**FOREWORD** **INTRODUCTION**

# N,N-DICYCLOHEXYL-2-BENZOTHIAZOLESSULFENAMIDE

**CAS N°: 4979-32-2**

# SIDS Initial Assessment Report

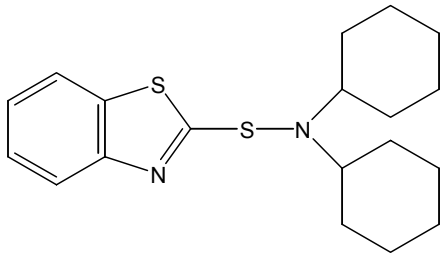
# For

# SIAM 18

Paris, France, 20-23 April 2004

| | |
|---|---|
| **1. Chemical Name:** | N, N-Dicyclohexyl-2-benzothiazolesulfenamide |
| **2. CAS Number:** | 4979-32-2 |
| **3. Sponsor Country:** | Japan<br>Contact Point:<br>Mr. Motohiko Katho<br>Director<br>Second International Organizations Division<br>Ministry of Foreign Affairs, Japan |
| **4. Shared Partnership with:** | |
| **5. Roles/Responsibilities of the Partners:**<br>Name of industry sponsor /consortium<br>Process used | |
| **6. Sponsorship History**<br>How was the chemical or category brought into the OECD HPV Chemicals Programme? | |
| **7. Review Process Prior to the SIAM:** | Expert committee performed spot checks on randomly selected endpoints and compared original studies with data in SIDS dossier. |
| **8. Quality check process:** | |
| **9. Date of Submission:** | January 15, 2004 |
| **10. Comments:** | The SIDS documents for N,N-dicyclohexyl-2-benzothiazolesulfenamide were discussed at SIAM 11 and the conclusion and recommendation on the human health part were agreed. With regard to the environment part SIAM 11 recommended that some additional studies (e.g. water solubility and identification of major metabolites as a result of hydrolysis) should be preformed. |

## SIDS INITIAL ASSESSMENT PROFILE

| | |
|---|---|
| **CAS No.** | 4979-32-2 |
| **Chemical Name** | N,N-Dicyclohexyl-2-benzothiazolesulfenamide |
| **Structural Formula** |  |

### SUMMARY CONCLUSIONS OF THE SIAR

**Human Health**

The acute toxicity of N,N-dicyclohexyl-2-benzotazothiazolesulfenamide (DCBS) is low. The oral $LD_{50}$ in rats is greater than 1,000 mg/kg and the dermal $LD_{50}$ in rabbits is more than 2,000 mg/kg. This chemical is moderately irritating to skin and slightly irritating to eyes but no sensitizing to skin.

In an oral study with rats according to the OECD combined repeated dose and reproductive/developmental toxicity screening test [TG 422], the major toxicities were found in clinical observation and histopathological examination in kidneys. Salivation in males at 400 mg/kg bw/day and decreased locomotor activity in females at 100 and 400 mg/kg bw/day were noted. Histopathological examination revealed hyaline droplets in the renal tubular epithelia in males and fatty degeneration of the renal tubular epithelia in females at 100 and 400 mg/kg bw/day. In addition, adrenal enlargement with vacuolation of the adrenocortical cells and atrophy of spleen in females at 100 and 400 mg/kg bw/day were observed. A NOAEL for repeat dose toxicity was established at 25 mg/kg bw/day for both sexes.

In the above screening test [OECD TG 422], the toxic effects were revealed in females and pups at the dose of 400 mg/kg bw/day. There was a decreased number of corpus lutea accompanied with decreases in the number of implantation sites and litter size. Three dams died on the expected delivery day or on the following day. All dams at 400 mg/kg bw/day lost their litters at delivery or by day 4 of lactation. There were no effects on the mating and fertility, and morphogenesis in pups at and below 100 mg/kg bw/day. A NOAEL for reproductive/developmental toxicity was established at 100 mg/kg bw/day.

The genotoxic potential of this chemical was mostly negative with and without an exogenous metabolic activation system in bacteria as well as mammalian cells, while the cytogenetic effect was judged to be positive in *in vitro* tests without an exogenous metabolic activation because of a slight increase of polyploid cells and induction of micronucleus cells. However, this chemical did not induce cytogenetic effects in an *in vivo* bone marrow chromosome test although a standard method was not used. The weight of evidence suggests this chemical may not be genotoxic *in vivo*.

**Environment**

DCBS is a white powder with a water solubility of $1.9 \times 10^{-3}$ mg/L at 25 °C, a melting point of 99 °C at 1013 hPa and a vapour pressure of $< 7.0 \times 10^{-5}$ Pa at 100 °C. A measured log Kow value of >4.8 suggests that this chemical is suspected to have a high bioaccumulation potential. This chemical is hydrolysed in water and its half-lives at 25 °C have been measured as 4.92 day at pH 4.0, 18.6 days at pH 7.0 and 112 days at pH 9, producing two major metabolites (dicyclohexylamine and 2-mercaptobenzothiazole). However actual hydrolysis rates are uncertain since the test was conducted above the water solubility limit. Environmental distribution using a Mackay level III model indicates that if the substance is released into water or soil it tends to remain its in original compartment whereas if released into air the substance is distributed into air (42.8 %) and soil (52.9 %). This substance is not readily

biodegradable. In the atmosphere the substance is indirectly photodegraded by reaction with OH radicals with a half-life of 2.26 hrs. One of the degradation products, 2-mercaptobenzothiazole is non-volatile and not readily biodegradable whereas dicyclohexylamine is non-volatile but readily biodegradable in the environment.

In acute toxicity tests with fish, daphnids and algae, no effects were observed at the limit of solubility of the substance [96 h LC50 of > 0.0344 mg/L (*Orizias latipes*, OECD TG 203); 48 h EC50 of > 0.0314 mg/L (*Daphnia magna*, OECD TG 202, Immobilisation); 72 h EC of > 0.0118 mg/L (*Selenastrum capricornutum*, OECD TG 201, both biomass method and growth rate method) were reported].
Also in chronic toxicity tests with daphnids and algae, no effects were observed at the limit of solubility of the substance. A 21 d NOEC of >= 0.0331 mg/L (*Daphnia magna,* OECD TG 211, reproduction) and a 72 h NOEC of >= 0.0118 mg/L (*Selenastrum capricornutum*, OECD TG 201) were reported.

**Exposure**

Annual production volume of DCBS in Japan was about 1,900 tonnes in 2000-2003, and there is no information on import and export volumes.
In Japan, DCBS is solely used as an accelerator of vulcanization and is completely reacted in the vulcanizing process. During vulcanisation processes and the use of rubber products, some degradation products (e.g. mercaptobenzothiazole: CAS No. 149-30-4 or di(benzothiazoyl-2)disulfide: CAS No. 120-78-5) may appear and they can be released into the environment. It is reported that during the vulcanization the unstable sulphur-nitrogen-bond of DCBS is split with the intermediate formation of mercaptobenzothiazol radicals. Products resulting from the process are the basic amines, benzothiazole derivatives and further reaction products. As further degradation products, benzothiazole, 2-methylbenzothiazole and 2-benzothiazolone and 2-methylthiobenzothiazole are reported.
Occupational exposures at production sites may occur by the inhalation route during bag filling operation. No actual workplace concentration data was available. Workers wear dust respirator and body-covering clothing and local exhaust ventilation system is operated during the filling process.

## RECOMMENDATION

The chemical is a candidate for further work.

## RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

**Human Health**
The chemical possesses a hazard for human health (repeated dose toxicity). An exposure assessment and, if necessary risk assessments for workers and consumers should be performed taking into account possible breakdown products.

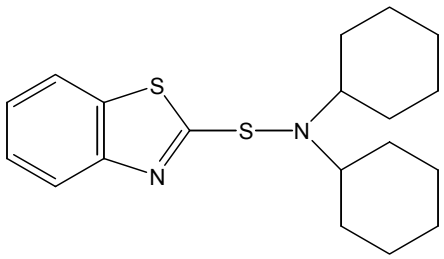
**Environment**
The chemical did not show any adverse effects in several acute and chronic toxicity tests with aquatic organisms. During the use of the substance several degradation products are formed which possess properties indicating a hazard for the environment. These degradation products are present 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 degradation products should be performed. The currently on-going assessment of di(benzothiazoyl-2)disulfide (CAS No. 120-78-5), of N-cyclohexylbenzothiazole-2-sulfenamide (CAS No. 95-33-0) and of the sulfenamide accelerator category should be taken into account.

# SIDS Initial Assessment Report

## 1 IDENTITY

### 1.1 Identification of the Substance

CAS Number: 4979-32-2
IUPAC Name: N, N-Dicyclohexyl-2-benzothiazolesulfenamide
Molecular Formula: $C_{19}H_{26}N_2S_2$
Structural Formula:




Molecular Weight: 346.59
Synonyms: N,N-dicyclohexylbenzothiazole-2-sulfenamide

### 1.2 Purity/Impurities/Additives

The purity of N, N-Dicyclohexyl-2-benzothiazolesulfenamide was > 99.2 %, and the major impurities were unknown.

### 1.3 Physico-Chemical properties

**Table 1 Summary of physico-chemical properties**

| Property | Value | Protocol (reference) or comment |
|---|---|---|
| Physical state | White powder | |
| Melting point | 99 °C | SRC online data base(2004) |
| Boiling point | > 300 °C (1013 hPa) | OECD TG 103 (MITI, 1994b) |
| Relative density | No data is available | |
| Vapour pressure | < 7.0 x $10^{-5}$ Pa at 100 °C | OECD TG 104 (MITI, 1994b) |
| Water solubility | 1.9 ug/L at 25°C | OECD TG 105 (CERI, 2001) |
| Partition coefficient n-octanol/water (log value) | > 4.80 at 25 °C | OECD TG 107 (METI, 1994b) |

# 2 GENERAL INFORMATION ON EXPOSURE

## 2.1 Production Volumes and Use Pattern

N,N-dicyclohexyl-2-benzothiazolesulfenamide is produced in a closed system with an annual production level of about 1,000 tons/year in 1990 – 1993 and 1,900 tonnes in 2000-2003 in Japan, and most of this amount was sold and handled in Japan. There is no information about imported volumes of N, N-dicyclohexyl-2-benzothiazolesulfenamide (MITI, 1994a). In Japan, it is reported that three companies produce the chemical, whereas two companies are known to produce the chemical in the US (CERI, 2004).

In Japan, N,N-dicyclohexyl-2-benzothiazolesulfenamide is used as an accelerator of vulcanization and is completely reacted in the vulcanizing process (CERI, 2004).

During vulcanisation, unstable sulphur-nitrogen-bond of the substance is split with the intermediate formation of mercaptobenzthiazole radical. Products resulting from the process are the basic amines, benzothiazole derivatives and further reaction products. As further degradation products, benzothiazole, 2-methylbenzothiazole and 2-menzothiazole can be formed. These breakdown products being formed during vulcanisation are included into the polymer matrix.

## 2.2 Environmental Exposure and Fate

### 2.2.1 Sources of Environmental Exposure

Although no monitoring data are available, it is assumed that emissions of N,N-dicyclohexyl-2-benzothiazolesulfenamide to waste water and air from production sites and downstream use are low in Japan because the substance is produced in a closed system and adequate measures to prevent any leakage are taken throughout the production process as well as during transport. However, during the production and the use of the substance several degradation products (e.g. benzothiazole; Cas No. 95-16-9, 2-mercaptobenzothiazole; Cas No. 149-30-4, 2-methylbenzothiazole; Cas No. 120-75-2, 2-methylthiobenzothiazole; Cas No. 615-22-5 and 2-benzothiazolone; Cas No. 934-34-9) may be formed. These degradation products are present in many rubber products (e.g. car tyres) and a release to the environment may occur.

### 2.2.2 Photodegradation

An indirect photodegradation of N,N-dicyclohexyl-2-benzothiazolesulfenamide with OH radicals is expected to occur in the atmosphere. The half-life of N,N-dicyclohexyl-2-benzothiazolesulfenamide is calculated to be 2.26 hours assuming an OH radical concentration of $1.5 \times 10^6$ molecules/$cm^3$ (CERI, 2003).

### 2.2.3 Stability in Water

It was reported that this chemical was hydrolyzed at pH 4 (half-life time: 4.92 days at 25 °C), 7 (half-life time: 18.6 days at 25 °C) and 9 (half-life time: 112 days at 25 °C). Although the study was well conducted and measurement conditions were reliable, the initial concentration was above the water solubility and the actual degradation rates might therefore be faster than the reported values. As a result of the hydrolysis, N,N-Dicyclohexyl-2-benzothiazolesulfenamide seems to be converted into two

major daughter chemicals which are possibly dicyclohexylamine and 2-mercaptobenzthiazole based on mass spectrometry (CERI, 2001). It is assumed that other metabolites can also be formed as a result of hydrolysis.

### 2.2.4 Transport between Environmental Compartments

The potential environmental distribution of N,N-dicyclohexyl-2-benzothiazolesulfenamide obtained from a generic level III fugacity model under three emission scenarios is shown in Table 2. The result shows that if N,N-dicyclohexyl-2- benzothiazolesulfenamide is released into the water compartment, this chemical has a tendency to stay in the water compartment, but if this chemical is released into the air compartment, this chemical is likely to be transported to other compartments.

A calculated log Koc (soil/sediment and water partition coefficient) value of 5.04 indicates that the chemical is likely absorbed to soil and sediment (CERI, 2003).

**Table 2: Environmental distribution using a generic fugacity model, Mackey level III**

| Compartment | Release: 100 % to air | Release: 100 % to water | Release: 100 % to soil |
|---|---|---|---|
| Air | 43.2 % | 0.2 % | 0.0 % |
| Water | 6.4 % | 87.2 % | 0.0 % |
| Soil | 49.5 % | 0.2 % | 100.0 % |
| Sediment | 0.9 % | 12.3 % | 0.0 % |

### 2.2.5 Biodegradation

A ready biodegradability test was conducted according to OECD TG 301 C (MITI, 1994b). Based on the BOD and HPLC analysis, only up to 6 % of biodegradation was determined.

### 2.2.6 Bioaccumulation

A measured log Kow value of > 4.8 suggests that this chemical is suspected to have a high bioaccumulation potential (MITI, 1994b).

## 2.3 Human Exposure

### 2.3.1 Occupational Exposure

Occupational exposures at production sites may occur by the inhalation route. No actual workplace concentration data are available. Dermal exposure was expected to be negligible because this chemical is solid at handling temperature and its partition coefficient is > 4.8. The estimated exposure concentration during bag filling according to the EASE model is 2-5 mg/m$^3$ during dry manipulation with local exhaust ventilation.

Based on this data, the estimated human exposure for bag filling (4 hours/day) without protective equipment EHE inhalation is 0.36 mg/kg/day. In Japan, workers wear dust respirator and body-covering clothing during the filling process. This substance is used as an accelerator of vulcanization in the rubber industry. No occupational exposure limit for this chemical was located.

### 2.3.2 Consumer Exposure

N,N-dicyclohexyl-2-benzothiazolesulfenamide is an accelerator of vulcanization and is completely reacted in the vulcanizing process. Therefore, this substance is not contained in rubber products, however some degradation products may be present in many rubber products.

# 3 HUMAN HEALTH HAZARDS

## 3.1 Effects on Human Health

### 3.1.1 Toxicokinetics, Metabolism and Distribution

There is no available information.

### 3.1.2 Acute Toxicity

Studies in Animals

Acute toxicity data are reported in the literature for rats and rabbits. The available results show a consistent picture of acute toxicity (Table 3). Among these studies, the study by MHW (1996) was identified as the key study because it was well conducted and used a current protocol. This study was conducted according to OECD TG 401.

The male and female rats received doses of 1077, 1401, 1821, 2367, 3077 and 4000 mg/kg by gavage. Fatalities occurred in males at doses of 2367 mg/kg and more and in females at doses of 1401 mg/kg and more. However, no dose-related increases in mortality were observed. Clinical signs such as tremor, convulsion, decreased locomotor activity, deep respiration, piloerection, chromodacryorrhea and perigenital region soiled with urine, as well as low body weight in comparison to control were observed in both sexes. No macroscopic abnormalities were observed in both sexes.

**Table 3 Acute toxicity of N,N-dicyclohexyl-2-benzothiasolesulfenamide in experimental animals**

| Route | Animals | Values | Type | References |
|---|---|---|---|---|
| Oral | | | | |
| | Rat | Males: 1,821mg/kg | $LD_0$ | MHW, Japan: 1996 |
| | | Female: 1,077mg/kg | | |
| | Rat | >5,000 mg/kg | $LD_{50}$ | Monsanto Study BD-84-217 |
| | Rat | 10,000 mg/kg | $LD_{50}$ | de Groot: 1975 |
| | Rat | 6,420 mg/kg | $LD_{50}$ | Marhold: 1986 |
| | Rat | 8,500 mg/kg | $LD_{50}$ | Vorobeva: 1968 |
| Dermal | | | | |
| | Rabbit | >2,000 mg/kg | $LD_{50}$ | Monsanto Study BD-84-217 |

Studies in Humans

There is no available information.

Conclusion

Acute toxicity of this chemical is low in rodents because $LD_{50}$ values via oral and dermal routes are high, greater than 1,000 mg/kg.

### 3.1.3 Irritation

Application of 20 mg/24h of N,N-dicyclohexyl-2-benzothiazolesulfenamide induced moderate skin irritation in rabbits [Marhold: 1986]. Application of 500 mg/24h induced slightly eye irritation in rabbits [Marhold: 1986].

### 3.1.4 Sensitisation

N,N-dicyclohexyl-2-benzothiazolesulfenamide was not sensitizing to skin in the maximization test using guinea pigs [Monsanto Study IR-84-230]. There is no available information on humans.

Conclusions*:*

This chemical is moderately irritating to skin and slightly irritating to eyes in rabbits, but not sensitizing to skin in guinea pigs.

### 3.1.5 Repeated Dose Toxicity

Studies in Animals

*Inhalation*

Repeated inhalation exposure of male rats for 15 days, daily, 2 hr/day, at doses of 350-400 $mg/m^3$ caused mucous membrane irritation [Vorobeva: 1968].

*Dermal*

There is no available information.

*Oral*

In two oral studies, rats received for 4 weeks 2000-10000 ppm (ca. 133-667 mg/kg bw/day) in feed [Monsanto Study BD-87-327] and for 3 months 2500 and 5000 ppm (ca.167, 333 mg/kg bw/day) in feed [Monsanto Study ML-88-180]. Reduced body weight gain and food consumption were observed. However no detailed information was published in these two studies. A study by MHW (1996) was identified as the key study because it was well conducted and used a current protocol. Details of the study are as follows.

Using an OECD combined repeat dose and reproductive/developmental toxicity screening test [TG 422], SD (Crj:CD) rats received doses of 0, 6, 25, 100 and 400 mg/kg/day by gavage [MHW, Japan: 1996]. Males were dosed for 44 days and females were dosed for 40-51 days from 14 days prior to mating to day 3 of lactation throughout the mating and pregnancy period.

During cage side observation, salivation was noted at 400 mg/kg in males, and decreased locomotor activity as well as blotted fur on the lower abdomen were noted at 100 and 400 mg/kg, and chromdacryorrhea at 400 mg/kg in females. Three females at 400 mg/kg died on the expected delivery day or on the following day. Urinalysis and blood chemistry revealed increases in urinary ketone bodies and serum inorganic phosphorus, and decreases in serum chloride in males at 400 mg/kg. There were no hematological changes in males. Macroscopic examination revealed cecum dilatation and thymus atrophy in both sexes and kidney swelling, adrenal enlargement and spleen atrophy in females at 400 mg/kg. Increased relative kidney weights and decreased absolute thymus weights were noted, in males and in both sexes at 400 mg/kg, respectively. In histopathological examination, fatty degeneration of the renal tubular epithelia, vacuolation of the adrenocortical cells and atrophy of the spleen were observed in females at 100 and 400 mg/kg. Also, increases of hyaline droplets in the renal tubular epithelia were observed in males at 100 and 400 mg/kg. The NOAEL for repeat dose toxicity was 25 mg/kg/day for both sexes.

Studies in Humans

There is no available information.

Conclusion

Major toxic effects in rats by oral administration were salivation and decreased locomotor activity as clinical signs and histopathological changes in the kidney. The NOAEL for repeat dose toxicity in rats was 25 mg/kg/day for both sexes**.**

### 3.1.6 Mutagenicity

*In vitro* Studies

**Bacterial test**

A reverse gene mutation assay was conducted according to OECD TG 471 [MHW, Japan: 1996]. This chemical was not mutagenic in Salmonella typhimurium TA100, TA1535, TA98, TA1537 and Escherichia coli WP2 uvrA at concentrations of up to 5000 ug/plate, with or without an exogenous metabolic activation system.

**Non-bacterial test in vitro**

A chromosomal aberration test according to OECD TG 473 was conducted in cultured Chinese hamster lung (CHL/IU) cells [MHW, Japan: 1996]. Structural chromosomal aberrations were not induced up to the concentration of 0.82 mg/ml resulting in 50% cell growth inhibition during continuous treatment or the limit concentration of 3.5mg/ml (10 mM) during short-term treatment with and without an exogenous metabolic activation system. Polyploidy was increased by continuous treatment without an exogenous metabolic activation system, and the maximum incidence was 6 % at the concentration of 0.41mg/ml after 48 hr. The cytogenetic effect of this substance was suggested to be equivocally positive.

An in vitro micronucleus test was conducted in cultured CHL/IU cells [MHW, Japan: 1996]. Micronucleus cells were induced at the concentration of 0.21 mg/ml or more after 48-hr continuous treatment without an exogenous metabolic activation system. Other studies show negative results such as a HGRPT assay using CHO cells with and without an exogenous metabolic activation [Monsanto Study PK-84-231] and an unscheduled DNA synthesis test in primary rat cultured hepatocytes [Monsanto Study SR-84-291].

*In vivo* Studies

**Genetic test *in vivo***

An *in vivo* bone marrow chromosome test was designed to evaluate the clastogenic potential of the test substance as measured by increases in numerical and structural chromosomal aberration in bone marrow cells from Crj:CD (SD) rats [Monsanto Study HL-84-293]. A single dose of the test substance was administered by gavage at 1,000 mg/kg. Animals were scheduled to be sacrificed at approximately 6, 24, and 48 hrs after administration. Although there is a limitation in this study because the guideline is unknown, clastogenicity and polyploidy were not induced.

All studies are summarized in the following Table 4.

**Table 4 Summary of genotoxicity studies**

| Type of test | Test system | Dose | Result | Reference |
|---|---|---|---|---|
| BACTERIAL TEST | | | | |
| Ames test *(reverse mutation)* | *S. typh.* (TA100, TA1535, TA98, TA1537), *E. coli* WP2 uvrA | 312.5, 625, 1250, 2500, 5000 ug /plate | Negative (+ & - MA) | MHW, Japan: 1996 |
| Ames test *(reverse mutation)* | *S. typh.* (TA100, TA98) | No data | Negative (+ & - MA) | You et al.: 1982 |
| Ames test *(reverse mutation)* | *S. typh.* (no data) | No data | Negative (+ & - MA) | Monsanto Study HL-84-292 |
| NON-BACTERIAL TEST *IN VTRO* | | | | |
| Chromosomal aberration test | CHL/IU cell | 0.9, 1.8, 3.5 mg/ml (short-term treatment) 0.21, 0.41, 0.82 mg/ml (continuous treatment) | Negative (+ & - MA) Equivocally positive* (- MA) | MHW, Japan: 1996 |
| *In vitro* micronucleus test | CHL/IU cell | 0.21, 0.41, 0.82 mg/ml | Positive (- MA) | MHW, Japan: 1996 |
| HGRPT Assay | CHO cell | Up to 500 ug/ml | Negative (+ & - MA) | Monsanto Study PK-84-231 |
| Unscheduled DNA synthesis | Primary rat hepatocytes | Up to and including 50 ug/ml | Negative (no data) | Monsanto Study SR-84-291 |
| GENETIC TOXICITY *IN VIVO* | | | | |
| Chromosomal aberration *in vivo* | Rat (SD) | 1000 mg/kg (by gavage) | Negative | Monsanto Study HL-84-293 |

MA: metabolic activation

* Structural chromosomal aberrations were not induced but polyploidy was increased by the maximum incidence of 6 %.

Conclusions:

The genotoxic potential of this chemical was mostly negative with and without an exogenous metabolic activation system in bacteria as well as mammalian cells, while the cytogenetic effect was judged to be positive in *in vitro* tests without an exogenous metabolic activation because of a slight increase of polyploid cells and induction of micronucleus cells. However, this chemical did not induce the cytogenetic effects in an *in vivo* bone marrow chromosome test although a standard method was not used. A weight of evidence suggests this chemical may not be genotoxic *in vivo*.

### 3.1.7 Carcinogenicity

The carcinogenic potential of N,N-dicyclohexyl-2-benzothiazolesulfenamide was investigated in a long-term study in Wistar rats of both sexes [Bayer AG: 1975]. Groups of 20 animals per sex were

injected once a week over 413 days at a total of 1,000 or 20,000 mg/kg bw. An increased number of sarcomas located at the injection site was observed in all dose groups, but no signs of systemic toxicity were reported, and there was no difference in the survival between the control group and the dose group. However, this study could not be assessed because there is no further detailed information.

Conclusion:

There is no available information on carcinogenicity.

### 3.1.8 Toxicity for Reproduction

The only available data are from the OECD combined repeat dose and reproductive toxicity study [OECD TG 422]. This study was identified to be well conducted and reported.

N,N-Dicyclohexyl-2-benzothiazolesulfenamide was administered to SD (Crj:CD) rats by gavage at doses of 0, 6, 25, 100 and 400 mg/kg for 44 days from 14 days prior to mating in males and for 40-51 days from 14 days prior to mating to day 3 of lactation throughout the mating and pregnancy period in females [MHW, Japan: 1996].

Toxic effects were revealed in females and pups only at 400 mg/kg. There was decreased number of the corpus lutea accompanied with decreases in number of implantation sites and litter size. Three dams died on the expected delivery day or on the following day. All dams lost their litters at delivery or by day 4 of lactation. Therefore, there were decreases in reproduction/development parameters such as gestation index, number of live pups at birth, live birth index and viability index on day 4 of lactation. The NOAEL for reproductive/developmental toxicity was 100 mg/kg/day. On the other hands, morphological changes were not observed in reproductive organs of male and females in all repeated and reproductive toxicity studies. Therefore, the observed reproductive effects are considered to follow maternal toxicities such as decreased locomotor activity and histological changes in kidneys, adrenals and spleen.

Conclusion

There are significant reductions of gestation index, number of live pups at birth, live birth index and viability index on day 4 of lactation at 400 mg/kg. A clear NOAEL of reproductive/developmental toxicity was established at 100 mg/kg/day.

### 3.1.9 Information on a structural related chemical

There are available toxicity information of N-cyclohexyl-2-benzothiazolesulfenamide (monocyclohexyl compound) (CAS No. 95-33-0), a structurally related chemical to N,N-dicyclohexyl-2-benzothiazolesulfenamide (dicyclohexyl compound) [MHW, Japan: 1997]. Both chemicals similarly show kidney toxicity but the toxicity level of monocyclohexyl compound is less than that of dicyclohexyl compound as follows.

The oral $LD_{50}$ value of monocyclohexyl compound is 5,300 mg/kg in rats. In a repeated dose study, the monocyclohexyl compound induces kidney toxicity in male rats, but this effect is considered to be an accumulation of $\alpha_{2u}$-globulin complex, which is specific to male rats. Based on marked decreases in body weight gain, the NOAEL is considered to be 250 mg/kg/day. As for developmental toxicity, the monocyclohexyl compound only induces decreased fetal body weight, which was a likely secondary effect of maternal toxicity. The NOAEL is considered to be 289 mg/kg/day. The monocyclohexyl compound is not mutagenic in a bacterial reverse mutation test

and a chromosomal aberration test *in vitro*. It is known that the monocyclohexyl compound could be a causative agent of allergic contact dermatitis to humans.

### 3.2 Initial Assessment for Human Health

There is no available information on kinetics and metabolism of N,N-dicyclohexyl-2-benzothiazolesulfenamide. The acute toxicity of this chemical is low based on an oral $LD_{50}$ of greater than 1,000 mg/kg in rats and a dermal $LD_{50}$ of more than 2,000 mg/kg in rabbits. This chemical is moderately irritating to skin and slightly irritating to eyes but not sensitizing to skin. In an oral rat study according to the OECD combined repeated dose and reproductive/developmental toxicity screening test [TG 422], the major toxicities were found in clinical observation and histopathological examination in kidneys. Salivation in 400 mg/kg males and decreased locomotor activity in 100 and 400 mg/kg females were noted. Histopathological examination revealed hyaline droplets in the renal tubular epithelia in males and fatty degeneration of the renal tubular epithelia in females at 100 and 400 mg/kg. In addition, adrenal enlargement with vacuolation of the adrenocortical cells and atrophy of spleen in females at 100 and 400 mg/kg were observed. A NOAEL for repeat dose toxicity was established at 25 mg/kg/day for both sexes.

In the above screening test [OECD TG 422], the effects were revealed in females and pups at a dose of 400 mg/kg. There was a decreased number of the corpus lutea accompanied with decreases in number of implantation sites and litter size. Three dams died on the expected delivery day or on the following day. All dams at 400 mg/kg lost their litters at delivery or by day 4 of lactation. There were no effects on the mating and fertility, and morphogenesis in pups at and below 100 mg/kg/day. A NOAEL for reproductive/developmental toxicity was established at 100 mg/kg/day.

Genotoxic potential of this chemical was mostly negative with and without an exogenous metabolic activation system in bacteria as well as mammalian cells, while the cytogenetic effect was judged to be positive in *in vitro* tests without an exogenous metabolic activation because of a slight increase of polyploid cells and induction of micronucleus cells. However, this chemical did not induce the cytogenetic effects in an *in vivo* bone marrow chromosome test although a standard method was not used. A weight of evidence suggests this chemical may not be genotoxic *in vivo*.

## 4 HAZARDS TO THE ENVIRONMENT

### 4.1 Aquatic Effects

The substance itself is not stable, however, during production, vulcanisation and environmental degradation (biotic and abiotic), several metabolites are formed such as benzothiazole and 2-methylbenzothiazole. For these degradation products only few effect data were available. For benzothiazole, *Cyprinodon variegatus*: 96h-LC50 = 58 mg/l (Evans et al., 2000) was available. For 2-methylbenzothiazole acute and chronic toxicity data were reported; *Oncorhynchus mykiss*: 96h-LC50 = 0.73 mg/l (Monsanto, 1981), 89d-NOEC = 0.041 mg/l (CMA, 1989), *Daphnia magna*: 48h-EC50 = 4.1 mg/l (Monsanto, 1979), 21d-NOEC = 0.22 mg/l (Bayer, 1987), *Selenastrum capricornutum*: 96h-ErC50 = 0.13 mg/l (Monsanto, 1978). The test with *Selenastrum* is considered not valid because 2-methylbenzothiazole is instable under the applied test conditions (photolysis), thus the effect value can only be considered as a nominal concentration.

Acute Toxicity Test Results

N,N-dicyclohexyl-2-benzothiazolesulfenamide has been tested in a limited number of aquatic species from three trophic level. Reliable acute toxicities results are shown in Table 5. There had been two series of tests conducted in 1994 and 2002 by E.A Japan and MOE Japan respectively. In the studies in 1994, the experimental conditions and results were well documented in these studies, although the tests were not conducted under GLP, the chemical concentrations in the medium had not been measured during the course of the experiments, and the toxicities were calculated based on nominal concentration. The estimated value of L(E)$C_{50}$ for fish, daphnids and algae were 96 h $LC_{50}$ > 1000 mg/L (*Orizias latipes*, OECD TG 203), 24 h $EC_{50}$ > 1000 mg/L (Daphnia magna, OECD TG 202 part 1, immobility) and 0-72 h $EC_{50}$ = 15 mg/L (*Selenastrum capricornutum*, OECD TG 201, biomass method), respectively. N,N-dicyclohexyl-2-benzothiazolesulfenamide has very low water solubility (1.9 ug/L at 25°C), the nominal exposure concentrations of each test were extremely higher than the water solubility using dispersants. Regarding to algal test, the growth inhibition had been observed at all concentration however the dose-response curve was irregular. Therefore the test result was considered to be not reliable for lack of confirmation of exposure concentration by analytical monitoring. Therefore these results should be regarded as invalid.

Later a set of experiments was conducted in 2002 in compliance with GLP by MOE, Japan. These tests were carried using a solvent instead of a dispersant, and the concentration had been set up corresponding to the solubility of this chemical in the dilution water. The test results were reliable because each toxicity result was calculated based on measured concentrations and the test was done in compliance with GLP. The preliminary investigation on the test showed that this substance was relatively unstable as the half life time was 30 hours under the test conditions and the solubility was 0.0400 mg/L in the test solution.

The test report from 2002 (MOE, Japan, 2002a-d) demonstrated that the solubility of this chemical in pure water was 39.1 ug/L, which was measured under the condition below by a Japanese contract laboratory.

| | | |
|---|---|---|
| Measurement conditions | Medium; | pure water |
| | Time for stirring; | 48 hrs |
| | Temperature; | 20 degree C |
| | Analysis; | HPLC |

This result was different from the presented value of 1.9 ug/L (Table 1) but consistent with an ECOSAR estimation of 54 ug/L. The ecotoxicological tests in 2002 were conducted according to a solubility value of 39.1 ug/L. Therefore the nominal concentration of 0.0400 mg/L may be above the solubility limit in the medium. And under each test condition the test concentration this chemical was shown to decrease at different rates.

In the fish and daphnids test the concentrations are shown below in mg/L

| | Fish test | Daphnids test | |
|---|---|---|---|
| Nominal concentration : | 0.0400 | 0.0400 (highest) | 0.005(lowest) |
| Initial concentration : | 0.0368 | 0.0385 | 0.00478 |
| After a 48/24 hours : | 0.0334(90%) | 0.0256 (66%) | 0.00218 (45% of initial) |
| Mean : | 0.0351 | 0.0314 | 0.00323 |
| Test Medium | Tap water | Elendt M4 medium | |

Comparing the above two results, stability seems to be different depending on the medium. In tap water (for fish test) the concentration is maintained at above 90% over 48 hours. But in the M4 medium the concentration falls to 45 – 66 % after 24 hrs.

*Fish*

An acute toxicity value of N,N-Dicyclohexyl-2-Benzothiazolesulfenamide to fish was reported (MOE, Japan, 2002a). The test in 1994 (EA, Japan 1994, unpublished data) was conducted at a concentration above the water solubility limit and the concentration of the vehicle was over the limit of 100 mg/L. Therefore the test was considered to be invalid.

The reliable toxicity for fish of 96 h $LC_{50}$ was > 0.0334 mg/L (MOE, Japan, 2002a). In this experiment no mortality was observed. Therefore the $LC_{50}$ of this substance is considered to be above the water solubility.

*Daphnids*

In the test performed in 2002 *Daphnia magna* was used and the tests was conducted according to OECD TG 203. In the test performed in 1994 (EA, Japan 1994, unpublished data) the daphnids were exposed at nominal concentrations ranging from 100 mg/L to 1000 mg/L using a mixture of DMSO and HCO40 as a vehicle and the concentration of the vehicle was above 100 mg/L. At the concentrations of 500 mg/L and 1000 mg/L 15 % immobility was observed. But as the concentrations were extremely higher than the water solubility, it was not clear whether the observed effects were due to toxic effects or physical effects. And also the exposure concentration was not confirmed by chemical analysis. For these reasons this test was considered to be invalid.

In the test performed in 2002, daphnids were exposed at concentrations ranging from 0.005 to 0.04 mg/L with analytical monitoring which showed a measured mean concentration of 0.00323 mg/L in the lowest to 0.00314 mg/L in the highest. The measured concentration was 44 % of the nominal in the lowest exposure and 64 % in the highest exposure in the test solution after 24 hours. In the test no toxic sign or mortality was observed at any concentration. Therefore the $LC_{50}$ of this substance is considered to be above the water solubility.

*Algae*

There is only one reliable test result available. The test was conducted with green algae, *Selenastrum capricornutum*, as a limit test with one nominal concentration of 0.04 mg/L according to OECD TG 203 and GLP by MOE Japan (2002c). The concentration of N,N-Dicyclohexyl-2-Benzothiazolesulfenamide in the test solution was measured to be 0.0335 mg/L at the beginning and 0.00415 mg/L at the end of the exposure. These analytical values seem to be reasonable compared to the water solubility in the pure water however the reason for the reduction (88% of initial concentration) during the exposure was not known. In the test no growth inhibition was observed; the cell density of the solvent control and exposed after 72 h were not different significantly. Therefore the $EC_{50}$ was > 0.0118 mg/L (geometric mean of the measured concentration).

**Table 5 Acute toxicity of N,N-Dicyclohexyl-2-Benzothiazolesulfenamide to aquatic organisms**

| Species | Method | Exposure | Result | Reference |
|---|---|---|---|---|
| Medaka<br>*Orizias latipes* | OECD TG 202<br>GLP test | 96 h<br>semistatic | $LC_{50}$ > 0.0334 mg/L<br>(measured concentration) | MOE, Japan<br>(2002a) |
| *Daphnia magna* | OECD TG 202<br>GLP test | 48 h<br>semistatic | (Immobilisation)<br>$EC_{50}$ > 0.0314 mg/L<br>(measured concentration) | MOE, Japan<br>(2002b) |
| *Selenastrum capricornutum* | OECD TG 201<br>GLP test | 72 h<br>static<br>open system | (rate method)<br>$EC_{50}$ > 0.0118 mg/L<br>(biomass method)<br>$EC_{50}$ > 0.0118 mg/L<br>(measured concentration) | MOE, Japan<br>(2002c) |

Chronic Toxicity Test Results

For daphnids and algae the chronic toxicity of N,N-Dicyclohexyl-2-Benzothiazolesulfenamide was investigated in 1994(E.A. Japan) and 2002 (MOE. Japan). The reliable data are shown in Table 6.

*Daphnids*

In the chronic study by E.A. Japan (1994d) the test (OECD TG 202 reproduction) result showed that the daphnids exposed to the substance at the nominal concentration of 100 mg/L had a significant decreasein the number of offspring compared to the control. Therefore a second experiment was carried out with exposure concentrations ranging from 5.6 mg/L to 56 mg/L with controls. The test resulted in a NOEC of 10 mg/L based on the nominal concentration. However these concentrations were extremely higher than the water solubility. The test should be considered as invalid as it is not clear whether toxic effects on the reproduction occurred at the water solubility level.

Therefore the experiment by MOE Japan in 2002 shown in the Table 6 was conducted to clarify the chronic toxicity at the lower level up to the water solubility. In the test daphnids were exposed to the nominal concentrations of up to 0.04 mg/L with a blank control and a vehicle control (100 ppm of a solvent of DMF). No significant difference was observed between the vehicle control and the exposed daphnids on reproduction and mortality of parental daphnids. Therefore the 21 d NOEC was 0.0331 mg/L estimated based on the mean measured concentration.

*Algae*

An algal growth inhibition test was conducted (*Selenastrum capricornutum*, OECD TG 201, MOE, Japan, 2002c) as a limit test with one nominal exposure concentration of 0.04 mg/L . In the test no difference was observed between the controls (blank and vehicle of 100 ppm DMF) and the exposed organisms, and the 72 h NOEC was determined to be 0.0118 mg/L based on the geometric mean of the initial and final measured concentrations.

**Table 6 Chronic toxicity of N,N-Dicyclohexyl-2-Benzothiazolesulfenamide to aquatic organisms**

| Species | Method | Exposure | Result | Reference |
|---|---|---|---|---|
| *Daphnia magna* | OECD TG 211<br>GLP test | 21 d<br>semistatic | (Reproduction)<br>$EC_{50}$ > 0.0331 mg/L<br>NOEC = 0.0331 mg/L<br>(measured concentration) | MOE, Japan (2002d) |
| *Selenastrum capricornutum* | OECD TG 201<br>GLP test | 72 h<br>static<br>open system | (growth rate method)<br>72 h NOEC = 0.0118 mg/L<br>(biomass method)<br>72 h NOEC = 0.0118 mg/L | MOE, Japan (2002c) |

### 4.2 Terrestrial Effects

No information was available.

### 4.3 Other Environmental Effects

No information was available

### 4.4 Initial Assessment for the Environment

This chemical may have a high bioaccumulation potential based on its Log Pow (>4.8), and it is not readily biodegradable. Although no biodegradability is observed, this chemical tends to be hydrolyzed under acidic and environmental conditions.

In acute toxicity tests with fish, daphnids and algae, no effects were observed at the limit of solubility of the substance [96 h LC50 of > 0.0344 mg/L (*Orizias latipes*, OECD TG 203); 48 h EC50 of > 0.0314 mg/L (Daphnia magna, OECD TG 202, Immobilisation); 72 h EC of > 0.0118 mg/L (*Selenastrum capricornutum*, OECD TG 201, both biomass method and growth rate method) were reported].

Also in chronic toxicity tests with daphnids and algae, no effects were observed at the limit of solubility of the substance. A 21 d NOEC of 0.0331 mg/L (*Daphnia magna,* OECD TG 211, reproduction) and a 72 h NOEC of 0.0118 mg/L (*Selenastrum capricornutum*, OECD TG 201) were reported.

## 5 RECOMMENDATIONS

The chemical is a candidate for further work.

**Human Health**

The chemical possesses a hazard for human health (repeated dose toxicity). An exposure assessment and, if necessary risk assessments for workers and consumers should be performed taking into account possible breakdown products.

**Environment**

The chemical did not show any adverse effects in several acute and chronic toxicity tests with aquatic organisms. During the use of the substance several degradation products are formed which possess properties indicating a hazard for the environment. These 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 degradation products should be performed. The currently on-going assessment of di(benzothiazoyl-2)disulfide (CAS No. 120-78-5), of N-cyclohexylbenzothiazole-2-sulfenamide (CAS No. 95-33-0) and of the sulfenamide accelerator category should be taken into account.

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MOE, Japan (2002 c): Unpublished.

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Monsanto Study HL-84-292, unpublished

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# SIDS DOSSIER

# N, N-Dicyclohexyl-2-Benzothiazolesulfenamide

# CAS No. 4979-32-2

Sponsor Country: Japan

DATE: September, 2000
DATE: April 2004 (Revised)

**1.0.1 SUBSTANCE INFORMATION**

**A. CAS-Number** 4979-32-2

**B. Name (IUPAC name)** N,N-Dicyclohexyl-2-benzothiazolesulfenamide

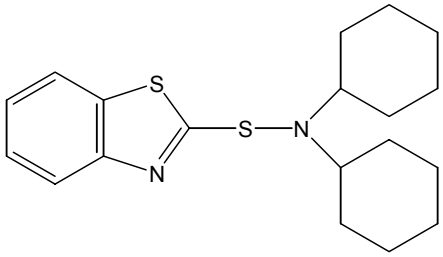
**C. Name** *(OECD name)* N,N-Dicyclohexyl-2-benzothiazolesulfenamide

**D. CAS Descriptor** Not applicable

**E. EINECS-Number** 225-625-8

**F. Molecular Formula** $C_{19}H_{26}N_2S_2$

**G. Structural Formula**




**H. Substance Group** Not applicable

**I. Substance Remark** None

**J. Molecular Weight** 346.59

**1.0.2 OECD INFORMATION**

**A. Sponsor Country:** Japan

**B. Lead Organization:**

Name of Lead Organization:
Ministry of Health, Labor and Welfare (MHLW)
Ministry of Economy, Trade and Industry (METI)
Ministry of Environment (MOE)
Contact person: Mr. Yasuhisa Kawamura
Director
Second International Organizations Division
Ministry of Foreign Affairs
Address: 2-2-1 Kasumigaseki, Chiyoda-ku
Tokyo 100-8919, Japan
TEL 81-3-3581-0018
FAX 81-3-3503-3136

**C. Name of responder**

Name: Same as above contact person
Address:

**1.1 GENERAL SUBSTANCE INFORMATION**

**A. Type of Substance**
element **[ ]**; inorganic **[ ]**; natural substance **[ ]**;
organic **[ X ]**; organometallic **[ ]**; petroleum product **[ ]**

**B. Physical State** gaseous **[ ]**; liquid **[ ]**; solid **[ X ]**

**C. Purity** > 99.2 %

**1.2 SYNONYMS** N,N-Dicyclohexyl-2-benzothiazolesulfenamide (DBTS)

**1.3 IMPURITIES** Unknown

**1.4 ADDITIVES** Unknown

**1.5 QUANTITY**

| Location | Production (tonnes) | Date |
|---|---|---|
| Japan | ca. 1,000/year | 1990-1993 |

Reference: MITI, Japan (1994a)

**1.6 LABELLING AND CLASSIFICATION**

None

**1.7 USE PATTERN**

**A. General**

| **Type of Use:** | **Category:** |
|---|---|
| Industry use | Accelerator of vulcanization |

Reference: MITI, Japan (1994a)
ECDIN Database

**B. Uses in Consumer Products**

None

**1.8 OCCUPATIONAL EXPOSURE LIMIT VALUE**

None

**1.9 SOURCES OF EXPOSURE**

**1.10 ADDITIONAL REMARKS**

**A. Options for disposal** Incineration

Reference: MITI, Japan (1994a)

**B. Other remarks** None

**2.1 MELTING POINT**

(a)
Value: 99 °C
Decomposition: Yes **[ ]** No **[ X ]** Ambiguous **[ ]**
Sublimation: Yes **[ ]** No **[ X ]** Ambiguous **[ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ X ]**
Reference: SRC online Data base, searched in January 2004
Reliability: (2) valid with restrictions
Scientifically acceptable estimation method.
Flag: Critical Study for SIDS

(b)
Value: 92 -93 °C
Decomposition: Yes **[ ]** No **[ X ]** Ambiguous **[ ]**
Sublimation: Yes **[ ]** No **[ X ]** Ambiguous **[ ]**
Method:
GLP: Yes **[ ]** No **[ ]** ? **[ X ]**
Reference: Bayer AG (1975)

(c)
Value: 101 - 102 °C
Decomposition: Yes **[ ]** No **[ X ]** Ambiguous **[ ]**
Sublimation: Yes **[ ]** No **[ X ]** Ambiguous **[ ]**
Method:
GLP: Yes **[ ]** No **[ X ]** ? **[ ]**
Reference: Torii et al., J. Org. Chem., 43, 3223 (1978)

**2.2 BOILING POINT**

Value: > 300 °C
Pressure: 1013 hPa
Decomposition: Yes **[ X ]** No **[ ]** Ambiguous **[ ]**
Method: OECD Guideline 103
GLP: Yes **[ ]** No **[ ]** ? **[ X ]**
Test substance: Source: Ouchishinko Chemical Industry Co., Ltd.
Purity: > 99 %
Remark: Test substance was decomposed at 300 °C
Reference: MITI, Japan (1994b)
Reliability: (2) valid with restrictions
Flag: Critical Study for SIDS

**2.3 DENSITY (Relative density)**

No data available

**2.4 VAPOUR PRESSURE**

Value: < 7.0 x $10^{-5}$ Pa
Temperature: 100 °C
Method: calculated **[ ]**; measured **[ X ]**
OECD Test Guideline 104 Gas saturation method
Test substance: Source: Ouchishinko Chemical Industry Co., Ltd.
Purity: > 99 %
Remark: High purity nitrogen gas was passed through the test substance at 100 °C for 21 hours. The test substance

transported in carrier gas was trapped in acetonitrile at outlet and quantified with HPLC. The maximum value of vapour pressure was calculated from the detection value of HPLC analysis and the volume of saturated gas passed through.

Result: Since the amount of the test substance trapped in acetonitile was less than detection limit of HPLC analysis (0.00123 mg/L), a limit value at 100 °C was reported .

GLP: Yes **[ ]** No **[ X ]** ? **[ ]**

Reference: MITI, Japan (1994b)

Reliability: (2) valid with restrictions

Flag: Critical Study for SIDS

## 2.5 PARTITION COEFFICIENT $log_{10}P_{ow}$

Log Pow: > 4.80

Temperature: 25 °C

Method: calculated **[ ]**; measured **[ X ]**
OECD Test Guideline 107

Test substance: Source: Ouchishinko Chemical Industry Co., Ltd.
Purity: > 99 %

Remark: After partition equilibrium of the test substance was established between n-octanol and water at three volume ratios, the concentrations of the test substance of both phases were determined with HPLC.

Result: Concentration in n-octanol and water phases were as follows (mg/L):

| Condition | Run 1 | | Run 2 | |
|---|---|---|---|---|
| | Water | Octanol | Water | Octanol |
| 1 | < 0.0195 | 1240 | < 0.0195 | 1220 |
| 2 | < 0.0196 | 612 | < 0.0196 | 615 |
| 3 | < 0.0185 | 305 | < 0.0185 | 314 |

Concentrations in water phase were less than detection limit of HPLC.

GLP: Yes **[ X ]** No **[ ]** ? **[ ]**

Reference: MITI, Japan (1994b)

Reliability: (2) valid with restrictions

Flag: Critical Study for SIDS

## 2.6 WATER SOLUBILITY

### A. Solubility

Value: 1.9 ug/L

Temperature: 25 °C

Description: Miscible **[ ]**; Of very high solubility **[ ]**;
Of high solubility **[ ]**; Soluble **[ ]**;
Slightly soluble **[ ]**; Of low solubility **[ ]**; Of very low solubility **[ X ]**; Not soluble **[ ]**

Method: OECD Test Guideline 105, Flask method

Test substance: Source: Ouchishinko Chemical Industry Co., Ltd.
Purity: > 99 %

Remark: 10 mg of the test substance and 20 ml of purified water were added in a glass vessel. The vessels were kept at

30 ºC with shaking for 24, 48 and 72 hours. Then the vessels were equilibrated for 24 hours at 25 ºC with occasional shaking. After the aqueous phase was filtered, the concentrations of the test substance in water were determined by LC-MS analysis.
Although the flask method can be applicable for those substances having a solubility above 0.02 g/l, the LC-MS analysis detected well below the reported concentration range.

Result:

| Time | Concentration (ug/l) | Average (ug/l) | pH |
|---|---|---|---|
| 24 | 1.5 | 1.7 | 6.6 |
| | 1.9 | | 6.4 |
| 48 | 2.0 | 2.1 | 6.3 |
| | 2.1 | | 6.4 |
| 72 | 1.9 | 2.1 | 6.4 |
| | 2.3 | 2.1 | 6.2 |

Total average concentration is 1.9 ug/l.

GLP: Yes **[ ]** No **[ X ]** ? **[ ]**
Reference: CERI (2001)
Reliability: (2) valid with restrictions
Flag: Critical Study for SIDS

**B. pH Value, pKa Value**

No data available

**2.7 FLASH POINT**

No data available

**2.8 AUTO FLAMMABILITY**

No data available

**2.9 FLAMMABILITY**

No data available

**2.10 EXPLOSIVE PROPERTIES**

No data available

**2.11 OXIDIZING PROPERTIES**

No data available

**2.12 OXIDATION: REDUCTION POTENTIAL**

No data available

**2.13 ADDITIONAL DATA**

**A. Partition co-efficient between soil/sediment and water (Kd)**

Log Koc: 5.04
Method: Estimated by PCKOCWIN v1.66.
Reliability: (2) valid with restriction.
Scientifically acceptable estimation method
Reference: EPI-PCKOCWIN v1.66, calculated in January 2004

**B. Other data**

None

## 3.1 STABILITY

### 3.1.1 PHOTODEGRADATION

(a) Type: Air [ X ]; Water [ ]; Soil [ ]; Other [ ]

Light source: Sun light [ ]; Xenon lamp [ ]; Other [ ]
Light spectrum: . . . . . nm
Relative intensity:
Spectrum of substance:
Concentration of Substance:
Temperature:
Indirect Photolysis:
Type of sensitizer: OH
Concentration of sensitizer: 1,500,000 molecules/$cm^3$
Rate constant (radical): 113.8512 x $10^{-12}$ cm3/molecule*sec
Degradation: 50% after 2.26 hours
Method: calculated [ X ]; measured [ ];
GLP: Yes [ ] No [ X ] ? [ ]
Test substance:

Remarks: The reaction rate constant with OH radical was estimated by SRC AOPWIN v1.90. The half-life (2.26 hours) was calculated based on the calculated rate rate constant and OH radical concentration in atmosphere of,500,000 molecules/$cm^3$.

Flag: Critical study for SIDS endpoint

Reliability:(2) valid with restriction.
Scientifically acceptable estimation method

Reference: EPI-AOPWIN v1.9, calculated in January 2004

(b) Type: Air **[ ]**; Water **[ X ]**; Soil; Other **[ ]**

Light source: Sunlight **[ X ]**; Xenon lamp **[ ]**; Other **[ ]**
Spectrum of substance: epsilon = 9.01 x $10^3$ at 300 nm
Estimated parameter for calculation:
Quantum yield 0.01
Concentration 5 x $10^{-5}$ M
Depth of water body 500 cm
Conversion constant 6.023 x $10^{20}$

Result: Degradation rate 2.10 x $10^{-11}$ mol/l/s
Half life 5.23 x $10^{-2}$ years
Reference: W. J. Lyman, W. F. Reehl and D. H. Rosenblatt, "Handbook of Chemical Property Estimation Method", McGraw Hill Book Co.1981.

### 3.1.2 STABILITY IN WATER

Type: Abiotic (hydrolysis) **[ X ]**; biotic (sediment) **[ ]**
Result: Half life 4.92 days at pH 4
18.6 days at pH 7
112 days at pH 9 at 25 °C
Method: OECD Test guideline 111
GLP: Yes **[ ]** No **[ X ]** ? **[ ]**
Test substance: Source: Ouchishinko Chemical Industry Co., Ltd.

Purity: > 99 %

Remark: 2 ml of 20 mg/l of the test substance solution dissolved in acetonitrile was added to 200 ml of the buffer at pH 4.0, 7.0 and 9.0 (nominal concentation = 0.2 mg/l). The solutions were kept at 50, 60 and 70 °C and the concentrations in each solution as a function of time were determined by HPLC analysis. The first-order rate constant of hydrolysis at 25 °C was calculated by extrapolating the linear regression equation between logarithm of the rate constant and reciprocal temperature. The half-life of the test substance at each pH was calculated from the rate constant. Dicyclohexylamine and 2-mercaptobenzthiazole were detected as major metabolites by LC-MS analysis. Mass balance is not known.

Result: N,N-Dicyclohexyl-2-Benzothiazolesulfenamide is hydrolysed with the rate depending on pH. The half-life time at 25 °C at pH 4.0, 7.0 and 9.0 are 4.92, 18.6 and 112 days, respectively.

Flag: Critical study for SIDS endpoint.

Reliability: (2) reliable with restriction.
Although the study was well conducted and testing conditions were reliable, initial concentration was above the water solubility and actual degradation rates might be faster than the reported values.

Reference: CERI (2001)

### 3.1.3 STABILITY IN SOIL

No data available

## 3.2 MONITORING DATA (ENVIRONMENT)

No studies located

## 3.3 TRANSPORT AND DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS INCLUDING ESTIMATED ENVIRONMENTAL CONCENTRATIONS AND DISTRIBUTION PATHWAYS

### 3.3.1 TRANSPORT

No data available

### 3.3.2 THEORETICAL DISTRIBUTION (FUGACITY CALCULATION)

The potential environmental distribution of N,N-Dicyclohexyl-2-benzothiazolesulfenamide obtained from a generic level III fugacity model under three emission scenarios is shown in Table.

| Compartment | Release: 100% to air | Release: 100% to water | Release: 100% to soil |
|---|---|---|---|
| Air | 43.2 % | 0.2 % | 0.0 % |
| Water | 6.4 % | 87.2 % | 0.0 % |
| Soil | 49.5 % | 0.2 % | 100.0 % |

| | | | |
|---|---|---|---|
| Sediment | 0.9 % | 12.3 % | 0.0 % |

Media: Air, Water, Soil and Sediment
Method: Generic level III fugacity model
Year: 2001
Remark: Following input parameters used for the calculation.
Molecular weight: 346.59
Melting point: 99 °C
Vapour pressure: 7.0 x $10^{-5}$ Pa
Water solubility: 1.9 ug/L
Log Kow: 4.8
Half life in air: 2.26 hrs
in water: 360 hrs
in soil: 360 hrs
in sediment 1440 hrs.
Flag: Critical study for SIDS endpoint
Reliability: (2) valid withrestriction
Reference: EA & MITI, Japan (1994)

## 3.4 IDENTIFICATION OF MAIN MODE OF DEGRADABILITY IN ACTUAL USE

No data available

## 3.5 BIODEGRADATION

Type: aerobic **[ X ]**; anaerobic **[ ]**
Inoculum: adapted **[ ]**; non-adapted **[ X ]**;
Concentration of the chemical: 100 mg/l related to Test Substance **[ X ]**
Medium: water**[ ]**;water-sediment**[ ]**;soil **[ ]**;
sewage treatment **[ ]**
other **[**Japanese standard activated sludge**]**
Degradation: Degree of degradation after 28 days
0, 0 and 0 % from BOD
4, 6 and 0 % from GC analysis
Results: Readily biodeg. **[ ]**; Inherently biodeg. **[ ]**;
under test condition no biodegradation observed **[ X ]**
Method: OECD Test Guideline 301 C
Test substance: Source: Ouchishinko Chemical Industry Co., Ltd.
Purity: > 99 %
Remark: 30 mg of the test substance or aniline (reference substance) and 9mg as MLSS of the activated sludge were added to 300 mL of test medium. The test and reference solutions were cultivated in BOD meter together with the inoculum blank and abiotic control ones at 25°C for 28 days, during which the oxygen consumption was continuously measured. After the termination of the test, the residual test substance in the test solution and abiotic control was extracted with chloroform and quantified with HPLC method. The biodegradability was calculated from the oxygen consumption and the residual amount.
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Reference: MITI, Japan (1994b)
Flag: Critical study for SIDS endpoint
Reliability: (1) valid without restriction

**3.6 $BOD_5$,COD OR RATIO $BOD_5$/COD**

Not applicable

**3.7 BIOACCUMULATION**

No data available

**3.8 ADDITIONAL REMARKS**

**A. Sewage treatment** None

**B. Other information** None

**4.1 ACUTE/PROLONGED TOXICITY TO FISH**

(a)
Type of test: static **[ ]**; semi-static **[X]**; flow-through **[ ]**; other **[ ]**
open-system **[X]**; closed-system **[ ]**
Species: *Oryzias latipes*
Exposure period: 96 hr
Results: $LC_{50}$ (24h) > 1000 mg/L > Water solubility
$LC_{50}$ (48h) > 1000 mg/L > Water solubility
$LC_{50}$ (72h) > 1000 mg/L > Water solubility
$LC_{50}$ (96h) > 1000 mg/L > Water solubility
Analytical monitoring: Yes **[ ]** No **[X]** ? **[ ]**
Method: OECD Test Guideline 203 (1981)
Year 1994
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: N,N-Dicyclohexyl-2-benzothiazolesulfenamide
purity = 99.9 %
Remarks:

Test Conditions.
Detail and discuss any significant protocol deviations, and detail differences from the guideline followed including the following as appropriate:
- Test fish (Age/length/weight, loading, pretreatment): Acclimated for several days before testing; any groups showing > 5 % mortality during 7 days were not used for testing
- Test conditions, e.g.
Details of test (static, semi-static, flow-through): Semi-static, open-system
Dilution water source: Purified tap water
Dilution water chemistry (hardness, alkalinity, pH, DOC, TSS, salinity): Not described
Stock and test solution and how they are prepared: Stock solution was prepared using methanol as solvent by ultrasonic (790 mg/L).
Concentrations dosing rate, flow-through rate, in what medium: Concentrations of 95, 170, 310, 560 and 1000 mg/L were tested.
Vehicle/solvent and concentrations: Final concentration of 500 mg/L methanol was used.
Stability of the test chemical solutions: Not described
Number of replicates, fish per replicate: 10 fish were used for each concentration.
Water chemistry in test (O2, pH) in the control and one concentration where effects were observed: DO and pH were measured daily; pH 6.8 - 7.2, DO = 4.5 - 9.2 mg/L
Test temperature range: 21 - 23 °C

Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.): Not described

Results.
Discuss if the effect concentration is greater than materials solubility. Describe additional information that may be needed to adequately assess data for reliability and use, including the following, if available:

- Biological observations: Not described

Table showing cumulative mortality:

| Test substance concentration (mg/L) | Cumulative mortality of each exposure (%) | | | |
|---|---|---|---|---|
| | 24 hours | 48 hours | 72 hours | 96 hours |
| 0 | 0 | 0 | 0 | 0 |
| 95 | 0 | 0 | 0 | 0 |
| 170 | 0 | 0 | 0 | 0 |
| 310 | 0 | 0 | 0 | 0 |
| 560 | 0 | 0 | 0 | 0 |
| 1000 | 0 | 0 | 0 | 0 |

- Lowest test substance concentration causing 100% mortality:
- Mortality of controls: 0 % during exposure period
- Abnormal responses: Not described
- Reference substances (if used) - results: $LC_{50}$ (96 hours) = 0.32 mg/L for sodium pentachlorophenol

Reference: Environment Agency Japan (1994): Investigation on the Ecotoxicological effects of OECD High Volume Chemicals (Phase 3)

Reliabilities: Klimisch Code: 3 = Invalid
This study was regarded invalid because the exposure concentrations were far above the water solubility. And extremely high concentration of methanol (500 mg/L) was used as a solvent.

(b)
Type of test: static **[ ]**; semi-static **[X]**; flow-through **[ ]**; other **[ ]**
open-system **[X]**; closed-system **[ ]**
Species: *Oryzias latipes*
Exposure period: 96 hr
Results: $LC_{50}$ (96h) > 0.0334 mg/L (>0.0351 mg/L mean measured conc.)
Analytical monitoring: Yes **[X]** No **[ ]** ? **[ ]**
Method: OECD Test Guideline 203, 1992
GLP: Yes **[X]** No **[ ]** ? **[ ]**
Test substance: other TS: N,N-Dicyclohexyl-2-benzothiazolsulfene amide (CAS No.: 4979-32-2, OUCHISHINKO CHEMICAL INDUSTRIAL CO., LTD.(Japan), Lot. No.: 112021, Purity = 99.8 %

Remarks
Method:
-Test Organisms:
a) Supplier: Test organisms were obtained from commercial fish shop (Kanagawa, Japan).
b) Size (length and weight): 2.06 cm (1.80 - 2.31 cm) in length; 0.122 g (0.066 - 0.173 g) in weight
c) Age: Not described
d) Any pretreatment: Test organisms were acclimated for one month before testing. During acclimination, test

fishes were fed with TETRAMINE equivalent to 2% of weight per day. These test organisms were not fed for 24 hours before the test started. The mortality of the test organisms for 7 days before testing was less than 5%. LC50(96 hr) for a reference substance (copper sulfate pentahydrate) was 1.2 mg/L.
-Test substance:
a) Empirical Formula: C19H26N2S2
b) Molecular Weight: 346.55 g/mol
c) Purity: =99.8 %
-Test Conditions:
a) Dilution Water Source: Dilution water was prepared from tap water. The tap water was dechlorinated and treated by activated carbon. Before using the dilution water, aeration was fully carried out.
b) Dilution Water Chemistry:
pH: = 7.8
Total hardness (as CaCO3): = 68 mg/L
c) Exposure Vessel Type: 5 L test solution in a 5 L glass beaker
d) Nominal Concentrations: control, solvent control and 0.04 mg/L. Test concentration was determined based on preliminary test result.
e) Vehicle/Solvent and Concentrations: Dimethylformamide was used as solvent. 100uL/L of dimethylformamide was contained in the each test soltion.
f) Stock Solutions Preparations and Stability: 0.2mg of test chemical was dissolved in the 5,000mL of dilution water contained 500uL of dimethylformamide. Infrared absorption spectrum of the refrigerated test chemical was detected at the start and the end of the test, and both spectrums are not contradictory to each other. Solvent was prepared as 40mg/10mL of dimethylformamide diluted to 10 times by dimethylformamide.
g) Number of Replicates: 1
h) Fish per Replicates: 10
i) Change Rate of Test Water: Test medium was renewed every 2 days.
j) Water Temperature: 24+/-1°C
k) Light Condition: 16:8 hours, light-darkness cycle (less than 1,000 lux)
l) Feeding: None
m) Aeration : Test solution was not aerated during the test period.
-Analytical Procedure: The tested concentrations were measured at the start and the 24th hour using HPLC.
-Statistical Method:
a) Data Analysis: All of test organisms were lived at the end of the test period, therefore the LC50 is more than the highest concentration.

b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): Geometric Mean

Result:
- Measured Concentrations: The test concentrations were measured at 0 h and 48 h. The measured concentration of 0.04mg/L at start of test was 0.0368 mg/L and decreased to 0.0334 at the end of the test. Mean measured

concentration was 0.0351 or 84% of nominal concentration.

| Nominal Conc. mg/L | Measured Conc. 0 Hour | Measured Conc. 48 Hours | Mean* Measured Conc. | Percent of Nominal 0 Hour | Percent of Nominal 48 Hours |
|---|---|---|---|---|---|
| Control | < 0.00001 | < 0.00001 | | --- | --- |
| Solvent Control | < 0.00001 | < 0.00001 | | --- | --- |
| 0.0400 | 0.0368 | 0.0334 | 0.0351 | 92 | 76 |

*: Geometric mean

- Water chemistry (pH and DO) and temperature in test: Water chemistry and temperature were measured for old and renewal solution with control and each concentration at the start of test and every 24 hours.
pH: 6.6 - 7.3
DO: 6.3 - 8.7 mg/L
Water Temperature: 23.5 - 24.0°C
-Effect Data(mortality):
LC50 (96hr) > 0.0334 mg/L (measured concentration after 48hr)
LC50(96hr) > 0.0351 mg/L(mean measured concentration)
LC100 (96hr) > 0.0334 mg/L (mc)
- Cumulative Mortality: None of test organisms were killed during exposure period at control and 0.0334 mg/L.

| Measured Conc. mg/L | Cumulative Number of Dead (Percent Mortality) 24hr | 48hr | 72hr | 96hr |
|---|---|---|---|---|
| Control | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| 0.0334 | 0 (0) | 0 (0) | 0 (0) | 0 (0) |

Other Effect: Toxicological symptom was not observed at any concentration.

| Measured Conc. mg/L | Symptoms 24hr | 48hr | 72hr | 96hr |
|---|---|---|---|---|
| Control | n | n | n | n |
| 0.0334 | n | n | n | n |

n: No abnormalities are detected
- Calculation of toxicity values: The calculation of toxicity values was the measured concentration. The reason is that some of the deviations from the nominal concentration were not less than +/-20%.

Reference: Ministry of Environment, Japan (2002a)
Reliabilities: (1) valid without restriction
Flag: Critical study for SIDS endpoint

**4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES**

**A. Daphnia**
(a)
Type of test:

static **[X]**; semi-static **[ ]**; flow-through **[ ]**;
other **[ ]**;open-system **[X]**; closed-system **[ ]**
Species: *Daphnia magna*
Exposure period: 24 hr
Results: $EC_{50}$ (24h) > 1000 mg/L >Water solubility
Analytical monitoring:
Yes **[ ]** No **[X]** ? **[ ]**
Method: OECD Test Guideline 202 (1984)
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: N,N-Dicyclohexyl-2-benzothiazolesulfenamide,purity=99.9%
Remarks:

Test Conditions.
- Test organisms:
· Source, supplier, any pretreatment, breeding method: supplied from National Institute for Environmental Science
· Age at study initiation: < 24 hours old
· Control group: 100, 180, 320, 560 and 1000 mg/L

- Test conditions
· Stock solutions preparation and stability: Stock solution was prepared with a mixture of a solvent and a detergent (DMSO: HCO-40 = 9:1), and the final concentration of the mixture was unknown.
· Test temperature range: 21 - 23℃
· Exposure vessel type: 50 mL-test solution in a glass beaker, 4 beakers per treatment
· Dilution water source: Reconstituted water
· Dilution water chemistry: hardness = 250 mg/L; pH 7.8; Ca/Mg ratio = 6.56; Na/K ratio = 6.54; alkalinity = 40.2 mg/L
· Lighting: Not described
Water chemistry in test: pH 7.2 - 7.7; DO = 7.2 - 8.5 mg/L
- Element (unit) basis: immobilization
-Test design: 20 Daphnia (4 replicates; 5 organisms per replicate) were exposed to each of 5 nominal concentrations (100, 180, 320, 560 and 1000 mg/L).
- Method of calculating mean measured concentrations: Not described
- Exposure period: 24 hours
- Analytical monitoring: Not described

Results:

- Biological observations
• Number immobilization as compared to the number exposed: The test was carried out with 20 Daphnia at start for each nominal concentration.

| Chemical Concentration (mg/L) | Immobility Number | Immobility Rate (%) |
|---|---|---|
| 0 | 0 | 0 |
| 100 | 0 | 0 |
| 180 | 0 | 0 |

| | | |
|---|---|---|
| 320 | 0 | 0 |
| 560 | 3 | 15 |
| 1000 | 3 | 15 |

Reference: EA, Japan (1994): Investigation on the Ecotoxicological effects of OECD High Volume Chemicals (Phase 3): p. 65 - 164.

Reliabilities: (3) invalid
This study was conducted at the concentrations far above the water solubility. Therefore, the toxicity value was not reliable, and the adverse effects was observed at the higher concentrations (560 mg/L and 1000 mg/L in nominal), but it could not distinguish the true toxic effect from physical effect of the test substance.

(b)
Type of test: static **[]**; semi-static **[X]**; flow-through **[ ]**; other **[ ]**; open-system **[X]**; closed-system **[ ]**
Species: *Daphnia magna*
Exposure period: 48 hr
Results: $EC_{50}$ (24h) > 0.0314
Analytical monitoring:
Yes **[X ]** No **[]** ? **[ ]**
Method: OECD Test Guideline 202 (1984)
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: other TS: N,N-Dicyclohexyl-2-benzothiazolsulfene amide (CAS No.: 4979-32-2, OUCHISHINKO CHEMICAL INDUSTRIAL CO., LTD.(Japan), Lot. No.: 112021, Purity = 99.8 %
Remarks:
Methods
Test Organisms:

(a) Age: < 24 hours old
(b) Supplier/Source: Test organisms were obtained from National Institute for Environmental Studies Japan) and had been reproduced in the testing laboratory for 7 years.
(c) Any pretreatment: Parental daphnids were acclimated for 28 days on test condition before testing. During acclimination, test daphnids were fed with Chlorella vulgaris, 0.2 mg carbon/day/individual. The mortality of the daphnids was less than 5% for 2 weeks before testing. Any resting-egg and male daphnia was not observed. EC50(48hr, immobility) for reference substance (potassium dichromate) was 0.89mg/L.

-Test substance:

(a) Empirical Formula: C19H26N2S2
(b) Molecular Weight: 346.55 g/mol
(c) Purity: =99.8 %

-Test Conditions:

(a) Dilution Water Source: Elendt M4 recommended by OECD TG 211 was used as dilution water.

(b) Exposure Vessel Type: 100 mL test solution in a 100 mL glass beaker
(c) Nominal Concentrations: control, solvent control, 0.005, 0.0084, 0.014, 0.024 and 0.04 mg/L
(d) Vehicle/Solvent and Concentrations: Dimethylformamide was used as solvent. 100uL/L of dimethylformamide was contained in the each test soltion.
(e) Stock Solutions Preparations and Stability: Test chemical was refrigerated. A 0.04mg test chemical in a solvent was dissolved in 1,000mL dilution water. Infrared absorption spectrum of the refrigerated test chemical was detected at the start and the end of the test, and both spectrums are not contradictory to each other. Solvent was prepared as 40mg/10mL of dimethylformamide diluted to 10 times by dimethylformamide.
(f) Number of Replicates: 4
(g) Individuals per Replicates: 5
(h) Water Temperature: 20+/-1°C
(i) Light Condition: 16:8 hours, light-darkness cycle
(j) Feeding: None
(k) Aeration : not described

- Analytical Procedure: The tested concentrations were measured at the start and the 24th hour using HPLC.
- Statistical Method:
(a) Data Analysis: Any abnormal behavior of the test animals was not observed at the end of the test period, therefore the LC50 is more than the highest concentration.
(b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): Geometric Mean

Results: Measured Concentrations: The test concentrations were measured at the start and the end of the test. Some of the deviations from the nominal were less not than +/- 20%.

| Nominal Conc. mg/L | Measured Conc. mg/L | | Geometric Mean During 24 hours (mg/L) | Percent of Nominal | |
|---|---|---|---|---|---|
| | 0 Hour Fresh | 24 Hour Old | | 0 Hour Fresh | 24 Hour Old |
| Control | <0.00001 | <0.00001 | | --- | --- |
| Solvent Control | <0.00001 | <0.00001 | | --- | --- |
| 0.005 | 0.00478 | 0.00218 | 0.00323 | 96 | 44 |
| 0.0084 | 0.00799 | 0.00342 | 0.00523 | 95 | 41 |
| 0.014 | 0.0125 | 0.00685 | 0.00925 | 89 | 49 |
| 0.024 | 0.0252 | 0.0144 | 0.0190 | 105 | 60 |
| 0.04 | 0.0385 | 0.0256 | 0.0314 | 96 | 64 |

Fresh: freshly prepared test solution.
Old: test solution after 48 hours exposure
- Water chemistry (pH and DO) and temperature in test: Water chemistry and temperature were measured for

control and each concentration at the start and the end of the test.
pH: 8.0 - 8.2
DO: 8.4 - 8.8mg/L
Water Temperature: 20.1 - 20.3°C
-Effect Data:
EC0 (48hr) > 0.0314 mg/L (mc)
EC50 (48hr) > 0.0314 mg/L (mc)
mc: based on the measured concentrations
-Mortality or Immobility: No test organism was Immobilized at any concentration.
Measured Cumulative Number of Dead or Immobilized Daphnids
(Percent Mortality or Immobility)

| Conc. mg/L | 24 Hour | 48 Hour |
|---|---|---|
| Control | 0 ( 0 ) | 0 ( 0 ) |
| Solvent Control | 0 ( 0 ) | 0 ( 0 ) |
| 0.00323 | 0 ( 0 ) | 0 ( 0 ) |
| 0.00523 | 0 ( 0 ) | 0 ( 0 ) |
| 0.0925 | 0 ( 0 ) | 0 ( 0 ) |
| 0.0190 | 0 ( 0 ) | 0 ( 0 ) |
| 0.0314 | 0 ( 0 ) | 0 ( 0 ) |

- Calculation of toxic values: Measured concentration

Reference: Ministry of Environment, Japan (2002b)
Reliabilities: (1)valid with restriction
Flag: Critical study for SIDS endpoint

**B. Other aquatic organisms**

No data available

**4.3 TOXICITY TO AQUATIC PLANTS e.g. Algae**

(a)
Species: *Selenastrum capricornutum* ATCC 22662
End-point: Biomass **[X]**; Growth rate **[ ]**; Other **[ ]**
Exposure period: 72 hours
Results: Biomass: $EC_{50}$ (72h) = 15 mg/l
Analytical monitoring: Yes **[ ]** No **[X]** ? **[ ]**
Method: open-system **[**X**]**; closed-system **[ ]**
OECD Test Guideline 201 (1984)
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: N,N-Dicyclohexyl-2-benzothiazolesulfenamide, purity=99.9%
Remarks:
Methods:
- Test organisms: Laboratory culture
Method of cultivation
Controls
- Test Conditions
Test temperature range: 23 - 25 °C
Growth/test medium: OECD medium
Shaking: Occasional shaking
Dilution water source: Not described
Exposure vessel type: 100 mL-medium in a 300 mL-Erlenmeyer flask with a silicon cap which allow ventilation

Water chemistry in test (pH) in at least one replicate of each concentration (at start and end of the test): Not described
Stock solutions preparation:
Stock solution was prepared with a mixture of DMSO and HCO-40 (9:1), and the final concentrations of this mixture in the test solutions were ranging 800-980 mg/L. Controls, with and without this vehicle, were taken for the test; however no detail information on the vehicle control were given in the original reports
Light levels and quality during exposure: 4000 - 7000 lx, continuous

-Test design:
Number of replicates: 3 parallel runs for each nominal concentration.
Concentrations: 20, 33, 50, 100, and 200 mg/L
Initial cell number in cells/m L: 1.0 X $10^4$

- Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.): Not described

Results:
- Biological observations
Cell density at each flask at each measuring point: Not described
Growth curves: Logarithmic growth until end of the test

Reference: Environment Agency Japan (1994): Investigation on the Ecotoxicological effects of OECD High Volume Chemicals (Phase 3): p. 1 - 64.
Reliability: (3) invalid
Remark for Reliability: For the reasons of no analytical monitoring, with the exposure extremely higher than the water solubility and the irregular dose-response curve this test has problems to assess the toxic value. It needs to verify the exact concentration in the test solution.

(b)
Species: *Selenastrum capricornutum* ATCC 22662
End-point: Biomass **[]**; Growth rate **[X ]**; Other **[ ]**
Exposure period: 72 hours
Results: $EC_{50}$ (72h) > 0.0118 mg/L
Analytical monitoring: Yes **[X ]** No **[]** ? **[ ]**
Method: open-system **[**X**]**; closed-system **[ ]**
OECD Test Guideline 201 (1984)
GLP: Yes **[X]** No **[]** ? **[ ]**
Test substance: other TS: N,N-Dicyclohexyl-2-benzothiazolsulfene amide (CAS No.: 4979-32-2, OUCHISHINKO CHEMICAL INDUSTRIAL CO., LTD.(Japan), Lot. No.: 112021, Purity = 99.8 %
Remarks:
Remarks for Methods:
- Test Organisms:
a) Supplier/Source: Obtained from American Type Culture Collection and reproduced in aseptic culture at 6th June 1996.
b) Method of Cultivation: Sterile
c) Stain Number: ATCC22662
d) Pre-culture (duration, medium, etc.): Test alga was

pre-incubated for 4 days under the same method of test in OECD medium. EbC50 (0-72 hr) for a reference substance (potassium dichromate) was 0.473 mg/L.

-Test substance:
a) Empirical Formula: C19H26N2S2
b) Molecular Weight: 346.55 g/mol
c) Purity: =99.8 %

- Test Conditions:
a) Medium: OECD medium
b) Exposure Vessel Type: 300mL Erlenmeyer flask
c) Nominal Concentrations: control solvent control and 0.04 mg/L
d) Vehicle/Solvent and Concentrations: Dimethylformamide was used as solvent. 100uL/L of dimethylformamide was contained in the each test soltion.
e) Stock Solutions Preparations and Stability: Test chemical was refrigerated. 0.04mg test chemical was dissolved in 1000mL OECD medium included 100uL/L of dimethylformamide and which was used as 0.04 mg/L test solution. Infrared absorption spectrum of the refrigerated test chemical was detected at the start and the end of the test, and both spectrums are not contradictory to each other. Solvent was prepared as 40mg/10mL of dimethylformamide diluted to 10 times by dimethylformamide.
f) Number of Replicates: 3
g) Initial Cell Number: 10,000 cells/Ml
h) Water Temperature: 23+/-2°C
i) Light Condition: 4,000 lux (+/- 20%), continuously
j) Shaking: 100 rpm

- Analytical Procedure: The tested concentrations were measured at the start and the 72nd hour using HPLC.

- Statistical Method:
a) Data Analysis: The calculated inhibition rate at the highest concentration based on growth rate inhibition and biomass were less than 50%, therefore the EC50 was more than the highest concentration. The NOEC values were determined by Student T-test.

b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.): Mean measured concentration was not calculated.

Results: - Measured Concentrations: The tested concentrations were measured at the start and the 72nd hour. At the start of the test, the deviations from the nominal concentration were less than +/-20%. Although at the end of the test, the measured concentration of 0.04mg/L of the test chemical was 0.00415mg/L or 10% of nominal concentration. The major reason for the decrementation of the test chemical was considered as hydrolysis of the test chemical and aclimination of test chmical to algae.

| Nominal conc. mg/L | Measured Conc., mg/L | | Percent of nominal | |
|---|---|---|---|---|
| | 0 Hour | 72 Hour | 0 Hour | 72 Hour |

| | | | | |
|---|---|---|---|---|
| Control | <0.00001 | <0.00001 | --- | --- |
| Solvent Control | <0.00001 | <0.00001 | --- | --- |
| 0.0400 | 0.0335 | 0.00415 | 84 | 10 |

- Water chemistry (pH) and temperature in test: pH was measured for control and each concentration at the start and the end of test period. Water temperature was measured every 24hours.

pH: 7.8 – 7.9 (at the start of the test)
9.3 – 9.7 (at the end of the test)
water temperature: 22.8 - 23.3°C

-Effect Data: biomass Area Method
EbC50(0-72hr) > 0.0118 mg/L (mc)
NOEbC (0-72hr) > 0.0118 mg/L (mc)
Rate Method
ErC50(24-48hr) > 0.0118 mg/L (mc)
NOErC (24-48hr) 0.0118 mg/L (mc)
ErC50(0-72hr) > 0.0118 mg/L (mc)
NOErC (0-72hr) > 0.0118 mg/L (mc)
mc: measured concentration
- Percent Growth Inhibition of Selenastrum capricornutum

| Nominal Conc. mg/L | Area under the growth curves (Average) Area A (0-72hr) | Inhibition (%)* IA (0-72hr) |
|---|---|---|
| Control | 40,545,000 | --- |
| Solvent Control | 40,240,000 | --- |
| 0.0400** | 39,539,000 | 1.7 |

Growth rates and percent inhibition (Average)

| Nominal Conc. mg/L | Rate u(24-48hr) | Inhibition(%)* Im(24-48hr) | Rate u(0-72hr) | Inhibition(%)* Im(0-72hr) |
|---|---|---|---|---|
| Control | 0.0843 | --- | 0.0800 | --- |
| Solvent Control | 0.0869 | --- | 0.0807 | --- |
| 0.0400 | 0.0860 | 1.0 | 0.0825 | -2.2 |

*: Values are the percent inhibition relative to solvent control.
**: The maximum attainable concentration under the present test conditions and preparation methods.

Growth Curves: During the test period algae grew almost linearly(log scale) in each concentration.

- Calculation of toxic values: Both Nominal concentration and measured concentration

Reference: Ministry of Environment, Japan (2002c)
Reliability: (1) valid without restrictions
Flag: Critical study for SIDS endpoint

**4.4 TOXICITY TO BACTERIA**

Test species: *Belebtschlamm*
Method: Test for Inhibition of Oxygen Consumption by Activated Sludge, ISO 8192

Type of test: static **[ ]**, semi-static **[ ]**, flow-through **[ ]** Other **[ ]**
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test results: EC50 (3 hr) > 10000 mg/l
Test substance: N,N-Dicyclohexyl-2-benzothiazolesulfenamide
Remarks: Direkteinwaage
Reference: Bayer AG
Reliability: (4)not assingnable
Details of the test result were not available, however the method was relevant as a test guide-line.

**4.5 CHRONIC TOXICITY TO AQUATIC ORGANISMS**

**4.5.1. CHRONIC TOXICITY TO FISH**

No data available

**4.5.2. CHRONIC TOXICITY TO AQUATIC INVERTEBRATES**

(a)Type of test: static **[ ]**; semi-static **[**X**]**; flow-through **[ ]**; other **[ ]**; open-system **[X]**; closed-system **[ ]**
Species: *Daphnia magna*
End-point: Mortality **[ ]**; Reproduction rate **[X]**; Other **[X]**
Exposure period: 21 days
Results:
Immobility: $EC_{50}$ (48 h) = > 1000 mg/l
$EC_{50}$ (21 d) = 140 mg/l (95% confidence limits: 110-170 mg/l)
Reproduction: $EC_{50}$ (21 d) = 40 mg/l (95% confidence limits: 35-47 mg/l)
NOEC = 10 mg/l ($p < 0.05$)
LOEC = 18 mg/l ($p < 0.05$)

Analytical monitoring: Yes **[ ]** No **[X]** ? **[ ]**
Method: OECD Test Guideline 202 (1984)
GLP: Yes **[ ]** No **[X]** ? **[ ]**
Test substance: N,N-Dicyclohexyl-2-benzothiazolesulfenamide, purity=99.9%
Remarks:
Remarks for Method:
- Test organisms:
Source, supplier, any pre-treatment, breeding method: supplied from National Institute for Environmental Studies, Japan
Age at study initiation: < 24 hours old

- Test conditions
Stock solutions preparation and stability: Stock solution was prepared with DMSO: HCO-40 = 9:1 (5.6 - 56 mg/L in the second experiment).
Test temperature range: 21 - 23 °C
Exposure vessel type: 400 mL-test solution in a 500 mL-glass beaker; 4 beakers per treatment
Dilution water source: Reconstituted water

Dilution water chemistry: hardness = 250 mg/L; pH 7.8; Ca/Mg ratio = 6.56; Na/K ratio = 6.54; alkalinity = 40.2 mg/L
CaCl2 2H2O : 294 mg/L
MgSO4 7H2O : 123 mg/L
NaHCO3 : 64.8 mg/L
KCl : 5.75 mg/L
Lighting: 16:8 hours; light-darkness cycle, 2000 lx
Water chemistry in test: DO and pH were measured when the test solution was exchanged.

DO: 6.6 – 9.0 mg/L, pH: 7.5-7.8
Feeding: Green algae, daily
– Element (unit) basis: Reproduction
– Test design: 40 Daphnia (4 replicates: 10 organisms per replicate) were exposed.
– Method of calculating mean measured concentrations (i.e. arithmetic mean, geometric mean, etc.): Not described
– Exposure period: 21 days
– Analytical monitoring: No

Remarks for Results

– Biological observations
Time of the first production of young (d): 8 - 12 days
Mean cumulative numbers of young produced per adult:

The first Experiment Result

| Chemical concentration (mg/L) | Cumulative no. of young per adult | |
|---|---|---|
| | 14 days | 21 days |
| 0 | 25.0 | 59.9 |
| 100 | 5.1 | 10.1* |
| 180 | 0 | 0 |
| 320 | 0 | 0 |
| 560 | 0 | 0 |
| 1000 | 0 | 0 |

The second Experiment Result

| Chemical concentration (mg/L) | Cumulative no. of young per adult | |
|---|---|---|
| | 14 days | 21 days |
| Control | | 61.24 |
| Vehicle control | | 68.52 |
| 5.6 | | 62.86 |
| 10 | | 60.90 |
| 18 | | 53.63* |
| 32 | | 36.52* |
| 56 | | 20.62* |

*: Significant difference ($p < 0.05$)

Reference: Environment Agency Japan (1994): Investigation on the Ecotoxicological effects of OECD High Volume Chemicals (Phase 3): p.65 - 164.

Reliability: (3) invalid
Remarks for Reliability:
For the reasons of no analytical monitoring, with the exposure extremely higher than the water solubility, it should be invalid unless the indicated toxicity was proved as true toxicity instead of physical effect due to non-soluble compartment.
Flag:

(b)
Type of test: static **[ ]**; semi-static **[**X**]**; flow-through **[ ]**; other **[ ]**; open-system **[X]**; closed-system **[ ]**
Species: *Daphnia magna*
End-point: Mortality **[ ]**; Reproduction rate **[X]**; Other **[X]**
Exposure period: 21 days
Results: Immobility: $EC_{50}$ (48 h) > 0.0331 mg/l
$EC_{50}$ (21 d) > 0.0331 mg/l
Reproduction: $EC_{50}$ (21 d) > 0.0331 mg/l
NOEC = 0.0331 mg/l
Analytical monitoring: Yes **[X ]** No **[]** ? **[ ]**
Method: OECD Test Guideline 211 (1998)
GLP: Yes **[X ]** No **[]** ? **[ ]**
Test substance: other TS: N,N-Dicyclohexyl-2-benzothiazolsulfene amide (CAS No.: 4979-32-2, OUCHISHINKO CHEMICAL INDUSTRIAL CO., LTD.(Japan), Lot. No.: 112021, Purity = 99.8 %
Remarks:
Method:
-Test Organisms:
a) Age: < 24 hours old
b) Supplier/Source: Test organisms were obtained from National Institute for Environmental Studies (Japan) and had been reproduced in the testing laboratory for 7 years.
c) Any pretreatment: Parental daphnids were acclimated for 21 days on test conditions before testing, any groups showing high mortality were not used for testing. The mortality of the daphnids was less than 5% for 2 weeks before testing. EC50(48 hr, immobility) for a reference substance (potassium dichromate) was 0.89 mg/L.

-Test substance:
a) Empirical Formula: C19H26N2S2
b) Molecular Weight: 346.55 g/mol
c) Purity: =99.8 %

- Test Conditions:
a) Dilution Water Source: Elendt M4 medium recommended by OECD TG 211 was used as dilution water.
b) Exposure Vessel Type: 80 mL test solution in a 100mL glass beaker
c) Nominal Concentrations: control, solvent control, 0.002, 0.0042, 0.009, 0.019 and 0.04 mg/L
d) Vehicle/Solvent and Concentrations: Dimethylformamide was used as solvent. 100uL/L of dimethylformamide was contained in the each test soltion.
e) Stock Solutions Preparations and Stability: Test chemical was refrigerated. Each test solution was

prepared as proper quantity of test chemical was dissolved in 1,000 mL of Elendt M4 contained dimethylformamide. Infrared absorption spectrum of the refrigerated test chemical was detected at the start and the end of the test, and both spectrums are not contradictory to each other. Solvent was prepared as 40mg/10mL of dimethylformamide diluted to 10 times by dimethylformamide.
f) Number of Replicates: 10
g) Individuals per Replicates: 10
h) Renewal Rate of Test Water: everyday
i) Water Temperature: 20+/-1oC
j) Light Condition: 16:8 hours, light-darkness
k) Feeding: 0.15 mg carbon/day/individual (Chlorella vulgaris: Green Algae)
l) Aeration: During test period, test solution did not aerated.

- Analytical Procedure: The test concentrations were measured for fresh test solution at the start, 7th and 14th day and old test solution at 1st, 8th and 15th day using HPLC.

- Statistical Method:
a) Data Analysis:
LC50 and EC50: During test period the any test organism was not killed in any concentration. The difference of reproduction was analyzed by log it method and its significant difference was not shown. From these reason LC50 and EC50 is more than highest concentration.
NOEC and LOEC: The cumulative number of juveniles produced per adult in control and test vessels after 21days was analyzed by Dunnett's Multicomparison Test, Snalysis of Variance (ANOVA) and Bartlett's Test.

b) Method of Calculating Mean Measured Concentrations (i.e. arithmetic mean, geometric mean, etc.):
Time-weighted Mean

Results:

- Effect: reproduction- Measured Concentrations: The test concentrations were measured for renewal and old test solution at the start of test and 1st, 8th, 9th, 16th and 17th day. Some of them, the deviations from the nominal were not less than +/-20%.

| Nominal Conc. | Measured Conc., mg/L | | | |
|---|---|---|---|---|
| mg/L Date | 0 | 1 | 7 | 8 |
| | Fresh | Old | Fresh | Old |
| Control | <0.00002 | <0.00002 | <0.00002 | <0.00002 |
| Solvent Control | <0.00002 | <0.00002 | <0.00002 | <0.00002 |
| 0.002 | 0.00191 | 0.00091 | 0.00184 | 0.00123 |
| 0.0042 | 0.00388 | 0.00142 | 0.00376 | 0.00212 |
| 0.009 | 0.00924 | 0.00309 | 0.00885 | 0.00421 |
| 0.019 | 0.0188 | 0.00737 | 0.0186 | 0.0136 |
| 0.04 | 0.0443 | 0.0238 | 0.0422 | 0.0227 |

| Nominal Conc. | | Measured Conc., mg/L | | | |
|---|---|---|---|---|---|
| mg/L | Date | 14 Fresh | 15 Old | TWM* mg/L | % of Nominal |
| Control | | <0.00002 | <0.00002 | --- | --- |
| Solvent Control | | <0.00002 | <0.00002 | --- | --- |
| 0.002 | | 0.00178 | 0.00069 | 0.00134 | 67 |
| 0.0042 | | 0.00395 | 0.00168 | 0.00265 | 63 |
| 0.009 | | 0.00887 | 0.00353 | 0.00589 | 65 |
| 0.019 | | 0.0199 | 0.00951 | 0.0141 | 74 |
| 0.04 | | 0.0493 | 0.0234 | 0.0331 | 83 |

Fresh: Start of renewal period
Old: End of renewal period
*: Time-weighted mean of measured concentration during 21 days

- Measured Concentration as a Percentage of Nominal

| Nominal Conc. | Measured Concentration as a Percentage of Nominal | | | | | |
|---|---|---|---|---|---|---|
| mg/L | Date | 0 | 1 | 7 | 8 | 14 | 15 |
| | | Fresh | Old | Fresh | Old | Fresh | Old |
| 0.002 | | 96 | 46 | 92 | 62 | 89 | 35 |
| 0.0042 | | 92 | 34 | 90 | 50 | 94 | 40 |
| 0.009 | | 103 | 34 | 98 | 47 | 99 | 39 |
| 0.019 | | 99 | 39 | 98 | 72 | 105 | 50 |
| 0.04 | | 111 | 60 | 106 | 57 | 123 | 59 |

Fresh: Start of renewal period
Old: End of renewal period

- Water chemistry (pH and DO) and temperature in test: Water chemistry and temperature were measured for control and each concentration at the start of test and before and after renewal of the test solutions.

pH: 7.4 – 8.5
DO: 7.4 - 8.8 mg/L
Water Temperature: 19.9 - 20.4°C

-Effect Data:
LC50 (21day) > 0.0331 (mc)
EC50 (21day) > 0.0331 (mc)
NOEC (21day) > 0.0331 (mc)
LOEC (21day) > 0.0331 (mc)
mc: based on the measured concentrations

- Cumulative Number of Died Parental Daphnids: No test organism was killed at control, 0.002, 0.0042, 0.009 and 0.04 mg/L. The lowest concentration that test organisms were dead was at solvent control after 3days.

| Nominal Conc. (mg/L) | Cumulative Number of Dead Parental Daphnids (days) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Solvent | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | | | | | | | | | | |
| 0.002 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.0042 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.009 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.019 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.04 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Nominal Conc. (mg/L) — Cumulative Number of Dead Parental Daphnids (days)

| | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Solvent | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Control | | | | | | | | | | | |
| 0.002 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.0042 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.009 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.019 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 0.04 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

-Effect Data(reproduction):Juveniles were first produced at 8th day at every concentration.

Nominal Conc. mg/L (days) — Mean Cumulative Numbers of Juveniles Produced per Adult

| | 0--7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|
| Control | 0--0 | 5.2 | 5.9 | 5.9 | 16.6 | 29.2 | 29.2 | 29.4 |
| Solvent | 0--0 | 7.6 | 7.6 | 7.6 | 17.0 | 35.8 | 35.8 | 38.4 |
| Control | | | | | | | | |
| 0.002 | 0--0 | 6.4 | 7.5 | 7.5 | 19.5 | 27.6 | 27.6 | 37.7 |
| 0.0042 | 0--0 | 4.9 | 4.9 | 4.9 | 18.9 | 29.6 | 29.7 | 37.8 |
| 0.009 | 0--0 | 5.1 | 5.8 | 5.8 | 12.8 | 27.7 | 27.8 | 33.1 |
| 0.019 | 0--0 | 6.0 | 6.0 | 6.0 | 19.9 | 28.7 | 29.0 | 34.0 |
| 0.04 | 0--0 | 5.6 | 7.0 | 7.0 | 20.1 | 26.0 | 26.0 | 35.0 |

Nominal Conc. mg/L — Mean Cumulative Numbers of Juveniles Produced per Adult (days)

| | 15 | 16 | 17 | 18 | 19 | 20 | 21 |
|---|---|---|---|---|---|---|---|
| Control | 53.6 | 53.6 | 53.6 | 73.8 | 76.7 | 76.7 | 86.8 |
| Solvent | 61.6 | 61.6 | 61.4 | 85.7 | 85.7 | 85.7 | 96.8 |
| Control | | | | | | | |
| 0.002 | 52.7 | 52.7 | 55.7 | 77.5 | 77.5 | 77.5 | 94.5 |
| 0.0042 | 54.4 | 54.4 | 57.0 | 80.5 | 80.6 | 80.6 | 95.5 |
| 0.009 | 53.9 | 53.9 | 57.0 | 78.3 | 78.3 | 78.3 | 95.6 |
| 0.019 | 53.2 | 53.2 | 53.2 | 79.9 | 79.9 | 79.9 | 96.1 |
| 0.04 | 48.6 | 48.6 | 53.9 | 75.5 | 75.5 | 75.5 | 96.0 |

-Cumulative numbers of juveniles produced per adult alive for 21days in each test vessels and results of statistical comparison of the mean values (by Dunnett's Multicomparison Test)

| Vessel No. | Control | Solv. Cont. | Nominal Conc., mg/L 0.002 | 0.0042 | 0.009 | 0.019 | 0.04 |
|---|---|---|---|---|---|---|---|
| 1 | M | 82 | 82 | 77 | 110 | 64 | 98 |
| 2 | 77 | 109 | 116 | 109 | 103 | 109 | 100 |
| 3 | 96 | 104 | 104 | 81 | 80 | 104 | 100 |
| 4 | 71 | 78 | 114 | 102 | 85 | 82 | 91 |
| 5 | 108 | 106 | 91 | 95 | 94 | 101 | 89 |
| 6 | 104 | 103 | 76 | 109 | 85 | D | 96 |
| 7 | 73 | 110 | 98 | 93 | 104 | 93 | 106 |
| 8 | 72 | 97 | 53 | 110 | 107 | 106 | 106 |
| 9 | 78 | 82 | 117 | 100 | 87 | 104 | 102 |
| 10 | 102 | D | 94 | 79 | 101 | 102 | 85 |
| Mean | 86.8 | 96.8 | 94.5 | 95.5 | 95.6 | 96.1 | 96.0 |
| S. D. | 15.4 | 12.7 | 20.2 | 12.8 | 10.7 | 14.5 | 8.0 |
| Inhibition rate(%) | | 2.4 | 1.3 | 1.2 | 0.7 | 0.8 | |
| Significant difference | | | | | | | |

M: Was treated as a missing value because the adult was pushed by pipett and damaged when transferring after 11-day exposure.
D: Were not included for calculation because parental Daphnia was dead during 21-day testing period.
- Calculation of toxicity values: The calculation of toxicity values was the measured concentrations.

Reference: Ministry of Environment, Japan (2002)
Reliability: (1) valid without restrictions
This test was conducted in 2002 in accordance with Guidance Document 23. The test chemical has a very low water solubility and is unstable in the test condition due to hydrolysis.
Flag: Critical study for SIDS endpoint

**4.6 TOXICITY TO TERRESTRIAL ORGANISMS**

**4.6.1 TOXICITY TO SOIL DWELLING ORGANISMS**

No data available

**4.6.2 TOXICITY TO TERRESTRIAL PLANTS**

No data available

**4.6.3 TOXICITY TO OTHER NON MAMMALIAN TERRESTRIAL SPECIES (INCLUDING AVIAN)**

No data available

**4.7 BIOLOGICAL EFFECTS MONITORING (INCLUDING BIOMAGNIFICATION)**

No studies located

**4.8 BIOTRANSFORMATION AND KINETICS IN ENVIRONMENTAL SPECIES**

No data available

**4.9 ADDITIONAL REMARKS** None

## 5.1 ACUTE TOXICITY

### 5.1.1 ACUTE ORAL TOXICITY

(a)
Type: $LD_0$ **[X]**; $LD_{100}$ **[ ]**; $LD_{50}$ **[ ]**; $LDL_0$ **[ ]**; Other **[ ]**
Species/strain: Rat / (SD)
Sex: Male & Female
Number of animals: 5 per sex per dose group
Vehicle: Sesame oil
Doses: 0, 1077, 1401, 1821, 2367, 3077, 4000 mg/kg/day (suspended in sesame oil)
Value: 1,821 mg/kg for males, 1,077 mg/kg for females
Method: OECD Test Guideline 401
Year: 1996
GLP: Yes **[X]**; No **[ ]**; ? **[ ]**
Test substance: Ouchi Shinko Chemistry, Lot No. 307021, Purity: 99.2 %, Kept at 4°C until use
Remarks: Test type: Single Dose Toxicity Test
Route of administration: Oral (by gavage)
Duration of test: 15 days
Doses per time period: Single dose
Control group and treatment: Concurrent vehicle
Post exposure observation period: 14 days
Test Subjects
Age at study initiation: 5 weeks old in both sexes
Study Design
Volume administration or concentration: 10 mL/kg bw
Satellite groups and reasons they were added: None
Clinical observations performed and frequency: General condition was observed frequently on day of treatment, and at least once after the day. Body weights were determined on days 1, 3, 7 and 14 after treatment.
Necropsy: All dead animals and all surviving animals killed under ether anesthesia at the termination of the observation period were subjected to necropsy.
Result: Fatalities occurred in males at doses of 2367 mg/kg and more and in females at doses of 1401 mg/kg, 2367 mg/kg and more. However no dose-related increase in mortalities were observed.
Number of deaths at each dose level:

| Dose (mg/kg): | 0 | 1077 | 1401 | 1821 | 2367 | 3077 | 4000 |
|---|---|---|---|---|---|---|---|
| Number of animals/sex: | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Male: | 0 | 0 | 0 | 0 | 2 | 2 | 1 |
| Female: | 0 | 0 | 1 | 0 | 4 | 1 | 4 |

Time of death: Dead animals were observed at day 1 after dosing for males, from day 1 to 3 after dosing for females.
Clinical signs (description, severity, time of onset and duration): Tremor and convulsion, as well as decreased locomotor activity, deep respiration, piloerection, chromodacryorrhea and soiling in the perigenital region with urine were approximately dose-dependently observed for one to seven days after dosing in all treated groups of both sexes.

Body weight: Body weights of the treated groups were lower than those Gross pathology incidence and severity: No macroscopic abnormalities that could be attributed to treatment with the test substance were seen.
Reliability: Valid without restriction
Reference: MHW, Japan: 1996

(b)
Type: $LD_0$ **[ ]**; $LD_{100}$ **[ ]**; $LD_{50}$ **[X]**; $LDL_0$ **[ ]**; Other **[ ]**
Species/strain: Rat
Value: > 5,000 mg/kg
Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Remarks: None
Reference: Monsanto Study BD-84-217

(c)
Type: $LD_0$ **[ ]**; $LD_{100}$ **[ ]**; $LD_{50}$ **[X]**; $LDL_0$ **[ ]**; Other **[ ]**
Species/strain: Rat
Value: 10,000 mg/kg
Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Remarks: None
Reference: de Groot: 1975

(d)
Type: $LD_0$ **[ ]**; $LD_{100}$ **[ ]**; $LD_{50}$ **[X]**; $LDL_0$ **[ ]**; Other **[ ]**
Species/strain: Rat
Value: 6,420 mg/kg
Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Remarks: None
Reference: Marhold: 1986

(e)
Type: $LD_0$ **[ ]**; $LD_{100}$ **[ ]**; $LD_{50}$ **[X]**; $LDL_0$ **[ ]**; Other **[ ]**
Species/strain: Rat
Value: 8,500 mg/kg
Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Remarks: None
Reference: Vorobeva: 1968

**5.1.2 ACUTE INHALATION TOXICITY**

No data available

**5.1.2 ACUTE DERMAL TOXICITY**

(a)
Type: $LD_0$ **[ ]**; $LD_{100}$ **[ ]**; $LD_{50}$ **[X]**; $LDL_0$ **[ ]**; Other **[ ]**
Species/strain: Rabbit
Value: > 2,000 mg/kg

Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Remarks: None
Reference: Monsanto Study BD-84-217

**5.1.3 ACUTE TOXICITY, OTHER ROUTES OF ADMINISTRATION**

(a)
Type: $LD_0$ **[ ]**; $LD_{100}$ **[ ]**; $LD_{50}$ **[X]**; $LDL_0$ **[ ]**; Other **[ ]**
Species/strain: Rat
Route of Administration: i.m. **[ ]**; i.p. **[ ]**; i.v. **[ ]**; infusion **[ ]**;
Exposure time:
Value: > 5,000 mg/kg
Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Remarks: N,N-Dicyclohexyl-2-benzothiazolesulfenamide (DCBS) of technical and analytical grade was tested
Reference: Bayer AG: 1975

**5.2 CORROSIVENESS/IRRITATION**

**5.2.1 SKIN IRRITATION/CORROSION**

(a)
Species/strain: Rabbit
Results: Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**;Irritating **[ ]**; Moderate irritating **[X]**; Slightly irritating **[ ]**; Not irritating **[ ]**
Classification: Highly corrosive (causes severe burns) **[ ]**; Corrosive (causes burns) **[ ]**; Irritating **[ ]**; Not irritating **[ ]**
Method: Standard Draize Test
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Remarks: Exposure: 20 mg/24h
Reference: Marhold: 1986

(b)
Species/strain: Rabbit
Results: Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[ ]**; Not irritating **[X]**
Classification: Highly corrosive (causes severe burns) **[ ]**; Corrosive (causes burns) **[ ]**; Irritating **[ ]**; Not irritating **[X]**
Classification: Unknown
Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Remarks: Exposure time : 24h
Reference: Monsanto Study BD-84-217

**5.2.2 EYE IRRITATION/CORROSION**

(a)
Species/strain: Rabbit

Results: Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[ ]**; Not irritating **[ ]*** Mild (IUCLID)
Classification: Irritating **[ ]**; Not irritating **[ ]**; Risk of serious damage to eyes **[ ]**
Method: Standard Draize test
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Remarks: Exposure: 500 mg/24h
Reference: Marhold: 1986

(b)
Species/strain: Rabbit
Results: Highly corrosive **[ ]**; Corrosive **[ ]**; Highly irritating **[ ]**; Irritating **[ ]**; Moderate irritating **[ ]**; Slightly irritating **[ ]**; Not irritating **[X]**
Classification: Irritating **[ ]**; Not irritating **[ ]**; Risk of serious damage to eyes **[ ]**
Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Remarks: Exposure time:24h
Reference: Monsanto Study BD-84-217

**5.3 SKIN SENSITISATION**

Type: Guinea pig maximization test
Species/strain: Guinea pig
Results: Sensitizing **[ ]**; Not sensitizing **[X]**; Ambiguous **[ ]**
Classification: Sensitizing **[ ]**; Not sensitizing **[ ]**
Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Santocure DCBS; purity: Unknown
Remarks:
Reference: Monsanto Study IR-84-230

**5.4 REPEATED DOSE TOXICITY (SIDS data)**

(a)
Type
Species/strain: Rat (SD)
Sex: Female**[ ]**; Male**[ ]**; Male/Female**[X]**; No data**[ ]**
Route of administration: Oral (by gavage)
Exposure period: Males; 44 days, Females; from 14 days before mating to day 3 of lactation
Frequency of treatment: Daily
Post exposure observation period: None
Doses: 6, 25, 100, 400 mg/kg/day (in sesame oil)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]** Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
NOAEL: Male: 25mg/kg/day; Female: 25mg/kg/day
LOAEL: Male: 100mg/kg/day; Female: 100mg/kg/day
Results: Body weight: Low body weight gain during the treatment period in males and at day 20 of pregnancy in females at 400 mg/kg (Dunnets test $p < 0.01$)

Food/water consumption: Low food consumption at day 1 of treatment in males (p<0.01), during premating period (p<0.05), and at days 0 and 20 of pregnancy (p<0.01) in females at 400 mg/kg. Water consumption was not determined.
Clinical signs (description, severity, time of onset and duration):In males, salivation after day 39 of treatment in two males at 400 mg/kg was detected. In females, decreased locomotor activity in one at 100 mg/kg and five at 400 mg/kg, soiling in the perigenital region with urine in one at 100mg/kg and in six at 400 mg/kg, and chromodacryorrhea in five at 400 mg/kg were detected during day 5 to 16 of treatment and during last stage of pregnancy to lactation period.
Urinalysis: Increase in urinary ketone body in males at 400 mg/kg.
Haematology:
Males: No stat. sig. Difference from controls.
Males: Increases of inorganic phosphorus and decreased Cl at 400 mg/kg (p<0.01).

| **Dose level (mg/kg/day)** | | | | |
|---|---|---|---|---|
| **0** | **6** | **25** | **100** | **400** |
| No. of animals | | | | |
| 10 | 10 | 10 | 10 | 10 |
| **Inorganic phosphorus** | | | | |
| (mg/dL, Mean,±SD) | | | | |
| 6.7 ± 0.4 | 6.3 ± 0.7 | 6.5 ± 0.6 | 6.4 ± 0.7 | 7.6 ± 0.5 |
| **Cl** (mEq/L, Mean ± SD) | | | | |
| 103 ± 1 | 103 ± 1 | 103 ± 1 | 102 ± 1 | 101 ± 1 |

Ophthalmologic findings: Not examined
Mortality and time to death: 30 % in females at 400 mg/kg (three out of ten animals died on the expected delivery day or on the following day)
Gross pathology incidence and severity: Cecum dilatation (male: 5/10, female: 1/10) and thymus atrophy (male: 4/10, female: 7/10), kidney swelling with pale in colour (female: 3/10), adrenal enlargement with pale in colour (female: 7/10) and spleen atrophy (female: 5/10) at 400 mg/kg.

Organ weight changes:
Male: Decreases in final body weight and thymus weight at 400 mg/kg (absolute) (p<0.01),increase in kidney and testes weight at 400 mg/kg (relative) (p<0.01)

| **Dose level (mg/kg/day)** | | | | |
|---|---|---|---|---|
| **0** | **6** | **25** | **100** | **400** |
| No. of animals | | | | |
| 10 | 10 | 10 | 10 | 10 |
| Final body weight | | | | |
| (g, Mean ± SD) | | | | |
| 467 ± 30 | 469 ± 33 | 478±17 | 476 ± 27 | 411 ± 18 |
| Absolute weight | | | | |
| thymus(g, Mean±SD) | | | | |
| 0.43 ± 0.07 | 0.37±0.08 | 0.36±0.08 | 0.40±0.10 | 0.31±0.09 |
| Relative weight | | | | |
| kidneys(g%, Mean±SD) | | | | |
| 0.66 ± 0.06 | 0.64±0.04 | 0.65±0.04 | 66±0.04 | 0.78±0.04 |
| testes(g%, Mean±SD) | | | | |

0.71±0.07 0.70±0.06 0.69±0.06 0.68±0.05 0.80±0.05

Female: Decrease in thymus weight at 400 mg/kg (absolute)

| **Dose level (mg/kg/day)** | | | | |
|---|---|---|---|---|
| **0** | **6** | **25** | **100** | **400** |
| No. of animals | | | | |
| 10 | 10 | 10 | 8 | 5 |
| Absolute weight thymus(g, Mean±SD) | | | | |
| 0.22±0.07 | 0.21±0.07 | 0.21±0.08 | 0.19±0.04 | .10±0.04 |

Histopathology (incidence and severity):
Male:
Kidney: Increase of hyaline droplets in the proximal tubular epithelium (100 and 400mg/kg):
Thymus: Increase of atrophy (400mg/kg):

| **Dose level (mg/kg/day) degree*** | | | | |
|---|---|---|---|---|
| **0** | **6** | **25** | **100** | **400** |
| No. of animals | | | | |
| 10 | 10 | 10 | 10 | 10 |
| Kidneys:Hyaline droplet, proximal **+~++** | | | | |
| 0 | 0 | 0 | 4 | 8 |
| tubular epithelium | | | | |
| Thymus: Atrophy + | | | | |
| 0 | 0 | 0 | 0 | 4 |

*degree: + slight, ++ moderate

Female:
Kidney: Increase of fatty degeneration of proximal tubular epithelium (100 and 400 mg/kg):
Adrenal: Increase of cortical cell vacuolization (400mg/kg):
Thymus: Increase of atrophy (400 mg/kg):
Spleen: Increase of atrophy (400 mg/kg):

| **Dose level (mg/kg/day) degree*** | **0** | **6** | **25** | **100** | **400** |
|---|---|---|---|---|---|
| No. of animals | 10 | 10 | 10 | 10 | 10 |
| Kidney: Fatty degeneration of proximal tubular epithelium | | | | | |
| +~++ | 0 | 0 | 0 | 3 | 4 |
| Adrenal:Cortical cell vacuolization | | | | | |
| +~++ | 0 | 0 | 0 | | 9 |
| Thymus: Atrophy +~+++ | 2 | 2 | 3 | 3 | 7 |
| Spleen: Atrophy +~+++ | 0 | 0 | 0 | 1 | 5 |

*degree: + slight, ++ moderate, +++marked

Remarks: Duration of test: 51 days
Test Subjects
Age at study initiation: 9 weeks old in males, 8 weeks old in females
Weight at study initiation: About 340 g for males, about 210 g for females
No. of animals per sex per dose: 10 per sex per dose group
Study Design
Vehicle: Sesame oil
Satellite groups and reasons they were added: None
Clinical observations performed and frequency: General condition was observed twice a day, body wt. and food consumption were determined once a week. For males only,

urinalysis was carried out at 42 days of chemical exposure, and haematology and biochemistry at time of necropsy after 44 days.
Organs examined at necropsy organ weight: Liver, kidney, thymus, testes, epididymis microscopic: Brain, heart, liver, kidney, adrenal, spleen, cecum, testis, ovary and mammary gland in all animals in control and 400 mg/kg, pituitary, epididymis, seminal vesicle, prostate, uterus and vagina in addition to those organs in six animals(one couple in 100mg/kg and two couples in 400mg/kg) failed to cause pregnancy, and in 6, 25 and 100mg/kg, kidney and thymus of both sex, and liver, spleen and adrenal of males which have histopathological changes at the higher doses.

Statistical methods: Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data

Method: OECD Combined Repeat Dose and reproductive/Developmental Toxicity Screening Test (OECD TG 422)
Year: 1996
GLP: Yes **[X]**; No **[ ]**; ? **[ ]**
Test substance: Ouchi Shinko Chemistry, Lot No. 307021, Purity: 99.2 %, Kept at 4°C until use

Reliabilities: Valid without restriction
Reference: MHW, Japan: 1996

(b)
Species/strain: Rats (SD)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Oral feed
Exposure period: 4 weeks
Frequency of treatment: Daily
Post exposure observation period: No data
Dose: 2000, 3000, 5000, 7500, 10000ppm (ca. 133, 200, 333, 500, 667mg/kg /day)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOAEL: Not established
LOAEL: Not established
Results: No significant changes related to treatment were found in hematology clinical chemistry evaluations, terminal organ/body weights, or organ/body weight ratios or gross necropsy examinations. Dose-related depression in body weight gain and reduced feed consumption was noted in all treatment groups in comparison to controls. There were no histopathological data.
Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Santocure DCBS, purity: unknown
Reference: Monsanto Study BD-87-327

(c)
Species/strain: Unspecified, probably rat
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Oral feed
Exposure period: 3 months
Frequency of treatment: Daily

Post exposure observation period: No data
Dose: 2500, 5000ppm (ca. 167, 333 mg/kg bw/day)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**
Concurrent no treatment **[X]**; Concurrent vehicle **[ ]**;
Historical **[ ]**
NOAEL: Not established
LOAEL: Not established
Results: Reduced body weight gain and reduced food consumption in both sexes in both treatment groups were observed; no target organ toxicity or histopathological findings were suggested.
Method: Unknown
GLP: Yes **[ ]**; No **[X]**; ? **[ ]**
Test substance: Santocure DCBS, purity: unknown
Remarks:
Reference: Monsanto Study ML-88-180

(d)
Species/strain: Rats
Sex: Female **[ ]**; Male **[X]**; Male/Female **[ ]**; No data **[ ]**
Route of Administration: Inhalation
Exposure period: 15 days
Frequency of treatment: Daily, 2 hr/day
Post exposure observation period: No data
Dose: 350 - 400 $mg/m^3$
Control group: Yes **[ ]**; No **[ ]**; No data **[X]**; no other data
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**;
Historical **[ ]**
NOAEL: Not established
LOAEL: 350 $mg/m^3$
Results: No effects except mucous membrane irritation were observed. No pronounced liver or kidney changes were observed.
Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Reference: Vorobeva: 1968

## 5.5 GENETIC TOXICITY IN VITRO

### A. BACTERIAL TEST

(a)
Type: Reverse mutation assay
System of testing: *S. typhimurium* TA100, TA1535, TA98, TA1537
*E. coli* WP2 *uvrA*
Concentration: -S9: 0, 312.5, 625, 1250, 2500, 5000 µg /plate
+S9: 0, 312.5, 625, 1250, 2500, 5000 µg /plate
Metabolic activation: With **[ ]**; Without **[ ]**;
With and Without **[X]**; No data **[ ]**
S9: Rat liver, induced with phenobarbital and 5,6-benzoflavone
Plate/test: 3
Number of replicates: 2
Results: Cytotoxicity conc.:With metabolic activation: Not observed
Without metabolic activation: Not observed
Precipitation conc.:312.5 µg/plate

Genotoxic effects: + ? -
With metabolic activation: **[ ] [ ] [X]**
Without metabolic activation: **[ ] [ ] [X]**
Methods: Guidelines for Screening Toxicity Testings of Chemicals, Reverse-mutation assay in bacteria, Japan, and OECD TG 471 and 472
Year: 1996
GLP: Yes **[X]**; No **[ ]**; ? **[ ]**
Test substance Ouchi Shinko Chemistry, Lot No. 304006, Purity: more than 99.5%, Kept at 4 °C until use
Remarks: Study Design:
Procedure: Pre-incubation
Solvent: DMSO
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)
Precipitation conc: 312.5 µg/plate
Reliabilities: Valid without restriction
Reference: MHW, Japan: 1996

(b)
Type: Ames test
System of testing: *S. typhimurium* TA98, TA100
Concentration: Unknown
Metabolic activation:
With **[ ]**; Without **[ ]**; With and Without **[X]**;
No data **[ ]**
S9: Unknown
Results: negative
Cytotoxicity conc.: Unknown
Precipitation conc.:Unknown
Genotoxic effects: + ? -
With metabolic activation: **[ ] [ ] [X]**
Without metabolic activation: **[ ] [ ] [X]**
Method: Unknown
GLP: Yes **[ ]** No **[ ]** ? **[X]**
Test substance: Purity: Unknown
Remarks:
Reference: You et al.: 1982

(c)
Type: Ames test
System of testing: *S. typhimurium* (no further data)
Concentration: Unknown
Metabolic activation:
With **[ ]**; Without **[ ]**; With and Without **[X]**;
No data **[ ]**
S9: Unknown
Results: Negative
Cytotoxicity conc : Unknown
Precipitation conc.: Unknown
Genotoxic effects: + ? -
With metabolic activation: **[ ] [ ] [X]**
Without metabolic activation: **[ ] [ ] [X]**
Method: Unknown
GLP: Yes **[ ]**; No **[ ]**; ? **[X]**
Test substance: Purity: Unknown
Remarks:
Reference: Monsanto Study HL-84-292

**B. NON-BACTERIAL IN VITRO TEST**

(a)
Type: Chromosomal aberration test
System of testing: Chinese hamster lung (CHL/IU) cell
Concentration: -S9 (continuous treatment): 0, 0.21, 0.41, 0.82 mg/ml
-S9 (short-term treatment): 0, 0.9, 1.8, 3.5 mg/ml
+S9 (short-term treatment): 0, 0.9, 1.8, 3.5 mg/ml
Metabolic activation:
With **[ ]**; Without **[ ]**; With and Without **[X]**;
No data **[ ]**
S9: Rat liver, induced with Phenobarbital and 5,6-benzoflavone
Plates/test: 2
Results: Cytotoxicity conc.: With metabolic activation: Not observed
Without metabolic activation: 0.41 mg/ml
Precipitation conc.: Not observed
Genotoxic effects: clastogenicity polyploidy

| | + | ? | - | + | ? | - |
|---|---|---|---|---|---|---|
| With metabolic activation: | **[ ]** | **[ ]** | **[X]** | **[ ]** | **[X]** | **[.]** |
| Without metabolic activation: | **[ ]** | **[ ]** | **[X]** | **[ ]** | **[X]** | **[ ]** |

Structural chromosomal aberrations were not induced in CHL/IU cells up to the concentration giving 50% cell growth inhibition or the limit concentration of mM, with and without metabolic activation. While, polyploid cells were induced by continuous treatment for 24- and 48-hr. The maximum incidence was 6 % of the concentration of 0.41mg/mL after 48-hr. The cytogenetic effects of the test substance were suggested to be equivocally positive.

| Concentration (mg/ml) | 0(vehicle) | 0.21 | 0.41 | 0.82 |
|---|---|---|---|---|
| Time of exposure (hr) | 24 | 24 | 24 | 24 |
| Polyploid (%) | 0.38 | 1.25* | 1.13 | 1.96* |
| Time of exposure (hr) | 48 | 48 | 48 | 48 |
| Polyploid (%) | 0.13 | 3.38* | 6.00* | 1.64* |

*: Significantly different from vehicle at $p<0.05$.
Method: Guidelines for Screening Toxicity Testings of chemicals, Chromosomal test in cultured mammalian cells, Japan and OECD TG 473

Year: 1996
GLP: Yes **[X]**; No **[ ]**; ? **[ ]**
Test substance: Ouchi Shinko Chemistry, Lot No. 304006,Purity: more than 99.5 %, Kept at 4 °C until use
Remarks: Study Design:
For continuous treatment, cells were treated for 24 or 48 hr without S9. For short-term treatment, cells were treated for 6 hrs with and without S9 and cultivated with fresh media for 18 hrs.
Maximum concentration for continuous treatment was giving 50% growth inhibition of the cells.
Maximum concentration for short-term treatment was the limit
Solvent: 0.5% CMCNa sol.
Positive controls:
Mitomycin C for continuous treatment
Cyclophosphamide for short-term treatment

Statistical methods: Fisher's exact analysis
Reliabilities: Valid without restriction
Reference MHW, Japan: 1996

(b)
Type: *In vitro* micronucleus test
System of testing: Chinese hamster lung (CHL/IU) cell
Concentration: -S9 (continuous treatment): 0, 0.21, 0.41, 0.82 mg/ml

Metabolic activation: With **[ ]**; Without **[X]**;
With and Without **[ ]**; No data **[ ]**
S9:
Plates/test: Unknown
Results: Cytotoxicity conc.: Without metabolic activation: more than 0.21 mg/ml
Precipitation conc.: Not observed
Genotoxic effects: At the concentration of 0.21mg/mL or more after 48-hr continuous treatment without S9 mix, number of micronucleus cells were significantly increased more than control (P<0.05).

| Without metabolic activation: | + **[X]** | ? **[ ]** | - **[ ]** |
|---|---|---|---|
| Concentration (mg/kg) 0(vehicle) | 0.21 | 0.41 | 0.82 |
| Time of exposure (hr) 48 | 48 | 48 | 48 |
| No. of cells analysed 2000 | 2000 | 2000 | 2000 |
| No. of cells with micronuclei 5 | 13* | 17* | 24* |
| No. of cells with multi nuclei 13 | 53* | 51* | 147* |

*: Significantly different from vehicle at p<0.05

Method: Unknown
GLP: Yes **[X]**; No **[ ]**; ? **[ ]**
Test substance: Ouchi Shinko Chemistry, Lot No. 304006, Purity: More than 99.5%, Kept at 4 °C until use
Remarks: Study Design:
Cells were treated for 48 hrs without S9.
Concentration: 0, 0.21, 0.41, 0.82 mg/ml
Plates/test: 2
Solvent: 0.5% CMCNa sol.
Positive controls: Mitomycin C
Micronuclei and multi nuclei were induced in CHL/IU cells by continuous treatment for 48-hr at concentrations of 0.21mg/ml or more. Judgement was done by statistical method of Kastenbaum and Bowman (*Mutation Res.*, 9, 527-549(1970))
Year: 1996
Reliabilities: Valid with restriction because of no information on Test Guideline and cytotoxicity.
Reference: MHW, Japan: 1996

(c)
Type: HGRPT Assay
System of testing: Chinese hamster ovary cells
Concentration: Up to 500 µg/ml
Metabolic activation:
With **[ ]**; Without **[ ]**; With and Without **[X]**;
No data **[ ]**
S9: Unknown

Result: Negative

Cytotoxicity conc.: Unknown
Precipitation conc.: Unknown

| Genotoxic effects: | + | ? | - |
|---|---|---|---|
| With metabolic activation: | **[ ]** | **[ ]** | **[X]** |
| Without metabolic activation: | **[ ]** | **[ ]** | **[X]** |

Method: Unknown
Test substance: Purity: unknown
Reference: Monsanto Study PK-84-231

(d)
Type: Unscheduled DNA synthesis
System of testing: Primary rat hepatocytes
Concentration: Up to and including 50 □g/ml
Metabolic activation:
With **[ ]**; Without **[ ]**; With and Without **[ ]**;
No data **[X]**
S9: Unknown
Results: Negative
Cytotoxicity conc.: Unknown
Precipitation conc.:Unknown

| Genotoxic effects: | + | ? | - |
|---|---|---|---|
| With metabolic activation: | **[ ]** | **[ ]** | **[X]** |
| Without metabolic activation: | **[ ]** | **[ ]** | **[X]** |

Method: Unknown
Test substance: Santocure DCBS; purity: Unknown
Reference: Monsanto Study SR-84-291

## 5.6 GENETIC TOXICITY IN VIVO

(a)
Type: Chromosomal aberration
Species/strain: Rat (SD) Sex:
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Oral (gavage)
Exposure period: Single administration
Doses: 1000 mg/kg
Results: Effects on mitotic index or PCE/NCE ratio by dose level by sex: None

| Genotoxic effects: | + | ? | - |
|---|---|---|---|
| | **[ ]** | **[ ]** | **[X]** |

NOAEL(C)/LOAEL(C): None
Statistical results: Chromosomal aberration: Results from the 48-hr sacrifice showed a statistically significant increase in percent aberrant cells (p=0.015) and mean aberrations per cell (p=0.015) when analysed using non-parametric analysis. Results of the ANOVA analysis showed a significant increase in percent aberrant cells (p=0.0189) when compared with concurrent controls, but no significant increase when compared to historical controls. Results of RIDIT analysis showed no significant increase when compared to concurrent (p=0.06067) or historical (p=0.06395) controls.

Aberration data of 48-hr sacrifice

| Treatment | Vehicle | 1,000mg/kg |
|---|---|---|
| No. of animals | 10 | 10 |
| No. of metaphases analysed | 488 | 500 |
| No. of aberrant cells | 0 | 5 |
| Percent aberrant cells | 0.00 | 1.00 |
| Total number of aberrations | 0 | 8 |
| Mean number of aberrations per cell | 0.000 | 0.016 |

Polyploidy: No statistically significant differences between the mean chromosome numbers of the test groupand the vehicle control were seen. Rats had a normal diploid chromosomal number of 42.
Clinical observation and Mortality: Abnormal clinical observations such as depression, red stains on nose/eyes, soft feces, slight depression, urine stains, and rough coat in both sexes at A significant decrease in mean body weights was seen for 1,000mg/kg group, 48-hr males ($p<0.0001$) and 48-hr females($p=0.0217$).
This chemical did not induce structural chromosomal aberrations and polyploidy in *in vivo* bone marrow chromosome test.
Method: Unknown

GLP: Yes **[X]**; No **[ ]**; ? **[ ]**
Test substance: Santocure DCBS, Purity: 96 % (adjusted to 100% for dosing purposes)
Remarks: Age at study initiation: approximately 58-63 days old
No. of animals per dose: 15/dose/sex
Vehicle: Corn oil
Duration of test: 2 days
Frequency of treatment: Once
Sampling times and number of samples: 6, 24 and 48 hrs after administration and 5 samples/dose/sex
Control groups: Vehicle
Clinical observation performed: Mortality, observation of general appearance, behaviour, toxic and pharmacological effects, and body weight.
Organs examined at necropsy: None
Criteria for evaluating results: Numbers and types of chromosomal aberrations, mitotic index, and chromosome number for each metaphase
Criteria for selection for M.T.D.: Frequency of chromosomal aberration
Statistical methods: The mean mitotic indices, mean chromosome numbers, percent aberrant cells and the mean number of aberrations per cell for each group were statistically compared using the kruskal-wallis nonparametric analysis of variance and nonparametric pairwise group comparisons (KW-ANOVA). The results for the 48-hour sacrifice were also analysed by Analysis of Variance with Dunnett's test and RIDIT analysis (Relative to and Identified Distribution) using both concurrent and historical controls. Body weight data was analysed by analysis of covariance (ANCOVA). All tests were one-tailed at the 95% confidence interval ($p<0.05$).
Year: 1985
Reliabilities: Valid without restriction
Reference: Monsanto Study HL-84-293

**5.7 CARCINOGENICITY**

Species/strain: Rats (Wistar)
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[X]**; No data **[ ]**
Route of Administration: Injection
Exposure period: 413 days
Frequency of treatment: Once a week
Post exposure observation period: Entire lifetime
Dose: 1,000 mg/kg bw, 20,000 mg/kg bw (total amount)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**;

Historical **[ ]**; Other **[X]**
Results: No signs of systemic toxicity were reported. There was no difference between the survival of the control group and the dose group. An increased number of sarcomas located at the injection site was observed in all dose groups.
Method: Other
GLP: Yes **[ ]**; No **[X]**; ? **[ ]**
Test substance: Purity: unknown
Remarks: 20 animals of each sex were administered.
Reference: Bayer AG: 1975

**5.8 TOXICITY TO REPRODUCTION**

Type: Fertility **[ ]**; One-generation study **[ ]**; Two-generation study **[ ]**;Other **[X]**
Species/strain: Rat (SD)
Sex: Female **[ ]**; Male**[ ]**; Male/Female **[X]**; No data **[ ]**
Route of administration: Oral (by gavage)
Exposure period: Males; 44 days
Females; from 14 days before mating to day 3 of lactation

Frequency of treatment: Daily
Duration of test: Male: for 44 days, female: for 40-51 days
Post exposure observation period: None
Doses: 6, 25, 100, 400 mg/kg/day (in sesame oil)
Control group: Yes **[X]**; No **[ ]**; No data **[ ]**
Concurrent no treatment **[ ]**; Concurrent vehicle **[X]**; Historical **[ ]**
Premating exposure period: Male: 14 days, Female: 14 days
NOAEL: 100 mg/kg for parents and F1
Results: NOAEL and LOAEL parental toxicity: NOAEL: 100mg/kg/day
LOAEL: 400mg/kg/day
NOAEL and LOAEL foetal toxicity: NOAEL: 100 mg/kg/day
LOAEL: 400mg/kg/day
Actual dose received by dose level by sex if available:
0, 6, 25, 100, 400 mg/kg/day for both sexes
Parental data with dose level (with NOAEL value): At 400 mg/kg, there were decreases in reproductive parameters (numbers of corpora lutea ($p<0.05$), implantation sites ($p<0.01$), pregnants with parturition ($p<0.05$) and pregnants with live pups on days 0 ($p<0.05$) or 4 ($p<0.01$).

| **Dose level (mg/kg/day)** | | | | |
|---|---|---|---|---|
| **0** | **6** | **25** | **100** | **400** |
| No. of animals | | | | |
| 10 | 10 | 10 | 10 | 10 |
| No. of corpora lutea (Mean±SD) | | | | |
| 19.7 ± 2.0 | 18.0 ± 2.3 | 18.5 ± 2.4 | 17.6 ± 1.3 | 16.3 ± 1.2 |
| No. of implantation sites (Mean±SD) | | | | |
| 18.8 ± 1.4 | 16.9 ± 2.3 | 17.4 ± 1.8 | 17.4 ± 1.4 | 15.3 ± 1.7 |
| No. of pregnants with parturion | | | | |
| 10 | 10 | 10 | 9 | 5 |
| No. of pregnants with living pups on day 0 | | | | |
| 10 | 10 | 10 | 9 | 3 |
| No. of pregnants with living pups on day 4 | | | | |
| 10 | 10 | 10 | 8 | 0 |

Foetal data with dose level (with NOAEL value): At 400 mg/kg, there were decreases in developmental parameters (total number of pups born ($p<0.01$), number of pups alive on day 0 ($p<0.01$) or 4 ($p<0.05$) of lactation and live birth index ($p<0.05$)).

**Dose level (mg/kg/day)**

| 0 | 6 | 25 | 100 | 400 |
|---|---|---|---|---|
| No. of pups born(Mean±SD) | | | | |
| 17.0 ± 2.9 | 15.8 ± 1.9 | 15.5 ± 1.7 | 16.1 ± 1.9 | 12.0 ± 3.1 |
| No. of pups alive on day 0 of lactation (Mean±SD) | | | | |
| 16.8 ± 2.9 | 15.4 ± 1.6 | 14.5 ± 2.0 | 15.4 ± 1.9 | 4.0 ± 3.8 |
| No. of pups alive on day 4 of lactation (Mean±SD) | | | | |
| 16.3 ± 2.9 | 15.3 ± 1.6 | 13.8 ± 3.8 | 13.9 ± 5.5 | 0 |
| Live birth index(%) | | | | |
| 98.8 ± 2.6 | 97.7 ± 4.0 | 94.0 ± 11.7 | 95.9 ± 4.3 | 31.4 ± 29 |

Note: Live birth index(%) = (No. of live pups born on day 0/No. of pups born)×100

Statistical results, as appropriate: All of the above changes were statistically significant as mentioned.
Mortality and day of death: At 400 mg/kg, three females died on day 22 or 23 of pregnancy.
Body weight: Low body weight gain during the treatment period in males and at day 20 of pregnancy in females at 400 mg/kg ($p<0.01$).
Food/water consumption: Low food consumption at day 1 of treatment in males ($p<0.01$), during premating period ($p<0.05$), and at days 0 and 20 of pregnancy($p<0.01$) in females at 400mg/kg.
Foetal data: All litters loss due to death after birth at 400mg/kg.
Grossly visible abnormalities, external, soft tissue and skeletal abnormalities: No statistically significant effects

Remarks: Vehicle: Sesame oil
Age at study initiation was 9 weeks old for males and 8 weeks old for females. The animals were sacrificed on the day 4 of lactation for females. Females with no delivery were killed 4 days after the delivery expected date.
Weight at study initiation: About 340 g for males, about 210 g for females
Number of animals per dose: 10 per sex per dose
Mating procedures: Male/female per cage; 1/1, length of cohabitation; 0 mg/kg, days 2.1 ± 1.0;6mg/kg, days 2.6 ± 1.4; 25mg/kg, days 2.7 ± 1.4; 100mg/kg, days 1.9 ± 1.0; 400mg/kg, days5.6 ± 3.9 (no stat, sig. difference from control) until proof of pregnancy (formation of vaginal closing or sperm detection in vagina)
Clinical observations performed and frequency:
Parent: General appearance twice a day
Foetus: General appearance once a day after birth
Urinalysis, and haematological and biochemical analysis: For males only, urinalysis was carried out at 42 days of treatment period, and haematology and biochemistry were carried out at time of necropsy after 44 days.
Parameters assessed during study: Body wt. (once a week), food/water consumption (once a week), No. of pairs with successful copulation, copulation index (No.

of pairs with successful copulation/No. of pairs mated x 100), pairing days until copulation, No. of pregnant females, fertility index = (No. of pregnant animals/No. of pairs with successful copulation x 100), No. of corpora lutea, No. of implantation sites, implantation index (No. of implantation sites/No. of corpora lutea x 100), No. of living pregnant females, No. of pregnant females with parturition, gestation length, No. of pregnant females with live pups on day 0, gestation index (No. of females with live pups/No. of living pregnant females x 100), No. of pregnant females with live pups on day 4, delivery index (No. of pups born/No. of implantation sites x 100), No. of pups alive on day 0 of lactation, live birth index (No. of live pups on day 0/No. of pups born x 100), sex ratio (Total No. of male pups/Total No. of female pups), No. of pups alive on day 4 of lactation, viability index (No. of live pups on day 4/No. of live pups on day 0 x 100), body wt. Of live pups (on day 0 and 4)
Organs examined at necropsy:
Parent: organ weight: Liver, kidney, thymus, testes, epididymis
Microscopic: Brain, heart, liver, kidney, adrenal, spleen, cecum, testis, ovary and mammary gland in all animals in control and 400 mg/kg, pituitary, epididymis, seminal vesicle, prostate, uterus and vagina in addition to those organs in six animals (one couple in 100mg/kg and two couples in 400mg/kg) failed to cause pregnancy, and in 6, 25 and 100mg/kg, kidney and thymus of both sex, and liver, spleen and adrenal of males which have histopathological changes at the higher doses.
Foetus: Full macroscopic examinations on all of pups
Statistical methods: Dunnett's or Scheffe's test for continuous data and Chi square test for quantal data

Method: OECD Combined Repeat Dose and Reproductive/Developmental Toxicity Screening Test (OECD TG 422)
Year: 1996
GLP: Yes **[X]**; No **[ ]**; ? **[ ]**
Test substance: Ouchi Shinko Chemistry, Lot No. 307021, Purity: 99.2%, Kept at 4 °C until use
Reliabilities: Valid without restriction

**5.9 DEVELOPMENTAL TOXICITY/ TERATOGENICITY**

Species/strain: Chicken
Sex: Female **[ ]**; Male **[ ]**; Male/Female **[ ]**; No data **[X]**
Duration of test: Unknown
Exposure period: Single injection
Frequency of treatment:
Dose: The highest dose tested was reported to be a saturated acetone solution corresponding to 0.5 umoles per egg (ca. 173 ug)
Control group: Yes **[ ]**; No **[ ]**; No data**[X]**
Concurrent no treatment **[ ]**; Concurrent vehicle **[ ]**; Historical **[ ]**
NOEL Maternal Toxicity: Unknown
NOEL Maternal Toxicity: Unknown
Results: The test substance did not produce any of evidence of embryotoxic or teratogenic effects.
Maternal general toxicity: unknown

| | |
|---|---|
| | Pregnancy/litter data: unknown |
| | Foetal data: unknown |
| Method: | Chicken embryo method |
| GLP: | Yes **[ ];** No **[ ];** ? **[X]** |
| Test substance: | Purity: unknown |
| Remark: | The test substance was injected into three day chicken embryos |
| Reference: | Korhonen et al.: 1982 |

**5.10 OTHER RELEVANT INFORMATION**

**A. Specific toxicities**

No available data.

**B. Toxicodynamics, toxicokinetics**

No available data.

**5.11 EXPERIENCE WITH HUMAN EXPOSURE**

No available data.

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