European Union Risk Assessment Report

N-Cyclohexylbenzothiazol-2-sulphenamide

CAS No: 95-33-0
EINECS No: 202-411-2

RISK ASSESSMENT

FINAL APPROVED VERSION
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RISK ASSESSMENT

May 2008
Germany

FINAL APPROVED VERSION

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<td>Review of report by MS Technical Experts</td>
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<td>2008</td>
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Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups. The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93 on the evaluation and control of the risks of “existing” substances. “Existing” substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as “Rapporteur”, undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94, which is supported by a technical guidance document. Normally, the “Rapporteur” and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the “Rapporteur” to develop a proposal for a strategy to limit those risks. The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.

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1 O.J. No L 084, 05/04/199 p.0001 – 0075
2 O.J. No L 161, 29/06/1994 p. 0003 – 0011
0. OVERALL RESULTS OF THE RISK ASSESSMENT

CAS Number: 95-33-0
EINECS Number: 202-411-2
IUPAC Name: N-Cyclohexylbenzothiazol-2-sulphenamide

Environment

Conclusion (i) There is a need for further information and/or testing.

Several tire recycling activities have been shown to cause exposure of the environment by benzothiazole derivatives. This exposure could not be quantified on the basis of available information. While tire recycling is increasing, exposure of aquatic and terrestrial environment from these activities should be investigated. These activities are especially tire shredding and uses of tire crumb in ground materials.

A number of benzothiazole derivatives were measured in road runoff, in receiving waters and in road border soil. The substances originate from tire abrasion. The measured data indicate that there may be risk in these receiving environments. The available data are, however, too few and no final conclusions should be based on them. Therefore measured data from water bodies receiving road runoff and soils in the vicinity of roads should be produced.

Landfills are according to the available few studies from leachate a source of benzothiazole derivative releases to aquatic environment. A major source of these substances are expected to be landfilled general rubber products and already deposited tires. Possible risks cannot be excluded due to the scarce and variable data. Measured data are needed to draw conclusions for landfills in general.

In most of the scenarios chronic ecotoxicity data on MeSBT, MeBT, BT and BTon might be able to refine the risk ratios. However, such tests should be considered only after the above required information is made available.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion concerns CBS emissions from the three CBS production sites to the aquatic environment as a source of CBS and waste water treatment plants of the sites. The conclusion also covers the secondary poisoning route of CBS with present exposure levels.

In addition, the combined exposure of CBS and its breakdown products in the aquatic environment and waste water treatment plants does not cause risks at any producer site. In rubber industry, no releases of vulcanisation agents to the surface waters occur. Consequently, no risks for aquatic environment are expected.

This conclusion covers also the exposure of soil for the CBS production and rubber industry (emissions to air).

Human health
Human health (toxicity)

Workers

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Two occupational exposure scenarios have been identified: (1) production of CBS in the large-scale chemical industry and (2) the use of CBS as vulcanisation accelerator in the rubber industry.

For CBS, systemic toxicity by repeated inhalation and skin sensitisation are the most relevant toxicological endpoints. For respiratory tract irritation a conclusion (on hold) was drawn.

With respect to systemic effects there is concern for repeated dose toxicity by inhalation for scenario 1 (production of CBS). A critical exposure level of 2 mg/m³ is proposed as reference for establishing an occupational exposure limit. It is assumed that adherence to this reference level will effectively minimise the risk for respiratory tract irritation as well.

For skin sensitisation, dermal contact results in concern for both scenarios; however, because of relevant control measures, the risk of allergic skin reactions during production of CBS (scenario 1) is considered relatively low.

Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
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1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 95-33-0
EINECS Number: 202-411-2
IUPAC Name: N-Cyclohexylbenzothiazol-2-sulphenamide
Molecular formula: C_{13}H_{16}N_{2}S_{2}

Molecular weight: 264.4 g/mol
Synonyms: 2-Benzothiazolesulfenamide, N-cyclohexyl-
Benzothiazyl-2-cyclohexylsulfenamide
2-(Cyclohexylaminothio)benzothiazole
CBS
N-cyclohexyl-2-benzothiazolesulfenamide

1.2 PURITY/IMPURITIES, ADDITIVES

Purity: \geq 96\%

Impurities:
disulphides and sulfinic acid derivatives of mercaptobenzothiazole, dimercaptobenzothiazoles
and methylmercaptobenzothiazoles \leq 3\%
di(benzothiazol-2-yl)disulphide \leq 0.5\%
cyclohexylamine \leq 0.5\%
water \leq 0.3\%

1.3 PHYSICO-CHEMICAL PROPERTIES

N-cyclohexylbenzothiazole-2-sulfenamide (CBS) is a grey or yellow powder with a slight
odour. Data on the physical and chemical properties are given in the following table:
Table 1.1 Summary of physico-chemical properties

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<tr>
<th>Property</th>
<th>Value</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Physical state</td>
<td>solid</td>
<td></td>
</tr>
<tr>
<td>Melting point</td>
<td>97.5-105 °C</td>
<td>Monsanto (1968)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bayer (1969)</td>
</tr>
<tr>
<td>Boiling point</td>
<td>Decomposition starts at 145 °C</td>
<td>Bayer (1997)</td>
</tr>
<tr>
<td>Relative density</td>
<td>1.286 at 20 °C to water at 4 °C</td>
<td>Bayer (1997)</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>1.5x10^{-8} hPa at 20 °C</td>
<td>Bayer (1988)</td>
</tr>
<tr>
<td>Water solubility</td>
<td>0.32 mg/l at 21 °C (pH 7)</td>
<td>Monsanto (1980a)</td>
</tr>
<tr>
<td>Partition coefficient</td>
<td>logPow 4.93</td>
<td>Monsanto (1980a)</td>
</tr>
<tr>
<td>n-octanol/water (log value)</td>
<td></td>
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<tr>
<td>Granulometry</td>
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<tr>
<td>Conversion factors</td>
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<tr>
<td>Flash point</td>
<td>not applicable (solid)</td>
<td></td>
</tr>
<tr>
<td>Autoflammability</td>
<td>no selfignition up to the melting point</td>
<td>Bayer (1997)</td>
</tr>
<tr>
<td>Flammability</td>
<td>not highly flammable</td>
<td>Bayer (1997)</td>
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<tr>
<td>Explosive properties</td>
<td>not explosive</td>
<td>due to structural reasons</td>
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<td>Oxidizing properties</td>
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<td>Viscosity</td>
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1) The vapour pressure was determined using the gas saturation method and confirmed by entries in safety data sheets of various companies.  
2) The water solubility was determined with the column elution method. Buffer solutions were used. The water solubility at pH 5 was 0.24 mg/l and 0.48 mg/l at pH 9 (21 °C).  
3) The shaking flask method was used for the determination of the partition coefficient n-octanol/water. The calculation with SRC-LOGKOW for Microsoft Windows resulted in a logPow of 3.47. For the risk assessment the experimental value is preferred.  
4) not relevant for the risk assessment

1.4 CLASSIFICATION

1.4.1 Current classification

Classification according to Annex I of Directive 67/548/EEC (25th ATP):

Xi, N
Xi Sensitizing
R43 Sensitizing: R 43 May cause sensitization by skin contact
On the basis of the available data the classification as Xi - sensitizing and labelling with R43 is confirmed.

Concerning the environment, according to the data presented below and the criteria of Directive 67/548/EEC, the classification of CBS according to Annex I as “Dangerous for the environment” and signed with the phrase R 50/53 (“Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment”) is confirmed.

1.4.2 Proposed classification

Category 3 reprotoxicity

R 62 Possible risk of impaired fertility

There are no fertility studies for CBS. However, repeated dose toxicity studies with the main hydrolysis product cyclohexylamine (CHA) revealed treatment related changes in testicular organ weights associated with histopathological changes (tubular atrophy, spermatotoxicity). Since histopathological changes were quite severe in some of the studies with repeated administration and occurred with a steep dose response curve, classification of CBS as reprotoxic category 3 and labelling with R62 is proposed.
2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

2.1.1 Production processes

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.

During these reactions hydrogen sulphide, benzothiazole, dimercaptobenzothiazole and sulphurous resins arise as by-products. MBT is separated from low-boiling components by dissolution in aqueous sodium hydroxide. This 10-40% solution of MBT-sodium salt (NaMBT) is treated with organic solvents (e.g. toluene, xylene) in order to extract the by-products. From the cleaned solution MBT is precipitated by acidification with mineral acids and/or the zinc salt (ZnMBT) by the addition of inorganic zinc salts.

If the NaMBT solution is oxidized (e.g. with chlorine, hydrogen peroxide, atmospheric oxygen) 2,2’-dithio-bis-benzothiazole (MBTS) precipitates (GDCh, 1991).

CBS and other benzothiazole sulphenamides are obtained by oxidation of a mixture of MBT or NaMBT and cyclohexylamine or other amines. The CBS precipitate is filtered, washed with water and extruded. The filtrate is distilled to regain the non-reacted cyclohexylamine. Remaining MBT is precipitated with mineralic acid. The water phase is discharged into the sewer.

2.1.2 Production capacity

World CBS production was estimated in 1993 at 44 000-45 000 t. It was expected to increase to about 53000 t by 1998 (Srour, 1994).

Originally ten companies notified production or import under the Regulation 93/793/EEC. Six companies have in the meantime ceased their activity. In the European Union (EU-15), CBS is produced and/or imported at the present at four sites. One of the notifiers is solely importing. According to the data supplied by these four producing and importing companies, 16101 t/a are produced, 431 t/a are imported and 10524 t/a exported outside of the EU. Thus 6008 t/a of the substance flow of the four companies are consumed within Europe. In addition, HPV-scale import not subject to the Regulation 93/793/EEC occurs in the EU-15. A total market volume of 20 000 t/a is assumed in this assessment.
2.2 USES

CBS is exclusively used as vulcanization accelerator in rubber goods manufacture. Vulcanization is a technical process in which the macromolecules of caoutchouc are cross-linked, mainly by sulphur. This process achieved at temperatures between 150 and 200°C transforms the rubber from the thermoplastic into the elastomeric state.

As vulcanization with sulphur is rather slow, accelerators are used. Accelerators serve to control time and the rate of vulcanization, and number and type of cross-links which determine the quality of the rubber goods. CBS is dosed with concentrations of 0.5–1% (ww) to the caoutchouc.

During vulcanization the unstable sulphur-nitrogen link of benzothiazole-sulphenamides is split and during a complex reaction sequence the rubber molecules are vulcanized with the intermediate formation of a 2-mercaptobenzothiazole (MBT) radical. Products resulting from the process are the basic amines, MBT (partly bound as “pending group”), and secondary reaction products which are described in section 3.1.1.1 (GDCh, 1991).

Figure 2.1: Reaction scheme of the transformation of CBS during vulcanization

In 1996, the world production of natural caoutchouc was about 6 million t and of synthetic caoutchouc about 9.1 million t. In Germany, about 1 million t automobile tires and 530.000 t other rubber goods were produced in 1995 (Baumann & Ismeier, 1998). The most important product of rubber industry is automobile tires with about 2/3 of the total production (OECD, 2004a). According to BLIC (2005), products for the automotive branch cover 65 % of the production volume of rubber goods other than tires. One kind of further quantitative breakdown of the non-tire area has been presented in the Emission Scenario Document on Additives in Rubber Industry (OECD 2004a) in the determination of the generic rubber industry point source.
2.3 FURTHER BENZOTHIAZOLE COMPOUNDS

As presented in section 3.1.1, production and use of CBS cause an environmental exposure of a number of benzothiazole derivatives. However, CBS is not the only source of these pollutants, further benzothiazole compounds used as vulcanization accelerators or for other applications cause an exposure of the same derivatives. In the following an overview is given of the other sources of benzothiazole derivative exposure.

Vulcanization Accelerators:

World rubber vulcanisation accelerators production was estimated for 1993 at about 175000 t. Of this total, benzothiazole and sulphenamide-type products accounted alone for nearly 79% or about 138500 t (Srour, 1994). The benzothiazole derivatives were consumed as accelerators in Western Europe in the amounts listed below:

Table 2.1 Western European Accelerators Demand 1993 (Srour, 1994)

<table>
<thead>
<tr>
<th>Substance</th>
<th>Demand [t/a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Mercaptobenzothiazole (MBT)</td>
<td>1500</td>
</tr>
<tr>
<td>2-Mercaptobenzothiazole zinc salt (ZnMBT)</td>
<td>1400</td>
</tr>
<tr>
<td>2,2'-Dithiobis-benzothiazole (MBTS)</td>
<td>5000</td>
</tr>
<tr>
<td>N-Cyclohexylbenzothiazole-2-sulphenamide (CBS)</td>
<td>15500</td>
</tr>
<tr>
<td>Morpholinobenzothiazole-2-sulphenamide (MBS)</td>
<td>1500</td>
</tr>
<tr>
<td>N,N-Dicyclohexylbenzothiazole-2-sulphenamide (DCBS)</td>
<td>1600</td>
</tr>
<tr>
<td>N-tert.-butylbenzothiazole-2-sulphenamide (TBBS)</td>
<td>3600</td>
</tr>
<tr>
<td>N-Diisopropylbenzothiazole-2-sulphenamide (DIPS)</td>
<td>300</td>
</tr>
<tr>
<td>TOTAL</td>
<td>28600</td>
</tr>
</tbody>
</table>

From the use of MBS, toxicity problems related to nitrosamines which are formed during vulcanization resulted in a dramatic decline in MBS production. MBS is gradually being replaced by TBBS and CBS (Srour, 1994).

Plant protection agents:

Methabenzthiazuron is a herbicide produced via MBT. In 1993 the European demand was 1800 t (Srour, 1994). It is included in the third priority list of the working program for evaluation of plant protection products under the Council Directive 91/414/EEC. The dossier for the evaluation was to be sent to the Rapporteur Member State Sweden by 30 November 2004. Sweden has not received the dossier and consequently the European market access of the substance will be denied in the near future.
Mefenacet (2-benzothiazol-2-yloxy-N-methylacetanilide) is a rice herbicide chiefly sold in Japan. The production in 1993 was estimated to 1700 t (Srour, 1994). Mefenacet was denied the access to the EU market by July 2003 while not notified by any company under the Directive 91/414/EEC.

**Biocides:**

2-2-(Thiocyanomethylthio)benzothiazole (TCMTB; CAS 21564-17-0) has been notified under the Biocides Directive 98/8/EEC for the use in nine product groups according to the Commission Regulation (EC) 2032/2003 and its amendment (EC) 1048/2005 i.a. for the use in slimicides, and conservation products. No use for wood preservation has been notified against the earlier information according to which TCMTB is also used as a fungicide by tanneries (Brownlee et al., 1992 and Srour, 1994). In addition, the registration as antifouling paint has been withdrawn.

```
  N               2-thiocyanatomethylbenzothiazole
   S     +---------------------
  |        |       (TCMTB)     |
   S        H2             C   S   CN
```

When released into the waste water, the substance hydrolyses to MBT (Brownlee et al., 1992), a compound which is also a degradation product of CBS (cf. section 3.1).

2-Mercaptobenzothiazole (MBT; CAS 149-30-4) has been notified under the Biocides Directive 98/8/EEC Commission Regulation (EC) 1048/2005 for the use in six product groups, i.a. for the use in slimicides and indoor desinfection of public buildings.

**Corrosion inhibitors:**

Reddy & Quinn (1997) measured benzothiazole compounds in antifreeze liquids of 5 different cars. From the measured concentrations, the use amount of antifreeze and estimated release factors, the annual releases for the USA were calculated to 1.5-4000 kg/a for Benzothiazole (BT) and Benzothiazolone (BTon), and 0.02-12 kg/a for Morpholinobenzothiazole-2-sulphenamide (MBS). These fluxes are several orders of magnitude smaller than the releases via tire particles.

**Intermediates in Chemical Synthesis:**

Benzothiazole (BT; CAS 95-16-9) is a technical product. According to the IUCLID database (last update 30 May 1995) 1000 – 5000 t were produced in Europe in 1994. The substance is exclusively used as an intermediate in chemical synthesis (see Appendix C).

2-Mercaptobenzothiazole (MBT; CAS 149-30-4) is produced according to the IUCLID (2000) in Europe in the volume of 10 000 – 50 000 t/a. It is the major raw material in the production of benzothiazole derivatives (see Appendix A).
Food Flavour:

Benzothiazole is included in the list of food flavours of the EU (European Commission, 1999). This use is also recorded by World Health Organization (2002). No information on the quantity used is available.

2.4 NATURAL BENZOTHIAZOLE COMPOUNDS

In the literature some benzothiazole derivatives occurring as natural products are described. From fermentation culture extracts of *Micrococcus sp.*, a marine bacterium obtained from tissues of the sponge *Tedania ignis*, 2-mercaptobenzothiazole (MBT), 2-methylbenzothiazole (MeBT), 2-benzothiazolone (BTon) and 6-hydroxy-3-methyl-2-benzothiazolone were detected (Stierle et al., 1991). Seifert and King Jr., (1982) found the fungi *Aspergillus clavatus* to form benzothiazole. Seeds, germinating seeds and roots of a tropic legume *Zapoteca formosa* were confirmed to release benzothiazole (Lane et al., 2004). As a further benzothiazole derivative, firefly luciferin is reported (De Wever and Verachtert, 1997a).

Several studies have identified benzothiazole in food like endive (Götz-Schmidt and Schreier, 1986), mangos (Engel and Tressl, 1983) and black tea (Vitzthum et al., 1975). These studies were aimed at to find naturally formed (aroma) compounds and therefore it was not further discussed whether the substances found actually were of natural or of anthropogenic origin. Also 2-methylthiobenzothiazole and 2-methylbenzothiazole have been found in food products (see Appendices III, V and VI).

![Firefly luciferin](image)

![6-hydroxy-3-methyl-2-benzothiazolone](image)

On the basis of the information presented above, it can be expected that small amounts of benzothiazole compounds are released from natural sources. These amounts are, however, not quantifiable and probably negligible. Natural sources can be assumed to have no impact on the local environmental concentrations in the scenarios included in this assessment.

2.5 LEGISLATIVE CONTROLS

CBS is not at present regulated in substance related legislation.
3 ENVIRONMENT

General

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. Detailed information on five relevant degradation products is included in the Appendices I-VI. Information on 2,2’-Dithiobis-benzothiazole (MBTS), which was subject of work in the OECD HPVC Programme, is included as well (cf. Appendix B). Production and use of MBTS cause an exposure of the same benzothiazole compounds as CBS and it is in some cases also formed as a degradation product of CBS.

According to the TGD, exposure to the degradation products has to be assessed in case these are stable enough in the environment. The shares of the different benzothiazole derivatives formed in the degradation of CBS are not well quantifiable which makes the exposure estimation difficult. In addition, there are other sources than CBS contributing to the measured levels of its degradation products. If measured data is used instead of calculated PECs, the risk characterization reflects in some scenarios risks arising not only from use of CBS but from the production and use of other benzothiazole derivatives as well.

Approach for evaluating risks caused by the combined exposure of CBS degradation products using measured data

Measured data from the environment is crucial for drawing conclusions in this assessment. Measured data on all relevant degradation products are used for risk characterization assuming that the response to the mixture of these substances in the environment obeys the theory of concentration addition. The risk from such substances can be evaluated in a conservative way by the simple method of adding the risk ratios of individual substances together as proposed in the TGD for petroleum substances. The background of this method is presented below.

This approach does not directly give the risk ratio caused from the use of CBS but it represents the exposure caused by the use of the whole product group of benzothiazole derivatives. CBS makes approximately half of this exposure. Since new measured data are scarce, the results have to be considered with caution. The risk ratios obtained with this method are presented in Chapter 3.3.

Multicomponent Exposure – Background

Two main approaches have been described to model the toxicological behaviour of chemical mixtures. These are the “concentration addition” (CA) (or: “dose addition”) and “independent action” (IA) (or: “response addition”) models (Greco et al., 1992). Both models assume that no interactions occur between the substances in question, i.e., no synergistic or antagonistic effects are expected.

The CA-model assumes that the effects are concentration additive. The original model is:
\[
\sum_{i=1}^{n} \frac{C_i}{EC_{x,i}} = 1,
\]

where \( C_i \) are the concentrations of components in a mixture, which causes \( X \% \) effects and \( EC_{x,i} \) are effect concentrations of each component alone that would produce \( X \% \) effect.

A CA-model can in theory be applied only when the substances have the same mode of action (same alterations of cellular or organ function) or more strictly defined the same mechanism of toxicity (same interaction at the target receptor). In addition, the relative toxic potencies of the substances should be the same in the whole range of concentrations for the given endpoint. This means that the dose-response curves should have similar forms and the substances cause effects as if they were dilutions of each other. Consequently, the method should be used to characterize the mixture toxicity only for one species at a time.

One commonly used application of the CA-model is the TEF (Toxic Equivalent Factors) for dioxins and furans. Also the hazard index (HI) used in the health risk assessments in the U.S. is based on concentration addition. A thorough overview of hazard index and other applications and prerequisites of the concentration addition models is given in the “Supplementary Guidance for Conducting Health Risk Assessments of Chemical Mixtures” (U.S. Environmental Agency, 2002). In addition, Calamari and Vighi (1992) base their proposal for water quality objective for mixtures on the concentration addition theory.

The IA-model (response addition model) can be applied for substances with dissimilar mode of actions. In brief, response addition assumes that the response to a given concentration of a mixture is determined by the responses to the components and the pairwise correlation coefficient of the components. The correlation coefficients reflect the probability of the test object to elicit both effects simultaneously (range from –1 to 1). The boundary condition is, that the response to the complete mixture (expressed as a probability) is equal or less to one. This method requires data on mode of action.

Vighi et al. (2003) compared the predictive capability of CA- and IA -models in a single-species algal bioassay (Scenedesmus vacuole) testing different mixtures of 22 substances and the substances separately. The substances used are known to have strictly dissimilar mode of action. The authors concluded, that the CA-model overestimated the mixture toxicity whereas the IA-model predicted the toxicity precisely.

Several models taking into account the interaction (synergy or antagonism) have also been presented (e.g., U.S. Environmental Agency, 2002; Rider and LeBlanc, 2005; Teuschler et al., 2005). In addition, a kind of a mixture toxicity model, where reduction in toxicity due to contaminant interaction is modelled, is the Biotic Ligand Model (BLM) used in the EU risk assessment of Zinc/Zinc oxide.

**Multicomponent Exposure and Risk Assessment of Existing Chemicals**

The additive risk characterization (ARC) model as introduced in the TGD for petroleum substances is a rather free application of the CA model:

\[
RCR_{\text{mixture}} = \frac{PEC_1}{PNEC_1} + \frac{PEC_2}{PNEC_2} + \frac{PEC_3}{PNEC_3} + \ldots + \frac{PEC_n}{PNEC_n}
\]
where risks of each “blocks” (in the case of this assessment substances) are added to obtain the final risk estimate. This model is almost identical with the Hazard Index –method used in the United States.

However, for the application above, departure from several assumptions behind the theory of concentration addition and behind the use of Hazard Index has occurred. The requirement for similar dose-response curves is not implemented since the component-PNECs may have been derived using critical data from different trophic levels (algae, fish, daphnids) or different species of the same trophic level, the critical studies may have measured different toxicity end-points, for some PNECs acute and for some PNECs chronic data may have been used. On the other hand, the assessment factors used to derive the PNECs cover most of this uncertainty. According to the TGD, the assessment factors try to cover even the risk of synergistic effects of the substance with other substances in the environment. The ARC-equation is a conservative approach to evaluate the risks due to multiple exposure.

In theory, a more suitable way to conduct the risk characterization would be e.g., to use the above equation to calculate risk ratios for each trophic level. This could be done using the lowest result of the trophic level in question but applying still the same assessment factors as for the general PNECs. Thus three trophic level specific mixture RCRs would result and the highest ratio would be used for the conclusions of the assessment. In addition, risk ratios for chronic and acute data should be calculated separately for the mixtures, where chronic exposure clearly causes different shape of dose-response curve than acute exposure for one or more of the components. Due to too few data, this trophic level specific approach is not possible to implement and therefore the applied approach as given by the equation above must be taken. The model gives more conservative risk estimates than the trophic level specific method.

This approach is recommended to be used by the TGD for petroleum substances, but it is applicable also to other similar exposure situations caused by a compact group of substances with baseline toxicity. In case the components do not all obey the same mode of action, the model still gives the upper limit of risk since the results from an IA-model are always lower.
cyclohexylamine. Remaining MBT is precipitated with mineralic acid. The water phase is discharged into the sewer. The data submitted by the 3 producers, gives an total annual release into the hydrosphere of <555 kg/a for the three plants (cf. 3.1.2.1).

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. According to the data submitted by the 3 producers, the total annual releases into the atmosphere amount to < 927 kg/a (cf. 3.1.3.1).

### 3.1.2.2 Release from industrial/professional use -use by rubber industry

**Breakdown of CBS during vulcanisation (cf. Figure 3.1 in Chapter 3.1.1.2.2)**

The breakdown of vulcanization accelerators is described in several publications. During vulcanization the unstable sulphur-nitrogen bond of CBS and other benzothiazole sulphenamides is split with the intermediate formation of a 2-mercaptopbenzothiazole (MBT) radical. Products resulting from the process are the basic amines, benzothiazole derivatives, and further reaction products. MBT is partly bound via sulphur or polysulphide groups at the polymer matrix, the so-called “pending group” (GDCh, 1991). As further degradation products, benzothiazole (BT), 2-methylbenzothiazole (MeBT), and 2-benzothiazolone (BTon) are reported (Baumann & Ismeier, 1998; Reddy & Quinn, 1997).

Gradwell & McGill (1994) studied the thermal decomposition of CBS (no additives present) and found at temperatures above 185°C 2-mercaptopbenzothiazole (MBT), the amine salt of MBT and 2-N-cyclohexylaminobenzothiazole (CB) as the main products. Oligo- and polysulphides of MBT, 2,2’-dithiobis-benzothiazole (MBTS) and CBS were detected as intermediates. The same products emerged from the decomposition of morpholinobenzothiazole-2-sulphenamide (MBS) and N-tert.-butylbenzothiazole-2-sulphenamide (TBBS).

The breakdown products being formed during vulcanization are included into the polymer matrix. Consequently, benzothiazole derivatives were frequently detected in rubber and tires. In table 3.1 some results are listed. The same substances were frequently measured in aqueous eluates, the results are included in the Appendices. In addition to the substances in the table, 2-methylbenzothiazole (MeBT) and 2-methylthiobenzothiazole (MeSBT) were detected in eluates.

**Table 3.1 Measurements of Benzothiazole Derivatives in Rubber Goods and Tires**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Medium</th>
<th>Concentration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Mercaptopbenzothiazole (MBT)</td>
<td>Rubber shoes</td>
<td>&lt;1-1910 mg/kg</td>
<td>Kaniwa et al. (1994)</td>
</tr>
<tr>
<td>2,2’-Dithiobis-benzothiazole (MBTS)</td>
<td>Rubber shoes</td>
<td>180-420 mg/kg</td>
<td>Kaniwa et al. (1994)</td>
</tr>
<tr>
<td>Benzothiazole (BT)</td>
<td>Tire wear particles</td>
<td>124.3 mg/kg</td>
<td>Rogge et al. (1993)</td>
</tr>
<tr>
<td></td>
<td>Crumb tire particles</td>
<td>171 mg/kg</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>Benzothiazolone (BTon)</td>
<td>Crumb tire particles</td>
<td>81 mg/kg</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
</tbody>
</table>
Releases into the atmosphere by rubber industry

As vulcanization is achieved at high temperature (150-200°C), volatile reaction products are released from the rubber mixture. In the exhaust gas a series of organic compounds were detected:

- With the amino moiety: cyclohexylamine, cyclohexanone, and cyclohexylisocyanate (Hilton & Altenau, 1973; Baumann & Ismeier, 1998). These compounds are included in the EINECS inventory as existing substances. In the present risk assessment they are not considered, it is proposed to assess their releases together with their production and use.

Dependent on the vulcanization procedure, the releases of organic carbon into the exhaust air amount to 20-150 mg/cm³. Meanwhile more than 100 compounds were identified. A series of methods for air cleaning is described in the literature, e.g. incineration, ionization filter, adsorption, biofilter and scrubber (Spendlin, 1992; Baumann & Ismeier, 1998). There is no quantitative information available about the effectiveness of the cleaning techniques.

Levin (1994) presented a summary of the “Nordic curing fume project” in which factory scale curing experiments with rotorcuring at 170 °C and laboratory scale curing experiments with gas transfer mold at 180 °C were run for six different rubber products. The authors concluded that in the factory trials the loss of weight during vulcanisation was 0.044 % whereas in the laboratory scale, the loss of weight was 0.40-0.80 % and the mean loss of weight including all experiments was approximately 0.05 %. For the release estimation to air the method and defaults of the emission scenario document on additives in rubber industry (OECD 2004) and the results described by Levin (1994) are applied.

Releases into the waste water by rubber industry

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 (BLIC 2004).

Cleaning of process equipment is a dry process carried out by dry blasting or by using ceramic, synthetic beads or dry carbon dioxide pellets in a closed system (BLIC 2004).

In addition, releases into waste water may occur in the following areas (GDCh, 1991; Baumann & Ismeier, 1998):

- Cleaning of workplaces,
- reprocessing of scrub rubber,
• the use of washing systems for cleaning exhaust air.

The emission scenario document on additives in rubber industry (OECD 2004) assumes based on data gathered for the document zero releases of vulcanisation agents into the waste water from the generic point source. Fraction of the vulcanisation agent remaining in the rubber is 1 according to the document.

3.1.2.3 Release from tires during service life

Tire Tread Abrasion

Benzothiazoles are released into the environment by abrasion of tire tread. Four automobile tires release per km about 60-80 mg particles with a diameter of 30-70 µm, 1-5 mg fine particles (< 30 µm) and 1-5 mg gas, mainly CO, CO₂, COS, SO₂, and hydrocarbons (Baumann & Ismeier, 1998).

The amount of tire abrasion occurring in the Federal Republic of Germany (former Western Germany) each year was estimated by the Federal Ministry of Transport as approx. 75,000 t for 1989. For the same year, the industry association of the caoutchouc industry estimated the amount to 80,000 t/a (GDCh, 1991).

Lower amounts were calculated by Baumann & Ismeier (1998). Abrasion tests with different tire types resulted in amounts of 20 mg/tire/km for automobiles, 36 mg/tire/km for trucks with a weight below 7.5 t, 21 mg/tire/km trucks with a weight above 7.5 t, 18 mg/tire/km for tractors, and 32 mg/tire/km for buses. Based on the number of vehicles, their number of tires, and the average traffic distance per year, the annual total abrasion was calculated to 61,360 t for 1995 and estimated to 65,000 t for 2000. The abrasion amount along the roads was estimated to 55 kg/km for roads within settled areas and to 175 kg/km outside urban districts.

Abraded tire particles mainly accumulate near roads. Cadle & Williams (1980) report measurements in soil near roadways, the concentrations are 2% at the shoulder and are decreasing exponentially with distance to less than 0.01% at 30 m. In a laboratory experiment on biological degradation of the particles in soil, a degradation rate of 0.15% per day was found. Several factors such as temperature, oxygen, ozone, light, humidity, and microorganisms affect the degradation process. To match the amount of rubber observed at road sites, the removal rate must be about 5-fold higher, indicating that other factors like oxygen attack, wind erosion and water runoff also contribute to the total removal.

Kim et al. (1990) demonstrated the importance of tire tread particles in urban air. From suspended particulate matter (SPM) sampled in the city of Tokyo, the tire fraction was estimated by GC measurement of benzothiazole generated by pyrolysis. The tire tread concentrations followed the trend of traffic density during the day, during a summer day 1-6.5 μg/m³ on the road level and 30% of these concentrations in 86 m height were detected. At a further site located 20 km from the city, the weight percentage of tire tread in SPM varied between 1.3 and 3% in winter and between 0.5 and 1.5% in spring.

Abraded tire tread particles are released into all environmental compartments; however, the distribution is only roughly quantifiable.
Migration and Leaching

Small-sized molecules like benzothiazoles are mobile in the rubber matrix, they can cross the surface into environmental matrices.

Different authors investigated the migration of benzothiazoles from rubber into water. Baumann & Ismeier (1998) produced artificial particles of different size from 6 different tires (automobile and truck tires, each new and old) which were shaken for 24 hours with both distilled (pH 7) and acidified water (pH 4) simulating acid rain. In the eluate, <0.5–884 µg/l 2-mercaptobenzothiazole (MBT), 23.1–1345 µg/l benzothiazole (BT), <2–254 µg/l 2-methylbenzothiazole (MeBT) and 7.69–263 µg/l 2-methylthio-benzothiazole (MeSBT) were detected. The detected concentrations were varied much between different tire types, and were generally higher in new than in old tires. They were not dependent on particle size and pH. In a further experiment 2 new automobile tires were dipped into neutral and acid water, the benzothiazoles were measured every 2 months. The BT concentrations were 367-1972 µg/l in neutral and 14.6-805 µg/l in acid water, while MBT, MeBT and MeSBT were not detected. More results of measurements in rubber eluates are included in the Appendices 1-6.

Summary of the Releases from Tires During Service Life

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. Volatile substances like benzothiazole (BT) and 2-methylbenzothiazole (MeBT) can also be assumed to be released directly from tires to the atmosphere. As these substances were detected in old tires, it can be concluded that their volatilization from tires is only partial.

The most important compounds which can be expected to be found in the environment due to CBS use as vulcanisation accelerator are 2-mercaptobenzothiazole (MBT), benzothiazole (BT), 2-benzothiazolone (BTon), 2-methylthio-benzothiazole (MeSBT), and 2-methylbenzothiazole (MeBT). In addition to CBS, other accelerators like 2-mercaptobenzothiazole (MBT), 2,2’-dithiobis-benzothiazole (MTS) and benzothiazole-sulphenamides are precursors of the same compounds. An attempt to quantify the release from tires is presented in Chapter 3.1.2.2.2 and in the Appendices III and V.

3.1.2.4 Release from general rubber goods during service life

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.

Baumann et al. (1998) examined the environmental relevance of 2-mercaptobenzothiazole (MBT) releases caused by the use of rubber for sports ground surfaces by i.a., “washing” 2m*2m pieces of the surface materials 24 h in deionised water. They found in all three analysed sport ground material eluates 2-mercaptobenzothiazole (MBT) in concentrations of 1.2 mg/l, 0.8 mg/l and 0.9 mg/l. MBT was not found in the investigated riding ground material eluate.
It is not possible to estimate the amount of release from rubber goods on the basis of the present literature. On the basis of the results by Bauman et al. (1998) it could be expected that a temporary local risk arises in a water body after an installation of new rubber materials, although the total release amount remains minor. Potential risks from this use can be assumed to be covered by the similar use of recycled tire crumb (see part e below).

On the other hand, some of the rubber products do not come into contact with water and thus releases are minor both in a local and regional scale.

### 3.1.2.5 Release from used tires and rubber goods - release from disposal

An overview of the disposal and recycling routes of tires and their shares in the EU for the year 2003 are presented in Table 3.2.

<table>
<thead>
<tr>
<th>Disposal and recycling routes</th>
<th>% in the year 1992 (EU-12)</th>
<th>% in the year 2003 (EU-15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Export for reuse outside the EU</td>
<td>62</td>
<td>13</td>
</tr>
<tr>
<td>Retreading</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Material recycling</td>
<td>13</td>
<td>26</td>
</tr>
<tr>
<td>Energy recovery</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>Landfill and other disposal</td>
<td>14</td>
<td>20</td>
</tr>
</tbody>
</table>

Although the emission scenario document of rubber industry (OECD 2004) has included retreading into the activities of a generic rubber industry plant, it has to be recognized that tire retreading is a separate industrial business. According to BLIC (2005) the emissions from this sector are expected to be similar to the emissions from rubber industry.

Tire recycling includes uses e.g., in steel production and cement kilns, erosion barriers, artificial reefs, roads and shoe soles (BLIC 2005). For recycling tires can be used as whole or shredded to particles. The size of the particle varies according to the recycling purpose.

Chien et al. (2003) assessed occupational health hazards in two similar tire shredding facilities in Taiwan with process loading of 26 500 kg/day and 55 000 kg/day, respectively. Both facilities can shred tires into chips of few square inches and to smaller rubber crumb particles of various sizes. The air of the main working areas were sampled and benzothiazole was found in all samples of the volatile organic carbon fraction and in particulate fraction. Total particulate levels ranged from 0.42 to 6.54 mg/m³ in all samples. Benzothiazole was not quantified from the samples. The dust concentration was low due to air-pollution control devices indoors. Several other substances were identified in VOC and particulates as well. It is not possible to estimate on the basis of this study the relevance of benzothiazole emissions to air from this activity.

Reddy and Quinn (1997) concluded based on their research on benzothiazoles in urban runoff and on laboratory weathering experiments of asphalt slab containing crumbed tire rubber that such an asphalt may initially contribute larger amounts of dissolved benzothiazoles into urban runoff than tires but the relative contribution will decrease rainstorm after rainstorm.

Azizian et al. (2003) carried out batch leaching experiments with crushed crumb rubber asphalt and distilled water. Particle size of the asphalt was ≤1/4 inch. Benzothiazole and
2(3H)-benzothiazolone were measured from the leachate. A 24h leaching sample contained 0.447 mg/l benzothiazole, 0.012 mg/l 2(3H)benzothiazolone, 1.870 mg/l aluminium and 0.023 mg/l mercury. The maximum leaching rate in the 168h –study occurred within the first 10h.

Dye et al. (2006) measured concentrations of VOCs in the air of three Norwegian indoor sport halls. Two of the halls (Manglerudhallen and Valhall) have artificial turf which contains shredded tire granules. The turf in Maglerudhallen was ca. one year old, whereas in Valhall it was newly filled. In the third hall (Østfoldhallen) the turf consisted of thermoplastic elastomer granules. The concentrations of benzothiazole in gas phase were in the first two halls between 4.5 and 31.7 µg m⁻³ for six samples in total covering several opening hours, different air temperatures and different ventilation conditions.

The highest concentrations were found in Valhall. In this hall turf had probably due to its novelty a film cover, the influence of which was investigated in laboratory scale by measuring in an adsorption chamber emissions of granules from the two first halls. When the granules were washed with cyclohexane, the relative source strength of Valhall granules was five times more than the emission of Maglerudhallen granules. The difference without washing was ca. 20 %. This suggests that the film acted at the point of air sampling in Valhall as a diffusion barrier and that concentrations in air may increase after wearing of the film. In Østfoldhallen the mean concentration of two sampling periods was 3.7 µg m⁻³ in gas phase. The results demonstrate that alternative material causing lower emissions to recycled tires is already available.

The study of Dye et al. (2006) also included an indirect measurement of several benzothiazoles in fine particulate matter fraction. 2-benzothiazolone (BTon) and 2-mercaptobenzothiazole (MBT) were measured in concentrations of up to 1 ng m⁻³ (sum of the concentrations in PM₂⋅₅ and PM₁₀). The other benzothiazoles found were 2-methylthiobenzothiazole (MeSBT), N-cyclohexyl-2-benzothiazole-sulphenamide (CBS), 2,2’-dithio-bis-benzothiazole (MBTS) and N-cyclohexyl-2-benzothiazolamine (an impurity in CBS), but their concentrations were 1 to two orders of magnitude lower. Concentrations in Østfoldhallen remained under the detection limits (all below 1 pg m⁻³).

Another Norwegian group (Plesser and Lund, 2004) investigated leaching of chemicals from three outdoor sport ground granule products, which were made of shredded tires. The test was conducted according to the standards EN 12457-4 and NT ENVIR 005. Leaching time in deionised water was 48 hours. They found in the leachate i.a. alkyl phenols (up to 4.7 µg l⁻¹ in total, PAHs (< 1 µg l⁻¹ in total), phthalates (up to 8.3 µg l⁻¹) and zinc (up to 2.3 mg l⁻¹). Benzothiazole derivatives were not analysed. Taking into account the tire crumb elution experiment results of Baumann and Ismaier (1998) and other elution experiments cited in Appendices I-VI, concentrations of benzothiazole derivatives in leachate of sport ground granules could be expected to be in the same range as concentrations of zinc. Källqvist (2005) derived based on the leaching experiments of Plesser and Lund (2004) estimates of annual emissions from an outdoor sport ground. For zinc an emission of ca. 19 kg a⁻¹ was obtained.

Birkholz et al. (2003) tested three tire crumb materials used in playgrounds. Water leachates were used for toxicity testing on *Vibrio fischeri*, *Daphnia magna*, *Pimephales promelas* and *Selenastrum capricornutum*. Different standard whole effluent testing methods for acute toxicity were applied but it was not indicated whether a static or flow-through method was applied, which is crucial information for the evaluation of validity. Concentrations of substances in leachate were not measured either. The authors observed moderate toxicity measured as toxic units in the initial leachates but saw also a reduction in response for aged materials.
The studies mentioned above confirm that tire recycling causes releases to water and air. The amount of open uses of shredded tires within a region or a Member State is not known. However, the amount could be expected to be high enough to cause relevant amount of regional releases. In addition, on the basis of the available studies, high local concentrations may be expected at least on a short term. Tire recycling is a growing branch and more information is needed in order to be able to quantify the releases from this sector.

The Directive 1999/31/EC on Landfill of Waste banned the disposal of whole tires by July 2003 and disposal of shredded tires by July 2006 excluding some minor tire products. Consequently it can be expected that the share of other routes than disposal into landfills will increase in the coming years.

Benzothiazole derivatives are released from landfills by migration/leaching from and by degradation of the rubber matrix. Releases into the hydrosphere via leachate can be expected, and consequently the compounds have also been detected there. Infiltration into groundwater can be expected from older landfills which do not have bottom lining. The targeted risk assessment of NiCd-batteries established a framework for estimating regional, continental and local exposure from operational landfills using measured concentration in leachate (TRAR 2003). The exposure assessment of benzothiazole derivatives for this endpoint is also based on measured data.

### 3.1.2.6 Other sources: releases to municipal waste water and a short evaluation of the relevance of municipal waste water as a combined source to the risk assessment

Several non-quantifiable sources of benzothiazole derivatives can be assumed to contribute to the releases into domestic waste water and waste water from small businesses. These are i.a. the use of BT as flavour and biocide use of MBT and TCMTB. Other contributors to municipal waste water are sources described in parts c to e above.

Klöpfer et al. (2005) measured samples pooled over 24 h from the influent and effluent of the largest municipal waste water treatment plant in Berlin. This large treatment plant treats relatively low amounts of industrial waste water and it receives storm water from the connected urban area. Two sampling campaigns (March-June 2002 = Ruh1; October-November 2003 = Ruh2) were carried out. Concentrations of benzothiazoles relevant for this assessment are presented in Table 3.3. In addition to these four substances, Benzothiazolsulphonic acid (BTSO_3H) and 2-aminobenzothiazole (ABT) were found. BTSO_3H was found in concentrations around 1-2 µg/l whereas concentrations of ABT were around its detection limit of 0.1 µg/l.

Klöpfer et al. investigated whether correlation between influent concentrations and storm events exist after finding benzothiazole derivatives in high concentrations in highway runoff from Berlin (see chapter 3.1.2.2.2). They did not see correlation but considered that possible concentration peaks during rain events may be diluted due to the large size of the connection area.

Benzothiazole derivatives were also found in domestic waste water in a separate sewer area (see Table 3.3) and the research group concluded that household waste water contributes relevant amounts of benzothiazoles into the municipal waste water stream. Again, BTSO_3H was found in the highest concentrations (mean 0.84 µg/l) of the benzothiazoles analysed. The same substances were also found within the study in the largest municipal waste water
treatment plant of Beijing, although with higher BT and BTon concentrations relative to other components.

Table 3.3  Mean influent and effluent concentrations in the largest municipal sewage treatment plant in Berlin, Germany and in domestic waste water from the connection area

<table>
<thead>
<tr>
<th></th>
<th>Ruh1 (µg/l) n=20</th>
<th>Ruh2 (µg/l) n=9</th>
</tr>
</thead>
<tbody>
<tr>
<td>2- Mercaptobenzothiazole</td>
<td>0.19</td>
<td>0.02</td>
</tr>
<tr>
<td>2- Mercaptobenzothiazole</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>2- Mercaptobenzothiazole</td>
<td>Not measured</td>
<td>Not measured</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>0.85</td>
<td>0.74</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>0.55</td>
<td>0.28</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>0.50</td>
<td>0.16</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>0.14</td>
<td>0.20</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>0.17</td>
<td>0.44</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>0.44</td>
<td>0.36</td>
</tr>
<tr>
<td>Benzothiazole</td>
<td>0.09</td>
<td></td>
</tr>
</tbody>
</table>

(Klöpfer et al, 2005)

The effluent flow rate of the sewage treatment plant of the study is 212 600 m³/d, which makes approximately ¼ of the effluent flow of the metropolitan area treatment plants in Berlin (Stadt Berlin, 2004). Using the effluent concentrations of the first sampling period (Ruh1), annual emission rates for MBT (1.6 kg/a), BT (42.7 kg/a), BTon (10.9 kg/a) and MeSBT (34.1 kg/a) are estimated. Using a dilution factor of the treatment plant effluent (receiving river Spree; D is approximately 3), the effluent concentrations of the first sampling period and the PNECs presented in chapter 3.2.5, it can be concluded that the concentrations in the receiving river are far below the PNEC of each substance and the sum of the risk ratios for the four substances is below 1 (0.06). Thus from this single plant no risks arise to the aquatic environment.

Based on the study of Klöpfer et al. (2005), on earlier studies from municipal stps presented in the Appendices I-V and on the presence of benzothiazoles in street runoff (chapter 3.1.2.2.2), it can be expected that benzothiazole derivatives are ubiquitously present in the effluents of municipal sewage treatment plants. Sources of benzothiazoles are household waste water, storm water and industrial waste water, whereas the exact shares vary probably from treatment plant to treatment plant due to the different shares of combined sewers in the connection areas and due to the type and size of industry connected.

No conclusions regarding to local risks from municipal treatment plants in general can be drawn at the moment. On the basis of the considerations regarding to local risks above, municipal treatment plants as a combined source of benzothiazole derivatives are not followed further in this assessment. Nevertheless, due to a high amount of municipal sewage treatment plants in each European river basin, significant amounts of benzothiazoles may be
emitted to the aquatic environment. Monitoring of long-term trends of the most persistent benzothiazoles BT, MeSBT and BTon in the aquatic environment should be considered in regional or national monitoring programs.

3.1.3 Environmental fate

3.1.3.1 Degradation in the environment

An overview of the degradation paths and relevant breakdown products is presented in Figure 3.1.

3.1.3.1.1 Biodegradation

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 (Monsanto, 1992).

BIOWIN v4.02 predicts that CBS is not readily biodegradable, but, on the other hand, the substance is not completely persistent to biodegradation, either. The models applied to the screening of persistency in the PBT assessment provide following estimates: BIOWIN2 = 0.32; BIOWIN 3 = 2.61 and BIOWIN6 = 0.03.

3.1.3.1.2 Abiotic Degradation

Hydrolysis of CBS was studied in deionized water at pH 7 using a phosphate buffer system. Water was filtered through a 0.45 μm filter to minimize particulate catalysed hydrolysis. A solution of 1 mg/l (carrier: acetonitrile) was stirred 20 min and divided into aliquots for analysis and stored at ambient temperature wrapped with aluminium foil to exclude photodegradation. The extracted samples were analyzed by HPLC/UV-detector (total recovery 89.8 – 92.8 %) and GC/MS. 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 (Monsanto, 1984).

Hansson & Agrup (1993) analyzed test solutions containing 500 µM CBS in 0.5 M phosphate buffer and 10% tetrahydrofuran at pH 6.5. After 2 hours reaction time 70% CBS, 5% 2,2’-dithiobis-benzothiazole (MBTS), and trace amounts of 2-mercaptopbenzothiazole (MBT) were detected. When the experiment was repeated with MBT as the test substance, 60% of the MBT were converted to MBTS after 2 hours (cf. appendix A section 2.3).

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). A control kept in the dark at 23°C had a half-life of 9.6 hours. In both experiments, one transformation product was detected by HPLC but not identified (Monsanto, 1980).

The Monsanto study reveals that benzothiazole (BT) is the major product of CBS hydrolysis. Hansson & Agrup detected smaller amounts of MBTS, which was probably formed from MBT being initially formed. Photolysis of CBS resulted in the same product as in the dark control, i.e. probably BT was formed as the main product.

In the further exposure assessment, a half-life of 12.5 h (k = 0.055 h⁻¹) for CBS hydrolysis is used. Photolytic degradation cannot be considered, as only rates for the upper water layer in summer sunlight were determined and a degradation rate valid for real environmental conditions cannot be derived.

The indirect atmospheric oxidation rate by reaction with OH-radicals was estimated with TGD defaults to be 79.5 * 10⁻¹² cm³ molecule⁻¹ s⁻¹ by AopWin v1.91 and half-life thus 0.202 days.

### 3.1.3.1.3 Further Degradation Products

The breakdown products being released into the waste water and the atmosphere undergo further degradation reactions. An overview of the known reaction pathways is given in Fig. 3.1. All compounds included in the overview are detected in waste water and in environmental compartments; therefore they are included into the CBS risk assessment. Their data of environmental relevance found in the literature are referred in the respective Appendices.

Figure 3.1: Degradation products of CBS
3.1.3.1.4 Overview of the breakdown products

In this assessment, the following benzothiazole derivatives are considered as relevant for environmental exposure:

2-Mercaptobenzothiazole (MBT, cf. Appendix A)

The available data on MBT biodegradation reveal that mineralization occurs only when the inoculi are adapted. Such adaptation processes may occur e.g., in industrial or municipal biological treatment plants. It can be concluded from the degradation tests as well as from the available monitoring data that MBT removal in treatment plants can be considerable but not complete. In addition to MBT, releases of the products of primary transformation are expected.
MBT is mineralized via the pathway 2-benzothiazolesulfonic acid (BTSO\textsubscript{3}H) $\rightarrow$ 2-benzothiazolone (BTon) $\rightarrow$ CO\textsubscript{2}, H\textsubscript{2}O etc. The results of the available laboratory tests are largely dependent on the test conditions, and a prediction to which extent MBT is mineralized in industrial treatment plants is not possible. Different microorganism species seem to be responsible for the individual reaction steps, in some cases the reactions are inhibited by MBT. Therefore, it cannot be excluded that the intermediates BTSO\textsubscript{3}H and BTon are released by industrial sources.

Oxidation of MBT to MBTS is only expected when two MBT molecules can meet, i.e. the MBT concentration is high enough. This reaction might occur in waste waters at concentrations above 75-100 mg/l, but not at environmental levels.

Beside oxidation, MBT can be methylated to 2-methylthiobenzothiazole (MeSBT). This reaction is confirmed to occur both in industrial plants and in environmental samples, leading to releases of MeSBT, a metabolite which is ubiquitously present in the environment. MeSBT can be further oxidized, the products are described in appendix V section 2.2.

When released into surface waters, photolysis of MBT could be an important process leading to 2-benzothiazolone (BTon) and benzothiazole (BT) as the products. The rate of photolysis under environmental conditions cannot be derived.

2,2'$'$-Dithio-bis-benzothiazole (MBTS, cf. Appendix B)

The available tests on biodegradation reveal that MBTS is not mineralized. Instead of biodegradation, hydrolysis is expected. The fact that no oxygen uptake was observed can be explained by the poor degradability of the hydrolysis products (cf. the appendices A MBT, C BT, D BTon).

It can be concluded from the dark control of a photolysis study that under environmental conditions MBTS in aqueous solutions is hydrolyzed within a few days. Because of the poor water solubility, the reaction rate in slurries is largely decreased. In concentrations levels relevant for environmental exposure, MBTS is expected to be a product, as the oxidation back to MBTS can be excluded. End products of MBTS degradation are BT and BTon. MBTS degradation is accelerated by sunlight, probably the same products are formed.

Benzothiazole (BT, cf. Appendix C)

According to two OECD screening tests on ready biodegradability no final conclusion about the degradability of BT is possible because of the contradictory results. Tests simulating more realistic environmental conditions showed also inconclusive results. Therefore, no conclusion about the degradation in environmental compartments is possible. Monitoring results support the assumption that BT is stable, as it was detected in many aqueous compartments. As BT is volatile from aqueous solutions, volatilization from the water compartment may be a significant elimination mechanism.

No final conclusion about the end products is possible. While in several investigations BT was mineralized, other authors found 2-benzothiazolone (BTon) as a product of primary transformation.
2-Benzothiazolone (BTon, cf. Appendix D)
The biodegradation of BTon was not examined in standard tests. BTon is formed from 2-mercaptobenzothiazole in biological treatment plants. From available tests performed with inoculi being pre-adapted under special conditions it can be concluded that BTon is partially mineralized, but the rate of mineralization in unclear. The main parameter determining degradation in treatment plants is the MBT concentration. Under environmental conditions, degradation within weeks or months is expected.

2-Methylthiobenzothiazole (MeSBT, cf. Appendix E)
The available studies on biodegradation reveal that MeSBT is not mineralizable. Products of primary oxidation were detected in the environment, generally in lower concentrations than the parent substance. MeSBT is resistant to both direct and sensitized photolysis by sunlight. The poor degradability of MeSBT is supported by monitoring results which demonstrate the ubiquitous presence of MeSBT.

2-Methylbenzothiazole (MeBT, cf. Appendix F)
There are no tests available on both biological and abiotical degradation, therefore a conclusion about the degradability is not possible.

In the literature, further benzothiazole derivatives are tentatively described. Their concentrations, are below the concentrations of the derivatives considered above

Benzothiazolsulphenic acid (BTSO₃H; CAS 941-57-1) is an intermediate of MBT biodegradation which is formed in industrial and municipal treatment plants and in the environment (Klöpfer, 2005). The substance is not in industrial use. The compound hydrolyses under the formation of BTon. In the study of Klöpfer (2005), BTSO₃H was found in domestic waste water, municipal STP effluent and influent and urban storm water in the highest concentrations of all analysed benzothiazoles. The mean concentrations of BTSO₃H varied in the effluent analysis campaigns in Berlin between 0.99 µg/l and 2.10 µg/l. The concentrations of other benzothiazoles were 0.55 µg/l or lower. Stormwater from a highway contained BTSO₃H up to 55 µg/l making ca. 60% of the total benzothiazole concentration on a mass basis. BTSO₃H has not been detected in most of the earlier studies on benzothiazoles due to the analytical problems. The method developed by Klöpfer et al. (2004) using solid-phase extraction and liquid chromatography with mass spectrometer enables a better detection of this polar substance.

Using the QSARs contained in EPI Suite v3.12 (U.S. Environmental Protection Agency and Syracuse Research Corporation, 2004), estimates of the key properties were obtained for BTSO₃H. It has a very high water solubility (1:1), a logKow of −0.99 (for the undissociated form) a very low volatility (1.77 * 10⁻⁸ Pa m³/mol) and according to the ECOSAR v0.99 it is not toxic to aquatic organisms (acute effect values ≥ 473 g/l). As a conclusion, BTSO₃H is not expected to elicit any effects in the concentration range detected and expected in the aquatic environment.

Benzothiazolsulphenic acid methyl ester (MeSOBT) and benzothiazolsulphonic acid methyl ester (MeSO₂BT) are formed by oxidation of MeSBT. Both compounds were detected in the urban environment (Schmegel, 1995), although generally in lower concentrations than the
parent substance. It is assumed that a possible risk raised by these compounds is covered by the MeSBT assessment.

2-Morpholinobenzothiazole was detected in the environment by several authors (e.g. Spies et al., 1987). It is formed from morpholinobenzothiazole-2-sulphenamide (MBS). From the use of MBS, toxicity problems related to nitrosamines which are formed during vulcanization resulted in a dramatic decline in MBS production. MBS is gradually being replaced by other benzothiazole sulphenamides (Srour, 1994).

2-N-cyclohexylaminobenzothiazole is a thermal decomposition product of CBS (Gradwell & McGill, 1994). The compound was detected in road dust, runoff- and river water particles and river sediments (Kumata et al., 2000). The concentrations in environmental compartments are much lower than of the relevant derivatives.

During vulcanization, cyclohexylamine is formed as a breakdown product of CBS. Releases of cyclohexylamine are not considered in the environmental assessment. This compound is a HPVC chemical. The production/import is according to the IUCLID (2000) 10 000 – 50 000 t/a.

3.1.3.2 Distribution

The distribution of CBS in a “unit world” was calculated according to the Mackay fugacity model level I (Mackay, 1991) considering the values for vapour pressure (1.5.10^{-6} \text{ Pa}), log Kow (4.93) and water solubility (0.32 mg/l). The main target compartments were estimated to be sediment (51.5 %), soil (44.2 %) and water (4.1 %).

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 m^3/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 hydrofobics).

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.

PCKOCWIN v1.66 predicts a Koc of 18 890 l/kg, but the first value is applied for the modelling.

Other Benzothiazole Derivatives

The studies about the distribution properties of the CBS degradation products are presented in the appendices I – VI. In the following table, important distribution parameters are summarized:

Table 3.4 Distribution parameters of CBS breakdown products

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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Air</td>
</tr>
</tbody>
</table>

RAPPORTEUR GERMANY

31

R035_HH_ENV_0805.DOC
2-Mercaptobenzothiazole (MBT) 118 (pH 7) <2.6·10⁻⁸ 1.4 56 <8 0% 99.6% 0.2% 0.2%
2,2’-Dithio-bis-benzothiazole (MBTS) <0.2 5.97·10⁻⁸ 4.5 2290 ≤51 <0.1% 17.1% 42.8% 40.0%
Benzothiazole (BT) 3000 14 2.0 115 ≤7.5 11.0% 86.8% 1.1% 1.0%
2-Benzothiazolone (BTon) 690 4.3·10⁻⁶ 1.76 86 5 0% 99.1% 0.5% 0.4%
2-Methylthiobenzothiazole (MeSBT) 125 0.1 3.1 429 49 1.4% 46.1% 51.4% 1.14%
2-Methylbenzothiazole (MeBT) 513 9.47 2.47 201 16 47.7% 50.1% 1.2% 1.1%

* PCKOCWIN predicts for the compounds significantly higher Koc-values than equations listed in the TGD.
** Level I uses an own Koc-estimate which may differ from the Koc chosen for the assessment.

3.1.3.3 Bioaccumulation

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 bioaccumulation potential of the CBS degradation products is evaluated in the Appendices I – VI; in Table 3.4 the BCF values are included.

3.1.4 Aquatic compartment (incl. sediment)

The log Kow of 4.93 indicates relevant accumulation potential of CBS in sediments. However, because of the rapid hydrolysis (half-life 12.5 h) real accumulation is not expected. Therefore a risk assessment for this sub-compartment is not necessary.

The exposure of sediments by the group of benzothiazole derivatives cannot be assessed because neither representative monitoring data in sediments nor effect tests on sediment organisms are available. Both exposure and environmental effects could be estimated using the equilibrium partitioning method. As this approach leads to the same PEC/PNEC ratios as in water, a specific assessment for sediment is not provided.

3.1.4.1 Calculation of predicted environmental concentrations (PEClocal)

3.1.4.1.1 Calculation of PEClocal for production

For calculating the Clocal,aqua for the sites which provided information, the dilution of the waste water in the river is considered according to

\[ C_{\text{local,water}} = C_{\text{local,eff}} \cdot D \quad \text{with} \quad D = \frac{Q_{\text{ww}}}{Q_{\text{ww}} + Q_{\text{river}}} \]
The PECregional was calculated to 0.011 µg/l (cf. 3.1.5). In the following table, the estimated concentrations, emission volumes, and the underlying specific data are presented:

### Table 3.5 PEClocal for CBS during production

<table>
<thead>
<tr>
<th>Site</th>
<th>Site-specific data</th>
<th>Defaults</th>
<th>Ceff [µg/l]</th>
<th>PEClocal water [µg/l]</th>
<th>Emission to surface water [kg/a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Effluent conc. (90%ile), production period, sewage and river flow</td>
<td>river</td>
<td>6</td>
<td>0.08</td>
<td>6.3</td>
</tr>
<tr>
<td>B</td>
<td>Effluent conc. (90%ile), sewage and river flow prod. period 300 d/a</td>
<td>river</td>
<td>&lt; 100</td>
<td>&lt; 0.13</td>
<td>&lt; 108</td>
</tr>
<tr>
<td>C</td>
<td>Effluent conc. (mean), production period, sewage and river flow</td>
<td>river</td>
<td>&lt; 10</td>
<td>0.03</td>
<td>6.2</td>
</tr>
</tbody>
</table>

The CBS emission into the hydrosphere at the 3 production sites makes together < 121 kg/a. Effluent concentration is used in all sites as PECstp.

For the calculation of PECregional, site specific information is used. Further information is confidential.

**Benzothiazole Derivatives**

All relevant benzothiazole derivatives have been measured recently in the effluents of plant A, B and plant C. In Table 3.6, effluent concentrations (90-P or maximum values) together with the resulting Clocals are presented.

### Table 3.6 Local concentrations for benzothiazole derivatives in surface water during CBS production

<table>
<thead>
<tr>
<th>Substance</th>
<th>Site</th>
<th>Ceffluent [µg/l]</th>
<th>Clocal [µg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Mercaptobenzothiazole (MBT)</td>
<td>A</td>
<td>&lt;20</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>6.4</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>183.8</td>
<td>0.477</td>
</tr>
<tr>
<td>2,2'-Dithio-bis-benzothiazole (MBTS)</td>
<td>A</td>
<td>&lt;20</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>106</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>&lt; 10</td>
<td>&lt;0.026</td>
</tr>
<tr>
<td>Benzothiazole (BT)</td>
<td>A</td>
<td>&lt;10</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>80</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>&lt; 10</td>
<td>&lt;0.026</td>
</tr>
<tr>
<td>2-Benzothiazolone (BT)</td>
<td>A</td>
<td>&lt;10</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>70</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>47.0</td>
<td>0.122</td>
</tr>
</tbody>
</table>
3.1.4.1.2 Calculation of PEC<sub>local</sub> during use

Rubber and Tire Industry

The emission scenario document on additives in rubber industry (OECD 2004) states based on underlying data that for vulcanisation accelerators releases into waste water do not occur. This result is based on the assumption that the fraction of accelerators remaining in vulcanised rubber is one. Correspondingly, no estimation on exposure is presented.

Benzothiazole Derivatives

There are some monitoring data of benzothiazole derivatives in waste water of rubber and tire manufacturers available:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Mercaptobenzothiazole (MBT)</td>
<td>0.59 mg/l (average)</td>
<td>Effluent, tire plant</td>
<td>CMA (1985)</td>
</tr>
<tr>
<td></td>
<td>30 µg/l</td>
<td>Effluent, tire plant</td>
<td>Jungclaus et al. (1976)</td>
</tr>
<tr>
<td>Benzothiazole (BT)</td>
<td>20 / 60 µg/l</td>
<td>Effluent from 2 tire plants</td>
<td>Jungclaus et al. (1976)</td>
</tr>
<tr>
<td>2-Benzothiazolone (BTon)</td>
<td>No concentration reported</td>
<td>Groundwater at a rubber producer</td>
<td>Lesage (1991)</td>
</tr>
</tbody>
</table>

This older monitoring data from processing plants don’t reflect the current situation in the EU where releases into waste water are not expected according to the emission scenario document on additives in rubber industry (cf. 3.1.1.1).

Use of tires

During vulcanization, CBS completely breaks down, therefore releases of CBS into the environment are not expected from tires. Benzothiazole derivatives included in the rubber matrix are released into the environment first in tire tread particles. The particles reach the hydrosphere with runoff and soil with drift caused by turbulence from wind and cars. The compounds enter the environment from the particles either by leaching with rainwater or by degradation of the rubber matrix.
No estimate of the total releases of benzothiazole derivatives from tires is available. For an estimation of CBS' contribution to the releases of these degradation products from tires several assumptions have to be made. In Germany, tire abrasion was estimated to be 65,000 t/a by Bauman and Ismeier (1998). For the regional model, the amount is divided by four (the approximate relation of the amount of German population of ca. 80 million and the population in the default region of 20 million). Thus the regional tire tread particle volume is 16,250 t/a. According to Table 2.2, CBS contributes 54% of the usage of benzothiazole and sulphenamide group as vulcanization accelerator. This substance group makes 79% of the total rubber accelerator usage. The amount of CBS added into the rubber during vulcanization is 0.75% (concentration range average). These shares are assumed to be representative also for an average composition of tire tread dust. Consequently, the annual regional release of abraded tire particles contains 52 t/a breakdown products calculated as added CBS. The annual release from tire dust particles is assumed to be roughly the same as the amount entering the road technosphere in tire dust.

For the continental release, the European sales figures and a tire abrasion rate (11.5%) from the report of Blok (2002) are used. A tire dust volume of 359,014 t/a results for Europe which corresponds to an amount of 11,49 t/a added CBS. The release amount for the continental technosphere is obtained by subtracting the European volume of 11,49 t/a by the regional volume of 52 t/a. Thus a continental volume of 1097 t/a as added CBS is gained. It has to be noted, that the estimate of Blok (2002) for the European abrasion amount would give approximately a too higher regional emission compared to the approach using German abrasion amount, if the default 10% of the European emissions is allocated to the region. This makes a difference in the screening approach of MeSBT for regional PEC in water, which would cause with this higher estimate a RCR of ca. 1. However, it does not change the conclusions regarding MeSBT and thus the German amounts are used further.

Blok (2002) considered the fate of Zn release from road. Both ZnO and vulcanization breakdown products can be assumed to initially be released in particle bound form. The vast majority of the emission of Zn from road comes from tires. Therefore the fate of Zn and decomposition products of CBS is to some extent comparable.

Zn was assumed for the model development of Blok (2002) to be emitted via runoff and via drift in the relation of 2:1 from the road. The proportion of solids and Zn emitted from roads via runoff and drift are varying considerably and it is difficult to estimate. The size of the load transported by runoff is best described according to the literature by the share of impervious surfaces of the catchment basin area (e.g. Novotny and Olem, 1993; Melanen, 1981). The POLMIT report on the emissions from highways (Transport Research Laboratory, 2002) saw in turn a relation between the traffic intensity and pollution load of the road side soils.

Only one attempt was found in the literature to describe the removal of solids from the street surface as a function of wind and traffic velocity. The equation presented by Novotny et al. (1985) was derived from measured data from Washington D.C. area and related the removal also to the height of the curb. The model was developed for the time scale of dry periods between rain events and is therefore not applicable for estimation of drift removal on an annual basis. For the estimation of regional and continental emissions from roads to soils and surface waters the relation chosen by Blok (2002) is assumed for this assessment. Correction regarding to drift depositing on hard shoulder of the road as presented by Blok (2002) is not considered in the total release estimation.

In Germany, ca. 50% of the urban areas are connected to separate storm water sewers, the rest of the rainwater is directed to the municipal stp. The EU does not have common
legislation in place for the treatment of urban storm water. Accordingly, many of the Member States do not have “best management practices” for urban storm water treatment in place systematically. Therefore for the regional and continental release via runoff 50 % of the release can be assumed to be directed into the storm sewers without treatment (thus directly into the surface waters) and 50 % to the municipal stp.

The regional and continental releases from tires are estimated according to the assumptions above in the two screening approaches which calculate PECs for Benzothiazole and 2-Methylthiobenzothiazole. The results are presented in the Appendices III and V.

Measured data

Klöpfer (2005) measured in August 2003 concentrations of benzothiazoles from highway runoff during and after one rain event. The highway has a traffic density of ca. 200 000 ADI and stormwater runoff is drained from the road area to an urban lake. Altogether 19 samples were taken. The maximum flow rate in the drainage line during the sampling time was 340 m³/h. The benzothiazoles measured were 2-mercaptopbenzothiazole, benzothiazole, 2-benzothiazolone, 2-methylthiobenzothiazole, aminobenzothiazole (ABT) and benzothiazolsulphonic acid (BTSA). Concentrations of BTSA were between 10.6 and 55.6 µg/l, whereas concentrations of the second most abundant substance BTon were between 5.1 and 12.0 µg/l. Measured values concerning the substances of this assessment are presented in Table 3.7 and in Appendices I-VI.

The concentrations of benzothiazole derivatives in road runoff and receiving waters were measured by several authors. Baumann & Ismeier (1998) sampled rainwater in the drainage system of a highway bridge during 3 rainfall events, from beginning of rainfall to 30 minutes after beginning. The concentrations of benzothiazole and 2-methylthiobenzothiazole increased with the duration of the rainfall, while 2-mercaptopbenzothiazole and 2-methylbenzothiazole were not detected.

Reddy & Quinn (1997) measured in the U.S. benzothiazole and 2-benzothiazolone in 11 samples of urban runoff draining into rivers collected during storms. During one storm, five sequential samples were collected, and the fluxes of the compounds were presented. For both compounds the fluxes were highest during the first 20 minutes and decreased slowly within 4 hours. Additionally, 7 samples were collected in a highway settling pond. They demonstrated that in highway settling points the concentrations of benzothiazole and 2-benzothiazolone are significantly lower than in road runoff. Dilution, distribution and degradation processes lead to the observed decrease in the concentration levels from road onwards. Similar results were obtained by Krumwiede & Jastorff (2002) which measured benzothiazole, 2-methylthiobenzothiazole and 2-benzothiazolone in urban road puddles and in road drainage canals in Bremen, Germany.

Schmegel (1995) measured a number of benzothiazole derivatives in different urban media. The highest concentrations were detected in the water phase of canalization sludge, the samples were collected in a cleaning vehicle. Further samples were collected in an urban park lake and in ditches with flowing water near highly frequented roads and highways. The duration since the last rainfall is not known.

Table 3.8 Concentrations of Benzothiazole Derivatives in Road Runoff and receiving waters [µg/l]:

<table>
<thead>
<tr>
<th>Medium</th>
<th>Benzothiazole (BT)</th>
<th>2-Benzothiazolone</th>
<th>2-Methylbenzothiazole</th>
<th>2-Methylthiobenzothiazole</th>
<th>Reference</th>
</tr>
</thead>
</table>
### Abbreviations

- MBT: 2-mercaptobenzothiazole
- BT: benzothiazole
- BTon: 2-benzothiazolone
- MeBT: 2-methylbenzothiazole
- MeSBT: 2-methylthiobenzothiazole

### Calculation of PEC<sub>local</sub> for disposal

#### Releases from tire recycling – tire crumb in ground materials

The findings of i.a. Dye et al. (2004), Azizian et al. (2003), Baumann and Ismaier (1998) and Baumann et al. (1998) show that benzothiazole derivatives can be expected to leach from ground materials containing tire crumb and that the concentrations may be in the mg per litre level (see details in chapter 3.1.1.1.e). Direct measurements of releases of tire crumb uses are, however, not available and a local assessment could not be conducted. Releases of 2-mercaptobenzothiazole, benzothiazole, 2-benzothiazolone, 2-methylthiobenzothiazole and 2-methylbenzothiazole can be expected. A leaching study measuring the release of these
substances from tyre crumb used in ground materials would be necessary to be able to estimate the exposure and total release. In addition, information on the amounts of recycled rubber used for these purposes is needed.

**Releases from Landfills**

Used tires and rubber goods have been partially deposited over the years into landfills. Deposition of whole tires was banned in the year 2003 and deposition of shredded tires from July 2006. Releases of CBS breakdown products into surface waters from leaching can be expected. Old landfills may cause releases into groundwater via infiltration. An overview of the conditions in operational and closed landfills in the EU is included in the targeted risk assessment of NiCd-batteries (TRAR 2003). Consequently the compounds were measured in landfill leachate. Data found in the literature is presented in Appendices III-VI.

Concentrations in the latest study from four German landfills were for BT <0.1-2.35 µg/l, for BTon < 1-525 µg/l, for MeSBT <0.1-1.38 µg/l and for MeBT <0.1 µg/l. The samples were all single samples (Krumwiede, 2002). Two samples originated from leachate, two from groundwater next to the landfill. The findings from groundwater confirm that leachate contained benzothiazole derivatives. Schmegel (1995) measured from a single sample from one of the landfills of the previous study concentrations of 65 µg/l (BT), 14 µg/l (BTon), 96 µg/l (MeSBT) and 80 µg/l (MeBT). The few available data from Europe and elsewhere indicate concentrations in the range of µg/l for the four substances but some significantly higher values are also available. However, the quality and amount of measured data are too low to conclude on a general level on the risks due to exposure from landfill leachate.

Regional and continental releases from landfills were not estimated. For such an estimation, more measured data should be made available. Considering the available data and the amount of landfills in Europe, total releases from landfills may be relevant for the ambient background concentrations.

### 3.1.5 Terrestrial compartment

#### 3.1.5.1 Calculation of PEC\(_{\text{local}}\)

#### 3.1.5.1.1 Calculation of PEC\(_{\text{local}}\) for production

Significant amounts of CBS dust are released during production. This dust will reach the soil in the vicinity of the production sites by wet and/or dry deposition. Because of the instability of CBS against hydrolysis the exposure is expected to be negligible. Exposure of soil due to deposition (no sludge application is assumed for production) is estimated for the two major degradation products benzothiazole and 2-methylthiobenzothiazole in Appendices III and V.

#### 3.1.5.1.2 Calculation of PEC\(_{\text{local}}\) during use

**Rubber industry**
During vulcanization at least benzothiazole (BT) and 2-methylbenzothiazole (MeBT) may be released into the atmosphere. Deposition from the atmosphere in the vicinity of rubber and tire manufacturers cannot be excluded. Exposure via sludge application is assumed to be zero according to the emission scenario document for rubber industry (OECD 2004), which assumes no releases into waste water. The exposure from emissions to air has been evaluated in the screening approaches (see Chapter 3.3 and Appendices III and V).

Use of tires

Abraded tire tread particles are transported via the air or rainwater runoff into soils near roads. Baumann & Ismeier (1998) measured benzothiazole derivatives in soil samples near 9 roads with high traffic density and at an international car racing track. The results from roads are presented in Table 3.11. The highest concentrations were detected at the edge of highways (samples from 8 roads). Parallel measurements of PAHs demonstrated that their concentrations at distances of 5-10 m were decreased to 1-2% of the concentrations at the edge (0 m distance), thus it can be assumed that the concentrations of the benzothiazole derivatives will also decrease largely with the distance. This assumption is also supported by the measured data presented in the Risk Assessment of Zinc.

**Table 3.9 Concentration of Benzothiazole derivatives near roads (Baumann & Ismeier, 1998):**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration [mg/kg dw]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzothiazole (BT)</td>
<td>&lt;0.5 – 17.4</td>
</tr>
<tr>
<td>2-Methylbenzothiazole (MeBT)</td>
<td>&lt;0.5 – 4.63</td>
</tr>
<tr>
<td>2-Methylthiobenzothiazole (MeSBT)</td>
<td>&lt;0.5 – 9.15</td>
</tr>
</tbody>
</table>

Following the decision of the Competent Authorities on the basis of the document JM/56/2003, the exposure assessment should be based on the concentrations measured at a distance of 5 – 6 m to the edge of a highway. Measurements at higher distances (5m and 10m) were performed at four roads. In these samples the concentrations were at, below but identified or far below the detection limit (LOD) of 0.5 mg/kg dw. The detection limit is still four times higher than the highest of the derived PNECs of the three analysed substances. Thus, results from the distance of 0 m are used for the assessment. It can be assumed that their concentrations decrease with the distance to the same extent as the PAH concentrations. Therefore, the initial exposure assessment is based on 2% of the measured concentrations at the edge. The 90-P values of the measurements are used as provisional PECs (values < LOD handled as 0.5 mg/kg dw).

**Table 3.10 PEClocal,soil for the road borders.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>90-P of measured concentrations [mg/kg dw]</th>
<th>PEClocal,soil [µg/kg dw]</th>
<th>PECporew. [µg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzothiazole (BT)</td>
<td>16.8</td>
<td>336</td>
<td>140</td>
</tr>
<tr>
<td>2-Methylbenzothiazole (MeBT)</td>
<td>1.7</td>
<td>34</td>
<td>13</td>
</tr>
<tr>
<td>2-Methylthiobenzothiazole (MeSBT)</td>
<td>4.3</td>
<td>86</td>
<td>10</td>
</tr>
</tbody>
</table>
No agreed model for the estimation of the concentrations in soil in the road border environment is available. The PECs from measured data should be considered as a rough estimate because they originate from the only available study. More measured data is indeed needed in order to get a better overview of the exposure in soils.

The total release estimates from roads for BT and MeSBT are presented in the screening approaches (see Appendices III and V). The assumptions for the calculation of the regional and continental releases are presented in detail in Chapter 3.1.2.2. It is assumed that the complete release transported via drift (1/3 of the total release from tires) is deposited onto soil near road border. This assumption is supported by the measured data from the road side soils (cf. e.g. the risk assessment of Zn, Draft of Dec 2004 or Blok, 2002). The fraction transported further by air is considered to be zero for the regional and continental exposure estimation.

3.1.6 Atmosphere

3.1.6.1 Calculation of PEC_{local}

3.1.6.1.1 Calculation of PEC_{local} for production

As the vapour pressure of CBS is very low, the substance (as acid or salt) can be released in dust form only. The site-specific data submitted by the producers are:

<table>
<thead>
<tr>
<th>Site</th>
<th>Release amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>780 kg/a</td>
</tr>
<tr>
<td>B</td>
<td>&lt; 147 kg/a</td>
</tr>
<tr>
<td>C</td>
<td>Negligible</td>
</tr>
</tbody>
</table>

The local exposure is calculated for the site A. The PEC_{air} is calculated with the defaults of the TGD for distribution on the basis of the information on production period:

- Release amount: 780 kg/a
- Production period: 330 d/a
- \( \rightarrow \) Daily release: 2.36 kg/d
- \( \rightarrow \) \( C_{local}^{air} \): 0.66 µg/m³

The default exposure scenario for the production step assumes no emission to air. The emission from the site A was allocated to regional model and the emission of 147 kg/a from site B was allocated to the continental model.

Other Benzothiazoles

During production of benzothiazole derivatives, volatile by-products can be released into the atmosphere. In Canada, benzothiazole (BT) was monitored in the vicinity of a site producing...
chemicals for the rubber industry, the detected concentrations 50 m downwind of the company buildings were maximum 500 ppb (2.8 mg/m3). The compound was being emitted from a vent stack approximately 15 m high (Lane et al., 1980; Lane, 1982).

All three CBS production sites have provided either measured data on BT in the surrounding air of the plant or information regarding to releases. A concentration of 0.03 mg/m3 is chosen as a representative realistic worst case estimate for the concentrations in surrounding air of CBS production sites.

### 3.1.6.1.2 Calculation of PEC_{local} for use

#### Rubber and tire industry

CBS is a non-volatile compound, therefore releases into the atmosphere are not expected. In the following a generic scenario and experimental data are presented.

The emission scenario document (ESD) on additives in rubber industry (OECD 2004a) proposes for vulcanization agents with a vapour pressure under 100 Pa an emission factor of 0.075 to air. Using the production volume of 55 000 kg/d proposed for a generic rubber industry site, content of 0.75 % of CBS in rubber and the equation 2 for emission calculation from the ESD, an emission of 31 kg/day is obtained. Using EUSES 2.0.3 with TGD’s default parameters for exposure estimation, a $C_{local\text{air}}$ of 8.62 µg/m3 results.

Assuming that 10 % of the CBS use in the rubber sector (totally 20 000 t/a) occurs in the region, a regional emission of 150 t/a and 1350 t/a continental emission are calculated for this generic scenario. It should be noted that this calculation assumes that undegraded CBS is emitted. While CBS is degraded during the curing process, these estimates are for information and for the use as the starting point in the screening assessments for BT and MeSBT, only.

#### Benzothiazole derivatives

During vulcanization volatile breakdown products are formed. In laboratory vulcanization experiments, benzothiazole (BT) and 2-methylbenzothiazole (MeBT) were detected in the exhaust gas. It is likely that the predicted CBS-emission above will enter the environment completely reacted to its breakdown products.

According to the information of BLIC (2005), BT and MeBT were measured in a “Nordic curing fumes” -project. The summary report of the project provided to the Rapporteur gave an estimation of the concentration of BT in vulcanization smoke in the factory area calculated for a factory producing 25 000 t rubber products in a year. The estimate was 0.06 µg m$^{-3}$ (gas phase). The concentration estimated for a day care centre at a distance of 200 m from the source was 0.04 µg m$^{-3}$in air (gas phase). Based on results, concentrations of MeBT in air can be assumed to be lower than the estimated concentrations of BT.

For comparison, emissions to air were estimated using the weight of loss (mean 0.05%) from the Nordic study, the defaults of the emission scenario document for rubber industry (OECD 2004a) and the emission scenario document for plastic additives (OECD 2004b). 0.6 t/a regional release, 5.0 t/a continental release and 35 kg/a local release and $C_{local\text{air}}$ of 0.03 µg m$^{-3}$. More details on the calculations are provided in Appendix C.
3.1.6.1.3 Calculation of PEC\textsubscript{local} for disposal

Releases from tire recycling – tire crumb in ground materials

On the basis of the study of Dye et al. (2006) on the concentrations of BT in sport halls air, a rough local assessment was conducted (see Appendix C). C\textsubscript{localair} of 0.023 µg m\textsuperscript{-3} at a distance of 100 m and an annual emission of 30 kg a\textsuperscript{-1} from one sport hall were estimated.

The other benzothiazole derivatives measured indirectly in the fine particulate matter in air were present in concentrations lower than 1 ng m\textsuperscript{-3} and can therefore be assumed to not cause relevant exposure of environment.

The results of the study of Dye et al. (2006) can be used to approximate roughly total release of BT to air from outdoor sport grounds, playgrounds and other similar uses. The amount of uses applying tire crumb is not available and the emission to the regional model could therefore not be calculated. It can be, however, expected that the amount of these uses is very high and they may be a relevant diffuse contributor to the background levels of BT.

3.1.7 Non compartment specific exposure relevant to the food chain

The log K\textsubscript{ow} of 4.93 indicates high accumulation potential of CBS via the food chain. However, because of the rapid hydrolysis (half-life 12.5 h) significant accumulation is not expected. Exposure via aquatic food chain is estimated for the situation based on the PEC\textsubscript{s} for plant A (PEC\textsubscript{local} = 0.1 µg/l; PEC\textsubscript{regional} = 0.01µg/l). According to the Technical Guidance Document (TGD), a BMF of 2 and BCF of 3094 are used for the calculation:

\[ \text{PEC}_{\text{oral,fish}} = 0.309 \text{ mg/kg} \]

From the benzothiazole derivatives found in the environment, only 2-methylthiobenzothiazole (MeSBT) could be relevant for this endpoint while having a log K\textsubscript{ow} of about 3.1 and measured BAFs for leeches between 100 and 400 (see Appendix V). Considering that the bioaccumulation potential of CBS is significantly higher than the bioaccumulation potential of MeSBT, and on the other hand, CBS is estimated to be very much more ecotoxic than MeSBT, it is assumed that the secondary poisoning assessment of CBS covers also the possible risks caused by MeSBT.

The other exposure-relevant derivatives have lower octanol-water partition coefficients and are not expected to bioaccumulate.

3.1.8 Calculation of PEC\textsubscript{regional} and PEC\textsubscript{continental}

For the regional exposure assessment of CBS, releases from production and use as vulcanization accelerator in rubber industry were considered in previous chapters. In the following table, the emissions and the resulting PEC\textsubscript{s} are presented:

<table>
<thead>
<tr>
<th></th>
<th>t/a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regional emission to air</td>
<td>150.7</td>
</tr>
<tr>
<td>Regional emission to surface water</td>
<td>Confidential</td>
</tr>
<tr>
<td>Regional emission to industrial soil</td>
<td>0.2</td>
</tr>
</tbody>
</table>
The CBS concentrations were calculated based on the default regional and continental scenarios as provided by EUSES 2.0.3 using site specific information from CBS production and the generic scenario for rubber industry. The regional exposure is presented below for the relevant compartments (see Table 3.13). It should be noted, that concentrations in water were obtained by taking into account the fast hydrolysis time. Thus on the top of these concentrations, other benzothiazole derivatives and further degradation products have been formed. Concentration in soil and in air is an overestimation due to the fact that the emission to air from rubber industry has here been calculated as CBS-emission. In reality, instead of CBS its breakdown products are formed in the curing process.

Table 3.13 Regional concentrations for CBS.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>PEC (µg/l or µg/kg wwt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regional PEC in surface water</td>
<td>0.01</td>
</tr>
<tr>
<td>Regional PEC in sea water</td>
<td>4 * 10^{-4}</td>
</tr>
<tr>
<td>Regional PEC in air</td>
<td>1.84 *10^{-3}</td>
</tr>
<tr>
<td>Regional PEC in agricultural soil</td>
<td>299</td>
</tr>
<tr>
<td>Regional PEC in pore water</td>
<td>1.4</td>
</tr>
<tr>
<td>Regional PEC in natural soil</td>
<td>310</td>
</tr>
<tr>
<td>Regional PEC in industrial soil</td>
<td>339</td>
</tr>
<tr>
<td>Regional PEC in sediment</td>
<td>5.7</td>
</tr>
<tr>
<td>Regional PEC in sea water sediment</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Other Benzothiazoles

Especially benzothiazole (BT) and 2-methylthiobenzothiazole (MeSBT) seem to be ubiquitously present in the regional environment according to the measured data, although mainly in low concentrations. They were detected in river water, river sediment, seawater and in the Antarctic glacier ice.

Alternative regional PECs for freshwater and seawater were derived on the basis of measured data on BT and MeSBT. The data used for surface water –PEC originates for BT from River Gallego (Spain), Rhein Delta (The Netherlands) and Elbe (Germany) and for MeSBT from the River Elbe (Germany), the River Rhine (the Netherlands) and the River Meuse (The Netherlands). The sample and site amounts are given in the Appendices III and V. The data for seawater –PEC originates from German Bight from six representative sampling locations.

Due to the small amount of data, the PECs were derived as 90-P of the results of all samples from all sites. The exception was Elbe data of Fooken et al., 1996, from which averages of each site were included. The non-detects were handled as zeros. The data from freshwater vary for BT between non-detects and 24 ng/l and for MeSBT between non-detects and 380 ng/l. For the seawater the variation range is for BT 0.03-1.23 ng/l and for MeSBT 0.16-1.37...
ng/l. All the measurements used were made in the early- or mid-1990s and it should be noted that the use of benzothiazole derivatives as vulcanisation accelerators has increased significantly since then. **PEC_{water}** is 23 ng/l for BT and 61 ng/l for MeSBT. **PEC_{marine}** is 0.82 ng/l for BT and 0.99 ng/l for MeSBT.

### 3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT ASSESSMENT)

#### 3.2.1 Aquatic compartment (incl. sediment)

For the effects assessment of CBS on aquatic organisms it has to be taken into account that the test substance concentrations were generally above the water solubility (0.32 mg/l). Only one test was conducted without carrier solvent and CBS was in this test observed to form a precipitate in the test chambers. In other tests using a solvent, the test substance can be assumed to have dissolved completely.

It was concluded in section 3.1.1.2 that dissolved CBS is rapidly degraded by hydrolysis and photolysis, the hydrolysis half-life was determined to 12.5 h and complete degradation was observed at 24 h. It is expected that in static tests the observed effects are caused partly by the degradation products rather than by the parent substance. Analytical measurements reveal that in a flow-through system the influence of hydrolysis and photolysis could be minimized (Monsanto, 1981).

If the present quality criteria are strictly applied, the static acute tests, where no monitoring of the test concentrations occurred, should be regarded as not valid. The main degradation products appearing during the time frame of acute tests are benzothiazole (BT) and benzothiazolone (BTon). BT and BTon are roughly one order of magnitude less toxic than CBS (see Appendices III and IV). Thus the results of the static tests with nominal concentrations can be considered as the upper limit for the actual effect value.

#### 3.2.1.1 Toxicity test results

##### 3.2.1.1.1 Fish

A static bioassay to *Pimephales promelas* was conducted by Monsanto (1979b). Duplicate samples with each 10 fish were exposed to the test substance solutions. The test concentrations were obtained by transferring the TS directly to the test chambers. The compound was observed to form a precipitate in the test chambers. Analytical control was not performed. After an exposure period of 96 h the LC50 value was above the highest nominal concentration (1000 mg/l). Because of precipitation and hydrolytic degradation of the test substance, this test is considered as not valid.

The toxicity of CBS to *Oncorhynchus mykiss* was tested in a flow-through system. The fish were exposed to 5 nominal test substance concentrations between 0 and 1.0 mg/l. The flow rate was 5 tanks volume/day. Acetone was used as carrier. Analytical measurements revealed a good agreement between nominal and actual levels during the test period. After 14 days of exposure the mortality was 20% at the highest concentration tested (0.96 mg/l effective
A LC50 cannot be determined because of missing data. A gradual increase in mortality with increase in exposure time was observed, indicating a potential for cumulative toxicity (Monsanto, 1981).

In addition, a static 96 hour acute toxicity test on *Salmo gairdneri* and *Lepomis macrochirus* has been reported by Monsanto (1976). However, the oxygen saturation in this study was at the end of the test below 30 % for both species and the study is considered to be not valid.

### 3.2.1.1.2 Aquatic invertebrates

The acute toxicity of CBS to *Daphnia magna* was determined in a static immobilization test after an exposure period of 48 h. Duplicate samples with each 10 individuals of less than 24 h old daphnids were exposed to five test substance concentrations. Acetone was used in preparation of all working stock solutions. Analytical control was not performed. The nominal LC50 was reported to be 18 mg/l, i.e. far above the water solubility of 0.32 mg/l (Monsanto, 1979a).

### 3.2.1.1.3 Algae

A phytotoxicity test was performed to determine the effect of CBS on the freshwater alga *Selenastrum capricornutum* (Monsanto, 1979c). Test concentrations used were 0.1, 0.3, 0.6, 1.0 and 3.2 mg/l. Triplicates were run for each test concentration and solvent control. Test temperature was 24 ± 1 °C and pH range was 7.2-7.5 measured at the start and the end of the test separately for each test concentration. Dimethylformamide (DMF) was used to prepare a primary stock solution. Student’s T-test was applied for the estimation of the results. The calculated 96h-EC50 were 1.1 mg/l (0.3-3.8 mg/l at the confidence level of 95 %) based on decrease of in vivo chlorophyll a and 0.9 mg/l (0.3-3.2 mg/l at the confidence level of 95 %) referring to the growth rate. NOECs or EC10 values were not determined. Decrease of chlorophyll a was 4% at 0.3 mg/l, the cell number decreased by 8% at the same concentration. All effect values are based on nominal concentration only. Taking into account the instability of CBS against hydrolysis and photolysis it can be assumed that after 96 hours of test duration only minimal amounts of CBS are available in the test system. Therefore it can be concluded that 96h-EC50 < 0.9 mg/l for CBS.

### 3.2.1.1.4 Microorganisms

An activated sludge respiration inhibition (OECD 209) test has been carried out with a commercial product sample of CBS. The substance was tested in the concentrations of 100 mg/l, 1000 mg/l and 10 000 mg/l. The test concentrations are above the water solubility of 0.32 mg/l. The test vessels had temperatures between 20.2 – 22.8 °C. Inhibition of the respiration rate of the activated sludge over 3 hours was determined. Two control and two reference substance vessels (3,5-dichlorophenol at concentrations of 1 and 20 mg/l) were included.

The EC50 for the reference substance was determined as ca. 6 mg/l, which is within the normal range of 5 to 30 mg/l for this test. A respiration inhibition of 4 % was observed in the test concentration of 1000 mg/l and an inhibition of 11 % in the concentration of 10 000 mg/l. Thus it can be concluded that EC50 > 10 000 mg/l.
3.2.1.2 Quantitative Structure-Activity Relationships (QSARs)

Due to the unstable sulphur-nitrogen bond CBS can be expected to be unselectively reactive in the target organism. Thus excess ecotoxicity compared to the baseline toxicity could be expected. The high logKow of 4.93 indicates also high toxicity.

Data on benzothiazole derivatives have so far not been used for the development of QSAR-models. Ecotoxicity of CBS was estimated with ECOSAR v0.99h (U.S.EPA, 2004) and according to the classification scheme of Verhaar et al. (1992). The results are presented in Table 3.15.

ECOSAR v0.99h assigns CBS to the group of neutral organics. Of the other groups, the equations of benzothiazolines (thiazolinones) could also be appropriate for CBS. The equations for benzothiazolines were derived for fish and daphnids based on one data point each, and thus the accuracy of the equations is questionable. The predictions are in the same range with predictions from neutral QSARs in Table 3.16.

The second way of estimation applies the approach of Verhaar et al. (1992). In this system, CBS is considered to belong to the class 3 (“reactive chemicals”) (Bol et al. 1993). The baseline toxicity was first calculated using appropriate QSARs for non-polar substances. For this purpose, the models of Verhaar et al. (1995) and Van Leeuwen et al. (1992) as recommended in the TGD (Part III, Section 4.1, Table 1) were used. The resulting estimates (see Table 3.16) were divided by the “toxicity range factors” of the class 3 (RF_{Tmax} = 10^4 and RF_{Tmin} = 10) to obtain the final toxicity range. Bol et al (1993) obtained using this method (with different baseline-QSARs) the worst case estimate of LC50 for fish of 0.59 µg/l.

In addition, the QSAR estimates of the Danish EPA (2006) are included. The program assigns CBS to the class of neutral organics.

**Table 3.14 QSAR – estimates for aquatic ecotoxicity.**

<table>
<thead>
<tr>
<th>Organism</th>
<th>ECOSAR v0.99h</th>
<th>TGD Part III, Section 4.1, Table 1</th>
<th>Danish EPA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute</td>
<td>Chronic</td>
<td>Acute</td>
</tr>
<tr>
<td>Fish</td>
<td>0.345</td>
<td>0.071</td>
<td>0.69</td>
</tr>
<tr>
<td>Daphnid</td>
<td>0.453</td>
<td>0.182</td>
<td>0.26</td>
</tr>
<tr>
<td>Algae</td>
<td>0.335</td>
<td>0.182</td>
<td>0.18</td>
</tr>
</tbody>
</table>

(1) These QSARs are for neutral organics and they have been used as the starting point for the approach of Verhaar et al. (1992)

(2) The acute toxicity range as the outcome of the method of Verhaar et al. (1992)

The acute tests indicate that the algae (96hEC50 ≤ 0.9 mg/l) would be the most sensitive species followed by fish (14d-LC20 = 0.96 mg/l) and by daphnids. This comparison is however hampered by the fact that all except one fish test were static tests with no analytical monitoring. The QSAR-predictions indicate that algae would be the most sensitive species. The L(E)C50-estimates for algae and fish are from ECOSAR v0.99h slightly lower than the test results but still above the water solubility. For daphnids, the ECOSAR v0.99h gives a significantly lower estimate than the test available. The “best case” estimates according to the method of Verhaar et al. (1992) are in the range of few ten µg per litre which is approximately one order of magnitude less than the lowest test results.
### 3.2.1.3 Calculation of Predicted No Effect Concentration (PNEC)

Results from short-term tests are available for freshwater species out of 3 trophic levels.

**Table 3.15 Overview of the toxicity of CBS to aquatic organisms**

<table>
<thead>
<tr>
<th>Species</th>
<th>Test type</th>
<th>Test conditions</th>
<th>Exposure time</th>
<th>Effect Conc.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>Static</td>
<td>22°C 8.2</td>
<td>96 h</td>
<td>LC50 &gt; 1000 mg/l (n)</td>
<td>Monsanto (1979b)</td>
</tr>
<tr>
<td><em>Pimephales promelas</em></td>
<td>Static</td>
<td>13°C 6.9-7.6</td>
<td>14 d</td>
<td>LC20 = 0.96 mg/l (e)</td>
<td>Monsanto (1981)</td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>Flow-through</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>Static</td>
<td>20°C 7.2-7.8</td>
<td>48 h</td>
<td>LC50 &lt; 18 mg/l (n)</td>
<td>Monsanto (1979a)</td>
</tr>
<tr>
<td><em>Selenastrum capricornutum</em></td>
<td>Static</td>
<td>24°C 7.3-7.5</td>
<td>96 h</td>
<td>ECµ50 &lt; 0.9 mg/l (n)</td>
<td>Monsanto (1979c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ECµ8 &lt; 0.3 mg/l (n)</td>
<td></td>
</tr>
</tbody>
</table>

(n): nominal concentrations  (e): effective concentrations

The lowest effect values were obtained in a flow-through test on the fish *Oncorhynchus mykiss* (14d-LC20 = 0.96 mg/l, effective concentration) and on the alga *Selenastrum capricornutum* (96h-ECµ50 < 0.9 mg/l, nominal concentration). The effect values are above the water solubility of 0.32 mg/l. The value obtained from the fish test reflects the toxicity of CBS as in the flow-through system the influence of hydrolysis and photolysis could be minimized. On the other hand, the result of the algae test can be considered as the upper boundary for the EC50, since the expected degradation products BT and BTon are less toxic than CBS. The QSARs predict for the baseline toxicity of CBS L(E)C50 -values around the water solubility and the prediction method of Verhaar et al (1992) predicts significantly lower effect concentrations.

Although the algae test is due to the degradation of CBS not valid, it is considered that no new algae test is necessary because a new test would not lead to a change of the conclusion. CBS degrades rapidly and therefore its relevant degradation products are main interest of this assessment. It is noted, that due to the rapid degradation of CBS, it would not be possible to conduct a valid static test.

As a pragmatic approach decided by the TC NES III’06, water solubility is used for the derivation of a tentative PNEC and the assessment factor of 1000 is applied.

**PNEC(tentative)water,freshwater = 0.32 µg/l**

No data on effects of marine organisms are available. Consequently, for the assessment of marine biota, an assessment factor of 10000 is used. The PNEC is calculated to
PNEC\(\text{tentative}\)\(_{\text{water,marine}} = 0.032 \mu g/l\)

It is noted, that the definition of “tentative” does not lead to any further testing. The flag is used to indicate, that a pragmatic approach for the derivation of PNEC was taken in the lack of completely valid base set data and that the PNEC is considered appropriately conservative for this rapidly degrading substance, whose risks are estimated in a conservative, combined way together with the relevant degradation products. The word “tentative” hence also points to the fact, that it is not possible at present to conduct a valid algae test.

Reading across from the properties of 2,2’-Dithio-bis-benzothiazole (MBTS; see Appendix B), inhibition of nitrification in the sewage treatment plant cannot be excluded. There is no study available for this type of effects for CBS. Due to similarities regarding structure and reaction routes, the PNEC for nitrification inhibition from MBTS will be applied for CBS:

\[
PNEC_{\text{microorganisms}} = 1.9 \text{ mg/l}.
\]

### 3.2.1.4 Calculation of Predicted No Effect Concentration (PNEC) for sediment organisms

There are no test results available with sediment dwelling organisms, therefore a PNEC for sediment was calculated with the equilibrium partitioning method. The PNEC\(\text{tentative}\)\(_{\text{sediment}}\) is 86.6 ng/kg wwt. Because of the rapid hydrolysis of CBS (half-life 12.5 h, cf. section 3.1.1.2.2) the substance cannot be tested in a water/sediment system. As relevant exposure is not expected, a refined risk assessment for this sub-compartment is not necessary.

### 3.2.2 Terrestrial compartment

There are no test results available with soil organisms. The PNEC\(\text{tentative}\)\(_{\text{soil}}\) of 70.1 ng/kg wwt calculated from PNEC\(\text{tentative}\)\(_{\text{water}}\) of 0.32 ng/l according to the equilibrium partitioning method is used. As relevant exposure is only expected for breakdown products, the assessment is considered only for background information.

### 3.2.3 Atmosphere

CBS is a non-volatile substance and it is released into the atmosphere only in dust form. Tests on plants are not available.

### 3.2.4 Non compartment specific effects relevant to the food chain

PNEC\text{Coral} is derived using the lowest NOAEL from relevant available studies presented in section 4. No studies on birds are available. In a developmental study (Ema et al. 1989) groups of 10 -17 mated female Wistar rats were administered CBS in form of Soxinol CZ-G (99% purity) via diet at dosage levels of 0, 0.001, 0.01, 0.1 and 0.5% from day 0 to day 20 of
pregnancy. The NOAEL derived from the results of this study for dams is 7.1 mg/kg bw/day (= 0.01% CBS in the diet) based on reductions in body weight gain. The NOAEL/developmental toxicity for the offspring is 69.6 mg/kg bw/day (= 0.1% CBS in the diet) and is based on decreased mean fetal body weights. Using the maternal NOAEL of 7.1 mg/kg bw/day and the relevant conversion factors (AF = 300, CONVmammal = 20) given in the TGD PNEC<sub>oral,mammal</sub> = 0.47 mg/kg food is obtained.

### 3.2.4.1 Other benzothiazole derivatives

The effect assessments of the environmentally relevant breakdown products of CBS are presented in the Appendices I-VI. Table 3.16 presents an overview of the PNECs for aquatic organisms.

**Table 3.16 Aquatic PNECs of CBS degradation products**

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Substance</th>
<th>PNEC&lt;sub&gt;water, freshwater&lt;/sub&gt; [µg/l]</th>
<th>Remarks</th>
<th>PNEC&lt;sub&gt;water, marine&lt;/sub&gt; [µg/l]</th>
<th>PNEC&lt;sub&gt;microorg&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2-Mercaptobenzothiazole (MBT)</td>
<td>0.82 (e)*</td>
<td>NOECs on long-term toxicity to fish and daphnids</td>
<td>0.082</td>
<td>1.0 mg/l</td>
</tr>
<tr>
<td>II</td>
<td>2,2'-Dithiobis-benzothiazole (MBTS)</td>
<td>0.6 (n)**</td>
<td>Effects caused partly by degradation products</td>
<td>0.06</td>
<td>1.9 mg/l</td>
</tr>
<tr>
<td>III</td>
<td>Benzothiazole (BT)</td>
<td>8.1 (e)</td>
<td>Acute tests on fish, invertebrates and algae</td>
<td>0.8</td>
<td>14.8 mg/l</td>
</tr>
<tr>
<td>IV</td>
<td>2- Benzothiazolone (BTon)</td>
<td>16.1 (e)</td>
<td>Acute tests on daphnia and algae</td>
<td>1.6</td>
<td>1.0 ***</td>
</tr>
<tr>
<td>V</td>
<td>2-Methylthiobenzothiazole (MeSBT)</td>
<td>3.4 (e)</td>
<td>Acute tests on daphnia and algae</td>
<td>0.3</td>
<td>1.0 ***</td>
</tr>
<tr>
<td>VI</td>
<td>2-Methylbenzothiazole (MeBT)</td>
<td>29.8 (e)</td>
<td>Acute tests on daphnia and algae</td>
<td>3.0</td>
<td>1.0 ***</td>
</tr>
</tbody>
</table>

* (e) indicates that the critical study result refers to effective concentration. ** (n) indicates that the critical study result refers to nominal concentration. *** as a tentative PNEC a read across from PNEC for MBT is used.

Valid tests on soil organisms are not available for the benzothiazole derivatives. As shown in section 3.1.3.3, benzothiazole (BT), 2-methylthiobenzothiazole (MeSBT) and 2-methylbenzothiazole (MeBT) were measured in soils near roads. In this report PNEC<sub>soil</sub> derived using the equilibrium partitioning method are used.

**Table 3.17 PNECs<sub>soil</sub> of CBS degradation products**

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Substance</th>
<th>PNEC&lt;sub&gt;soil&lt;/sub&gt; [mg kg wwt&lt;sup&gt;-1&lt;/sup&gt;]</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2-Mercaptobenzothiazole (MBT)</td>
<td>0.0228</td>
</tr>
<tr>
<td>II</td>
<td>2,2'-Dithiobis-benzothiazole (MBTS)</td>
<td>0.059</td>
</tr>
<tr>
<td>III</td>
<td>Benzothiazole (BT)</td>
<td>0.0172</td>
</tr>
<tr>
<td>IV</td>
<td>2- Benzothiazolone (BTon)</td>
<td>0.0367</td>
</tr>
</tbody>
</table>
Fumigation tests with benzothiazole derivatives are not available.

Tests on the toxicity to sewage treatment plant micro-organisms are not available for BToN, MeSBT and MeBT. For these substances, the lowest PNEC derived on the basis of test data is applied (PNEC_{stp, microorg.} for MBT: 1.0 mg/l). For substances, which are most toxic on the basis of ecotoxicity data from aquatic environment and which are expected to show excess toxicity, PNEC_{stp, microorg.} were derived from tests. Thus such cross-reading is considered a rather conservative approach and thus applied here.

The stable degradation products of CBS, which are relevant regarding the exposure in the environment (BT, BToN, MeSBT and MeBT), do not show high bioaccumulation potential on the basis of partition coefficients and available studies. Only MeSBT has logKow above 3, but for this substance, no data on effects from mammals or birds exist. However the generic secondary poisoning assessment of CBS is assumed to represent the worst case for all relevant degradation products.

### 3.3 RISK CHARACTERISATION

#### 3.3.1 Aquatic compartment (incl. sediment)

The CBS effects assessment for the hydrosphere resulted in PNEC(tentative)_{water} -values of 0.32 µg/l for freshwater and 0.032 µg/l for marine organisms. A PNEC for micro-organisms was based on read-across from MBTS and it is 1.9 mg/l.

#### 3.3.1.1 Production

The PECs were calculated on the basis of site-specific release data. The results are:

<table>
<thead>
<tr>
<th>Site</th>
<th>Release into</th>
<th>PEC_{local water} (µg/l)</th>
<th>PEC_{water} / PEC_{water}</th>
<th>C_{eff.} (µg/l)</th>
<th>C_{eff.} / PNEC_{microorg.}</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>river</td>
<td>0.1</td>
<td>0.3</td>
<td>6</td>
<td>0.003</td>
</tr>
<tr>
<td>B</td>
<td>river</td>
<td>&lt; 0.13</td>
<td>&lt; 0.4</td>
<td>&lt; 100</td>
<td>0.05</td>
</tr>
<tr>
<td>C</td>
<td>river</td>
<td>0.03</td>
<td>0.09</td>
<td>&lt; 10</td>
<td>0.005</td>
</tr>
</tbody>
</table>

---

4 Conclusion (i) There is a need for further information and/or testing.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion concerns the CBS production plants regarding CBS emissions to the aquatic environment. The PNEC is considered to be tentative while it is derived from the water solubility. The acute toxicity tests for daphnids and algae are not valid due to the degradation of CBS. However, there is no need for new tests. CBS is instable in the environment as confirmed by the degradation tests and monitoring data from industrial wwtp effluents. The conclusions are not expected to be changed by new tests.

In addition, CBS is not expected to cause risks to the waste water treatment plants of the production sites.

Benzothiazole Derivatives

During production, releases of a number of benzothiazole derivatives are expected which are breakdown products of CBS and/or being formed as metabolites and abiotic degradation products during biological waste water treatment. The risk characterization ratios (RCRs) are calculated on the basis of measured concentrations and PNECs listed in table 3.16.

Table 3.19 Risk characterisation for benzothiazole derivatives during CBS production

<table>
<thead>
<tr>
<th>Substance</th>
<th>Site</th>
<th>$C_{\text{local water}}$ (µg/l)</th>
<th>$C_{\text{water}} / PNEC_{\text{water}}$</th>
<th>$C_{\text{effl}}$ (µg/l)</th>
<th>$C_{\text{effl}} / PNEC_{\text{microorg.}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Mercaptobenzothiazole (MBT)</td>
<td>A</td>
<td>&lt; 0.3</td>
<td>&lt; 0.37</td>
<td>&lt; 20</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.008</td>
<td>0.09</td>
<td>6.4</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.478</td>
<td>0.583</td>
<td>183.8</td>
<td>0.18</td>
</tr>
<tr>
<td>2,2'-Dithio-bis-benzothiazole (MBTS)</td>
<td>A</td>
<td>&lt; 0.3</td>
<td>&lt; 0.5</td>
<td>&lt; 20</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.1</td>
<td>0.2</td>
<td>106</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>&lt; 0.026</td>
<td>&lt; 0.04</td>
<td>&lt; 10</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Benzothiazole (BT)</td>
<td>A</td>
<td>&lt; 0.2</td>
<td>&lt; 0.002</td>
<td>&lt; 10</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.13</td>
<td>0.02</td>
<td>80</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>&lt; 0.026</td>
<td>&lt; 0.003</td>
<td>&lt; 10</td>
<td>0.001</td>
</tr>
<tr>
<td>2-Benzothiazolone (BTon)</td>
<td>A</td>
<td>&lt; 0.2</td>
<td>&lt; 0.01</td>
<td>&lt; 10</td>
<td>&lt; 1*</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.12</td>
<td>0.007</td>
<td>70</td>
<td>&lt; 1*</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.122</td>
<td>0.008</td>
<td>47.0</td>
<td>&lt; 1*</td>
</tr>
<tr>
<td>2-Methylthiobenzothiazole (MeSBT)</td>
<td>A</td>
<td>&lt; 0.2</td>
<td>&lt; 0.06</td>
<td>&lt; 10</td>
<td>&lt; 1*</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.03</td>
<td>0.009</td>
<td>24</td>
<td>&lt; 1*</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>&lt; 0.026</td>
<td>&lt; 0.008</td>
<td>&lt; 10</td>
<td>&lt; 1*</td>
</tr>
<tr>
<td>2-Methylbenzothiazole (MeBT)</td>
<td>A</td>
<td>&lt; 0.2</td>
<td>&lt; 0.007</td>
<td>&lt; 10</td>
<td>&lt; 1*</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>0.07</td>
<td>0.002</td>
<td>29</td>
<td>&lt; 1*</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>&lt; 0.026</td>
<td>&lt; 0.001</td>
<td>&lt; 10</td>
<td>&lt; 1*</td>
</tr>
</tbody>
</table>

* RCR is calculated assuming that the PNEC of the specific substance is not lower than the PNEC of MBT (1.0 mg/l) which is the lowest of the PNECs derived on the basis of tests.
The additive risk characterization approach where the RCRs for individual degradation products are added together to give a single risk ratio for a local scenario gives the following results:

Site A: $\text{RCR} < 0.95$
Site B: $\text{RCR} = 0.25$
Site C: $\text{RCR} = 0.64$

When including the RCR of CBS to the sum, the results are as follows:

Site A : $\text{RCR} < 1.25$
Site B: $\text{RCR} < 0.65$
Site C: $\text{RCR} = 0.7$

**Conclusion (ii)**

There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

All sites have provided measured data on emissions to the aquatic environment for all the required six degradation products. For plants B and C no risks are expected even with the worst case additive approach of additive risk characterization. The risk ratio for plant A is slightly above 1 according to the additive approach. No risks are expected from site A on the basis of the measured data and confidential information.

No risks are expected at the waste water treatment plants on the basis of substance specific risk ratios for MBT, MBTS and BT. Information on the effects of BTop, MeSBT and MeBT to the treatment plants are not available. However, using PNECstp,microorg of MBT as a conservative estimate, risks are not expected for these substances. The conclusion applies also for the additive risk characterization approach.

**3.3.1.2 Rubber and Tire Industry**

According to the emission scenario document on additives in rubber industry, no emissions to waste water occur.

**Conclusion (ii)**

There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
3.3.1.3 Use in Tires

Benzothiazole derivatives are released into surface waters via abraded tire tread particles. Based on very scarce measured data, provisional PECs were determined for water bodies receiving runoff from roads. Table 3.19 presents the resulting risk ratios.

Table 3.20 Risk characterization of CBS breakdown products for use in tires.

<table>
<thead>
<tr>
<th>Product</th>
<th>Preliminary PEC&lt;sub&gt;water&lt;/sub&gt; [µg/l]</th>
<th>PEC&lt;sub&gt;water&lt;/sub&gt;/PNEC&lt;sub&gt;water&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzothiazole (BT)</td>
<td>0.6</td>
<td>0.07</td>
</tr>
<tr>
<td>2-Benzothiazolone (BTon)</td>
<td>0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>2-Methylbenzothiazole (MeBT)</td>
<td>0.6</td>
<td>0.02</td>
</tr>
<tr>
<td>2-Methylthiobenzothiazole (MeSBT)</td>
<td>5.6</td>
<td>1.65</td>
</tr>
</tbody>
</table>

The additive risk characterization approach where the RCRs for individual degradation products are added together gives a RCR of 1.75. In addition, Methylthiobenzothiazole alone causes already risk.

Conclusion (i) There is a need for further information and/or testing.

The use of CBS as vulcanisation accelerator causes releases of its breakdown products, which are due to the use in tires widely distributed in the environment. The exposure estimate is based on one study only but it is supported by some measured data from road runoff. Measured data from water bodies receiving directly road runoff should be produced for the degradation products. Water and sediment samples from at least 10 sites from urban and rural areas should be included covering representative rain events and situation between rain events. In case the concentrations prove to be as high as those used for the risk characterization, tests on chronic toxicity on aquatic organisms should be considered on the compounds with highest RCRs.

3.3.1.4 Releases from tire recycling – tire crumb in ground materials

Results of leaching tests of tires, rubberised asphalt and novel rubber material for sport grounds give reason to expect that runoff discharged to surface waters from sport fields and other outdoor sites using tire crumb in the ground material may cause local risk to aquatic environment. Local assessment could not be conducted for sport fields as direct measured data from leachate or receiving waters is lacking. In addition, information to estimate total releases was not available.

Conclusion (i) There is a need for further information and/or testing.

A study to investigate leaching of CBS degradation products from tyre crumb used for ground materials or measured data from surface waters receiving runoff from sports grounds are needed for the relevant CBS degradation products. In addition, information on the total volume of tire crumb in outdoor uses is needed in order to estimate the total release. It is noted, that releases of the breakdown products from tire recycling are not concentrating to few local situations but there is a large number of potential sources which hence lead to a wide distribution of the breakdown products of CBS.
### 3.3.1.5 Releases from Landfills

Releases of benzothiazole derivatives into surface waters occur from used tires and rubber goods deposited into landfills.

Based on the available measured data, no conclusions regarding to the general exposure level can be drawn. Since some of the measured concentrations are rather high, high exposure situations cannot be excluded.

**Conclusion (i)** There is a need for further information and/or testing.

More measured data on landfill leachates are needed to improve the exposure estimation.

### Sediment

Because of the rapid hydrolysis accumulation of CBS in sediments is not expected. Therefore a risk assessment for this sub-compartment is not necessary.

For CBS breakdown products neither representative monitoring data in sediments nor effect tests on sediment organisms are available. Both exposure and environmental effects could be estimated using the equilibrium partitioning method. This approach leads to the same PEC/PNEC ratios and conclusions as in the aquatic risk assessment.

### 3.3.2 Terrestrial compartment

A $\text{PNEC}_{\text{soil}}$ of 70.1 µg kg wwt$^{-1}$ (79.4 µg kg dw$^{-1}$) has been derived by the equilibrium partitioning method from $\text{PNEC}_{\text{water}}$. $\text{PNEC}_{\text{soil}}$ for Benzothiazole (17.2 µg kg wwt$^{-1}$, 20 µg kg dw$^{-1}$), for 2-Methylthiobenzothiazole (26.3 µg kg wwt$^{-1}$, 29.9 µg kg dw$^{-1}$) and for 2-Methylbenzothiazole (69.9 µg kg wwt$^{-1}$, 79.2 µg kg dw$^{-1}$) have also been determined by the equilibrium partitioning method.

### 3.3.2.1 Production

Significant amounts of CBS dust are released during production. This dust will reach the soil in the vicinity of the production sites by wet and/or dry deposition. Because of the instability of CBS against hydrolysis there is an exposure of benzothiazole as the main hydrolysis product. The screening approach for benzothiazole covers the exposure due to dust emissions from CBS production. The results from the screening approach do not imply risks to soil for the production sites.

**Conclusion (ii)** There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
3.3.2.2 Rubber Industry

Relevant amount of deposition (0.269 mg/m²/d) from the atmosphere in the vicinity of rubber and tire manufacturers has been predicted (as CBS) in this assessment. While CBS breaks down in the curing process other benzothiazole derivatives are formed. The exposure of the terrestrial compartment are likely to be caused by the degradation products. The screening approach on BT shows risk of 1.13 and MeSBT show no concern for terrestrial compartment.

On the basis of the information provided by rubber industry on BT and MeBT, the experimental emission factor to air would be two orders of magnitude lower than the default estimate. Therefore, no local risks are expected for soil from rubber industry.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

3.3.2.3 Use in Tires - road borders

Benzothiazole derivatives accumulate in soils near roads where abraded tire tread particles are deposited. The risk characterization is based on PECs derived from one study only using 90-percentiles of measured concentrations at the road border of eight German roads assuming that 2% of the concentration are still present at the distance of 5 m from the road border.

Table 3.21 Risk characterization of CBS breakdown products for road border soil.

<table>
<thead>
<tr>
<th>Compound</th>
<th>PECsoil [µg/kg dw]</th>
<th>PEC/PNEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzothiazole (BT)</td>
<td>336</td>
<td>16.8</td>
</tr>
<tr>
<td>2-Methylbenzothiazole (MeBT)</td>
<td>34</td>
<td>0.44</td>
</tr>
<tr>
<td>2-Methylthiobenzothiazole (MeSBT)</td>
<td>86</td>
<td>2.9</td>
</tr>
</tbody>
</table>

The additive risk characterization approach where the RCRs for individual degradation products are added together gives a RCR of 20. BT and MeSBT seem to cause already alone risk at the 5 meter distance of the studied roads. The other exposure relevant substances which have been found recently in highway runoff are BTOn and MBT. For these substance, measured data is lacking from road border soil.

Conclusion (i) There is a need for further information and/or testing.

Measured data from soil borders on the CBS degradation products should be produced in order to derive the final PECsoil. Samples from at least ten sites at highway road borders and urban areas should be provided at the distances according to the principles of the road border document JM/56/2003. In case the results show very high exposure, tests on terrestrial organisms could be considered. The analytical method should reach for each substance a lower limit of quantisation than the value of the corresponding PNEC.

3.3.3 Atmosphere

No tests on plants for the exposure route air are available on CBS.
3.3.3.1 Production

A Clocal$_{\text{air}}$ of 0.66 µg/m$^3$ was obtained from the provided information on CBS releases in dust as the highest representative for the production sites. CBS is emitted from the production plants in dust and dust releases lead to an exposure of soils and will be assessed in section 3.34.

On the basis of the site specific information provided from CBS production plants on their BT-emissions, highest concentration in air expected in the surrounding area is 0.03 mg/m$^3$. This is a level of exposure where a plant fumigation test is not seen necessary.

**Conclusion (ii)** There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion concerns CBS and BT emissions to air from all production sites.

3.3.3.2 Rubber and Tire Industry

CBS is a non-volatile compound. Therefore releases into the atmosphere are not expected. On the other hand, according to the available emission scenario document, vulcanisation agent emissions to air from this business are relatively large. During vulcanization volatile breakdown products are formed. In laboratory vulcanization experiments, benzothiazole (BT) and 2-methylbenzothiazole (MeBT) were detected in the exhaust gas. Using information from a vulcanization study, local releases and local concentrations in air were estimated to be low (Clocal is ca. 0.06 µg m$^3$ for BT and even lower for MeBT) and plant-air route is considered not to be relevant.

**Conclusion (ii)** There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion applies for the emissions of CBS and its degradation products to air.

3.3.3.3 Releases from tire recycling – tire crumb in ground materials

Use of tire crumb in the synthetic turf material causes emissions of benzothiazole. These emissions can be expected to be released from indoors and outdoors sport halls, playgrounds and asphalt. Due to a probably high amount of these uses, the regional release may be relevant for the assessment. However, due to the lack of information on the total demand of tire crumb materials, no estimation of total release could be conducted.

**Conclusion (i)** There is a need for further information and/or testing.

There is need for information on the market volume of tire crumb used for ground materials.
3.3.4 Secondary poisoning

A PECoral, fish of 0.309 mg/kg wwt was obtained for a scenario were the predator feeds half of the time from the recipient of plant A. A PNECoral, mammal of 0.47 mg/kg food was obtained from a NOAEL of 7.1 mg /kg bw/d for maternal toxicity. A risk ratio of 0.66 results for this scenario. For birds, no toxicity data was found.

ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already

The conclusion concerns secondary poisoning of mammals via aquatic food chain. Due to the instability of CBS, risks in other food chains are not expected either.

Of the degradation products of CBS only 2-methylthiobenzothiazole (MeSBT) could be relevant for this endpoint. Due to the lack of toxicity data an assessment of MeSBT cannot be performed directly. However, the bioaccumulation potential of CBS is very much higher than the bioaccumulation potential of MeSBT. In addition, based on the aquatic ecotoxicity and the reactivity of CBS, the toxicity of CBS in the upper food chain can be expected to be significantly higher than the toxicity of MeSBT. Hence it is assumed, that the possible risks due to the exposure to MeSBT are covered by the secondary poisoning assessment of CBS.

The octanol-water partition coefficients of the other exposure-relevant derivatives are below 3, thus these compounds are not expected to accumulate via the food chain.
4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General discussion

In Western Europe N-Cyclohexylbenzothiazol-2-sulphenamide (in the following CBS) is exclusively used as a vulcanisation accelerator in rubber goods manufacture (Bayer, 1997). CBS is produced by four companies in the EU. In 1993 the demand of CBS in Western Europe was estimated at 15,500 t which is about 50% of the total accelerators demand. The world CBS production is estimated to 44,000 – 45,000 t for 1993 (Srour, 1994). It is expected that the CBS demand will increase in the next years, because CBS in contrast to other vulcanisation accelerators cannot form toxic nitrosamine (Bayer, 1997).

CBS is produced in a closed system. Thus, unit operations are usually carried out with negligible exposure. Storage and conveying CBS is performed in largely automated equipment. Where skin contact can occur, employees are supplied with work dress, safety shoes, gloves and protecting glasses.

CBS is a slight greyish powdery substance (vapour pressure $1.5 \times 10^{-8}$ hPa at $20^\circ$C) which decomposes under the influence of heat. According to information provided by industry CBS is mainly used in dust suppressed forms (granulates or master batches). But the provided information is non-sufficient, so that exposure due to the handling the powdery substance cannot be excluded.

Detailed information see chapter 2 (general information on exposure).

Due to the physico-chemical properties of the substance inhalation and dermal exposure to dusts during the handling of CBS are expected to be the main source of exposure for workers.

There are no data available and no consumer products listed in the Swedish product register and in other data bases (e.g. Nordic Product Register SPIN).

4.1.1.2 Occupational exposure

Industrial activities using CBS present opportunities for occupational exposure. Exposure ranges depend on the particular operation and the risk reduction measures in use. Occupational exposure limits for CBS have not been established in Western Europe and USA (Ariel, 2003).

The following scenarios are regarded to be relevant for occupational exposure:
Scenario 1: Production of CBS (4.1.1.2.1)
Scenario 2: Use of CBS as a vulcanisation accelerator in the rubber industry (e.g. rubber goods, tires) (4.1.1.2.2)

During the vulcanisation (curing) process, CBS like any other vulcanising agent is reacting for at least 95%. Taking into account that the maximum concentration of CBS in the uncured compounds is 3.5% (technical rubber), the amount of CBS that can be retained in the finished product is limited to 0.2% (BLIC, 2004). Due to the resulting low concentration of CBS, a considerable exposure to CBS during the processing of rubber goods is not expected. Therefore, the processing of rubber, e.g. cutting, melting, is not considered in this report.

The assessment of inhalation exposure is mainly based on measured exposure levels from which – if possible – 90th percentiles are derived as representing reasonable worst case situations.

If available, only data measures later than 1990 are used in exposure assessment. Scenarios are clustered as far as possible to make the description transparent. If quantitative exposure data is not available, model estimates are taken.

Beside inhalation exposure, dermal exposure is assessed for each scenario. Two terms can be used to describe dermal exposure:

Potential dermal exposure is an estimate of the amount of a substance landing on the outside of work wear and on the exposed skin.

Actual dermal exposure is an estimate of the amount of a substance actually reaching the skin.

Within the framework of existing substances there is an agreement between the EU member states, to assess - as a rule - dermal exposure as exposure to hands and parts of the forearms. In this, the main difference between both terms – potential and actual - is the protection of hands and forearms by work wear and – more important – the protection by gloves. Within this exposure assessment, the exposure reducing effect achievable by gloves is only considered if information is provided indicating that, for a certain scenario, gloves are a widely accepted protective measure and that the gloves are fundamentally suitable for protection against the substance under consideration. As a measure for the latter, tests according to DIN EN 374 are taken as a criterion. For most downstream uses it is commonly known that gloves are not generally worn. In these cases, dermal exposure is assessed as actual dermal exposure for the unprotected worker. Since quantitative information on dermal exposure is often not available, the EASE model is mostly used for assessing dermal exposure.

4.1.1.2.1 Occupational exposure from production

CBS is produced in the chemical industry in large-scale sites within closed systems. Manufacture can be done by oxidative coupling of sodium mercaptobenzothiazole (NaMBT) with cyclohexylamine or by reacting dithiobisbenzothiazole (MBTS) with excess cyclohexylamine. After filtering the resulting CBS suspension, the raw material is washed with water. For the ease of dust-free handling, technical grade CBS powder is dust suppressed with small amounts of paraffin oil or anionic surfactants. The product is dried, packaged (paper bags or big bags) and transported on pallets (Bayer, 1997). In addition master batches, in which CBS is blended with polymers (about 50 - 80 % CBS) are placed on the market as well as powdery CBS. Detailed information about powdery CBS is not available.
For the large-scale chemical industry high standards of control at the workplaces are assumed to be practised even if the containment is breached, e.g. during filling, cleaning, maintenance, repair work and sampling. Inhalation exposure in other fields is normally minimised by technical equipment (e.g. special designed filling stations, local exhaust ventilation).

Inhalation exposure

**Measured data**

**Table 4.1: Total dust and CBS exposure at workplaces during production**

<table>
<thead>
<tr>
<th>Job category / activities</th>
<th>Years of measurement</th>
<th>Number of samples</th>
<th>Measurement data (Total dust) [mg/m³]</th>
<th>Measurement data (CBS) [mg/m³]</th>
<th>50th percentile (Total dust) [mg/m³]</th>
<th>90th percentile (Total dust) [mg/m³]</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-h TWA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Production</td>
<td>1992 - 1/1996</td>
<td>28 (p)</td>
<td>&lt; 0.1 - 5.5</td>
<td>-</td>
<td>-</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>10/1996</td>
<td>6 (p)</td>
<td>&lt; 0.1 - 0.3, 2.7, 7.6</td>
<td>&gt; 0.1, 3.2</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12/1996</td>
<td>4 (p)</td>
<td>0.1 - 1.0</td>
<td>&lt; 0.1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Production (milling, blending)</td>
<td>1994 - 1998</td>
<td>23 (p)</td>
<td>0.1 - 4.5</td>
<td>-</td>
<td>0.9</td>
<td>2.0</td>
</tr>
</tbody>
</table>

<: no data available
p: personal sampling

Airborne concentrations of total dust are determined by gravimetric analysis and concentration of CBS is detected by high pressure liquid chromatography (Bayer, 1996). The analytical data on CBS concentrations are to be considered as preliminary as the method used is no standard method and has not been completely validated.

Two producers provided reliable measurement results regarding exposure to dust (total dust) and, in part, to CBS (c.f. table 4.1). For two measurement collectives, the 90th percentiles for exposure to dust are about 2 mg/m³. Measurements carried out 10/1996 reveal that exposure during handling and filling of CBS are relatively high (up to 7.6 mg/m³ total dust and up to 3.2 mg/m³ CBS). Repeated measurements (12/1996) at those workplaces with highest exposure did not confirm the exposure levels and yielded < 2 mg/m³ total dust and < 0.1 mg/m³ CBS.

A third company provided a few measurement results with incomplete information, total dust concentration of 4.4 mg/m³ and 7.1 mg/m³ were submitted.

As seen at table 4.1, only one company determined the concentration of CBS after sampling total dust. The measurements reveal that the CBS concentration amounts to up to 50% of the total dust concentration in 10/1996 and up to 10% in 12/1996. Detailed information on the circumstances leading to relatively high or low CBS percentages in the dust is not available. It can be seen, that the 90th percentiles of total dust (2 mg/m³) are below the highest measurement of CBS 3.2 mg/m³. In conclusion, the 90th percentile from two measurement collectives of 2.0 mg/m³ should be taken as representing the reasonable worst case situation for the production of CBS.

Since 2 of 3 companies submitted useful measurement results regarding to total dust which cover different activities, the measurement results are assumed to be representative.
According to the information provided by three manufactures in the production process of CBS a total of 93 workers are employed. Workers normally use PPE (gloves, goggles etc.).

Summary of the exposure level

Inhalation exposure (dust) has to be assessed for production of CBS.

For the assessment of health risks from daily inhalation exposure to CBS during the production an 8-h time weighed average concentration (8-h TWA) of 2 mg/m³ should be taken as representing the reasonable worst case situation.

It is to be assumed that the substance is processed daily. Consequently, the duration and the frequency of exposure to CBS are assumed to be daily and for the entire length of shift.

Dermal exposure

When producing CBS (dust-suppressed, powder) dermal exposure could occur during activities like drumming, sampling, cleaning, maintenance and repair work.

Modelled data

For the unprotected worker, according to the EASE model, potential dermal exposure is assessed as follows:

Input parameters: Non dispersive use, direct handling, intermittent
Level of exposure: 0.1 – 1 mg/cm²/day.

Considering an exposed area of 420 cm² (palms of hands) the model yields an exposure level of 42 - 420 mg/person/day.

For assessing actual dermal exposure levels, it has to be considered that the substance is manufactured primarily in closed systems and that the use of personal protection equipment (PPE, here gloves and eye protection) is highly accepted in the large-scale chemical industry. The extent of protection by PPE (here gloves) depends inter alia on the suitability of the recommended material with regard to the permeation properties of substance. For the handling of powdery substances, as a rule, the suitability of the gloves can be assumed. As a rough estimation, a protection efficiency of 90 % achieved by suitable gloves is taken resulting in dermal exposure of 4.2 – 42 mg/person/day. The upper value is regarded to represent the reasonable worst case situation.

Summary of the exposure level

For assessing the health risks from daily dermal exposure in the area of production (scenario 1), an exposure level of 42 mg/person/day should be taken. This exposure assessment is based on the assumption that gloves are suitable for the protection against powder.

Exposure to the eyes is largely avoided by using eye protection.

4.1.1.2.2 Occupational exposure from formulation

CBS is exclusively used as a vulcanisation accelerator in rubber goods manufacture, mostly in combination with other sulfenamide-accelerators and with curing retarders to achieve the
optimum balance of vulcanisation behaviour. Information from 16 rubber processing companies in Germany reveals, that dust suppressed CBS is mostly used and that companies using powdery CBS apply the substance in rather low quantities (< 2000 kg/a).

The amount of CBS in the tyres is between 0.5 – 1.5 % and in technical rubber products between 0.4 – 3.5 %. (BLIC, 2004)

According to descriptions given in the literature (Ullmann, 1998, Winnacker-Küchler, 1983), rubber manufacture comprises four main steps:

1) Blending (transfer and mixing of starting materials)
2) Transfer and storage
3) Forming (e.g. extruding)
4) Vulcanising

If moulding techniques are applied, forming and vulcanising are performed in one step. The 3rd step „forming“ (extruding, calendaring, moulding) and step 4 „vulcanisation“ are regarded to be not relevant for occupational exposure to CBS and are therefore not described here.

Blending

Usually CBS is transported to customer’s warehouses by truck. For the purpose of weighing, bags are opened and emptied. CBS is then transferred to mixing rooms, where mixtures (“batches”) are produced by means of a multi step process (BAYER, 1997).

The equipment used for mixing the compounds may be broken down in two categories: open mill mixing and internal mixing. In the rubber industry internal mixing is used mostly.

For open mill mixing (two roll mill) generally a two stage mixing process is used. The first step is to load rubber and add the compounding agents, e.g. fillers, extender oils, plasticizers and softeners. Afterwards these mixtures are fed to the mixer again where the curing agents and accelerators are added. The mixing can be done continuously or batch-wise. For internal mixing, batch-type mixing devices are used consisting essentially of a completely enclosed mixing chamber. The production size can vary from 50 to 500 kg.

Transfer

After blending the batches could be fed directly to the forming devices, but in the rubber industry it is more frequent to store the batches before forming. When protected from heat, CBS remains inactive in such compounds and enables limited shipping and storage before final use.

On account of the low vapour pressure of the substance (1.5 x 10⁻⁸ hPa at 20°C), exposure to dust during step 1 is regarded to be the main source of exposure. Activities like opening and emptying bags, transfer, weighing, mixing, cleaning and repair works are exposure relevant.

The weighing process is in general an automated process. While the mixing, i.e. the homogenisation of the viscous rubber with the additives is achieved in a dry mixing process in a closed chamber. Potential exposure to dust is only possible during the filling of the mixer and release of the batch (BLIC, 2004).

Transfer and storage (step 2) of the resulting mixtures (containing < 3% CBS) are judged to be of minor relevance.
It is to be assumed that gloves and eye protection are not regularly worn and that both, immediate dermal contact and exposure to eyes caused by hand-eye contacts occur.

There is no detailed information on duration and frequency of exposure relevant activities. But there are no facilities where CBS is continuously weighed throughout the entire duration of a shift and CBS is only representing 10 % of all weighed chemicals (BLIC, 2004). It is to be assumed that exposure relevant activities are carried out for a limited duration and in case not daily. This holds true especially for use of the powdery substance, which companies apply in small quantities (< 2000 kg/a).

**Inhalation exposure**

**Measured data**

CBS specific monitoring data for workplace exposure are available from the Goodyear Tire & Rubber Company for seven production sites of tyres in different European countries. The used CBS is oil coated, or in form of pellets (Flexsys, 2004). Only in three of 48 cases CBS was detectable. The measurement results range from <0.02 to 1.0 mg/m³ (Goodyear, 1996).

Measurement data of some BLIC (European Association of the Rubber Industry) members have also indicated that the actual exposure level is lower than 0.5 mg/m³ (BLIC, 2004).

Due to the fact that the data refer to the large scale production of tyres only, measurement results regarding total dust exposure in the rubber industry are taken in addition to describe occupational exposure. Workplace measurements in the rubber industry were performed in The Netherlands (Vermeulen et al., 2000) and UK (Dost et al., 2000).

In the rubber manufacturing industry in The Netherlands, the 8-h TWA medians of airborne particulate exposure range between 0.85 mg/m³ and 1.5 mg/m³ (10 plants in 1997) for compounding, mixing, pre-treating and engineering services (e.g. maintenance).

In rubber goods companies in UK, the medians of total dust (rubber process dust) range between 0.8 and 4.2 mg/m³ (personal 8-h TWA, n = 82, 1996 - 1997) for weighing, mixing and milling activities. In new tyres companies, the median of exposure levels obtained during weighing, milling and mixing is 1.6 mg/m³ (personal 8-h TWA, n = 22, 1996 - 1997).

Additional data from 1994 provided by the monitoring authorities of the Federal States (Länder) of Germany in the rubber tire production are located at < 0.6 mg/m³ total dust (8-h TWA).

In all described studies it is stated that dust-suppressed powders were most commonly found to be use.

**Analogous data for the use of powdery substances**

Exposure scenarios with the handling of rather low quantities of powdery substances in formulating processes are taken for analogy considerations. Such workplaces were subject of an BAuA study on the EASE model (Bredendiek-Kämper, 1999). It turned out, that exposure levels are below 1 mg/m³ (8-h TWA), if low amounts of powdery substances are handled. This was shown at workplaces in the textile industry, where printing inks are mixed by adding and mixing powdery substances (colour kitchen, typical amounts a few kg).
Modelled data

EASE for Windows 2.0, Aug. 1997 was used.

a) EASE estimation for the use of dust-suppressed (coated with paraffin oil or anionic surfactants) CBS:

Input parameters:  
- $T = 20 \degree C$, exposure-type is dust, low dust techniques, LEV present
- Level of exposure:  
  - 0 - 1 mg/m$^3$

b) EASE estimation for the use of dust-suppressed (coated with paraffin oil or anionic surfactants) CBS:

Input parameters:  
- $T = 20 \degree C$, exposure-type is dust, low dust techniques, LEV absent
- Level of exposure:  
  - 0 - 5 mg/m$^3$

It is to be considered that CBS is not continuously weighed throughout the entire duration of a shift and CBS is only representing 10% of all weighed chemicals (BLIC, 2004). Therefore the daily duration of 1 h is assumed. This reduces the exposure levels to 0.13 – 0.63 mg/m$^3$.

The use of powdery CBS is of minor relevance but it is also assessed. The EASE estimation leads to:

c) EASE estimation for the use of powdery CBS at workplaces with local exhaust ventilation (LEV):

Input parameters:  
- $T = 20 \degree C$, exposure-type is dust, dry manipulation, LEV present
- Level of exposure:  
  - 2 - 5 mg/m$^3$

d) EASE estimation for the use of powdery CBS at workplaces without LEV:

Input parameters:  
- $T = 20 \degree C$, exposure-type is dust, dry manipulation, LEV absent
- Level of exposure:  
  - 5 - 50 mg/m$^3$

Considering a daily duration of 1 h, exposure levels reduce to 0.63 – 6.3 mg/m$^3$.

Summary of the exposure level

Measurement results provided by one company at tyre producing sites are < 1 mg/m$^3$ CBS.

According to industry information CBS is mainly used in dust suppressed form, only a small amount is used as a powder. Inhalation to CBS dusts occur predominately at weighing, dosing and filling. These exposure relevant tasks are assumed to be performed for 1 h/day leading to an exposure level of 0.6 mg/m$^3$ (EASE estimation, 8-h TWA) for dust suppressed CBS.

If CBS powder is applied, exposure levels might be up to 6.3 mg/m$^3$ (EASE estimation). This is plausible, if high amounts of dusty materials are handled at workplaces without LEV. At handling smaller quantities < 0.1 t/shift it was found within the framework of an EASE validation study that the EASE model overestimates exposure levels. Due to the low amounts of powdery CBS marketed (<2000 kg/a) it is probable, that low amounts of the powdery CBS are applied on a daily scale. According to the above mentioned study (Bredendiek-Kämper, 1999) this situation would lead to daily exposure levels below 1 mg/m$^3$ (8-h TWA) independent from installed LEV.
Dermal exposure

Industry described that workers belonging to the rubber industry usually wear gloves. But a comprehensive study on exposure to particulate in the rubber industry in The Netherlands reveals, that only limited numbers of workers wear gloves: 16 – 73 % of the workers in the area of compounding, mixing, pre-treatment and engineering services use gloves (Vermeulen, 2000).

Analogous data

Dermal exposure due to blending (mixing) of zinc oxide (in a not very dusty powder form) has been measured in the RISKOFDERM project at rubber companies in The Netherlands (RISKOFDERM, 2003). Amounts of 50 – 500 kg zinc oxide were added manually to hoppers from bags in 2 – 11 minutes. Exposure to the hands was measured for four workers each in three different factories using a hand washing technique on a surface area of 820 cm². The workers did not use gloves. The range of measured values was 21 – 211 mg zinc oxide, with a 90th percentile of approximately 100 mg (RISKOFDERM, 2003; Marquart, 2006). Assuming that mixing CBS occurs for two batches per worker per day, the dermal exposure is 200 mg/person/day. During the production of rubber goods in The Netherlands, measurements of dermal exposure were performed by means of personal sampling of cyclohexane soluble material (CSM) using dermal pad samplers (Vermeulen, 2000). The pad samplers were worn on the lower part of the wrist of the hand. CSM on the pad was determined by means of a NIOSH method (NIOSH, 1977). The median exposures at compounding / mixing (weighing, empty bags, internal mill, open mill) and pre-treatment (repair buffing) range from 0.02 – 0.07 mg/cm² (8-h TWA, n = 172) and 0.18 mg/cm² (8-h TWA) for engineering services (e.g. maintenance, bench fitting; n = 55). It is stated that high dermal exposure of workers in the engineering services is caused by works at lubricating machineries without gloves, by breakdown work and by operating lathes. It is not clear if gloves were used during measurements at compounding, mixing and pre-treatment. Taking the highest value of 0.18 mg/cm² and an exposed skin area of 820 cm², a value of 148 mg/person/day is obtained.

Summary of the exposure level

Taking both sets of analogous data into account for assessing the health risks of daily dermal exposure in the rubber industry (scenario 2), an exposure level of 200 mg/person/day should be taken. This exposure assessment is based on the assumption that suitable gloves are not regularly worn (Vermeulen, 2000).

It cannot be presupposed that eye protection is regularly used. For assessing the risks, hand eye contacts as well as possible contacts to the eye should be considered.

4.1.1.2.3 Summary

Based on the available information, the exposure assessment reveals that handling CBS during production and its use as a vulcanisation accelerator in the rubber manufacture industry are the main sources for occupational exposure.

For occupational exposure there are two scenarios:
Scenario 1: Production of CBS (4.1.1.2.1)
Scenario 2: Use of CBS as a vulcanisation accelerator in the rubber industry (4.1.1.2.2)

During the vulcanisation (curing) process, CBS like any other vulcanising agent is reacting for at least 95 %. Taking into account that the maximum concentration of CBS in the uncured compounds is 3.5 % (technical rubber), the amount of CBS that can be retained in the finished product is limited to 0.2 % (BLIC, 2004). Due to the resulting low concentration of CBS, a considerable exposure to CBS during the processing of rubber goods is not expected. Therefore, the processing of rubber, e.g. cutting, melting, is not considered in this report (see chapter 4.1.1.2.2).

During the vulcanisation (curing) process, CBS like any other vulcanising agent is reacting for at least 95 %. Taking into account that the maximum concentration of CBS in the uncured compounds is 3.5 % (technical rubber), the amount of CBS that can be retained in the finished product is limited to 0.2 % (BLIC, 2004). Due to the resulting low concentration of CBS, a considerable exposure to CBS during the processing of rubber goods is not expected. Therefore, the processing of rubber, e.g. cutting, melting, is not considered in this report.

During the curing process, the majority of the additives are chemically reacting and therefore are no longer present in the finished articles.

Relevant inhalation and dermal exposure levels are given in table 4.1.a and b, respectively.

For the large-scale chemical industry, it is assumed that the production and further processing of CBS is mainly performed in closed systems. Exposure occurs if the systems are breached for certain activities, e.g. filling (scenario 1).

As concerning dermal exposure, for the handling of solid substances, as a rule, the suitability of the gloves can be presupposed. This is considered in assessing dermal exposure during production using the EASE model assuming that single dermal contacts can occur although suitable gloves are used (scenario 1).

In the rubber processing industry mainly dust suppressed and to a minor extent powdery CBS are handled. The main source of exposure occurs during emptying scales, weighing and filling (scenario 2). Here it is to be assumed that CBS represents only 10 % of all weighed chemicals and the weighing process is in general an automated process. For this scenarios dermal exposure is assessed for the unprotected worker (Vermeulen, 2000).
Table 4.2: Conclusions of the inhalation exposure assessment

<table>
<thead>
<tr>
<th>Scenario number, area of production and use</th>
<th>Form of exposure</th>
<th>Activity</th>
<th>Duration [h/day]</th>
<th>Frequency [days/year]</th>
<th>Shift average concentration [mg/m³]</th>
<th>Method</th>
<th>Short-term concentration [mg/m³]</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production</td>
<td>dust</td>
<td>charging, drumming, cleaning, repair, maintenance</td>
<td>shift length (assumed)</td>
<td>daily</td>
<td>2</td>
<td>workplace measurements (total dust)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1. Production of CBS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uses</td>
<td>dust</td>
<td>dosing, weighing, transfer, filling</td>
<td>1h / day</td>
<td>daily</td>
<td>0.6 ¹ ²</td>
<td>EASE (low dust technique) analogous data, powdery substance</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2. Use as a vulcanisation accelerator in the rubber industry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹: Use of high amounts of dust suppressed CBS
²: Compared to use of dust suppressed CBS, minor relevant
### Table 4.3: Conclusions of the dermal exposure assessment

<table>
<thead>
<tr>
<th>Scenario number, area of production and use</th>
<th>Form of exposure</th>
<th>Activity</th>
<th>Frequency [days/year]</th>
<th>Contact level (1)</th>
<th>Level of exposure [mg/cm²/day]</th>
<th>Exposed area [cm²]</th>
<th>Shift average [mg/person/day]</th>
<th>Method (use of gloves)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production</td>
<td>dust</td>
<td>charging, drumming, cleaning, repair, maintenance</td>
<td>daily</td>
<td>incidental</td>
<td>0 - 0.1</td>
<td>420</td>
<td>42</td>
<td>EASE (90 % protection by suitable gloves)</td>
</tr>
<tr>
<td>2. Use as a vulcanisation accelerator in the rubber industry</td>
<td>dust</td>
<td>dosing, weighing, transfer, filling</td>
<td>daily</td>
<td>-</td>
<td>-</td>
<td>820</td>
<td>200</td>
<td>analogous data (without gloves)</td>
</tr>
</tbody>
</table>

1): Contact level according to the EASE model
4.1.1.2.4 Summary of occupational exposure

[click here to insert data; consider using a table; see example below]
### Table 4.4: Conclusions of the occupational exposure assessment

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Activity 1</th>
<th>Frequency Days/year</th>
<th>Duration Hours/day</th>
<th>Inhalation</th>
<th>Dermal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Reasonable worst case</td>
<td>Typical concentration</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Unit</td>
<td>Method 2</td>
</tr>
<tr>
<td>Production</td>
<td></td>
<td></td>
<td></td>
<td>Measured</td>
<td></td>
</tr>
<tr>
<td>Subscenario 1</td>
<td>Full shift</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscenario 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscenario 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscenario 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscenario 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscenario 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uses</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscenario 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscenario 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subscenario 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1: Full shift, short term, etc.
2: Measured, EASE, Expert judgment, Calculated, etc.
4.1.1.3 Consumer exposure

Almost all rubber compounds on the market contain rubber accelerator as CBS in a wide range of products, but it is difficult to know which rubber product contain which rubber accelerants. Therefore the use of CBS in consumer product cannot be ruled out completely. However, based on relevant databases such as SPIN and Nordic database no direct consumer exposure seems to occur. In addition, based on a search in Google, Current Contents and Toxline we found no indication for exposure to CBS through the use of gloves, rubber, toys and household products. Therefore consumer exposure is thought to be minimal and does not need to be further characterized.

4.1.1.3.1 Summary of consumer exposure

In conclusion, consumer exposure is considered to be negligible.

4.1.1.4 Humans exposed via the environment

A realistic worst case exposure scenario was derived for CBS exposure for conditions combined from production site A (emission to air) and B (emission to water). Regional exposure to CBS can be expected to be negligible.

Table 4.5 Indirect local exposure to CBS in the impact area of a CBS production plant.

<table>
<thead>
<tr>
<th>Intake media</th>
<th>Local concentrations at site A</th>
<th>mg/kg bw/d</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water</td>
<td>6 *10^-4</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Fish</td>
<td>3.8*10^-3</td>
<td>15.3</td>
<td></td>
</tr>
<tr>
<td>Leaf crops</td>
<td>0.0113</td>
<td>46.3</td>
<td></td>
</tr>
<tr>
<td>Root crops</td>
<td>8.5*10^-3</td>
<td>34.8</td>
<td></td>
</tr>
<tr>
<td>Meat</td>
<td>4.2*10^-4</td>
<td>1.7</td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>2.5*10^-4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>1.6*10^-4</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>Total intake</td>
<td>0.0245</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The concentration of CBS in air outside the plant A is 0.66 µg m⁻³.

One of the most relevant stable degradation products of CBS is benzothiazole (BT). Degradation products mercaptobenzothiazole (MBT) and 2,2’-dithio-bis-benzothiazole (MBTS), which are probably more toxic than BT, are not stable. BT is in general measured in the highest concentrations of all six included degradation products in environmental samples (with some exceptions). Thus indirect exposure estimate for BT reflects the major part of the exposure caused by CBS production (and use). In addition, BT is the degradation product, which is expected due to its volatility to have largest emissions to air of the six degradation products included in the assessment and consequently all exposure routes are equally relevant for it. For BT, indirect exposure was calculated for production site A using the screening approach described in Appendix C. Concentration of BT in air outside sport halls was
estimated at 0.023 µg m\(^{-3}\) at a distance of 100 m (see chapter 3.1.3.3). This type of exposure is local of nature. The BT concentration outside CBS production site is three orders of magnitude higher. Thus the calculation below covers also risks possibly caused by emissions to air from sport halls.

Table 4.6  **Indirect local exposure to BT in the impact area of CBS production plant.**

<table>
<thead>
<tr>
<th>Intake media</th>
<th>Local concentrations at site A</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water</td>
<td>2.6 *10(^{-3})</td>
<td>67.4</td>
</tr>
<tr>
<td>Fish</td>
<td>1.13*10(^{-3})</td>
<td>29</td>
</tr>
<tr>
<td>Leaf crops</td>
<td>5.8*10(^{-5})</td>
<td>1.5</td>
</tr>
<tr>
<td>Root crops</td>
<td>8.9*10(^{-6})</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Meat</td>
<td>5.6*10(^{-6})</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Milk</td>
<td>3.4*10(^{-7})</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Air</td>
<td>7.3*10(^{-5})</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>Total intake</strong></td>
<td><strong>0.004</strong></td>
<td></td>
</tr>
</tbody>
</table>

The concentration of BT in air used for the calculation is 0.03 mg m\(^{-3}\). Some monitoring data of BT in food are presented in Appendix C. These data show that BT is present in food. BT has been measured in food mainly in connection of studies searching for aroma components and these studies have not considered the actual source of BT. In addition, mainly qualitative detections are available.

Barnes et al. (2003) looked for BT and MBT in 236 liquid food and drink samples in the United Kingdom. The study was originally set up for studying possible migration from rubber which could have been in contact with the samples. The authors did not find any traces of these substances. Due to rather high limits of detection this study cannot be used for excluding the presence of BT and MBT in food and drinks via indirect exposure.

In the absence of quantitative monitoring data, EUSES estimates in Table 4.1 and 4.2 are used for the assessment.

4.1.1.4.1  **Exposure via air**

4.1.1.4.2  **Exposure via food and water**

A realistic worst case exposure scenario was derived for CBS exposure for conditions combined from production site A (emission to air) and B (emission to water). Regional exposure to CBS can be expected to be negligible.

Table 4.7: **Indirect local exposure to CBS in the impact area of a CBS production plant.**

<table>
<thead>
<tr>
<th>Intake media</th>
<th>Local concentrations at site A</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water</td>
<td>6 *10(^{-5})</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
Fish \[3.8 \times 10^{-3}\] 15.3
Leaf crops \[0.0113\] 46.3
Root crops \[8.5 \times 10^{-3}\] 34.8
Meat \[4.2 \times 10^{-4}\] 1.7
Milk \[2.5 \times 10^{-4}\] 1
Air \[1.6 \times 10^{-4}\] <1
Total intake \[0.0245\]

One of the most relevant degradation products of CBS is benzothiazole (BT). For BT, indirect exposure was calculated for production site A using the screening approach described in Chapter 3 and Appendix C.

Table 4.8: Indirect local exposure to BT in the impact area of CBS production plant.

<table>
<thead>
<tr>
<th>Intake media</th>
<th>Local concentrations at site A</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water</td>
<td>[2.6 \times 10^{-3}]</td>
<td>67.4</td>
</tr>
<tr>
<td>Fish</td>
<td>[1.13 \times 10^{-3}]</td>
<td>29</td>
</tr>
<tr>
<td>Leaf crops</td>
<td>[5.8 \times 10^{-5}]</td>
<td>1.5</td>
</tr>
<tr>
<td>Root crops</td>
<td>[8.9 \times 10^{-6}]</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Meat</td>
<td>[5.6 \times 10^{-6}]</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Milk</td>
<td>[3.4 \times 10^{-7}]</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Air</td>
<td>[7.3 \times 10^{-6}]</td>
<td>1.9</td>
</tr>
<tr>
<td>Total intake</td>
<td>[0.004]</td>
<td></td>
</tr>
</tbody>
</table>

Some monitoring data of BT in food are presented in Appendix C. These data show that BT is present in food. BT has been measured in food mainly in connection of studies searching for aroma components and these studies have not considered the actual source of BT. In addition, mainly qualitative detections are available.

Barnes et al. (2003) looked for BT and MBT in 236 liquid food and drink samples in the United Kingdom. The study was originally set up for studying possible migration from rubber which could have been in contact with the samples. The authors did not find any traces of these substances. Due to rather high limits of detection this study cannot be used for excluding the presence of BT and MBT in food and drinks via indirect exposure.

In the absence of quantitative monitoring data, EUSES estimates in Table 4.1 and 4.2 are used for the assessment.
4.1.1.5 Combined exposure

4.1.2 Effects assessment: Hazard identification and dose (concentration)-response (effect) assessment

N-Cyclohexylbenzothiazol-2-sulphenamide (CBS) can undergo hydrolysis to 2-mercaptobenzothiazole (MBT) and cyclohexylamine (CHA). (Hansson and Agrup, 1993). Therefore, information about these two hydrolysis products is also considered for the toxicological effect assessment, if appropriate. According to Annex I of Directive 67/548/EEC MBT is labelled with R43 (May cause sensitization by skin contact) and CHA is labelled with R 21/22 (Harmful in contact with skin and if swallowed) and with R 34 (Causes burns).

4.1.2.1 Toxicokinetics, metabolism and distribution

Limited data are available on the kinetics and metabolism of CBS which are restricted to some oral studies in the rat. CBS can undergo hydrolysis to 2-mercaptobenzothiazole (MBT) and cyclohexylamine (CHA) (Hansson and Agrup, 1993). Therefore, toxicokinetic information about these two hydrolysis products is also included in this chapter. Toxicokinetics and metabolism of MBT has been investigated after oral, dermal and i.v. application in male Fischer 344 rats and after dermal application in guinea pigs.

4.1.2.1.1 Studies in animals

In vivo studies

Inhalation

There are no studies with CBS applied via inhalation.

Dermal

There are no studies with dermally applied CBS.

2-Mercaptobenzothiazole (MBT)

Toxicokinetics and metabolism of dermally applied MBT has been reviewed by BG Chemie (2000) and the investigations described based on an EPA report, which was not available. 14C-2-MBT has been applied occlusively to intact and scarificated skin of male Hartley guinea pigs and Fischer 344 rats (36.1 µg [5.01 µCi] of 14C-2-MBT for 96 hours). 16.1 to 17.5 % of the applied radioactivity was absorbed in the rat, whereas 33.4 % of the applied radioactivity was absorbed in the guinea pig. Higher absorption rates in the guinea pigs have been discussed as being due to species differences and differences in application area (5 cm² in guinea pigs vs. 2 cm² in rats). After absorption, MBT was distributed in the whole body. 13.1 – 32.6 % of the applied radioactivity was excreted with the urine, urinary excretion of
Radioactivity was maximal at 3-6 hours after application. 0.04 – 1.26 % of the applied radioactivity was excreted with feces.

**Oral**

Two publications on the fate of $^{14}$C-radiolabelled CBS in rats were obviously based on the same experiments (Adachi et al., 1989; published in Japanese with abstract and tables in English; Fukuoka et al., 1995). In these experiments rats were given single oral doses of 250 mg/kg $^{14}$C-CBS. The amounts of radioactivity recovered from urine and from the faeces were dependent on the position of the radioactivity label in the parent compound. Following administration of CBS radiolabeled in the cyclohexyl moiety $^{14}$C-radioactivity was recovered to 89.6% of the dose within three days. The extent of radioactivity was 65.4% in urine and 24.2% in faeces. As the biliary excretion amounted to 5% it might be concluded that at least 70% of the radioactivity was absorbed from the gastro-intestinal tract. When the substance was $^{14}$C-labeled in the C-2 position of the thiobenzothiazole, 92.3% of the radioactivity was recovered within three days and similar amounts of radioactivity were found in urine (46.9%) as in faeces (45.4%). In the urine, 2-mercaptobenzothiazol and cyclohexlyamine were identified as metabolites of CBS. The results indicate intensive metabolism of CBS. As hydrolysis to 2-mercaptobenzothiazol and cyclohexlyamine 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 thus explaining the different recovery rate in urine and in faeces with different positions of the $^{14}$C label.

**2-Mercaptobenzothiazole (MBT)**

Toxicokinetics and metabolism of orally applied MBT has been reviewed by BG Chemie (2000). After single (55.5 mg/kg bw [0.0497 mCi] and 0.592 mg/kg bw of $^{14}$C-2-MBT) and repeated (0.509 mg/kg bw of non-labelled MBT for daily for 14 days, followed by 0.503 mg/kg bw [0.0586 mCi] of $^{14}$C-2-MBT) oral applications (gavage) to male and female Fischer 344 rats, about 96% (males) and 101% (females) of the applied radioactivity was excreted with the urine within 96 h. About 10% (males) and 5% (females) of the applied radioactivity was excreted into the feces within 96 h. Most of the radioactivity was excreted within the first 24 h after application. This points to rapid and almost complete absorption of MBT from the gastrointestinal tract. After absorption, a wide distribution of radioactivity was observed. Two main urinary metabolites and probably 2-5 further metabolites could be differentiated. One of the main metabolite has been identified as the thioglucuronide of MBT, the other main metabolite has not been identified (most probably, it seems to be a compound in which the thiol group has been oxidized). After a single oral dose of MBT, unchanged MBT could be identified in the urine of one female animal. However, after repeated oral doses, unchanged MBT could not be determined in the urine of the treated animals. Identification and quantification of urinary metabolites has not been performed.

After oral application of 50 mg $^{35}$S-MBT to male Wistar rats, unchanged MBT, $^{35}$S-MBT-sulfate, $^{35}$S-MBT-glucuronide and non-labelled Benzothiazolmercapturic acid could be identified. Quantification of metabolites has not been performed.

**Cyclohexylamine (CHA)**

The metabolism of $^{14}$C-CHA hydrochloride has been investigated mice and Wistar and DA rats after application with the diet (400 mg/kg bw/day). Wide species differences in the
metabolism of CHA, indicated as differences of concentration of hydroxylated metabolites in plasma and testes could be observed. This information is taken from an abstract without detailed information (Roberts et al., 1989).

In vitro studies

Fukuoka et al. (1995) showed in investigations with artificial gastric juice, that after 5 min of incubation only 47% of originally applied CBS could be detected. The artificial gastric juice consisted of sodium chloride, pepsin, hydrochloric acid and water. It was discussed by the authors that pepsin might be involved in the cleavage of CBS.

A hydrolysis study investigated the hydroxylation rate of CBS under acid conditions. CBS was dissolved in 0.1 molar hydrochloric acid and heated to 35 °C. The pH was maintained at 1.0. Over a period of 156 h (6.5 d) 9% and subsequently 2% of CBS were hydrolyzed per day resulting in the reaction products cyclohexylamin, mercaptobenzothiazol and mercaptobenzothiazyl disulfide. Information on GLP compliance is not available to the rapporteur. (Lanxess 2007).

4.1.2.1.2 Studies in humans

There are no human data on toxicokinetics of CBS after inhalation, oral or dermal uptake.

4.1.2.1.3 Summary of toxicokinetics, metabolism and distribution

The results after oral administration to rats indicate that CBS is readily absorbed and that intensive metabolism of CBS takes place. As hydrolysis to 2-mercaptobenzothiazol and cyclohexylamine was shown in vitro and may 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. An absorption of 100% for the oral route is proposed to be taken for the risk characterisation, whereas dermal and inhalation absorption is assumed to be 100% (defaults).

No data are available for the dermal route. Therefore, a default value for dermal absorption should be applied. Based on the physico-chemical properties of CBS (molecular weight: 264.4 g/mol; log Pow 3.47; water solubility: 0.32 mg/l) a default value of 100% would be derived. However, this default value does not reflect the toxicity data (low toxicity via the dermal route). Therefore, an extent of absorption of 10% will be assumed for dermal risk characterisation purposes (cf. 4.1.3.2).

4.1.2.2 Acute toxicity

[click here to insert text]
4.1.2.2.1 Studies in animals

In vivo studies

Inhalation

No data available.

Dermal

Acute dermal toxicity is low as judged by a study with rabbits, demonstrating a dermal LD50 value > 7940 mg/kg bw: Three rabbits were applied doses of 5010 mg/kg (1 animal) or 7940 mg/kg (2 animals) of CBS as 40% substance suspension in corn oil directly to the clipped, intact skin for 24 hours using semi-occlusive dressings. No mortalities occurred within a 14-day observation period. Clinical signs included reduced appetite and reduced activity for 3-5 days. Viscera of animals appeared normal at sacrifice (Randall and Bannister 1990).

Oral

Acute oral toxicity of CBS is very low, with oral LD50 values reported for rats and mice higher than 5000 mg/kg bw:

An oral LD50 of 5300 mg/kg resulted from a study with four groups of five rats each receiving 3980, 5010, 6310 and 7940 mg/kg of a 25% suspension of CBS in corn oil. Deaths occurred at doses of 5010 mg/kg (1 rat died), 6310 mg/kg (4 rats died) and 7940 mg/kg (4 rats died). Clinical signs included reduced appetite, reduced activity, increasing weakness, ocular discharge, slight tremors, collapse and death. Necropsy of decedents showed lung and liver hyperemia and acute gastrointestinal inflammation. Viscera of surviving animals appeared normal at sacrifice after a seven days observation period (Randall and Bannister 1990).

An oral LD50 value of 6850 mg/kg was detected in a second study with rats: Seven groups of 3-5 rats each were administered doses of 1400, 2100, 3200, 4700, 7000, 10000 and 16000 mg/kg as a 20% suspension of CBS in 1.5% aqueous solution of methylcellulose. In this study deaths occurred at doses of 3200 mg/kg (1/5 rats died after 21 days), 4700 mg/kg (2/5 rats died after 4 resp. 14 days), 10000 mg/kg (3/4 rats died within 6 hours to 19 days) and 16000 mg/kg (3/3 rats died within 14 days). No clinical signs were observed after administration of 2100 mg/kg. Clinical signs at higher doses included initial lessening of motor activity, followed by a period of hyperactivity, an increase in the respiratory movements, mild tremors, moderate clonic convulsions, frequent uncoordinated motions of the head, feet and tail, occasionally mild tonic convulsions, gradually failing respiration, and terminal coma. The survivors generally lost body weight during the first week; such losses were regained during the third week. At necropsy, animals demonstrated hemorrhagic edema or congestion of the lungs and moderate to severe parenchymatous degeneration of the brain, liver, heart and kidneys (Kettering Laboratory 1951).

In a third study with rats, an oral LD50 >5000 mg/kg bw was stated: "N-Cyclohexyl-2-benzothiazyl sulfenamide commercial grade" was suspended in corn oil and orally administered to three groups of 10 males and 10 females each (doses of 1000, 2500 and 5000 mg/kg). No clinical signs were observed after administration of 1000 mg/kg bw, deaths occurred at 5000 mg/kg (2 males and 3 females died within 3 days). Clinical signs at 2500 mg/kg bw and 5000 mg/kg bw included irregular respiration, dyspnea, hypersensitivity and
ataxia, reversible within 5 days in surviving animals. No remarkable changes were found in any groups at necropsy (Sumitomo Chemical Co. 1977).

The oral LD50 for mice was detected as > 8000 mg/kg bw: 4000 mg/kg bw or 8000 mg/kg bw of a substance called "Soxinol" was applied orally to 24 male mice as a suspension in 5% aqueous solution of Arabian rubber. No deaths and no changes of general conditions were observed (National Institute of Health Sciences, Japan 1995).

In vitro studies
No data available.

**4.1.2.2 Studies in humans**
No data available.

**4.1.2.3 Summary of acute toxicity**
The acute toxicity of CBS in animals is very low after oral and dermal administration; LD50 values of >5000 mg/kg bw were obtained. Data on inhalation toxicity and human data are not available.

**4.1.2.3.1 Skin**

Studies in animals
0.5 g of finely ground CBS moistened with saline was applied under semi-occlusive dressings to clipped, intact and abraded skin of each of six rabbits (three males and three females) for 24 hours. There were four application sites in each rabbit (two sites for intact, two sites for abraded skin). 24 hours post application, 4 erythema grade 1.0 were observed in 3 animals at the intact application sites which had resolved at the 72 hours observation time (Monsanto Comp. 1982).

Studies in humans
Products called „Santocure“ (CBS, purity approximately 96-98%) have been tested in patch tests with humans. No skin irritation was observed after dermal applications for 24 or 48 hours: 200 volunteers were patch tested with „Santocure, full strength" using distilled water as vehicle. None of the volunteers demonstrated irritation after removal of the patch test after 24 hours (test site was observed at 24 and 48 hours after patch removal; Monsanto Company 1950). In a repeated insult patch test with 51 persons using a 70% substance preparation in petrolatum, N-cyclohexyl-2-benzothiazole sulfenamide induced irritation in 8/51 individuals.

Criteria to assess symptoms of irritation (and sensitisation) include erythema. In addition to erythema, skin reactions other than erythema were considered (e.g. cracking, drying, fissuring, glazing itching and so on). When evaluating irritating responses, also confounding factors were considered. These included: adhesive, washing solvents, trauma and dermatitis...
coincidental with the procedure (Monsanto Company 1982). In five of these individuals, minimal erythema was observed sporadically and was not considered of any significance.

4.1.2.3.2 Eye

Studies in animals

100 mg of finely ground CBS was placed into the conjunctival sac of each of six rabbits (3 males, 3 females). At the 24 hours observation time slight conjunctival irritation was detected (no detailed grading given). This irritation had resolved at 48 hours observation time (Monsanto Comp. 1973).

Studies in humans

No data available.

4.1.2.3.3 Respiratory tract

Occasional signs of mild nasal irritation were observed in a 28-day inhalation toxicity study in Sprague-Dawley CD rats, which were whole body exposed to atmospheric concentrations of 0.0043, 0.0144 and 0.048 mg/l CBS for 6 hours per day and 5 days per week for a period of four consecutive weeks (Monsanto, 1981a). The rats exhibited occasional nasal irritation, which appeared to be concentration related in terms of incidence and severity (no more data). Signs of nasal irritation were observed immediately after the 6-hour exposure period, disappeared by the next morning, and did not correlate to histopathologic effects (c.f. 4.1.2.6.1). The condition was usually observed at the end of exposure but was not observed the following morning (c.f. 4.1.2.6.1). In light of the fact that CBS has shown slight irritations at the eye of rabbits in an eye irritation test it seems plausible that CBS leads also to slight irritations at the mucous membranes of the respiratory tract after inhalation.

4.1.2.3.4 Summary of irritation

CBS has demonstrated few cases of skin irritation in human patch tests with the commercial product, when using petrolatum as a vehicle. In animal tests CBS caused slight irritation on the skin and on the conjunctivae of the eye of rabbits. Occasional signs of mild nasal irritation were observed in some Sprague-Dawley CD rats immediately after the 6-hour exposure period with atmospheric concentrations up to 0.048 mg/l 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. However, these data cannot be used to conclude a potential of CBS to cause acute respiratory irritation relevant for classification and labelling.

4.1.2.4 Corrosivity

CBS is not a corrosive substance.
4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

Skin

In vivo studies

„Santocure Duplex Process” (no data on CBS content) has not demonstrated any delayed hypersensitivity in a Buehler test with guinea pigs: Based upon the results of a dose-range-finding study with guinea pigs and in discussion with the sponsor, the highest non-irritating dose chosen for induction was 25%. S-Duplex was dermally applied to twenty guinea pigs (10 males and 10 females) for a total of three 6-hour insult periods at a concentration of 25%. An additional group of ten guinea pigs was treated with 1-chloro-2,4-dinitrobenzene (DNCB, positive control). In order to assess the influence of the vehicle on sensitization, four guinea pigs were treated with 80% ethanol during the induction period. Fourteen days after the last induction period, all animals were challenged at a naive site. A positive response was elicited in the animals receiving DNCB, no response was observed in the vehicle induced animals challenged with the test article at a 25% concentration, no positive response was observed in the experimental group receiving the test article at 25% concentration (Pharmacon Research International 1982).

In vitro studies

No data available.

Respiratory tract

No data available.

4.1.2.5.2 Studies in humans

Skin

In vivo studies

In a repeated insult patch test with 51 persons using a 70% substance preparation in petrolatum, N-cyclohexyl-2-benzothiazole sulphenamide acted as a sensitizer in 5/51 individuals (Monsanto Company 1982).

CBS is reported frequently to be a causative agent of allergy to rubber additives: Rubber additives (mainly vulcanizers and antioxidants) are increasingly a cause of contact dermatitis in Spain. Among 7000 patients seen during a 10-year period, 686 had one or more positive reactions to rubber additives. These 686 patients were also patch tested separately with 18 individual rubber chemicals at 1% concentration in petrolatum. Patch tests were removed at
48 hours, and evaluation was performed 48 and 96 hours after application. 34 of the 686 patients (4.9%) reacted positive when tested with CBS (Conde-Salazar et al. 1993).

In Poland occupational allergic contact dermatitis was diagnosed in 334 out of 1697 patients with suspected occupational dermatitis examined between January 1989 and March 1994. This research included 46 patients who were sensitive to rubber products. These 46 patients were patch tested using Finn Chambers and an application time of two days. The results were stated on the second and third days after application. 4/19 females and 7/27 males reacted positive when tested with CBS (Kiec-Swierczynska 1995). A second study on sensitivity to rubber additives demonstrated CBS as cause of contact dermatitis in 2.3% of 1538 patients: 1538 patients of a dermatologic clinical department were tested with 1% CBS in petrolatum, 2.3% reacted positive (Rudzki et al. 1976).

In Denmark research on causative substances of thiuram-sensitivity revealed that CBS caused contact dermatitis in 1/15 patients: 15 of 22 thiuram-sensitized patients were tested with CBS at 1% in petrolatum using Finn Chambers and 2 days-occlusion. Reading was performed on days 2, 3 and 7 and demonstrated 1/15 patients positive when tested with CBS (Knudsen et al. 1993).

In India research on causative substances of shoe dermatitis revealed that CBS caused contact dermatitis in 2/47 patients: 105 patients with foot dermatitis were patch tested with various shoe allergens: 47 of them showed a positive reaction to one or more allergens, two out of them demonstrated positive reactions when tested with CBS (Bajaj 1988).

Data on inhalation sensitization are not available. However, no cases of inhalation sensitization have been reported in the area of occupational exposure.

**In vitro studies**

No data available.

**Respiratory tract**

No data available.

### 4.1.2.5.3 Summary of sensitisation

Data on sensitization caused by inhalation are not available. As no cases of respiratory sensitization after occupational exposure have been reported yet, it can be assumed that CBS does not induce sensitization via the inhalation route. CBS did not cause skin sensitization in guinea pigs. In contrast, there was one well conducted human patch testing study which clearly demonstrated contact sensitization in humans. Data from epidemiological studies are difficult to assess, but also indicate some skin sensitizing potential of CBS. In consequence, existing classification with R 43 is confirmed.

### 4.1.2.6 Repeated dose toxicity

The available repeated dose toxicity studies in experimental animals used the inhalation, dermal and oral (feeding, gavage) route of exposure to CBS. They were accepted for the
requirements of the Regulation 793/93/EEC according to the Annex VIIA, 92/32/EEC and the methods of the Annex V, 67/548/EEC.

4.1.2.6.1 Studies in animals

In vivo studies

Inhalation

In a 28-day inhalation toxicity study groups of Sprague-Dawley CD rats (10/sex/group) were whole body exposed to atmospheric concentrations of CBS of 0.0043, 0.0144 and 0.048 mg/l (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 (Monsanto, 1981a). The equivalent aerodynamic diameter of the CBS dust generated was 7.6 µm with a geometric standard deviation of 2.71 µm. This distribution of CBS particle sizes was above the recommendations in common inhalation guideline tests. Two further groups of rats (10/sex) acting as control groups were exposed to compressed, filtered air alone. There were no treatment-related premature deaths. The rats exhibited occasional nasal irritation which appeared to be concentration related in terms of severity and number of animals exhibiting the sign (no more data). This finding was observed immediately after the 6-hour exposure period, and disappeared by the next morning. Since the animals recovered from this lesion within 24 hours and these findings could not be correlated to histopathologic effects, the observation of nasal irritation in few CBS-exposed animals was not considered to be toxicologically adverse.

No concentration dependent effects were noted for general appearance, behaviour, body weight, food consumption, hematology, urinalysis, gross pathology and absolute and relative organ weights. Clinical biochemistry revealed concentration-related statistically significant increase of serum glutamic oxalacetic transaminase (SGOT) values in males and females exposed to 0.0144 and 0.048 mg/l compared to control groups. There were no changes in organ weights and macroscopic examination that were considered to be an effect of exposition with CBS. A slight increased incidence of brown pigment within sinusoidal macrophages in lymph nodes was present in 2/10 males and 4/10 females exposed to 0.048 mg/l compared to controls (3/20 females). Very slight to slight increase of hemosiderin storage in the spleen was observed in 5/10 females of the 0.048 mg/l concentration group. This finding was not observed in male and female rats exposed to the mid and low concentration of 0.0144 and 0.0043 mg/l, or in any of the 20 males and 20 females of the internal controls. No other CBS-related microscopic lesions were reported. Since a clear increase in hemosiderin deposition was observed in female rats exposed to 0.048 mg/l compared to the internal control group (0/10) this finding was considered as a CBS-related effect. Deposition of hemosiderin pigments in the spleen is generally considered to be a sign of increased erythrocyte destruction and indicates that hemoglobin released after hemolysis has reached this organ. It is known that extensive hemosiderosis can lead to organ damage through the release of free iron and subsequent free radical production. Since the observed increase in hemosiderosis is determined without any other indications of hemolysis including e.g. changes in hematology or clinical biochemistry parameters, the isolated finding of hemosiderosis in the spleen is considered to be of minimal toxicological significance and not indicative of an adverse effect. Therefore, the no-observed-effect-concentration (NOEC) for systemic effects is 0.0144 mg/l and the systemic NOAEC which could be derived from this study is 0.048 mg/l. No lower systemic NOAEC was considered because the increased SGOT levels at 0.0144 mg/l were not
confirmed by microscopic changes in the liver and should therefore not be regarded as an adverse effect.

A further microscopic finding was present in the eyes of a few animals exposed to the highest concentration of 0.048 mg/l. There was a slightly increased incidence and severity of conjunctivitis in each of 2/10 males and females when compared to controls (1/20 males). Because a limited number of animals were affected this finding was considered to be incidental and spontaneous in nature. Microscopy of nasal turbinates, olfactory bulb and the lungs showed a number of histopathologically findings. Their type, incidence and severity did not distinguish CBS-exposed rats from controls. Based on the fact that the signs of nasal irritation observed at clinical examination were only short in duration and could not be correlated to histopathologic effects, no toxicological significance was attached to this finding. So, the NOAEC for local effects on the respiratory tract in rats was 0.048 mg/l. Overall, the NOAEC for systemic and local effects for CBS in Sprague-Dawley CD rats was 0.048 mg/l, 6h/d, 5 d/wk (Monsanto, 1981a).

2-Mercaptobenzothiazole (MBT)

In rats, the inhalation of 300 to 400 mg MBT (dust)/m³ air for two hours daily for 15 days led to slight reduced body weight and slight transient changes in the functional state of the nervous system in the majority of the animals. No further data available (Varobeva and Mezentserva, 1962).

Dermal

In a dermal 21-day toxicity study according to OECD TG 410 CBS was applied daily to the intact and abraded skin in doses of 125, 500 and 2000 mg/kg bw/d to each of five male and female adult New Zealand White rabbits for a period of 3 weeks. Saline was used as the control. Ground CBS was applied onto the skin moistened and covered with occlusive dressing. No data were available indicating the administered concentrations or surface area doses of CBS. 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. One male control rat was found dead on day 12 and one male treated with 2000 mg/kg bw/d on day 13, respectively. Neither death was attributed to treatment with CBS. A third rabbit, a male treated with 125 mg/kg bw/d, was sacrificed in extremis on day 18 due to a fracture of the femur on the right hind leg. No dose dependent effects were noted for general appearance, behaviour, body weight, hematology, blood biochemistry, gross pathology, absolute and relative organ weights and histopathology. During the study period a few rabbits in each of the CBS dose groups exhibited very slight to slight erythema on the application area of the skin, which was not observed in any CBS-treated animal at the end of the treatment period. The incidence of very slight to slight desquamation seen as slight scaling was increased in animals of the CBS-dose groups when compared to controls. But a dose-response relationship was not noticed. At microscopy of the skin slight hyperkeratosis and acanthosis in the epidermis and infiltration of inflammatory infiltrate in the dermis were observed. These 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 (Monsanto, 1981b).
Oral

In a 28-day (feeding) toxicity study groups of Sprague-Dawley CD rats (5/sex/group) were administered at dietary concentrations of 0, 100, 250, 500, 1000, and 3000 mg/kg bw/d (corresponding to mean compound consumption of 0, 101, 249, 500, 961, and 3208 mg/kg bw/d in males; and 0, 92.5, 229, 459, 910, and 2769 mg/kg bw/d in females) CBS for a period of 28 consecutive days. Three female rats given 3000 mg/kg bw/d died during the treatment period. Two deaths occurred during study week two and one death occurred during study week four. CBS-related effects were noted at the highest dose level of 3000 mg/kg bw/d and included hair loss, laboured breathing, yellowish staining of the anogenital region and distension of the abdomen. Less frequent findings were abdominal masses and decrease of motor activity. In rats of both sexes statistically significant body weight reduction and a decrease in food consumption were observed at ≥500 mg/kg bw/d CBS. No blood biochemistry and hematology parameters were examined, and no histopathology was performed in this study because this study was intended to serve as a range-finding study for chronic and reproductive toxicity studies. The NOAEL for systemic effects which could be derived from this study is approximately 250 mg/kg bw/d and is based on reduced body weight gain and food consumption. The lack of blood biochemistry, hematology and histopathology data diminishes the validity of this NOAEL though food consumption and body data are generally recognized as sensitive indicators of systemic toxicity (Monsanto, 1980).

In a 28-day (gavage) toxicity study mostly according to OECD TG 407 groups of Crj:CD (SD) rats (6/sex/group) were tested at dosages of 0, 25, 80, 250, and 800 mg/kg bw/d CBS (purity 98.8%) for a period of 28 consecutive days; additional 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. This report was written in Japanese with an abstract in English, but included expressive tables with results of hematology, clinical biochemistry, assessment of organ weights and incidences of histopathological findings. Therefore, these data were used for characterisation of the repeated dose toxicity of CBS. No mortality occurred during the study period. After administration of 800 mg/kg bw/d signs of loss of general conditions like piloerection and solid fur were observed in female rats. Suppression of food consumption and body weight gain was observed in females given ≥250 mg/kg bw/d and in males at 800 mg/kg bw/d. Hematology revealed statistically significant shortening of the prothrombin time in males given ≥250 mg/kg bw/d, and statistically significant decreases in hematocrit value, reticulocyte count and platelet count for the 800 mg/kg bw/d females. Clinical biochemistry revealed statistically significant decreases in alanine aminotransferase (ALAT) for males of all CBS treated groups and for females given ≥80 mg/kg bw/d, in total protein for males treated at ≥250 mg/kg bw/d, in chloride levels in males and females at 800 mg/kg bw/d, and in sodium levels in females at 800 mg/kg bw/d, additionally calcium concentration was elevated in females at the 800 mg/kg bw/d dose group. Urinalysis revealed an increase of ketone bodies in males treated with ≥250 mg/kg bw/d. The statistically significant increase in relative kidney weights in the 800 mg/kg bw/d males was considered to be associated with an increase in deposition of hyaline droplets in the proximal tubular epithelium of the kidneys in male rats evident at 800 and 250 mg/kg bw/d. This effect in male kidneys showed a clear tendency towards reversibility. All other changes were completely reversible after recovery period of 14 days.

In summary 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 NOAEL for systemic effects in Crj:CD (SD) rats is 80 mg/kg bw/d; the NOAEL for local effects after administration via gavage is 800 mg/kg bw/d (Chemicals Investigation Promoting Council, 1997c).

2-Mercaptobenzothiazole (MBT)
Under the carcinogenicity testing program of the NTP MBT had also been investigated in toxicity studies with different duration of treatment, and additionally in carcinogenicity tests in rats and mice (NTP 1988). These studies were also reviewed in BG Chemie (2000).

In range-finding experiments studying subchronic and chronic toxicity, groups of five male and five female rats (F344/N, 6 weeks old) and five male and five female mice (B6C3F1, 6-8 weeks old) were administered MBT (96-97% purity) in corn oil by gavage 12 doses over 16 days (on 5 days/week). The following doses were used: 0, 156, 313, 625, 1250, or 2500 mg/kg bw/d for rats; and 0, 188, 375, 750, 1500, or 3000 mg/kg bw/d for mice. There were no MBT-related deaths in rats. Mean body weight gain in rats of each sex given 2500 mg/kg bw/d was reduced by 8-14%.

In the mice, 4/5 males and 5/5 females receiving 3000 mg/kg bw/d and 4/5 females receiving 1500 mg/kg bw/d died before the end of the study. Mice given 1500 or 3000 mg/kg bw/d were lethargic after Day 1. Final mean body weights were not adversely affected by MBT, and no MBT-related findings were observed at necropsy.

For selection of doses for the 2-year studies groups of F344/N rats (10/sex/group) were administered 0, 188, 375, 750, or 1500 mg/kg bw/d MBT (96-97% purity) in corn oil by gavage 5 days per week for a period of 13 consecutive weeks. Groups of 10 B6C3F1 mice of each sex were administered 0, 94, 188, 375, 750, or 1500 mg/kg bw/d MBT on the same schedule.

There were no MBT-related deaths in rats. The animals displayed irritable behaviour that was more pronounced with increasing dose and was characterized as resistance to gavage. A delay in body weight gain was reported in females ≥750 mg/kg bw/d and in males after administration of 1500 mg/kg bw/d. Liver weight and liver to body weight ratios were increased in dosed rats with the greatest change occurring at 750 and 1500 mg/kg bw/d. No gross or microscopic effects could be related to MBT administration. So, the NOAEL for systemic effects of MBT in F344/N rats in this subchronic study was 375 mg/kg bw/d.

Premature deaths occurred in the mice study: 5/10 males and 7/10 females received dosages of 1500 mg/kg bw/d. Two of the deaths were related to gavage technique mistake. MBT administration did not affect body weight gain in mice. Liver weight to body weight ratios was higher than those of the vehicle controls. Clonic seizures, lacrimation, and salivation were observed in the 750 and 1500 mg/kg bw/d groups. Lethargy and rough coats were observed at 375 and 750 mg/kg bw/d. No MBT-related gross or microscopic pathologic effects were observed in mice. The NOAEL for systemic effects of MBT in B6C3F1 mice was 375 mg/kg bw/d. No association between the observed clinical signs of toxicity in rats and mice and gross or microscopic pathologic effects were observed. Thus, from these subchronic studies there is no indication for specific toxic properties of MBT in mice and rats.

In a following carcinogenicity test, 50 male F344/N rats were administered at 0, 375, and 750 mg/kg bw/d MBT in corn oil by gavage 5 days per week for 103 weeks; 50 female F344/N rats were administered with 0, 188, or 375 mg/kg bw/d, respectively. 50 B6C3F1 mice of each sex were given 0, 375, and 750 mg/kg bw/d MBT.

Administration of MBT resulted in decreased survival in dosed male rats (vehicle control, 42/50; low dose, 22/50; high dose, 20/50) and in the high dose group of female mice (37/50;
39/50; 22/50) but not in female rats (28/50; 31/50; 25/50) or in male mice (38/50; 33/50; 30/50). No effect on body weight gain in MBT-dosed male and female rats was observed; in MBT-dosed male and female mice, minor reductions occurred between weeks 3 and 64, with recovery thereafter. Postgavage lethargy and prostration occurred frequently in MBT-dosed rats and mice. The principal nonneoplastic lesions of MBT seen in these studies were nephropathy and inflammation and ulceration of the stomach in male and female rats. The severity of nephropathy, characterized by tubular degeneration and regeneration, was increased in MBT-dosed male rats. There were no increases of nonneoplastic lesions in mice which were considered to be MBT-related.

Cyclohexylamine (CHA)

Cyclohexylamine was tested by oral administration at several dose levels in several tests on different duration in several strains of rats and mice.

In a subchronic toxicity study groups of 15 male and 15 female CFE rats were administered 0, 600, 2000, or 6000 ppm cyclohexylamine hydrochloride in the diet for a period of 13 consecutive weeks. These dose levels were equivalent to approximately 0,41, 143, 468 mg/kg bw/d. The study included a paired feeding study: Groups of five rats/sex were fed at 0 or 6000 ppm CHA with an amount of feed that was equal to that consumed by rats of the main study in the previous 24 hours. Body weight gain and food intake were significantly reduced at ≥2000 ppm cyclohexylamine hydrochloride. No changes in hematology, clinical biochemistry or urinary excretion values were associated with cyclohexylamine hydrochloride treatment. In the group fed with 6000 ppm absolute and relative testes weights were decreased (also in paired fed littermates) and tubular atrophy in the tested were observed. Reduced spermatogenesis and tubular atrophy in the testes were in 4/11 males treated with 2000 ppm, whereas this was not seen in rats fed 600 ppm (for more details see chapter 4.1.2.9.1). Apart from the effects on the testis noted at ≥2000 ppm, no changes were seen in other organs and tissues examined by microscopy. The NOAEL for systemic effects was detected in CFE rats fed cyclohexylamine hydrochloride at 600 ppm, a level equivalent to an intake of approximately 41 mg/kg bw/d (Gaunt et al., 1974).

In a long-term toxicity study groups of 48 male and 48 female Wistar rats were fed diets containing 0, 600, 2000 or 6000 ppm cyclohexylamine hydrochloride (corresponding to about 0, 24, 82 and 300 mg/kg bw/day in males; and 0,35, 120 and 440 mg/kg bw/d in females) for 104 weeks. Parameters of toxicity investigated included behaviour and general appearance, body weight gain and body weights, food and water consumption, limited parameters in hematology, urinalysis and tests of kidney function, necropsy and microscopy of a large number of tissues. Effects attributed to treatment with cyclohexylamine hydrochloride included a dose-related decrease in mortality, decreased body weight gain and decreased terminal body weights. There was a dose-related reduction in the rate of body weight gain throughout the study, significantly at 2000 and 6000 ppm. Compared with the controls, males and females given 6000 ppm showed respectively a 31 and 41% reduction in body weight at wk 101. In the rats given 2000 ppm the corresponding reductions were 14 and 24%, while at the lowest level of treatment (600 ppm) the reductions were 7 and 11%. Food consumption was decreased at ≥2000 ppm. At ≥2000 ppm females had elevated relative thyroid weights; and minor hematological effects were noted in both sexes. In male rats a reduction of absolute testes weights was observed in the 2000 ppm dose group (relative weights unchanged). Severe testicular degeneration was seen after treatment with 6000 ppm. At the same dose level an increased frequency of pulmonary alveoli with foamy macrophages was observed in rats of both sexes. The effects on body weight gain and body weights were observed in both sexes in
all treated groups throughout the experiment. However, there were no statistically significant increases in the incidence of testicular lesions and no other relevant systemic effect in rats given the lowest dose level of 600 ppm. Therefore, the NOAEL for systemic effects of cyclohexylamine hydrochloride in male and female Wistar rats in this study was 600 ppm, corresponding to about 24 mg/kg bw/d in males and 35 mg/kg bw/d in females (Gaunt et al., 1976).

In a long-term study groups of 48 male and 50 female ASH-CS1 mice were fed diets containing 0, 300, 1000, or 3000 ppm cyclohexylamine hydrochloride (corresponding to about 0, 40, 140, or 400 mg/kg bw/d in both sexes) for a period of 80 weeks. There were no effects attributable to treatment with cyclohexylamine hydrochloride in the number of deaths, rate of body-weight gain, food intake, and hematology. The occurrence of toxic effects in testes in male mice treated with cyclohexylamine hydrochloride was not reported. The only cyclohexylamine hydrochloride-related effect was an increased incidence of minor hepatic changes in females (cell vacuolization or polyploidy) given 3000 ppm. Therefore, the NOEL for systemic effects of cyclohexylamine hydrochloride was considered to be 1000 ppm, corresponding to about 140 mg/kg bw/d (Hardy et al., 1976).

In three long-term studies groups of 50 male and 50 female Swiss (SPF derived, outbred) mice were fed at dietary concentration of 0.5% cyclohexylamine sulfate (equivalent to about 500 mg/kg bw/d in both sexes, calculated on an assumed body weight of 20 g and a mean daily food intake of 2 g for mice) of over a period of 18 or 21 months. The whole experiment included several other chemicals. The body weight, mortality and food intake were recorded regularly. Hematology was carried out in 5 males and 5 females at an age of 12 months and at the end of the study. Microscopy was performed in several organs and tissues. Special attention has been given to the urinary system. The body weights of the cyclohexylamine sulfate-treated mice were significantly lower compared to the controls. The survival rate of mice of the cyclohexylamine groups was higher as compared to the controls. Hematology revealed no significant differences between cyclohexylamine sulfate groups and controls. The observed pathologically findings were equally distributed over the control group and the cyclohexylamine sulfate-treated groups. The occurrence of toxic effects in testes in male mice treated with cyclohexylamine sulfate was not reported. Changes frequently observed were leukemia, amyloid nephrosis and proliferative alterations in the lungs. These changes are normally seen in Swiss mice. Overall, no indications for systemic toxic effects were found in Swiss mice after long-term feeding at a dietary concentration of 0.5% cyclohexylamine sulfate (Kroes et al., 1977).

In vitro studies
No data available.

4.1.2.6.2 Studies in humans
No data available.

4.1.2.6.3 Summary of repeated dose toxicity
Human toxicity data after repeated exposure to CBS is not available.
The data on repeated dose toxicity in experimental animals with CBS included studies with three routes of administration: inhalation, dermal, oral. These studies were accepted for the requirements of the Regulation 793/93/EEC according to the Annex VIIA, 92/32/EEC. So, the available data permit the derivation of a NOAEC for repeated dose toxicity by inhalation, and a NOAEL by dermal exposure and by oral administration.

Repeated exposure by inhalation to 0.048 mg/l CBS for 28 days caused clear increases in hemosiderin deposition in the spleen of female rats, however without any other indications of hemolysis including e.g. changes in hematology or clinical biochemistry parameters. No local effects on the respiratory tract could be observed at this concentration. The aerodynamic diameter (MMAD) of the CBS dust tested was 7.6µm with a GSD of 2.7µm. This distribution of CBS particle sizes was above the recommendations in common inhalation guideline tests. Thus the reliability of the study is limited because inhalation of these CBS particles is reduced compared to particles with a MMAD lower than about 4µm. There is no information available whether a smaller particle size of CBS is known compared to the particle size of CBS in the study of Monsanto (1981a). No systemic or local effects were noted in New Zealand White rabbits after repeated dermal exposure for three weeks with 2000 mg/kg bw/d. The oral administration by gavage of ≥250 mg/kg bw/d CBS for a period of 28 days caused coagulopathy of the blood in male and female Crj:CD (SD) rats and effects in the kidney of males. No relevant systemic toxic effects were observed in male and female rats after oral administration of 80 mg/kg bw/d CBS and no local toxic effects on the digestive tract were determined in animals of both sexes treated with 800 mg/kg bw/d.

No-observed-adverse-effect-level or concentration (NOAEL/NOAEC):

Inhalation
With repeated inhalation exposure to 0.048 mg/l CBS for 28 days clear increases in hemosiderin deposition in the spleen were observed in 5/10 female rats. Such finding was neither observed in the lower test group nor in the internal control group. Therefore, this finding was considered as an exposure-related effect to CBS. Since the observed increase in hemosiderosis is determined without any other indications of hemolysis including e.g. changes in hematology or clinical biochemistry parameters, the isolated finding of hemosiderosis in the spleen is considered to be of minimal toxicological significance and not indicative of an adverse effect on health. So, the highest tested concentration of 0.048 mg/l is considered as NOAEC for systemic effects. For local effects on the respiratory tract a NOAEC could be derived from the same subacute inhalation toxicity study. No relevant local effects were observed at 0.048 mg/l.
28-day study/ Sprague-Dawley CD rat
NOAEC(sys, local): 0.048 mg/l, 6 hours/d, 5 days/week (Monsanto, 1981a)

Dermal
In a subacute dermal toxicity study using doses of 125, 500 and 2000 mg/kg bw/d no systemic or local effects were noted in New Zealand White rabbits after repeated dermal exposure for three weeks with 2000 mg/kg bw/d.
21 day-study/ New Zealand White rabbits
NOAEL(sys, local): 2000 mg/kg bw/d (Monsanto, 1981b)
Oral

For the oral route of exposure two subacute oral toxicity studies are available, a feeding study and a gavage study. From the feeding study with Sprague-Dawley CD rats a NOAEL of approximately 250 mg/kg bw/d could be derived based on reduced body weight gain and food consumption. However, no blood biochemistry and hematology parameters were examined, and no histopathology was performed in this feeding study. The gavage study was performed mostly in accordance to the regulation requirements (EEC method B.7) and provided data on hematology, clinical biochemistry, organ weights and incidences of histopathological findings. In a 28-day (gavage) toxicity study signs of coagulopathy of the blood were observed in both male and female Crj:CD (SD) rats. In males shortening of the prothrombine time (statistically significant) was noted after oral administration of \( \geq 250 \) mg/kg bw/d CBS, and in females statistically significant decreased platelet count was noted at 800 mg/kg bw/d. CBS-related effects were present in male and female Crj:CD (SD) rats at \( \geq 250 \) mg/kg bw/d. There were signs of a coagulopathy of the blood in both sexes and effects in the kidney of male rats. No relevant systemic toxic effects were observed in male and female rats after repeated oral administration of 80 mg/kg bw/d CBS. Considering the toxicological relevance of the different NOAELs, the NOAELsys of 80 mg/kg bw/d seemed to be the most relevant one, because the subacute feeding (28-day) study does not fulfil the EEC Annex V criteria. In the same study no local toxic effects on the digestive tract were noted in males and females after repeated administration of 800 mg/kg bw/d (NOAELlocal).

28-day (gavage) study/ Crj:CD (SD) rat

NOAELsys: 80 mg/kg bw/d (Chemicals Investigation Promoting Council, 1997c)
NOAELlocal: 800 mg/kg bw/d (Chemicals Investigation Promoting Council, 1997c)

A lower NOAELsys of 7.1 mg/kg bw/d (= 0.01% CBS in the diet) for dams was derived from the results of a developmental study based on reductions in body weight gain (Ema et al., 1989). At present there is no reasonable explanation to the unexpected low LOAEL of 69.6 mg/kg bw/d (= 0.1% CBS in the diet) for weight gain impairment in this dietary study (c.f. 4.1.2.9.2). Two other developmental toxicity studies with gavage administration as well as the above reported guideline according repeated dose toxicity study via gavage (28-day) consistently gave considerable higher LOAEL. Furthermore, weight gain impairment in the study of Ema et al. (1989) did not show a dose response and was not seen at the respective dose level in the two comparable developmental studies as well as in the above reported guideline according repeated dose toxicity study. Thus, the lower maternal NOAEL in the study of Ema et al. (1989) is supposed to be not related to a higher sensitivity of pregnant rats but rather be explained from the administration route or probably the rat strain (Wistar rats (Kar:Wistar, Keari Co., Osaka).

In summary, the NOAELsys for systemic effects for CBS of 80 mg/kg bw/d was derived from the 28-day (gavage) study in Crj:CD (SD) rats and the most sensitive NOAECsys of 0.048 mg/l (highest concentration tested; 6h/d, 5 d/wk) from a standard 28-day inhalation study in Sprague-Dawley CD rats, respectively.

On the basis of the data submitted, classification of CBS as “harmful” according to the criteria given in Directive 67/548/EEC is not warranted. Inhalation, dermal and oral route of exposure did not show any local or systemic effect at critical dose levels.

This is supported by repeated dose toxicity data for the hydrolysis products MBT and cyclohydroxylamine:
In toxicity studies with MBT on different duration of treatment, and in addition in carcinogenicity tests in rats and mice of several strains, body weight reduction and microscopically visible changes in the kidneys were observed. After long-term administration decreased survival was noted in dosed male and female rats given doses up to 375 mg/kg bw/d in female rats and 750 mg/kg bw/d in male rats, and nephropathy in males and ulcers and inflammation in the forestomach in males and females, respectively. There were no increases of nonneoplastic lesions in mice given up to 375 mg/kg bw/d MBT for a period of two years.

Oral administration of cyclohexylamine (at different doses and exposure levels) in several strains of rats and mice in tests on different duration revealed that the testes is the most sensitive organ to the toxicological effects of cyclohexylamine. No relevant toxic effects were observed in male and female rats given 600 ppm (approx. 24 mg/kg bw/d in males and 35 mg/kg bw/d in females)) in the diet for two years. Cyclohexylamine appeared to be somewhat less toxic in mice. Dietary levels of up to 3000 ppm (approx. 400 mg/kg bw/d) did not influence the mortality, rate of body-weight gain, food and water intake, and hematology parameters in mice treated for 80 weeks. No indications for systemic toxicity were found in Swiss mice after long-term feeding at a dietary concentration of 0.5% cyclohexylamine (approx. 500 mg/kg bw/d).

4.1.2.7 Mutagenicity

Some papers which mention genotoxicity data on CBS were not considered because the information on genotoxicity of CBS is too limited (Donner et al. 1983; Rannug et al. 1984; You et al. 1982).

4.1.2.7.1 Studies in vitro

CBS was negative in well-conducted gene mutation assays employing Salmonella typhimurium tester strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 and Saccharomyces cerevisiae strain D4 with and without S-9 mix for doses ranging from 0.1 to 500 µg/plate; toxic effects were induced by the highest doses (Monsanto Report 1976).

Furthermore, another bacterial gene mutation test - written in Japanese with an abstract in English - was negative. Here the tester strains Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537 and Escherichia coli WP2 uvrA were used. Treatment by the preincubation method was done for doses up to 5000 µg/plate. Toxicity effects varied in a strain-specific manner; at high doses from 200 µg/plate upwards precipitations were observed (Chemicals Investigation Promoting Council, 1997b).

A well-conducted mouse lymphoma assay with L5178Y cells was negative with S-9 mix up to a dose of 75.0 µg/ml and without S-9 mix up to a dose of 30.0 µg/ml; toxic effects were found with and without S-9 mix in high doses (Monsanto Report 1979). No differentiation on colony-size was made.
An in vitro chromosomal aberration test - written in Japanese with an abstract in English - was weakly positive (Chemicals Investigation Promoting Council, 1997c). The test was run with CHL cells with and without S-9 mix.

In the absence of S-9 mix no induction of structural chromosomal aberrations was found for doses ranging from 10 to 41 µg/ml; no strong cytotoxicity was induced, but higher doses led to total cytotoxicity; various treatment and sampling times were used (24/24 h; 48/48 h and 6/18 h).

In the presence of S-9 mix weakly increased frequencies of structural chromosomal aberration were found in two experiments after 6 h treatment and 18 h sampling. Again no strong cytotoxicity was induced but higher doses led to total cytotoxicity. The main results were as follows:

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Concentration (µg/ml)</th>
<th>Frequency of Chromosomal Aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment I</td>
<td>41 µg/ml CBS</td>
<td>3.5% chromosomal aberrations excl. Gaps</td>
</tr>
<tr>
<td>Experiment I</td>
<td>81 µg/ml CBS</td>
<td>2.0% chromosomal aberrations excl. Gaps</td>
</tr>
<tr>
<td>Experiment II</td>
<td>41 µg/ml CBS</td>
<td>1.0% chromosomal aberrations excl. Gaps</td>
</tr>
<tr>
<td>Experiment II</td>
<td>81 µg/ml CBS</td>
<td>3.5% chromosomal aberrations excl. Gaps</td>
</tr>
</tbody>
</table>

Since all negative and solvent controls ranged from 0.5 to 1.0% structural chromosomal aberrations excl. Gaps, this is interpreted as weak evidence for a clastogenic potential.

2-Mercaptobenzothiazole (MBT)
The genotoxicity of MBT was reviewed in BG Chemie (2000).

MBT was negative in a number of bacterial gene mutation tests and in two mammalian cell gene mutation tests (HPRT tests on V79 and CHO cells).

According to a NTP report (1989) MBT induced structural chromosomal effects in CHO cells in presence of S-9 mix in the dose range 351 to 451 µg/ml; negative results were obtained without S-9 mix.

In the same NTP report a weak positive result was reported in a mouse lymphoma assay (again only in presence of S-9 mix). According to BG Chemie (2000) two further mouse lymphoma assays led to a weak positive and a negative result.

SCE tests in CHO cells (induction of sister-chromatid exchanges) resulted in marginal positive findings (BG Chemie 2000).

Cyclohexylamine (CHA)
CHA was negative in a number of in vitro genotoxicity tests: bacterial gene mutation tests (Herbold 1981; Mortelmans et al. 1986); in vitro chromosomal aberration test (Brewen et al. 1971); mammalian cell gene mutation test (HPRT test on CHO cells; Brusick et al. 1989); test for induction of unscheduled DNA synthesis in freshly isolated rat hepatocytes (Brusick et al. 1989).

4.1.2.7.2 Studies in vivo
Embryonic mortality of CBS was investigated in rats. However, no reliable data on dominant lethal mutations can be derived because test methodology and data presentation are inadequate (Aleksandrov 1982).
In a dose of 2000 mg/kg the unspecified test substance "santocure" was injected twice into the stomach of males (with an interval of 3 days) and females (1st and 3rd days of estrus). No visible toxic signs were observed. The total embryonic mortality rate was increased (ca. doubling) but not the postimplantation embryonic mortality. The study was run without a positive control. Description of methodology and results is incomplete. The test result cannot be evaluated adequately.

2-Mercaptobenzothiazole (MBT)
According to BG Chemie (2000) MBT led to negative results in a number of in vivo tests: micronucleus tests with mice, (intraperitoneal application), micronucleus tests with rats (intraperitoneal application), dominant lethal test with rats (oral application by gavage). DNA binding study on various tissues including liver in rats (oral application by gavage).

Cyclohexylamine (CHA)
A majority of in vivo genotoxicity tests with CHA was negative: Two bone marrow chromosomal aberrations with Chinese hamsters and rats (Brewen et al. 1971; Dick et al. 1974); a chromosomal aberration test with spermatogonia of Chinese hamsters (Machemer et al. 1976); a dominant lethal test with mice (Epstein et al. 1972); and a Drosophila test (Brusick et al. 1989). In contrast, positive findings were described for chromosomal aberration tests with bone marrow cells and spermatogonia of rats; however, these data are of a low reliability due to the lack of negative and positive controls for both mutagenic endpoints (Legator et al. 1969). However, Dick et al. (1974) showed that the same treatment regimen as in the Legator study did not induce chromosomal aberrations in bone marrow cells in rats; in this study positive and negative controls are available. The other reliable chromosomal aberration tests by Brewen at al. (1971) with bone marrow cells of the Chinese hamster and with spermatogonia by Machemer et al. (1976) were also negative. A marginal positive result was obtained in a mouse spot (Fahrig 1982).

4.1.2.7.3 Summary of mutagenicity
CBS was negative in gene mutation assays employing various tester strains of Salmonella and one of Saccharomyces; also a mouse lymphoma assay was negative. An in vitro chromosomal aberration test gave weak evidence for a clastogenic potential. The only in vivo test (on embryonic mortality) cannot be assessed adequately due to insufficient data reporting. There is no relevant evidence for mutagenicity of CBS. This is supported by genotoxicity data for the hydrolysis products MBT and CHA.
Classification of CBS as a mutagen is not warranted.

4.1.2.8 Carcinogenicity
There are no studies available in experimental animals according to the current criteria for the testing of carcinogenicity by inhalation, dermal or oral application route to CBS. However, there are two types of old long term studies in mice of two strains: B6C3F1 and B6AKF1 (NCI 1968). The test procedures of both studies presented are in accordance with generally accepted scientific standards, however, differ in some respects from the published guidelines. The following significant study deficiencies exist: size of experimental groups, only one dose level tested, and in the general experimental design (animal housing and bedding, environmental conditions in animal rooms). Nevertheless, the data submitted are considered
useful in assessing the carcinogenic potential of CBS. Several long-term studies with oral application in rats and mice are also reported in 4.1.2.6 and 4.1.2.9.1., for more details see there.

### 4.1.2.8.1 Studies in animals

#### In vivo studies

**Inhalation**

No data available.

**Dermal**

No data available.

**Oral**

Groups of 18 mice per sex of the B6C3F1 strains and the B6AKF1 strains, were given 215 mg/kg bw/d CBS by oral gavage repeatedly for 21 days followed by exposure for nearly 17 months to diet containing CBS at a concentration equivalent to 90 mg/kg bw/d (corresponding to a time-weighted average dose of 95.3 mg/kg bw/d). An equal number of mice served as untreated controls, and another as vehicle controls. Survival of the CBS-treated mice of both strains was comparable to the control groups (vehicle control B6C3F1: male 14/18, female 18/18; B6AKF1 male: 18/18, female: 17/18; CBS B6C3F1: male 14/18, female 18/18; CBS B6AKF1: male 16/18, female 14/18). No adverse effects were reported. Tumours were found in all groups but there were no statistically significant differences between CBS-treated and control mice in the incidence of the individual types of tumour. The total number of tumours was not statistically significant different in CBS-treated and control mice (vehicle control B6C3F1 male/female: 4/0, B6AKF1 male/female: 1/1; CBS B6C3F1 male/female: 8/6, CBS B6AKF1 male/female: 3/3). In summary there was no evidence of a carcinogenic effect in both strains of mice (NCI 1968).

#### 2-Mercaptobenzothiazole (MBT)

In a carcinogenicity study MBT (96-97% purity) was administered by gavage 5 days per week in a corn oil vehicle to groups of F344/N rats and B6C3F1 mice of each sex for two years.

**Rats**

Groups of 50 male rats were administered at 0, 375, and 750 mg/kg bw/d and groups of 50 female F344/N rats at 0, 188, or 375 mg/kg bw/d, respectively. Administration of MBT resulted in significantly decreased survival in dosed male rats (vehicle control: 42/50; low dose: 22/50; high dose: 20/50) but not in female rats (28/50; 31/50; 25/50). No effect on body weight gain in MBT-dosed rats was observed. Post application of MBT lethargy and prostration occurred frequently in dosed rats of both sexes.

The incidences of a variety of tumours were increased in rats dosed with MBT; some of the increased incidences were not always dose-related. For example, the mean incidence (P<0.01) for mononuclear cell leukaemia and pancreatic acinar cell adenomas was only significantly increased in low dose male rats. The mean incidences in male rats were 7/50 (control), 16/50
(low dose), 3/50 (high dose) for mononuclear cell leukemia; and 2/50 (control), 13/50 (low dose), 6/49 (high dose) for pancreatic acinar cell adenomas, respectively. The incidence of leukemia in low dose males exceeded the high level for the historical corn oil vehicle control range, however, that was not noted for the incidence of pancreatic acinar cell adenomas. But it is known that the administration of corn oil by gavage increases the incidence of pancreatic acinar cell adenomas in rats. It is also stated that the survival of the low and high dose group of male rats was significantly lower than that of the vehicle controls. Comparable numbers of male rats were at risk at the end of the study (low dose: 22, and high dose: 20), so it is doubtful that survival rates affected the dose-response relationship for neoplasms. Increased tumour incidences with dose-related trends (P < 0.05) included pituitary gland adenomas in females (15/49; 24/50; 25/50), preputial gland adenomas or carcinomas (combined) in males (1/50; 6/50; 5/50), adrenal gland pheochromocytomas or malignant pheochromocytomas (combined) in males (18/50; 27/50; 24/49), and pheochromocytomas in females (1/50; 5/50; 6/50). These tumours were observed at significantly greater incidences (P <= 0.05) in the high dose groups than in the vehicle controls. The incidence of these tumours did also not exceed the values for historical controls. Moreover, these responses suggested that MBT expressed some carcinogenic activity in rats at doses sufficient to accelerate mortality.

In summary, under the conditions of this 2-year gavage study, there was some evidence of carcinogenic activity of MBT for male F344/N rats, indicated by increased incidences of mononuclear cell leukemia, pancreatic acinar cell adenomas, adrenal gland pheochromocytomas, and preputial gland adenomas or carcinomas (combined). There was some evidence of carcinogenic activity for female F344/N rats, indicated by increased incidences of adrenal gland pheochromocytomas and pituitary gland adenomas.

Mice

Groups of 50 B6C3F1 mice of each sex were given 0, 375, and 750 mg/kg bw/d MBT for 103 weeks. Administration of MBT resulted in decreased survival in the high dose females (vehicle control: 35/50; low dose: 39/50; high dose: 22/50) but not in males (38/50; 33/50; 39/50). In male mice, minor reductions in body weight gain (high dose: 6%-14%; low dose: 4%-8%) occurred between weeks 3 and 64, with recovery thereafter. Post application of MBT mice of both sexes were frequently lethargic.

The mean incidence (P=0.028) of hepatocellular adenomas or carcinomas (combined) was increased only in low dose females (4/50; 12/49; 4/50) and was clearly not dose-dependent. In comparison with historical control data, this incidence was also within the normal range. It is possible that low survival in the high dose group of female mice prevented the expression of hepatocellular tumourigenicity, since this is a late-appearing neoplasm in mice. No significant increases in tumour incidences were seen in male mice when compared with the internal controls.

Therefore, it was concluded that no evidence of carcinogenic activity of MBT was noted for male B6C3F1 mice dosed with 375 or 750 mg/kg bw/d. There was equivocal evidence of carcinogenic activity for female B6C3F1 mice, indicated by increased incidences of hepatocellular adenomas or carcinomas, combined. Overall, male and female rats and female mice showed mostly non-dose-dependent changes in tumour incidences of individual organs. The two species were affected by types of tumour which are known to occur spontaneously and their incidence in the treated animals was within the range of historical control data. In addition, systemic toxic effects of MBT were observed for both the administered doses in male rats and for the high dose in female mice, i.e. the maximum tolerated dose was
exceeded. In summary, careful evaluation of the presented result yields no findings which could be regarded as indicative of a carcinogenic potential of MBT (NTP 1988).

Cyclohexylamine (CHA)

Groups of 48 male and 48 female Wistar rats were fed diets containing 0, 600, 2000 or 6000 ppm cyclohexylamine hydrochloride (corresponding to about 0, 24, 82 and 300 mg/kg bw/d in males; and 0, 35, 120 and 440 mg/kg bw/d in females) for 104 weeks. Histopathology examinations were conducted on most tissues, including the urinary bladder, from the animals in all groups. The total number of tumours found was lower in the rats given 6000 ppm than in the controls; indeed, most of the tumours occurred in the controls alone or with a similar frequency in treated and control rats. In no case was there a significant increase in tumour incidence in treated rats compared with the controls. Only three tumours were present in rats given 6000 ppm without parallel findings in controls. These were a basal-cell carcinoma of the skin and an osteosarcoma of the skull in males and a glioma in the brain of a female. These three tumours encountered in this study, although less common in untreated rats of this laboratory, have been described in untreated rats by other workers. A range of tumours was identified in rats given lower levels of cyclohexylamine hydrochloride without comparable findings in the controls or the highest treatment dose. In view of the established spontaneous incidence of the individual tumours and their absence from the rats given the highest level of cyclohexylamine hydrochloride, it is considered that the occurrence of these tumours represents the normal incidence in rats rather than an effect of the treatment with cyclohexylamine hydrochloride. Based on the data obtained in this study, it is concluded that no carcinogenic effect could be detected in rats given cyclohexylamine hydrochloride in their diet at levels up to 6000 ppm (corresponding to about 300 mg/kg bw/d in males and 440 mg/kg bw/d in females (Gaunt et al., 1976).

Cyclohexylamine hydrochloride was fed to groups of FDRL (Wistar derived) rats (30/sex/group) for two years provide doses of 0, 15, 50, 100 or 150 mg/kg bw/d. Microscopy was made on at least 20 organs from 15 to 20 rats of each sex in the control and highest dose group and on 8 major organs from 10 or more rats of each sex in the other groups. There was a statistically significant decrease in the rate of body weight gain in males treated at 100 and 150 mg/kg bw/d and in females treated at ≥50 mg/kg bw/d. This effect was attributed to decreased food intake. Extensive examinations of the urinary bladders revealed no tumours, and the incidences of the other observed tumours were similar in all groups, including the controls (Oser et al., 1976).

Groups of 48 male and 50 female ASH-CS1 mice were fed diets containing 0, 300, 1000, or 3000 ppm cyclohexylamine hydrochloride (corresponding to about 0, 40, 140, or 400 mg/kg bw/d in both sexes) for a period of 80 weeks. Relatively complete histopathological examinations were conducted on the tissues of the mice from all treatment groups. There were no statistically significant differences in the tumour incidences of the cyclohexylamine hydrochloride-treated and control groups (Hardy et al., 1976).

Groups of 50 male and 50 female Swiss (SPF derived, outbred) mice were fed at dietary concentration of 0.5% cyclohexylamine sulfate (equivalent to about 500 mg/kg bw/d in both sexes, calculated on an assumed body weight of 20 g and a mean daily food intake of 2 g for mice) over a period of 18 or 21 months. Cyclohexylamine sulfate did not show any carcinogenic effect in mice (Kroes et al., 1977).
Other
Groups of 18 B6C3F1 and 18 B6AKF1 mice of each sex received a single subcutaneous injection in 0.05 ml suspension (1000 mg/kg) CBS in the nape of the neck with sacrifice at ca. 17 months after injection (no more data available). An equal number of mice of each strain served as untreated controls, and another as vehicle controls. Survival of the CBS-treated mice was comparable to the control groups in both strains. No adverse effects were reported and no statistically increases in tumour incidences were observed in mice of both strains. The total number of tumours was similar in treated and control mice (vehicle control B6C3F1 male/female: 4/0, B6AKF1 male/female: 1/1; CBS B6C3F1 male/female: 3/1, CBS B6AKF1 male/female: 0/2). In summary there was no evidence of a carcinogenic effect of CBS in both strains of mice (NCI 1968).

In vitro studies
No data available.

4.1.2.8.2 Studies in humans
No data available.

4.1.2.8.3 Summary of carcinogenicity
The existing two long-term studies on CBS in mice are not in accordance with the current testing procedures as proposed by guidelines on carcinogenicity and/or combined chronic toxicity/carcinogenicity (EEC methods, B.32, 33). 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 the both hydrolysis products, MBT and cyclohexylamine, have 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 cyclohexylamine 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. Cyclohexylamine 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.

Currently, the available data of CBS and its hydrolysis products are insufficient to justify the evaluation as an human carcinogen according to the EEC criteria for classification and labelling requirements for dangerous substances (EEC Directive 2001/59/EEC, Annex VI of the Directive 67/548/EEC). Therefore, there is no need for classification and labelling of CBS as a carcinogen.
4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Effects on fertility

Studies in animals

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.

The 28-day repeated dose toxicity studies on rats (c.f. 4.1.2.6) with oral dietary (Monsanto, 1980) and inhalatory (Monsanto, 1981a) exposure of CBS could not be exploited for information and data on effects on the reproductive organ system, because organ weight determinations and histopathology on organs of the reproductive system had not been performed during these studies. Likewise, also the available long-term studies performed on mice (NCI, 1968; c.f. 4.1.2.8) do not provide information with regard to reproductive organs.

Data on organ weights and histopathology of reproductive organs were available from an oral (gavage) 28-day repeat dose toxicity test (c.f. 4.1.2.6) written in Japanese with an abstract in English (Chemicals Investigation Promoting Council, 1997c). Groups of 6 Crj:CD (SD) rats per sex were treated with 0, 25, 80, 250 and 800 mg CBS/kg bw/d (purity 98.8%). No treatment related changes in absolute and relative weights of ovaries and absolute weights of testis were observed. Relative weights of testis were significantly higher (p<0.001) in animals treated with 800 mg/kg bw/day than in control animals concomitant to decreased body weights. In the recovery group in one out of 6 rats histopathological changes were seen after a 14 day recovery period at 800 mg/kg bw/d (moderate diffuse atrophy of seminiferous tubuli, moderate diffuse hyperplasia of interstitial cells and marked epididymal decrease in sperm number). No such effects were observed in males terminated immediately after the administration period. Histopathology on female reproductive organs was not provided. Since it was not clear why the findings on histopathology were only present in the recovery group and only one of 12 animals of the highest dose group was affected, no NOAEL could be derived from this study.

Systemically toxic effects (statistically significant decrease of bodyweight gain), signs of coagulopathy of the blood and hyaline droplets in kidney proximal tubular epithelia were found at a LOAEL of 250 mg/kg bw/d resulting in a NOAEL for systemic toxicity of 80 mg/kg bw/d.

2-Mercaptobenzothiazole (MBT)

With MBT a two-generation feeding study had been performed (Springborn Laboratories Inc., 1990) with Sprague Dawley rats. In this study groups of 28 male and female animals of the F0 and F1 parental generation were fed the basal diet or diet containing MBT (98.2-98.5% purity) at concentrations of 2500, 8750, and 15000 ppm for a minimum of 70 days (F0 parental animals) or 88 days (F1 parental animals) prior to mating and continued until sacrifice. Selection of the F1 generation was performed at weaning of the F0 offspring with 28 males and 28 females randomly selected from each group.
F0 and F1 parental animals were observed daily for signs of overt toxicity, morbidity or mortality. Body weight was measured weekly for the males throughout the study. For females, body weights were measured weekly prior to confirmation of mating and at specified intervals during gestation (gestational day 0, 7, 14, and 20) and lactation (postnatal day 1, 7, 14, and 21). Food intake was measured on the same days as body weights. F0 and F1 parents were sacrificed and necropsied following weaning of their offspring. Liver, kidney testes and ovary organ weights were taken from all F0 and F1 parents when sacrificed for scheduled necropsy. Microscopic examination was performed on all tissues collected (epididymides, kidneys, liver, ovaries, pituitary, prostate, seminal vesicles, testes, uterus, vagina) for the control and the high dose (15000 ppm) group. Kidneys from the F0 parents of all treated groups and kidneys and livers from the F1 parents from all treated groups were also examined microscopically.

For F0 offspring (F1 pups) and F1 offspring (F2 pups) pup viability was determined daily throughout lactation. On lactation day 4, litters were culled to a maximum of 8 pups (4/sex). Detailed examination of the pups was performed on lactation days 0, 4, 7, 14, and 21. The sex of the pups was determined on lactation day 0 and verified on lactation days 4 and 21. Individual pup weights were measured on lactation days 1, 4, 7, 14, and 21. Nonselected F1 pups were sacrificed at weaning and examined. F2 pups were sacrificed and necropsied on lactation day 21.

Survival was 100% in all F0 and F1 parental animals. There were no treatment-related clinical signs observed in any of the MBT exposed groups. Food intake (g/kg bw/d) was significantly reduced in the 8750 and 15000 ppm groups of the F0 generation during the first week of treatment. Thereafter, food intake was equal or greater than in the control group. Body weight gain was dose-dependent and significantly reduced in F0 males during the first week of treatment. In F0 females body weight gain was reduced in the mid and high exposure groups during the first week of treatment. Weight gain remained to be slightly reduced for approximately 10 weeks for males but not for females. Offspring body weights were significantly reduced beginning from day 14 of lactation in the mid and high dose group F1 pups correlating with the onset of food intake. Likewise body weight was reduced in F2 pups beginning from day 14 of lactation, however at all dose groups. Treatment-related histopathological changes were seen in the kidneys of both F0 and F1 parental animals. Brown pigment was observed in the lumen and epithelial cells of the proximal convoluted tubules in males and females in the mid and high dosage group, with a greater incidence in the males than in females. Cortical tubular basophilia and alpha 2 µ-globulin inclusions in the epithelial cells of the proximal convoluted tubules occurred in males from all groups with a higher incidence in the treated groups. In addition, absolute and relative kidney weight was significantly increased for F0 and F1 males in the mid and high dosage group. In the livers microscopic changes consisting of hepatocyte hypertrophy were observed in F1 animals from the 8750 and 15000 ppm groups. Histopathological changes correlated with a significant increase of liver organ weights in the males of the mid and high dosage groups and for females in the high dosage group. All parameters related to reproductive functions, including precoital interval, copulation and fertility indices, pregnancy rates and gestation length, were similar in the control and the treated animals in both generations. Litter size and pup viability was also comparable between the control and the MBT exposed groups of the F0 and the F1 generations.

In summary, from this study no effects adverse to reproductive capability and capacity could be demonstrated for dietary exposures of up to and including 15000 ppm (according to an intake of approximately 1200 mg/kg bw/day with the assumption of a mean body weight of 250 g and a mean daily food intake of 20 g for rats). 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 is no indication for inherent reproductive toxicity of MBT even at systemic toxic dose levels.

MBT has also been investigated with repeated dose application for a period of 13 weeks in rats and mice during a study, which was part of an NTP study on toxicology and carcinogenesis (c.f. chapter 4.1.2.8). During this repeated dose toxicity study groups of 10 F344/N rats/sex were administered 0, 188, 375, 750, or 1500 mg MBT/kg bw (96-97% purity) in corn oil by gavage 5 days per week. Groups of 10 B6C3F1 mice/sex were administered 0, 94, 188, 375, 750, or 1500 mg MBT/kg bw on the same schedule (NTP TR 332/NIH Publication No. 88-2588, 1988). Animals were checked two times per day. Individual animal body weights were recorded weekly. At the end of the 13-week study, survivors were killed. A necropsy was performed on the animals of all groups. Histopathology examinations were performed on all animals from all groups on a couple of tissues and organs for instance including mammary gland, ovaries, uterus, testes and prostate. With the investigation on rats, there were no compound related deaths. Body weight gain was reduced with increasing doses, with a maximum change of -15% compared with vehicle controls. Liver weight and liver to body weight ratios were increased in dosed rats with the greatest change occurring in the two highest dose groups (750 and 1500 mg/kg bw/d). No gross or microscopic effects could be related to chemical administration. In the mice study five of ten males and 7/10 females that received dosages of 1500 mg/kg bw/d died before the end of the study. Two of the deaths were related to gavage technique. Chemical administration did not affect body weight gain in mice. Liver weight to body weight ratios were higher than those of the vehicle controls. Clonic seizures, lacrimation, and salivation were observed in the 750 and 1500 mg/kg groups. Lethargy and rough coats were observed in the 375 and 750 mg/kg dose groups. However, at necropsy after 13 weeks of administration no compound related gross or microscopic pathologic effects were observed in the animals. Thus, from this study there is no indication for specific toxic properties of MBT in mice and rats adverse to organs of the reproductive system even at systemic toxic dose levels.

Cyclohexylamine (CHA)

Data on investigations on the toxicological properties of CHA including aspects of reproduction and development have been summarised in two reviews (IARC, 1980; Bopp et al., 1986). It is reported that during studies with repeated administration for 90 days with food concentrations of CHA ranging from 0.01 to 1.0% that effects on the male reproductive organs had been observed in test animals.

In a first study from which no data are available to the rapporteur (Collings and Kirkby, 1974), groups of 15 or 16 male rats were given diets containing CHA hydrochloride at concentrations of 0.01, 0.05, 0.1, 0.2, 0.5, or 1.0% for 90 days. Body weight gain and food intake were significantly decreased at dietary levels of 0.2% and above. The absolute testicular weights were depressed at the two highest concentrations, while the relative weight was increased at 0.5%, but decreased at 1%. Degeneration of tubular epithelium was seen in both testes of 13 out of 15 rats given 1% CHA hydrochloride, with ≥ 95% of the tubules being affected in 8 rats, ≥ 70% in 4 rats, and ≥ 40% in 1 rat. The incidence of other histopathological changes in the testes (i.e., reduced spermatogenesis, intertubular oedema, tubules with luminal debris, or hypertrophic degeneration) was generally similar in the control and treated groups, although two rats in the 0.5% group exhibited some evidence of greater hydropic degeneration.
In another study (Gaunt et al., 1974) groups of 15 male CFE rats were given a diet containing 0, 600, 2000 and 6000 ppm CHA hydrochloride according to intakes of approximately 0, 41, 143 and 468 mg/kg bw/d in male rats for a 13 week period as part of a paired-feeding study. Body weight gain and food intake was significantly reduced at 2000 and 6000 ppm in the diet. Absolute and relative testicular weights were only decreased in the high dose group. Initially, histopathological examination of the testes revealed reduction in spermatogenesis and tubular atrophy in 4 of 11 rats at 2000 ppm and in 18 of 20 rats at 6000 ppm. However, in 1975 the original slides and freshly prepared slides were re-examined independently by two pathologists to better assess the incidence of the lesions. The agreement between the two pathologists was reasonably good, and this second evaluation indicated that the incidence of the lesions was only increased in the rats receiving 6000 ppm CHA hydrochloride in the diet. However, rats treated with even the highest concentration for months remained fertile.

In a further study (Mason and Thompson, 1977), groups of 25 male Wistar as well as Sprague-Dawley rats were fed diets containing 600, 2000 and 6000 ppm CHA hydrochloride for 90 days. CHA intakes calculated from the available data on mean body weight and total food consumption were 0, 56, 178 and 486 mg/kg bw for Wistar rats and 0, 55, 176 and 494 mg/kg bw/d for Sprague Dawley rats. Paired fed and paired- weight control groups were included to assess the effects of decreased food consumption on the development of the testicular lesions. Body weight and food intake were depressed at 2000 and 6000 ppm in both strains compared to the ad libitum fed control groups. The weights of the high dose animals were also significantly lower than those of the paired-fed controls, but did not differ from the paired-weight groups. Testicular effects were only seen in the 6000 ppm groups, as exemplified by the reductions in the absolute weights of testes, the sperm count and sperm motility, and histologically by an increased incidence of impaired spermatogenesis. In the affected animals there was a marked reduction or the complete absence of spermatogenesis in many of the tubules. The only identifiable cell types remaining in these tubules were the Sertoli cells and a few spermatogonia; multinucleated cells were occasionally present. The basement membrane did not appear to be thickened nor were the Leydig cells involved. In contrast to these affected tubules, spermatogenesis appeared to be occurring in a normal fashion in other tubules. The absence of any effect in the paired-fed and paired-weighted control groups clearly indicated that the testicular changes were not due to initiation, but were directly attributable to the highest concentration of CHA.

In a further study (Brune et al., 1978), details on data of which are not available to the rapporteur, it is reported that groups of 100 young male Sprague-Dawley rats were given diets providing daily doses of 50, 100, 200, or 300 mg CHA base per kilogram. Ad libitum and paired-fed control groups were also included. At the end of the 3-month study, the body weights of the rats in all the CHA treated groups were significantly decreased in comparison to the ad libitum fed control group. However, significant decreases in body weight were only found in the 200 and 300 mg/kg/day groups when compared with their respective paired-fed groups. The testicular weights were significantly lower in the 200 and 300 mg/kg/day groups than the nontreated controls, but compared to the paired-fed controls, a significant effect was only seen with the highest dose. Three sections from each testis were examined microscopically for tubular alterations which were scored on a 0 to 4 scale. A pathologist who was not aware of the treatment the animal had received examined all slides. No differences in testicular scores were seen at 100 mg/kg/day, but scores in the 200 and 300 mg/kg/day groups were significantly higher than those of the ad libitum fed control groups and the corresponding paired-fed groups. The increased testicular scores primarily resulted from a
small number of animals that were severely affected, rather than from slight changes in a large number of rats. The most severe lesions consisted of degenerative changes in the tubules, giant cell formation, and complete tubular atrophy. In some cases only Sertoli cells remained within the affected tubules. Thus, this study demonstrated that 100 mg/kg/day was the no-adverse effect dose for testicular effects and based upon the slight changes at 200 mg/kg/day and marked effects at 300 mg/kg/day, suggested that the dose-response curve was quite steep.

Testicular effects of CHA have also been investigated in chronic studies. In a long-term toxicity study (Gaunt et al., 1976) diets containing 6000, 2000 and 600 ppm according to intakes of 300, 82 and 24 mg CHA/kg bw/d were fed to Wistar rats. The incidence of bilateral testicular atrophy (39%) was significantly increased in the treated males with 6000 ppm, but not with 2000 or 600 ppm CHA in the diet for 2 years. The similarity of the effects in the 2-year study and the 90-day study conducted in the same laboratory suggested that the lesions probably developed relatively early and did not become progressively more severe with continued treatment. For further information on the study and on systemic toxic effects see 4.1.2.6. No such testicular effects, however, were seen in a comparable long-term study performed on mice from the same laboratory (Hardy et al., 1976), during which groups of 48 males and of 50 females had been fed diets with 0.03, 0.1, or 0.3 % CHA for 80 weeks.

In a further study with Wistar rats (Khera et al., 1971) male and female animals were treated continuously from 17, respectively 21 days before mating, during mating and gestation and throughout lactation and weaning to a total of up to 119 days orally via gavage with doses of 22, 44, 89 or 178 mg CHA sulfate/kg bw/day. The were no changes in female fertility observed during this study, whereas fertility in treated males (no quantitative data provided) appeared to be slightly lower during the first of the three mating trials, however without any relation to dose. No adverse effects were seen on embryo viability, perinatal viability, litter size, litter weight, postnatal viability, or the weight gain of pups. Visceral and skeletal examinations of neonates did not reveal any treatment-related changes.

Studies in humans

No data available.

4.1.2.9.2 Developmental toxicity

Studies in animals

In a developmental study (Ema et al. 1989) groups of 10 -17 mated female Wistar rats were administered CBS in form of Soxinol CZ-G (99% purity) via diet at dosage levels of 0, 0.001, 0.01, 0.1 and 0.5% from day 0 to day 20 of pregnancy. According to the authors the average daily intake amounted to 0, 0.7, 7.1, 69.6 and 288.8 mg/kg, respectively. Significantly (p < 0.05) lower maternal body weight gain during pregnancy was noted in the 0.5% group (91 ± 9 g) and the 0.1% group (73 ± 15 g) in comparison to the control group (108 ± 26 g). Also food consumption in the 0.5% group (302 ± 30 g) differed significantly (p < 0.05) from that of controls (355 ± 52 g). Neither death nor clinical signs of toxicity were reported for the pregnant females of any group. 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 (p < 0.05) lower body weights of male (3.89 ± 0.29 g in comparison to 4.43 ± 0.38 g in the controls) and female (3.77 ± 0.34 g in comparison to 4.17
+ 0.36 g in the controls) fetuses and of the placentae (0.42 + 0.07 g in comparison to 0.60 + 0.15 g in the controls) were noted at the highest dose level. Extensive external, internal and skeletal examinations (of the 92 to 150 fetuses per dose group) did not reveal any malformations nor any changes in the incidence of skeletal variations at any dosage level. The NOAEL derived from the results of this study for dams is 7.1 mg/kg bw/day (= 0.01% CBS in the diet) based on reductions in body weight gain. The NOAEL/developmental toxicity for the offspring is 69.6 mg/kg bw/day (= 0.1% CBS in the diet) and is based on decreased mean fetal body weights.

In a guideline-according teratology study (Monsanto 1981c) which was performed with Charles river COBS CD rats groups of 20 to 25 pregnant females were treated by gavage with CBS in form of „Santocure“ (purity not specified) at dose levels of 100, 300, 500, and 900 mg/kg bw per day during g.d. 6 to g.d. 15. The control group received corn oil. Due to excessive toxicity and death the 900 mg/kg dose group had to be ceased before the end of the study. One maternal death and a severe decrease in mean maternal body weight gain and adjusted body weight on g.d. 20 were observed at the 500 mg/kg dose level. A slight to moderate decrease in mean maternal body weight gain was noted in the 300 mg/kg dose group. Hairloss was noted in all groups, most frequently in the 500 mg/kg dose group. There were no dose dependent and biologically meaningful effects on mean numbers of corpora lutea, total implantations, postimplantation loss, viable fetuses, fetal sex distribution in any of the treated groups when compared to controls. There was a very slight increase in the number of fetuses and litters with malformations in the 500 mg/kg dose group due primarily to three fetuses, each from a different litter, with a thread-like tail and a small anus. However, overall, there were no statistically significant differences in the number of litters with malformations in any of the treated groups when compared to the control groups. Further effects observed in the offspring were related to fetal body weight: A slight decrease in mean fetal body weight was observed in the 100 and 300 mg/kg dose group which was statistically significantly different from controls for the 500 mg/kg dose group. The NOAEL derived from this study for maternal toxicity is 100 mg/kg bw/day and is based on a decrease in mean body weight gain. The NOAEL for the offspring is 300 mg/kg bw/day and is based on a decreased mean fetal body weight.

In a further teratology study (Sitarek et al. 1996) which was performed with Imp: DAK rats (Institute’s own breeding colony) groups of 17 to 22 mated females were treated by gavage with sulfenamide TS (purity not specified) at dose levels of 50, 150, and 450 mg/kg bw per day during g.d. 6 to g.d. 15. The control group received sunflower oil. No significant differences in the appearance and behaviour were noted between test and control pregnant females. Dams in the high-dose group (450 mg/kg bw) exhibited significantly decreased body weight gain and a significant increase in relative kidney weights and in absolute spleen weights. Also the placental weight was found to be significantly lower than in unexposed rats. The high-dose group (450 mg/kg bw) also exhibited embryo-/fetotoxic effects in terms of a significant increase in the frequency of litters with early resorptions, an increase of the mean value of late resorptions per litter and of postimplantation losses as well as decreased fetal body weight and crown-rump length. 192 to 213 fetuses were examined for external, skeletal and visceral abnormalities. From visceral examinations an increased number of fetuses with internal hydrocephalus was diagnosed for the high dose group but also for the 150 mg/kg dose group. However, the numbers of fetuses with enlarged cerebral ventricles (about 20% of all fetuses affected) and/or renal pelvis were obviously elevated in this study across all treatment groups including the controls. However, this kind of observations was not obtained from the two other studies and is difficult to put into perspective, since there are no historical data
available for the particular rat strain used in this study. Therefore, these observations are not considered as being indicative for a substance specific teratogenic potential.

Studies in humans
No data available.

Other information on CBS

CBS in form of „Santocure“ produced some rather unspecific effects only at concentrations near saturation in studies with chick embryos in ovo (Korhohnen et al. 1982, 1983). The authors explained this by poor diffusion of the substance to the embryo, indicating that the chick embryo in ovo as a developmental model might not be very suitable. These data are not considered adequate for hazard identification purposes.

2-Mercaptobenzothiazole (MBT)

MBT had been investigated for developmental and teratogenic effects in a study on Sprague-Dawley rats (Crl:COBS®CD®BR®VAF®) with the oral (gavage) route of administration (Springborn Laboratories Inc., 1989a). 26 pregnant rats per dose group had been treated with doses of 300, 1200, or 1800 mg MBT/kg bw/day during the critical period of organogenesis from gestation day 6 through gestation day 15. MBT (98.1% purity) had been suspended in corn oil (Mazola®) and administered at a volume of 10 ml/kg. Control animals received corn oil at an equivalent dosage volume. During the experimental period all animals were observed daily for mortality and clinical abnormalities. Individual body weights were measured on gestation days 0, 6, 9, 12, 16, and 20. Individual food consumption was measured daily and reported for the specific intervals. All animals were sacrificed on gestation day 20 and were subjected to a gross post mortem examination. Necropsy examination included evaluation of the external surfaces, orifices, and viscera. Emphasis was placed on morphopathological changes that could interfere with survival and fetal development. The numbers of corpora lutea, early and late resorptions, as well as of viable fetuses were recorded. Fetuses were examined for fetal body weight and for external, visceral and skeletal abnormalities.

No treatment-related deaths were found during this study, however, clinical observations were made in dams of the 1200 and 1800 mg/kg/day dose groups including salivation, dark material around the mouth and urine staining. Several rats in the 1800 mg/kg/day dose group also displayed decreased activity. Calculations on maternal body weights revealed significant weight loss in the 1800 mg/kg/day dose group between gestation day 6 to 9 and statistically significantly reduced mean body weights on gestation day 9. Also food intake was statistically significantly reduced in this dose group during gestation days 6-9. Body weights and body weight changes as well as food consumption in the control, 300, and 1200 mg/kg/day dose groups were in general comparable. No treatment-related morphopathological changes in dams were observed at necropsy. There were no substance-related effects on the number of viable fetuses, on fetal sex ratio or on fetal body weights across groups. Mean number of postimplantation-loss in the 1800 mg/kg/day dose group was slightly but statistically significantly increased (1.7) in comparison to the control group (0.8) and was only slightly outside the range of laboratory historical control data (0.6-1.4). No other statistically or biologically significant differences were noted between the control and the MBT treated groups with regard to fetal malformations or variations.
MBT has been further investigated for developmental and teratogenic effects in a study on New Zealand White rabbits with the oral (gavage) route of administration (Springborn Laboratories Inc., 1989b). 20 artificially inseminated does per group had been treated with doses of 50, 150, or 300 mg MBT/kg bw/day (98.1% purity) during the critical period of organogenesis from gestation day 6 through gestation day 18. MBT had been suspended in 1% methylcellulose and administered at a volume of 2 ml/kg. Control animals received 1% aqueous solution of methylcellulose at an equivalent dosage volume. All animals were observed daily for signs of overt toxicity. Body weights were measured on gestation days 0, 6, 9, 12, 15, 19, 24, and 29. Food consumption was measured daily and reported for the specific intervals. Caesarean section was performed on gestation day 29. Intrauterine survival was evaluated and fetuses were examined for body weights and for external, visceral, and skeletal anomalies.

No substance-related deaths occurred during the conduct of the study and no substance-related clinical signs were observed. Mean maternal body weight gain during the treatment period was lower in the dosed groups in comparison to the vehicle controls amounting to 99, 107, and 53 grams in the 50, 150 and 300 mg/kg dose groups as compared to 145 grams in the controls. Mean food intake during the treatment period was similar in the dosed and in the vehicle control groups amounting to 150, 141, 146, and 149 grams in the control, 50, 150, and 300 mg/kg dose groups. At scheduled necropsy no treatment-induced lesions were noted in the dosed groups. A single incidence of abortion was noted in the 150 mg/kg/day group on gestation day 19. One doe delivered prematurely on gestation day 29 before the scheduled Caesarean section. Pregnancy percentages were 95, 90, 85 and 100 in the control, 50, 150, and 300 mg/kg/day groups respectively. Parameters measured at the caesarean section, including numbers of corpora lutea, implantation sites, early as well as late resorptions, pre- and post-implantation losses, viable and dead fetuses, fetal sex ratio and fetal weights were similar in all groups. Fetal morphological evaluations did not reveal any statistically or biologically significant differences between the vehicle control and the MBT treated groups with regard to malformations or variations.

Cyclohexylamine (CHA)

During a long-term study with continuous breeding over six generations (Kroes et al., 1977) amongst other substances also CHA sulfate was fed to Swiss mice at dietary concentrations of 0.5%, the only dose level tested (according to an intake of approximately 300 mg/kg bw/day with the assumption of mean body weight of 20 g and a mean daily food intake of 2 g for mice). As breeding proceeded, results were noted for pregnancy rate, the number of live-born fetuses, the sex ratio, the postnatal survival at day 5 and day 20 and the pup weight at day 5 and day 20. Part of the litters were studied prenatally with their dams killed on gestational day 20 and the pups examined for gross and skeletal abnormalities. Part of the litters was studied postnatally with pups and their dams killed after the weaning period. Growth retardation was reported for the treated adult animals, more pronounced in the first generations and especially seen in the females (no food consumption or body weight data provided from the study). Histological examination of the liver, kidneys, urinary bladder or any of the other organs did not reveal any abnormalities, which could be attributed to the treatment. The breedings from the treated animals had lower mean numbers of liveborn fetuses in most of the generations in comparison to the concurrent controls and lower numbers of mean postnatal day 20 survivors, however any quantitative and litter data are not provided from the study. Any teratogenic effects were not revealed during the study.
During a further long-term (2-year) study with continuous breeding over several generations (Oser et al., 1976) CHA hydrochloride was fed to rats (FRDL strain) at dietary concentrations to provide 0, 15, 50, 100, and 150 mg CHA base/kg/day. Observations included growth, feed efficiency, clinical and hematological tests, reproduction, teratology, mortality and gross and microscopic pathology. Rats from the first litters of each generation from F0 through F4 were mated to produce the next succeeding generation. Those from the second litters of F1 through F4 were also mated, half the dams being delivered by Caesarean section on day 20 of pregnancy to permit examination of the fetuses for possible teratogenic effects, while litters from the other half were raised to maturity. Except for some non-progressive growth retardation in the higher dosage groups, due to lower food consumption, the physical and clinical observations in the test groups were within normal limits and were not significantly different from the controls. Almost every mating resulted in birth of a live litter, demonstrating that treatment with CHA at even the highest dosage caused no impairments on fertility or gestation. Reproductive performance through 3 successive matings revealed no adverse effects. A falling off of fertility and gestation was observed at the 6th matings of the F0 generation in all groups including the controls, when the rats were old (dams were about 66 weeks of age). The data for litter production in 5 consecutive generations showed no gradual diminution in fertility rate and only in the highest dose groups was there a reduction in litter size. A higher incidence of testicular atrophy was observed in the F0 group at the highest dose level, which is not uncommon for aged rats. However, in view of the lack of evidence of spermatogenic arrest and since these males continued to be fertile even at the 6th mating these findings were not regarded as significant.

CHA had been further investigated in a FDA guideline according study for developmental and teratogenic effects on two species with rats as well as with mice (Lorke and Machemer, 1983). Groups of 25 inseminated virgin NMRI mice or Long Evan rats per test group were treated orally from gestation days 6 to 15 with dosages of 0 (control), 10, 30, and 100 mg/kg bw/day by gavage. The test substance was dissolved in demineralised water. The administration volume was 10 ml/kg body weight in each group. General behaviour of the mothers and their weight were recorded. Caesarean sections on female mice and rats were performed on the 18th and 20th day of pregnancy, respectively. The following investigations were carried out: determination of the numbers of implantations, of living and dead fetuses and of dead and resorbed embryos and fetuses, of the litter weight and average fetus weight per litter, of the number of runts, of the average placenta weight per litter, and examination of about 1/3 of the fetuses for visceral deformations and of the remaining fetuses assessment of the abdominal and thoracic organs and subsequent examination for skeletal deformations. Oral doses of CHA of up to and including 100 mg/kg bw/day were tolerated by the dams without impairment of their external appearance and behaviour and were not lethal. In the rats 100 mg/kg/day led to significantly reduced weight gain (of 39.9 g) during the treatment period in comparison to the controls (56.6 g) and during the entire pregnancy, whereas no adverse effects of weight gain were revealed for mice. Pregnancy ratios of mice and of rats were unaffected by treatment with doses up to and including 100 mg/kg bw/day. The average numbers of implantations, resorption rate, sex ratio of the fetuses, average fetus weight, average placenta weight, frequency of fetuses with signs of slight retardation in the skeleton, runts and malformation rate were not affected in mice at all dose levels and in rats up to and including 30 mg/kg bw/day. Reduced average fetus and placenta weights were found in rats after treatment with the high dose of 100 mg/kg bw/day. The rat revealed to be the more sensitive species from this study. A NOAEL/maternal toxicity and developmental toxicity of 100 mg/kg/day can be derived for mice and of 30 mg/kg bw/day can be derived for rats from this study.
Visceral and skeletal examinations of neonates did not reveal any treatment-related changes in a further study with Wistar rats (Khera et al., 1971). Details of the study are described in chapter 4.1.2.9.

**Studies in humans**

No data available.

**4.1.2.9.3 Summary of toxicity for reproduction**

**Fertility**

From an oral 28-day repeated dose toxicity test with CBS in rats data on reproductive organ toxicity were available. Atrophy of seminiferous tubuli, hyperplasia of interstitial cells and decrease in epididymal sperm numbers were found at a dose of 800 mg/kg bw/d. No NOAEL could be derived from this study since it was unclear why the histopathological findings only were observed in the recovery group and in one of 12 animals of the highest dose group. Additional data were available from investigations on the products of hydrolysis of CBS. For mercaptobenzothiazole (MBT), there is no indication for reproductive organ toxicity or for functional impairment of reproductive capacity and capability.

From investigations with cyclohexylamine (CHA) with repeated administration over longer periods, testicular effects in terms of weight and morphological changes had been revealed. Tubular atrophy and reductions in spermatogenesis had been demonstrated repeatedly in several independent studies. Results from the studies with rats with repeat administration are summarised in Table 4.9.

A NOAEL/testicular toxicity of 100 mg CHA base/kg bw/day is derived from the 3-months study of Brune et al., 1978, and a NOAEL of 82 mg/kg bw/d from the chronic study of Gaunt et al., 1976. With the assumption of hydrolysis of CBS to equimolar amounts of CHA and MBT and with molecular weights of 99.18 for CHA free base and of 264.5 for CBS these values are converted to a NOAEL/reproductive organ toxicity of 267 mg/kg bw/day (Brune et al., 1978) and 218 mg/kg bw/d (Gaunt et al., 1976) for CBS. The absence of testicular effects at these dose levels is supported by the 28 day study with CBS (Chemicals Investigation Promoting Council, 1997c) showing effects at a dose of 800 mg/kg bw/d, whereas no histopathological effects on testes were found with a dosage of 250 mg/kg bw/d.

Taking into account the available database of both CHA and CBS an overall NOAEL (reproductive organ toxicity) of 218 mg/kg bw/d is recommended for use for quantitative risk assessment of CBS.

**Table 4.9: Cyclohexylamine (CHA) - Summary of data on reproductive organ toxicity from repeated dose toxicity tests**

<table>
<thead>
<tr>
<th>Reference species/strain route/duration of exposure</th>
<th>LOAEL/ testes effects</th>
<th>LOAEL/ syst tox effects</th>
<th>T: NOAEL/ testes effects</th>
<th>S: NOAEL/ syst tox effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collings et al. 1974</td>
<td>1000 mg/kg/d</td>
<td>200 mg/kg/d</td>
<td>T 500 mg/kg/d</td>
<td></td>
</tr>
</tbody>
</table>
rat, strain not known

<table>
<thead>
<tr>
<th>Diet Study</th>
<th>Treatment</th>
<th>Testis Weight</th>
<th>Weight Gain</th>
<th>Dietary Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaunt et al. 1974</td>
<td>468 mg/kg/d</td>
<td>abs. and rel. testis weight decreased and tubular degeneration in 13/15 rats</td>
<td>143 mg/kg/d</td>
<td>S 100 mg/kg/d</td>
</tr>
<tr>
<td>CFE rat diet/ 13 weeks</td>
<td>abs. and rel. testis weight reduced and tubular atrophy and reduced spermatogenesis</td>
<td>decreased bw gain and food intake</td>
<td>T 143 mg/kg/d</td>
<td>S 41 mg/kg/d</td>
</tr>
<tr>
<td>Mason et al. 1977</td>
<td>486 mg/kg/d</td>
<td>reduction in abs. weights of testis, tubular atrophy, lower sperm count and motility, impaired spermatogenesis</td>
<td>486 mg/kg/d</td>
<td>T 178 mg/kg/day</td>
</tr>
<tr>
<td>Wistar rat diet/ 90 days</td>
<td>494 mg/kg/d</td>
<td>reduction in abs. weights of testis, tubular atrophy, lower sperm count and motility, impaired spermatogenesis</td>
<td>494 mg/kg/d</td>
<td>T 176 mg/kg/day</td>
</tr>
<tr>
<td>Mason et al. 1977</td>
<td>200 mg/kg/d</td>
<td>reduced testis weight tubular alterations</td>
<td>200 mg/kg/d</td>
<td>T 100 mg/kg/d</td>
</tr>
<tr>
<td>Brune et al. 1978</td>
<td>300 mg/kg bw/d</td>
<td>testicular atrophy</td>
<td>82 mg/kg bw/d</td>
<td>T 82 mg/kg/day</td>
</tr>
</tbody>
</table>

Dietary concentrations (% or ppm) were converted to daily oral intake values (mg/kg bw/day) with the assumption of a mean body weight of 300 g and mean daily food intake of 10% of body weight for male rats if no details on CHA intakes were available from the study descriptions.

**Developmental toxicity**

The available animal data for the hazard assessment of CBS with respect to developmental toxicity are recruited from experiments with rats with the oral route of exposure. Studies with other routes of administration or with other species were not identified in the available database. Results from the three available oral developmental toxicity studies are summarised in Table 4.10. The studies consistently demonstrated that CBS induces maternal toxicity in terms of impairment of maternal weight gain during gestation and signs of fetal growth retardation in terms of reduced mean fetal body weight. Fetal body weight impairment, however, was exclusively observed at oral dosages associated with significantly reduced maternal weight gain of 15-30%. A substance-related specific embryotoxic and/or teratogenic potential was not revealed from the available studies. Therefore, there is no need for classification and labelling as a reproductive toxicant with regard to developmental toxicity.
Quantitative risk assessment for CBS with respect to developmental toxicity should be based on the data of the study of Ema et al., 1989, with a NOAEL/developmental toxicity of 70 mg/kg bw/day. However it should be recognised that this recommendation is a rather conservative approach. At present there is no reasonable explanation to the unexpected low LOAEL for weight gain impairment in the dietary study of Ema et al., 1989. Two other developmental toxicity studies with gavage administration and a repeated dose toxicity study via gavage (28 day study) consistently gave considerable higher LOAEL values. The lower maternal NOAEL in the study of Ema et al. (1989) with a 20 day dietary exposure is thus supposed to be not related to a higher sensitivity of pregnant rats but rather be explained from the administration route or probably the rat strain (Wistar rats (Kar:Wistar, Keari Co., Osaka)).

### 4.1.3 Risk characterisation

#### 4.1.3.1 General aspects

**Summary of toxicological effects**

CBS is readily absorbed after oral administration to rats and intensive metabolism takes place. As hydrolysis to 2-mercaptopbenzothiazol (MBT) and cyclohexylamine (CHA) was shown *in vitro* and may 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. There are no experimental data on dermal route of administration or exposure by inhalation. Absorption of 100% for the oral route is proposed to be taken for the risk characterisation, whereas dermal and inhalation absorption is assumed to be 100% (defaults). No data are available for the dermal route. Therefore, a default value for dermal absorption should be applied. Taking into account the physico-chemical data and the experimental data indicating a low toxicity via the dermal route an absorption rate of 10% will be assumed for dermal risk characterisation purposes.
The acute toxicity of CBS in animals is very low after oral and dermal administration; LD50 values of >5000 mg/kg bw were obtained. Data on inhalation toxicity and human data are not available.

CBS has demonstrated few cases of skin irritation in human patch tests with the commercial product. In animal tests CBS caused slight irritation on the skin and on the conjunctivae of the eye of rabbits. CBS is not a corrosive substance.

Limited information on respiratory tract irritation results from the 28-day rat inhalation study. Rats exhibited occasional nasal irritation which appeared to be concentration-related in terms of incidence and severity. The effects were usually observed at the end of the exposure but were not observed the following morning. A NOAEC or LOAEC cannot be derived. Any further information on these local effects is not available.

Data on sensitisation caused by inhalation is not available. As no cases of respiratory sensitisation after occupational exposure have been reported yet, it can be assumed that CBS does not induce sensitisation via the inhalation route. CBS did not cause skin sensitisation in guinea pigs. 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 consequence, classification with R 43 is warranted.

Concerning repeated dose toxicity studies in humans are not available. Exposure by inhalation of rats to the highest concentration of 0.048 mg/l CBS for 28 days caused no adverse systemic effects (NOAEC<sub>sys</sub>) and no adverse local effects on the respiratory tract (NOAEC local) in animals of both sexes. The reliability of the study is limited because the aerodynamic diameter (MMAD) of the CBS dust tested was 7.6µm with a GSD of 2.7µm. This distribution of CBS particle sizes will result in a lower inhalation as compared to particles with a MMAD lower than about 4µm.

After dermal exposure of rabbits for three weeks with 2000 mg/kg bw/d CBS no systemic or local effects were noted.

Oral administration by gavage of ≥250 mg/kg bw/d CBS over a period of 28 consecutive days caused coagulopathy of the blood in male and female Crj:CD (SD) rats and effects in the kidney of males. No relevant systemic toxic effects were observed in male and female rats after repeated oral administration (gavage) of 80 mg/kg bw/d CBS (NOAEL<sub>sys</sub>). In the same study the repeated treatment with 800 mg/kg bw/d showed no local toxic effects on the digestive tract in animals of both sexes.

CBS did not induce mutagenic effects in gene mutation assays employing various Salmonella strains and a Saccharomyces strain; also a mouse lymphoma assay was negative. An in vitro chromosomal aberration test gave weak evidence for a clastogenic potential. The only in vivo test (on embryonic mortality) cannot be assessed adequately due to insufficient reporting. There is no relevant evidence for mutagenicity of CBS. This is supported by genotoxicity data for the hydrolysis products MBT and CHA.

The existing two long-term studies on CBS 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, have 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, respectively. CHA is not carcinogenic in rats at doses up to 450 mg/kg bw/d and in mice up to 500 mg/kg bw/d, respectively.

Data on reproductive toxicity of CBS in humans are not available. Several oral developmental toxicity studies with CBS on rats did not reveal a specific embryo-/fetotoxic or teratogenic potential (NOAEL/developmental toxicity 70 mg/kg bw/d). Fetal growth retardation in terms of reduced mean fetal body weight was observed at dose levels that also significantly reduced maternal weight gain during pregnancy. Data on investigations of reproductive organ toxicity is available from an oral 28-day repeated dose toxicity test with CBS in rats and from several repeated dose studies with MBT and CHA. While for MBT there is no indication for reproductive organ toxicity or for functional impairment of reproductive capacity and capability testicular toxicity had been observed for CHA. Since the available study with CBS did not allow derivation of a no observed adverse effect level, a NOAEL of 218 mg/kg bw/d was extrapolated from a chronic study with CHA which should be used for risk characterisation purposes.
Table 4.11: Toxicological hazard identification

<table>
<thead>
<tr>
<th>N-Cyclohexylbenzothiazol-2-sulphenamide (CAS-No.: 95-33-0)</th>
<th>Inhalation</th>
<th>Dermal</th>
<th>Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute toxicity</td>
<td>no data</td>
<td>no hazard identified (animal data: LD50/rabbits, &gt;7940 mg/kg)</td>
<td>no hazard identified (animal data: LD50/rats, &gt;5000 mg/kg)</td>
</tr>
<tr>
<td>Irritation / corrosivity</td>
<td>skin: no hazard identified (animal and human data [human patch test]) eye: no hazard identified (animal data) respiratory tract: no data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sensitisation</td>
<td>skin: concern due to positive human patch test; R43 confirmed respiratory tract: no data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repeated dose toxicity (local)</td>
<td>no specific toxic effect up to the highest tested concentration (NOAEC of 0.048 mg/l, 6 h/d, 5 days/week, 28-day study, rats)</td>
<td>no specific toxic effect up to the highest tested dose (NOAEL of 2000 mg/kg bw/d, 21 day-study, rabbits)</td>
<td>no specific toxic effect up to the highest tested dose (NOAEL of 800 mg/kg bw/d, 28-day study, rat)</td>
</tr>
<tr>
<td>Repeated dose toxicity (systemic)</td>
<td>no specific toxic effects up to the highest tested concentration (NOAEC of 0.048 mg/l, 6 h/d, 5 days/week, 28-day study, rats)</td>
<td>no specific toxic effect up to the highest tested dose (NOAEL of 2000 mg/kg bw/d, 21 day-study, rabbits)</td>
<td>coagulopathy and kidney lesions at 250 mg/kg (NOAEL of 80 mg/kg bw/d, 28-day study, rats)</td>
</tr>
<tr>
<td>Mutagenicity</td>
<td>no relevant evidence for mutagenicity from a number of in vitro and in vivo studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td>no data</td>
<td>no data</td>
<td>no carcinogenic effect up to 95.3 mg/kg bw/d [time-weighted average dose] (mouse long term studies)</td>
</tr>
<tr>
<td>Fertility impairment</td>
<td>no data</td>
<td>no data</td>
<td>testicular toxicity (tubular atrophy, impaired spermatogenesis) - NOAEL (systemic tox. eff.) 67 mg/kg bw/d * - NOAEL (reprod. organ tox.) 218 mg/kg bw/d</td>
</tr>
<tr>
<td>Developmental toxicity</td>
<td>no data</td>
<td>no data</td>
<td>no specific toxic effects adverse to development - NOAEL (maternal toxicity) 7 mg/kg bw/d - NOAEL (developmental toxicity) 70 mg/kg bw/d</td>
</tr>
</tbody>
</table>

* This oral NOAEL/systemic toxicity of 67 mg/kg/d was extrapolated from a 2-year study with the hydrolysis product cyclohexylamine and is not selected for risk characterisation on repeated dose toxicity of CBS. Due to possible toxicokinetic reasons and implications like duration of the experiments (life long versus young adult) a slightly different NOAEL to that of 80 mg/kg bw/d with CBS in the 28-day study might occur. Thus, the data with CBS as parent substance from the 28-day study will be used for risk characterisation purposes.
4.1.3.2 Workers

Introductory remarks

CBS (N-cyclohexylbenzothiazol-2-sulfenamide) is a powdery substance with a very low vapour pressure. CBS is soluble in organic solvents whereas solubility in water is low. In Western Europe, CBS is exclusively used as a vulcanisation accelerator in rubber goods manufacture. For occupational risk assessment the MOS approach as outlined in the TGD (Human Health Risk Characterisation, Final Draft) is applied. This occupational risk assessment is based upon the toxicological profile of CBS (chapter 4.1.2) and the occupational exposure assessment (chapter 4.1.1.2). The threshold levels identified in the hazard assessment are taken forward to this occupational risk assessment.

This introductory remark specifies the route-specific information on CBS absorption and compares the route-specific CBS results of experimental animal testing in order to describe the relative toxic potency for different routes of exposure. In addition, a short introduction to the MOS approach is given.

Absorption and bioavailability for different routes of exposure

Based on experimental data, oral absorption is assumed to be 100%. There are no experimental absorption data for dermal and inhalation exposure; with reference to the TGD for these routes of exposure default values of 100% are proposed (see effects assessment).

In general, route-to-route extrapolation is considered to be a poor substitute for toxicity data obtained using the appropriate route of exposure. For CBS, toxicity data for the oral, dermal and inhalation route are available (table 4.1.3.2.A). Comparisons of the route-specific experimental results are only possible with restrictions, because for dermal and inhalation testing the highest tested doses did not result in adverse effects and thus corresponding LOAELs are not available.

For inhalation exposure, the default value of 100% absorption is used for calculation of the internal body burden. Experimental results of subacute inhalation testing are not contradictory to this assumption.

The comparison of the subacute oral and dermal data (see Table 4.1.3.2.A) indicates that toxic potency of CBS by dermal contact is significantly lower than the corresponding oral toxic potency. While the oral rat NOAEL is 80 mg/kg/d, the dermal rabbit NOAEL is 2,000 mg/kg/d (or higher). The direct comparison of these route-specific data indicates, that toxic potency by the dermal route might be 25-times lower than toxic potency by the oral route of application. When assuming a 100% oral bioavailability this relationship corresponds to a dermal bioavailability of 4%. For different reasons, this direct comparison of route-specific toxicity data is not sufficiently justified: Different species (rats, rabbits) are compared; furthermore, the rabbit data do not result in any adverse effect. Special importance should be given to the general empirical evidence, that for many substances tested there is a rather strong indication of an inverse relationship between relative dermal absorption and surface area dose. Thus it should be assumed for CBS risk assessment that the dermal availability following relatively low surface area doses in the occupational setting is higher than the dermal availability of CBS at the rather high surface area dose in experimental animal testing. However, based on the CBS data available there is no firm justification for a specific value of
dermal bioavailability to be chosen. In order to account for the clear experimental evidence of a lower toxic potency by dermal contact on the one hand and to prevent an underestimation of dermal risk (in case of using the 4% value) on the other hand, it is concluded that a 10% dermal bioavailability percentage might be an adequate assumption for dermal risk assessment.

Table 4.12: Route-specific toxic potency of CBS

<table>
<thead>
<tr>
<th>Route of application</th>
<th>Species</th>
<th>Duration frequency</th>
<th>LOAEL/C (original dose unit)</th>
<th>NOAEL/C (original dose unit)</th>
<th>Systemic effects</th>
<th>LOAEL (external)</th>
<th>NOAEL (external)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dermal</td>
<td>rabbit</td>
<td>21 days</td>
<td>-</td>
<td>2,000 mg/kg/d</td>
<td>-</td>
<td>-</td>
<td>2,000 mg/kg/d</td>
</tr>
<tr>
<td>oral</td>
<td>rat</td>
<td>28 days</td>
<td>250 mg/kg/d</td>
<td>80 mg/kg/d</td>
<td>Reduced bodyweight gain, coagulopathy</td>
<td>250 mg/kg/d</td>
<td>80 mg/kg/d</td>
</tr>
<tr>
<td>inhalation</td>
<td>rat</td>
<td>28 days, 6 h/d</td>
<td>-</td>
<td>48 mg/m³</td>
<td>-</td>
<td>-</td>
<td>14 mg/kg/d(1)</td>
</tr>
</tbody>
</table>

(1) 48 mg/m³ x 10⁻³ m³/l x 0.8 l/min/kg x 360 min

In table 4.12 the exposure levels of table 4.1 are summarised and the route-specific and total internal body burdens are identified. Risk assessment for combined exposure requires the calculation of a total internal body burden; for this calculation a 100% systemic availability by inhalation and a 10% systemic availability for dermal contact is assumed (see previous chapter).

Table 4.13: Occupational exposure levels and internal body burden (CBS)

<table>
<thead>
<tr>
<th>Exposure scenario</th>
<th>Inhalation</th>
<th>Dermal contact</th>
<th>Internal body burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/m³</td>
<td>mg/kg/d</td>
<td>mg/p/d</td>
</tr>
<tr>
<td>Inhalation(1)</td>
<td>Dermal(2)</td>
<td></td>
<td>Combined</td>
</tr>
<tr>
<td>Production of CBS</td>
<td>2</td>
<td>0.3</td>
<td>42(3)</td>
</tr>
<tr>
<td>Use as a vulcanisation accelerator in the rubber industry</td>
<td>1</td>
<td>0.14</td>
<td>200(4)</td>
</tr>
</tbody>
</table>

(1) based on the assumption of 100% bioavailability for inhalation and a breathing volume of 10 m³ per shift
(2) based on the assumption of 10% bioavailability following dermal contact
(3) EASE (90 % protection by suitable gloves)
(4) Analogous data (without gloves)
MOS Approach

The MOS approach for human risk characterisation is described in detail in the TGD (Human Health Risk Characterisation, Final Draft). The following chapter contains a short introduction to the MOS approach used. The basic principle of the MOS approach is a comparison of scenario-specific MOS values (the relationship between the experimental NOAEL respectively the adjusted starting point and the exposure level) with a reference MOS (product of various assessment factors).

MOS calculation and the adequate starting point

Basically, MOS values are calculated as quotient of a relevant NOAEL from experimental animal testing or human studies and actual workplace exposure levels. In specific situations, the MOS approach requires to convert the original NOAEL into an adequate starting point or corrected NOAEL previously to MOS calculation in order to be directly comparable to the exposure assessment. If the route of application in animal or human studies is different from the actual occupational exposure, the dose units of the experimental data should be converted to the dose unit of the exposure data. Additionally, possible differences in bioavailability between routes, as well as possible differences in bioavailability between animals and humans should be accounted for the calculation of the corrected NOAEL. In the absence of any route-specific information on oral and inhalation absorption, the TGD recommends to assume a 50% oral absorption and a 100% inhalation absorption. For CBS, route-specific data on subacute toxicity support specific assumptions on route-specific bioavailability (see above).

For occupational risk assessment, the corrected inhalatory NOAEC accounts for the difference of the standard respiratory volume (6.7 m³) and the respiratory volume for light activity (10 m³).

MOS values are calculated for different routes of exposure and for different toxicological endpoints. The routes of exposure specifically considered in occupational risk assessment are exposure by inhalation and dermal contact.

In addition, for risk assessment of combined exposure (exposure by inhalation and dermal contact) an adequate internal NOAEL is derived from external NOAELs and specific information on route-specific absorption. For MOS calculation, the adjusted internal starting point is divided by the internal body burden. Depending on route-specific exposure and absorption, inhalation exposure and/or dermal exposure may contribute to the internal body burden. With respect to the possible outcome of an assessment for combined risks, interest focuses on scenarios with conclusion ii at both exposure routes. Based on theoretical considerations, combined exposure will not increase the most critical route-specific risk component more than twice.

Reference MOS

The MOS values calculated have to be compared with a reference MOS. The reference MOS is an overall assessment factor, which is obtained by multiplication of individual assessment factors. The Technical Guidance Document emphasises several aspects which are involved in the extrapolation of experimental data to the human situation. For these assessment factors,
default values are recommended. It is important to point out that any relevant substance-specific data and information may overrule the defined default values.

Interspecies extrapolation on the one hand is based on allometric scaling (factor 4 for rats and factor 2 for rabbits). For remaining interspecies differences the TGD proposes an additional factor of 2.5.

For workers, an adjustment factor for intraspecies differences of 5 is recommended. Based on an evaluation of empirical data by Schneider et al. (2004) it is anticipated that a factor of 5 will be sufficient to protect the major part of the worker population (about 95%).

For chemical substances it is usually expected that the experimental NOAEL will decrease with increasing duration of application. Furthermore, other and more serious adverse effects may appear with prolonged exposure duration. For duration adjustment, a default factor of 6 is proposed for extrapolation from a subacute to chronic exposure. The duration adjustment factor is lower (a factor of 2) for the transition from subchronic experimental exposure to chronic exposure. For CBS, for duration adjustment of subacute toxicity data for the purpose of RDT risk assessment the default factor of 6 is used.

The TGD defines two further adjustment factors (uncertainty in route-to-route extrapolation and dose-response relationship including severity of effect). In specific cases these factors may be different from one. For CBS, no such factors are used.

*Comparison of MOS and reference MOS*

The MOS values for different toxicological endpoints and different exposure scenarios are compared with the substance- and endpoint-specific reference MOS. MOS values clearly above the reference MOS do not lead to concern, whereas MOS values that are clearly below the reference MOS are cause for concern. There may be various risk-related aspects which are not covered by default assessment factors. These additional qualitative aspects should be carefully considered when performing a risk assessment and should have adequate influence on finding of conclusions.

*Critical Exposure Levels*

In a parallel procedure, which gives identical but more direct results, the adjusted toxicological starting point is directly divided by the reference MOS. As a result, an exposure level (in mg/m³ or mg/kg/d) is identified, which may serve as a direct trigger for decisions when compared with the occupational exposure levels. In the context of this risk assessment report this trigger value is called “critical exposure level”. Concern will be expressed for scenarios with occupational exposure levels higher than the relevant “critical exposure level”.

**4.1.3.2.1 Acute toxicity**

*Systemic effects (inhalation)*

Human or animal data with acute exposure by inhalation are not available. In animals, acute oral toxicity was detected to be very low, with oral LD 50 values reported for rats and mice higher than 5,000 mg/kg. With reference to these acute toxicity studies, no clinical signs were observed at 2,100 mg/kg.
Because of the specified uncertainties in route-to-route extrapolation, it should be tried to use the results of the 28-day inhalation study for acute toxicity assessment as well. The aerodynamic diameter (MMAD) of the CBS dust tested was 7.6µm with a geometric standard deviation (GSD) of 2.7µm. This distribution of CBS particle sizes limits the reliability of the study because inhalation of these CBS particles is reduced compared to particles with a MMAD lower than about 4µm.

For the highest dose group of 48 mg/m³ no adverse systemic effects were reported. In general, threshold levels for acute toxicity are anticipated to be higher than corresponding levels for subacute toxicity. Thus it is assumed, that an acute NAEC (rat) is greater than 48 mg/m³. Without any further exposure-related adjustments, this exposure level is directly used as conservative starting point for acute risk assessment.

Based on an interspecies factor of 2.5 (remaining differences) and an intraspecies factor of 5 a reference MOS of 12.5 is calculated. The corresponding critical inhalation exposure level for acute toxicity in humans calculates to about 4 mg/m³ (48 / 12.5).

Compared to this critical exposure level of 4 mg/m³ the scenario-specific exposure levels for both occupational scenarios are slightly lower (2 mg/m³ for scenario 1 and 1 mg/m³ for scenario 2). Additionally, because the chosen approach is considered to be a conservative approach (use of a subacute NOAEL without experimental verification of a LOAEL), there is no concern for acute inhalation toxicity (see table 4.14).

Conclusion: ii

Systemic effects (dermal)

Acute dermal toxicity is considered to be very low. The dermal LD50 for rabbits was determined to be greater than 7,940 mg/kg. At this dose level there was no lethality and the only signs of toxicity reported were reduced appetite and activity. Also in a 21-day dermal study (see repeated dose toxicity) no signs of systemic toxicity up to a dose of 2,000 mg/kg/d was observed in rabbits. This study will be used as a provisional basis for acute dermal risk assessment as well.

Without any further adjustment the NOAEL of 2,000 mg/kg is used as adequate starting point. The default factor for interspecies adjustment for rabbits is $2 \times 2.5$, possible intraspecies variation is accounted for by a factor of 5. The corresponding reference MOS is 25 ($2 \times 2.5 \times 5$). The corresponding critical exposure level calculates to 80 mg/kg (2,000/25).

There is no concern for acute dermal toxicity (see table 4.14) for both occupational scenarios. The acute dermal risks for both scenarios are considered even lower, because it is assumed, that the use of a subacute NOAEL is a very conservative approach for acute risk assessment.

Conclusion: ii

Combined exposure

With reference to the semi-quantitative route-specific risk assessments for acute toxicity (inhalation, dermal) it is considered evident, that combined risk assessment for acute toxicity is mainly triggered by exposure by inhalation and is not significantly influenced by dermal exposure. As for acute inhalation toxicity, no concern is reached for combined exposure.

Conclusion: ii
Table 4.14:  Acute toxicity, systemic effects

<table>
<thead>
<tr>
<th></th>
<th>Inhalation</th>
<th>Dermal</th>
<th>Combined exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting point for MOS calculation</td>
<td>48 mg/m³</td>
<td>2,000 mg/kg/d</td>
<td>See text</td>
</tr>
<tr>
<td>Reference MOS</td>
<td>12.5</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Critical exposure level</td>
<td>4 mg/m³</td>
<td>80 mg/kg</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exposure</th>
<th>MOS</th>
<th>Conclusion</th>
<th>Exposure</th>
<th>MOS</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Production of CBS</td>
<td>2</td>
<td>24</td>
<td>0.6</td>
<td>3333</td>
<td>ii</td>
</tr>
<tr>
<td>2. Use as a vulcanisation accelerator in the rubber industry</td>
<td>1</td>
<td>48</td>
<td>3</td>
<td>667</td>
<td>ii</td>
</tr>
</tbody>
</table>

4.1.3.2.2 Irritation and corrosivity

Skin, Eye

In human patch tests with the commercial product, CBS has not demonstrated any skin irritation. In animal tests CBS caused slight skin and eye irritation. The observed effects are not considered sufficient for classification. There is no concern for dermal or eye irritation at the workplace.

Conclusion: ii

Respiratory tract

The only information on respiratory tract irritation results from the 28-day rat inhalation study (see 4.1.2.6). Rats exhibited occasional nasal irritation which appeared to be concentration-related in terms of incidence and severity. The effects were usually observed at the end of the exposure but were not observed the following morning. The report does not contain any further information on these local effects. A NOAEC or LOAEC cannot be derived. Based on this rather limited information, further taking into account the missing irritating potential of CBS in the subacute dermal rabbit study, it is assumed that respiratory tract irritation in the range of airborne CBS concentrations tested is mild. In principle, there is the option of further testing of respiratory tract irritation. Further testing, however, is not considered of priority, because it is assumed that adherence to the critical CBS exposure level of 2 mg/m³, which is specifically derived for systemic effects (see 4.1.3.2.4), effectively reduces the risk of respiratory tract irritation as well. The highest exposure level to be evaluated is 2 mg/m³ for scenario 1 (production of CBS). This judgement on respiratory tract irritation and the corresponding risk is considered to be adequately expressed as conclusion i (on hold).
4.1.3.2.3 Sensitisation

Skin

In a test with guinea pigs no sensitising properties were detected but a repeated insult human patch test with 51 persons using a 70% preparation of CBS demonstrated contact sensitivity in 5/51 individuals. In addition some of the patients with contact dermatitis did show a positive skin reaction on challenge with CBS in skin tests. Based on these data CBS is classified as a skin sensitiser; an effect threshold cannot be estimated.

Allergic contact dermatitis is considered to be a severe health problem. It is assumed that in the chemical industry (scenario 1) the use of gloves is highly accepted, but single dermal contacts may occur although suitable gloves are used. In the rubber processing industry (scenario 2) dermal exposure is assessed for the unprotected worker. Because of relevant personal protection measures, for scenario 1 the risk for skin sensitisation is lower than for scenario 2. However, because the available data do not allow to derive a NOAEL, a general concern is expressed for both scenarios.

Conclusion: iii

Respiratory tract

No information on respiratory sensitisation is available. Some potential of CBS to cause respiratory sensitisation cannot be excluded with certainty since in human skin tests the substance demonstrated allergenic properties. However, because there are no specific case reports on human respiratory sensitisation, concern is not expressed.

Conclusion: ii

4.1.3.2.4 Repeated dose toxicity

Local effects by dermal contact

In a subacute dermal toxicity study using doses of up to 2,000 mg/kg/d no substance-specific systemic or local effects were noted in rabbits. Based on these results conclusion ii is reached for primary skin irritation based on repeated dermal exposure. However, it should be recognized, that acute and repeated dermal exposure should be avoided because of the skin sensitising potential of CBS.

Conclusion: ii

Local effects by inhalation

The only information available on local effects by inhalation is already described in chapter 4.12. With reference to this chapter, conclusion i (on hold) is drawn for local effects by inhalation as well.
Conclusion: i (on hold)

Repeated dose toxicity, systemic effects

The relevant experimental studies to be used for RDT risk characterisation are summarized in table 4.1.3.2.A (see chapter on “absorption and bioavailability for different routes of exposure”). It is relevant to emphasize, that only testing by the oral route resulted in an experimental effect threshold (oral NOAEL of 80 mg/kg/d, oral LOAEL of 250 mg/kg/d). For the dermal and inhalation route of exposure the highest dosages tested did not yield an adverse effect. For that reason, the results of subacute oral testing in rats are especially relevant for RDT risk characterisation.

Systemic effects by inhalation

The calculation of the internal starting point is based on the oral rat study. For the oral route a 100% absorption is taken forward to risk characterisation. Thus, the oral NOAEL of 80 mg/kg/d is transformed to an internal starting point of 80 mg/kg/d.

Assuming a 100% absorption by inhalation, the relevant inhalatory dose is identical to the internal starting point of 80 mg/kg/d. The inhalatory dose of 80 mg/kg/d is divided by a factor of 0.38 m³/kg (rat breathing volume during 8 hours) and is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. This results in an inhalative starting point of 141 mg/m³ (80 x 1/0.38 x 6.7/10).

For the identification of the reference MOS adjustment factors for interspecies and intraspecies differences, and for differences in frequency of duration are applied. For interspecies differences the default factor is 2.5 (the factor for allometric scaling is already implicitly applied), for intraspecies differences (workers) the default factor is 5. The default factor of 6 is used to adjust for possible differences of thresholds for short-term and chronic exposure. Thus the reference MOS calculates to 75 (2.5 x 5 x 6). Based on oral study results, the critical inhalation exposure level at the workplace is identified as 2 mg/m³ (141 / 75).

For comparison, the result of the subacute inhalation study is translated into the corresponding critical inhalation exposure level: Rats were exposed to CBS concentrations of 4.3, 14.4 and 48 mg/m³ for 6 hours per day, 5 days per week. There were no adverse effects at the highest concentration tested. The experimental NOAEC of 48 mg/m³ is (1) adapted by a factor of 6/8 to account for differences between the experimental inhalation duration of 6 hours per day and the average working day of 8 hours per day, and (2) is multiplied by a factor of 6.7/10 for activity-driven differences of respiratory volumes in workers. This results in an adjusted inhalation starting point of 24 mg/m³ (48 x 6/8 x 6.7/10). With a reference MOS of 75 (2.5 x 5 x 6) the corresponding inhalation exposure level is identified as 0.3 mg/m³ (24 / 75).

Because oral testing (and not testing by the inhalation route) resulted in an experimental effect threshold, and because of the limited reliability of the subacute inhalation study (see “local effects by inhalation”) the critical inhalation exposure level of 2 mg/m³ is taken forward to risk characterisation. Although being a borderline situation, concern is expressed for scenario 1 (production of CBS with an exposure level of 2 mg/m³). For the use of CBS as vulcanisation accelerator (scenario 2) the exposure level of 1 mg/m³ is taken forward to risk characterisation. This value refers to low amounts of powdery CBS marketed. According to industry information, CBS however is mainly used in dust suppressed form leading to an exposure level of 0.6 mg/m³ (see 4.1.1.2). Based on this exposure information, in comparison
to the critical inhalation exposure level of 2 mg/m³, conclusion ii for scenario 2 is considered adequate. For corresponding MOS values see table 4.15.

Conclusion: iii

Systemic effects by dermal exposure

For dermal risk assessment a CBS study with rabbits is available. Experimental animals were exposed daily for 21 days for 6 hours per day to 125, 500 and 2,000 mg/kg/d. No dose-dependent systemic effects were noted. The dermal NOAEL was derived to be 2,000 mg/kg/d, keeping in mind that the “real NOAEL” might be higher than 2,000 mg/kg/d. There is no obvious reason to dismiss the results of this dermal rabbit study for dermal risk assessment. This dermal study indicates that dermal exposure (to rabbits) is significantly less effective than oral application (to rats) (see table 4.12 and corresponding discussion).

There are differences in daily (hours per day) and weekly (days per week) duration of exposure for the experimental animals and workers. These differences for daily exposure (6/8) and for weekly exposure (7/5) balance each other. For MOS calculation, the experimental NOAEL of 2,000 mg/kg/d is directly used as starting point.

For the calculation of the reference MOS an interspecies factor of 5 (a factor of 2 for metabolic rate scaling from rabbit to humans and of 2.5 for remaining interspecies differences) and an intraspecies factor of 5 is used. Additionally a factor of 6 for duration adjustment (subacute versus chronic) is applied.

Altogether the reference MOS results in 150 (5 x 5 x 6). The corresponding critical dermal exposure level calculates to 13 mg/kg/d (2,000 / 150). It should be kept in mind that there are two factors which are not quantitatively taken into account in this calculated critical dermal exposure level and which will be effective in the opposite direction: (1) the actual dermal NOAEL for rabbits might be higher than 2,000 mg/kg/d, (2) the dermal NOAEL for lower surface area doses might be lower than for the high surface area dose tested. The highest dermal exposure level at the workplace is 3 mg/kg/d (scenario 2). Based on the considerations outlined above, there is neither concern for scenario 1 (production of CBS) nor for scenario 2 (use as a vulcanisation accelerator). For MOS values see table 4.15.

Conclusion: ii

Systemic effects by combined exposure

The assessment of systemic effects by combined exposure is based on the subacute oral rat study and on corresponding calculations of internal doses (internal NOAEL and total internal body burden).

The internal starting point (based on the oral data) is 80 mg/kg/d. The reference MOS is calculated to be 300 (4 x 2.5 for interspecies differences, 5 for intraspecies differences, and 6 for duration adjustment). The corresponding critical internal exposure level is 0.27 mg/kg/d (80 / 300). Total internal body burdens for both scenarios are calculated from external exposure levels and the assumption of 100% systemic availability by inhalation and 10% systemic availability by dermal contact (table 4.13). The comparison of the critical internal exposure level of 0.27 mg/kg/d with the internal body burdens for both occupational scenarios (0.36 and 0.44 mg/kg/d) indicates concern for combined exposure. It is recognized that the concern for both scenarios is borderline.
Table 4.15: Repeated dose toxicity, systemic effects

<table>
<thead>
<tr>
<th>Exposure (mg/m³)</th>
<th>MOS</th>
<th>Conclusion</th>
<th>Exposure (mg/kg/day)</th>
<th>MOS</th>
<th>Conclusion</th>
<th>Exposure (mg/kg/day)</th>
<th>MOS</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>141 mg/m³</td>
<td>141</td>
<td>ii</td>
<td>2,000 mg/kg/d</td>
<td>2,000</td>
<td>ii</td>
<td>80 mg/kg/d (internal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td></td>
<td></td>
<td>150</td>
<td></td>
<td></td>
<td>300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 mg/m³</td>
<td></td>
<td></td>
<td>13 mg/kg/d (external)</td>
<td></td>
<td></td>
<td>0.27 mg/kg/d (internal)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Production of CBS

- Conclusion: iii

2. Use as a vulcanisation accelerator in the rubber industry

- Conclusion: iii

(1) Conclusion iii already results from inhalation exposure

4.1.3.2.5 Mutagenicity

Based on available data, there is no relevant evidence for mutagenicity of CBS. This is supported by mutagenicity data for the hydrolysis products MBT and CHA.

Conclusion: ii

4.1.3.2.6 Carcinogenicity

Two long-term studies with CBS in mice did not provide evidence for a carcinogenic potential of CBS. Results of experimental studies (mice, rats) with the hydrolysis products of CBS (MBT and CHA) did not result in carcinogenic effects either. Based on these experimental data an occupational risk concerning carcinogenicity is not anticipated.

Conclusion: ii

4.1.3.2.7 Toxicity for reproduction

Effects on fertility

A rat NOAEL of 218 mg/kg/d for testis toxicity is used for risk characterisation, because testis toxicity may lead to impaired fertility in humans. Testicular toxic effects in rats were observed following oral application both of CBS itself and of its metabolite CHA. For CBS,
there are no fertility studies in rats. The rat NOAEL for testis toxicity (218 mg/kg/d) is higher than the rat NOAEL for general subacute toxicity (80 mg/kg/d).

Inhalation

The calculation of the internal starting point is based on the oral rat studies on testis toxicity. For the oral route a 100% absorption is taken forward to risk characterisation. Thus, the oral NOAEL of 218 mg/kg/d is directly transformed to an internal starting point of 218 mg/kg/d.

The internal NOAEL for testis toxicity is converted into a NOAEC (rat, in mg/m³). Systemic availability by inhalation is assumed to be 100%. The internal NOAEL of 218 mg/kg/d is divided by a factor of 0.38 m³/kg (rat breathing volume during 8 hours) and multiplied by a factor of 6.7/10 (activity-driven differences of respiratory volumes in workers). This results in an adjusted rat NAEC of 384 mg/m³ (218 x 1/0.38 x 6.7/10).

For the identification of the reference MOS an interspecies factor of 2.5 (for remaining differences) and an intraspecies factor of 5 is taken. Accordingly the reference MOS results in 12.5 x (5 x 2.5). The corresponding critical inhalation exposure level calculates to 31 mg/m³ (384 / 12.5).

The highest exposure level is 2 mg/m³ for scenario 1. Compared to the critical inhalation exposure level of 31 mg/m³ there is no concern for both scenarios (production of CBS and use as a vulcanisation accelerator).

Conclusion: ii

Dermal contact

Subacute testing of CBS (rabbit, dermal) revealed a general NOAEL of 2,000 mg/kg/d. However, there is no specific experimental information on a NOAEL/LOAEL for testis toxicity by dermal contact.

For dermal risk characterisation of fertility impairment it is assumed, that there are the same route-specific potency differences as for RDT and that the relationship of general toxicity to testis toxicity for the oral route is valid for dermal contact as well. Because of the assumption of a 10% systemic availability by dermal contact, the internal NOAEL (testis toxicity) of 218 mg/kg/d is equivalent to an external dermal starting point of 2,180 mg/kg/d (218 x 10).

The reference MOS of 50 consists of the standard interspecies factor of 4 x 2.5 and the intraspecies factor of 5. The corresponding critical dermal exposure level calculates to 44 mg/kg/d (2,180 / 50). Since dermal exposure for both exposure scenarios is relatively low, there is no indication of a risk for fertility impairment at the workplace (see MOS values in table 4.16).

Conclusion: ii

Combined exposure

Risk assessment for combined exposure again starts with the internal NAEL of 218 mg/kg/d. The reference MOS of 50 is the same as for dermal risk assessment. The corresponding critical internal exposure, which is to be compared to the calculated internal body burden, calculates to 4.4 mg/kg/d (218 / 50). There is no additional concern for combined exposure (see MOS values in table 4.16).
Developmental toxicity

The developmental rat toxicity studies with CBS do not indicate a specific embryotoxic, fetotoxic or teratogenic potential (for further information see chapter 4.1.2.9). Fetal growth retardation was exclusively observed at dose levels associated with significantly reduced maternal weight gain of 15 to 30%. Based on the discussion on the experimental NOAELs for maternal toxicity and developmental toxicity (see chapter 4.1.2.9.3) the lowest NOAEL for developmental toxicity of 70 mg/kg/d is a very conservative approach.

Because a specific risk of developmental damage is not anticipated, a specific concern for developmental toxicity is not expressed.

Conclusion:  

4.1.3.2.8 Summary of risk characterisation for workers

Systemic CBS toxicity has been tested by different routes of application (oral, dermal, by inhalation). Only subacute toxicity testing by the oral route of application resulted in adverse effects with a NOAEL of 80 mg/kg/d.

Table 4.16: Fertility impairment

<table>
<thead>
<tr>
<th></th>
<th>Inhalation</th>
<th>Dermal</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting point for MOS calculation</td>
<td>384 mg/m³</td>
<td>2,180 mg/kg/d</td>
<td>218 mg/kg/d (internal)</td>
</tr>
<tr>
<td>Reference MOS</td>
<td>12.5</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Critical exposure level</td>
<td>31 mg/m³</td>
<td>44 mg/kg/day</td>
<td>4.4 mg/kg/d (internal)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exposure (mg/m³)</th>
<th>MOS</th>
<th>Conclusion</th>
<th>Exposure (mg/kg/day)</th>
<th>MOS</th>
<th>Conclusion</th>
<th>Internal body burden (mg/kg/day)</th>
<th>MOS</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Production of CBS</td>
<td>2</td>
<td>192</td>
<td>ii</td>
<td>0.6</td>
<td>3,663</td>
<td>ii</td>
<td>0.36</td>
<td>606</td>
</tr>
<tr>
<td>2. Use as a vulcanisation accelerator in the rubber industry</td>
<td>1</td>
<td>384</td>
<td>ii</td>
<td>3</td>
<td>727</td>
<td>ii</td>
<td>0.44</td>
<td>495</td>
</tr>
</tbody>
</table>
In the subacute dermal rabbit study the highest dose level tested (2,000 mg/kg/d) did not result in adverse effects. Dermal risk assessment is based either on the result of the subacute dermal rabbit study or on the subacute oral toxicity data in combination with the assumption of a 10% dermal systemic availability. For justification of the 10% value see chapter 4.1.3.2 (the section on absorption and systemic availability before 4.1.3.2.1).

In the subacute rat inhalation study the highest dose level tested (48 mg/m³) did not result in adverse effects either. The corresponding dose by inhalation (14 mg/kg/d, see table 4.1.3.2.A) is lower than the oral NOAEL of 80 mg/kg/d. The actual inhalatory dose is considered even lower because the particle size distribution tested results in a reduced inhalability of the particles. Because of the limited relevance of the CBS inhalation data, risk assessment for exposure by inhalation is based on the subacute oral toxicity data combined with the assumption of a 100% absorption by inhalation.

Based on the marginal information on respiratory tract irritation in the 28-day rat inhalation study (see chapter 4.1.2.6) it is assumed that respiratory tract irritation in the range of CBS concentrations tested is mild. Further testing is not considered of priority, because it is assumed that adherence to the critical CBS exposure level of 2 mg/m³, which is specifically derived for systemic effects (see 4.1.3.2.4) effectively reduces the risk of respiratory tract irritation. Based on these considerations, conclusion i (on hold) was drawn for local effects in the respiratory tract.

Table 4.17 summarizes the endpoint-specific and scenario-specific conclusions for CBS. Conclusion iii is enlisted in case of concern for at least one occupational exposure scenario.
Table 4.17: Summary on occupational risk assessment

<table>
<thead>
<tr>
<th>Toxicological endpoints</th>
<th>General conclusion</th>
<th>Exposure Scenarios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute toxicity</td>
<td>inhalation</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>dermal</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>combined</td>
<td>ii</td>
</tr>
<tr>
<td>Irritation/ Corrosivity</td>
<td>dermal</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>eye</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>acute respiratory tract</td>
<td>i (on hold)</td>
</tr>
<tr>
<td>Sensitisation</td>
<td>skin</td>
<td>iii</td>
</tr>
<tr>
<td></td>
<td>respiratory</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>inhalation, local</td>
<td>i (on hold)</td>
</tr>
<tr>
<td></td>
<td>inhalation, systemic</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>dermal, local</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>dermal, systemic</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>combined, systemic</td>
<td>iii</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1, 2</td>
</tr>
<tr>
<td>Repeated dose toxicity</td>
<td></td>
<td>ii</td>
</tr>
<tr>
<td>Mutagenicity</td>
<td></td>
<td>ii</td>
</tr>
<tr>
<td>Carcinogenicity</td>
<td></td>
<td>ii</td>
</tr>
<tr>
<td>Fertility impairment</td>
<td>inhalation</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>dermal</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>combined</td>
<td>ii</td>
</tr>
<tr>
<td>Developmental toxicity</td>
<td>inhalation</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>dermal</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>combined</td>
<td>ii</td>
</tr>
</tbody>
</table>

(1) conclusion iii already results from inhalative exposure, therefore no specific concern for the combined exposure scenario is indicated

With respect to systemic effects there is concern for repeated dose toxicity (inhalation) for scenario 1 (production of CBS). The lowest critical endpoint-specific exposure level is 2 mg/m³ which is derived for systemic effects by repeated inhalation (table 4.18). This level should be used as reference for establishing an occupational exposure limit. It is assumed that adherence to this reference level will effectively minimise the risk for respiratory tract irritation as well.

Dermal contact is without concern regarding general systemic effects; but may elicit allergic skin reactions due to the skin sensitising potential of CBS. With respect to skin sensitisation, there is a general concern for all dermal exposure scenarios; however, because of routinely implemented control measures, the corresponding concern for scenario 1 (production of CBS) is relatively low.
### Table 4.18: Ranking of CBS health risks for workers (inhalation)

<table>
<thead>
<tr>
<th>Exposure Scenario</th>
<th>Exposure level in mg/m³</th>
<th>Repeated dose toxicity, systemic</th>
<th>Fertility impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production of CBS</td>
<td>2</td>
<td>iii</td>
<td>ii</td>
</tr>
<tr>
<td>Use as a vulcanisation accelerator in the rubber industry</td>
<td>1</td>
<td>ii</td>
<td>ii</td>
</tr>
</tbody>
</table>
4.1.3.3 Consumers

Consumer exposure to this chemical is considered to be negligible.

Therefore human health risk regarding to acute toxicity, irritation, corrosivity, sensitisation, repeated dose toxicity, mutagenicity, carcinogenicity and reproductive toxicity is considered to be negligible.

4.1.3.3.1 Acute toxicity

4.1.3.3.2 Irritation and corrosivity

4.1.3.3.3 Sensitisation

4.1.3.3.4 Repeated dose toxicity

4.1.3.3.5 Mutagenicity

4.1.3.3.6 Carcinogenicity

4.1.3.3.7 Toxicity for reproduction

4.1.3.3.8 Summary of risk characterisation for consumers

Consumer exposure to this chemical is considered to be negligible.

Therefore human health risk regarding to acute toxicity, irritation, corrosivity, sensitisation, repeated dose toxicity, mutagenicity, carcinogenicity and reproductive toxicity is considered to be negligible.

Conclusion ii)

There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

4.1.3.4 Humans exposed via the environment
4.1.3.4.1 Exposure via air

The contribution of emissions to air to the indirect local exposure (cf. 4.1.1.4, Table 4.3) is considered to be negligible for risk characterisation.

4.1.3.4.2 Exposure via food and water

The exposure estimations for humans via the environment are summarised in section 4.1.1.4 (Table 4.3). A total daily intake of $2.45 \times 10^{-2}$ mg/kg bw/d was calculated for the local scenario. The main contributions are the $\text{DOSE}_{\text{fish}}$, $\text{DOSE}_{\text{leave crops}}$ and $\text{DOSE}_{\text{root crops}}$ with fractions of 15.3, 46.3% and 34.8%, respectively, for the oral route. Regional exposure can be expected to be negligible.

Comparison of exposure and effects

When considering possible risks of CBS to human health arising from indirect exposure via the environment the key areas of concerns may be for repeated dose toxicity, mutagenicity, carcinogenicity, and reproductive toxicity.

MOS for the local exposure scenario

Repeated dose toxicity (Animal studies with oral administration)

In a gavage toxicity study groups of Crj:CD (SD) rats (6 animals/sex/group) were tested at dosages of 0, 25, 80, 250, and 800 mg/kg bw/d CBS for a period of 28 consecutive days (Chemicals Investigation Promoting Council, 1997c; cf. 4.1.2.6). CBS-related effects were observed in male and female rats at $\geq 250$ mg/kg bw/d (signs of a coagulopathy of the blood in rats of both sexes, and hyaline droplets in kidney proximal tubular epithelia of male rats). No relevant CBS-related toxic effects were observed in animals of both sexes at 80 mg/kg bw/d (NOAEL for systemic effects). From a 28-day feeding study on Sprague-Dawley CD rats a NOAEL of approximately 250 mg/kg bw/d could be derived based on reduced body weight gain and food consumption (Monsanto 1980). Data on blood biochemistry, hematology, and histopathology are lacking in this study.

A NOAEL of 7.1 mg/kg bw/d (0.01% CBS in the diet) for dams was derived from a developmental study on Wistar rats based on reductions in body weight gain at of 69.6 mg/kg bw/d (0.1 % CBS in the diet) (Ema et al., 1989).

Margin of safety (MOS)

In the following the data base on repeated dose toxicity of CBS is considered to explain the conclusion about the appropriateness of the MOS for this endpoint.

- Overall confidence in the database

The data taken into account for performing the risk characterisation have been evaluated with regard to their reliability, relevance and completeness according to the TGD. The data were published in peer reviewed journals or submitted to the Competent Authority in private
reports being adequately detailed and in accordance with internationally recognised guidelines and to GLP.

The findings of all studies are in principle not contradictory so that the judgement can be based on the database (cf. sections 4.1.2.6, 4.1.2.9.2 and 4.1.2.9.3).

There are no reasons to assume limited confidence.

- Uncertainty arising from the variability in the experimental data

The studies cited above allow to conclude on the NOAEL of severe toxicity from CBS on rats. The main findings of the various studies (cf. 4.1.2.6 and 4.1.2.9.2) which are not in full compliance to current test guidelines showed good consistency.

However, at present there is no reasonable explanation to the unexpected low LOAEL of 69.6 mg/kg bw/d for weight gain impairment in the dietary developmental study by Ema et al. (1989) (cf. 4.1.2.9.2). Two other developmental toxicity studies with gavage administration as well as the above reported 28-day repeated dose toxicity studies via gavage and feeding gave consistently considerably higher LOAEL values. Furthermore, weight gain impairment in the study of Ema et al. (1989) did not show a dose response and was not seen at the respective dose level in the two other developmental studies as well as in the above reported repeated dose toxicity studies. Therefore, the low maternal NOAEL in the study of Ema et al. (1989) will not be used for risk characterisation purposes.

Thus, a certain uncertainty of a very minor extent might be remaining which should be considered in borderline situations.

- Intra- and interspecies variation

Data on kinetics of the substance do not allow to calculate the intraspecies and interspecies variability by applying modern approaches.

- Dose response relationship

There is no reason to assume a special concern.

- Nature and severity of the effect

CBS orally administered to rats on 28 days induced signs of a coagulopathy of the blood in rats of both sexes. These effects are considered to be severe health effects. There are no reasons to assume that the effects shown in the animal experiments are limited to the species tested, thus being not of relevance for humans.

- Differences in exposure (route, duration, frequency and pattern)

The estimated total daily intake (with an assumed absorption of 100%) is compared with an oral NOAEL from a 28-day study. There are no reasons to assume that special concern can be derived from this procedure.
- The human population to which the quantitative and/or qualitative information on exposure applies

Following the exposure scenario there is no reason to assume a special risk for elderly or children.

- Other factors

There are no other factors known requiring a peculiar high margin of safety.

**MOS for the local exposure scenario**

The daily intake was calculated to be 0.0245 mg/kg bw/d. The margin of safety between the exposure level of 0.0245 mg/kg bw/d and the oral NOAEL of 80 mg/kg bw/d is judged to be sufficient. Thus, with respect to repeated dose effects CBS is considered to be of no concern in relation to local indirect exposure via the environment (conclusion (ii)).

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

**Mutagenicity**

CBS was negative in gene mutation assays employing various Salmonella strains and a Saccharomyces strain; also a mouse lymphoma assay was negative. An in vitro chromosomal aberration test gave weak evidence for a clastogenic potential. The only in vivo test (on embryonic mortality) cannot be assessed adequately due to insufficient data reporting. There is no relevant evidence for mutagenicity of CBS (conclusion (ii)). This is supported by genotoxicity data for MBT and CHA as hydrolysis products of CBS.

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

**Carcinogenicity**

The two long-term studies on CBS in mice showed that CBS is not carcinogenic at a dose of 95.3 mg/kg bw/d (time-weighted average dose). In addition, results of oral long-term carcinogenicity studies of both CBS hydrolysis products, MBT and cyclohexylamine, clearly demonstrated that both MBT and cyclohexylamine 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.
The margin of safety between the NOAEL of 95.3 mg/kg bw/d based on data from the 107-week inhalation study on B6C3F1 mice (CIIT 1993). and the daily intake of 0.0245 mg/kg bw/d is judged to be sufficient to conclude on no concern for tumour formation in relation to local indirect exposure via the environment (MOS of about 3900, conclusion (ii)).

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

**Toxicity for reproduction**

**Effects on fertility**

Data on reproductive organ toxicity is available from an oral 28-day repeated dose toxicity study in rats and from several repeated dose studies with the metabolites MBT and cyclohexylamine. For MBT there is no indication for reproductive organ toxicity or for functional impairment of reproductive capacity and capability. Testicular toxic effects in rats were observed following oral application both of CBS itself and of the metabolite CHA. Findings were tubular atrophy, hyperplasia of interstitial cells, reduced spermatogenesis and changes in absolute and relative testis weights. For CBS, there are no studies on fertility impairment in rats available. The NOAEL for testis toxicity (218 mg/kg/d) which is derived from the study with CHA on rats by Gaunt et al. (1976) is higher than the rat NOAEL for general subacute toxicity (80 mg/kg/d, cf. 4.1.2.6).

**Margin of safety (MOS)**

For the decision on the MOS, the following aspects have been considered and taken into account:

- Uncertainty arising from the variability in the experimental data
  There are no reasons to assume an important uncertainty which has to be taken into account. The findings of all studies on CBS or its metabolite CHA are not contradictory (cf. 4.1.2.9.3).

- Overall confidence in the database
  There are no reasons to assume no confidence.

- Intra- and interspecies variation
  Data on kinetics of the substance do not allow to calculate the intra- and interspecies variability by applying modern approaches.

- Dose response relationship
  The histopathological changes were quite severe in some of the studies with repeated administration and occurred with a steep dose response curve.

- Nature and severity of the effects
  In all studies, testicular effects were only found at dose levels that also led to reduction in food intake and/or body weight gain. However, from those studies including paired-fed controls it became obvious, that the testicular effects were not simply secondary to overall
impairment of the animals but rather directly attributable to substance administration. CHA treatment related testicular changes, however, did not obviously change reproductive capability and capacity of the rats in the studies of Gaunt et al. (1974, 1976) and Mason et al. (1977), since most of the treated males remained fertile. However, this must not be the case in humans.

- Differences in exposure (route, duration, frequency and pattern)

The estimated total daily intake with an assumed absorption rate of 100% is compared with an oral NOAEL of 218 mg/kg bw/d for fertility. There are no reasons to assume a special concern from this procedure.

- The human population to which the quantitative and/or qualitative information on exposure applies

There is no reason to assume a special risk for children.

- Other factors

There are no other factors known requiring a peculiar margin of safety.

MOS for the local exposure scenario

The calculated daily intake is 0.0245 mg/kg bw/d. The margin of safety between the exposure level of 0.0245 mg/kg bw/d and the NOAEL (oral) of 218 mg/kg bw/d is judged to be sufficient even taking into account the steep dose response. Thus, with respect to fertility effects the substance is considered to be of no concern in relation to local indirect exposure via the environment (conclusion (ii)).

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Developmental toxicity

Several oral developmental toxicity studies with CBS on rats did not reveal a specific embryotoxic, fetotoxic or teratogenic potential. Fetal growth retardation was exclusively observed at dose levels associated with significantly reduced maternal weight gain of 15 to 30% during pregnancy. Based on considerations on the experimental NOAELs for maternal toxicity and developmental toxicity (cf. 4.1.2.9.3) the value of 70 mg/kg bw/d (Ema et al., 1989) will be used as NOAEL developmental toxicity for risk characterisation.

Margin of safety (MOS)

For the decision on the MOS, the following aspects have been considered and taken into account:
- Uncertainty arising from the variability in the experimental data
There are no reasons to assume an important uncertainty which has to be taken into account. The findings of all studies are not contradictory (cf. 4.1.2.9.3).

- Overall confidence in the database
There are no reasons to assume no confidence.

- Intra- and interspecies variation
Data on kinetics of the substance do not allow to calculate the intra- and interspecies variability by applying modern approaches.

- Dose response relationship
There is no reason to assume a special concern.

- Nature and severity of the effects
Significantly lower body weights of male and female fetuses and of the placentae were noted at the highest dose level of 289 mg/kg bw/d. However, no substance-related specific embryotoxic and/or teratogenic potential was revealed from the available studies.

- Differences in exposure (route, duration, frequency and pattern)
The estimated total daily intake with an assumed absorption rate of 100% is compared with an oral NOAELs of 70 mg/kg bw/d for developmental toxicity. There are no reasons to assume a special concern from this procedure.

- The human population to which the quantitative and/or qualitative information on exposure applies
There is no reason to assume a special risk for children.

- Other factors
There are no other factors known requiring a peculiar margin of safety.

**MOS for the local exposure scenario**
The calculated intake is 0.0245 mg/kg bw/d. The margin of safety between the exposure level of 0.0245 mg/kg bw/d and the NOAEL (oral) of 70 mg/kg bw/d is judged to be sufficient. Thus, with respect to developmental effects the substance is considered to be of no concern in relation to local indirect exposure via the environment (conclusion (ii)).

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.
MOS for the regional exposure scenario

Given the negligible exposure for the regional scenario there will be no concern in relation to repeated dose toxicity, mutagenicity, carcinogenicity, and reproductive toxicity due to regional indirect exposure of humans via the environment (conclusion (ii)).

ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

4.1.3.4.3 Summary of risk characterisation for exposure via the environment

Following the exposure estimations for humans via the environment there is no concern for indirect local and regional exposure to CBS.

Conclusion ii)

There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

4.1.3.5 Combined exposure

[click here to insert text; consider whether appropriate to conduct such an assessment, in particular when considering a consumer exposure of a similar order of magnitude to an environmental exposure]

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Exposure assessment

4.2.1.1 Workers

4.2.1.2 Consumers

4.2.1.3 Humans exposed via the environment
4.2.2 Effects assessment: Hazard identification

4.2.2.1 Explosivity

CBS is not explosive.

4.2.2.2 Flammability

CBS is not highly flammable.

4.2.2.3 Oxidizing potential

Due to its chemical structure, CBS is not expected to possess any oxidising properties.

4.2.3 Risk characterisation

4.2.3.1 Workers

not applicable conclusion: ii

4.2.3.2 Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

4.2.3.3 Humans exposed via the environment
5 RESULTS

5.1 INTRODUCTION

5.2 ENVIRONMENT

Conclusion (i) There is a need for further information and/or testing.

Conclusion (i) applies to following scenarios:

Several tire recycling activities have been shown to cause exposure of the environment by benzothiazole derivatives. This exposure could not be quantified on the basis of available information. While tire recycling is increasing, exposure of aquatic and terrestrial environment from these activities should be investigated. These activities are especially tire shredding and uses of tire crumb in ground materials.

A number of benzothiazole derivatives were measured in road runoff, in receiving waters and in road border soil. The substances originate from tire abrasion. The measured data indicate that there may be risk in these receiving environments. The available data are, however, too few and no final conclusions should be based on them. Therefore measured data from water bodies receiving road runoff and soils in the vicinity of roads should be produced.

Landfills are according to the available few studies from leachate a source of benzothiazole derivative releases to the aquatic environment. Major sources of these substances are expected to be landfilled general rubber products and already deposited tires. Possible risks cannot be excluded due to the scarce and variable data. Measured data are needed to draw conclusions for landfills in general.

In most of the scenarios chronic ecotoxicity data on MeSBT, MeBT, BT and BTon might be able to refine the risk ratios. However, such tests should be considered only after the above required information is made available.

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

This conclusion concerns CBS emissions from the three CBS production sites to the aquatic environment as a source of CBS and waste water treatment plants of the sites. The conclusion also covers the secondary poisoning route of CBS with present exposure levels.

In addition, the combined exposure of CBS and its breakdown products in the aquatic environment and waste water treatment plants does not cause risks at any producer site. In

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5 Conclusion (i) There is a need for further information and/or testing.
Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.
Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.
rubber industry, no releases of vulcanisation agents to the surface waters occur. Consequently,
no risks for aquatic environment are expected.

This conclusion covers also the exposure of soil for the CBS production and rubber industry
(emissions to air).

5.3 **HUMAN HEALTH**

5.3.1 **Human health (toxicity)**

5.3.1.1 **Workers**

**Conclusion (iii)** There is a need for limiting the risks; risk reduction measures which are
already being applied shall be taken into account.

Two occupational exposure scenarios have been identified: (1) production of CBS in the
large-scale chemical industry and (2) the use of CBS as vulcanisation accelerator in the rubber
industry.

For CBS, systemic toxicity by repeated inhalation and skin sensitisation are the most relevant
toxicological endpoints. For respiratory tract irritation a conclusion i (on hold) was drawn.

With respect to systemic effects there is concern for repeated dose toxicity by inhalation for
scenario 1 (production of CBS). The critical exposure level of 2 mg/m³, which is derived for
systemic effects by repeated inhalation, is proposed as reference for establishing an
occupational exposure limit. It is assumed that adherence to this reference level will
effectively minimise the risk for respiratory tract irritation as well.

For skin sensitisation, dermal contact results in concern for both scenarios; however, because
of relevant control measures, the risk of allergic skin reactions during production of CBS
(scenario 1) is considered relatively low.

5.3.1.2 **Consumers**

**Conclusion (ii)** There is at present no need for further information and/or testing and no
need for risk reduction measures beyond those which are being applied already.

5.3.1.3 **Humans exposed via the environment**

**Conclusion (ii)** There is at present no need for further information and/or testing and no
need for risk reduction measures beyond those which are being applied already.
6 REFERENCES


Ariel (2003): Ariel Insight 2.0, For survival in a world of expanding chemical regulations, April 2003

Bajaj AK, Gupta SC, Chatterjee AK and Singh KG (1988). Shoe dermatitis in India. Contact Dermatitis 19, 372-375


Berufsgenossenschaft der chemischen Industrie (1990). 2-Mercaptobenzothiazol. Toxikologische Bewertung; Ausgabe 05/90. BG Chemie Heidelberg


BLIC (2004): Information from the European Association of the Rubber Industry, 03.11.2004


RAPPORTEUR GERMANY 138 R035_HH_ENV_0805.DOC
CJ and Hernems JLM, (1993). Predictions of the Aquatic Toxicity of High-Production-Volume-Chemicals. Part A: Introduction and Methodology. Research Institute of Toxicology, Utrecht University, Utrecht, the Netherlands


Brownlee et al. (1992): Environ. Toxicol. Chem. 11, 1153-1168


De Wever & Verachtert (1997a): Water Res. 31(11), 2673-2684


Flexsys (2004): Information from the company Flexsys NV/SA, 05.08.2004


Goodyear Tire & Rubber Company (1996): CBS specific monitoring data from tire manufacturing, FAX from 03.06.1996


IARC (1980). IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans; Some Non-Nutritive Sweeting Agents Volume 22

Jungclaus et al. (1976): Analyt. Chem. 48 (13), 1894-1896


Kaniwa et al. (1994): Contact Dermatitis 30(1), 26-34


Lorke D and Machemer L (1983). The effect of cyclohexylamine on the embryo following oral administration to mice and rats. Toxicol. Letters 17, 137-143


Mason PL and Thompson GR (1977). Testicular effects of cyclohexylamine hydrochloride in the rat. Toxicology 8, 143-156


Monsanto (1968), Patent: GB 1106577; Chem. Abstr. 68, 96660


Monsanto (1979a): Acute Toxicity of Santocure to Daphnia magna. Report No. AB-79-305

Monsanto (1979b): Acute Toxicity of Santocure to Fathead Minnows (Pimephales Promelas). Report No. AB-79-0306

Monsanto (1979c): Toxicity of Santocure to the Freshwater Alga Selenastrum Capricornutum. Report No. BP-79-7-108


Monsanto (1992): Environmental persistence screening of selected rubber chemicals, Mo-92-9056


Oser BL, Carson S, Cox GE, Vigin EE and Sternberg SS (1976). Long-term and multigeneration toxicity studies with cyclohexylamine hydrochloride. Toxicology 6, 47-65


Springborn Laboratories Inc (1989a). Teratology Study in Rats with MBT. SLS Study No. 3205.2

Springborn Laboratories Inc (1989b). Teratology Study in Rabbits with MBT. SLS Study No. 3205.4

Springborn Laboratories Inc (1990). SLS Study No. 3205.5


Vermeulen, R., de Hartog, J., Swuste, P., Kromhout H. (2000): Trends in Exposure to Inhalable Particulate and Dermal Contamination in the Rubber Manufacturing Industry:

Varobeva RS, Mezentseva NV (1962). Toxicity of Sulphenamide derivatives of MBT used as vulcanisation. Soviet Rubber Technology 21, 14-15


ABBREVIATIONS

[update the list to correspond to the substance RAR]

ADI  Acceptable Daily Intake
AF  Assessment Factor
ASTM  American Society for Testing and Materials
ATP  Adaptation to Technical Progress
AUC  Area Under The Curve
B  Bioaccumulation
BBA  Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF  Bioconcentration Factor
BMC  Benchmark Concentration
BMD  Benchmark Dose
BMF  Biomagnification Factor
bw  body weight / Bw, b.w.
C  Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
CA  Chromosome Aberration
CA  Competent Authority
CAS  Chemical Abstract Services
CEC  Commission of the European Communities
CEN  European Standards Organisation / European Committee for Normalisation
CMR  Carcinogenic, Mutagenic and toxic to Reproduction
CNS  Central Nervous System
COD  Chemical Oxygen Demand
CSTEE  Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT\textsubscript{50}  Clearance Time, elimination or depuration expressed as half-life
d.wt  dry weight / dw
dfi  daily food intake
DG  Directorate General
DIN  Deutsche Industrie Norm (German norm)
DNA  DeoxyriboNucleic Acid
DOC  Dissolved Organic Carbon
DT\textsubscript{50}  Degradation half-life or period required for 50 percent dissipation / degradation
DT\textsubscript{90}  Period required for 50 percent dissipation / degradation
E  Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
EASE  Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50  Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC  European Communities
EC10  Effect Concentration measured as 10% effect
EC50  median Effect Concentration
ECB  European Chemicals Bureau
ECETOC  European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM  European Centre for the Validation of Alternative Methods
EDC  Endocrine Disrupting Chemical
EEC  European Economic Communities
EINECS  European Inventory of Existing Commercial Chemical Substances
ELINCS  European List of New Chemical Substances
EN  European Norm
EPA  Environmental Protection Agency (USA)
ErC50  Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD  Emission Scenario Document
EU  European Union
EUSES  European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)  (Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
FAO  Food and Agriculture Organisation of the United Nations
FELS  Fish Early Life Stage
GLP  Good Laboratory Practice
HEDSET  EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM  Helsinki Commission - Baltic Marine Environment Protection Commission
HPLC  High Pressure Liquid Chromatography
HPVC  High Production Volume Chemical (> 1000 t/a)
IARC  International Agency for Research on Cancer
IC  Industrial Category
IC50  median Immobilisation Concentration or median Inhibitory Concentration
ILO  International Labour Organisation
IPCS  International Programme on Chemical Safety
ISO  International Organisation for Standardisation
IUCLID  International Uniform Chemical Information Database (existing substances)
IUPAC  International Union for Pure and Applied Chemistry
JEFFCA  Joint FAO/WHO Expert Committee on Food Additives
JMPR  Joint FAO/WHO Meeting on Pesticide Residues
Koc  organic carbon normalised distribution coefficient
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>Kow</td>
<td>octanol/water partition coefficient</td>
</tr>
<tr>
<td>Kp</td>
<td>solids-water partition coefficient</td>
</tr>
<tr>
<td>L(E)C50</td>
<td>median Lethal (Effect) Concentration</td>
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<tr>
<td>LAEL</td>
<td>Lowest Adverse Effect Level</td>
</tr>
<tr>
<td>LC50</td>
<td>median Lethal Concentration</td>
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<tr>
<td>LD50</td>
<td>median Lethal Dose</td>
</tr>
<tr>
<td>LEV</td>
<td>Local Exhaust Ventilation</td>
</tr>
<tr>
<td>LLNA</td>
<td>Local Lymph Node Assay</td>
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<tr>
<td>LOAEL</td>
<td>Lowest Observed Adverse Effect Level</td>
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<td>LOEC</td>
<td>Lowest Observed Effect Concentration</td>
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<td>LOED</td>
<td>Lowest Observed Effect Dose</td>
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<td>Lowest Observed Effect Level</td>
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<tr>
<td>MAC</td>
<td>Maximum Allowable Concentration</td>
</tr>
<tr>
<td>MATC</td>
<td>Maximum Acceptable Toxic Concentration</td>
</tr>
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<td>MC</td>
<td>Main Category</td>
</tr>
<tr>
<td>MITI</td>
<td>Ministry of International Trade and Industry, Japan</td>
</tr>
<tr>
<td>MOE</td>
<td>Margin of Exposure</td>
</tr>
<tr>
<td>MOS</td>
<td>Margin of Safety</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular Weight</td>
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<td>Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)</td>
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<td>NAEL</td>
<td>No Adverse Effect Level</td>
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<td>NOAEL</td>
<td>No Observed Adverse Effect Level</td>
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<tr>
<td>NOEL</td>
<td>No Observed Effect Level</td>
</tr>
<tr>
<td>NOEC</td>
<td>No Observed Effect Concentration</td>
</tr>
<tr>
<td>NTP</td>
<td>National Toxicology Program (USA)</td>
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<td>O</td>
<td>Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)</td>
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<tr>
<td>OECD</td>
<td>Organisation for Economic Cooperation and Development</td>
</tr>
<tr>
<td>OEL</td>
<td>Occupational Exposure Limit</td>
</tr>
<tr>
<td>OJ</td>
<td>Official Journal</td>
</tr>
<tr>
<td>OSPAR</td>
<td>Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic</td>
</tr>
<tr>
<td>P</td>
<td>Persistent</td>
</tr>
<tr>
<td>PBT</td>
<td>Persistent, Bioaccumulative and Toxic</td>
</tr>
<tr>
<td>PBPK</td>
<td>Physiologically Based PharmacoKinetic modelling</td>
</tr>
<tr>
<td>PBTK</td>
<td>Physiologically Based ToxicoKinetic modelling</td>
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<tr>
<td>PEC</td>
<td>Predicted Environmental Concentration</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
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<td>--------------</td>
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<tr>
<td>pH</td>
<td>logarithm (to the base 10) (of the hydrogen ion concentration {H⁺})</td>
</tr>
<tr>
<td>pKa</td>
<td>logarithm (to the base 10) of the acid dissociation constant</td>
</tr>
<tr>
<td>pKb</td>
<td>logarithm (to the base 10) of the base dissociation constant</td>
</tr>
<tr>
<td>PNEC</td>
<td>Predicted No Effect Concentration</td>
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<tr>
<td>POP</td>
<td>Persistent Organic Pollutant</td>
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<td>PPE</td>
<td>Personal Protective Equipment</td>
</tr>
<tr>
<td>QSAR</td>
<td>(Quantitative) Structure-Activity Relationship</td>
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<td>R phrases</td>
<td>Risk phrases according to Annex III of Directive 67/548/EEC</td>
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<td>RAR</td>
<td>Risk Assessment Report</td>
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<td>RC</td>
<td>Risk Characterisation</td>
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<td>RfD</td>
<td>Reference Dose</td>
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<td>RNA</td>
<td>Ribonucleic Acid</td>
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<tr>
<td>RPE</td>
<td>Respiratory Protective Equipment</td>
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<td>RWC</td>
<td>Reasonable Worst Case</td>
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<td>S phrases</td>
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<tr>
<td>SAR</td>
<td>Structure-Activity Relationships</td>
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<td>SBR</td>
<td>Standardised birth ratio</td>
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<tr>
<td>SCE</td>
<td>Sister Chromatic Exchange</td>
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<tr>
<td>SDS</td>
<td>Safety Data Sheet</td>
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<tr>
<td>SETAC</td>
<td>Society of Environmental Toxicology And Chemistry</td>
</tr>
<tr>
<td>SNIF</td>
<td>Summary Notification Interchange Format (new substances)</td>
</tr>
<tr>
<td>SSD</td>
<td>Species Sensitivity Distribution</td>
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<tr>
<td>STP</td>
<td>Sewage Treatment Plant</td>
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<tr>
<td>T(+)</td>
<td>(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)</td>
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<tr>
<td>TDI</td>
<td>Tolerable Daily Intake</td>
</tr>
<tr>
<td>TG</td>
<td>Test Guideline</td>
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<tr>
<td>TGD</td>
<td>Technical Guidance Document</td>
</tr>
<tr>
<td>TNSG</td>
<td>Technical Notes for Guidance (for Biocides)</td>
</tr>
<tr>
<td>TNO</td>
<td>The Netherlands Organisation for Applied Scientific Research</td>
</tr>
<tr>
<td>UC</td>
<td>Use Category</td>
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<tr>
<td>UDS</td>
<td>Unscheduled DNA Synthesis</td>
</tr>
<tr>
<td>UN</td>
<td>United Nations</td>
</tr>
<tr>
<td>UNEP</td>
<td>United Nations Environment Programme</td>
</tr>
<tr>
<td>US EPA</td>
<td>Environmental Protection Agency, USA</td>
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<tr>
<td>UV</td>
<td>Ultraviolet Region of Spectrum</td>
</tr>
<tr>
<td>UVCB</td>
<td>Unknown or Variable composition, Complex reaction products of Biological material</td>
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</table>
vB  very Bioaccumulative
vP  very Persistent
vPvB very Persistent and very Bioaccumulative
v/v  volume per volume ratio
w/w  weight per weight ratio
WHO World Health Organization
WWTP Waste Water Treatment Plant
Xn Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
Xi Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
APPENDIX A: 2-MERCAPTOBENOTHIAZOLE (MBT)

1.1 Identification of the Substance

Name: 2-Mercaptobenzothiazole

Synonyms: 2-(3H)-Benzothiazolethione
2-Benzothiazolethiole
MBT

CAS No.: 149-30-4

Empirical Formula: C₇H₅NS₂

Molecular weight: 167.25 g/mol

Structural Formula for the undissociated tautomers:

\[ \text{H} \quad \text{S} \quad \text{S} \quad \text{N} \quad \text{SH} \]

MBT is a weak acid with a pKₐ of ca. 7. Two tautomers are known for the undissociated (neutral) form (see above) and for the anionic form, each. A second dissociation constant (pKₐ of 3.08) has been reported, too, under which MBT is protonised. Around the pH of 7 the neutral thione form and the anionic form of the thiol tautomer are present in about similar amounts (Schmegel 1995).

1.2 Physico-Chemical Data

Tab. 1.1: Physico-Chemical Data of MBT

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>180.2°C</td>
<td>Weast (1979)</td>
</tr>
<tr>
<td>Boiling point</td>
<td>Decomposition above 260°C</td>
<td>GDCh (1991)</td>
</tr>
<tr>
<td>Solubility in water</td>
<td>51 mg/l (25°C; pH 5)</td>
<td>Monsanto (1980a)</td>
</tr>
<tr>
<td></td>
<td>118 mg/l (25°C; pH 7)</td>
<td>Brownlee et al. (1992);</td>
</tr>
<tr>
<td></td>
<td>905 mg/l (25°C; pH 9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Test conducted in creek water:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>190 mg/l (24°C; pH 6.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>230 mg/l (24°C; pH 7.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>260 mg/l (24°C; pH 8.5)</td>
<td></td>
</tr>
<tr>
<td>Property</td>
<td>Value</td>
<td>Source</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>pH</td>
<td>7.03 (aqueous buffer)</td>
<td>GDCh (1991)</td>
</tr>
<tr>
<td></td>
<td>3.08</td>
<td>Shulman et al. (1971)</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>&lt; 2.6 x 10^{-8} Pa (25°C)</td>
<td>Monsanto (1980a)</td>
</tr>
<tr>
<td>Log Kow (measured)</td>
<td>2.41 (neutral form); T: 24 °C</td>
<td>Brownlee et al. (1992)</td>
</tr>
<tr>
<td></td>
<td>Test conducted in creek water:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.43 (pH 6.5; T: 24 °C)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.38 (pH 7.5; T: 24 °C)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.95 (pH 8.5; T: 25 °C)</td>
<td></td>
</tr>
<tr>
<td>Log Kow (estimated)</td>
<td>1.61 (pH 7.4)</td>
<td>Illing &amp; Benford (1976)</td>
</tr>
<tr>
<td></td>
<td>2.86 (neutral form)</td>
<td>KOWWIN v1.67</td>
</tr>
<tr>
<td></td>
<td>2.29 (neutral form; estimated from measured $K_w$)</td>
<td>Schmegel (1995)</td>
</tr>
</tbody>
</table>

### 2. Exposure

#### 2.1 Production and Use

In 1993, 38,000 t MBT were manufactured within Western Europe. The production process is described in the CBS report section 2.2. MBT and its zinc salt are used as vulcanization accelerators in rubber industry, the amounts in Western Europe were 1500 t MBT and 1400 t ZnMBT in 1993 (Srour, 1994).

In addition, the majority of the technically relevant benzothiazole derivatives are produced via MBT. It is an intermediate for different benzothiazole sulphanamides and 2,2'-dithiobis-benzothiazole (MBTS) which are exclusively used as vulcanization accelerators in rubber industry. Further end products are methabenzthiazuron which is used as a pesticide in agriculture and 2-(thiocyanomethylthio)benzothiazole (TCMTB) which is used as a biocide. For methabenzthiazuron, the European demand was 1800 t in 1993 (Srour, 1994). It is included in the third priority list of the working program for evaluation of plant protection products under the Council Directive 91/414/EEC. The dossier for the evaluation was due to be sent to the Rapporteur Member State Sweden by 30 November 2004. Sweden has not received the dossier. Hence, the use of methabenzthiazuron as plant protection product will end in Europe.

TCMTB has been notified under the Biocides Directive 98/8/EEC for the use in ten product groups in the Commission Regulation 2003/2032/EC, i.a. for the use in antifouling paints, slimicides, and conservation products. No use for wood preservation has been notified against the earlier information according to which TCMTB is also used as a fungicide by tanneries (Brownlee et al., 1992 and Srour, 1994).

MBT itself has been notified under the Biocides Directive 98/8/EEC for the use in seven product groups, i.a. for the use in slimicides, wood preservatives and indoor disinfection of public buildings. The tonnage is unknown but can be assumed to be small compared to the industrial use.

Minor amounts are used in photography, in flotation of ores, as corrosion inhibitor in drilling and cutting oils, and in antifreeze fluids for automobiles (GDCh, 1991). The Nordic chemical
product database SPIN showed for the year 2003 use in the categories “process regulators”, “reprographic agents”, “adhesives, binding agents”, “cleaning, washing agents” and “surface treatment”.

### 2.2 Sources of Exposure

#### Chemical Industry

MBT emissions can be expected to occur within chemical industry during its production and use in the synthesis of benzothiazole derivatives. Table 2.1 presents the local concentrations in surface water based on measured data from the CBS production plants in the EU-15. Emissions to surface water from the three plants which provided data are around 1 t/a.

**Table. 2.1. Local concentration of MBT during CBS production (90-P or maximum)**

<table>
<thead>
<tr>
<th>Site</th>
<th>Ceffluent [µg/l]</th>
<th>Clocal [µg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt;20 (DL)</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>B</td>
<td>6.4</td>
<td>8*10^-3</td>
</tr>
<tr>
<td>C</td>
<td>183.8</td>
<td>0.48</td>
</tr>
</tbody>
</table>

For the background information, the available measured emission data from around the world are presented in Table 2.3. Some of this data are old and represent the past emission situation. The Toxic Release Inventory of the United States (U.S. Environmental Protection Agency, 2005) shows for the complete chemicals industry (reporting class “SIC28”) a discharge of 32 t to surface water and an emission of 1.8 t to air for the year 2003.

#### Rubber Industry

MBT, its zinc salt, MBTS and the benzothiazole sulphenamides are used as vulcanization accelerators in rubber goods manufacture. The substances are dosed in concentrations of 0.25-1% (ww) to the caoutchouc. During vulcanization (achieved at temperatures between 150 and 200°C) the unstable sulphur-nitrogen links of benzothiazole-sulphenamides resp. the S-S link of MBTS are split and during a complex reaction sequence the rubber molecules are vulcanized with the formation of other benzothiazole compounds. The vulcanization process is further described in the CBS report section 2.3. An overview about the MBT content in rubber goods and aqueous eluates is presented in Table 2.2.

Old measured data (cf. Table 2.3) reveal that MBT occurred in the waste water of tire manufacturers. In the USA the substance was detected in concentrations up to 0.59 mg/l (CMA, 1985). However, according to the emission scenario document for additives in rubber industry (OECD 2004) no emissions of vulcanisation accelerators are expected into waste water at the present.

#### Tire Tread Abrasion

Abraded tire particles accumulate near roads, leading to an exposure of soils in the vicinity. Discharges of abraded particles into the hydrosphere are directed via rainwater runoff from roads. Lower amounts of abraded particles are released into the atmosphere. In the environment, the particles are degraded by biotic and abiotic processes, leading to the release of the included monomers like MBT.

Rubber goods and automobile tires contain MBT; the substance was frequently detected in extracts and eluates (see Table 2.2). Therefore releases by tire tread abrasion would be
expected. A series of benzothiazole compounds but not MBT was quantified in road runoff, its concentration in highway runoff and in snow near a road with high traffic density was below the detection limit of 0.2 µg/l (Baumann & Ismeier, 1998). Klöpfer (2005) found MBT in road runoff from a highway drainage area. The concentrations measured were 0.1-0.3 µg/l during one rain event. The cause of the low concentration may be rapid degradation in road zones, leading to releases of MBT transformation products.

Use of biocides

Use of MBT for the notified applications under the Biocides Directive 98/8/EEC causes emissions to the environment. In addition, the use of TCMTB cause exposure of MBT and other benzothiazole derivatives which are degradation products of TCMTB. In this report, releases caused by biocidal use of MBT and TCMTB use are not assessed. Compared to the use amounts of vulcanization accelerators, this release source is expected to be of minor importance and the local risks will be assessed under the Biocides Directive.

Measured data

Table 2.3. presents a compilation of available measured data of MBT in environmental samples and releases.
**Table 2.2: Monitoring of MBT in Rubber Products and Eluates**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubber shoes</td>
<td>&lt;1-1910 mg/kg</td>
<td>5</td>
<td></td>
<td>Kaniwa et al. (1994)</td>
</tr>
<tr>
<td>Eluate from rubber sports place</td>
<td>&lt;0.05-1.2 mg/l</td>
<td>4</td>
<td>Exposure with water for 24 h</td>
<td>Baumann et al. (1998)</td>
</tr>
<tr>
<td>surface</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eluate from rubber tubes</td>
<td>≤2.17 mg/l</td>
<td>6</td>
<td></td>
<td>Baumann et al. (1998)</td>
</tr>
<tr>
<td>Eluate from latex glove</td>
<td>9.5 mg/l</td>
<td>1</td>
<td></td>
<td>Baumann et al. (1998)</td>
</tr>
<tr>
<td>Eluate from teat rubber</td>
<td>4.7 mg/l</td>
<td>1</td>
<td>Rubber pulverized, 24 h exposure with water</td>
<td>Baumann et al. (2000)</td>
</tr>
<tr>
<td>Eluate from artificial tire tread</td>
<td>&lt;0.5–884 µg/l</td>
<td>25</td>
<td>6 different tires (automobile and truck tires, each new and old)</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Eluate from complete tires</td>
<td>&lt;0.5 µg/l</td>
<td>2</td>
<td>Sampled after 2 month exposure in a water bath</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Eluate from complete tires or dust</td>
<td>≤80 mg/l</td>
<td></td>
<td>6 tires, extracted at pH 4.0, 7.0, 9.0 after 2, 4, 7 days</td>
<td>CMA (1985)</td>
</tr>
<tr>
<td>Medium</td>
<td>Concentrations</td>
<td>Number of Samples</td>
<td>Remark</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>----------------</td>
<td>-------------------</td>
<td>----------------------------------------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Effluent MBTS producer</td>
<td>4.2 mg/l (average)</td>
<td>4</td>
<td>(USA)</td>
<td>CMA (1985)</td>
</tr>
<tr>
<td>Municipal plant effluent</td>
<td>11 µg/l (average)</td>
<td>4</td>
<td>Origin: MBTS producer (USA)</td>
<td>CMA (1985)</td>
</tr>
<tr>
<td>Municipal waste water (influent)</td>
<td>191 ng/l (average, SD: 70 ng/l)</td>
<td>20</td>
<td>Average of 20 composite samples of 24 h collected over three months in 2002 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2004), Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.02 µg/l (average, SD: 0.03 µg/l)</td>
<td>20</td>
<td>Average of 20 composite samples of 24 h collected over three months in 2002 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (influent)</td>
<td>0.02 µg/l (2 samples &gt; LOD)</td>
<td>9</td>
<td>Average of 9 composite samples of 24 h collected over one month in 2003 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.01 µg/l (average; SD: 0.01 µg/l)</td>
<td>9</td>
<td>Average of 9 composite samples of 24 h collected over one month in 2003 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.01 µg/l</td>
<td>1</td>
<td>Year 2003 (Berlin, Schönerlinde, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (influent)</td>
<td>1.12 µg/l (average; SD: 1.26 µg/l)</td>
<td>3</td>
<td>Pooled samples (GaoBeiDian, Beijing, China)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.04 µg/l (average; SD: 0.01 µg/l)</td>
<td>4</td>
<td>Pooled samples (GaoBeiDian, Beijing, China)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Effluent tire plant</td>
<td>0.59 mg/l (average)</td>
<td>4</td>
<td>(USA)</td>
<td>CMA (1985)</td>
</tr>
<tr>
<td>Effluent from a tire manuf.</td>
<td>30 µg/l</td>
<td>1</td>
<td>MBT not identified at a second plant</td>
<td>Jungclaus et al. (1976)</td>
</tr>
<tr>
<td>Untreated tannery wastewater</td>
<td>655 µg/l</td>
<td></td>
<td>Origin: TCMTB</td>
<td>Fiehn et al. (1994)</td>
</tr>
<tr>
<td>Highway runoff</td>
<td>&lt;0.2 µg/l</td>
<td>8</td>
<td>Sampled during 3 rainfalls, from beginning to 30 min after beginning</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Highway runoff</td>
<td>0.2 µg/l (average) 0.2 µg/l (90-P) 0.1-0.3 µg/l (min-</td>
<td>19</td>
<td>Outlet of a highway section drainage basin (ca. 200 000 ADI), year 2003 (Berlin, Germany)</td>
<td>Klöpfer (2005)</td>
</tr>
<tr>
<td>Medium</td>
<td>Concentrations</td>
<td>Number of Samples</td>
<td>Remark</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>----------------</td>
<td>-------------------</td>
<td>--------------------------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td>Snow near a highly frequented road</td>
<td>&lt;0.2 µg/l</td>
<td>3</td>
<td>Sampled 14 days after snowfall</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Water</td>
<td>&lt;0.021 µg/l (1977)</td>
<td>12</td>
<td>Sampled in Japan, “water” not further characterized</td>
<td>JETOC (1993)</td>
</tr>
<tr>
<td>Sediment</td>
<td>&lt;37 µg/kg (1977)</td>
<td>12 111</td>
<td>Not detected in fish in 90 samples (det. limit 0.002-1 mg/kg)</td>
<td>JETOC (1993)</td>
</tr>
<tr>
<td>River water</td>
<td>0.50 µg/l</td>
<td>1</td>
<td>River Dee (UK)</td>
<td>Rennie (1988)</td>
</tr>
<tr>
<td>River sediment</td>
<td>0.16-1.62 mg/kg</td>
<td>10</td>
<td>Sampled at Grand Calumet River, Indiana (USA), bulk sediment, dry weight</td>
<td>Hoke et al. (1993)</td>
</tr>
<tr>
<td>River sediment</td>
<td>4.6-44 mg/kg</td>
<td></td>
<td>Sampled in Japan</td>
<td>Shinohara et al. (1978)</td>
</tr>
<tr>
<td>Lake water</td>
<td>&lt;1 µg/l</td>
<td>4</td>
<td>Lake containing two tire reefs (USA)</td>
<td>CMA (1985)</td>
</tr>
<tr>
<td>LANDFILL LEACHATE</td>
<td>&lt; 1 µg/l</td>
<td>4</td>
<td>(USA)</td>
<td>CMA (1985)</td>
</tr>
</tbody>
</table>
Natural Occurrence

In the literature some benzothiazole derivatives occurring as natural products are described. From fermentation culture extracts of Micrococcus sp., a marine bacterium obtained from tissues of the sponge Tedania ignis, 2-mercaptobenzothiazole (MBT), 2-methylbenzothiazole (MeBT), 2-benzothiazolone (BTon) and 6-hydroxy-3-methyl-2-benzothiazolone were detected (Stierle et al., 1991). There is an agreement in the scientific literature that the natural occurrence of benzothiazoles is rare and the compounds detected in environmental compartments are mainly of anthropogenic origin.

Hallberg & Larsson (1999) measured MBT in the seawater of a Norwegian fjord. Depending on water depth (0-150 m) the reported concentrations are up to 40 µM (6.7 mg/l). As there is no anthropogenic outlet into the Fjord the authors considered MBT to be naturally produced, however the source could not be identified. Compared with the toxicity data referred in section 3.1, the reported concentrations appear improbable. In addition, concentrations of the further metabolites of MBT, e.g. 2-methylthiobenzothiazole (MeSBT), would be expected in the same order of magnitude; actually they were measured in seawater in the ng/l range.

2.3 Degradation

Biodegradation

The biodegradation of MBT has been determined according to an OECD standard test. In the MITI Test (OECD 301 C), a concentration of 100 mg MBT/l was examined. The inoculum concentration was 30 mg/l, degradation was monitored by biological oxygen demand (BOD) analysis, as prescribed in the guideline. Accordingly, MBT was degraded to 2.5% within a period of 14 days. Therefore, MBT is classified as not readily biodegradable (CITI, 1992).

A biodegradation test was also conducted according to a TSCA (Toxic Substances Control Act) test guideline. During an exposure of 28 days with an inoculum mixture of soil, activated sludge and raw wastewater, which was adapted to increasing MBT concentrations of 8.14 to 16.29 mg/l during 14 days, MBT biodegradation was analysed by radioactivity measurements. The control substance was radiolabelled glucose. A formation of 0.1-0.2% of radiolabelled CO₂ was determined, therefore, no ultimate MBT biodegradation took place (CMA, 1989b).

Using a shake flask procedure (draft method no 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 a SCAS supernatant was incubated during 35 days with MBT concentrations of 17 and 18 mg/l. The CO₂ evolution in percent of the ThCO₂ was reported to be 2% and MBT is considered to be persistent (Monsanto, 1992).

Tomlinson et al. (1966) tested MBT concentrations in the effluents after an exposure of 7 weeks in adapted activated sludge using a „bioassay“ method based on short term toxicity test (nitrifying activity). There was no evidence that MBT was decomposed.

While in ultimate biodegradation standard tests no biodegradation was shown, the transformation and degradation processes of MBT have been examined in an exhaustive manner in adapted and sometimes complex feeding test systems.
Chudoba (1977) examined MBT degradation in adapted activated sludge. The inoculum was adapted to five different thiazoles during 76 days before use. The incubation period was 30 days, sampling was performed every 2 days and the COD was determined. At the end of incubation period, UV spectroscopy of the filtrates was performed. It was revealed by COD determination (values only shown in a graph), that MBT was extremely resistant, the COD remained stable, confirmed by UV spectroscopy.

A certain degradation of MBT was reported by manometric determination of O₂ consumption. The inoculi used were industrial and adapted to benzothiazole-2-sulphonic acid and a mixed culture isolated from the adapted sludge. 1 µmol of MBT was tested in a final volume of 3 ml (56 mg/l). An oxygen consumption of 85 µl in the mixed culture and of 30 µl in the adapted sludge were determined at a temperature of 30 °C (values taken from a graph), from which a biodegradation of 34 % and 12 % according to the two different inocula can be calculated. In a parallel experiment benzothiazole-2-sulphonic acid was tested: in the mixed culture 60% of the theoretical oxygen amount were taken up (Mainprize et al., 1976).

More data are documented in an unpublished report from Bayer AG (1988a). In a pilot plant of 35 m³ flow and with adapted sludge, and with a MBT concentration of 200 mg/l in the influent, a concentration of 10 - 60 mg/l in the effluent was determined. Biodegradation was tested by total organic carbon (TOC) analysis. It is however not known whether MBT was degraded biologically or eliminated by chemical oxidation (leading to MBTS) only.

Partial degradation of MBT was found in laboratory-scale fed-batch systems (DeWever & Verachtert, 1994; 1997). The activated sludge used as inoculum was taken from rubber chemicals wastewater treatment plants. The inoculum concentration was very high, 3-5 g/l, the exposure time 61 days, and concentrations up to 200 mg/l MBT were tested. MBT disappearance was determined by UV spectrometry and HPLC analysis. MBT was best degraded by a mixture of MBT-history and non-MBT-history sludge. The substance was never completely oxidized, but partially transformed to different end-products: with MBT concentrations up to 75 mg/l, 2-benzothiazolesulfonic acid (BTSO₃H) and a group of unidentified polar products, the latter are not further degraded. Above the MBT toxicity threshold (75-100 mg/l) 2,2’-dithiobis-benzothiazole (MBTS) was detected; this compound is insoluble and accumulated in sludge. Further experiments on BTSO₃H degradation had incoherent results: when present as the sole compound, its removal was rather slow. In the presence of MBT, BTSO₃H disappeared rapidly in the first phase, but after further addition of MBT BTSO₃ was degraded much more slowly. MBT seems to slow down BTSO₃H removal, but on the other hand changes BTSO₃H degradation resulting in a more complete breakdown.

De Wever (1998) isolated an axenic culture capable to the further degradation of BTSO₃H which was identified as *Rhodococcus erythropolis*. The isolate also degraded 2-benzothiazolone (BTon) and benzothiazole (BT), but not MBT, which was found to inhibit the biodegradation of BTon, BT, and BTSO₃H. Under anaerobic conditions, BTSO₃H was transformed into BTon in stochiometric amounts.

De Vos et al. (1993) examined parameters affecting the degradation of benzothiazoles in activated sludge systems. By complex feeding procedures to adapted sludge, [benzothiazole (BT) and MBT were added at different time, at different concentrations, two different industrial sludges were further adapted to MBT, BT and 2-benzothiazolone (BTon)], it was shown that MBT inhibited the oxidation of BT and BTon, and was further toxic to all sludges. BT and BTon at 2 mg/l were completely degraded, but the degradation of MBT was negligible at all concentrations. Only with sludge which was adapted during several weeks in the fed-batch adaptation system, MBT was slowly removed. When sludges were incubated with higher MBT concentrations, 2,2’-dithiobis-benzothiazole (MBTS) was formed at acid
pH; at alkaline pH, BTSO$_3$H was formed. As the MBT disappearance was determined in all different systems, it is assumed that only primary degradation processes are revealed, although the changes in chemical oxygen demand (COD) were reported to be analogous to the changes in concentration of the compound under investigation (data are not shown in the publication).

Klöpfer et al, (2005) monitored the removal of MBT in a large municipal waste water treatment plant of Ruhleben, Berlin, Germany. The removal regarding the concentration difference of influent and effluent of ca. 90% was observed in a three months sampling program which took into account the variation in the influent flow conditions. The authors observed a clear connection in the removal of MBT and formation of MeSBT. Taking into account the distribution properties of MBT (see Chapter 2.4), it can be assumed that much of the removal observed in this study was caused by biodegradation.

Reemtsma (1994) assessed the biodegradation of 2-thiocyanatomethylthio-benzothiazole (TCMTB) with microorganisms from a municipal wastewater treatment plant. MBT is the hydrolysis product of this substance which is mainly used as a biocidal product in tanneries, therefore the fate of MBT has been examined. An aerobic degradation test of MBT at an initial concentration of 10 mg/l (2 l batch systems with the addition of 115 mg/l yeast extract as an additional carbon source according to ISO 9888), revealed that after 28 days 10% were methylated to 2-methylthiobenzothiazole (MeSBT), and 87% was not methylated (determined by HPLC, UV spectroscopy). In the abiotic control, no transformation was found. MeSBT was not further degradable.

In a pilot treatment plant fed with wastewater from two tanneries, containing 1.2 and 0.55 mg MBT/l as the predominant benzothiazole derivative. It was found that benzothiazole (BT) is formed from unknown precursors in the anaerobic treatment, whereas MeSBT is significantly diminished. MBT is refractory against anaerobic treatment. During subsequent aerobic treatment, MBT and BT are substantially eliminated, while MeSBT is formed. Finally, MBT accounted for 40% of the benzothiazoles and was exceeded by BT in the treated tannery I wastewater (53%) and MeSBT in tannery II wastewater (57%). Adsorption onto biomass appeared not to be a major elimination process for all examined derivatives. No correlation were detected between the decrease of one and the increase of another benzothiazole derivative. In conclusion, it was found that benzothiazole derivatives are not completely removable by biological treatment, and will be released into surface waters (Reemtsma et al., 1995).

Therefore, the aerobic transformation of MBT and its related benzothiazoles was assessed also on model sediment columns at significantly lower concentrations simulating the mixture of surface water with industrial effluent (0.022 - 0.033 mg/l). While, initially, small amounts of MBT were detected in the column effluents, in contrast to the batch test, MBT was completely methylated within the residence time of about 2 days (Reemtsma, 1994). This results are in coherence with the results found by Brownlee et al. (see below) who observed methylation after mixing MBT with sedimentary matter.

Drotar et. al. (1987) examined biomethylation of MBT by different bacteria (one Corynebacterium and three Pseudomonas strains) containing thiol methyltransferase activity. This enzyme system is widely distributed in nature, thus biomethylation has been pointed out as a very important biotransformation pathway of MBT in many ecosystems.

Gaja & Knapp (1998) noted that MBT can be removed in the presence of both live and dead activated sludges. In die-aways with “live” industrial activated sludge from a rubber chemicals manufactory, MBT decreased within 4 days, while similar results were obtained
after the sludge was heat-killed. They found that removal is unlikely due to adsorption, instead of this non-enzymic reactions which are not characterized are proposed. Transformation products were not determined. No evidence could be obtained for truly degradative removal.

Kondo et al. (1988) examined by a cultivation method the degradation of MBT in surface waters. MBT at a concentration of 20 mg/l was exposed to non adapted surface waters of two river and two sea water samples during 3 days in the dark, at 30 ° Celsius. Growth of microorganisms was judged by turbidity measurements at 610 nm, and the disappearance of MBT was analysed by „suitable methods“ . A very variable biodegradation of aniline according to the different testing laboratories and incubation medium is documented. According to the classification of Kondo, MBT has to be considered as to be of „hard degradability“.

In a study performed by Monsanto (1980), river water which passes through a city and may be exposed to thiazoles, has been incubated with 1 mg/l MBT in the dark during 8 weeks at room temperature. The disappearance of MBT was monitored by HPLC analysis. Whereas the control substance quinoline disappeared within weeks, MBT was not biodegraded. Although MBT concentrations decreased, in abiotic controls, MBT concentration decreased also, therefore a non-biotic process is assumed.

Brownlee et al. (1992) determined MBT concentrations by HPLC in sediment samples. The sediment was adapted because thiazoles were detected in the creek where the samples were taken. The sediment was spiked with 6 mg/l MBT. The flasks were shaken for 12 hours at room temperature and after a subsequent standing for 12 hours, extraction was performed at pH 2 and pH 8. MBT was the only compound detected in the pH 2 extract, at pH 8, MBT and 2-methylthiobenzothiazole (MeSBT) was found. Methylation is reported to be biologically mediated.

Anaerobic degradation was examined in surface water (river water) by Karelova and Tomasovicova (1988) in the presence and absence of glucose. The inoculum was adapted during 10 days. The incubation with a MBT concentration of 5 and 10 mg/l took place during 6 weeks at 25°C. MBT degradation was monitored by UV spectroscopy. At both MBT concentrations tested degradation was slowed down by the addition of glucose. Without glucose at an initial MBT concentration of 5 mg/l, 44 % MBT disappeared whereas at MBT concentration of 10 mg/l, 74% MBT disappeared. With glucose, 13% disappeared at an initial concentration of 5 mg/l and 9% at 10 mg/l. Only primary degradation was monitored in this study and it is not clear which metabolites have been formed. It seems nevertheless, that MBT was accepted by bacteria as sole carbon source when no glucose was available.

BIOWIN v4.02 predicts that MBT is not readily biodegradable but not persistent to biodegradation, either. Following specific results are estimated: BIOWIN2 = 0.65, BIOWIN3 = 2.83 and BIOWIN6 = 0.14.

Abiotic Degradation

No experimental data are available about the photodegradation of MBT in the atmosphere. Because of the extreme low volatility of the substance, this pathway is of little environmental relevance.

In aqueous solutions, MBT can be oxidized by atmospheric oxygen to 2,2'-dithiobisbenzothiazole (MBTS). Hansson & Agrup (1993) analyzed test solutions containing 500 µM (84 mg/l) MBT in 0.5 M phosphate buffer and 10% tetrahydrofuran at pH 6.5. After 2 hours reaction time 60% of the MBT was converted to MBTS. The MBT solution was stable after
addition of the reducing agent glutathione. When the same experiment was run with a MBTS solution, only trace amounts were converted to MBT without glutathione after 2 hours, while after glutathione addition MBTS was completely reduced to MBT. The results reveal that the equilibrium between MBT and MBTS is largely influenced by the redox status of the medium.

The UV spectrum of MBT indicates that photodegradation under environmental conditions is possible. Irradiation of a solution of 0.37 mg MBT/l in phosphate buffer with sunlight (July, 42° latitude) revealed a half-life of the direct photodegradation of 31 min. With the addition of humic acids the half-life was 27 min indicating that direct photolysis is more important than indirect degradation (CMA, 1989c). Similar results were obtained in a further experiment (Monsanto, 1980a): a 1.1 mg/l solution irradiated with sunlight at midday in August had a half-life of 2.7 hours. These values refer 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 certainly longer.

Brownlee et al. (1992) identified the reaction products of photolysis: after irradiation of a MBT solution in phosphate buffer with sunlight, 2-benzothiazolone (BT(on)) and benzothiazole (BT) were detected with yields of 4% and 11%, respectively. Acidification yielded in further 18% BT. Similar results were obtained in the presence of dissolved organic matter. The authors conclude that BT and BT(on) were stable products of MBT photolysis in aquatic environments.

Summary of MBT degradation

The available data on MBT biodegradation reveal that under standard conditions the substance is not mineralized, therefore MBT could be classified as not biodegradable. Mineralization occurs only when the inoculi are adapted. Such adaptation processes may occur e.g. in industrial or municipal biological treatment plants. It can be concluded from the degradation tests as well as from the available monitoring data that MBT removal in treatment plants can be considerable but not complete. In addition to MBT, releases of the products of primary transformation are expected.

The pathways of MBT degradation are presented in figure 1. MBT is mineralized via the pathway 2-benzothiazolesulfonic acid (BTSO3H) → 2-benzothiazolone (BT(on)) → → CO2, H2O etc. The results of the available laboratory tests are largely dependent on the test conditions, and a prediction to which extend MBT is mineralized in industrial treatment plants is not possible. Different microorganism species seem to be responsible for the individual reaction steps, and some cases the reaction are inhibited by MBT. Therefore, it cannot be excluded that the intermediates BTSO3H and BT(on) are released by industrial sources.

Oxidation of MBT to MBTS is only expected when two MBT molecules can meet, i.e. the MBT concentration is high enough. This reaction might occur in waste waters at concentrations above 75-100 mg/l, but not at environmental levels.

Beside oxidation, MBT can be methylated to 2-methylthiobenzothiazole (MeSBT). This reaction is confirmed to occur both in industrial plants and in environmental samples, leading to releases of MeSBT, a metabolite which is ubiquitously distributed in the environment. MeSBT can be further oxidized, the products are described in appendix V, section 2.2.

When released into surface waters, photolysis of MBT could be an important process leading to 2-benzothiazolone (BT(on)) and benzothiazole (BT) as the products. The photolysis rate cannot be determined.
### Figure 1: Metabolization pathway of MBT

![Metabolization pathway of MBT](image)

2.4 Distribution

The molecular structure and thus the distribution properties of MBT are largely determined by the pH of the medium. MBT is in the environmentally relevant pH range a weak acid with a pKa of about 7, i.e. in the environment both the acid and the basic form can exist. MBT can form complexes with heavy metals, e.g. copper, nickel, zinc or cobalt (Schmegel, 1995).

Based on a water solubility of 118 mg/l (pH 7) and a vapour pressure of \(< 2.6 \times 10^{-8} \text{ Pa} \) (25°C), the Henry’s law constant is calculated to \(< 3.7 \times 10^{-8} \text{ Pa m}^{3}/\text{mol} \). The value indicates that MBT is not volatile from aqueous solutions.

The distribution between the organic phase of soil or sediment solids and porewater can be calculated from the octanol/water partitioning coefficient: with a log Kow of 1.4 and the equation \( \log Koc = 0.52 \log Kow + 1.02 \) (TGD, 1996) the Koc value is calculated to 56 l/kg. PCKOCWIN v1.66 gives in turn 1613 l/kg. Values in line with the latter QSAR-result were obtained from two laboratory tests. CMA (1989a) determined the adsorption onto 3 soil and 3 sediment types in batch equilibrium studies. Kd values of 0.799-5.73 l/kg for the soils and
18.3-23.0 l/kg for the sediments were obtained. Considering the organic carbon content, the Koc constants were 326-1360 l/kg for the soils and 2130-3560 l/kg for the sediments. Similar values resulted from a study from Monsanto (1980b): using 4 different soils, the Koc values were 863-1829 l/kg (Kd 7.5-18 l/kg). Extraction of MBT from the soils resulted in recoveries as low as 40-60%, the authors conclude that MBT either reacted with soils or was irreversibly adsorbed. For further calculations, the mean Koc value of 1570 l/kg found for totally 7 soils and 3 sediments is used.

From AOPWIN v1.9, a half-life of 9.5 h for reaction with hydroxyl radicals results (5.0 * 10^6 OH/cm^3, 24 h reaction/day).

The distribution of MBT in a “unit world” is calculated according to the Mackay fugacity model Level I, considering the values for vapour pressure (2.6*10^-8 Pa), log Kow (1.4) and water solubility (118 mg/l). The main target compartments is estimated to be water with 99.6% and 0.2% for both soil and sediment. The adsorption coefficient of MBT is underestimated in this model, consequently the fractions for soil and sediment should actually be higher.

SimpleTreat in EUSES 2.0 gives as the distribution in (local) stp the following: 0% (air), 83.8% (water), 16.2% (sludge).

2.4 Bioaccumulation

MBT is included in the MITI list. Following the OECD 305 C guideline, a BCF of < 0.8 and < 8 has been determined according to the exposure concentrations, respectively. The concentrations of 0.1 and 0.01 mg/l have been tested in the carp and the exposure period was 6 weeks (CITI, 1992).

In a metabolism study with carp, where ^14C-MBT was administered by gavage into the anterior intestine, only negligible radioactivity was found in the different organs and tissues after 72 hours, It is concluded that ca. 100 % of the dose was excreted into the water (Hashimoto, K. et al., 1978).

Based on these results, the potential of MBT to bioaccumulate in fish can be estimated to be low and there is no need to estimate the risks for the secondary poisoning routes.
3. Effects Assessment

3.1 Aquatic Compartment

For the effects assessment of MBT the degradation properties in aqueous solutions have to be taken into account. Under summer sunlight, MBT undergoes photolytical degradation with half-lives of about 30 min at the water surface. Static tests conducted without analytical control should be interpreted carefully, because the test substance concentrations probably decrease below 80% of the nominal as demanded by the test guidelines. Even in flow-through tests, the measured concentrations dropped by up to 50% of the nominal (e.g. CMA, 1989 d,e). If the present quality criteria are strictly applied, the acute tests, where no monitoring of the test concentrations occurred, should be regarded as not valid. Considering that in the applied test conditions the biological degradation is unlikely, the main degradation products appearing during the time frame of acute tests are benzothiazole (BT) and benzothiazolone (BTon). These are formed via photolysis. BT and BTon are approximately one order of magnitude less toxic than MBT based on the available data (see Appendices III and IV). Without knowing the rate of photolysis, it can only be concluded that the real L(E)C50-values for MBT are in these tests below the reported values and the results are used as background information for the assessment.

In some test reports the occurrence of precipitates is reported, their nature was not examined. A plausible cause is the oxidation of MBT by atmospheric oxygen to the insoluble 2,2’-dithiobis-benzothiazole (MBTS).

In the tests either MBT or its sodium salt was used as the test substance. The toxicity of both substances is expected to be identical and only dependent on pH. Below the effect values are referred as MBT.

3.1.1 Toxicity to Fish

Tests on acute toxicity to fish are available for 3 freshwater species. An overview is presented in Table 3.1.

Static tests on the acute toxicity to *Brachydanio rerio* and *Pimephales promelas* resulted in 96h-LC50 values of 1.6 mg/l for *B. rerio* (geometric mean of LC0 and LC100) and 11 mg/l for *P. promelas* (Bayer AG, 1988b; Monsanto, 1979a). The results should be interpreted with caution because no analytical monitoring was performed and the test substance concentrations were probably not stable due to photolytical degradation of MBT during the test period.

A flow-through test to juvenile *Oncorhynchus mykiss* was conducted by Monsanto (1981). The fish were exposed to nominal concentrations between 0.06 and 1.00 mg/l, the flow rate was set to provide five tank volumes per day. The analytical control (RP-LC) suggest that, except for the lowest concentration of 0.06 mg/l, agreement between nominal and actual test concentrations was reasonable good. The test was terminated after 8 days of exposure, at which time no death had occurred for three consecutive days. The resulting LC50 values (based on measured concentrations) were 0.73 mg/l after 4 days and 0.67 mg/l after 8 days. The authors interpreted the relatively small increase in mortality between 4 and 8 days and the fact that no death occurred on day 6-8 suggest that the material does not show substantial cumulative toxicity under conditions of acute exposure.
### Table 3.1: Toxicity of MBT to Fish

<table>
<thead>
<tr>
<th>Species</th>
<th>Test type</th>
<th>Test conditions</th>
<th>Exposure time</th>
<th>Effect conc.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brachydanio rerio</em></td>
<td>static</td>
<td>21-23°C, pH 6.4-6.9</td>
<td>96 h</td>
<td>LC0 = 0.8 mg/l (n)</td>
<td>Bayer AG (1988b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LC50 = 1.6 mg/l (n)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LC100 = 3.2 mg/l (n)</td>
<td></td>
</tr>
<tr>
<td><em>Pimephales promelas</em></td>
<td>static</td>
<td>22°C, pH 8.2</td>
<td>96 h</td>
<td>LC50 = 11 mg/l (n)</td>
<td>Monsanto (1979a)</td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>flow-through</td>
<td>13°C, pH 7.1-8.6</td>
<td>96 h, 192 h</td>
<td>LC50 = 0.73 mg/l (e)</td>
<td>Monsanto (1981)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LC50 = 0.67 mg/l (e)</td>
<td></td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em> (Embryo-larval-test)</td>
<td>flow-through</td>
<td>21-2°C, pH 6.9-7.4</td>
<td>89 d, 192 h</td>
<td>NOEC = 0.041 mg/l (e)</td>
<td>CMA (1989d)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LOEC = 0.078 mg/l (e)</td>
<td></td>
</tr>
</tbody>
</table>

(n): nominal concentrations  (e): effective concentrations
The most reliable study available is an embryo-larval test conducted in a flow-through system (CMA, 1989d). Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed for 89 days (60 days post-hatch). The selected nominal concentrations between 24 and 380 µg MBT/l were based on the results of a preliminary flow-through test. Clear yellow-coloured diluter stock solution of 21.4 mg/l (nominal) was prepared weekly for the definitive exposure by diluting MBT with acetone. Weekly analysis of the test solution was performed, based on the measured concentrations the treatment levels were between 21 and 320 µg/l.

Observations were made on embryo viability, survival of organisms at hatch and survival and growth (wet weight and total length) of larvae after 60 days post-hatch exposure. All concentrations tested did not adversely affect embryo viability which ranged from 91 to 97% at the completion of the hatching period (test day 31). The survival of the rainbow trout embryos at hatch in any concentration tested ranged from 86% in the highest treatment level (320 µg/l) to 89% in the 21 µg/l concentration. Statistical analyses demonstrated that there were no treatment level effects for this biological endpoint. At test termination (60 day post-hatch), exposure to all tested concentration of MBT ranged from 90 to 95% and was statistically comparable to the survival of the pooled control larvae, 94%.

In the concentration tested, statistical comparison of the growth parameters indicated that larval length was the most sensitive indicator of the toxicity of MBT. The mean total length of larvae exposed to concentrations ≥ 78 µg/l was significantly reduced. Based on the larval length following 60 days post-hatch exposure to concentrations of ≥ 78 µg/l the LOEC was determined to 78 µg/l and the NOEC to 41 µg/l (CMA, 1989d).

### 3.1.2 Toxicity to Invertebrates

Static tests on the acute toxicity to *Daphnia magna* using MBT (Monsanto, 1979b) and NaMBT (Monsanto, 1978a) as test substances resulted after 48 h exposure in LC50 values of 4.1 and 8.4 mg/l, respectively. Acetone was used in the preparation of all working stock solutions. Analytical control was not performed, therefore the effect values are based on nominal concentrations.

A semistatic test on *Daphnia magna* reproduction according to OECD 202 was conducted by Bayer AG (1987). 5 concentrations of test compound with 10 daphnids (first instar less than 18 hours old) in duplicate vessels were applied. Acetone was used in the preparation of all working stock solutions. The NOEC regarding reproduction (without statistical assessment) was estimated at 0.22 mg/l (nominal concentration).

In test conducted in a flow-through system (CMA, 1989e), *Daphnia magna* were exposed for 21 days. The selected nominal concentrations between 31 and 500 µg MBT/l were based on the results of a preliminary test. Clear yellow-coloured diluter stock solution of 29 mg/l (nominal) was prepared weekly for the definitive exposure by diluting MBT with acetone (e.g. 1.46 g as active ingredient with 100 ml acetone). Weekly analysis of the test solution was performed, based on the measured concentrations the treatment levels were between 29 and 470 µg/l. This test was performed under the most valid conditions and is therefore used for the PNEC derivation.
### Table 3.2: Toxicity of MBT to Invertebrates

<table>
<thead>
<tr>
<th>Species</th>
<th>Test type</th>
<th>Test conditions</th>
<th>Exposure time</th>
<th>Effect conc.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daphnia magna</td>
<td>static 19</td>
<td>7.7-8.1</td>
<td>48 h</td>
<td>LC50 = 8.4 mg/l (n)</td>
<td>Monsanto (1978a)</td>
</tr>
<tr>
<td></td>
<td>static 20+-1</td>
<td>7.4</td>
<td>48 h</td>
<td>LC50 = 4.1 mg/l (n)</td>
<td>Monsanto (1979b)</td>
</tr>
<tr>
<td></td>
<td>semistatic 20</td>
<td></td>
<td>21 d</td>
<td>NOEC = 0.22 mg/l (n)</td>
<td>Bayer AG (1987)</td>
</tr>
<tr>
<td></td>
<td>flow-through 20+-2</td>
<td>7.5-8.2</td>
<td>21 d</td>
<td>NOEC = 0.24 mg/l (e)</td>
<td>CMA (1989e)</td>
</tr>
</tbody>
</table>

(n): nominal concentrations  
(e): effective concentrations
During the chronic exposure, control daphnids had begun to release offspring by test day 7. The time for release of first brood offspring by daphnids in the exposure solutions  \( \leq 240 \mu \text{g/l} \) occurred generally by day 7 and was not adversely affected by the concentration of MBT tested. On day 21, 58% survival was observed among daphnids at the highest concentration (470 \( \mu \text{g/l} \)). Survival at the remaining treatment levels (240 - 29 \( \mu \text{g/l} \)) ranged from 93 to 98% and was statistically comparable to the survival of the control organisms. Adult organisms in the highest treatment level (470 \( \mu \text{g/l} \)) were observed to be pale in colour, small in body size and exhibit erratic swimming behaviour. No significant sublethal effects were observed at the remaining lower levels (240 - 29 \( \mu \text{g/l} \)). Analyses of the reproduction data for this study (days 0 - 21) established that MBT did not adversely affect daphnids reproduction at concentrations which did not affect organism survival. Daphnids exposed to \( \leq 240 \mu \text{g/l} \) MBT released between 89 - 138 offspring per female which was not significantly different from the number released by the control. Based on the observed effects of MBT on daphnids survival, the NOEC was estimated to 240 \( \mu \text{g/l} \).

### 3.1.3 Toxicity to Algae

A phytotoxicity test was performed to determine the effect of MBT on the freshwater alga *Selenastrum capricornutum* (Monsanto, 1979c). Dimethylformamide (DMF) was used to prepare a primary stock solution. The calculated 96h-EC50 were 0.23 mg/l based on decrease of in vivo chlorophyll a and 0.25 mg/l referring to the growth rate. NOECs or EC10 values were not determined. Decrease of chlorophyll a was 12% at 0.1 mg/l, the cell number decreased by 8% at 0.06 mg/l. All effect values are based on nominal concentration only. Taking into account the instability of MBT against photolysis it can be assumed that after 96 hours of test duration only minimal amounts of MBT are available in the test system.

Similar experiments using a 50% aqueous solution of NaMBT resulted in 96h-EC50 values (referred as MBT) of 0.18 mg/l based on decrease of in vivo chlorophyll a and 0.13 mg/l referring to the growth rate (Monsanto, 1978b).

In both tests NOEC or EC10 values were not determined. From reported effects at lower concentrations and considering that MBT is photolysed during the tests, a NOEC of < 60 \( \mu \text{g/l} \) (nominal concentration) can be estimated.

### 3.1.4. Quantitative Structure-Activity Relationships (QSARs)

Already due to its polarity and the thiol group MBT can be assumed to cause excess ecotoxicity compared to the baseline toxicity. The metal complex building property of MBT is likely to cause enhanced ecotoxicity as well. On the other hand, MBT's ecotoxicity can be expected to decrease with increasing pH above the pH of 7 due to the increasing portion of its dissociated form, which has a higher water solubility and a lower lipophilicity.

Data on MBT or other benzothiazole derivatives have so far not been used for the development of QSAR-models. MBT's ecotoxicity was estimated with ECOSAR v0.99h (U.S.EPA, 2004), according to the classification scheme of Verhaar et al. (1992) and with the model of Pavan et al. (2005). The results are presented in Table 3.3. The predictions were calculated with the logKow of the undissociated form and with the logKow at the pH 7. The domains of the all three mentioned models are applicable for MBT.

ECOSAR v0.99h automatically allocates MBT to the group of phenols. In this group, the equation for acute algae toxicity is the same as used for the neutral organics (baseline toxicity). The equations of the program for the group of thiols/mercaptans were also used to
obtain estimates, while this group is as (or more) suitable to MBT as the group of phenols. A
third group in ECOSAR, which can be used for MBT, is the group of thiazolinones/benzothiazolines. These equations give estimates which are lower than using
the phenol-group QSARs, but higher than the results of the thiol-group models.

The second way of estimation applies the approach of Verhaar et al. (1992). According to
their guidance, MBT can be assigned to the class 3 (reactive substances). The baseline
toxicity was first calculated using appropriate QSARs for non-polar substances. For this
purpose, the models of Verhaar et al. (1995) and Van Leeuwen et al. (1992) as recommended
in the Technical Guidance Document (Part III, Section 4.1, Table 1) were used. The resulting
estimates (see Table 3.3) were divided by the “toxicity range factors” of the class 3 \( R_{T_{\text{max}}}=10^4 \) and \( R_{T_{\text{min}}}=10 \) to obtain the final toxicity range.

Finally, the acute toxicity to fish was calculated for a comparison using the model of Pavan et
al. (2005):

\[
\log L_{\text{C}50} = -0.574 \log K_{\text{ow}} + 0.454 \text{ELUMO} - 2.445,
\]

where \text{ELUMO} is the energy of the lowest unoccupied molecular orbital. The equation is an
adjusted version from the original model of Veith and Mekeneyan (1993) for “mixed” mode
of action. The model was developed for aromatic substances which are considered to act by
several modes of action including narcosis and unspecific reactivity due to electrophilic or
nucleophilic reactions.

<table>
<thead>
<tr>
<th>Table 3.3. QSAR –estimates for aquatic ecotoxicity.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Organism</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Fish ((1))</td>
</tr>
<tr>
<td>Fish ((2))</td>
</tr>
<tr>
<td>Daphnid ((1))</td>
</tr>
<tr>
<td>Daphnid ((2))</td>
</tr>
<tr>
<td>Algae ((1))</td>
</tr>
<tr>
<td>Algae ((2))</td>
</tr>
</tbody>
</table>

(1) Values calculated for the pH of 7 with the logKow of 1.4
(2) Values calculated for the undissociated form with the logKow of 2.68
(3) Result(phenol-QSAR) / result(thiols-QSAR)
(4) These QSARs are for neutral organics and they have been used as the starting point for the approach of Verhaar et
al. (1992)
(5) The acute toxicity range as the outcome of the method of Verhaar et al. (1992)
(6) The used \text{ELUMO} =-0.6 \text{ eV} (Schmegel 1995)

The acute tests indicate that the algae (96hEC50 \( \leq 0.23 \) mg/l) would be the most sensitive
species followed by fish (96hLC50 = 0.73 mg/l) and by daphnids. The QSAR-predictions for
the pH of 7 give different sensitivity orders for different QSARs. The predictions from
ECOSAR v0.99h using the phenol equations and the model of Pavan et al. seem to
underestimate the toxicity. It should be noted that on the contrary to the impression gained
from the available acute tests, none of the models have predicted daphnids to be at pH of 7 the
least sensitive group.

### 3.1.5 Determination of the PNECwater
Results from long-term tests are available for freshwater species out of 3 trophic levels. An embryo-larval test to *Oncorhynchus mykiss* carried out in a flow-through system resulted in a NOEC of 41 µg/l. From the flow-through test on *Daphnia magna* a NOEC of 240 µg/l was obtained, while for algae (*Selenastrum capricornutum*) the NOEC was estimated to < 60 µg/l (nominal concentration). Because MBT can be expected to be unstable against photolysis in the reported test conditions, the effective concentration may be significantly lower in the algae test. While PNECs are derived in this assessment separately for the main degradation products of CBS, the effect caused by MBT, not its degradation products, is searched. In addition, the acute test results indicate that algae would be the most sensitive group. Due to the considerations above an assessment factor of 50 is applied to the result from the fish test. The PNEC is determined to:

\[
PNEC_{\text{freshwater}} = \frac{41 \, \text{µg/l}}{50} = 0.82 \, \text{µg/l}
\]

The PNEC could be improved by an algae growth test with analytical control of the test substance concentration.

For the assessment of marine biota, an assessment factor of 500 is used. The PNEC is calculated to

\[
PNEC_{\text{marine}} = 0.082 \, \text{µg/l}
\]

### 3.1.6 Microorganisms

Tests on the inhibition of sludge respiration with both MBT, its sodium- and zinc-salt are available (Bayer AG, 1990a,b). In some cases, the test substance concentration exceeded the water solubility (118 mg MBT/l at pH 7). The EC10 values (referred as MBT) obtained are in the range of 111-279 mg/l. Applying an assessment factor of 10 to the lower value, the PNEC for sludge respiration is determined to 11 mg/l.

The available tests on sludge nitrification reveal that the inhibition is dependent on the adaptation (cf. Table 3.3). Tomlinson (1966) studied the inhibition of the first nitrification step (oxidation of NH₄ to NO₂) and obtained after 9 weeks exposure EC75 values of 44 mg/l for adapted sludge and 2.5 mg/l for non-adapted. For the hazard assessment values obtained from tests with adapted sludge are used, as MBT occurs exclusively in industrial waste waters and the biological treatment plants are adapted to MBT releases. Generally an EC50 value is divided by an assessment factor of 10, as only EC75 values are available a factor of 20 is chosen. Based on the EC75 of 44 mg/l, the PNEC for nitrification is determined to 2.2 mg/l.

A nitrification inhibition test was also performed with *Nitrosomas* isolated from wastewater. NH₄ removal was analysed in a model of sediment columns charged with quartz sand. Effects were found with MBT concentrations of 0.25 mg/l (Reemtsma, 1994). Because the test design does probably not reflect the conditions in a waste water treatment plant, the results are not used for the PNEC derivation.

A large number of tests to bacteria are available. Foltinova & Blöckinger (1970) tested the effect of MBT on pathogenic bacteria. Some organisms were affected by concentrations of 50 mg/l were obtained. Reemtsma (1994) examined the growth of *Vibrio fischeri* and
obtained an EC20 of 4 mg/l. For the assessment of microorganisms in treatment plant these
tests are probably less relevant, therefore they are not used for the PNEC derivation.

Table 3.4: Toxicity of MBT to Microorganisms

<table>
<thead>
<tr>
<th>Species</th>
<th>Dur.</th>
<th>Effects</th>
<th>Endpoint</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge, municipal</td>
<td>3 h</td>
<td>EC10 = 279 mg/l</td>
<td>respiration</td>
<td>Bayer AG (1990a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EC50 = 3301 mg/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activated sludge from laboratory facility</td>
<td>3 h</td>
<td>EC10 = 111 mg/l</td>
<td>respiration</td>
<td>Bayer AG (1990b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EC50 = 1020 mg/l (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activated sludge from laboratory facility</td>
<td>3 h</td>
<td>EC10 = 211 mg/l</td>
<td>respiration</td>
<td>Bayer AG (1990b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EC50 = 757 mg/l (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activated sludge, non-adapted</td>
<td>27 h</td>
<td>EC50 = 1-10 mg/l</td>
<td>nitrification</td>
<td>Baumann et al. (1998)</td>
</tr>
<tr>
<td>Activated sludge, non-adapted</td>
<td>9 w</td>
<td>EC75 = 2.5 mg/l</td>
<td>nitrification</td>
<td>Tomlinson (1966)</td>
</tr>
<tr>
<td></td>
<td>2-4 h</td>
<td>EC75 = 3 mg/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activated sludge, adapted</td>
<td>9 w</td>
<td>EC75 = 44 mg/l</td>
<td>nitrification</td>
<td>Tomlinson (1966)</td>
</tr>
<tr>
<td>Nitrosomas (isolated from wastewater)</td>
<td></td>
<td>ECxxx = 0.25 mg/l</td>
<td>Nitrification (3)</td>
<td>Reemtsma (1994, 1995)</td>
</tr>
<tr>
<td>Pseudomonas putida</td>
<td>18 h</td>
<td>EC0 &gt; 1000 mg/l</td>
<td>growth</td>
<td>Bayer AG (1990a)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>28 d</td>
<td>Part. inhib. 50 mg/l</td>
<td>growth</td>
<td>Foltinova &amp; Blöckinger (1970)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EC100 = 250 mg/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>28 d</td>
<td>Part. inhib. 50 mg/l</td>
<td>growth</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>EC100 = 250 mg/l</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium tuberculosis (H37 Rv)</td>
<td>28 d</td>
<td>EC100 = 250 / 100 mg/l (2 media)</td>
<td>growth</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium tuberculosis (H37 Rv IR⁺)</td>
<td>28 d</td>
<td>EC100 = 500 / 100 mg/l (2 media)</td>
<td>growth</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium tuberculosis (H37 Rv SR⁺)</td>
<td>28 d</td>
<td>EC100 = 500 / 250 mg/l (2 media)</td>
<td>growth</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium bovis (deficient strain of M.</td>
<td>28 d</td>
<td>EC100 = 100 / 50 mg/l (2 media)</td>
<td>growth</td>
<td></td>
</tr>
<tr>
<td>tuberculosis)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mycobacterium bovis</td>
<td>28 d</td>
<td>EC100 = 250 / 100 mg/l (2 media)</td>
<td>growth</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium avium</td>
<td>28 d</td>
<td>EC100 = 500 / 250 mg/l (2 media)</td>
<td>growth</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium kansaii</td>
<td>28 d</td>
<td>EC100 = 500 / 250 mg/l (2 media)</td>
<td>growth</td>
<td></td>
</tr>
<tr>
<td>Mycobacterium phlei</td>
<td>28 d</td>
<td>EC100 &gt; 1000 / &gt; 500 mg/l (2 media)</td>
<td>growth</td>
<td></td>
</tr>
<tr>
<td>Vibrio fischer</td>
<td>20 h</td>
<td>EC20 = 4 mg/l</td>
<td>growth</td>
<td>Reemtsma (1994, 1995)</td>
</tr>
<tr>
<td>Tetrahymena pyriformis</td>
<td>24 h</td>
<td>EC50 = 10 mg/l</td>
<td>growth</td>
<td>Yoshioka et al. (1985)</td>
</tr>
</tbody>
</table>

All effects refer to nominal concentrations
(1): Test substance: Zn-MBT (values referred as MBT)
(2): Test substance: Na-MBT (values referred as MBT)
(3): Test conducted in a sediment column
A growth inhibition tests for the protozoa *Tetrahymena pyriformis* resulted in an EC50 of 10 mg/l. The organisms were cultivated at 30°C for 24 h without agitation, then the number of cells was counted (Yoshioka et al., 1985). Applying an assessment factor of 10, the PNEC for protozoa is determined to 1.0 mg/l.

For the assessment of microorganisms in biological treatment plants, the lowest PNEC (obtained from the test to the protozoa *Tetrahymena pyriformis*) is selected:

PNEC_{microorg.} = 1.0 \text{ mg/l}

### 3.2 Terrestrial Compartment

Bremner & Krogmeier (1989) examined the influence of MBT on seeds germination of 7 different plants. The seeds of alfalfa (*Medicago sativa*), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*), oats (*Avena sativa*) and corn (*Zea mays*) were placed into two soils with an organic carbon content of 3.3 and 6.6% and kept in the dark at 20°C for 9 days. Radicle or root emergence were not affected by the three applied MBT concentrations (12.5, 125 and 625 mg/kg air-dried soil).

Only one reliable laboratory test to terrestrial organisms is available. As no effects were observed, a PNEC for terrestrial organisms cannot be determined from the test. Applying the equilibrium partitioning approach according to the Technical Guidance Documents, a PNEC_{soil} of 0.0228 mg/kg wwt is calculated based on a Koc of 1570 l/kg and a PNEC_{water} of 0.82 µg/l.
4. Summary

MBT is a High Production Volume Chemical, 38000 t MBT were manufactured within Western Europe in 1993. The majority of the production amount is processed to benzothiazole sulphenamides and 2,2'-dithiobis-benzothiazole (MBTS) which are exclusively used as vulcanization accelerators by rubber and tire industry. Minor amounts of MBT (and its salts) are used as vulcanization accelerators without further processing.

During production and processing, MBT is released into the waste water of chemical facilities where it was measured in concentrations up to 0.47 mg/l. MBT is a breakdown product of the benzothiazole sulphenamides and MBTS, consequently it was frequently measured in rubber and tire extracts or eluates. In highway runoff concentrations of 0.1-0.3 µg/l were recently measured. In addition, MBT is present in the influents and effluents of municipal treatment plants. Average effluent concentrations up to ca 0.02 µg/l have been detected.

The available data on MBT biodegradation reveal that mineralization occurs only when the inoculi are adapted under special conditions. Such processes are expected in biological treatment plants, but not in the environment. It can be concluded from the degradation tests as well as from the available monitoring data that MBT removal in waste water treatment plants is not complete. In addition to MBT, releases of the products of primary transformation are expected. Intermediates or products of MBT biodegradation are: 2-benzothiazolesulfonic acid (BTSO3H), 2-benzothiazolone (BTon), 2-methylthiobenzothiazole (MeSBT). At higher concentrations (above 75-100 mg/l), 2,2'-dithiobis-benzothiazole (MBTS) as a product of abiotic oxidation is formed, which is mainly adsorbed onto sludge.

When released into surface waters, photolysis of MBT could be an important process leading to 2-benzothiazolone (BTon) and benzothiazole (BT) as the products.

In the environment, MBT is distributed mainly into the hydrosphere. Experimentally determined Koc values in the range of 326 to 1829 l/kg indicate that adsorption onto soil or sediment solids occurs.

Experimentally determined BCFs of <8 indicate a low bioaccumulation potential to fish.

The ecotoxicity of MBT was determined in long-term tests to three trophic levels. An embryo-larval test to Oncorhynchus mykiss carried out in a flow-through system resulted in a NOEC of 41 µg/l. From the flow-through test on Daphnia magna a NOEC of 240 µg/l was obtained, while for algae (Selenastrum capricornutum) the NOEC was < 60 µg/l (nominal concentration). Using the result from the fish test and an assessment factor of 50, the PNEC is determined to 0.82 µg/l.

For the assessment of microorganisms in biological treatment plants, the PNEC (obtained from a test to the protozoa Tetrahymena pyriformis) is determined to 1.0 mg/l.

Risk characterisation for measured exposure of MBT is presented in the main report.
5. References


Bayer AG (1987). Interne Untersuchung zur chronischen Daphnientoxizität


Bayer AG (1990b) [Interne Untersuchung der Bakterientoxizität von Vulkazit ZM (Werk Leverkusen, Institut für Umweltanalyse und Bewertungen)] unpublished


CMA (1989b). Chemical Manufacturer’s Association, 2-Mercaptobenzothiazole - Determination of the aerobic aquatic degradation following TSCA Test Guideline § 796-3100, SLI Report 89-9-3087


CMA (1989d). Chemical Manufacturer’s Association, 2-Mercaptobenzothiazole – The Toxicity to Rainbow Trout During an Early Life-Stage Exposure

CMA (1989e). Chemical Manufacturer’s Association, 2-Mercaptobenzothiazole – Chronic Toxicity to Daphnia magna under flow-through conditions


Foltinova P and Blöckinger G (1970). Biologia (Bratislava) 25, 175-180


Kaniwa et al. (1994): Contact Dermatitis 30(1), 26-34


Monsanto (1978a). Acute Toxicity of NaMBT (50%) to Daphnia magna. Report No. Microfiche No. OTS 84003A

Monsanto (1978b). Toxicity of NaMBT (50%) to the Freshwater Alga Selenastrum Capricornutum. Report No. BP-78-9-156


Monsanto (1979c). Toxicity of Thiotax to the Freshwater Alga Selenastrum Capricornutum. Report No. BP-79-7-107

Monsanto (1980a). Selected Environmental Fate Studies on nine Chemical Compounds. SRI Project No. 8669, prepared by Chou et al.


Monsanto (1992). Environmental persistence screening of selected rubber chemicals, Mo-92-9056


Shinohara et al. (1978). Bunseki Kagaku 27, 716-722


APPENDIX B: 2,2’-DITHIO-BIS-BENZOTHIAZOLE (MBTS)

1.1 Identification of the substance

Synonyms: Dibenzothiazole disulfide
Mercaptobenzothiazole disulfide
MBTS

CAS No.: 120-78-5

Empirical Formula: C_{14}H_{8}N_{2}S_{4}

Molecular weight: 332.42 g/mol

Structural Formula:

1.2 Physico-Chemical Data

Table 1.1: Physico-Chemical Data of MBTS

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>168°C</td>
<td>Keith &amp; Walters (1987)</td>
</tr>
<tr>
<td></td>
<td>&gt; 169°C</td>
<td>Bayer AG (1993a)</td>
</tr>
<tr>
<td></td>
<td>180°C</td>
<td>Windholz (1983)</td>
</tr>
<tr>
<td>Boiling point decomposition</td>
<td></td>
<td>Keith &amp; Walters (1987)</td>
</tr>
<tr>
<td>Solubility in water</td>
<td>&lt; 0.2 mg/l (20°C)</td>
<td>Bayer AG (1993a)</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>5.97·10^{-8} Pa (20°C)</td>
<td>Bayer AG (1992a)</td>
</tr>
<tr>
<td>Log Kow (measured)</td>
<td>4.5</td>
<td>Monsanto (1980)</td>
</tr>
</tbody>
</table>
2. Exposure

2.1 Production, Use and Formation

MBTS is industrially produced by oxidation of an aqueous solution of 2-mercaptobenzothiazole sodium salt (NaMBT) with chlorine, hydrogen peroxide, or atmospheric oxygen (GDCh, 1993).

MBTS is used exclusively as vulcanization accelerator in rubber industry, the demand in Western Europe was 5000 t in 1993 (Stour, 1994). The compound is added to the rubber mixtures in concentrations of 0.05-2% w/w. Of the total amount, about 30% were processed in tire production, and about 70% in the manufacture of technical rubber goods (packings, cable coverings, bicycle tires, tubes, handles, parts of home appliance, straps, conveying belts, transportation rollers, plugs, shoes). MBTS employed in tire production is used in the "inner liner". The inner liner hermetically seals the tire from within and protects it against moisture (GDCh, 1993).

When benzothiazole sulphenamides are used as accelerators, MBTS can be formed as an intermediate in an early phase of the vulcanization process. In subsequent reaction steps MBTS breaks down to a large extent. In a laboratory vulcanization test, residual MBTS contents of 0-40% (average ca. 10%) were found with regard to the sulphenamides used and 0-49% (average ca. 12%) with regard to the MBTS intermediately formed (Hann et al., 1991).

In aqueous solutions MBTS can be formed by oxidation of 2-mercaptobenzothiazole (MBT). The equilibrium between MBT and MBTS is largely influenced by concentration and the redox status of the medium (cf. the study of Hansson & Agrup 1993, cited below). Oxidation of MBT to MBTS is only expected when two MBT molecules meet, i.e. the MBT concentration is high enough. In waste waters this reaction was observed at concentrations above 75-100 mg/l, but not at environmental levels (cf. appendix A MBT).

2.2 Sources of Exposure

Production

As MBTS is produced in aqueous solutions, releases of MBTS containing waste waters into the sewage are expected. Because of the instability of the substance and the high adsorption onto sludge (see below) MBTS releases into the hydrosphere are unlikely, however releases of the breakdown products are expected.

MBTS is a breakdown product of CBS. Therefore the CBS-production sites A and B have measured MBTS in the treatment plant effluent. The results and Clocals derived for water are presented in the table below.

Table 2.1. WWTP effluent and resulting local concentrations of the CBS production plants.

<table>
<thead>
<tr>
<th>Site</th>
<th>C_{effluent} [µg/l]</th>
<th>C_{local water} [µg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt;20</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>B</td>
<td>106</td>
<td>0.1</td>
</tr>
<tr>
<td>C</td>
<td>&lt;10</td>
<td>&lt;0.026</td>
</tr>
</tbody>
</table>
Rubber Industry

MBTS is used as vulcanization accelerator in rubber goods manufacture. The substance is dosed in concentrations of 0.05-2.0% (w/w) to the caoutchouc (GDCh, 1993). During vulcanization (achieved at temperatures between 150 and 200°C) the unstable S-S link of MBTS is split and during a complex reaction sequence the rubber molecules are vulcanized with the formation MBT and other products. MBTS is also an intermediate decomposition product of N-cyclohexylbenzothiazol-2-sulphenamide (CBS). The vulcanization process is further described in the CBS report section 2.3.

Within the rubber industry releases into waste water occur in parts. The emission scenario document on additives in rubber industry states that vulcanisation accelerators are not released into waste water. In addition, relevant emissions of MBTS to air are not expected due to a very low vapour pressure.

Releases from Rubber Goods

MBTS is a content of rubber goods, in 4 shoes concentrations of 180-420 mg/kg were detected (cf. table A2.2). Releases from rubber goods into the environment occur due to tire tread abrasion and leaching by water. Because of the instability of MBTS under environmental conditions, the breakdown products instead of the parent substance are released. Consequently, the breakdown products but not MBTS were detected in environmental samples.

Table 2.2: Measured data of MBTS in Rubber Products and in Environmental Samples

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rubber shoes</td>
<td>180-420 mg/kg</td>
<td>4</td>
<td></td>
<td>Kaniwa et al. (1994)</td>
</tr>
<tr>
<td>Water</td>
<td>not detected (dl = 0.5 µg/l)</td>
<td>12</td>
<td>Sampled in Japan, “water” not further characterized</td>
<td>JETOC (1993)</td>
</tr>
<tr>
<td>Sediment</td>
<td>not detected (dl = 0.05-0.17 mg/kg)</td>
<td>12</td>
<td>Sampled in Japan</td>
<td>JETOC (1993)</td>
</tr>
</tbody>
</table>

2.3 Degradation

Biodegradation

In a MITI II test on inherent biodegradability no oxygen uptake was observed during 28 days of incubation (Bayer AG, 1988a). In a manometric respirometry test according to EEC 79/831, the oxygen uptake was 2% of the theoretical amount. The inoculum was adapted to MBTS for 4 weeks before the test was started (Bayer AG, 1992b).

BIOWIN v4.02 predicts that MBTS is not readily biodegradable but not persistent to biodegradation, either. Following estimates are provided: BIOWIN2 = 0.15, BIOWIN3 = 2.46 and BIOWIN6 = 0.002.
Abiotic Degradation

Hansson & Agrup (1993) analyzed test solutions containing 500 µM (170 mg/l) MBTS in 0.5 M phosphate buffer and 10% tetrahydrofuran at pH 6.5. After 2 hours reaction time a sediment of MBTS was formed and only trace amounts of MBTS were converted to MBT. After addition of the reducing agent glutathione, MBT was formed quantitatively within 10 minutes. When the same experiment was run with a MBT solution, 60% were converted to MBTS without glutathione after 2 hours, while after glutathione addition MBT was stable. The results reveal that the equilibrium between MBT and MBTS is largely influenced by the redox status of the medium.

The dependency of MBTS hydrolysis on temperature, pH and particle size was studied by Monsanto (1984). A slurry of 1650 mg MBTS/l in 1M phosphate buffer treated at a pH of 9.8 and a temperature of 58°C resulted after 65 h in a hydrolysis of 6.7%, with an MBT formation of 4.75%. Benzothiazole sulfinic acid and benzothiazole sulfonic acid were detected as further reaction products. While MBT was oxidized back to MBTS, the acids were further hydrolyzed to benzothiazole (BT) and benzothiazolone (BTon). In summary, MBTS is transformed by a combination of hydrolysis and oxidation to BT and BTon. The rate of hydrolysis increases with rising temperature, rising pH and decreasing particle size.

The UV spectrum of MBTS indicates that photodegradation under environmental conditions is possible. In a photolysis screening test, a 0.9 mg/l solution in water containing 10% acetonitril as a cosolvent was exposed to sunlight at midday in August. A half-life of 3 hours 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). A control kept in the dark at 24°C had a half-life of 19 hours, while a dark control in ice showed no transformation. In both experiments, three transformation products were detected by HPLC but not identified (Monsanto, 1980).

Conclusions

The available tests on inherent biodegradability reveal that MBTS is not mineralized. Instead of biodegradation, hydrolysis is expected. The fact that no oxygen uptake was observed can be explained by the poor degradability of the hydrolysis products (cf. the appendices I MBT, III BT, IV BTon).

It can be concluded from the dark control of the Monsanto (1980) photolysis study that under environmental conditions MBTS in aqueous solutions is hydrolysed within a few days. Because of the poor water solubility, the reaction rate in slurries is largely decreased. In concentrations levels relevant for environmental exposure, MBT is expected to be a product, as the oxidation back to MBTS can be excluded. End products of MBTS degradation are BT and BTon. MBTS degradation is accelerated by sunlight, probably the same products are formed.

2.4 Distribution

The distribution of MBTS between aqueous solution and air is described by the Henry’s law constant. From water solubility and vapour pressure, a value of $< 9.9 \times 10^{-9}$ Pa.m$^3$/mol is calculated, indicating that the substance is not volatile from aqueous solutions.
The distribution between the organic phase of soil or sediment solids and porewater can be calculated from the octanol/water partitioning coefficient. Using a log Kow of 4.5 and the equation $\log K_{oc} = 0.52 \log Pow + 1.02$, a $K_{oc}$ value of 2290 l/kg is obtained, indicating a high sorption potential. PCKOCWIN v1.66 calculates a $K_{oc}$ of 755 000.

The distribution of MBTS in a "unit world" is calculated according to the Mackay fugacity model level I, considering the values for vapour pressure ($5.97 \times 10^{-8}$ Pa), log Pow (4.5) and water solubility (0.2 mg/l). The main target compartments are estimated to be water with 17.1%, 42.8% for soil and 40.0% for sediment.

2.5 Accumulation

Using the log Kow of 4.5 a BCF of 1330 l/kg results from the QSAR of Veith et al. (1979) whereas BCFWIN 2.15 gives a BCF of 582 l/kg.

In contrast, available experimental values are considerably lower. The bioconcentration in fish (Cyprinus carpio) was determined in a test according to OECD guideline 305 C. BCFs in the range of 1.0-7.2 l/kg with 0.2 mg MBTS/l resp. <1.4-51 l/kg with 0.02 mg MBTS/l were reported (MITI, 1992). Only a general description of the test procedure is given without mentioning experimental details. Furthermore, the high variation of results is not discussed in the test protocol. Due to the fast hydrolysis of MBTS it can be assumed that the test data are closer to the reality than the QSAR-results above. MBTS is not expected to accumulate in the food chain.
3. Effects Assessment
3.1 Aquatic Compartment

General

For the effects assessment of MBTS on aquatic organisms it has to be taken into account that the MBTS concentrations tested were far above water solubility (< 0.2 mg/l). Even with the use of co-solvent the test material was observed to precipitate in the test chambers. It is expected that only a small fraction of the applied test substance was dissolved. In section 2.3 it was concluded that dissolved MBTS is hydrolyzed rapidly.

If the present quality criteria are strictly applied, the static acute tests, where no monitoring of the test concentrations occurred, should be regarded as not valid. The main degradation products appearing during the time frame of acute tests are benzothiazole (BT) and benzothiazolone (BTon). BT and BTon are roughly one order of magnitude less toxic than MBTS (see Appendices III and IV). Thus the results of the static tests with nominal concentrations can be considered as the upper limit for the actual effect value.

Bayer AG (1993b) conducted a test to *Daphnia magna* with analytical monitoring of the test substance. At a nominal concentration of 1000 mg/l, the substance could not be detected in the filtrated medium with a detection limit of 0.1 mg/l. It can be concluded that the observed effects are caused either by suspended MBTS, or its water-soluble impurities, or by MBTS degradation products.

Quantitative Structure-Activity Relationships (QSARs)

Due to the unstable sulphur-sulphur bond MBTS can be expected to be unselectively reactive in the target organism. Thus excess ecotoxicity compared to the baseline toxicity could be expected. The high logKow of 4.5 indicates also high toxicity.

Data on MBTS or other benzothiazole derivatives have so far not been used for the development of QSAR-models. The ecotoxicity of MBTS was estimated with ECOSAR v0.99h (U.S.EPA, 2004), according to the classification scheme of Verhaar et al. (1992) and with the model of Pavan et al. (2005). The results are presented in Table 3.1. The domains of the all three mentioned models are applicable for MBTS.

ECOSAR v0.99h allocates MBTS to the group of neutral organics. Groups included in ECOSAR with structural similarities are benzothiazolines and mercaptans.

The second way of estimation applies the approach of Verhaar et al. (1992). According to their guidance, MBTS can be assigned to the class 3 (reactive substances). To this conclusion for MBTS came already Bol, et al. (1993). The baseline toxicity was first calculated using appropriate QSARs for non-polar substances. For this purpose, the models of Verhaar et al. (1995) and Van Leeuwen et al. (1992) as recommended in the Technical Guidance Document (Part III, Section 4.1, Table 1) were used. The resulting estimates (see Table 3.1) were divided by the “toxicity range factors” of the class 3 (RF_{max} = 10^4 and RF_{min} = 10) to obtain the final toxicity range (see Table 3.1).

Finally, the acute toxicity to fish was calculated for a comparison using the model of Pavan et al. (2005):

\[
\text{LogLC}_{50} = - 0.574 \times \log \text{Kow} + 0.454 \times \text{ELUMO} - 2.445,
\]
where $ELUMO$ is the energy of the lowest unoccupied molecular orbital. The equation is an adjusted version from the original model of Veith and Mekeneyan (1993) for “mixed” mode of action. The model was developed for aromatic substances which are considered to act by several modes of action including narcosis and unspecific reactivity due to electrophilic or nucleophilic reactions.

Table 3.1. QSAR –estimates for aquatic ecotoxicity.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Acute $L(E)C50$ (mg/l)</th>
<th>Chronic $L(E)C50$ (mg/l)</th>
<th>Acute $L(E)C_{min}-L(E)C_{max}$ (µg/l)</th>
<th>Pavan et al. (2005)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>1.1</td>
<td>0.2</td>
<td>2.0</td>
<td>0.2 – 200</td>
</tr>
<tr>
<td>Daphnid</td>
<td>1.4</td>
<td>0.2</td>
<td>0.8</td>
<td>0.08 – 80</td>
</tr>
<tr>
<td>Algae</td>
<td>1</td>
<td>0.4</td>
<td>0.6</td>
<td>0.06 – 60</td>
</tr>
</tbody>
</table>

(1) These QSARs are for neutral organics and they have been used as the starting point for the approach of Verhaar et al. (1992)

(2) The acute toxicity range as the outcome of the method of Verhaar et al. (1992)

(3) The used $ELUMO = -0.86eV$ (Schmegel 1995)

The acute tests indicate that the algae (96hEC50 = 0.6 mg/l) would be the most sensitive species. The baseline toxicity predictions for fish and daphnids are lower than the test results and close to the predictions for algae.

Derivation of PNEC for aquatic environment

An overview about the available effect tests is presented in table 3.1. There are tests on the acute toxicity to freshwater species out of 3 trophic levels available. The lowest observed effect value is the ECµ50 of 0.6 mg/l from a growth inhibition test to the algae Selenastrum capricornutum. Using an assessment factor of 1000, the PNEC is determined to

$$\text{PNEC}_{\text{freshwater}} = \frac{600 \, \mu g/l}{1000} = 0.6 \, \mu g/l$$

It should be kept in mind that the determined PNEC value probably refers either to suspended MBTS, or its water-soluble impurities, or to MBTS degradation products.

For the assessment of marine biota, an assessment factor of 10000 is used. The PNEC is calculated to

$$\text{PNEC}_{\text{marine}} = 0.06 \, \mu g/l$$
Tab. 3.1: Toxicity of MBTS to Aquatic Organisms

<table>
<thead>
<tr>
<th>Species</th>
<th>Test type</th>
<th>Exposure time</th>
<th>Effect conc.</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pimephales promelas</td>
<td>static</td>
<td>96 h</td>
<td>LC₅₀ &gt; 1000 mg/l (n)</td>
<td>DMF used as co-solvent</td>
<td>Monsanto (1979a)</td>
</tr>
<tr>
<td>Oryzias latipes</td>
<td>static or semi-static</td>
<td>48 h</td>
<td>LC₅₀ = 19 mg/l (n)</td>
<td>DMF used as co-solvent</td>
<td>MITI (1992)</td>
</tr>
<tr>
<td>Daphnids</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daphnia magna</td>
<td>static</td>
<td>48 h</td>
<td>LC₅₀ = 82 mg/l (n)</td>
<td>DMF used as co-solvent</td>
<td>Monsanto (1979b)</td>
</tr>
<tr>
<td></td>
<td>static</td>
<td>48 h</td>
<td>LC₅₀ = 211 mg/l (n)</td>
<td>no co-solvent used</td>
<td>Bayer AG (1993b)</td>
</tr>
<tr>
<td>Algae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selenastrum capricornutum</td>
<td>static</td>
<td>96 h</td>
<td>EC₅₀ = 0.6 mg/l (n)</td>
<td>DMF used as co-solvent</td>
<td>Monsanto (1979c)</td>
</tr>
<tr>
<td>Scenedesmus subspicatus</td>
<td>static</td>
<td>72 h</td>
<td>EC₅₀ &gt; 40 mg/l (n)</td>
<td>no co-solvent used</td>
<td>Bayer AG (1992c)</td>
</tr>
</tbody>
</table>

All referred concentrations are nominal
Microorganisms

In a respiration inhibition test using activated sludge similar to OECD 209, no effects were observed in concentrations up to 10 g/l after 3 h of incubation (Bayer AG, 1988b). Applying an assessment factor of 10, the PNEC for sludge respiration inhibition is determined to be >1 g/l.

Tomlinson (1966) studied the inhibition of the first nitrification step (oxidation of NH₄ to NO₂) and obtained a 4 h-EC75 value of 38 mg/l. Generally an EC50 value is divided by an assessment factor of 10, as only EC75 values are available a factor of 20 is chosen. The PNEC for nitrification is determined to 1.9 mg/l.

For the assessment of microorganisms in biological treatment plants, the lowest PNEC is selected:

PNECmicroorg. = 1.9 mg/l

As well as the aquatic PNEC, the determined PNEC for microorganisms probably refers either to suspended MBTS, or its water-soluble impurities, or to MBTS degradation products.
4. Summary

MBTS is a High Production Volume Chemical, 5000 t MBTS were consumed within Western Europe in 1993. The substance is exclusively used as vulcanization accelerator by rubber and tire industry.

As MBTS is produced in aqueous solutions, releases of MBTS containing waste waters into the sewage are possible. Because of the instability of the substance and the expected high adsorption onto sludge MBTS releases into the hydrosphere are likely to be small, however releases of the breakdown products are expected.

During vulcanization MBTS decomposes almost completely to MBT and other products. Releases of MBTS into waste water are not expected according to the emissions scenario document for rubber industry. Emissions of the more volatile breakdown products to air may occur. MBTS was detected in rubber goods. Releases into the environment are expected due to tire tread abrasion and leaching by water. Because of the instability of MBTS under environmental conditions, the breakdown products instead of the parent substance are released. Consequently, the breakdown products but not MBTS were detected in environmental samples.

The available data on biodegradation reveal that MBTS is not mineralizable. Instead of biodegradation, hydrolysis and photolysis occur with expected half-lives of several days. The calculated log Kow indicates a high adsorption and accumulation potential.

The ecotoxicity of MBTS was determined in short-term tests to three trophic levels. For the effects assessment of MBTS on aquatic organisms it has to be taken into account that the MBTS concentrations tested were far above water solubility (< 0.2 mg/l). The lowest observed effect value was the 96h-ECµ50 of 0.6 mg/l from a growth inhibition test to the algae Selenastrum capricornutum. Using an assessment factor of 1000, the PNEC is determined to 0.6 µg/l. From a test on inhibition of nitrification a 4 h-EC75 value of 38 mg/l was obtained. Using an assessment factor of 20, the PNEC for microorganisms is determined to 1.9 mg/l. Both values probably refer either to suspended MBTS, or its water-soluble impurities, or to MBTS degradation products.

As relevant releases of MBTS into the environment are not expected, a risk for ecosystems can be excluded. However, releases of breakdown products are expected both during production, use by rubber industry and via tire tread abrasion. The same breakdown products occur during production and use of benzothiazole sulphenamides. Possible risks occurring from those compounds are assessed in the risk assessment report of N-Cyclohexyl-benzothiazole-2-sulphenamide (CBS).

The risk characterisation for measured exposure concentrations is presented in the main report.
5. References

Bayer AG (1988a). MITI (Abbau)-Test nach Dr. Painter, unpublished report
Hann et al. (1991). Vulcanization Chemistry; Comparison of the new Accelerator N-t-Butyl-Benzothiazole Sulfenamide (TBSI) with N-t-Butyl-2-Benzothiazole Sulfenamide (TBBS). Presented at a meeting of the Rubber Division, American Chemical Society, Detroit, Michigan, October 8-11, 1991
Hansson C and Agrup G (1993). Stability of the mercapto benzothiazole compounds. Contact Dermatitis 28, 29-34
Kaniwa et al. (1994). Contact Dermatitis 30(1), 26-34
MITI (1992). Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan, Compiled under the Supervision of Chemical Products Safety Division, Basic Industries Bureau MITI, Ed. by CITI, October 1992
Monsanto (1979c). Toxicity of Thiofide to the Freshwater Alga Selenastrum Capricornutum. Report No. BP-79-7-105
Monsanto (1980). Selected Environmental Fate Studies on nine Chemical Compounds. SRI Project No. 8669, prepared by Chou et al.
Monsanto (1984). Thiofide Hydrolysis. DOC ID. 87821067


APPENDIX C: BENZOTHIAZOLE (BT)

1.1 Identification of the substance

Name: Benzothiazole
Synonyms: 1-Thia-3-azaindene
Benzosulfonazole
CAS No.: 95-16-9
Empirical Formula: C₇H₅NS
Molecular weight: 135.19
Structural Formula:

1.2 Physico-Chemical Data

Table 1.1: Physico-Chemical Data of Benzothiazole

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>2°C</td>
<td>Weast (1979)</td>
</tr>
<tr>
<td>Boiling point</td>
<td>227-228°C, 231°C</td>
<td>Brownlee et al. (1981), Weast (1979)</td>
</tr>
<tr>
<td>Solubility in water</td>
<td>3000 mg/l at 24°C, 3590</td>
<td>Brownlee et al. (1992), Monsanto (1978)</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>0.14 hPa</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>Log Kow (measured)</td>
<td>1.99 (24°C), 2.04, 2.03, 1.94</td>
<td>Brownlee et al. (1992), Brownlee et al. (1981), Brownlee et al. (1981), Monsanto (1978)</td>
</tr>
</tbody>
</table>

2. Exposure

2.1 Production, Use and Formation

Benzothiazole is produced by chemical industry. According to the public IUCLID from the year 2000, three European companies have notified the substance. The substance is according to the notifiers exclusively used as an intermediate in chemical synthesis. There is no information available about the subsequent technical product(s) and about releases.

According to WHO (e.g., WHO 2002) BT is used as flavour in food and foodstuff. In addition, BT is included in the list of food flavours of the EU (Commission Decision 27.3.1999).
Benzothiazole (BT) was found to be formed during vulcanization in rubber goods manufacture. According to Eholzer & Kempermann (1983) the precursors are 2-mercaptobenzothiazole (MBT), its zinc salt, 2,2’-dithiobis-benzothiazole (MBTS) or benzothiazole sulphenamides which are used as vulcanization accelerators. Measurements of the BT content in rubber and tires are presented in Table 2.4. BT was detected in the exhaust gas of laboratory vulcanization experiments (Hilton & Altenau, 1973; Fraser & Rappaport, 1976, Levin, 1994).

A further formation pathway is the photolytical degradation of 2-mercaptobenzothiazole (MBT): Brownlee et al. (1992) found BT as the main photolysis product of a MBT solution in natural water. The authors conclude that BT beside minor amounts of 2-benzothiazolone (BTon) were stable products of MBT photolysis in aquatic environments.

BT was identified as the main product of hydrolysis and photolysis of N-cyclohexyl-benzothiazole-2-sulphenamide (CBS). The studies are described in section 3.1.1.2.2 of the CBS report. Because of the relative low release amounts of CBS, this source is of smaller importance than releases from vulcanisation process or from rubber products.

Seifert and King Jr., (1982) found the fungi Aspergillus clavatus to form benzothiazole. Gallois et al. (1990) measured BT in the culture media of 2 strains of the fungi Polyporus frondosus in a concentration range of 0-50 µg/l. In the media of further 27 fungi the compound was not found. The authors assume that BT is formed as a cleavage product of thiamine. Seeds, germinating seeds and roots of a tropic legume Zapoteca formosa were confirmed to release benzothiazole (Lane et al., 2004). Several studies (see table 2.7) have identified benzothiazole in food like endive (Götz-Schmidt and Schreier, 1986), mangos (Engel and Tressl, 1983) and black tea (Vitzthum et al., 1975). These studies were aimed at to find naturally formed (aroma) compounds and therefore it was not further discussed whether the substances found actually were of natural or of anthropogenic origin. Based on the studies above, natural formation of benzothiazole seems to occur. This formation cannot be quantified but it can be concluded that natural formation does not affect the local scenarios presented in the assessment.

2.2 Degradation

Biodegradation

The biodegradation of benzothiazole (BT) has been determined in two OECD standard tests:

In the OECD 301 D (closed bottle test), a concentration of 0.8 mg of BT was examined. A degradation of > 65% after 21 days is reported (Bayer AG, 1990). The test is poorly documented, and a final judgement about the validity is not possible.

In the MITI Test (OECD 301 C), a concentration of 100 mg of BT was examined. The inoculum concentration was 30 mg/l, degradation was monitored by biological oxygen demand (BOD) analysis, as prescribed in the guideline. Accordingly, BT was degraded at 0% within a period of 28 days (MITI, 1992).

Based on these contradictory results from two OECD standard tests, no conclusion in regard to biodegradability can be made and other results of publications and non standardized studies are assessed for biodegradation properties of BT.

In 1976, degradation of BT was reported by manometric determination of O₂ consumption. The inoculum used was industrial and adapted to benzothiazole-2-sulphonic acid and a mixed culture isolated from the adapted sludge. A concentration of 1 µmol of BT was tested in a
final volume of 3 ml. The volumetric O₂ consumption corresponds to a biodegradation of 57% and 48% within three hours according to the two different inocula can be calculated (Mainprize et al., 1976).

An aerobic degradation test of BT (2 litre batch systems with the addition of 115 mg/l yeast extract as an additional carbon source according to ISO 9888) revealed that at an initial concentration of 9.7 mg/l, BT was completely degraded after a 8 day lag phase and within another 8 days. As this degradation was monitored by HPLC analysis, it is possible that only primary degradation was shown. Moreover, the degradation pathway could not be determined. In a pilot treatment plant fed with wastewater from two tanneries, it was found that BT is formed from unknown precursors in the anaerobic treatment; by aerobic treatment, BT again was substantially eliminated. Transformation of BT and related benzothiazoles was assessed also on model sediment columns at significantly lower concentrations simulating the mixture of surface water with industrial effluent (13-27 µg/l). BT was not detectable in the effluent of the columns, and as BT exhibits a low Kow value, adsorption to the column is unlikely. Moreover, extraction of the upper 50 g of the column material did not provide any BT (Reemtsma, 1994; Reemtsma et al. 1995).

DeWever and Verachtert published results of the influence of 2-mercaptobenzothiazole (MBT) on BT degradation in laboratory fed batch systems. The activated sludge used as inoculum was a mixture of adapted and non adapted sludge. The sludge samples were taken from rubber chemicals wastewater treatment plants. The inoculum concentration was very high, 3-5 g/l and the exposure time 61 days. To study the effects on BT degradation, the reactors were fed in pulses to obtain MBT and BT concentrations of 50 up to 150 mg/l, respectively. As a result, both compounds disappeared quite efficiently. At higher BT concentrations, BT degradation was slower (DeWever and Verachtert, 1994). Again, as BT degradation was monitored by UV spectrometry and HPLC analysis, and no details are given on degradation products, it is assumed that no ultimate mineralization has been taking place.

De Vos et al. (1993) examined as well parameters which affect the degradation of benzothiazoles in activated sludge systems. By complex feeding procedures to adapted sludge, (BT and MBT were added at different time, at different concentrations, two different industrial sludges were further adapted to MBT, BT and BTon), it was shown that BT at 2 mg/l was completely degraded. The exposure time was up to 100 days. BT degradation was monitored by UV spectrometry and HPLC analysis, and no details are given on degradation products, therefore, this degradation is in fact again „disappearance“ only, and no mineralization half lives can be deduced.

Gaja & Knapp (1997) isolated a strain of Rhodococcus capable of growing on BT from a treatment plant of a facility manufacturing benzothiazole based rubber additives. In an oxygen uptake test using Rhodococcus previously grown on BT, 39% of the theoretical O₂ amount were taken up during 4-5 hours. When activated sludge from 2 municipal treatment plants receiving domestic and industrial waste waters was used, BT was degraded completely in 15-20 days after a lag period of about 5 days. In river water samples, no degradation occurred over a 101 day period.

To simulate realistic environmental conditions, BT (15 mg/l) was spiked into water collected in September from a highway settling pond. After 8 days of incubation 60% of the BT had transformed into 2-benzothiazolone (BTon). In water collected in November and December, however, BT disappeared completely and only traces of BTon could be detected. The authors assume that these different results may due to different bacteria populations or other seasonal factors like road salts (Reddy & Quinn, 1997). BTon was also found to be an intermediate of
BT degradation in a laboratory test. Benzothiazole-degrading bacteria isolated from sludge obtained from a plant treating waste-waters from rubber chemical production and from a lab-scale reactor fed with benzothiazoles were capable to transform BT to BTon, which was further metabolized (De Wever et al., 1998).

Brownlee et al. (1992) carried out field studies with a creek receiving an input of variable quantities of BT and other benzothiazole derivatives. Monthly sampling of the sediment at 6-7 different sampling sites were all collected on the same day, and frozen prior use. The extracts were analysed by GC. BT showed very good first order disappearance kinetics corresponding to half lives of 3 hours. This field results are interpreted that BT can be quite rapidly removed in adapted sediment probably by a combination of biodegradation and volatilisation.

BIOWIN v4.02 predicts that the substance is not readily biodegradable but on the other hand not likely to be persistent to biodegradation, either. The following estimates for screening of persistency are provided: BIOWIN2 = 0.75, BIOWIN3 = 2.9 and BIOWIN6 = 0.32.

Photolysis

BT was found to be a product of 2-mercaptobenzothiazole (MBT) photolysis (Brownlee et al., 1992). After irradiation of a MBT solution in phosphate buffer with sunlight, BT was detected with a yield of 11%; 2-benzothiazolone (BTon) was a further product. Acidification yielded in further 18% BT. Similar results were obtained in the presence of dissolved organic matter. The authors conclude that BT and BTon were stable products of MBT photolysis in aquatic environments.

Summary of BT degradation

According to the two OECD screening tests on ready biodegradability no final conclusion about the degradability of BT is possible because of the contradictory results. Tests simulating more realistic environmental conditions indicate that BT is degraded by adapted organisms at least primarily, but the rate of degradation cannot be quantified. Monitoring results (Table 2.6) support the assumption that the rate of primary degradation is not fast because BT was detected in many aqueous environments. As BT is volatile from aqueous solutions, volatilisation from the water compartment may be a significant elimination mechanism.

No final conclusion about the end products is possible. While in several investigations BT was mineralised, other authors found 2-benzothiazolone (BTon) as a product of primary transformation.

For the use of the screening approach for exposure due to the use of CBS, it is assumed that BT is persistent.

2.3 Distribution

With the physico-chemical data presented in section 1.2 and the equations provided by the Technical Guidance Document, the following distribution parameters are calculated:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henry’s law constant</td>
<td>0.63 Pa.m^3/mol</td>
</tr>
<tr>
<td>Koc</td>
<td>115 l/kg</td>
</tr>
</tbody>
</table>
Volatilisation (Henry’s Law Constant) was also determined experimentally by Monsanto (1978) at a temperature of 25 ±1 °C. A HLC of 0.365 Pa*m³/mol resulted as a mean of two systems. This value is used for the exposure estimations. A vapour pressure of 8.11 Pa calculated back from the measured HLC was applied for the Level I model above. The Henry’s law constant indicates that volatilisation can be a significant removal mechanism from the hydrosphere. Reddy & Quinn (1997) estimated a volatilization rate of 15 days in 1 m of stagnant water at 25°C with the wind blowing above the surface at 1 m/s. When the half-lives were recalculated at a water depth more realistic of urban runoff (0.1 cm and 1 m/s, respectively), the half-life became 30 min.

PCKOCWIN v1.66 gives a Koc of 996.2. The distribution of BT in a “unit world” according to the Mackay fugacity model level I based on the physico-chemical properties listed in Table 1.1 and using the experimentally determined HLC reveal that the main target compartments are water (86.8%) and the atmosphere (11.0%). EUSES 2.0 calculates the distribution in (local) stp as follows: 0.7 % (air), 97.9 % (water), 1.4 % (sludge).

### 2.4 Accumulation

Based on the equation log BCFfish = 0.85. log Pow – 0.70 (Veith, et al. 1979), a BCF of 10 l/kg is calculated from the log Kow of 2.0. BCFWIN 2.15 gives a BCF of 7 l/kg. Note: BCFWIN experimental database contains BCF for BT (BCF = 5.6). These values are in accordance with the experimentally determined value included in the MITI list (MITI, 1992). Following the OECD 305 C guideline, bioconcentration factors for the carp (*Cyprinus carpio*) over a 6 weeks exposure to concentrations of 0.2 mg/l and 0.02 mg/l have been determined. The BCF values ranged from 2.1 - 5.1 with a concentration of 0.2 mg/l and from < 4.1 - 7.5 with 0.02 mg/l.

Low to moderate bioaccumulation in leeches was found in a field experiment (Metcalfe et al., 1988). From measurements in a Canadian creek receiving waste water from a chemical facility and in three leechspecies, average “BCF” -values of 350 for *Dina dubia* and 250 for *Erpobdella punctata* were calculated. In *Helobdella stagnalis* the BT concentration was below the detection limit, i.e. the “BCF” was below ca. 35. Depuration experiments resulted in half-lifes of 9.5 days for *D. dubia* and 7 days for *E. punctata*. This study was strictly speaking measuring bioaccumulation, although uptake from water probably caused the largest part of the concentration difference between leeches and water.

### 2.5 Sources of exposure and estimated environmental concentrations

**Chemical Industry -production of CBS**
Three CBS-production sites have provided measured data from their emissions to water (see Table 2.1).

*Table 2.1. Local concentrations for BT during CBS production (90-P or maximum)*

<table>
<thead>
<tr>
<th>Site</th>
<th>Ceffluent [µg/l]</th>
<th>Clocal [µg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt;10</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>B</td>
<td>80</td>
<td>0.13</td>
</tr>
<tr>
<td>C</td>
<td>&lt;10</td>
<td>&lt;0.026</td>
</tr>
</tbody>
</table>

In Canada BT was monitored in the vicinity of a site producing chemicals for the rubber industry, the detected concentrations 50 m downwind of the company buildings were maximum 500 ppb (2.8 mg/m³). The compound was being emitted from an approximately 15 m high vent stack (Lane et al., 1980; Lane, 1982). These older data should be considered only as giving indication on the BT-emissions to air.

The CBS-producers have provided information regarding their BT-emissions to air. Measured data provided by site B gives a 90-P concentration of ca.0.03 mg/m³ (max. = 0.03 mg/m³) in air downwind direction of the site. Site C has estimated its annual emission to air to be in the worst case not more than 24 kg. Using EUSES 2.0, a Clocalair of 0.02 µg/m³ results. According to the process description of site A, a BT emission to air is negligible (less than emission from site C).

**Chemical industry- BT production and intermediate use**

Assuming a production volume of 2500 t/a and using the default emission and exposure estimation of the TGD for production (under IC 3/UC 33) with the parameters presented below, following local concentrations result.

*Table 2.2. Exposure estimation from production of BT. Model parameters and results.*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF to waste water</td>
<td>0.003</td>
</tr>
<tr>
<td>EF to air</td>
<td>0.0001</td>
</tr>
<tr>
<td>(EF to soil)</td>
<td>0.0001</td>
</tr>
<tr>
<td>T</td>
<td>300</td>
</tr>
<tr>
<td>Effluent flow rate</td>
<td>10 000 m³/s</td>
</tr>
<tr>
<td>D</td>
<td>40</td>
</tr>
<tr>
<td>f</td>
<td>1</td>
</tr>
<tr>
<td>Ceffluent</td>
<td>2.45 mg/l</td>
</tr>
<tr>
<td>Clocalwater</td>
<td>61.2 µg/l</td>
</tr>
<tr>
<td>Clocalair</td>
<td>2.32 *10⁻⁴ mg/m³</td>
</tr>
</tbody>
</table>

Assuming that the whole production volume of 2500 t/a is used as an intermediate off-site, and using the default emission and exposure estimation of the TGD for chemicals in synthesis (under IC 3/UC 33) with the parameters presented below, following local concentrations result.

*Table 2.3. Exposure estimation for the intermediate use of BT. Model parameters and results.*
EF to waste water | 0.007
---|---
EF to air | 0.001
(EF to soil | 0.0001)
T | 150
Effluent flow rate | 10 000 m³/s
D | 40
f | 0.3
C_{effluent} | 3.4 mg/l
C_{local\text{water}} | 86 µg/l
C_{local\text{air}} | 1.39 \times 10^{-3} \text{mg/m}^3

The calculations above are for background information only, and they are not followed further in this assessment.

The screening approach – local exposure caused by CBS-production

The maximum possible level of environmental exposure from CBS-production has been screened by assuming that all CBS-releases are immediately hydrolysed to BT in the environment. The local BT-emission from CBS-production has been calculated based on the generic scenario. The release into surface water was estimated at 89 kg/d as CBS. Since the generic scenario predicts no release to air, the release to air from the site A (2.4 kg/day as CBS) was used. After converting the release amount into BT according to the relation of the molar masses, a $C_{\text{local\text{water}}}$ of 111 µg/l and a $C_{\text{local\text{air}}}$ of 0.31 µg/m³ result.

Rubber and Tire Industry

In the USA BT was measured in the effluent of 2 tire manufacturers in concentrations of 20 and 60 µg/l (Jungclaus et al., 1976). The emission scenario document on additives in rubber industry (OECD 2004a) indicates that no releases of vulcanisation accelerators to waste water are caused by rubber and tire industry. Thus the exposure in agricultural soil is affected solely by the emission to air and by the regional background.

Levin (1994) presented a summary of the “Nordic curing fume project” in which factory scale curing experiments with roto-curing at 170 °C and laboratory scale curing experiments with gas transfer mold at 180 °C were run for six different rubber products to determine the total loss of weight in the curing process. In addition, concentrations of several substances in exhaust gas were measured. The author concluded that in the factory trials the loss of weight was 0.044 % whereas in the laboratory scale, the loss of weight was 0.40-0.80 % and the mean loss of weight including all experiments was approximately 0.05 %.

According to BLIC (2004), BT was found in the Nordic study in vulcanisation gas in concentrations of 70-270 ng/l and MeBT in concentrations below 50 ng/l (LOD). Levin (1994) reported that a concentration of 0.06 µg/m³ in air (gas phase) was estimated for a factory area producing 25,000 t rubber products in year based on the vulcanisation experiment results and
a specific local distribution model. A concentration of 0.04 µg/m³ in air (gas phase) was estimated for a daycare center of a specific factory located at a distance of 200 m.

A release estimation to air was derived applying the method and defaults of the emission scenario document on additives in rubber industry (OECD 2004a), the emission scenario document for plastic additives, part antioxidants (OECD 2004b) and the results described by Levin (1994).

The value for total loss of weight during vulcanisation (0.05 %) is used as the emission factor for curing phase. The emission scenario document for plastic additives, part antioxidants (OECD 2004b), suggests an emission factor of 0.005 % for the conversion phase (low volatility group, open process, solid articles) and that for raw materials handling no emissions are expected. Assuming that all CBS used is converted in curing process to BT, using the CBS market volume of 20 000 t/a and the portion of CBS in rubber (0.75 %) an emission of 11 t/a as CBS results. This would correspond to 5.6 t/a BT emission to air. Applying the 10 % rule, a regional emission of 0.6 t/a and a continental emission of 5.0 t/a are obtained. The local emission for the generic rubber factory producing 55,000 kg/d rubber 330 d/year is 39 kg/a as BT. Using this local emission, Clocal,air of 0.033 µg/m³ (total) was derived applying EUSES 2.0.3.

The screening approach - exposure caused by CBS used in rubber and tire industry

The maximum possible level of environmental exposure from use of CBS in rubber industry has been screened by assuming that all CBS used in vulcanization is converted into BT. Thus already the emissions consist of BT only. The site scenario is the generic scenario for rubber industry as presented in the emission scenario document. The generic site incorporates production of general rubber goods and tires. The rubber production volume of 55 000 kg/d, emission factor of 0.075 for air, emission factor of 0.0001 for soil, Freceipe of 1 and the content of 0.75 % in rubber (that of CBS) were used for the estimation. The resulting emission of 31 kg/day (as CBS) was converted to BT in the molar ratio (0.5113) resulting an emission of 15.9 kg/d. The distribution in the environment was calculated using the defaults as presented in the TGD. For the regional and continental emission calculation, the regional emission (150 t/a) and the continental emission (1350 t/a) of CBS was converted to BT in the molar ratio giving 76.7 t/a for the region and 690.3 t/a for the continent. As a result a Clocal of 0.44 µg/m³ was obtained.

Tire Tread Abrasion – road borders

Abraded tire particles accumulate near roads, leading to an exposure of soils at the road borders. Discharges of abraded particles into the hydrosphere are expected via rainwater runoff from roads. Lower amounts of abraded particles are almost completely emitted to the road border soil by drift due to turbulence caused by cars and wind. In the environment, the particles are degraded by biotic and abiotic processes, leading to the release of the monomers therein.

Rubber goods and automobile tires contain BT, the substance was frequently detected in extracts and eluates (see Table 2.4). Consequently, the substance was also observed in the drainage system of highways and in surface waters receiving road runoff, in snow near a road with high traffic density, and in soil near roads (Table 2.6).

The screening approach for exposure from tire abrasion
The maximum possible BT exposure from tires has been screened by assuming that all CBS added into tire rubber is converted in vulcanisation to BT. The main report (Chapter 3.1.2.2.2) considers the regional and continental releases as added CBS. Conversion to BT results in 6.1 t/a BT going to the regional STPs, surface waters and soil, respectively. The continental releases are 190 t/a going to the continental STPs, surface waters and soil, respectively.

Tire recycling – use of tire crumb in ground materials

On the basis of the study of Dye et al. (2006; see main report chapter 3.1.1.1 for study details) on the concentrations of BT in sport halls, air, a preliminary local assessment can be conducted. Using equations 40 and 41 of the TGD and the maximum BT-concentration measured in the halls (31.7 µg m\(^{-3}\)) a \(C_{\text{local,air}} = 0.023 \ \mu g \ m^{-3}\) at a distance of 100 m is derived. For the emission estimation, turf area of 67 m \(\times\) 105 m from the study of Dye et al. (2006), a height estimate of 10 m and ventilation rate of 1.5 per hour is used (several sources give approximately this ventilation rate for sport halls). Assuming that the ventilation and concentration in air remain same over the day and year, a release of 30 kg a\(^{-1}\) to air (gas phase) from one sport hall can be derived.

Several other benzothiazole derivatives originating from rubber material were measured indirectly in the fine particulate matter in air. They were present in concentrations lower than 1 ng m\(^{-3}\) and can be therefore assumed to not cause relevant exposure of environment.

The results of the study of Dye et al. (2006) can be used to approximate emissions to air from outdoor sport grounds, playgrounds and other similar uses. The amount of uses applying tire crumb is not available and the emission to the regional model could therefore not be calculated. It can be, however, expected that the amount of these uses is high and they may be a relevant diffuse contributor to the background levels of BT.

Further Sources

Beside caoutchouc chemicals, the biocide 2-(thiocyanomethylthio)benzothiazole (TCMTB; CAS 21564-17-0) is a further source for BT exposure. 2-2-(Thiocyanomethylthio)benzothiazole (TCMTB; CAS 21564-17-0) has been notified under the Biocides Directive 98/8/EEC for the use in nine product groups according to the Commission Regulation (EC) 2032/2003 and its amendment (EC) 1048/2005 i.a. for the use in slimicides, and conservation products. No use for wood preservation has been notified against the earlier information according to which TCMTB is also used as a fungicide by tanneries (Brownlee et al., 1992 and Srour, 1994). In addition, the registration as antifouling paint has been withdrawn.

2-Mercaptobenzothiazole (MBT; CAS 149-30-4) has been notified under the Biocides Directive 98/8/EEC Commission Regulation (EC) 1048/2005 for the use in six product groups, i.a. for the use in slimicides and indoor desinfection of public buildings.

Use of BT as food flavour may be an additional source of releases into environment. There is no information available on the use volume for this use. The use as food additive may in theory contribute to the exposure from landfills and from municipal sewage treatment and to the ambient regional concentrations of benzothiazoles. The major scenarios of the CBS-assessment are not affected by this use.
Regional concentrations for the screening approach

The default regional distribution taking into account the scenarios of CBS –production, use of CBS in rubber industry and releases from tires was calculated with EUSES to be as follows: PEC\textsubscript{regional\_water} is 0.6 µg/l, PEC\textsubscript{regional\_soil\_agr} is 1.17 µg/kg wwt and PEC\textsubscript{regional\_air} 4 \times 10^{-3} µg/m³.

Measured data

Throughout the world, BT has been detected in all environmental compartments as well as in industrial emissions. Tables 2.4-2.7 present the data available.
### Table 2.4: Measured data of BT in Rubber Products and Eluates

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tire wear particles</td>
<td>124.3 mg/kg</td>
<td></td>
<td>Particles from a tire testing machine</td>
<td>Rogge et al. (1993)</td>
</tr>
<tr>
<td>Crumb tire particles</td>
<td>171 mg/kg</td>
<td>2</td>
<td>Particles 150 µm generated from used tires</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>Eluate from artificial tire tread</td>
<td>23.1–1345 µg/l</td>
<td>25</td>
<td>6 different tires (automobile and truck tires, each new and old)</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Eluate from complete tires</td>
<td>14.6-1972 µg/l</td>
<td>2</td>
<td>Sampled after 2 month exposure in a water bath</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Vial Closures</td>
<td>5-10 mg/l</td>
<td>5</td>
<td>Measured in ethyl acetate extracts</td>
<td>Pattinson &amp; Wilkins (1989)</td>
</tr>
<tr>
<td>Medium</td>
<td>Concentrations</td>
<td>Number of Samples</td>
<td>Remark</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-------------------------</td>
<td>-------------------</td>
<td>---------------------------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Effluent from 2 tire manuf.</td>
<td>20 / 60 µg/l</td>
<td>2</td>
<td>Country: USA</td>
<td>Jungclaus et al. (1976)</td>
</tr>
<tr>
<td>Pulp and paper mill wastewater</td>
<td>10 – 30 µg/l</td>
<td></td>
<td>Sampled in USA</td>
<td>Petermann et al. (1978):</td>
</tr>
<tr>
<td>Untreated tannery wastewater</td>
<td>10.5 µg/l</td>
<td></td>
<td>Origin: TCMTB</td>
<td>Fiehn et al. (1994)</td>
</tr>
<tr>
<td>Municipal treatment plant effluents</td>
<td>qualitat. det.</td>
<td>5</td>
<td>Country: Illinois (USA)</td>
<td>Ellis et al. (1982)</td>
</tr>
<tr>
<td>Municipal treatment plant effluents</td>
<td>qualitat. det.</td>
<td>5</td>
<td>Country: Germany</td>
<td>Elsäßer et al. (1992)</td>
</tr>
<tr>
<td>Municipal waste water (influent)</td>
<td>0.85 µg/l (average; SD: 0.20)</td>
<td>20</td>
<td>Average for 20 composite samples of 24 h collected over three months in 2002 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.55 µg/l (average; SD: 0.19 µg/l)</td>
<td>20</td>
<td>Average of 20 composite samples of 24 h collected over three months in 2002 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (influent)</td>
<td>0.74 µg/l (average; SD: 0.24)</td>
<td>9</td>
<td>Average of 9 composite samples of 24 h collected over one month in 2003 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.28 µg/l (average; SD: 0.08 µg/l)</td>
<td>9</td>
<td>Average of 9 composite samples of 24 h collected over one month in 2003 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.07 µg/l</td>
<td>1</td>
<td>Year 2003 (Berlin, Schönerlinde, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (influent)</td>
<td>22.87 µg/l (average; SD: 2.43 µg/l)</td>
<td>3</td>
<td>Pooled samples (GaoBeiDian, Beijing, China)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>2.26 µg/l (average; SD: 0.27 µg/l)</td>
<td>4</td>
<td>Pooled samples (GaoBeiDian, Beijing, China)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Groundwater at a rubber</td>
<td>qualitat. det.</td>
<td></td>
<td>Ontario (Canada)</td>
<td>Lesage (1991)</td>
</tr>
<tr>
<td>Medium</td>
<td>Concentrations</td>
<td>Number of Samples</td>
<td>Remark</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------</td>
<td>-------------------</td>
<td>-------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>producer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surface water after a tire dump fire</td>
<td>2.5-720 µg/l</td>
<td>3</td>
<td>Virginia (USA)</td>
<td>Peterson et al. (1986)</td>
</tr>
<tr>
<td>Exhaust air from municipal treatment plant</td>
<td>qualitat. det.</td>
<td></td>
<td>Country: Switzerland</td>
<td>Hanggartner (1979)</td>
</tr>
<tr>
<td>Atmosphere 50 m downwind of a chemical manufacturer</td>
<td>max. 2.8 mg/m³</td>
<td></td>
<td>Country: Canada</td>
<td>LANE ET AL. (1980); LANE (1982)</td>
</tr>
<tr>
<td>Particulates released from an industrial plant</td>
<td>qualitat. det.</td>
<td></td>
<td>Plant producing carbon electrodes, Italy</td>
<td>Ciccioli et al. (1986)</td>
</tr>
<tr>
<td>Waste gas condensates</td>
<td>2.2 / 6.3 µg/l</td>
<td>2 plants</td>
<td>Country: Czechoslovakia</td>
<td>Bezacinsky et al. (1984)</td>
</tr>
</tbody>
</table>
Table 2.6: Measured data of BT in Environmental Compartments. * = used for derivation of PECregional (see the main report).

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Road dust, residential</td>
<td>78.7 µg/kg</td>
<td>1</td>
<td>Country: USA</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>Road dust, highway</td>
<td>149 µg/kg</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Road dust, urban</td>
<td>4.4 mg/kg</td>
<td></td>
<td>Collected in Pasadena (USA)</td>
<td>Rogge et al. (1993)</td>
</tr>
<tr>
<td>Urban particulate matter (road dust)</td>
<td>393 – 813 µg/kg</td>
<td>2</td>
<td>Country: USA</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>Soil immediately near roads</td>
<td>&lt;0.5-17.4 mg/kg dw</td>
<td>10</td>
<td>Highest concentration detected near a highway</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Soil, creosote contaminated</td>
<td>0.119 / 0.079 mg/kg</td>
<td>2</td>
<td></td>
<td>Brumley et al. (1991)</td>
</tr>
<tr>
<td>Landfill leachate</td>
<td>65 µg/l</td>
<td>3</td>
<td>Sampled in Germany</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Landfill leachate</td>
<td>16.9-281 ng/l</td>
<td>8</td>
<td>Country: Japan</td>
<td>Yasuhara et al. (1997)</td>
</tr>
<tr>
<td>Landfill leachate</td>
<td>qualitat. det.</td>
<td></td>
<td>Country: Canada</td>
<td>Barker et al. (1988)</td>
</tr>
<tr>
<td>House dust</td>
<td>qualitat. det.</td>
<td></td>
<td>Country: USA</td>
<td>Greenlaw et al. (1990)</td>
</tr>
<tr>
<td>Municipal treatment plants</td>
<td>0.11-0.60 µg/l</td>
<td>6</td>
<td>Sampled in Bremen (Germany)</td>
<td>Krumwiede &amp; Jastorff (2002)</td>
</tr>
<tr>
<td>Urban canalization sludge</td>
<td>60 µg/l</td>
<td>3</td>
<td>Sample from cleaning vehicle</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Highway runoff</td>
<td>&lt;2-11.8 µg/l</td>
<td>8</td>
<td>Sampled during 3 rainfalls, from beginning to 30 min after beginning</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Urban runoff</td>
<td>0.38 – 1.21 µg/l</td>
<td>10</td>
<td>Dissolved fraction</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>Urban park lake</td>
<td>0.6 µg/l</td>
<td>3</td>
<td>Sampled in Bremen (Germany)</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Road puddle, urban</td>
<td>0.49 / 2.42 µg/l</td>
<td>2</td>
<td>Sampled in Bremen (Germany)</td>
<td>Krumwiede &amp; Jastorff (2002)</td>
</tr>
<tr>
<td>Road drainage, urban</td>
<td>0.16-2.27 µg/l</td>
<td>9</td>
<td>Sampled in Bremen (Germany)</td>
<td>Krumwiede &amp; Jastorff (2002)</td>
</tr>
<tr>
<td>Highway settling pond</td>
<td>&lt;0.05 µg/l</td>
<td>7</td>
<td>Water phase</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>5 Ditches with highway runoff</td>
<td>0.2 – 1.4 µg/l</td>
<td>15</td>
<td>Sampled in Germany</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Highway runoff</td>
<td>2.2 µg/l (average)</td>
<td>19</td>
<td>One rain event, traffic density ca. 200 000 vph, Berlin, Germany</td>
<td>Klöpfer (2005)</td>
</tr>
<tr>
<td></td>
<td>3.0 µg/l (90-P)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>Concentrations</td>
<td>Number of Samples</td>
<td>Remark</td>
<td>Reference</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>---------------------------------------</td>
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<td>---------------------------------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>Rainwater</td>
<td>qualitat. det. 1.1-4.0 µg/l (min-max)</td>
<td></td>
<td>Country: UK</td>
<td>Welch &amp; Watts (1990)</td>
</tr>
<tr>
<td>Rainwater</td>
<td>22.13-50.62 ng/l</td>
<td>3 sites</td>
<td>Great Lakes (Canada)</td>
<td>Scott et al. (1996)</td>
</tr>
<tr>
<td>Snow near a road with high traffic density</td>
<td>9.9-71.2 µg/l</td>
<td>3</td>
<td>Sampled 14 days after snowfall</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Snow</td>
<td>0.05-0.59 µg/kg detected in 3 of 10 samples</td>
<td></td>
<td>Countries: Russia and Finland</td>
<td>Poliakova et al. (2000)</td>
</tr>
<tr>
<td>Pack ice</td>
<td>8-12 ng/l</td>
<td>3 sampling stations</td>
<td>Antarctica</td>
<td>Desideri et al. (1991)</td>
</tr>
<tr>
<td>River water</td>
<td>~0.05 µg/l &lt;20 µg/kg</td>
<td>2</td>
<td>Water phase Sediment</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>River water*</td>
<td>22-24 ng/l</td>
<td>detected at 3 from 8 sites</td>
<td>River Rhine, The Netherlands</td>
<td>Hendriks et al. (1994)</td>
</tr>
<tr>
<td>River water</td>
<td>“major contaminant”, not quantified</td>
<td></td>
<td>Delaware River, Philadelphia, USA</td>
<td>Brownlee et al. (1981)</td>
</tr>
<tr>
<td>River water</td>
<td>2 µg/l</td>
<td>1</td>
<td>not detected in sediment</td>
<td>Jungclaus et al. (1978)</td>
</tr>
<tr>
<td>River water</td>
<td>qualitat. det.</td>
<td></td>
<td>River Trent, UK</td>
<td>Drage et al. (1998)</td>
</tr>
<tr>
<td>River water*</td>
<td>0.38 / 2.231 ng/l</td>
<td>2</td>
<td>Gallego River (Spain)</td>
<td>Infante et al. (1993)</td>
</tr>
<tr>
<td>River water</td>
<td>0.378 / 0.081 µg/l</td>
<td>2 rivers</td>
<td>Japan</td>
<td>Koroma (2001)</td>
</tr>
<tr>
<td>Creek water</td>
<td>3.02 µg/l</td>
<td>3</td>
<td>Creek receives wastewater from a chemical facility</td>
<td>Metcalfe et al. (1988)</td>
</tr>
<tr>
<td>Surface water</td>
<td>qualitat. det.</td>
<td>5 sites</td>
<td>Origin: The Netherlands</td>
<td>Geerdink et al. (1999)</td>
</tr>
<tr>
<td>Lake water</td>
<td>0.2-242 ng/l</td>
<td>33</td>
<td>Great Lakes (Canada)</td>
<td>Scott et al. (1996)</td>
</tr>
<tr>
<td>Water</td>
<td>not det. in 1983 (dl = 0.1-0.5 µg/l)</td>
<td>30</td>
<td>Sampled in Japan, “water” not further characterized</td>
<td>JETOC (1993)</td>
</tr>
<tr>
<td>Well water</td>
<td>0.005-0.26 µg/l</td>
<td>96</td>
<td>Japan</td>
<td>Kadokami et al. (1995)</td>
</tr>
<tr>
<td>Sediment</td>
<td>1.6-3.3 µg/kg (dl 1.5-50 µg/kg)</td>
<td>detected in 4 from 30</td>
<td>Sampled in Japan</td>
<td>JETOC (1993)</td>
</tr>
<tr>
<td>Medium</td>
<td>Concentrations</td>
<td>Number of Samples</td>
<td>Remark</td>
<td>Reference</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------</td>
<td>-------------------</td>
<td>-------------------------</td>
<td>----------------------------</td>
</tr>
<tr>
<td>Sea sediment</td>
<td>2.5-2.6 mg/kg</td>
<td>detected at 2</td>
<td>New York Bight (USA)</td>
<td>Friedman (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>from 9 sites</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seawater*</td>
<td>0.66-2.27 ng/l (1990)</td>
<td>6 sampling stations</td>
<td>North Sea, German Bight</td>
<td>Bester et al. (1997)</td>
</tr>
<tr>
<td></td>
<td>0.03-1.23 ng/l (1995)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seawater</td>
<td>3-29 ng/l</td>
<td>8 sampling stations</td>
<td>Antarctica</td>
<td>Desideri et al. (1989)</td>
</tr>
<tr>
<td>Seawater</td>
<td>6-37 ng/l (water)</td>
<td>10 sampling stations</td>
<td>Antarctica</td>
<td>Desideri et al. (1991)</td>
</tr>
<tr>
<td></td>
<td>4-11 ng/l (particulate)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban Air</td>
<td>0.48-56.24 (Ø 18.04) ng/m³</td>
<td>12</td>
<td>Smog episode, Los Angeles (USA)</td>
<td>Fraser et al. (1998)</td>
</tr>
<tr>
<td>Atmosphere</td>
<td>Qualitative determ.</td>
<td></td>
<td>Forest sites</td>
<td>Dommröse &amp; Figge (1988)</td>
</tr>
</tbody>
</table>
Table 2.7: Monitoring of BT in Food and Biota

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water</td>
<td>qualit. det.</td>
<td></td>
<td>Sampled in Cincinnati (USA)</td>
<td>Coleman et al. (1980)</td>
</tr>
<tr>
<td>Drinking water</td>
<td>0.1-1.0 ng/l</td>
<td>3</td>
<td>5 rural and municipal sites</td>
<td>Petrick et al. (1992)</td>
</tr>
<tr>
<td>Boiled Beef</td>
<td>qualit. det.</td>
<td></td>
<td>Origin: USA</td>
<td>Liebich et al. (1972)</td>
</tr>
<tr>
<td>Roasted beef</td>
<td>qualit. det.</td>
<td></td>
<td>Origin: USA</td>
<td>Hartmann et al. (1983)</td>
</tr>
<tr>
<td>Vine</td>
<td>0.24-1.09 µg/l</td>
<td>12</td>
<td>Commercial Italian vines (the origin of BT in vine is not fully clarified)</td>
<td>Bellavia et al. (2000)</td>
</tr>
<tr>
<td>Fish tissue</td>
<td>qualit. det.</td>
<td></td>
<td>Cooked catfish</td>
<td>Grimm (2000)</td>
</tr>
<tr>
<td>Fish (Cyprinus carpio)</td>
<td>qualit. det.</td>
<td></td>
<td></td>
<td>Runge &amp; Steinhart (1990)</td>
</tr>
<tr>
<td>Fish liver (Morone saxatilis)</td>
<td>qualit. det.</td>
<td></td>
<td>Captured in California (USA)</td>
<td>Cashman et al. (1992)</td>
</tr>
<tr>
<td>Leeches (3 species)</td>
<td>&lt;0.1-1.4 mg/kg</td>
<td>6</td>
<td>Collected in a creek receiving wastewater from a chemical facility</td>
<td>Metcalfe et al. (1988)</td>
</tr>
<tr>
<td>Prawn (Panaeus japonicus)</td>
<td>qualit. det.</td>
<td></td>
<td></td>
<td>Pan et al. (1997)</td>
</tr>
<tr>
<td>Guava (Psidium guajava)</td>
<td>qualit. det.</td>
<td></td>
<td>Origin: Brazil</td>
<td>Idstein &amp; Schreier (1983)</td>
</tr>
<tr>
<td>Cherry fruits</td>
<td>qualit. det.</td>
<td></td>
<td>Origin: Germany</td>
<td>Karrer et al. (1990)</td>
</tr>
<tr>
<td>Mangos</td>
<td>10 µg/kg</td>
<td>6</td>
<td>Origin: Egypt</td>
<td>Engel &amp; Tressl (1983)</td>
</tr>
<tr>
<td>Potatoes (Solanum tuberosum)</td>
<td>qualit. det.</td>
<td></td>
<td>Origin: UK</td>
<td>Meigh et al. (1973)</td>
</tr>
<tr>
<td>Endive (Cichorium endivia)</td>
<td>qualit. det.</td>
<td></td>
<td>Origin: Germany</td>
<td>Götz-Schmidt &amp; Schreier (1986)</td>
</tr>
<tr>
<td>Black tea</td>
<td>qualit. det.</td>
<td></td>
<td></td>
<td>Vitzthum et al. (1975)</td>
</tr>
</tbody>
</table>
3. Effects Assessment

3.1 Aquatic Compartment

For the effects assessment of BT on aquatic organisms the volatility from aqueous solutions has to be taken into account, particularly in tests with open systems and longer exposure periods. The flow-through test with analytical monitoring conducted by Geiger et al. (1990) states that the concentrations decreased to about 80-90% of the nominal concentrations. In static tests it is expected that after several days the concentrations decrease below 80% of the nominal. This means that the results from longer static tests where no monitoring of test substance occurred should be considered on the basis of present quality criteria as not valid. The main degradation product of BT emerging first is expected to be BTon, which seems to be on the basis of QSARs and valid tests slightly more toxic than BT. Results of static acute tests without analytical monitoring can thus be considered as useful background information.

3.1.1 Toxicity to Fish

A flow-through test on the acute toxicity of BT to *Pimephales promelas* was conducted by Geiger et al. (1990). The fish were exposed in Lake Superior water to 5 test substance concentrations in the range of 27.7 to 155 mg/l. Analytical measurements revealed that the applied concentrations were >80% of the nominal during the test period. Based on measured concentrations a 96h-LC50 of 64 mg/l was obtained. The affected fish lost schooling behaviour, were hypoactive and underreactive to external stimuli, were darkly coloured and lost equilibrium prior to death. Considering these sub-lethal effects, a 96h-EC50 of 60.7 mg/l was determined.

Evans et al. (2000) tested lethal and sublethal effects of BT on larvae of sheepshead minnow (*Cyprinodon variegatus*). The fish were exposed over 5 days to 5 concentrations in the range of 3.7 to 60.0 mg/l. The 96h-LC50 value was calculated to 53 mg/l. Significant decreases in larval growth were noted at all exposure concentrations, the weight ranged from 53.6 to 64.9% of control. It has to be noted that larval growth was not dependent to the applied test substance concentrations. Analytical measurements were not performed, because of the volatility of BT the effective concentrations are expected to be lower.

A flow-through test on the acute toxicity of BT to juvenile *Oncorhynchus mykiss* was carried out by SRI International (1981). No standard guideline was applied but the study is adequately documented. The measured test concentrations were 0 mg/l, 1.6 mg/l, 4.4 mg/l, 8.1 mg/l, 14.9 mg/l and 22.9 mg/l and the duration altogether 14 days. The measured concentrations were over 95 % of the nominal concentrations. For each dose level and controls two test tanks were run with 10 fish each. Temperature varied between 13.0-14.0 °C, Hardness was 28 mg CaCO3/l, alkalinity 29 mg CaCO3/l and the pH varied during the test between 6.8 and 8.2. In the control tanks, no mortality was observed. Oxygen concentration varied between 9.4 and 10.8 mg/l. The fish were adapted to the test conditions for two weeks prior to test. An LC50 of 8.1 mg/l at 96 h and an LC50 of 5.6 mg/l at day 14 resulted.

Table 3.1 presents an overview of the studies which are considered plausible. In addition to the tests prescribed above, four such static acute fish tests are available where mortality was observed as the endpoint and only nominal concentrations were reported. These tests are also presented in brief in Table 3.1. It should be noted, that their validity is questionable while test concentrations may be < 80 % of the nominal concentrations.
3.1.2 Toxicity to Invertebrates

A static 96 h test with *Mysidopsis bahia* was conducted by the Analytical Bio-Chemistry Laboratories Inc. (1985) according to the U.S. Congress (1976). Test concentrations were 0, 10, 18, 32, 56 and 100 mg/l. Test conditions were as follows: temperature 25±2 ºC, O2: 5.6-7.0 mg/l (84-104 %; corrected for salinity), pH: 8.2-8.3 and salinity 27-28 %/00. LC50 at 96 h was 28 mg/l (calculated). Test concentrations were not monitored analytically. In addition to mortality, also quiescence and sinking to the bottom were observed at the test concentrations from 32 mg/l upwards. No effects were observed at the test concentration of 18 mg/l.

*Daphnia magna* was tested by Analytical Bio-Chemistry Laboratories, Inc.(1978) according to a guideline of U.S. Environmental Protection Agency (1975). A 48 h static test with test concentrations of 0, 1.0, 3.2, 5.6, 10, 32 and 56 mg/l, and two beakers each with ten daphnia (1st instar < 18 hours old) per beaker was conducted. No analytical monitoring was carried out. Water quality was as follows: T: 19 ±1 ºC, pH 7.7-7.9; O2 > 7.0 mg/l (observed at the end). LC50 of 20 mg/l (nominal) was obtained. No lethality was observed at the concentration of 5.6 mg/l.

3.1.3 Toxicity to Algae

EG&G Bionomics (1978) conducted a static 96 h *Selenastrum capricornutum* –test according to the guideline of U.S. Environmental Protection Agency (1971). Triplicates for the concentrations 6, 10, 32, 56, 100 mg/l, control and solution control were run in temperature of 24±1 ºC, pH 7.7-8.1 and 4000 lux light intensity. No analytical monitoring of the test concentrations was carried out. EC50 of 67 mg/l expressed as 50 % difference of chlorophyll a concentration and EC50 of 64 mg/l expressed as 50 % difference of cell numbers were obtained. It is not possible to calculate growth rate while the observation sheets are not documented.

Geurts & Kluskens (2004) conducted an algal growth inhibition test with *Pseudokirchneriella subcapitata*, basically according to OECD Test Guideline 201 and EEC Test Method C.3. BT concentrations were measured at test initiation and after 48h in samples from the lowest, one intermediate, and the highest test concentration. All measured BT concentrations ranged between 95 and 102% of the nominal concentrations.

The results of this study have to be used with special care as the control cultures did not grow exponentially. There was almost no measurable growth during the first day and significantly different growth rates during the second day (2.8/d) compared to the third day (1.7/d) of the test. A re-evaluation of the raw data was done for the period 24h – 72h, resulting in E2C20 and E2C50 values quite close to (slightly higher than) the corresponding values provided in the study report.

For the effects assessment, the reported values E2C20 = 40.3 mg/l and E2C50 = 50.8 mg/l are provisionally used with reservation.
### Table 3.1: Toxicity of BT to Fish and Algae

<table>
<thead>
<tr>
<th>Species</th>
<th>Test type</th>
<th>Exposure time</th>
<th>Effect conc.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>fish</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pimephales promelas</em></td>
<td>flow through</td>
<td>96 h</td>
<td>LC50 = 64.0 mg/l (e)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>EC50 = 60.7 mg/l (e)</td>
<td>Geiger et al. (1990)</td>
</tr>
<tr>
<td><em>Brachydanio rerio</em></td>
<td>static</td>
<td>96 h</td>
<td>LC0 = 65.5 mg/l (n)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>LC100 = 66.0 mg/l (n)</td>
<td>Bayer AG (1984)</td>
</tr>
<tr>
<td><em>Oryzias latipes</em></td>
<td>static</td>
<td>48 h</td>
<td>LC50 = 67.2 mg/l (n)</td>
<td></td>
</tr>
<tr>
<td><em>Cyprinodon variegatus</em></td>
<td>static or semi-static</td>
<td>96 h</td>
<td>LC50 = 53 mg/l (n)</td>
<td></td>
</tr>
<tr>
<td><em>Cyprinodon variegatus</em></td>
<td>static</td>
<td>96 h</td>
<td>LC50 = 36 mg/l (n)</td>
<td></td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>flow-through</td>
<td>96 h</td>
<td>LC50 = 8.1 mg/l (e)</td>
<td></td>
</tr>
<tr>
<td><em>Oncorhynchus mykiss</em></td>
<td>flow-through</td>
<td>14 d</td>
<td>LC50 = 5.6 mg/l (e)</td>
<td></td>
</tr>
<tr>
<td><em>Pimephales promelas</em></td>
<td>static</td>
<td>96 h</td>
<td>LC50 = 47 mg/l (n)</td>
<td></td>
</tr>
<tr>
<td><strong>invertebrates</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Mysisodopsis bahia</em></td>
<td>static</td>
<td>96 h</td>
<td>LC50 = 28 mg/l (n)</td>
<td></td>
</tr>
<tr>
<td><em>Daphnia magna</em></td>
<td>static</td>
<td>48 h</td>
<td>LC50 = 20 mg/l</td>
<td></td>
</tr>
<tr>
<td><strong>algae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Selenastrum capricornutum</em></td>
<td>static</td>
<td>96 h</td>
<td>EC50 = 64 mg/l (n)</td>
<td></td>
</tr>
<tr>
<td><em>Pseudokirchneriella subcapitata</em></td>
<td>static</td>
<td>72 h</td>
<td>Use with reservation, growth of control cultures not exponential</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E, C20 = 40.26 mg/l (e)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>E, C50 = 50.81 mg/l (e)</td>
<td>Geurts &amp; Kluskens (2004)</td>
</tr>
</tbody>
</table>

(n): nominal concentrations  (e): effective concentrations
3.1.4. Quantitative Structure-Activity Relationships (QSARs)

Data on benzothiazole derivatives have so far not been used for the development of QSAR-models. BT’s ecotoxicity was estimated with ECOSAR v0.99h (U.S.EPA, 2004), according to the classification scheme of Verhaar et al. (1992) and with the model of Pavan et al. (2005). The results are presented in Table 3.2.

BT is a non-polar substance, and ECOSAR v0.99h automatically allocates BT to the group of neutral organics (baseline toxicity).

The second way of estimation applies the approach of Verhaar et al. (1992). According to their guidance, BT can be assigned to the class 1 (non-polar substances) meaning that no excess toxicity is expected. The baseline toxicity was calculated using the models of Verhaar et al. (1995) and Van Leeuwen et al. (1992) as recommended in the Technical Guidance Document (Part III, Section 4.1, Table 1).

Finally, the acute toxicity to fish was calculated for a comparison using the model of Pavan et al. (2005):

\[
\text{LogLC50} = -0.574 \text{ LogKow} + 0.454 \text{ ELUMO} - 2.445
\]

where \( \text{ELUMO} \) is the energy of the lowest unoccupied molecular orbital. The equation is an adjusted version from the original model of Veith and Mekeneyan (1993) for “mixed” mode of action. The model was developed for aromatic substances which are considered to act by several modes of action including narcosis and unspecific reactivity due to electrophilic or nucleophilic reactions.

The acute tests indicate that fish (96hLC50 8.1 mg/l) would be the most sensitive species, whereas the baseline-QSARs predict algae to be the most sensitive group. The baseline-QSARs seem to slightly underestimate the toxicity of BT.

### Table 3.2. QSAR –estimates for aquatic ecotoxicity.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Acute EC50 (mg/l)</th>
<th>Chronic (mg/l)</th>
<th>Acute LC50 (mg/l)</th>
<th>LC50 (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>100.2</td>
<td>12.9</td>
<td>109.9</td>
<td>24.44</td>
</tr>
<tr>
<td>Daphnid</td>
<td>107.4</td>
<td>5.5</td>
<td>81.5</td>
<td></td>
</tr>
<tr>
<td>Algae</td>
<td>67.1</td>
<td>6.7</td>
<td>79.6</td>
<td></td>
</tr>
</tbody>
</table>

(1) These QSARs are for neutral organics
(2) The used \( \text{ELUMO} = -0.33 \text{ eV} \) (Schmegel 1995)

### 3.1.3 Determination of the PNECwater

Plausible acute tests are available from all three trophic levels. The fish test with lowest LC50 (8.1 mg/l) has been conducted in a flow through test with analytical control. The lowest algae EC50 (50.8 mg/l) has been obtained from a static test with analytical control. The lowest LC50 from the two invertebrate tests (20 mg/l) had no analytical control, but due to the short test duration it can be assumed that > 80 % of the nominal concentration remained in the solution. The fish study (SRI International, 1981) will be used for the derivation of PNEC. Assessment factors of 1000 (freshwater) and 10 000 (marine water) will be applied. Therefore,
\[ PNEC_{freshwater} = \frac{8.1 \text{ mg/l}}{1,000} = 8.1 \text{ µg/l} \]

and

\[ PNEC_{marine} = \frac{8.1 \text{ mg/l}}{10,000} = 0.81 \text{ µg/l} \]

### 3.1.4 Microorganisms

In a respiration inhibition test using activated sludge according to OECD 209, a 3h-EC50 of 650 mg/l was obtained (Yoshioka et al., 1986). A similar test according to a national guideline resulted in a 3h-EC50 of 635 mg/l and a EC10 of 274 mg/l (Bayer AG, 1990). Applying an assessment factor of 10 to the EC10, the PNEC for sludge respiration is determined to 27.4 mg/l.

A nitrification inhibition test was performed with *Nitrosomas* isolated from wastewater. NH\textsubscript{4} removal was analysed in a model of sediment columns charged with quartz sand. Effects were found with BT concentrations of 0.2 mg/l (Reemtsma, 1994). Because the test design does probably not reflect the conditions in a waste water treatment plant, the results are not used for the PNEC derivation.

Results from 2 growth inhibition tests for *Tetrahymena pyriformis* are available. EC50 values of 135 and 160 mg/l were obtained. Applying an assessment factor of 10 to the mean value (148 mg/l), the PNEC for protozoa is determined to 14.8 mg/l.

For the assessment of microorganisms in biological treatment plants, the lowest PNEC is selected:

\[ \text{PNEC}_{microorg.} = 14.8 \text{ mg/l} \]

**Table 3.3: Toxicity of BT to Microorganisms**

<table>
<thead>
<tr>
<th>Species</th>
<th>Dur.</th>
<th>Effects</th>
<th>Endpoint</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated sludge from laboratory facility</td>
<td>3 h</td>
<td>EC10 = 274 mg/l</td>
<td>Respiration</td>
<td>Bayer AG (1990)</td>
</tr>
<tr>
<td>Activated sludge, domestic</td>
<td>3 h</td>
<td>EC50 = 635 mg/l</td>
<td>Respiration</td>
<td>Yoshioka et al. (1986)</td>
</tr>
<tr>
<td><em>Nitrosomas</em> (isolated from wastewater)</td>
<td></td>
<td>ECxxx = 0.2 mg/l</td>
<td>Nitrification (1)</td>
<td>Reemtsma (1994, 1995)</td>
</tr>
<tr>
<td><em>Pseudomonas putida</em></td>
<td>18 h</td>
<td>EC0 = 50 mg/l</td>
<td>Growth inhibition</td>
<td>Bayer AG (1990)</td>
</tr>
<tr>
<td><em>Tetrahymena pyriformis</em></td>
<td>40 h</td>
<td>EC50 = 135 mg/l</td>
<td>growth</td>
<td>Schultz (1997)</td>
</tr>
<tr>
<td><em>Tetrahymena pyriformis</em></td>
<td>24 h</td>
<td>EC50 = 160 mg/l</td>
<td>growth</td>
<td>Yoshioka et al. (1986)</td>
</tr>
</tbody>
</table>

All effect values refer to nominal concentrations

(1): Test conducted in a sediment column
4. Summary
Benzothiazole is a High Production Volume Chemical, 1000 - 5000 t were manufactured within Western Europe in 1994. The substance is exclusively used as an intermediate in chemical synthesis.

BT is formed during vulcanization from benzothiazole derivatives being used as vulcanization accelerators. The compound was detected in the exhaust gas of a chemical plant producing benzothiazole derivatives and in the waste water of tire manufacturers. BT is a content in rubber and tires. It is released into the environment by tire tread abrasion and by migration and leaching from the rubber matrix. A further exposure source is the photolytical degradation of 2-mercaptobenzothiazole which is released by industrial sources and by tire tread abrasion. BT was monitored in highway runoff, in soils near roads, in river and seawater. The use of 2-(thiocyanomethylthio)benzothiazole (TCMTB) and 2-Mercaptobenzothiazole (MBT) as biocides is probably a source of minor importance.

From the available studies on biodegradation no final conclusion about the biological degradability is possible. In the hydrosphere BT is resistant to photolysis by sunlight. Monitoring results support the assumption that BT is stable, as it was detected in many aqueous environments. As BT is volatile from aqueous solutions, volatilization from the water compartment may be a significant elimination mechanism.

In the environment, BT is mainly distributed into the hydrosphere and the atmosphere. Because of its volatility, BT is transported in the atmosphere over long distances. This is demonstrated by measured data from the atmosphere, rainwater and antarctic glacier ice. The calculated Koc value of 115 l/kg indicates a moderate sorption potential. In a laboratory experiment with fish bioconcentration factors of 2.1 – 7.5 l/kg were determined showing low potential for bioaccumulation.

The acute ecotoxicity of BT was determined for 6 fish species, with LC50s in the range between 8.1 – 87mg/l, for two invertebrates and in two algal growth inhibition tests. PNEC of 8.1 µg/l was derived from a flow-through fish test. Based on a growth inhibition test on *Tetrahymena pyriformis* a PNEC<sub>microorg.</sub> of 14.8 mg/l is determined. PNEC<sub>soil</sub> was estimated with the equilibrium partitioning method to be 0.0172 mg/kg wwt.

The risk ratios based on measured data are presented in the main report.
5. References


Bayer AG (1990). Internal study, unpublished


Eholzer & Kempermann (1983). GAK 9, 470-480


Fraser MP and Rappaport (1976). Environmental Health Perspectives 17, 45-53


Meinhart E and Schreier P (1986). Study of flavour compounds from Parmigiano Reggiano cheese. Milchwissenschaft 41(11), 689-691


Pan BS, Tsai JR, Chen LM. and Wu CM (1997). Llipxyxygenase and Sulfur-Containing Amino Acids in Seafood Flavor Formation. ACS Symp. Ser. 674, 64-75


APPENDIX D: 2-BENZOTHIAZOLONE (BTON)

1.1 Identification of the Substance

Name: 2(3H)-Benzothiazolone

Synonyms:
- 2-Hydroxybenzothiazole
- 2-Benzothiazolone
- BTon
- OBT

CAS No.: 934-34-9

Empirical Formula: C₇H₅NOS

Molecular weight: 151.19 g/mol

Structural Formula:

\[
\begin{array}{c}
\text{N} \\
\text{H} \\
\text{S} \\
\text{O}
\end{array}
\quad \stackrel{\text{\rightarrow}}{\quad} \quad
\begin{array}{c}
\text{O} \\
\text{H}
\end{array}
\]

The compound can occur in two tautomeric forms. Both in solution as well as in the crystal the equilibrium is almost completely on the (left) side of 2-benzothiazolone.

1.2 Physico-Chemical Data

Tab. 1.1: Physico-Chemical Data of BTon
<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>138 °C</td>
<td>Weast (1979); Reddy (1997)</td>
</tr>
<tr>
<td>Boiling point</td>
<td>360 °C</td>
<td>Weast (1979); Reddy (1997)</td>
</tr>
<tr>
<td>Solubility in water</td>
<td>690 mg/l</td>
<td>Reddy (1997)</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>4.3 $10^{-6}$ Pa</td>
<td>Reddy (1997)</td>
</tr>
<tr>
<td>Log Kow</td>
<td>1.76</td>
<td>Reddy (1997); Klamer (1995)</td>
</tr>
</tbody>
</table>
2. Exposure

2.1 Sources of Exposure

BTon is not manufactured by chemical industry. This compound is formed from chemicals mainly used as vulcanization accelerators in rubber manufacture.

BTon is an intermediate during biodegradation of 2-mercaptobenzothiazole (MBT). The studies on MBT degradation are referred in Appendix A section 2.3. MBT is released into the wastewater by chemical industry during production of benzothiazole derivatives. All three European CBS-production plants provided recently measured data on BTon from emissions to surface water (see Table 2.1).

<table>
<thead>
<tr>
<th>Site</th>
<th>Ceffluent [µg/l]</th>
<th>Clocal [µg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt; 10</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>B</td>
<td>70</td>
<td>0.1</td>
</tr>
<tr>
<td>C</td>
<td>47.0</td>
<td>0.122</td>
</tr>
</tbody>
</table>

Table. 2.1. Local concentrations for BTon during CBS-production

BTon was found to be an intermediate of benzothiazole (BT) degradation. Benzothiazole-degrading bacteria isolated from sludge obtained from a plant treating wastewater from rubber chemical production and from a lab-scale reactor fed with benzothiazoles were capable to transform BT to BTon, which was further metabolized (De Wever et al., 1998). This process was also found in the environment: Reddy & Quinn (1997) spiked water collected from a highway settling-pond spiked with BT and found that 8 days later 60% of the BT had transformed into BTon, which was further degraded within 30 days. This process occurred only in water samples collected in September but not in water collected in winter, the authors assume that the process is dependent on seasonal factors like different populations or road salts.

Reddy & Quinn (1997) measured BTon in recycled automobile tires and related media. Extraction of a crumb rubber sample (about 150 µm diameter) resulted in a BTon content of 81 mg/kg tire particles. Rinsing the rubber particles with deionized water (4 g rubber with 100 ml water) for 5 consecutive times approximately 50% of the compound was solubilized, the equilibrium was reached after 24 hours. From a typical loss of 90 mg of tire/km, the authors estimated the annual flux of BTon into the environment in the USA to 110 t/a. As a further source of BTon...
Antifreeze fluids were identified: in antifreeze collected from the radiators of 5 cars the concentrations were 0.117 to 20.1 mg/l (average 7.23 mg/l) were measured. The annual flux was estimated to 1.5 – 4000 kg/a.

A formation mechanism in the environment is the photolytical degradation of 2-mercaptobenzothiazole (MBT): Brownlee et al. (1992) found BTon as a photolysis product of a 1.9 mg/l MBT solution in natural water with a yield of 5%. Further photolysis of BTon was not observed (Reddy & Quinn, 1997).

Further sources of probably minor importance are:

- BTon was identified as a component in waste gas condensates from coal power plants in the Czechoslovakia. In a sample from one plant, the concentration in the condensate was 28.2 µg/l, while in a second plant the substance was not detected (Bezacinsky et al., 1984).
- BTon was isolated from fermentation culture extracts of *Micrococcus sp.*, a marine bacterium obtained from tissues of the sponge *Tedania ignis* (Stierle et al., 1991).
- BTon is a metabolite of the pesticide Methabenzthiazuron (Schmegel, 1995).

**Table 2.2: Monitoring of BTon Related to Industrial Sources and Wastewater**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundwater at a rubber producer</td>
<td>No concentr. reported</td>
<td>Ontario (Canada)</td>
<td>Lesage (1991)</td>
</tr>
<tr>
<td>Pulp and paper mill wastewater</td>
<td>10 – 30 µg/l</td>
<td>Sampled in USA</td>
<td>Petermann et al. (1978):</td>
</tr>
<tr>
<td>Wasted industrial sludge</td>
<td>97 – 310 µg/kg</td>
<td>Tentatively identified</td>
<td>Legiec &amp; Kosson (1988)</td>
</tr>
</tbody>
</table>
Table 2.3: Monitoring of BTon in environmental compartments

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urban canalization sludge</td>
<td>19 µg/l</td>
<td></td>
<td>Sample from cleaning vehicle</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Road puddle, urban</td>
<td>1.85 / 6.90 µg/l</td>
<td>2</td>
<td>Sampled in Bremen (Germany)</td>
<td>Krumwiede &amp; Jastorff (2002)</td>
</tr>
<tr>
<td>Road drainage, urban</td>
<td>1.51-13 µg/l</td>
<td>9</td>
<td>Sampled in Bremen (Germany)</td>
<td>Krumwiede &amp; Jastorff (2002)</td>
</tr>
<tr>
<td>Urban runoff</td>
<td>0.72 – 6.9 µg/l</td>
<td></td>
<td>Dissolved fraction Particulates</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>Urban park lake</td>
<td>0.2 µg/l</td>
<td>3</td>
<td>Sampled in Bremen (Germany)</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Urban particulate matter (road dust)</td>
<td>696 – 893 µg/kg</td>
<td>2</td>
<td></td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>Road dust, residential</td>
<td>24.6 µg/kg</td>
<td>1</td>
<td></td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>Road dust, highway</td>
<td>90.2 µg/kg</td>
<td>1</td>
<td></td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>Highway settling pond</td>
<td>&lt;0.05 – 0.516 µg/l</td>
<td>7</td>
<td>Water phase Sediment</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td>4 Ditches with highway runoff</td>
<td>0.2 – 0.5 µg/l</td>
<td>12</td>
<td>Sampled in Germany</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Municipal treatment plants</td>
<td>&lt;0.1-52 µg/l</td>
<td>6</td>
<td>Sampled in Bremen (Germany)</td>
<td>Krumwiede &amp; Jastorff (2002)</td>
</tr>
<tr>
<td>Municipal waste water (influent)</td>
<td>0.50 µg/l (average; SD: 0.16)</td>
<td>20</td>
<td>Average for 20 composite samples of 24 h collected over three months in 2002 (Berlin, Ruhleben, Germany)</td>
<td>Kloepfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.14 µg/l (average, SD: 0.08 µg/l)</td>
<td>20</td>
<td>Average of 20 composite samples of 24 h collected over three months in 2002 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (influent)</td>
<td>0.16</td>
<td>9</td>
<td>Average of 9 composite samples of 24 h collected over one month in 2003 (Berlin,</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Medium</td>
<td>Concentrations</td>
<td>Number of Samples</td>
<td>Remark</td>
<td>Reference</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>-------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Municipal waste water</td>
<td>0.20 µg/l (average; SD: 0.06 µg/l)</td>
<td>9</td>
<td>Average of 9 composite samples of 24 h collected over one month in 2003 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>(effluent)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Municipal waste water</td>
<td>0.27 µg/l</td>
<td>1</td>
<td>Year 2003 (Berlin, Schönerlinde, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>(effluent)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Municipal waste water</td>
<td>2.29 µg/l (average; SD: 1.64 µg/l)</td>
<td>3</td>
<td>Pooled samples (GaoBeiDian, Beijing, China)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>(influent)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Municipal waste water</td>
<td>1.54 µg/l (average; SD: 0.66 µg/l)</td>
<td>4</td>
<td>Pooled samples (GaoBeiDian, Beijing, China)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>(effluent)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highway runoff</td>
<td>7.0 µg/l (average)</td>
<td>19</td>
<td>Outlet of a highway section drainage basin (ca. 200 000 ADI), year 2003 (Berlin, Germany)</td>
<td>Klöpfer (2005)</td>
</tr>
<tr>
<td></td>
<td>9.9µg/l (90-P)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.1-12.0 µg/l (min-max)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>River water</td>
<td>&lt;0.05 µg/l</td>
<td>2</td>
<td>Water phase</td>
<td>Reddy &amp; Quinn (1997)</td>
</tr>
<tr>
<td></td>
<td>&lt;20 – 31 µg/kg</td>
<td>10</td>
<td>Sediment</td>
<td></td>
</tr>
<tr>
<td>River sediment</td>
<td>≤ 300 µg/kg dw</td>
<td>5</td>
<td>Identified in 1 of 5 river sed.</td>
<td>Fabacher et al. (1991)</td>
</tr>
<tr>
<td>Great Lakes tributaries</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(USA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Groundwater from 2</td>
<td>No concentr. reported</td>
<td></td>
<td>Tentatively identified</td>
<td>Betowski et al. (1996)</td>
</tr>
<tr>
<td>Superfund sites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leachate from a landfill</td>
<td>14 µg/l</td>
<td>3</td>
<td>Sampled in Germany</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Leachate from a municipal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>landfill</td>
<td>No concentr. reported</td>
<td>3</td>
<td>Sampled in Sweden</td>
<td>Oman &amp; Hynning (1993)</td>
</tr>
</tbody>
</table>
Table 2.4: Monitoring of BTon in Food and Biota

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water</td>
<td>No concentr. reported</td>
<td>Sampled in Cincinnati(USA)</td>
<td>Coleman et al. (1980)</td>
</tr>
<tr>
<td>Drinking water</td>
<td>No concentr. reported</td>
<td>Sampled in UK</td>
<td>Crathorne et al. (1984)</td>
</tr>
<tr>
<td>Red wine</td>
<td>7.7 µg/l</td>
<td>Wine bottled in France (no clarification of origin of BT)</td>
<td>Boison &amp; Tomlinson (1990)</td>
</tr>
</tbody>
</table>
2.2 Degradation

Biodegradation

The biodegradation of 2(3H)-Benzothiazolone (BTon) was not examined by OECD standard tests. Several investigations are available which examined the degradability in pre-adapted inoculi.

Mainprize et al. (1976) studied biodegradation of BTon by manometric determination of O₂ consumption. The inoculi used were industrial (adapted to benzothiazole-2-sulphonic acid) and a mixed culture isolated from the adapted sludge. A concentration of 1 µmol of BTon was tested in a final volume of 3 ml, with an incubation temperature of 30 °C. From the oxygen uptake after 3 hours (values taken from a graph), a biodegradation of 73 % in the mixed culture and 78 % in the adapted sludge can be calculated.

Chudoba (1977) examined BTon degradation in activated sludge. The inoculum was adapted to five benzothiazole derivatives during 76 days before use. The incubation period was 30 days, sampling was performed every 2 days and COD was determined. At the end of incubation period, UV spectroscopy of the filtrates was performed. It was shown that BTon (100 mg/l) was biochemically completely degraded after 15 days (lag phase 10 days) confirmed by UV spectroscopy. Under these conditions, 2-mercaptobenzothiazole (MBT), benzothiazole-2-sulfonic acid (BTSO₃), and 2-methylthiobenzothiazole (MeSBT) were not degraded.

De Vos et al. (1993b) examined by BOD measurements with sludge obtained from a plant treating waste-waters from rubber chemical production. The sludge had been adapted to BT using a laboratory system operated for several weeks. After 5 days BTon was degraded to 10% (starting concentration 19 mg/l), 50% (3.8 mg/l), resp. 100% (1.9 mg/l). When combinations of benzothiazoles were used, it was found that 2-mercaptobenzothiazole (MBT) inhibited the oxidation of BTon. During fed-batch adaptation to benzothiazole derivatives [BT and MBT were added at different time, at different concentrations, two different industrial sludges were further adapted to MBT, BT and BTon], the sludge degraded readily BTon in approximately 10 days.

In a second publication of De Vos et al. (1993b), it was shown that from the laboratory scale activated sludge systems adapted to benzothiazole derivatives for 2 months, isolation of bacterial strains in BTon media was possible. The strain was able to degrade BTon within 6 - 8 days. The results confirm that after a long adaptation period, BTon may biodegrade as bacterial strains could be isolated from the BTon adapted inoculum.

Benzothiazole-degrading bacteria were identified as Rhodococcus erythropolis (De Wever et al., 1998). The bacteria were isolated from sludge obtained from a plant treating waste-waters from rubber chemical production and from a lab-scale reactor fed with benzothiazoles. The isolate was capable to degrade BTon and benzothiazole (BT), but not 2-mercaptobenzothiazole (MBT) which was found to inhibit BTon degradation. As a product of BTon degradation, dihydroxybenzothiazole was formed, which is in turn further degraded.

To simulate realistic environmental conditions, BTon (15-25 mg/l) was spiked into water collected from a highway settling pond. Incubation at room temperature resulted in degradation within 30 days. Kinetic data are not reported (Reddy & Quinn, 1997).

Degradation experiments in soil demonstrated a slow mineralization (Cheng et al., 1978). In soil samples incubated with benzo[2-¹⁴C]-thiazolone, 12% of the applied radioactivity was detected as ¹⁴CO₂ after 10 weeks.
BIOWIN v4.02 predicts that the substance is not readily biodegradable but not persistent to biodegradation, either. The following estimates are provided: BIOWIN2 = 0.87, BIOWIN3 = 2.82 and BIOWIN6 = 0.28.

Photolysis

BTon was found to be a product of 2-mercaptobenzothiazole (MBT) photolysis (Brownlee et al., 1992). After irradiation of a MBT solution in phosphate buffer with sunlight, BTon was detected with a yield of 4%; benzothiazole (BT) was a further product. Similar results were obtained in the presence of dissolved organic matter. The authors conclude that BT and BTon were stable products of MBT photolysis in aquatic environments.

Summary of BTon degradation

The biodegradation of BTon was not examined in standard tests. BTon is formed from 2-mercaptobenzothiazole in biological treatment plants. From available tests performed with inoculi being pre-adapted under special conditions it can be concluded that BTon is partially mineralized, but the rate of mineralization is unclear. The main parameter determining degradation in treatment plants is the MBT concentration. Under environmental conditions, degradation within weeks or months is expected.

2.3 Distribution

With the physico-chemical data presented in section 1.2 and the equations provided by the Technical Guidance Document, the following distribution parameter are calculated:

<table>
<thead>
<tr>
<th>Henry’s law constant</th>
<th>9.41 \times 10^{-7} \text{ Pa m}^2/\text{mol}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koc</td>
<td>86 l/kg</td>
</tr>
<tr>
<td>Mackay level I</td>
<td></td>
</tr>
<tr>
<td>Air</td>
<td>0.0%</td>
</tr>
<tr>
<td>Water</td>
<td>99.1%</td>
</tr>
<tr>
<td>Soil</td>
<td>0.45%</td>
</tr>
<tr>
<td>Sediment</td>
<td>0.42%</td>
</tr>
<tr>
<td>Susp. Sed.</td>
<td>0.0%</td>
</tr>
<tr>
<td>Fish</td>
<td>0.03%</td>
</tr>
</tbody>
</table>

The distribution of BTon in a “unit world” according to the Mackay fugacity model level I based on the physico-chemical properties listed in table 1.1 reveal that the main target compartment is water (99.1%). The calculated Henry’s law constant of 9.41 \times 10^{-7} \text{ Pa m}^2/\text{mol} indicates that the substance is not volatile from aqueous solutions. The Koc of 86 l/kg indicates a low sorption onto soil or sediment solids.

2.4 Accumulation

Based on the equation log BCFfish = 0.85 \times \log \text{Pow} - 0.70 (Veith et al 1979), a BCF of 6.3 l/kg is calculated from a \log \text{Kow} of 1.76. BCFWIN 2.15 gives a BCF of 5.
3. Effects Assessment

3.1 Aquatic Compartment

3.1.1 Toxicity to Invertebrates

Geurts & Kluskens (2004a) conducted a 48h acute immobilisation test with Daphnia magna under static conditions, basically according to OECD Test Guideline 202 and EEC Test Method C.2. BTon concentrations were measured at test initiation and after 48h in samples from the lowest, one intermediate, and the highest test concentration. All measured BTon concentrations ranged between 96 and 101% of the nominal concentrations.

The water fleas reacted with a quite steep concentration response curve, with no effects up to the second test concentration (9.89 mg/l), 80% immobilisation in the third concentration level (19.78 mg/l), and 100% immobilisation in the fourth and fifth concentration levels (39.56 mg/l and 79.12 mg/l, respectively). The 48h EC50 of 16.1 mg/l was calculated using the trimmed Spearman-Kärber method.

3.1.2 Toxicity to Algae

Geurts & Kluskens (2004b) conducted an algal growth inhibition test with Pseudokirchneriella subcapitata, basically according to OECD Test Guideline 201 and EEC Test Method C.3. BTon concentrations were measured at test initiation and after 48h in samples from the lowest, one intermediate, and the highest test concentration. All measured BTon concentrations ranged between 95 and 102% of the nominal concentrations.

The results of this study have to be used with special care as the control cultures did not grow exponentially. There was almost no measurable growth during the first day and significantly different growth rates during the second day (2.8/d) compared to the third day (1.7/d) of the test. A re-evaluation of the raw data was done for the period 24h – 72h, resulting in ErC20 and ErC50 values quite close to (slightly higher than) the corresponding values provided in the study report.

For the effects assessment, the reported values ErC20 = 14.44 mg/L and ErC50 = 22.42 mg/L are provisionally used with reservation.

3.1.4. Quantitative Structure-Activity Relationships (QSARs)

Data on BTon or other benzothiazole derivatives have so far not been used for the development of QSAR-models. BTon is a polar substance, and thus some excess toxicity compared to the baseline toxicity is expected. BTon is in the environmentally relevant pH range in neutral undissociated form. BTon’s ecotoxicity was estimated with ECOSAR v0.99h (U.S.EPA, 2004), according to the classification scheme of Verhaar et al. (1992) and with the model of Pavan et al. (2005). The results are presented in Table 3.1.

ECOSAR v0.99h automatically allocates BTon to the group of thiazolinones/benzothiazolines. These equations are based on one data point each and thus the results should be considered with caution.

The second way of estimation applies the approach of Verhaar et al. (1992). According to their guidance, BTon can be assigned to the class 2 (polar substances). The baseline toxicity was first calculated using appropriate QSARs for non-polar substances. For this purpose, the models of Verhaar et al. (1995) and Van Leeuwen et al. (1992) as recommended in the Technical Guidance Document (Part III, Section 4.1, Table 1) were used. The resulting
estimates (see Table 3.1) were divided by the “toxicity range factors” of the class 2 ($RF_{T_{max}} = 10$ and $RF_{T_{min}} = 5$) to obtain the final toxicity range.

Finally, the acute toxicity to fish was calculated for a comparison using the model of Pavan et al. (2005):

$$\text{LogLC50} = -0.574 \text{LogKow} + 0.454 \text{ELUMO} - 2.445,$$

where $\text{ELUMO}$ is the energy of the lowest unoccupied molecular orbital. The equation is an adjusted version from the original model of Veith and Mekeneyan (1993) for “mixed” mode of action. The model was developed for aromatic substances which are considered to act by several modes of action including narcosis and unspecific reactivity due to electrophilic or nucleophilic reactions.

Table 3.1. QSAR –estimates for aquatic ecotoxicity.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Acute EC50 (mg/l)</th>
<th>Chronic EC50 (mg/l)</th>
<th>Acute EC50 (mg/l)</th>
<th>Chronic EC50 (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>4.8</td>
<td>0.3</td>
<td>196.6</td>
<td>19.7-39.3</td>
</tr>
<tr>
<td>Daphnid</td>
<td>55.5</td>
<td>1.4</td>
<td>154.0</td>
<td>15.4-30.8</td>
</tr>
<tr>
<td>Algae</td>
<td>2.2</td>
<td>0.5</td>
<td>154.7</td>
<td>15.5-30.9</td>
</tr>
</tbody>
</table>

(1) These QSARs are for neutral organics and they have been used as the starting point for the approach of Verhaar et al. (1992)

(2) The acute toxicity range as the outcome of the method of Verhaar et al. (1992)

(3) The used $\text{ELUMO} = -0.06$ eV (Schmegel 1995)

ECOSAR v0.99h predicts lower toxicity for algae than measured. In addition, it predicts lower toxicity to fish (no test data available) than to daphnids. ECOSAR shows similar acute toxicity to fish and algae (values in the range of few mg/l) but one magnitude level less sensitivity regarding acute toxicity to Daphnids. Thus the tests seem to have covered the most sensitive species (algae).

3.1.3 Determination of the PNECwater

Available test data from a 48h daphnia immobilisation test and from an algal growth inhibition study allow a provisional PNEC derivation. Reservation regards the lack of any study with fish as a third trophic level and the limited validity of the algae test.

Read-across to other CBS metabolites and CBS, in particular to the more reliable test data obtained with the sufficiently water soluble metabolites, provide strong evidence that there is no specific toxicity to any trophic level’s standard test species. In all cases with test concentrations well below the limits of water solubility, the factors between the highest and the lowest $E(L)C50$ were < 10. Regarding the most sensitive EC-values, no trophic level’s standard test species prevails. Used EC-values from the weak algal study are backed by re-evaluation of the raw data.

In conclusion it appears justifiable to provisionally derive aquatic PNECs, applying assessment factors of 1,000 for the freshwater PNEC and 10,000 for the marine PNEC. The algal growth inhibition test (Geurts & Kluskens 2004b) resulted in an $E(C50)$ of 22.42 mg/l (effective). The acute daphnia immobilisation test (Geurts & Kluskens 2004a) resulted in a 48h EC50 = 16.1 mg/l (effective). Therefore,
PNEC_{freshwater} = 16.1 \text{ mg/l} / 1,000 = 16.1 \mu g/l

and

PNEC_{marine} = 16.1 \text{ mg/l} / 10,000 = 1.6 \mu g/l

3.1.4 Toxicity to Microorganisms

There are no valid tests on micro-organisms available, therefore a PNEC_{microorg.} cannot be estimated directly. For benzothiazoles, which are most toxic on the basis of ecotoxicity data from aquatic environment and which are expected to show excess toxicity, PNECs were derived from tests (CBS, MBTS, MBT). Of these data, the lowest PNEC_{stp, microorg} was derived for MBT (1.0 mg/l) and it is applied for BTon.

4. Summary

BTon is not produced industrially. This compound origins from benzothiazole derivatives being used as vulcanization accelerators in rubber manufacture.

BTon is a content in rubber and tires. The substance is released into the environment by tire tread abrasion and by migration and leaching from the rubber matrix. Further exposure sources are the biological degradation of 2-mercaptobenzothiazole and benzothiazole which are both released by industrial sources and by tire tread abrasion.

The available studies on biodegradation allow no clear conclusion about the rate of mineralization in biological treatment plants. Under environmental conditions, degradation within weeks or months is expected.

In the environment, BTon is mainly distributed into the hydrosphere (99.1%). The calculated Koc value of 86 l/kg indicates a low sorption potential. The calculated BCF of 6.3 l/kg indicates low bioaccumulation potential.

There are toxicity tests on daphnids and algae available. On this basis provisional PNECs for the aquatic compartment (freshwater and marine) are estimated as PNEC_{water, freshwater} = 16.1 \mu g/l and PNEC_{water, marine} = 1.6 \mu g/l. Due to lack of toxicity data a PNEC for microorganisms cannot be estimated directly, but a PNEC_{stp, microorg} of MBT (1.0 mg/l) is applied as a conservative read-across solution.

The risk characterisation for the measured exposure is presented in the main report.
5. References


Legiec Ia and Kosson DS (1988). In-Situ Extraction of Industrial Sludges. Environ. Progress 7(4), 270-278


Weast (1979). CRC Handbook of Chemistry and Physics, 59th edition
APPENDIX E: 2-METHYLTHIOBENZOTHIAZOLE (MESBT)

1.1 Identification of the substance

Name: 2-Methylthiobenzothiazole

CAS No.: 615-22-5

Empirical Formula: C₈H₇NS₂

Molecular weight: 181.28

Structural Formula:

![Structural Formula of 2-Methylthiobenzothiazole](image)

1.2 Physico-Chemical Data

*Table 1.1: Physico-Chemical Data of MeSBT*

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>52°C</td>
<td>Brownlee et al. (1981)</td>
</tr>
<tr>
<td>Boiling point</td>
<td>310 °C</td>
<td>MPBPWIN v1.41</td>
</tr>
<tr>
<td>Solubility in water</td>
<td>125 mg/l (24°C)</td>
<td>Brownlee et al. (1992)</td>
</tr>
<tr>
<td>Vapour pressure</td>
<td>0.1024 Pa</td>
<td>MPBPWIN v1.41</td>
</tr>
</tbody>
</table>
2. Exposure

2.1 Sources

MeSBT is not manufactured by chemical industry. The compound is formed from chemicals mainly used as vulcanization accelerators in rubber manufacture.

Baumann & Ismeier (1998) measured MeSBT in the eluate of artificial tire abrasion particles (cf. Table 2.4). Abraded tire particles accumulate near roads, leading to an exposure of soils and surface waters in the vicinity. In the environment, the particles are degraded by biotic and abiotic processes, leading to the release of included compounds like MeSBT. In the soil near a highway the substance was measured in concentrations up to 9.15 mg/kg. Discharges of abraded particles into the hydrosphere occur via rainwater runoff from roads. Consequently, MeSBT was detected in highway runoff, in the drainage system of highways and in surface waters receiving road runoff.

A further source is biological methylation of 2-mercaptobenzothiazole (MBT) which is released into the environment by industrial sources (production of benzothiazole derivatives, rubber industry), by tire tread abrasion and from the use of MBT as biocide. This reaction is confirmed to occur both in industrial plants and in environmental samples. The studies on MBT methylation are described in appendix A section 2.3.

2-Mercaptobenzothiazole (MBT; CAS 149-30-4) has been notified under the Biocides Directive 98/8/EEC for the use in seven product groups, i.a. for the use in slimicides, wood preservatives and indoor disinfection of public buildings.

The biocide 2-(thiocyanomethylthio)benzothiazole (TCMTB; CAS 21564-17-0) is a further source for MeSBT exposure. TCMTB has been notified under the Biocides Directive 98/8/EEC for the use in ten product groups in the Commission Regulation 2003/2032/EC, i.a. for the use in antifouling paints, slimicides, and conservation products. No use for wood preservation has been notified against the earlier information according to which TCMTB is also used as a fungicide by tanneries (Brownlee et al., 1992 and Srour, 1994)

In aqueous media TCMTB hydrolyses to 2-mercaptobenzothiazole (MBT) which can be subsequently methylated. MeSBT was detected in tannery and paper mill waste waters (cf. Table 2.5).

Because of the low use amounts, the biocidal use of MTB and TCMTB appears to be of minor importance compared to the rubber chemicals. Exposure from these uses is not estimated in this assessment.

2.2 Degradation

Biodegradation

The biodegradation of 2-methylthiobenzothiazole (MeSBT) was studied in tannery waste waters (Reemtsma 1994; et al., 1995). An aerobic degradation test of MeSBT (2 litre batch systems with the addition of 115 mg/l yeast extract as an additional carbon source according to the ISO 9888), revealed that after 28 days MeSBT at an initial concentration of 9 mg/l was not further degradable. In a pilot treatment plant fed with wastewater from two tanneries, it was found that benzothiazole (BT) is formed from unknown precursors in the anaerobic treatment, whereas MeSBT is significantly diminished, and 2-mercaptobenzothiazole (MBT) is refractory against anaerobic treatment. By aerobic treatment, MBT and BT are substantially eliminated, while MeSBT is formed.
In conclusion, it was found that benzothiazole derivatives are not completely removable by biological treatment, and will be discharged into surface waters. Therefore, MeSBT was assessed also on model sediment columns at significantly lower concentrations simulating the mixture of surface water with industrial effluent (0.1 - 0.2 µmol/l level corresponding to 0.018 - 0.036 mg/l). Initially, MeSBT was adsorbed for a 20 day period, but was then displaced by other organic matter. Steady state was achieved after 70 days of MeSBT addition (Reemtsma 1994; et al., 1995).

Using soil columns to determine breakthrough profiles, Hutchins et al. (1984) could not detect any mineralization of MeSBT. By labelled test compound analysis only 0.4% of the label was mineralized, and even this amount may have resulted from degradation of labelled impurities rather than of the parent compound.

In other studies a partial oxidation of MeSBT was observed. Chudoba (1977) examined MeSBT degradation in adapted activated sludge. It was revealed by COD determination (values only shown in a graph) that MeSBT was partially oxidized. The reaction was considered to be chemically as oxidation also occurred without addition of inoculum.

Carey & Thomson (1983) found primary MeSBT degradation in an experiment with activated sludge from a facility manufacturing benzothiazole derivatives. Disappearance of the test substance was observed using GC/MS analysis. MeSBT reacted faster when fulvic acids were added. The reaction product was brightly yellow coloured, and the authors assume that the product might be a phenol or quinone.

Theoretical considerations resulted that MeSBT can be oxidized either at the phenyl ring or at the exocyclic sulphur, the latter leading to benzothiazol sulphenic acid methylester (MeSOBT) and benzothiazol sulphonic acid methylester (MeSOOBT). Schmegel (1995) detected both compounds in different samples collected in the city of Bremen (Germany), in most samples their concentrations were below the MeSBT level. Jop et al. (1991) identified an oxidation product of the phenyl ring (4- or 7-hydroxy-2-methylbenzothiazole) in an effluent of a chemical plant, the compound could not be quantified.

The available studies reveal that MeSBT is not mineralizable. Products of primary oxidation were detected in the environment, generally in lower concentrations than the parent substance. The poor degradability of MeSBT is supported by monitoring results (cf. Table 2.3) which demonstrate the ubiquitous distribution of MeSBT.

BIOWIN v4.02 predicts that the substance is not readily biodegradable but not persistent to biodegradation, either. The following estimates are provided: BIOWIN2 = 0.61, BIOWIN3 = 2.80 and BIOWIN6 = 0.10.

Photolysis

Brownlee et al. (1992) irradiated a MeSBT solution in phosphate buffer with sunlight. The test substance concentration remained unchanged after the equivalent of 5 cloudless summer days of solar irradiation. After addition of dissolved organic matter no photolysis products could be detected. The authors conclude that MeSBT is resistant to both direct and sensitized photolysis by sunlight.

2.3 Distribution
Henry’s law constant was calculated to give 0.146 Pa m³/mol, meaning that MeSBT is slightly volatile.

Using the octanol-water partitioning coefficient log Kow of 3.1 and the equation log Koc = 0.52 log Kow + 1.02 of the TGD, the Koc value is calculated to 429 l/kg, indicating a moderate sorption potential. The Kpsoil is calculated to 8.6 l/kg. PCKOCWIN v1.66 calculates a Koc of 3118 l/kg. Using the Koc of 429 l/kg, EUSES 2.0 calculates the fate in the (local) stp as follows: 0.3 % (air), 94.7 % (water), 5.1 % (sludge).

2.4 Accumulation

Based on the equation log BCFfish = 0.85 log Pow − 0.70 (Veith, et al. 1979), a BCF of 86 l/kg is calculated from the log Kow of 3.1. BCFWIN 2.15 gives a BCF of 49. The latter is used for modelling purposes (BCFWIN’s experimental database is larger and contains benzothiazoles).

Moderate accumulation in leeches was found in a field experiment (Metcalf et al., 1988). From measurements in a Canadian creek receiving waste water from a chemical facility and in three leech species, “BCF” values of 400 for *Dina dubia*, “200” for *Erpobdella punctata*, and “100” for *Helobdella stagnalis* were calculated. Depuration experiments resulted in half-lives of 1.5 days for *D. dubia*, 2.5 days for *E. punctata*, and <1 day for *H. stagnalis*. This study was strictly speaking measuring bioaccumulation, although uptake from water probably caused the largest part of the concentration difference between leeches and water.

2.5. Measured data

Throughout the world, MeSBT has been detected in all environmental compartments as well as in industrial emissions. Tables 2.1-2.5 present the data available.

*Table 2.1: Local concentration of MeSBT during CBS production (90-P or maximum)*

<table>
<thead>
<tr>
<th>Site</th>
<th>Ceffluent [µg/l]</th>
<th>Clocal [µg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt; 10</td>
<td>&lt;0.151</td>
</tr>
<tr>
<td>B</td>
<td>24</td>
<td>0.030</td>
</tr>
<tr>
<td>C</td>
<td>&lt; 10</td>
<td>&lt;0.026</td>
</tr>
</tbody>
</table>
Table 2.2: Monitoring of MeSBT in Rubber Products and Eluates

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluate from artificial tire tread</td>
<td>7.69-263 µg/l</td>
<td>25</td>
<td>6 different tires (automobile and truck tires, each new and old)</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Eluate from complete tires</td>
<td>&lt;0.2 µg/l</td>
<td>2</td>
<td>Sampled after 2 month exposure in a water bath</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
</tbody>
</table>
### Table 2.3: Monitoring of MeSBT Related to Industrial Sources and Wastewater

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wastewater of a chemical facility</td>
<td>10-50 µg/l</td>
<td>3</td>
<td>USA</td>
<td>Lopez-Avila &amp; Hites (1980)</td>
</tr>
<tr>
<td>Untreated tannery wastewater</td>
<td>39 µg/l</td>
<td></td>
<td>Origin: TCMTB</td>
<td>Fiehn et al. (1994)</td>
</tr>
<tr>
<td>Tannery wastewater</td>
<td>not quantified</td>
<td>3</td>
<td>Sampled in Spain</td>
<td>Castillo et al. (1999)</td>
</tr>
<tr>
<td>Treated paper mill waste water</td>
<td>25-35 µg/l</td>
<td>4</td>
<td>Sampled in USA</td>
<td>Keith (1976)</td>
</tr>
<tr>
<td>Pulp and paper mill wastewater</td>
<td>10 – 40 µg/l</td>
<td></td>
<td>Sampled in USA</td>
<td>Petermann et al. (1978):</td>
</tr>
<tr>
<td>Municipal effluent</td>
<td>0.193 µg/l</td>
<td>13</td>
<td>USA</td>
<td>Amato et al. (1992)</td>
</tr>
<tr>
<td>Municipal effluent</td>
<td>0.6-57 µg/l</td>
<td>14</td>
<td>3 treatment plants, USA</td>
<td>Clark et al. (1991)</td>
</tr>
<tr>
<td>Municipal treatment plant effluents</td>
<td>qualitat. det.</td>
<td>5</td>
<td>Country: Illinois (USA)</td>
<td>Ellis et al. (1982)</td>
</tr>
<tr>
<td>Municipal treatment plant effluents</td>
<td>qualitat. det.</td>
<td>5</td>
<td>Country: Germany</td>
<td>Elsäßer et al. (1992)</td>
</tr>
<tr>
<td>Municipal effluent</td>
<td>0.5 / 1.5 µg/l</td>
<td>2 plants</td>
<td>Country: Germany</td>
<td>Fooken et al. (1996)</td>
</tr>
<tr>
<td>Municipal waste water (influent)</td>
<td>0.17 µg/l (average; SD: 0.06)</td>
<td>20</td>
<td>Average for 20 composite samples of 24 h collected over three months in 2002 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.44 µg/l (average, SD: 0.09 µg/l)</td>
<td>20</td>
<td>Average of 20 composite samples of 24 h collected over three months in 2002 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (influent)</td>
<td>0.44 µg/l (average; SD: 0.39)</td>
<td>9</td>
<td>Average of 9 composite samples of 24 h collected over one month in 2003 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.36 µg/l (average; SD: 0.06 µg/l)</td>
<td>9</td>
<td>Average of 9 composite samples of 24 h collected over one month in 2003 (Berlin, Ruhleben, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---------------------------------</td>
<td>---</td>
<td>--------------------------------------------------------------------------------------------------------------------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.40 µg/l</td>
<td>1</td>
<td>Year 2003 (Berlin, Schönnerlinde, Germany)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (influent)</td>
<td>0.53 µg/l (average; SD: 0.15 µg/l)</td>
<td>3</td>
<td>Pooled samples (GaoBeiDian, Beijing, China)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
<tr>
<td>Municipal waste water (effluent)</td>
<td>0.55 µg/l (average; SD: 0.13 µg/l)</td>
<td>4</td>
<td>Pooled samples (GaoBeiDian, Beijing, China)</td>
<td>Klöpfer et al. (2005)</td>
</tr>
</tbody>
</table>
Table 2.4: Monitoring of MeSBT in Environmental Compartments. * = used for derivation of PECregional (see the main report).

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highway runoff</td>
<td>&lt;2-7.90 µg/l</td>
<td>8</td>
<td>Sampled during 3 rainfalls, from beginning to 30 min after beginning</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Snow near a road with high traffic density</td>
<td>&lt;2-15.9 µg/l</td>
<td>3</td>
<td>Sampled 14 days after snowfall</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Soil immediately near roads</td>
<td>&lt;0.5-9.15 mg/kg dw</td>
<td>10</td>
<td>Highest concentration detected near a highway</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Urban park lake</td>
<td>5.6 µg/l</td>
<td>3</td>
<td>Sampled in Bremen (Germany)</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Urban canalization sludge</td>
<td>60 µg/l</td>
<td>3</td>
<td>Sample from cleaning vehicle</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Road drainage, urban</td>
<td>0.14-2.29 µg/l</td>
<td>9</td>
<td>Sampled in Bremen (Germany)</td>
<td>Krumwiede &amp; Jastorff (2002)</td>
</tr>
<tr>
<td>Ditch with highway runoff</td>
<td>0.9 µg/l</td>
<td>3</td>
<td>Sampled in Germany</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Highway runoff</td>
<td>0.3 µg/l (average)</td>
<td>19</td>
<td>One rain event, traffic density ca. 200 000 vph, Berlin, Germany</td>
<td>Klöpfer (2005)</td>
</tr>
<tr>
<td></td>
<td>0.7 µg/l (90-P)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2-1.5 µg/l (min-max)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Municipal treatment plants</td>
<td>0.45-1.77 µg/l</td>
<td>6</td>
<td>Sampled in Bremen (Germany)</td>
<td>Krumwiede &amp; Jastorff (2002)</td>
</tr>
<tr>
<td>Municipal waste water</td>
<td>167 ng/l</td>
<td>20</td>
<td>Average for 20 composite samples of 24 h collected over three months</td>
<td>Kloepfer et al. (2004)</td>
</tr>
<tr>
<td>Landfill leachate</td>
<td>96 µg/l</td>
<td>3</td>
<td>Sampled in Germany</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Landfill leachate</td>
<td>0.6 µg/l</td>
<td></td>
<td>Sampled in Italy</td>
<td>Galassi &amp; Benfenati (2000)</td>
</tr>
<tr>
<td>Landfill leachate</td>
<td>0.1-1.2 ng/l</td>
<td>8</td>
<td>Country: Japan</td>
<td>Yasuhara et al. (1997)</td>
</tr>
<tr>
<td>River water*</td>
<td>&lt;4-44 ng/l</td>
<td>6 sites</td>
<td>River Elbe, Germany</td>
<td>IKSE (1997)</td>
</tr>
<tr>
<td>Medium</td>
<td>Concentrations</td>
<td>Number of Samples</td>
<td>Remark</td>
<td>Reference</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>---------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>River water*</td>
<td>10-380 ng/l each 6 samples from 7 sites</td>
<td></td>
<td>River Elbe, Germany</td>
<td>ARGE Elbe (1996); Fooken et al. (1996)</td>
</tr>
<tr>
<td>River water*</td>
<td>27-71 ng/l detected at 6 from 8 sites</td>
<td></td>
<td>River Rhine, The Netherlands</td>
<td>Hendriks et al. (1994)</td>
</tr>
<tr>
<td>River water*</td>
<td>9 ng/l</td>
<td></td>
<td>River Meuse</td>
<td>Hankemeier et al. (1999)</td>
</tr>
<tr>
<td>River water</td>
<td>1-3 µg/l</td>
<td>3</td>
<td>USA</td>
<td>Lopez-Avila &amp; Hites (1999)</td>
</tr>
<tr>
<td>River water</td>
<td>not quantified</td>
<td></td>
<td>Delaware River, Philadelphia, USA</td>
<td>Brownlee et al. (1981)</td>
</tr>
<tr>
<td>River water</td>
<td>not quantified</td>
<td></td>
<td>Haw River, USA</td>
<td>Dietrich et al. (1988)</td>
</tr>
<tr>
<td>River water</td>
<td>0.378 / 0.081 µg/l</td>
<td></td>
<td>Japan</td>
<td>Koroma et al. (2001)</td>
</tr>
<tr>
<td>Creek water</td>
<td>23.27 µg/l</td>
<td>3</td>
<td>Creek receives wastewater from a chemical facility</td>
<td>Metcalfe et al. (1988)</td>
</tr>
<tr>
<td>River sediment of Great Lakes tributaries (USA)</td>
<td>&lt;0.1 mg/kg dw</td>
<td>5</td>
<td>Identified in 1 of 5 river sed.</td>
<td>Fabacher et al. (1991)</td>
</tr>
<tr>
<td>Seawater</td>
<td>0.04-1.06 ng/l (1990) 0.044-1.37 ng/l (1995)</td>
<td></td>
<td>North Sea, German Bight</td>
<td>Bester et al. (1997)</td>
</tr>
<tr>
<td>Seawater</td>
<td>2-17 ng/l</td>
<td></td>
<td>Antarctica</td>
<td>Desideri et al. (1989)</td>
</tr>
<tr>
<td>Groundwater from land application of domestic wastewater</td>
<td>0.03-0.15 µg/l detected at 2 from 4 sites</td>
<td></td>
<td></td>
<td>Hutchins et al. (1983); Hutchins &amp; Ward (1984)</td>
</tr>
<tr>
<td>Bank filtrate</td>
<td>“main component”, conc. not reported</td>
<td></td>
<td>River Rhine, Germany</td>
<td>Kölle et al. (1972)</td>
</tr>
</tbody>
</table>
### Table 2.5: Monitoring of MeSBT in Food and Biota

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drinking water qualit. det.</td>
<td></td>
<td></td>
<td>Sampled in Cincinnati (USA)</td>
<td>Coleman et al. (1980)</td>
</tr>
<tr>
<td>Leeches (3 species)</td>
<td>2.2-10.9 MG/KG</td>
<td>6</td>
<td>Collected in a creek receiving wastewater from a chemical facility</td>
<td>Metcalfe et al. (1988)</td>
</tr>
<tr>
<td>Cheese</td>
<td>qualit. det.</td>
<td></td>
<td>ORIGIN: ITALY</td>
<td>Meinhart &amp; Schreier (1986)</td>
</tr>
<tr>
<td>Endive (Cichorium endivia)</td>
<td>qualit. det.</td>
<td></td>
<td>Origin: Germany</td>
<td>Götz-Schmidt &amp; Schreier (1986)</td>
</tr>
<tr>
<td>Guava (Psidium guajava)</td>
<td>qualit. det.</td>
<td></td>
<td>Origin: Brazil</td>
<td>Idstein &amp; Schreier (1985)</td>
</tr>
<tr>
<td>Black tea</td>
<td>qualit. det.</td>
<td></td>
<td></td>
<td>Vitzthum et al. (1975)</td>
</tr>
</tbody>
</table>
3. Effects Assessment

3.1 Aquatic Compartment

3.1.1 Toxicity to Invertebrates

Geurts & Kluskens (2004a) conducted a 48h acute immobilisation test with *Daphnia magna* under static conditions, basically according to OECD Test Guideline 202 and EEC Test Method C.2. MeSBT concentrations were measured at test initiation and after 48h in samples from the lowest, one intermediate, and the highest test concentration. All measured MeBTS concentrations ranged between 83 and 97% of the nominal concentrations.

The water fleas reacted with a quite steep concentration response curve, with no effects in the first test concentration (3.3 mg/l), 30% immobilisation in the second concentration level (7.29 mg/l), and 100% immobilisation in the three highest concentration levels (16.04 mg/l, 35.38 mg/l, and 77.76 mg/l, respectively). The 48h EC50 of 8.5 mg/l was calculated using the trimmed Spearman-Kärber method.

3.1.2 Toxicity to Algae

Geurts & Kluskens (2004b) conducted an algal growth inhibition test with *Pseudokirchneriella subcapitata*, basically according to OECD Test Guideline 201 and EEC Test Method C.3. MeBTS concentrations were measured at test initiation and after 48h in samples from the lowest, one intermediate, and the highest test concentration. All measured MeSBT concentrations ranged between 87 and 92% of the nominal concentrations.

The results of this study have to be used with special care as the control cultures did not grow exponentially. There was only limited growth during the first day (growth rate 0.88/d) and significantly different growth rates were measured during the second day (2.5/d) compared to the third day (1.5/d) of the test. A re-evaluation of the raw data was done for the period 24h – 72h, resulting in higher ErC20 (ca. 3.1 mg/l) and ErC50 (ca. 6.1 mg/l) than the corresponding values provided in the study report.

For the effects assessment, the reported lower values ErC20 = 1.44 mg/L and ErC50 = 3.43 mg/L are considered sufficiently protective and provisionally used with reservation.

3.1.4. Quantitative Structure-Activity Relationships (QSARs)

Data on benzothiazole derivatives have so far not been used for the development of QSAR-models. MeSBT’s ecotoxicity was estimated with ECOSAR v0.99h (U.S.EPA, 2004), according to the classification scheme of Verhaar et al. (1992) and with the model of Pavan et al. (2005). The results are presented in Table 3.1.

MeSBT is a non-polar substance, and ECOSAR v0.99h automatically allocates it to the group of neutral organics (baseline toxicity).

The second way of estimation applies the approach of Verhaar et al. (1992). According to their guidance, MeSBT can be assigned to the class 1 (non-polar substances) meaning that no excess toxicity is expected. The baseline toxicity was calculated using the models of Verhaar et al. (1995) and Van Leeuwen et al. (1992) as recommended in the Technical Guidance Document (Part III, Section 4.1, Table 1).

Finally, the acute toxicity to fish was calculated for a comparison using the model of Pavan et al. (2005):
LogLC50 = - 0.574 LogKow + 0.454 ELUMO - 2.445,

where ELUMO is the energy of the lowest unoccupied molecular orbital. The equation is an adjusted version from the original model of Veith and Mekeneyan (1993) for “mixed” mode of action. The model was developed for aromatic substances which are considered to act by several modes of action including narcosis and unspecific reactivity due to electrophilic or nucleophilic reactions.

Table 3.1. QSAR –estimates for aquatic ecotoxicity.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Acute</th>
<th>Chronic</th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>12.4</td>
<td>1.9</td>
<td>17.1</td>
<td>6.76</td>
</tr>
<tr>
<td>Daphnids</td>
<td>14.4</td>
<td>1.2</td>
<td>9.8</td>
<td></td>
</tr>
<tr>
<td>Algae</td>
<td>9.6</td>
<td>1.8</td>
<td>8.5</td>
<td></td>
</tr>
</tbody>
</table>

(1) These QSARs are for neutral organics and they have been used as the starting point for the approach of Verhaar et al. (1992)

(2) The used ELUMO = -0.45 eV (Schmegel 1995)

The baseline-QSARs predicted ecotoxicity in the level of the two available studies. The QSARs indicate algae to be the most sensitive group.

3.1.3 Determination of the PNECwater

Available test data from a 48h daphnia immobilisation test and from an algal growth inhibition study allow a provisional PNEC derivation. Reservation regards the lack of any study with fish as a third trophic level and the limited validity of the algae test. ECOSAR v0.99 results do not give further guidance on which of the trophic levels is most sensitive.

However, read-across to other CBS degradation products and CBS, in particular to the more reliable test data obtained with the sufficiently water soluble benzothiazole derivatives, provide strong evidence that there is no specific toxicity to any trophic level’s standard test species. In all cases with test concentrations well below the limits of water solubility, the factors between the highest and the lowest E(L)C50 were < 10. Regarding the most sensitive EC-values, no trophic level’s standard test species prevails. Used EC-values from the weak algal study are hardly backed by re-evaluation of the raw data, but as lower figures considered sufficiently protective.

In conclusion it appears justifiable to provisionally derive aquatic PNECs, applying assessment factors of 1,000 for the freshwater PNEC and 10,000 for the marine PNEC. The acute daphnia immobilisation test (Geurts & Kluskens 2004a) resulted in a 48h EC50 = 8.5mg/l (effective). The algal growth inhibition test (Geurts & Kluskens 2004b) resulted in an ErC50 of 3.43mg/l (effective). Therefore,

\[
PNEC_{freshwater} = \frac{3.43 \text{ mg/l}}{1,000} = 3.4 \mu g/l
\]

and

\[
PNEC_{marine} = \frac{3.43 \text{ mg/l}}{10,000} = 0.34 \mu g/l
\]
3.1.4 Toxicity to Microorganisms

There are no valid tests on microorganisms available, therefore a PNECmicroorg. cannot be estimated directly. For benzothiazoles, which are most toxic on the basis of ecotoxicity data from aquatic environment and which are expected to show excess toxicity, PNECs were derived from tests (CBS, MBTS, MBT). Of these data, the lowest PNECstp,microorg was derived for MBT (1.0 mg/l) and it is applied for MeSBT.
4. Summary

MeSBT is not produced industrially. This compound originates from benzothiazole derivatives being used as vulcanization accelerators in rubber manufacture.

MeSBT can be found in rubber and tires. The substance is released into the environment by tire tread abrasion and by migration and leaching from the rubber matrix. A further exposure source is the biological methylation of 2-mercaptobenzothiazole which is released by industrial sources and by tire tread abrasion. The use of 2-(thiocyanomethylthio)benzothiazole (TCMTB) and 2-mercaptobenzothiazole (MBT) as a biocides is of minor importance regarding exposure.

The available studies reveal that MeSBT is not mineralizable. Products of primary oxidation are benzothiazolsulphenic acid methylester (MeSOBT) and benzothiazolsulphonic acid methylester (MeSO₂BT), both compounds were detected in the environment. In the hydrosphere MeSBT is resistant to photolysis by sunlight.

With a calculated Koc of 429 l/kg the substance has a moderate adsorption potential in soils or sediment solids and with the Henry’s law constant of 0.12 Pa m³/mol it is slightly volatile. A bioconcentration factor of 49 has been estimated.

MeSBT was detected in the waste water of chemical facilities. MeSBT is ubiquitous in the aquatic environment in concentrations which are mainly in the ng/l range.

There are toxicity tests on daphnids and algae available. On this basis provisional PNECs for the aquatic compartment (freshwater and marine) are estimated as PNECwater,freshwater = 3.4 µg/l and PNECwater,marine = 0.34 µg/l. Due to lack of toxicity data a PNEC for microorganisms cannot be estimated directly, but a PNECstp,microorg of MBT (1.0 mg/l) is applied as a conservative read-across solution.

The risk ratios based on measured data are presented in the main report.
5. References


ARGE Elbe (1996): Sonderuntersuchungsprogramm


Meinhart E and Schreier P (1986). Study of flavour compounds from Parmigiano Reggiano cheese. Milchwissenschaft 41(11), 689-691


Platford RF (1983). The octano-water partitioning of some hydrophobic and hydrophilic compounds. Chemosphere 12(7/8), 1107-1111


APPENDIX F: 2-METHYLBENZOTHIAZOLE (MEBT)

1.1 Identification of the substance

Name: 2-Methylbenzothiazole
CAS Nr.: 120-75-2
Empirical Formula: C₈H₇NS
Molecular weight: 149.22
Structural Formula:

```
\text{N} \\
\text{S} \\
\text{CH₃}
```

1.2 Physico-Chemical Data

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melting point</td>
<td>12-15.4°C</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Boiling point</td>
<td>266 °C</td>
<td>MPBPWIN v1.41</td>
</tr>
<tr>
<td>Solubility in water (calc.)</td>
<td>513 mg/l</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Log Kow (calc.)</td>
<td>2.47 *</td>
<td>Schmegel (1995)</td>
</tr>
</tbody>
</table>

* mean value from different calculation methods
2. Exposure

2.1 Sources

MeBT is not manufactured by chemical industry. The compound is formed from chemicals used as vulcanization accelerators in rubber manufacture.

MeBT was found to be formed during vulcanization in rubber goods manufacture. Badura et al. (1989) analyzed vulcanization fumes directly at production sites and found MeBT as a volatile breakdown product of N-cyclohexylbenzothiazole-2-sulphenamide (CBS). MeBT was also found in waste water of two sites producing CBS (see Table 2.1).

Table 2.1. Local concentrations for MeBT during CBS production (90-P or maximum)

<table>
<thead>
<tr>
<th>Site</th>
<th>Ceffluent [µg/l]</th>
<th>Clocal [µg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&lt;10</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>B</td>
<td>29</td>
<td>0.04</td>
</tr>
<tr>
<td>C</td>
<td>&lt;10</td>
<td>0.026</td>
</tr>
</tbody>
</table>

Baumann & Ismeier (1998) measured MeBT in the eluate of artificial tire abrasion particles (cf. table 2.2). Abraded tire particles accumulate near roads, leading to an exposure of soils in the vicinity. In the soil near a highway the substance was measured in concentrations up to 4.62 mg/kg. Discharges of abraded particles into the hydrosphere are expected via rainwater runoff from roads. MeBT was not found (detection limit 2 µg/l) in the runoff of a highway but was detected in the snow near a highly frequented road 14 days after snowfall. Further monitoring data in environmental compartments are referred in table 2.4.

MeBT was isolated from fermentation culture extracts of Micrococcus sp., a marine bacterium obtained from tissues of the sponge Tedania ignis (Stierle et al., 1991). Compared to the rubber chemicals, this source appears to be of minor importance. Vitzthum et al. (1975) found MeBT in black tea but the amount was not quantified. The study focused on finding aroma compound in tea and hence it was not further analysed whether MeBT was of natural or of anthropogenic origin.
### Table 2.2: Monitoring of MeBT in Rubber Products and Eluates

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluate from artificial tire tread</td>
<td>&lt;2-254 µg/l</td>
<td>25</td>
<td>6 different tires (automobile and truck tires, each new and old)</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Eluate from complete tires</td>
<td>&lt;0.2 µg/l</td>
<td>2</td>
<td>Sampled after 2 month exposure in a water bath</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
</tbody>
</table>

### Table 2.3: Monitoring of MeBT Related to Industrial Sources and Wastewater

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Municipal effluent</td>
<td>4 µg/l</td>
<td>1</td>
<td>Identified in 1 of 3 treatment plants, USA</td>
<td>Clark et al. (1991)</td>
</tr>
<tr>
<td>Industrial effluent</td>
<td>≤167 µg/l</td>
<td></td>
<td>Detected in 7 of 26 samples of 7 plants</td>
<td>Gaffney (1976)</td>
</tr>
</tbody>
</table>
Table 2.4: Monitoring of MeBT in Environmental Compartments

<table>
<thead>
<tr>
<th>Medium</th>
<th>Concentrations</th>
<th>Number of Samples</th>
<th>Remark</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highway runoff</td>
<td>&lt;2 µg/l</td>
<td>8</td>
<td>Sampled during 3 rainfalls, from beginning to 30 min after beginning</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Snow near a highly frequented road</td>
<td>&lt;2-14.2 µg/l</td>
<td>3</td>
<td>Sampled 14 days after snowfall</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Soil immediately near roads</td>
<td>&lt;0.5-4.63 mg/kg dw</td>
<td>10</td>
<td>Highest concentration detected near a highway</td>
<td>Baumann &amp; Ismeier (1998)</td>
</tr>
<tr>
<td>Urban park lake</td>
<td>0.6 µg/l</td>
<td>3</td>
<td>Sampled in Bremen (Germany)</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Urban canalization sludge</td>
<td>60 µg/l</td>
<td>3</td>
<td>Sample from cleaning vehicle</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>River water</td>
<td>not quantified</td>
<td></td>
<td>Delaware River, USA</td>
<td>Brownlee et al. (1981)</td>
</tr>
<tr>
<td>River water</td>
<td>&lt; 1 ng/l</td>
<td>2 rivers</td>
<td>Japan</td>
<td>Koroma et al. (2001)</td>
</tr>
<tr>
<td>River sediment of Great Lakes tributaries (USA)</td>
<td>1 mg/kg dw</td>
<td>1</td>
<td>Identified in 1 of 5 river sed.</td>
<td>Fabacher et al. (1991)</td>
</tr>
<tr>
<td>Landfill leachate</td>
<td>80 µg/l</td>
<td>3</td>
<td>Sampled in Germany</td>
<td>Schmegel (1995)</td>
</tr>
<tr>
<td>Landfill leachate</td>
<td>2.8 ng/l</td>
<td>8</td>
<td>Country: Japan</td>
<td>Yasuhara et al. (1997)</td>
</tr>
<tr>
<td>Landfill leachate</td>
<td>0.08 µg/l</td>
<td></td>
<td>Sampled in Italy</td>
<td>Galassi &amp; Benfènati (2000)</td>
</tr>
</tbody>
</table>
2.2 Degradation
There are no tests available on both biological and abiotical degradation, therefore a conclusion about the degradability is not possible. BIOWIN v4.02 predicts that the substance is not readily biodegradable but not persistent to biodegradation, either. The following estimates are provided: BIOWIN 2 = 0.81, BIOWIN 3 = 2.79 and BIOWIN 6 = 0.27.

2.3 Distribution
With the physico-chemical data presented in section 1.2 and the equations provided by the Technical Guidance Documents, the following distribution parameter are calculated:

<table>
<thead>
<tr>
<th>Henry’s law constant</th>
<th>2.75 Pa m³/mol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koc</td>
<td>201 l/kg</td>
</tr>
<tr>
<td></td>
<td>4.0 l/kg</td>
</tr>
<tr>
<td>Kpsoil</td>
<td>201 l/kg</td>
</tr>
<tr>
<td></td>
<td>4.0 l/kg</td>
</tr>
<tr>
<td>Mackay level I</td>
<td>Air 47.7%</td>
</tr>
<tr>
<td></td>
<td>Water 50.1%</td>
</tr>
<tr>
<td></td>
<td>Soil 1.17%</td>
</tr>
<tr>
<td></td>
<td>Sediment 1.09%</td>
</tr>
<tr>
<td></td>
<td>Susp. Sed. 0.002%</td>
</tr>
<tr>
<td></td>
<td>Fish 0.0007%</td>
</tr>
</tbody>
</table>

The distribution in a “unit world” according to the Mackay fugacity model level I based on the physico-chemical properties listed in table 1.1 reveal that the main target compartments of MeBT are water (50.1%) and the atmosphere (47.7%).

The calculated Henry’s law constant of 2.75 Pa m³/mol indicates that volatilization can be a significant removal mechanism from the hydrosphere.

From the octanol/water partitioning coefficient log Kow of 2.47 and the equation log Koc = 0.52 log Kow + 1.02 (TGD, 1996) the Koc value is calculated to 201 l/kg, indicating a moderate sorption potential.

2.4 Accumulation
Based on the equation log BCFfish = 0.85 log Pow – 0.70 (Veith et al 1979), BCF of 25 l/kg is calculated from the log Kow of 2.47. BCFWIN 2.15 gives a BCF of 16 l/kg. Experimental data are not available.

3. Effects
3.1 Aquatic Compartment
3.1.1 Toxicity to Invertebrates
Geurts & Kluskens (2004a) conducted a 48h acute immobilisation test with Daphnia magna under static conditions, basically according to OECD Test Guideline 202 and EEC Test Method C.2. MeBT concentrations were measured at test initiation and after 48h in samples
from the lowest, one intermediate, and the highest test concentration. All measured MeBT concentrations ranged between 89 and 99% of the nominal concentrations.

The water fleas reacted with a quite steep concentration response curve, with no effects up to the fourth test concentration level (16.48 mg/l), 75% immobilisation in the fifth concentration level (36.36 mg/l), and 100% immobilisation in the highest concentration level (79.2 mg/l). The 48h EC50 of 29.8 mg/l was calculated using the trimmed Spearman-Kärber method.

3.1.2 Toxicity to Algae

Geurts & Kluskens (2004b) conducted an algal growth inhibition test with *Pseudokirchneriella subcapitata*, basically according to OECD Test Guideline 201 and EEC Test Method C.3. MeBT concentrations were measured at test initiation and after 48h in samples from the lowest, one intermediate, and the highest test concentration. All measured MeBT concentrations ranged between 93 and 98% of the nominal concentrations.

The results of this study have to be used with special care as the control cultures did not grow exponentially. There was no measurable growth during the first day and significantly different growth rates during the second day (2.2/d) compared to the third day (1.3/d) of the test. A re-evaluation of the raw data was done for the period 24h – 72h. The resulting EC20 value (ca. 22 mg/l) is higher than the corresponding 11.65 mg/l provided in the study report. Due to inconsistent inhibition percentages in the lower test concentration range, especially the EC50 derived with the applied calculation method (45.2 mg/l) does not appear reliable enough for risk assessment purposes. According to re-evaluation of the raw data and expert judgement, an EC50 = ca. 32 mg/l is considered more defensible.

For the effects assessment, the reported EC20 = 11.65 mg/L and the re-evaluated EC50 = 32 mg/L are provisionally used with reservation.

3.1.4. Quantitative Structure-Activity Relationships (QSARs)

Data on benzothiazole derivatives have so far not been used for the development of QSAR-models. MeBT’s ecotoxicity was estimated with ECOSAR v0.99h (U.S.EPA, 2004), according to the classification scheme of Verhaar et al. (1992) and with the model of Pavan et al. (2005). The results are presented in Table 3.1.

MeBT is a non-polar substance, and ECOSAR v0.99h automatically allocates it to the group of neutral organics (baseline toxicity).

The second way of estimation applies the approach of Verhaar et al. (1992). According to their guidance, MeBT can be assigned to the class 1 (non-polar substances) meaning that no excess toxicity is expected. The baseline toxicity was calculated using the models of Verhaar et al. (1995) and Van Leeuwen et al. (1992) as recommended in the Technical Guidance Document (Part III, Section 4.1, Table 1).

Finally, the acute toxicity to fish was calculated for a comparison using the model of Pavan et al. (2005):

\[
\text{LogLC50} = - 0.574 \text{LogKow} + 0.454 \text{ELUMO} - 2.445,
\]

where \text{ELUMO} is the energy of the lowest unoccupied molecular orbital. The equation is an adjusted version from the original model of Veith and Mekeneyan (1993) for “mixed” mode of action. The model was developed for aromatic substances which are considered to act by
several modes of action including narcosis and unspecific reactivity due to electrophilic or nucleophilic reactions.

Table 3.1. QSAR –estimates for aquatic ecotoxicity.

<table>
<thead>
<tr>
<th>Organism</th>
<th>ECOSAR v0.99h</th>
<th>TGD Part III, Section 4.1, Table 1(1) Pavan et al. (2005) (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acute L(E)C50 (mg/l)</td>
<td>Chronic L(E)C50 (mg/l)</td>
</tr>
<tr>
<td>Fish</td>
<td>40</td>
<td>5.6</td>
</tr>
<tr>
<td>Daphnid</td>
<td>44.3</td>
<td>2.8</td>
</tr>
<tr>
<td>Algae</td>
<td>28.4</td>
<td>3.7</td>
</tr>
</tbody>
</table>

(1) These QSARs are for neutral organics and they have been used as the starting point for the approach of Verhaar et al. (1992)
(2) The used $E_{LUMO} = -0.36$ eV (Schmegel 1995)

The baseline-QSARs predicted ecotoxicity approximately in the level of the two available studies. The QSARs indicate algae to be the most sensitive group.

3.1.3 Determination of the PNECwater

Available test data from a 48h daphnia immobilisation test and from an algal growth inhibition study allow a provisional PNEC derivation. Reservation regards the lack of any study with fish as a third trophic level and the limited validity of the algae test.

However, read-across to other CBS metabolites and CBS, in particular to the more reliable test data yielded with the sufficiently water soluble metabolites, provide strong evidence that there is no specific toxicity to any trophic level’s standard test species. In all cases with test concentrations well below the limits of water solubility, the factors between the highest and the lowest E(L)C50 were < 10. Regarding the most sensitive EC-values, no trophic level’s standard test species prevails. The raw data of the weak algal study have been re-evaluated and the resulting lower E,C50 was considered sufficiently protective and is used in the risk assessment.

In conclusion it appears justifiable to provisionally derive aquatic PNECs, applying assessment factors of 1,000 for the freshwater PNEC and 10,000 for the marine PNEC. Re-evaluation of the algal growth inhibition test (Geurts & Kluskens 2004b) resulted in an E,C50 of ca. 32 mg/l (effective). The acute daphnia immobilisation test (Geurts & Kluskens 2004a) resulted in a 48h EC50 = 29.8 mg/l (effective). Therefore,

$$\text{PNEC}_{\text{freshwater}} = 29.8 \text{ mg/l} / 1,000 = 29.8 \mu\text{g/l}$$

and

$$\text{PNEC}_{\text{marine}} = 29.8 \text{ mg/l} / 10,000 = 3 \mu\text{g/l}$$

3.1.4 Toxicity to Microorganisms

There are no valid tests on micro-organisms available, therefore a PNECmicro-org cannot be estimated directly. For benzothiazoles, which are most toxic on the basis of ecotoxicity data
from aquatic environment and which are expected to show excess toxicity, PNECs were derived from tests (CBS, MBTS, MBT). Of these data, the lowest PNECstp,microorg was derived for MBT (1.0 mg/l) and it is applied for MeBT.

4. Summary

MeBT is not produced industrially. This compound origins from benzothiazole derivatives being used as vulcanization accelerators in rubber manufacture.

MeBT is contained in rubber and tires. The compound was measured in the exhaust gas of vulcanization, therefore releases into the atmosphere during rubber manufacture are expected.

The substance is released into the environment by tire tread abrasion and by migration and leaching from the rubber matrix. MeBT was quantified in soils and snow near highly frequented roads.

In the environment, MeBT is mainly distributed into the hydrosphere (50.1%) and the atmosphere (47.7%). The calculated Koc value of 201 l/kg indicate a moderate sorption potential. The calculated bioconcentration factor (BCF) is 16 l/kg.

There are no experimental data about degradation available.

There are toxicity tests on daphnids and algae available. On this basis provisional PNECs for the aquatic compartment (freshwater and marine) are estimated as PNECfreshwater = 29.8 µg/l and PNECmarine = 3.0 µg/l. Due to lack of toxicity data a PNEC for microorganisms cannot be estimated directly, but a PNECstp,microorg of MBT (1.0 mg/l) is applied as a conservative read-across solution.

Risk characterisation of the measured exposure is presented in the main report.
5. References


Gaffney (1976). J. Water Pollut. Control Federation 48, 2590-2598


The report provides the comprehensive risk assessment of the substance N-Cyclohexylbenzothiazol-2-sulphenamide. It has been prepared by Germany in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to man and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined. For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The environmental risk assessment for CBS concludes that there is a need for further information on risks from breakdown products of CBS to the aquatic and terrestrial compartments for tyre recycling activities, tyre abrasion and landfills. There is no concern for the environment from CBS production and use of CBS in the rubber industry.

For human health, there is concern for workers, for which further information and/or testing is needed, but no concern is expressed for consumers and for humans exposed via the environment.