
0	284.2 ± 23.2 (10)	276.6 ± 37.3 (6)	283.6 ± 26.2 (13)	262.2 ± 27.4 (8)
4	302.5 ± 14.1 (10)	308.1 ± 29.3 (6)	301.5 ± 20.3 (13)	284.9 ± 34.6 (8)

Values are expressed as Mean ± S.D. in grams.

Parenthesis indicates number of animals

Table 3: Food consumption of male rats treated orally with N-tert-butyl-2-benzothiazolesulfenamide in the combined repeat dose and reproductive/developmental toxicity screening test

Days of administration	Dose (mg/kg)			
	0	40	200	1000
1 – 8	202.8 ± 22.7	205.7 ± 10.9	211.1 ± 13.3	181.9 ± 15.5**
8 – 15	202.9 ± 19.4	200.1 ± 11.1	215.4 ± 13.5	191.1 ± 21.3
29 – 36	197.4 ± 20.4	205.2 ± 21.8	211.4 ± 18.5	192.7 ± 25.4
36 – 42	174.9 ± 18.6	177.9 ± 19.6	179.6 ± 14.3	165.8 ± 20.3

Values are expressed as Mean ± S.D. in grams – 13 animals used in each group throughout the study

**: significant difference from control, $p<0.01$

Table 4: Food consumption of female rats treated orally with N-tert-butyl-2-benzothiazolesulfenamide in the combined repeat dose and reproductive/developmental toxicity screening test

Days of administration (pre-mating period)	Dose (mg/kg)			
	0	40	200	1000
1 – 8	132.5 ± 7.3 (13)	130.8 ± 8.9 (13)	133.2 ± 9.3 (13)	116.7 ± 8.0** (13)
8 – 15	137.3 ± 10.8 (13)	134.1 ± 12.9 (13)	136.8 ± 11.7 (13)	124.7 ± 12.5* (13)
Days of pregnancy				
0 – 7	166.8 ± 16.9 (10)	165.3 ± 29.1 (7)	164.2 ± 16.5 (13)	133.4 ± 25.8* (9)
7 – 14	177.9 ± 15.2 (10)	180.9 ± 29.4 (7)	177.9 ± 15.2 (13)	161.2 ± 16.3 (9)
14 – 20	140.9 ± 8.8 (10)	138.3 ± 9.8 (7)	143.3 ± 11.7 (13)	122.4 ± 16.3* (9)
Days of lactation				
0 – 4	122.3 ± 21.9 (10)	117.6 ± 15.0 (6)	108.8 ± 25.8 (13)	121.2 ± 25.3 (8)

Values are expressed as Mean ± S.D. in grams.

Parenthesis indicates number of animals
*: significant difference from control, $p<0.05$
**: significant difference from control, $p<0.01$

Table 5: Hematological findings of male rats treated orally with N-tert-butyl-2-benzothiazolesulfenamide in the combined repeat dose and reproductive/developmental toxicity screening test

	Dose (mg/kg)			
	0	40	200	1000
		Red Blood Cells		
Count ($X10^4/mm^3$)	805 ± 35	814 ± 40	807 ± 31	774 ± 40
Hemoglobin (g/dl)	15.4 ± 0.6	15.5 ± 0.7	15.3 ± 0.5	14.5 ± 0.4**
Hematocrit (%)	44.4 ± 1.6	44.7 ± 1.9	43.7 ± 1.7	41.6 ± 1.6**
MCV (μm^3)	55.2 ± 1.7	55.0 ± 2.1	54.2 ± 1.1	53.7 ± 1.1
MCH (pg)	19.1 ± 0.7	19.0 ± 0.8	18.9 ± 0.4	18.7 ± 0.5
MCHC (%)	34.6 ± 0.6	34.6 ± 0.4	35.0 ± 0.5	34.8 ± 0.5
		White Blood Cells		
Count ($X10^2/mm^3$)	116 ± 20	111 ± 23	101 ± 19	86 ± 28**
Band neutrophil (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Segmented neutrophil (%)	10 ± 4	10 ± 5	15 ± 10	16 ± 11
Eosinophil (%)	0 ± 1	1 ± 1	1 ± 1	1 ± 1
Basophil (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Monocyte (%)	2 ± 2	2 ± 2	1 ± 1	2 ± 2
Lymphocyte (%)	87 ± 5	87 ± 6	83 ± 10	81 ± 11
		Platelet		
Count ($X10^4/mm^3$)	114.1 ± 8.2	112.5 ± 10.1	111.9 ± 8.9	115.8 ± 8.1

Values are expressed as Mean ± S.D. – 13 animals used in each group throughout the study

**: significant difference from control, p<0.01

Table 6: Blood chemical findings of male rats treated orally with N-tert-butyl-2-benzothiazolesulfenamide in the combined repeat dose and reproductive/developmental toxicity screening test

	Dose (mg/kg)			
	0	40	200	1000
Total protein (g/dl)	5.5 ± 0.3	5.6 ± 0.3	5.8 ± 0.2	5.7 ± 0.4
Albumin (g/dl)	2.8 ± 0.2	2.9 ± 0.3	3.0 ± 0.1	3.0 ± 0.1
A/C	1.05 ± 0.08	1.06 ± 0.15	1.08 ± 0.12	1.10 ± 0.15
BUN (mg/dl)	17 ± 2	17 ± 2	17 ± 2	18 ± 2
Creatinine (mg/dl)	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.0	0.7 ± 0.1
Glucose (mg/dl)	137 ± 13	157 ± 14**	141 ± 12	120 ± 15**
Total cholesterol (mg/dl)	59 ± 13	58 ± 11	59 ± 8	70 ± 12*
Total bilirubin (mg/dl)	0.07 ± 0.02	0.09 ± 0.02	0.09 ± 0.02**	0.09 ± 0.02**
Na (mEq/l)	144.5 ± 1.1	144.4 ± 0.9	144.6 ± 0.9	145.6 ± 0.7*
K (mEq/l)	3.77 ± 0.23	3.76 ± 0.13	3.83 ± 0.22	3.91 ± 0.2
Cl (mEq/l)	106.6 ± 1.4	105.8 ± 0.9	105.9 ± 1.0	106.0 ± 1.2
Ca (mg/dl)	8.6 ± 0.4	8.6 ± 0.5	8.7 ± 0.2	8.9 ± 0.2
Inorg. phos. (mg/dl)	6.2 ± 0.6	6.2 ± 0.5	5.7 ± 0.4	5.8 ± 0.5
ALP (U/l)	199 ± 41	218 ± 54	220 ± 38	205 ± 65
GOT (U/l)	58 ± 7	59 ± 7	60 ± 6	59 ± 10
γ. GTP (U/l)	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Values are expressed as Mean ± S.D. – 13 animals used in each group throughout the study

*: significant difference from control, p<0.05

**: significant difference from control, p<0.01

Table 7: Absolute and relative organ weights of rats treated orally with N-tert-butyl-2-benzothiazolesulfenamide in the combined repeat dose and reproductive/developmental toxicity screening test

Sex	Dose (mg/kg)	0		40		200		1000	
Final body weight (g)		447.2 ± 35.0	(13)	458.4 ± 36.1	(13)	455.9 ± 29.2	(13)	416.5 ± 42.4	(13)
	Liver (g)	12.57 ± 1.73[a]	(13)	14.21 ± 1.78	(13)	13.65 ± 0.98	(13)	13.41 ± 1.53	(13)
		2.80 ± 0.21[b]		3.09 ± 0.22**		3.00 ± 0.15*		3.22 ± 0.22**	
	Kidneys (g)	2.94 ± 0.32	(13)	3.03 ± 0.26	(13)	3.16 ± 0.27	(13)	3.27 ± 0.25*	(13)
		0.66 ± 0.06		0.66 ± 0.05		0.69 ± 0.05		0.79 ± 0.06**	
	Spleen (g)	0.77 ± 0.11	(13)	0.76 ± 0.07	(13)	0.76 ± 0.09	(13)	0.73 ± 0.10	(13)
Male		0.17 ± 0.02		0.17 ± 0.02		0.17 ± 0.02		0.18 ± 0.02	

Thymus (mg)	389.8 ± 122.5	(13)	370.1 ± 55.2	(13)	375.7 ± 67.3	(13)	313.5 ± 97.5	(13)
	86.8 ± 25.7		80.8 ± 10.8		82.5 ± 14.9		74.3 ± 18.3	
Testes (g)	3.04 ± 0.29	(13)	3.01 ± 0.21	(13)	3.02 ± 0.38	(13)	3.14 ± 0.21	(13)
	0.68 ± 0.06		0.66 ± 0.07		0.66 ± 0.07		0.76 ± 0.09*	
Epididymides (g)	1.10 ± 0.12	(13)	1.04 ± 0.10	(13)	1.07 ± 0.13	(13)	1.06 ± 0.10	(13)
	0.24 ± 0.03		0.23 ± 0.03		0.23 ± 0.03		0.26 ± 0.04	
Final body weight (g)	302.5 ± 14.1	(10)	308.1 ± 29.3	(6)	301.5 ± 20.3	(13)	284.9 ± 34.6	(8)
Liver (g)	12.87 ± 1.01	(10)	13.16 ± 1.49	(6)	14.15 ± 2.26	(13)	14.59 ± 3.05	(8)
	4.26 ± 0.38		4.27 ± 0.29		4.70 ± 0.67		5.10 ± 0.68*	
Kidneys (g)	2.09 ± 0.24	(10)	2.00 ± 0.28	(6)	2.14 ± 0.24	(13)	2.09 ± 0.11	(8)
Female	0.69 ± 0.08		0.65 ± 0.07		0.71 ± 0.07		0.74 ± 0.06	
Spleen (g)	0.66 ± 0.09	(10)	0.60 ± 0.10	(6)	0.56 ± 0.07	(13)	0.57 ± 0.11	(8)
	0.22 ± 0.03		0.19 ± 0.01		0.19 ± 0.02		0.20 ± 0.03	
Thymus (mg)	190.5 ± 49.5	(10)	168.7 ± 39.2	(6)	177.5 ± 76.6	(13)	154.7 ± 68.4	(8)
	62.8 ± 15.0		55.1 ± 13.4		58.5 ± 24.5		52.6 ± 19.5	

Values are expressed as Mean ± S.D.

Parenthesis indicates number of animals

[a]: absolute weight

[b]: relative weight (g or mg per 100 g body weight)

*: significant difference from control, $p<0.05$

**: significant difference from control, $p<0.01$

Table 8: Histopathological findings of rats treated orally with N-tert-butyl-2-benzothiazolesulfenamide in the combined repeat dose and reproductive/developmental toxicity screening test

Organ [number of animals examined]		Sex:	male				female			
Findings, grade and number of animals		Dose (mg/kg):	0	40	200	1000	0	40	200	1000
Liver			[13]	[13]	[13]	[13]	[13]	[13]	[13]	[13]
Hypertrophy, hepatocyte,	total		0	0	2	8^{oo}**	0	0	4*	5*
centrilobular	±		0	0	2	6	0	0	4	5
	+		0	0	0	2	0	0	0	0
Fatty change, periportal	total		13	13	13	13**	5	9	2	1
	±		0	2	5	13	5	9	2	1
	+		13	11	8	0	0	0	0	0
Microgranuloma	total		13	13	13	13	13	13	13	13
	±		11	11	11	11	13	11	13	12
	+		2	2	2	2	0	2	0	1
Cyst, bile duct	total		0	0	0	0	1	0	0	0
	++						1	0	0	0
Cellular infiltration,	total		0	0	0	0	0	1	0	0
lymphocyte	±						0	1	0	0
Hematopoiesis,	total		0	0	0	0	0	1	0	0
extramedullary	±						0	1	0	0
Kidney			[13]	[13]	[13]	[13]	[13]	[13]	[13]	[13]
Eosinophilic body	total		6	13^{oo}	13^{oo}	13^{oo}**	0	0	0	0
	±		2	5	6	1				
	+		1	7	5	2				
	++		3	1	1	10				
	+++		0	0	1	0				
Basophilic tubule cortex	total		9	9	7	8	4	3	6	1
	±		7	9	5	8	3	3	6	1
	+		2	0	1	0	1	0	0	0
	++		0	0	1	0				
Degeneration, vacuolar,	total		0	0	0	0	0	0	3	3
proximal tubule	±						0	0	3	1
	+						0	0	0	2
Cellular infiltration,	total		5	0^{o}	0^{o}	0^{o}	1	0	0	1
lymphocyte	±		4	0	0	0	1	0	0	1
	+		1	0	0	0				

Cyst	total	1	0	0	0	0	0	0	0
	±	1	0	0	0				
Haemorrhage, renal	total	0	0	1	0	0	0	0	0
tubule	±	0	0	1	0				
Proteinus cast	total	0	0	2	0	0	0	0	0
	±	0	0	2	0				
Cyst, multiple	total	0	0	0	0	1	0	0	0
	++					1	0	0	0
Mineralization	total	0	0	0	0	0	0	0	1
cortico-medullary junction	±					0	0	0	1

Organ [number of animals examined]		Sex:	male				female			
Findings, grade and number of animals		Dose (mg/kg):	0	40	200	1000	0	40	200	1000
Spleen			[13]	[13]	[13]	[13]	[13]	[13]	[13]	[13]
Hematpoiesis,	total		13	13	13	13	13	13	13	13
extramedullary	±		10	8	8	12	1	3	2	0
	+		3	5	5	1	9	7	9	12
	++						3	3	2	1
Deposit, pigm ent, brown	total		13	13	13	13**	13	13	13	13
	±		11	5	7	2	2	4	0	1
	+		2	8	6	11	11	9	13	7
	++						0	0	0	5
Berlin blue stain,	total		13	13	13*	13**	13	13	13	13
granules, positive	±		4	4	2	0	3	3	2	1
	+		9	7	3	5	7	5	9	6
	++		0	2	8	7	3	2	2	6
	+++		0	0	0	1	0	3	0	0
Thymus			[13]	[0]	[0]	[13]	[13]	[0]	[0]	[13]
Atrophy	total		1			2	6			8
	±		1			2	4			3
	+		0			0	1			4
	++						1			1
Haemorrhage	total		1			1	2			2
	±		0			0	2			0
	+		1			1	0			1
	++						0			1
Heart			[13]	[0]	[0]	[13]	[13]	[0]	[0]	[13]
Myocardial	total		1			0	0			0
Degeneration/fibrosis	±		1			0				
Adrenal gland			[13]	[0]	[0]	[13]	[13]	[0]	[0]	[13]
Necrosis, cortex	total		0			0	1			1
	+						0			1
	++						1			0
Testis			[13]	[0]	[0]	[13]	[0]	[0]	[0]	[0]
Spermatogenesis	total		4			5				
decreased, focal	±		2			5				
	+		2			0				
Epididymis			[13]	[0]	[0]	[13]	[0]	[0]	[0]	[0]

Cell debris, germ cell	total	2			0				
	±	2			0				
Ovary		[0]	[0]	[0]	[0]	[3]	[6]	[0]	[4]
Atretic follicle, increased	total					1	1		0
	±					0	1		0
	+					1	0		0

±: very slight; +: slight; ++: moderate; +++: severe

*: significant difference from control, p<0.05 (Mann-Whitney U test)

**: significant difference from control, p<0.01 (Mann-Whitney U test)

°: significant difference from control, p<0.05 (Fisher exact test)

°°: significant difference from control, p<0.01 (Fisher exact test)

Reliability : (1) valid without restriction
The full study report is not available. This summary is derived from a detailed summary of the report.

04.12.2002 (9)

Type :
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 4 weeks
Frequency of treatm. : daily
Post exposure period : none
Doses : 0, 10, 50, 300, 1000, 3000 mg/kg bw/day
Control group : yes
NOAEL : = 1000 mg/kg bw
LOAEL : = 3000 mg/kg bw
Method : other: no information available
Year : 1978
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Result : Dose level:
1000 mg/kg bw/day: No effects observed
3000 mg/kg bw/day: LOAEL. Body weight decreased. Food consumption decreased.
Test condition : Animals: 5 Male, 5 Female per dose
Conclusion : Under the conditions of the test the substance is considered not to be harmful by prolonged oral exposure.
Reliability : (4) not assignable
Secondary literature.

04.12.2002 (6)

Type :
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage

Exposure period	:	30 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0, 100, 300, 1000, 3000 mg/kg bw/day
Control group	:	yes
LOAEL	:	= 100 mg/kg bw
Method	:	other: Bio/dynamics Laboratory method
Year	:	1981
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Mortality: All female rats in the 3000 mg/kg bw/day group and 1 male rat in the 3000 mg/kg bw/day died by Day 6. All other 3000 mg/kg bw/day male rats were sacrificed on Day 6 in a moribund condition. Dose level: 100 mg/kg bw/day: LOAEL. Heart weight decrease in females. 300 mg/kg bw/day: Body weight decrease in males. Heart weight decrease in females. Kidney and liver weight increase in females. 1000 mg/kg bw/day: Body weight decrease in males. Heart weight decrease, kidney and liver weight increase in females. 3000 mg/kg bw/day: Mortality.
Test condition	:	Animals: 5 animals of each sex per dose level.
Conclusion	:	Under the conditions of the test the substance is considered not to be harmful by prolonged oral exposure.
Reliability	:	(4) not assignable Secondary literature.

04.12.2002 (6)

Type	:	
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	90 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0, 100, 300, 1000 mg/kg bw/day
Control group	:	yes
NOAEL	:	= 100 mg/kg bw
LOAEL	:	= 300 mg/kg bw
Method	:	other: Monsanto Laboratory method
Year	:	1982
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Dose level: 100 mg/kg bw/day: NOEL, NOAEL. No effects observed. 300 mg/kg bw/day: LOAEL. Body weight decrease in males 1000 mg/kg bw/day: Body weight decrease in males. Liver and kidney weight, cholesterol in the serum and the specific gravity of urine all increased in females
Test condition	:	Animals: 5 animals of each sex per dose level.
Conclusion	:	Under the conditions of the test the substance is considered not to be harmful by prolonged oral exposure.
Reliability	:	(4) not assignable

Secondary literature.

04.12.2002 (6)

Type	:	
Species	:	rat
Sex	:	male
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	1-3 days
Frequency of treatm.	:	multiple daily doses (varied)
Post exposure period	:	up to 14 days
Doses	:	up to 40 grams total dose
Control group	:	no
NOAEL	:	> 40000 mg/kg bw
Method	:	other: screening study
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Test condition	:	Animals: 1 male per dose Doses: Ranged from 15 to 40 g/kg bw
Conclusion	:	Under the conditions of the study, the substance is not considered harmful at an oral dose level of 40000 mg/kg bw. The validity of the study should be considered with regard to to the dosing regime, the number of animals used and the exposure time.
Reliability	:	(4) not assignable Secondary literature.

04.12.2002 (6)

Type	:	
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	1-5 days
Frequency of treatm.	:	multiple daily doses
Post exposure period	:	up to 14 days
Doses	:	up to 6000 mg/kg bw/day
Control group	:	no
NOAEL	:	> 6000 mg/kg bw/day
Method	:	other: no information available
Year	:	
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	All animals survived.
Test condition	:	Animals: 1-2 males or females Doses: multiple daily doses for up to 5 days and up to a total of 60 grams/kg of test material.
Conclusion	:	Under the conditions of this test the substance is considered not to be harmful at an oral dose level of 60 grams/kg. The validity of this test should be considered with regard to the dose frequency, the number of animals treated and the exposure period.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

Type	:	
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	4 weeks
Frequency of treatm.	:	6 hours/day, 5 days/week
Post exposure period	:	no
Doses	:	0, 0.0024, 0.029, 0.084 mg/l
Control group	:	yes
NOAEL	:	= .029 mg/l
Method	:	other : Monsanto laboratory method
Year	:	1978
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Dose level: 0.0024 mg/l: No effects observed 0.029 mg/l: NOAEL. Blood, aspartate amino transferase increase 0.084 mg/l: Blood, aspartate amino transferase increase. Bodyweight decrease. Liver, histopath. Lymph nodes, histopath.
Conclusion	:	Under the conditions of the test the substance is considered to be toxic by prolonged inhalation. The report was only available from the secondary literature; it must be presumed that the elevated aspartate aminotransferase at 0.029 mg/L was judged not to be an adverse alteration of morphology, functional capacity, growth, development or lifespan, the criteria given in IPCS Environmental Health Criteria 170 for defining an adverse effect and therefore the NOAEL was considered to be 0.029 mg/L.
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

Type	:	
Species	:	rabbit
Sex	:	male/female
Strain	:	New Zealand white
Route of admin.	:	dermal
Exposure period	:	21 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0, 125, 500, 2000 mg/kg bw/day
Control group	:	yes
NOAEL	:	> 2000 mg/kg bw
Method	:	other: IRDC Laboratory method
Year	:	1979
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	Dose level: 125 mg/kg bw/day: Slight skin irritation. 500 mg/kg bw/day: Slight skin irritation. 2000 mg/kg bw/day: NOAEL. Slight skin irritation.
Test condition	:	Animals: 10 animals of each sex per dose level.
Conclusion	:	Under the conditions of the test the substance is considered

not to be harmful by prolonged dermal exposure.

Reliability : (4) not assignable
Secondary literature

04.12.2002 (6)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : Salmonella typhimurium strains TA98, TA100, TA1535, TA1537.
Escherichia coli WP2 uvrA
Test concentration : see Test Conditions
Cycotoxic concentr. : see Result
Metabolic activation : with and without
Result : negative
Method : Guidelines for screening mutagenicity testing of chemicals, JAPAN
Year :
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Result : CYTOTOXIC CONCENTRATION:
Toxicity was observed at 25 µg/plate (TA1537), 62,5 µg/plate (TA1535, TA98) and 2500 µg/plate (TA100) without an S9 mix, and at 1000 µg/plate (TA1537) with an S9 mix. Toxicity was not observed in the other cases.

The substance did not induce mutations in the S. typhimurium and E. coli strains.

Test condition : DOSES:
-S9 mix
0, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 µg/plate(TA1537)
0, 3.91 - 500 µg/plate(TA98)
0, 15.6 - 500 µg/plate(TA1535)
0, 39.1 - 2500 µg/plate(TA100)
0, 313 - 5000 µg/plate(WP2)
+S9 mix
0, 62.5 - 2000 µg/plate(TA1537)
0, 313 - 5000 µg/plate(TA100, TA1535, TA98, WP2)

METABOLIC ACTIVATION: S9 - Rat liver, induced with phenobarbital and 5,6-benzoflavone

POSITIVE CONTROLS:
-S9 mix: 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (TA100, TA98, WP2), sodium azide (TA1535) and 9-aminoacridine (TA1537).
+S9 mix: 2-aminoanthracene (five strains)

PLATES/TEST: 3

REPLICATES: 2

Test substance : PURITY: 96%

Table 1 Mutagenicity of N-*tert*-butyl-2-benzothiazolesulfenamide in reverse mutation test on bacteria without metabolic activation – Experiment 1

Dose (µg/plate)	Number of revertants (number of colonies/plate, mean ± S.D.)				
	Base-pair substitution type			Frameshift type	
	TA100	TA1535	WP2 uvrA	TA98	TA1537
0	103 115 100 (106 ± 7.9)	8 8 11 (9 ± 1.7)	21 30 16 (22 ± 7.1)	19 18 17 (18 ± 1.0)	11 5 8 (8 ± 3.0)
1.56					9 10 12 (10 ± 1.5)
3.13					6 11 8 (8 ± 2.5)
3.91		ND		18 18 18 (18 ± 0.0)	
6.25					12 11 4 (9 ± 4.4)
7.81		ND		32 24 22 (26 ± 5.3)	
12.5					9 13 10 (11 ± 2.1)
15.6		10 10 11 (10 ± 0.6)		26 23 20 (23 ± 3.0)	
25					5* 11* 4* (7 ± 3.8)
31.3		2 7 8 (6 ± 3.2)		33 24 25 (27 ± 4.9)	
39.1	127 121 119 (122 ± 4.2)		ND		
50					4* 3* 4* (4 ± 0.6)
62.5		6 6 4 (5 ± 1.2)		15 22 13* (17 ± 4.7)	
78.1	116 137 144 (132 ± 14.6)		ND		
100					8* 3* 8* (6 ± 2.9)
125		7 12 11 (10 ± 2.6)		16* 23* 17* (19 ± 3.8)	
156	104 117 114 (112 ± 6.8)		ND		
250		8*,5*,7* (7 ± 1.5)		16*,18*,20* (18 ± 2.0)	
313	119 102 93		21 17 20		

	(105 ± 13.2)		(19 ± 2.1)		
500 c		6* 6* 9* (7 ± 1.7)		12* 18* 17* (16 ± 3.2)	
625 c	92 92 84 (89 ± 4.6)		20 19 20 (20 ± 0.6)		
1000 c		6* 5* 6* (6 ± 0.6)			
1250 c	98 89 103 (97 ± 7.1)		29 25 14 (23 ± 7.8)		
2500 c	103* 77* 118* (99 ± 20.7)		23 22 11 (19 ± 6.7)		
5000 c			21 10 15 (15 ± 5.5)		
Positive Controls					
Chemical	AF2	SA	AF2	AF2	2AA
Dose (µg/plate)	0.01	0.5	0.01	0.1	80
Number of colonies/plate	624 743 740 (702 ± 67.9)	382 328 300 (337 ± 41.7)	270 237 270 (259 ± 19.1)	629 623 632 (628 ± 4.6)	368 455 369 (397 ± 49.9)

AF2: 2-(2-Furyl)-3-(5-nitro-furyl)acrylamide, 2AA: 2-Aminoanthracene, SA: Sodium azide

*: Inhibition was observed against growth of bacteria. c: Precipitate was observed on the surface of agar plates ND: Not done

Purity was 96.0% and 1.56 wt% dibenzothiazyl disulfide was contained as impurity.

Table 2 Mutagenicity of N-*tert*-butyl-2-benzothiazolesulfenamide in reverse mutation test on bacteria without metabolic activation – Experiment 2

Dose (µg/plate)	Number of revertants (number of colonies/plate, mean ± S.D.)				
	Base-pair substitution type			Frameshift type	
	TA100	TA1535	WP2 uvrA	TA98	TA1537
0	111 91 119 (107 ± 14.4)	12 10 6 (9 ± 3.1)	22 23 21 (22 ± 1.0)	25 20 27 (24 ± 3.6)	6 5 5 (5 ± 0.6)
1.56					14 12 17 (14 ± 2.5)
3.13					21 15 17 (18 ± 3.1)
3.91		18 11 13 (14 ± 3.6)		19 31 27 (26 ± 6.1)	
6.25					18,15,10 (14 ± 4.0)
7.81		11 9 5 (8 ± 3.1)		29 33 41 (34 ± 6.1)	

12.5				11 9 9 (10 ± 1.2)
15.6		8 7 6 (7 ± 1.0)	25 24 24 (24 ± 0.6)	
25				9 9 6 (8 ± 1.7)
31.3		5 4 7 (5 ± 1.5)	20 28 28 (25 ± 4.6)	
39.1	92 102 95 (96 ± 5.1)	ND		
50				4* 6* 2* (4 ± 2.0)
62.5		12* 8* 7* (9 ± 2.6)	22 35 28 (28 ± 6.5)	
78.1	114 156 115 (128 ± 24.0)	ND		
100				9* 4* 6* (6 ± 2.5)
125		11* 7* 11* (10 ± 2.3)	26* 29* 32* (29 ± 3.0)	
156	120 94 90 (101 ± 16.3)	ND		
250		6* 9* 8* (8 ± 1.5)	27* 33* 30* (30 ± 3.0)	
313	69 83 101 (84 ± 16.0)	22 26 22 (23 ± 2.3)		
500		9* 6* 5* (7 ± 2.1)	28* 21* 30* (26 ± 4.7)	
625 c	106 119 117 (114 ± 7.0)	17 22 22 (20 ± 2.9)		
1250 c	96 86 91 (91 ± 5.0)	18 9 20 (16 ± 5.9)		
2500 c	98* 65* 80* (81 ± 16.5)	22 11 16 (16 ± 5.5)		
5000 c		17 16 25 (19 ± 4.9)		
Positive Controls				
Chemical	AF2	SA	AF2	AF2	2AA
Dose (µg/plate)	0.01	0.5	0.01	0.1	80
Number of colonies/plate	848 756 734 (779 ± 60.5)	315 319 336 (323 ± 11.2)	235 253 233 (240 ± 11.0)	724 638 737 (700 ± 53.8)	1002 1120 887 (1003 ± 116.5)

AF2: 2-(2-Furyl)-3-(5-nitro-furyl)acrylamide, 2AA: 2-Aminoanthracene, SA: Sodium azide
*: Inhibition was observed against growth of bacteria. c: Precipitate was observed on the surface of agar plates ND: Not done
Purity was 96.0% and 1.56 wt% dibenzothiazyl disulfide was contained as impurity.

Table 3 Mutagenicity of N-*tert*-butyl-2-benzothiazolesulfenamide in reverse mutation test on bacteria with metabolic activation - Experiment 1

Dose (µg/plate)	Number of revertants (number of colonies/plate, mean ± S.D.)				
	Base-pair substitution type			Frameshift type	
	TA100	TA1535	WP2 uvrA	TA98	TA1537
0	135 117 120 (124 ± 9.6)	7 9 11 (9 ± 2.0)	37 24 17 (26 ± 10.1)	31 37 29 (32 ± 4.2)	20 16 16 (17 ± 2.3)
62.5					11 8 11 (10 ± 1.7)
125					8 9 11 (9 ± 1.5)
250					16 11 17 (15 ± 3.2)
313	116 122 107 (115 ± 7.5)	16 9 12 (12 ± 3.5)	19 23 19 (20 ± 2.3)	23 25 29 (26 ± 3.1)	
500 c					16 8 11 (12 ± 4.0)
625 c	114 110 129 (118 ± 10.0)	14 8 20 (14 ± 6.0)	19 17 16 (17 ± 1.5)	26 17 20 (21 ± 4.6)	
1000 c					7* 6* 16* (10 ± 5.5)
1250 c	120 108 94 (107 ± 13.0)	15 13 13 (14 ± 1.2)	17 25 14 (19 ± 5.7)	19 16 26 (20 ± 5.1)	
2000 c					8* 5* 12* (8 ± 3.5)
2500 c	101 127 102 (110 ± 14.7)	13 14 12 (13 ± 1.0)	16 12 24 (17 ± 6.1)	13 24 22 (20 ± 5.9)	
5000 c	81 104 88 (91 ± 11.8)	17 15 7 (13 ± 5.3)	16 19 15 (17 ± 2.1)	18 21 27 (22 ± 4.6)	
Positive Controls					
Chemical	2AA	2AA	2AA	2AA	2AA
Dose (µg/plate)	1	2	10	0.5	2
Number of colonies/plate	313 467 416 (399 ± 78.4)	233 256 232 (240 ± 13.6)	393 421 453 (422 ± 30.0)	447 333 323 (368 ± 68.9)	307 314 309 (310 ± 3.6)

2AA: 2-Aminoanthracene *: Inhibition was observed against growth of bacteria.
c: Precipitate was observed on the surface of agar plates.
Purity was 96.0% and 1.56 wt% dibenzothiazyl disulfide was contained as impurity.

Table 4 Mutagenicity of N-*tert*-butyl-2-benzothiazolesulfenamide in reverse mutation test on bacteria with metabolic activation - Experiment 2

Dose (µg/plate)	Number of revertants (number of colonies/plate, mean ± S.D.)				
	Base-pair substitution type			Frameshift type	
	TA100	TA1535	WP2 uvrA	TA98	TA1537
0	94,118,113 (108 ± 12.7)	18,8,18 (15 ± 5.8)	37,40,24 (34 ± 8.5)	27,31,31 (30 ± 2.3)	19,16,10 (15 ± 4.6)
62.5					7,17,12 (12 ± 5.0)
125					12,17,15 (15 ± 2.5)
250					14,9,9 (11 ± 2.9)
313	110,117,118 (115 ± 4.4)	10,9,14 (11 ± 2.6)	19,32,39 (27 ± 6.8)	28,16,21 (22 ± 6.0)	
500 c					10,22,10 (14 ± 6.9)
625 c	82,125,101 (103 ± 21.5)	8,13,9 (10 ± 2.6)	12,14,20 (15 ± 4.2)	17,19,25 (20 ± 4.2)	
1000 c					17,7,5 (10 ± 6.4)
1250 c	93,131,103 (109 ± 19.7)	11,17,15 (14 ± 3.1)	16,19,22 (19 ± 3.0)	15,28,26 (23 ± 7.0)	
2000 c					10*,6*,10* (9 ± 2.3)
2500 c	102,71,82 (85 ± 15.7)	19,11,7 (12 ± 6.1)	24,21,20 (22 ± 2.1)	23,13,23)20 ± 5.8)	
5000 c	103,89,85 (92 ± 9.5)	13,10,17 (13 ± 3.5)	17,19,9 (15 ± 5.3)	18,29,30 (26 ± 6.7)	
Positive Controls					
Chemical	2AA	2AA	2AA	2AA	2AA
Dose (µg/plate)	1	2	10	0.5	2
Number of colonies/plate	912,959,942 (938 ± 23.8)	289,281,310 (293 ± 15.0)	521,748,741 (670 ± 129.1	354,335,352 (347 ± 10.4)	453,468,388 (436 ± 42.5)

2AA: 2-Aminoanthracene *: Inhibition was observed against growth of bacteria.
c: Precipitate was observed on the surface of agar plates.
Purity was 96.0% and 1.56 wt% dibenzothiazyl disulfide was contained as impurity.

Conclusion : The substance is not mutagenic with or without metabolic activation under the conditions of the test.
Reliability : (1) valid without restriction
The full study report is not available. This summary is derived from a detailed summary of the report.
04.12.2002 (10)

Type : Chromosomal aberration test
System of testing : Chinese Hamster CHL/IU cells
Test concentration : 0.015, 0.03 and 0.06 mg/ml
Cycotoxic concentr. :
Metabolic activation : with and without
Result : positive
Method : OECD Guide-line 473
Year : 1988
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : METHOD FOLLOWED:
Test method: OECD Guideline No. 473 and Guidelines for Screening Mutagenicity Testing of Chemicals (Japan)

DEVIATIONS FROM GUIDELINE: At the first fixation time cells were fixed 6 hours post-dosing instead of at the protocolled 4 hours post-dosing. However this amendment was not considered to seriously affect the experimental set-up of the study.

STATISTICAL METHODS;
Statistical significance of the incidence of cells with chromosomal abnormality between the control group and the test groups was analyzed using Fisher's direct probability test (5% of significant level was applied taking multiplicity into consideration). Cochran-Armitage's trend test ($p<0.05$) was performed on dose dependency when statistical significance was seen in the Fisher's test.

Result : GENOTOXIC EFFECTS:
No induction effect on structural abnormality or polyploid cells was observed in any groups treated continuously for 24 or 48 hr after adding the test substance.
In the short time treatment, structural abnormalities of chromosomes (including gaps) were induced in 5% and 6% of cells observed under the presence of S9 mix in middle concentration (0.2 mg/ml) and high concentration groups (0.4 mg/ml), respectively.
In addition, polyploid cells were observed in 1.13% and 1.5% in middle concentration (0.1 mg/ml) and high concentration groups (0.2 mg/ml), respectively, in the absence of S9 mix and 1.13% and 1.63% in middle concentration (0.2 mg/ml) and high concentration groups (0.4 mg/ml), respectively, in the presence of S9 mix. However the induction of polyploid cells was judged to be biologically negative since the incidence was low.

Test condition : SYSTEM OF TESTING:
Examining for the appearance of structural abnormalities such as gaps, fragmentation, exchange of chromosomal pattern and chromatid pattern, and the appearance of polyploid cells.

No of cells examined: 200 per group.
No of metaphases analysed: 800 per group.
ADMINISTRATION:
Dosing:
24 and 48 hours, -S9: 0.0038,0.0075, 0.015, 0.03 and 0.06 mg/mL
6 hours, +S9: 0.025, 0.05, 0.1, 0.2 and 0.4 mg/mL
6 hours, -S9: 0.013, 0.025, 0.05, 0.1 and 0.2 mg/mL
Positive and negative control groups and treatment:
Negative control: The vehicle of the test article.
Positive controls:
24 and 48 hour studies - Mytomycin C
6 hour studies - Cyclophosphamide
The positive controls were both applied at the concentrations which are known to induce chromosomal aberrations.

Test substance : PURITY:96%
MANUFACTURER: Ouchi-Shinko Chemical Industry Co. Ltd.
Lot No.: 508048
IMPURITIES: dibenzothiazylsulfide (1.56%)

Table 1: Chromosome analysis of Chinese hamster cells (CHL/IU) continuously treated with N-tert-butyl-2-benzothiazolesulfenamide (BBTSA)* without S9 mix

Group	Concent-ration (mg/ml)	Time of exposure (hr)	No. of cells analysed	No. of structural aberrations							Others[3]	No. of cells with aberrations		Polyploid[4]	Trend test[5]		Concurrent cytotoxicity[6]
				gap	ctb	cte	csb	cse	mul[2]	total		TAG (%)	TA (%)	(%)	SA	NA	(%)
Control			200	0	1	0	2	0	0	3	0	8 (1.5)	8 (1.5)	0.18			
Vehicle[1]	0	24	200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.36			100.0
BBTSA	0.015	24	200	0	2	0	0	0	0	2	0	2 (1.0)	2 (1.0)	0.00			82.0
BBTSA	0.030	24	200	0	1	0	1	0	0	2	0	2 (1.0)	2 (1.0)	0.13	NT	NT	62.5
BBTSA	0.060	24	200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.80			38.0
MC	0.00005	24	200	6	40	107	2	2	0	157	1	96 (48.0)	92 (45.0)	0.88			
Vehicle[1]	0	48	200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.25			100.0
BBTSA	0.015	48	200	0	0	1	0	0	0	1	0	1 (0.5)	1 (0.5)	0.25			85.5
BBTSA	0.030	48	200	0	0	0	1	0	0	1	0	1 (0.5)	1 (0.5)	0.13	NT	NT	85.0
BBTSA	0.060	48	38	1	0	0	0	0	0	1	0	1 (0.5)	1 (0.5)	0.50			60.5
MC	0.00005	48	200	6	18	55	11	3	10	103	7	66 (33.0)	62 (31.0)	0.25			

Abbreviations: Gap : chromatid gap and chromosome gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange (dicentric and ring, etc), mul: multiple aberrations, TAG: Total no. of cells with aberrations, TA: Total No. of cells with aberrations except gap, SA: structural aberration, NA: numerical aberration, MC: mitomycin C,NT: Not tested. 1) 0.5% carboxymethylcellulose was used as vehicle. 2) More than nine aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature

chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group. 5) Cochrain & Armitage's trend test was done ($p < 0.05$) when the incidence of TAG or polyploid in the treatment groups was significantly different to historical controls ($p < 0.05$) by Fischers exact test. 6) Cell confluency, representing cytotoxicity was measured with Monocollater™. *: Purity was 96%, Dibenzothiazyl disulfide (1.56 wt%) was contained as impurity.

Table 2 Chromosome analysis of Chinese hamster cells (CHL/IU) continuously treated with N-tert-butyl-2-benzothiazolesulfenamide (BBTSA)** with and without S9 mix

Group	Concent-ration (mg/ml)	S9 mix	Time of exposure (hr)	No. of cells analysed	No. of structural aberrations							Others[3]	No. of cells with aberrations		Polyploid[4]	Trend test[5]		Concurrent cytotoxicity
					gap	ctb	cte	csb	cse	mul[2]	total		TAG (%)	TA (%)	(%)	SA	NA	(%)
Control				200	2	2	0	0	0	0	4	0	4.3 (2.0)	2 (10.0)	0.25			
Vehicle[1]	0	-	6 - (18)	200	0	0	0	1	0	0	1	0	1 (0.5)	1 (0.5)	0.25			100.0
BBTSA	0.050	-	6 - (18)	200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.50			66.0
BBTSA	0.10	-	6 - (18)	200	0	0	0	5	0	0	5	0	1 (0.5)	1 (0.5)	1.13*	NT	+	42.5
BBTSA	0.20	-	6 - (18)	200	1	1	0	0	0	0	2	0	2 (1.0)	1 (0.5)	1.50[6]			28.8
CPA	0.005	-	6 - (18)	200	0	0	0	0	0	0	0	0	0 (0.0)	0 (0.0)	0.13			–
Vehicle[1]	0	+	6 - (18)	200	1	1	2	0	0	0	4	0	4 (2.0)	3 (1.5)	0.25			100.0
BBTSA	0.10	+	6 - (18)	200	2	1	2	1	0	0	6	1	4 (2.0)	2 (2.0)	0.88			221.6[7]
BBTSA	0.20	+	6 - (18)	200	3	2	3	3	0	10	21	0	10* (5.0)	7 (3.5)	1.19*	+	+	222.8[8]
BBTSA	0.40	+	6 - (18)	200	2	9	8	2	0	0	21	0	12*(6.0)	10 (6.0)	1.63*			102.0[9]
CPA	0.005	+	6 - (18)	200	10	122	354	4	2	70	562	0	174(87.0)	174(87.0)	0.00			-

Abbreviations: gap : chromatid gap and chromosome gap, ctb: chromatid break, cte: chromatid exchange, csb: chromosome break, cse: chromosome exchange (dicentric and ring, etc), f: acentric fragment (chromatid type), mul: multiple aberrations, TAG: Total no. of cells with aberrations, TA: Total No. of cells with aberrations except gap, SA: structural aberration, NA: numerical aberration, CPA: cyclophosphamide NT: Not toxic. 1) 0.5% carboxymethylcellulose was used as vehicle. 2) More than nine aberrations in a cell were scored as 10. 3) Others, such as attenuation and premature chromosome condensation, were excluded from the no. of structural aberrations. 4) Eight hundred cells were analysed in each group. 5) Cochrain & Armitage's Trend test done ($p < 0.05$). 6) Cell confluency, representing cytotoxicity was measured with Monocollater™. 7), 8) and 9) Increasing percentages were caused by adhesion of S9 or test substance to the culture dishes. *: Significantly different from historical control data ($p<0.05$) by Fischer's exact test using a Bonferroal correction for multiple comparisons. **: Purity was 96%, Dibenzothiazyl disulfide (1.56 wt%) was contained as impurity.

Conclusion : From the above results, it was concluded that the substance induces chromosomal abnormalities in CHL/IU cells in vitro in the presence of S9 metabolic activation.

Reliability : (1) valid without restriction

Reliable study conforming to Japanese Guidelines similar to OECD Guideline 473

04.12.2002 (11)

Type : Ames test
System of testing : Salmonella typhimurium strains TA 98, 100, 1535, 1537, 1538
Test concentration :
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: no information available
Year : 1982
GLP : no data
Test substance : other TS

Test substance : Unknown manufacturers substance.
Reliability : (4) not assignable
Secondary literature

04.12.2002 (22)

Type : Ames test
System of testing : Salmonella typhimurium strains TA98, 100, 1535, 1537, 1538
Test concentration : Up to 500 µg/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Bionetics method
Year : 1975
GLP : no data
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
Secondary literature

04.12.2002 (6)

Type : Ames test
System of testing : Salmonella typhimurium strains TA98, 100, 1535, 1537, 1538
Test concentration : up to 5000 µg/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Bionetics method
Year : 1975
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
Secondary literature

04.12.2002 (6)

Type : Bacterial gene mutation assay
System of testing : Salmonella typhimurium TA98, 100, 1535, 1537, 1538
Test concentration : up to 3000 µg/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: no information available
Year :
GLP : no data

Test substance	:	other TS
Test substance	:	Unknown manufacturers substance.
Reliability	:	(4) not assignable Secondary literature
04.12.2002		(6)
Type	:	DNA damage and repair assay
System of testing	:	E. coli WP2uvrA- (WU -)
Test concentration	:	up to 1000 µg/plate
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	ambiguous
Method	:	other: Bionetics method
Year	:	1956
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(4) not assignable Secondary literature
04.12.2002		(6)
Type	:	Escherichia coli reverse mutation assay
System of testing	:	E. coli W3110(polA+), p3078(polA-)
Test concentration	:	up to 1000 µg/plate
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: Bionetics method
Year	:	1971
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(4) not assignable Secondary literature
04.12.2002		(6)
Type	:	HGPRT assay
System of testing	:	CHO cells
Test concentration	:	
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: no information available
Year	:	1984
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(4) not assignable Secondary literature
04.12.2002		(6)
Type	:	Mammalian cell gene mutation assay
System of testing	:	CHO cells
Test concentration	:	up to 10 µg/ml
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: Bionetics method

Year : 1961
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Reliability : (4) not assignable
Secondary literature
04.12.2002 (6)

Type : Mammalian cell gene mutation assay
System of testing : BALB/3T3 cells
Test concentration : up to 35 µg/ml
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Bionetics method
Year : 1987
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Test condition : Cytotoxicity was observed at a 46% rate at 35 µg/ml
Reliability : (4) not assignable
Secondary literature
04.12.2002 (6)

Type : Mouse lymphoma assay
System of testing : L5178Y
Test concentration : up to 15 µg/ml without S9 and 60 µg/ml with S9
Cycotoxic concentr. :
Metabolic activation : with and without
Result : positive
Method : other: Bionetics method
Year : 1975
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Result : -S9: negative
+S9: positive
Reliability : (4) not assignable
Secondary literature
04.12.2002 (6)

Type : Mouse lymphoma assay
System of testing : L5178Y
Test concentration : up to 12.5 µg/ml without S9 and 50 µg/ml with S9
Cycotoxic concentr. :
Metabolic activation : with and without
Result : positive
Method : other: Bionetics method
Year : 1975
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Result : -S9: negative
+S9: positive
Reliability : (4) not assignable
Secondary literature
04.12.2002 (6)

Type : Mouse lymphoma assay

System of testing	:	L5178Y
Test concentration	:	up to 100 µg/ml
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: SRI method
Year	:	1978
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	-S9: negative +S9: positive
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

Type	:	Mouse lymphoma assay
System of testing	:	L5178Y
Test concentration	:	up to 100 µg/ml
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: SRI method
Year	:	1978
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	-S9: negative +S9: positive
Reliability	:	(4) not assignable Secondary literature

04.12.2002 (6)

Type	:	Yeast gene mutation assay
System of testing	:	S. cerevisiae strain D4
Test concentration	:	up to 500 µg/plate
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: Bionetics method
Year	:	1976
GLP	:	no data
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(4) not assignable Secondary literature

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5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male
Strain	:	CD-1
Route of admin.	:	i.p.
Exposure period	:	24 and 48 hours
Doses	:	500, 1000, 2000 mg/kg
Result	:	negative

Method	:	OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year	:	2002
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	EFFECT ON PCE/NCE RATIO: A statistically significant decrease in the PCE/NCE ratio was seen in the 48-hour 2000 mg/kg and 24-hour 500 mg/kg test material groups when compared to their concurrent vehicle control groups. The PCE/NCE ratios for the 1000 and 2000 mg/kg 24-hour test material dose groups were also lower than their concurrent vehicle control. GENOTOXIC EFFECTS: Negative. There were no statistically significant increases in the frequency of micronucleated PCEs in any of the test material dose groups when compared to their concurrent vehicle control groups. The positive control group showed a marked increase in the incidence of micronucleated polychromatic erythrocytes hence confirming the sensitivity of the system to the known mutagenic activity of cyclophosphamide under the conditions of the test. MORTALITY: none CLINICAL SIGNS: Clinical signs were observed in animals dosed with test material at and above 500 mg/kg and included as follows: hunched posture, pilo-erection, lethargy and ptosis.
Test condition	:	AGE: 5 -8 weeks old ANIMALS/DOSE: 7 VEHICLE: Arachis oil FREQUENCY OF TREATMENT: Single dose CONTROL GROUPS: 2 Control groups dosed i.p with Arachis oil (vehicle control) and 1 group dosed orally with cyclophosphamide (positive control). CLINICAL OBSERVATIONS: All animals observed for signs o f overt toxicity 1 hour after dosing and then once daily as applicable and immediately prior to termination. ORGANS EXAMINED AT NECROPSY: None CRITERIA FOR EVALUATING RESULTS: The incidence of micronucleated cells per 2000 polychromatic erythrocytes was scored. In addition, the number of normochromatic erythrocytes associated with 1000 erythrocytes were counted. CRITERIA FOR SELECTION OF M.T.D.: Range-finding toxicity study with animals dosed ip (1 male/1 female) and orally (1 male/1 female) at 2000 mg/kg. No deaths, one ip animal demonstrated diuresis as the only clinical sign.

Table 1: Summary of Group Mean Data

Treatment Group	Number of PCE with Micronuclei per 2000 PCE		PCE/NCE Ratio	
	Group Mean	SD	Group Mean	SD
Vehicle control, 48-hr sampling time	2.0	1.2	0.88	0.21
Vehicle control, 24-hr sampling time	2.6	2.3	0.85	0.26
Positive control, 24-hr sampling time	34.00***	18.3	1.56	0.63
2000 mg/kg, 48-hr sampling time	1.4	1.3	0.59*	0.25
2000 mg/kg, 24-hr sampling time	2.4	2.6	0.71	0.38
1000 mg/kg, 24-hr sampling time	2.1	2.3	0.62	0.32
500 mg/kg, 24-hr sampling time	1.6	1.4	0.53*	0.27

PCE = Polychromatic erythrocytes
NCE = Normochromatic erythrocytes
SD = Standard deviation
* = P<0.05
** = P<0.001

Reliability : (1) valid without restriction
Study conducted to standard test method under GLP

04.12.2002 (3)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : Males 42 days: Females from 14 days prior to mating to Day 3 of lactation.
Frequency of treatm. : daily
Duration of test : 42 days
Doses : 40, 200 and 1000 mg/kg/day
Control group : yes, concurrent vehicle

NOAEL maternal tox. : = 40 mg/kg bw
NOAEL teratogen. :
Method : other: OECD 422
Year :
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Method : METHOD FOLLOWED:
OECD 422 Combined Repeat Dose and Reproductive/Development Toxicity Screening Test
METHOD OF CALCULATION:
Copulation index, Ovulation index, Fertility index,
Parturition and lactation changes.
Offspring: Viability, sex ratio and body weight.

Test condition : TEST ORGANISMS:
Number:104
Sex per dose: 13 animals of each sex per dose level.
ADMINSTRATION/EXPOSURE:
Treatment:
Males: Duration of administraion was 42 days
Females: From 14 days prior to mating to Day 3 of lactation.
Termination:
Males: Day 43
Females: Day 4 of lactation
Vehicle: 5% gum arabic
Doses: 40, 200 and 1000 mg/kg/day

Test substance : PURITY: 96.4%

Table 1 Summary of reproductive performance in parental rats treated orally with TBBS in the combined repeat dose and reproductive/development toxicity screening test

	Dose (mg/kg)			
	0	40	200	1000
Number of mated pairs	13	13	13	13
Number of copulated pairs	12	12	13	13
Copulation index [A]	92.3	92.3	100	100
Number of pregnant animals	10	7	13	9
Fertility index [B]	83.3	58.3	100	69.2
Pairing days until copulation Mean ± S.D.	3.2 ±2.1	2.3 ± 1.1	3.7 ± 1.7	3.0 ± 2.8
Frequency of vaginal estrus Mean ± S.D.	1.1 ± 0.3	1.0 ± 0.0	1.2 ± 0.4	1.0 ± 0.0

Copulation index = (Number of copulated pairs/Number of mated pairs) X 100: %
Fertility index = (Number of pregnant animals/Number of copulated pairs) X 100: %

Table 2: Summary of development of pups from dams treated orally with TBBS in the combined repeat dose and reproductive/developmental toxicity screening test

	Dose (mg/kg)			
	0	40	200	1000
Number of pregnant females	10	7	13	9
Number of pregnant females with pups alive	10	6	13	8
Gestation index [A]	100	85.7	100	88.9
Gestation length in days	22.3±0.5 (10)	22.5±0.5 (6)	22.4±0.5 (13)	22.5±0.5 (8)
Number of corpora lutea	16.5±2.0 (10)	15.9±2.3 (7)	17.2±2.2 (13)	15.7±2.6 (9)
Number of implantation sites	14.0±4.0 (10)	14.0±5.5 (7)	14.5±5.3 (13)	12.8±5.8 (9)
Implantation index [B]	84.3±19.6 (10)	85.7±31.1 (7)	83.0±26.6 (13)	78.5±30.2 (9)
Day 0 of lactation				
Number of pups born	13.0±4.1 (10)	13.3±6.0 (7)	13.8±5.2 (13)	11.0±5.8 (9)
Delivery index [C]	92.7±9.0 (10)	83.0±36.7 (7)	94.9±5.6 (13)	75.8±31.5 (9)
Number of pups alive	12.9±4.0 (10)	13.1±5.9 (7)	13.5±4.9 (13)	10.9±5.8 (9)
Birth index [D]	92.1±8.6 (10)	82.2±36.4 (7)	93.2±4.8 (13)	75.1±31.8 (9)
Live birth index [E]	99.4±1.9 (10)	99.1±2.3 (6)	98.3±2.7 (13)	98.9±3.2 (8)
Pup weight in grams				
Male	6.8±0.9 (10)	6.5±0.3 (6)	6.7±1.0 (13)	6.5±0.9 (8)
Female	6.5±1.0 (10)	6.2±0.4 (5)	6.5±0.9 (13)	6.2±0.7 (8)
Sex ratio [F]	52.7±14.7 (10)	49.2±9.3 (6)	48.9±7.8 (13)	44.3±10.1 (8)
Day 4 of lactation				
Number of pups alive	12.7±4.1 (10)	13.0±5.9 (7)	13.3±4.8 (13)	10.6±5.5 (9)
Viability index [G]	97.8±5.4 (10)	98.8±2.9 (6)	99.1±2.2 (13)	97.8±6.2 (8)
Pup weight in grams				
Male	11.0±1.8 (10)	9.6±0.8 (6)	10.3±2.6 (13)	9.9±2.0 (8)
Female	10.5±1.8 (10)	9.5±0.7 (6)	9.8±2.3 (13)	9.5±1.6 (8)

Values are expressed as Mean ± S.D.

Parenthesis indicates the number of litters evaluated

Gestation index = (Number of pregnant females with pups alive/Number of pregnant females) X 100: %

Implantation index = (Number of implantation sites/Number of corpora lutea) X 100: %

Delivery index = (Number of pups born/Number of implantation sites) X 100: %

Birth index = (Number of pups alive on day 0/Nmber of implantation sites) X 100: %

Live birth index = (Number of pups alive on day 0/Number of pups born) X 100: %

Sex ratio = (Number of male pups alive on day 0/Number of pups alive on day 0) X 100: %

Viability index = (Number of male pups alive on day 4/Number of male pups alive on day 0) X 100: %

Conclusion : The test substance at the dose levels applied did not exert adverse effects on copulation and ovulation. Both male and female reproductive tissues were well examined and no abnormalities were observed. Changes in fertility index were observed at 40 and 1000 mg/kg

bw/day, but not at 200 mg/kg bw/day.
No dose-related abnormalities were observed with regard to parturition or lactation. There were no adverse affects on the viability, the sex ratio or body weights of pups in any treated group. The test substance at the dose levels applied did not demonstrate teratogenicity.

Reliability : (1) valid without restriction
Guideline study conducted to GLP

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Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	6-15 days of gestation
Frequency of treatm.	:	daily
Duration of test	:	
Doses	:	0, 50, 150, 500 mg/kg bw/day
Control group	:	yes
NOAEL maternal tox.	:	> 500 mg/kg bw
NOAEL teratogen.	:	> 500 mg/kg bw
Result	:	No effects observed on parent or offspring.
Method	:	other: IRDC method
Year	:	1978
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Result : Dose level:
50 mg/kg bw/day: No effects observed
150 mg/kg bw/day: No effects observed
500 mg/kg bw/day: No effects observed. Maternal: NOEL, NOAEL
Offspring: NOEL, NOAEL

Conclusion : Under the conditions of the test the substance is considered not to have any effect on reproduction.

Reliability : (4) not assignable
Only secondary literature available (IUCLID data set)

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5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

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