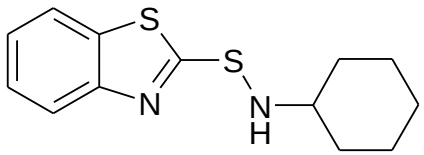
SIDS INITIAL ASSESSMENT PROFILE

CAS No.	95-33-0
Chemical Name	N-cyclohexylbenzothiazole-2-sulphenamide
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Analogue rationale

The main hydrolysis products of N-cyclohexylbenzothiazole-2-sulphenamide (CBS) are mercaptobenzothiazole (MBT), CAS-No. 149-30-4, and cyclohexylamine (CHA), CAS-No. 108-91-8, had been used as supporting substances for the hazard identification in different toxicological endpoints, e.g. mutagenicity and reproductive toxicity.

Physical-chemical properties

CBS is a grey or yellow powder with a slight odour, a melting point of 97.5-105 °C and a measured vapour pressure of 1.5x10-8 hPa at 20 °C. Decomposition starts at 145 °C. The measured partition coefficient (log Kow) is 4.93, and the water solubility is 0.32 mg/L at 21 °C (pH 7).

Human Health

The results after oral administration to rats indicate that N-cyclohexylbenzothiazole-2-sulphenamide (CBS) is readily absorbed and that intensive metabolism of CBS takes place. As hydrolysis to 2-mercaptobenzothiazol (MBT) and cyclohexlyamine (CHA) was shown *in vitro* and will occur in the gastrointestinal tract, presystemic metabolism may play a role in the fate of CBS with different kinetic fate of the metabolic breakdown products. For oral absorption a value of 100%, and for the inhalation absorption a value of 100% (defaults), are expected. Taking into account the available physical-chemical and toxicodynamic information, a dermal penetration value of 10% is proposed.

The acute toxicity of CBS in rats and mice is very low after oral and dermal administration; LD_{50} values >5000 mg/kg bw were obtained. Dermal administration in rabbits led to a LD_{50} value >7940 mg/kg bw. Data on inhalation toxicity and human data are not available.

CBS is not a corrosive substance. CBS has demonstrated few cases of skin irritation in human patch tests with the commercial product, when using petrolatum as a vehicle. CBS caused slight irritation on the skin in rabbits (Draize assay) and on the conjunctivae of the eye of rabbits (OECD TG 405). Occasional signs of mild nasal irritation were observed in rats immediately after the 6-hour exposure period with atmospheric concentrations up to 48 mg/m^3 CBS 5 days per week in a 28-day inhalation toxicity study. The animals recovered from symptoms within 24 hours and these findings did not correlate to histopathologic effects. In light of the fact that CBS has shown slight irritations at the eye of rabbits it seems plausible that CBS leads also to slight irritations at the mucous membranes of the respiratory tract after inhalation.

Data on sensitisation caused by inhalation are not available. CBS did not cause skin sensitisation in guinea pigs (Buehler test). In contrast, there was one well conducted human patch testing study which clearly demonstrated contact sensitisation in humans. Data from epidemiological studies are difficult to assess, but also indicate some skin sensitising potential of CBS.

In a 28-day inhalation toxicity study groups of rats (10/sex/group) were whole body exposed to atmospheric concentrations of CBS of 4.3, 14.4 and 48 mg/m3 (analytic values which represent approx. 9% of the nominal values) for 6 hours per day and 5 days per week for a period of four consecutive weeks. There were no treatment-related premature deaths. No concentration dependent effects were noted for general appearance, behaviour, body weight, food consumption, hematology, urinalysis, gross pathology and absolute and relative organ weights. There were no toxicologically significant changes in clinical chemistry parameters, and no changes in organ weights and macroscopic examination that were considered to be an effect of exposition with CBS. Therefore, the no-observed-adverse effect-concentration (NOAEC) for systemic effects was 48 mg/m^3.

In a 28-day (gavage) toxicity study mostly according to OECD TG 407 groups of rats (6 /sex/group) were tested at dosages of 0, 25, 80, 250, and 800 mg/kg bw/d CBS (purity 98.8%); additionally six animals per sex in the control and high dose groups were treated for 28 days and then allowed a 14-day treatment-free recovery period before sacrifice. CBS-related effects were present in males and females at ≥250 mg/kg bw/d. There were signs of a coagulopathy of the blood in males and females and effects in the kidney of male rats. No relevant CBS-related toxic effects were observed in animals of both sexes at 80 mg/kg bw/d. Therefore, the oral NOAEL in rats was 80 mg/kg bw/d.

In a dermal 21-day toxicity study according to OECD TG 410 CBS was applied daily to the intact and abraded skin at doses of 125, 500 and 2000 mg/kg bw/d to each of five male and female adult rabbits. The exposure time was 6 hours per day for a 7-day per week basis, for a period of 21 consecutive days. Two rabbits died during the course of study but neither death was attributable to CBS treatment. Experimental findings were present in both CBS treated animals and those of controls. Their incidence and severity did not distinguish CBS-treated rabbits from controls. Therefore, the NOAEL for systemic effects and local effects in rabbits after repeated dermal exposure was 2000 mg/kg bw/d.

Human toxicity data after repeated exposure to CBS is not available.

CBS was negative in well-conducted gene mutation assays employing various tester strains of Salmonella and one each of E. coli and Saccharomyces. A well-conducted mouse lymphoma assay was also negative. An *in vitro* chromosomal aberration test gave weak evidence for a clastogenic potential. Overall, CBS is not considered to be genotoxic which is in line with the overall negative genotoxicity data for the CBS hydrolysis products, MBT and CHA.

The existing two long-term studies on CBS in mice are not in accordance with the current testing procedures as proposed by OECD guidelines on carcinogenicity and/or combined chronic toxicity/carcinogenicity. However, they are performed in accordance with generally accepted scientific standards. The results have shown that CBS is not carcinogenic in mice at a dose of 95.3 mg/kg bw/d (time-weighted average dose). In addition, the carcinogenicity of both hydrolysis products, MBT and CHA, has been investigated in a number of long-term oral studies, involving a variety of strains of rats and mice. Results of these animal studies have clearly demonstrated that MBT and CHA are not carcinogenic in rats and mice. MBT is not carcinogenic in mice and male rats at a dose of 750 mg/kg bw/d and in female rats at a dose of 350 mg/kg bw/d. CHA is not carcinogenic in rats at doses up to 440 mg/kg bw/d and in mice up to 500 mg/kg bw/d, respectively.

Generation studies, respectively fertility studies are not available for CBS. Data from adequate repeated dose toxicity studies (90 days) to supplement the available developmental toxicity studies for hazard evaluation for reproductive toxicity at a screening level are neither available. Additional data were available from investigations on the hydrolysis products of CBS.

From the two-generation study with MBT no effects adverse to reproductive capability and capacity could be demonstrated for dietary exposures of up to and including 15000 ppm (corresponding to an intake of approximately 1200 mg/kg bw/day). Reductions in offspring body weights were correlated with the onset of food intake and were thus possibly related to a palatability problem. However, long-term dietary exposures revealed toxic effects at the liver and kidney organ systems which had been induced at even lower dosages of 8750 ppm (according to 700 mg/kg bw/day). Thus, from this study there was no indication for inherent reproductive toxicity of MBT even at systemic toxic dose levels.

CHA caused tubular atrophy and reductions in spermatogenesis in rats following oral administration at doses of approximately 200 mg/kg bw/day and above, in studies of 3-months to 2 years duration. NOAELs of 100 mg/kg bw/day and 82 mg/kg bw/day respectively were identified from a 3-month and 2-year study. These NOAELs are estimated to be equivalent to 276 and 218 mg/kg bw/day of CBS. As CBS is predicted to be metabolised rapidly and extensively to CHA, these findings suggest that CBS may cause similar testicular effects. The oral (gavage) 28-d study with CBS gave some limited support to the above findings due to the

induction of testicular effects in a single rat at a dose of 800 mg/kg bw/day and after recovery only that are absent at 250 mg/kg bw /day.

Results from oral developmental toxicity studies are available. Groups of 10-17 mated female Wistar rats were administered via diet with CBS at dosage levels of 0, 0.7, 7.1, 69.6 and 288.8 mg/kg bw/day from day 0 to day 20 of pregnancy. General toxicity was noted by significantly lower maternal body weight gain during pregnancy in the two highest dose group and reduced food consumption in the highest dose group.. Neither death nor clinical signs of toxicity were reported for the pregnant females of any group. Therefore the NOAEL derived from the results of this study for dams is 7.1 mg/kg bw/day in the diet based on reductions in body weight gain. There were no significant compound related effects on pre- and postimplantation losses, the number of live fetuses per litter or the sex ratio of live fetuses. However, significantly lower body weights of male and female fetuses and of the placentae were noted at the highest dose level. The NOAEL/developmental toxicity of 69.6 mg/kg bw/day is based on decreased mean fetal body weights at the highest dose level. These data were further supported by a guideline-compliant teratology study with rats groups of 20 to 25 pregnant females treated by gavage with CBS at dose levels of 100, 300, 500, and 900 mg/kg bw per day during gestation from day 6 to 15 and in a teratology study with groups of 17 to 22 mated females treated by gavage with CBS at dose levels of 50, 150, and 450 mg/kg bw/day during gestation days 6 to 15. Overall, fetal body weight impairment noted in the above studies was exclusively observed at oral dosages associated with significantly reduced maternal weight gain of 15-30%. Substance-related specific embryotoxic and/or teratogenic potential were not revealed from the available studies.

Environment

Hydrolysis of CBS was studied in deionized water at pH 7 using a phosphate buffer system. A half-life of 12.5 h was determined and hydrolysis was observed to be complete at the end of the study (24h). Benzothiazole was found to be the sole hydrolysis product, cyclohexylamine as a potential degradation product was not identified.

The UV spectrum of CBS indicates that photodegradation under environmental conditions is possible. In a photolysis screening test, a 1 mg/l solution in water containing 1% acetonitrile as a cosolvent was exposed to sunlight at midday in August. A half-life of 26 minutes was obtained (this value refers to the top millimetres of a water body in summer, because of factors like cloudiness, shadowing effects of vegetation, absorption and scattering of light by suspended solids etc. the actual environmental lifetime is substantially higher).

The indirect atmospheric oxidation rate by reaction with OH-radicals was estimated with $79.5 * 10^{-12}$ cm^3 $molecule^{-1}$ s^{-1} by AopWin v1.91 and half-life thus 0.202 days.

The biodegradation of CBS has been determined in a shake flask procedure (draft method n° 2 for the proposed standard for the determination of the ultimate degradability of organic chemicals, August 1979, ASTM committee). An inoculum of a bacterial suspension originating from raw sewage, soil and activated sludge was incubated during 35 days with CBS concentrations of 20 and 30 mg/l. The CO2 evolution was reported to be ca. 0% for the vessels with inoculum (duplicate) and 4 % for the sterile control. CBS is considered to be persistent to biological degradation

A level III fugacity model calculation with equal and continuous distributions to air, water and soil compartments suggests that CBS will distribute mainly to the soil (71.1 %) and sediment (18.9%), compartments with minor distribution to the water (9.65%) and negligible amount in the air (0.3%) compartment.

The distribution of CBS between aqueous solutions and air can be calculated from water solubility and vapour pressure. Using the same values, a Henry's law constant of 0.0017 Pa.m3/mol is obtained, indicating that the substance is not volatile from aqueous solutions.

The distribution between the organic phase of soil or sediment solids and pore water can be calculated from the octanol/water partitioning coefficient. Using a log Kow of 4.93, according to EUSES a Koc value of 12 400 l/kg is calculated (class: predominantly hydrophobics).

According to the SIMPLETREAT model in EUSES 2.0, 33.9 % of CBS are directed to water, 54.7 % adsorbed onto sludge, and 11.4 % are degraded (hydrolysis half-life 12.5 h) in municipal stp.

There are no experimental data on bioaccumulation available. Using the equation log BCF = 0.85 log Kow – 0.70 and a log Kow of 4.93, a BCF of 3094 l/kg is obtained. BCFWIN 2.15 gives a BCF of 1248 l/kg. The

results indicate high bioaccumulation potential for CBS.

The following acute toxicity test results have been determined for aquatic species:

Fish [*Oryzias latipes*]; 96 h LC_{50} = 2.1 mg/L (flow-through system, measured)
Invertebrate [*Daphnia magna*] 48 h EC_{50} = 0.79 mg/L (semistatic, measured)
Algae [*Selenastrum capricornutum*] 72 h E_rC50 > 0.15 mg/L ; NOEC=0.0084 mg/L(measured)

The following chronic test result has been determined for aquatic species:

Invertebrate [*Daphnia magna*] 21 d NOEC = 0.058 mg/L (reproduction, measured)

QSAR-Data (ECOSAR v0.99h)

Organism	Acute L(E)C50 (mg/L)	Chronic (mg/L)
Fish	0.345	0.071
Daphnid	0.453	
Algae	0.335	0.182

The effect values are above or in the range of the water solubility of 0.32 mg/L. for the effects assessment of CBS on aquatic organisms. The test substance concentrations were generally above the water solubility (0.32 mg/l). The observed effects in the static tests are thought to be caused partly by the degradation products rather than the parent substance.

Exposure

CBS is commercially produced with an annual production volume of 16000 tonnes in the EU 15. Worldwide production volume is estimated to be around 53000 tonnes in 1998. Total volume of manufactured and imported CBS in Japan seems to be 1000 – 10000 ton in 2004. The synthesis of CBS is carried out via 2-mercaptobenzothiazole (MBT). MBT is manufactured at temperatures ranging from 220-350°C and pressures up to approx. 13 MPa through the conversion of aniline, carbon disulphide (CS2) and sulphur; benzothiazole and sulphur or aniline, carbon disulphide (CS2), benzothiazole and sulphur.

CBS is exclusively used as vulcanization accelerator in rubber goods manufacture.

Production and use of CBS cause an environmental exposure of a number of benzothiazole derivatives which are formed as abiotic breakdown products in vulcanisation, waste water and in the environment. In addition, some of these derivatives are formed as metabolites in waste water and in the environment. Within the rubber industry releases into waste water occur only in parts. Tires and larger rubber articles are manufactured in a dry process, where the water used is not in contact with rubber. Wet process is used for extruded rubber, handmade rubber clothing, rubberised fabrics and some technical rubber products vulcanised in autoclaves. In such a process water normally contains special additives and the water is run in closed circuits where only evaporation losses are replaced by new water. Benzothiazole derivatives are released into the environment by tire tread particles. The particles accumulate in soil near roads, reach the hydrosphere via rainwater runoff, and occur as dust into the atmosphere. The particles were measured in all compartments. The benzothiazoles enter the environment from tire particles either by leaching with rainwater or by degradation of the rubber matrix. Consequently, the compounds have been detected in the hydrosphere and in soils. Releases of benzothiazole derivatives from rubber goods other than tires can be expected to occur by migration and leaching. Coming into contact with water, small-sized molecules like benzothiazole can cross the rubber surface into the environmental compartments. Because of the low vapour pressure, gaseous CBS releases into the atmosphere can be excluded. Some companies report about dust particle emissions into the atmosphere.

Occupational exposure by inhalation or dermal contact is possible.

Consumer exposure is considered to be negligible, since there is no evidence available on the use of CBS in consumer products.

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

Human Health

The chemical is of low priority for further work. The chemical possesses properties indicating a hazard for human health (skin sensitisation and repeated dose toxicity by inhalation). However, the

exposure situation at the workplace is controlled and adequate risk management measurements are in place. Individual countries may wish to carry out their own exposure assessments, relevant for their own industrial scenarios followed by a risk assessment.

Environment

The chemical is a candidate for further work. The substance has properties indicating a hazard for the environment (aquatic toxicity < 1 mg/l invertebrate and algae). Member countries are invited to perform an exposure assessment and, if necessary, a risk assessment (mainly needed for sites without a biological waste water treatment plant). If the exposure assessment shows significant exposure to the sediment, further testing of the toxicity on sediment dwelling organism is recommended.

Note: A risk assessment performed in the context of the EU Existing Substances Regulation (793/93/EEC) in the European Union is available.