

European Union Risk Assessment Report
BIS(HYDROXYLAMMONIUM)SULPHATE

CAS No: 10039-54-0

EINECS No: 233-118-8

RISK ASSESSMENT

FINAL APPROVED VERSION

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Luxembourg: Office for Official Publications of the European Communities, [ECB:
year]
ISBN [ECB: insert number here]

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Printed in Italy

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CAS No: 10039-54-0

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RISK ASSESSMENT

only environment

Final report, May 2008

Germany

FINAL APPROVED VERSION

Rapporteur for the risk assessment of Bis(hydroxylammonium)sulphate is Germany

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Date of Last Literature Search : [insert year]
Review of report by MS Technical Experts finalised: [insert month and year]
Final report: [year]

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) 793/93¹ 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) 1488/94², which is supported by a technical guidance document³. 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.

¹ O.J. No L 084, 05/04/199 p.0001 – 0075

² O.J. No L 161, 29/06/1994 p. 0003 – 0011

³ Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT⁴

CAS Number: 10039-54-0
EINECS Number: 233-118-8
IUPAC Name: Bis(hydroxylammonium)sulphate

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.

Conclusion (ii) applies to releases into water, soil and air by production of bis(hydroxylammonium)sulphate and processing at the production sites. In addition this conclusion can also be drawn for releases into air and soil resulting from the use of the substance.

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

Conclusion (i) applies to releases into the aquatic environment resulting from all uses of bis(hydroxylammonium)sulphate. Refinement with more specific data might be possible in all cases. Since it is no longer possible to submit this information under the Existing Substances Regulation, it is proposed to give further consideration to the substance under the REACH regulation.

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.

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EUSES Calculations can be viewed as part of the report at the website of the European Chemicals Bureau:
<http://ecb.jrc.it>

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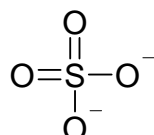
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1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 10039-54-0
 EINECS Number: 233-118-8
 IUPAC Name: Bis(hydroxylammonium)sulphate
 Molecular formula: $\text{H}_8\text{N}_2\text{O}_6\text{S}$
 Structural formula:



Molecular weight: 164.14 g/mol
 Synonyms: Hydroxylammonium sulfate (HAS)
 CA-Index-name: Hydroxylamine, sulfate (2:1) (salt)

1.2 PURITY/IMPURITIES, ADDITIVES

Purity: ca. 99 % w/w
 Impurities: < 0.1 % water
 < 1 % ammonium sulfate
 < 2.5 % sulfuric acid
 Additives: none

1.3 PHYSICO-CHEMICAL PROPERTIES

Bis(hydroxylammonium)sulphate is a white crystalline powder with a typical odour. The substance is water-soluble and only of very low solubility in organic solvents. Data on the physical and chemical properties are given in the following table:

Table 1-1 Summary of physico-chemical properties (experimentally determined)

Property	Value	Reference
Physical state	at 20 °C, 1013 hPa: white crystalline powder	
Melting point	decomposition above 120 °C ¹⁾	Sorbe, 1996
Boiling point	not applicable	
Relative density	1.883 at 20 °C	BASF AG, 1986
Vapour pressure	not determined	no test conducted because of the salt character
Water solubility	587 g/l at 20 °C ²⁾	BASF AG, 1972
Partition coefficient n-octanol/water (log value)	Log Pow -3.6 at pH 3.2 and 20 °C ³⁾	BASF AG, 1997
Dissociation constant	pKa 5.8	Hollemann-Wiberg, 1976
Conversion factors		
Flash point	not determined	substance is a solid
Autoflammability	no selfignition up to decomposition (120 °C)	Chemsafe, 2001
Flammability	non flammable ⁴⁾	Chemsafe, 2001
Explosive properties	explosive	BAM, 1980
Oxidizing properties	no oxidising properties	no test conducted because of structural reasons
Viscosity		
Henry's constant		
Surface tension	not determined	not relevant for the risk assessment

¹⁾ At temperatures above 130 °C the substance decomposes explosion likely

²⁾ The results for the water solubility found in the literature differed considerably. Sorbe for example cited a water solubility of 685 g/l at 25 °C without further information regarding the test substance and test method. It can be stated that there is a high temperature dependence of the solubility of Bis(hydroxylammonium)sulphate.

³⁾ The shaking flask method was used. While there is a protolytic balance the partition coefficient depends from the pH value considerably.

⁴⁾ Tests according to A.12 and A.13 were not conducted because of structural reasons.

In aqueous environment, Bis(hydroxylammonium)sulphate is expected to dissociate to $[\text{NH}_3\text{OH}]^+$ and $[\text{SO}_4]^{2-}$. The hydroxyl-ammonium ion is converted to hydroxylamine (CAS-No. 7803-49-8). The free hydroxylamine base is very reactive and is expected to decompose further by abiotic processes and nitrification. The expected degradation products are ammonia, nitrogen and water.

Table 1-2 Summary of physico-chemical properties of hydroxylamine

Property	Value	
Physical state	at 20 °C, 1013 hPa: large white flakes or needles	Merck , 1983
Melting point	33 °C	Merck , 1983
Boiling point	58 °C	Merck , 1983
Relative density	1.204 at 20 °C	Merck , 1983
Vapour pressure	7.1 kPa at 32 °C	Ullmann, 2002
Water solubility	Readily soluble	Ullmann, 2002
Partition coefficient-octanol/water (log value)	logPow -1.5	IPCS, CEC 2005
Explosive properties	May explode on heating at 129 °C	IPCS, CEC 2005

1.4 CLASSIFICATION

Bis(hydroxylammonium)sulphate is included in Annex I of Directive 67/548/EEC (30th ATP) and classified for environmental effects : N, R 50. (Very toxic to aquatic organisms)

The complete classification is:

E; R2
 Carc. Cat. 3; R40
 Xn; R21/22-48/22
 Xi; R36/38
 R43
 N; R50

And the labelling:

E; Xn; N
 R: 2-21/22-36/38-40-43-48/22-50
 S: (2-)36/37-61

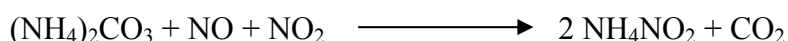
2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

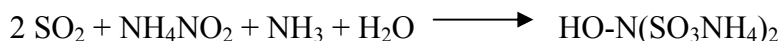
2.1.1 Production processes

Bis(hydroxylammonium)sulphate (HAS) can be produced by two methods: The Raschig process and a catalytic hydrogenation of nitric oxide.

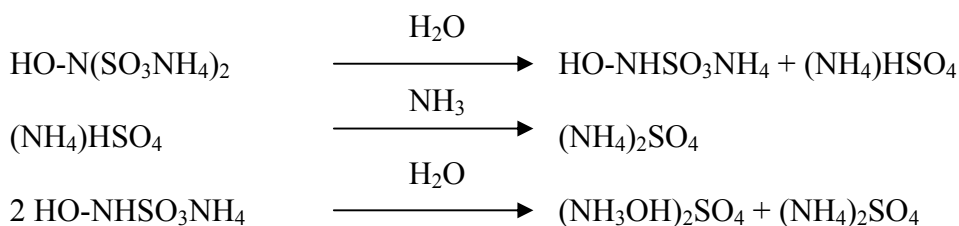
In the Raschig process, water, ammonia, and carbon dioxide react together in an absorption column. The product is a solution of ammonium carbonate, which forms an alkaline solution of ammonium nitrite with nitrogen oxides at low temperatures.



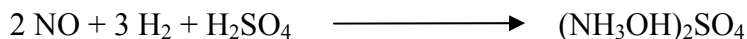
In a further step, the ammonium nitrite is converted to ammonium hydroxylamine disulfonate with sulfur dioxide.



The ammonium hydroxylamine disulfonate is then drawn off and the salt is hydrolyzed and neutralized. The final product are bis(hydroxylammonium)sulphate and ammonium sulphate.



For many years the Raschig process was the main production method. In the 1950s and 60s, the Raschig method was replaced by the catalytic hydrogenation process. In this method, purified nitric oxide is converted to hydroxylamine by reaction with hydrogen below 50 °C over a suspension of partially poisoned platinum catalyst in sulfuric acid.



In contrast to the Raschig process, only small amounts of ammonium as by-product were produced from the catalytic hydrogenation method.

2.1.2 Production capacity

According to the available information, bis(hydroxylammonium)sulphate is produced by two companies at 3 production sites in the European Union (EU 15). One is located in Germany, the remaining are in Belgium. The production at a fourth site in Belgium was discontinued in 1997. According to IUCLID (2000) the maximum production capacity is 500,000 t/a. More specific data were delivered by the lead company BASF (1997, 2003). According to this information, the production volumes range from approximately 120,000 to 250,000 t/a for each site. The volume exported to outside EU is 4,500 t/a. Import data are not available. The total consumption within the EU is estimated to be 490,000 t/a.

The three producers use the main fraction of the substance directly as intermediate for further processing at the production site. In addition to that, bis(hydroxylammonium)sulphate is used for processing by several unknown companies. In table 2.1 data about production and processing are compiled. This information is used as starting point for the exposure estimation.

In table 2.1 data on production are compiled.

Table 2-1: Production volumes in 2002

Site	Production [t/a]	Source
A	120,000	Industry
B	220,000	Industry
C	150,000	Industry
Total	490,000	Industry

At site A, production follows still the Raschig-method. At sites B and C, bis(hydroxylammonium)sulphate is produced by a catalytic hydrogenation process as described before. The catalytic process is operated in a closed cascade system. Depending on the technology implemented, the primary product is an aqueous solution of approximately 10 - 40 % bis(hydroxylammonium)sulphate. This solution is either directly used for further processing or stored in tanks. For trade or at-side processing the stored material is directed through a pipeline to an evaporation plant (closed system). Then the dry material is packed under local exhaust ventilation (LEV) into 25 kg polyethylene bags or big bags (BASF 1996 BASF, 2001).).

The substance is placed on the market as aqueous solution and as crystalline salt. Aqueous bis(hydroxylammonium)sulphate solution is transported via ship, road or rail in stainless steel or polyethylene tanks or containers (BASF, 1996; BASF, 2001; BASF, 2003).

2.2 USES

2.2.1 Introduction

The main amount of the bis(hydroxylammonium)sulphate (> 90 %) is used directly at the production site as intermediate for the production of cyclohexanone oxime or caprolactam (large-scale chemical industry). With cyclohexanon, bis(hydroxylammonium)sulphate is converted to cyclohexanonoxim and further to caprolactam (Beckmann transformation). The residual concentration of bis(hydroxylammonium)sulphate in caprolactam is below the detection limit (= 1 ppm).

In addition, bis(hydroxylammonium)sulphate is used in many branches of the chemical industry. Some applications include the following (Ullmann, 2002):

- Chemical industry: Intermediate for the production of pharmaceuticals, e.g. antibiotics and tranquilizers, Intermediate for the production of active ingredient for plant protection products, like insecticides and herbicides, and of sweeteners for the food industry

- Photographic industry: stabilizers for developers, additive in emulsions for colour films (containing 1 – 30 % bis(hydroxylammonium)sulphate)
- Rubber industry: accelerator for the vulcanizing of synthetic rubber, antioxidant for natural rubber
- Soap: auxiliary for refining fats for soap production
- Plastics: regulator and inhibitor in various polymerisations
- Metallurgy: additive for surface treatment of steel
- Nuclear industry: auxiliary for separation of uranium and plutonium
- Textile industry: auxiliary for specific dyeing processes; fixative for textile dyes

According to the information of the lead company (BASF, 2003), the contingent sold as intermediate e. g. for the production of oximes, active ingredients in plant protection products, and pharmaceuticals is 10,850 t/a. Minor amounts are used in textile and metal finishing industry and for photographic processing solutions. Furthermore, the usage of approximately 2,000 t/a is not known by the producers because it is sold for trade.

Table 2-2: Use categories for the uses of bis(hydroxylammonium)sulphate

Use	Industry category	Use category	Quantity used [t/a]	Percentage of total use
Intermediate (caprolactam)	003	033	476,000	97.2
Intermediate (other)	003	033	10,850	2.2
Metal finishing	008	009	260	5.4 E-04
Textile finishing	013	021	300	6.2 E-04
Trade	Trade	Unknown	2,000	4,3 E-03
Total			489,000	99.5

2.2.2 Scenarios

See chapter 3.

2.3 TRENDS

No information available.

2.4 LEGISLATIVE CONTROLS

No legislative controls implemented.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

3.1.2 Environmental releases

3.1.2.1 Release from production

In the EU (EU 15), bis(hydroxylammonium)sulphate is produced at three sites with a total production in 2002 of approximately 490,000 t/a. Depending on the producer, the main quantity (83 – 100 %) of the production is used directly at-site for further processing to cyclohexanone oxime or caprolactam.

According to the specific properties, the substance is expected to be released into the environment during production and processing mainly via waste water.

The exposure estimation for the aquatic environment for the 3 European production sites is presented in section 3.1.4.1.1. Effluents of the production sites are discharged into rivers. Since two sites are situated directly at the estuary region of the river, releases into the sea cannot be excluded.

Direct releases to the soil compartment via sludge application are not expected due to the negligible sorption potential and the incineration of the sludge at the production sites.

Releases into the atmosphere via volatilisation are not relevant, because in the aqueous phase, bis(hydroxylammonium)sulphate is dissociated and hence not volatile. During production and use, in solid form, releases to the air compartment are possible as dust. One producer provided data concerning releases into air as dust released during packaging of the dried substance which is considered for the exposure estimation in section 3.1.6.

3.1.2.2 Release from formulation

Not required, no formulation.

3.1.2.3 Release from industrial/professional use

Residues of bis(hydroxylammonium)sulphate in the processed compounds, cyclohexanone oxime or caprolactam are not expected due to complete transformation during processing. Downstream users might release residues from processing and use of bis(hydroxylammonium)sulphate as aqueous waste.

3.1.2.4 Release from private use

Not required, no private use.

3.1.2.5 Release from disposal

Since bis(hydroxylammonium)sulphate is transformed completely during processing, releases of the substance itself from disposal can be excluded.

3.1.2.6 Summary of releases

Bis(hydroxylammonium)sulphate is produced and processed at three European sites. The main contingent (> 83 %) is also processed at these sites. Releases into the environment during production and processing are mainly expected via waste water. In addition to that, residues from processing and use of bis(hydroxylammonium)sulphate might be released as aqueous waste by several downstream users.

Releases to air and soil are not expected due to negligible sorption potential and volatility.

3.1.3 Environmental fate

3.1.3.1 Degradation in the environment

Bis(hydroxylammonium)sulphate is a crystalline salt. In the aqueous environment, the substance is expected to dissociate to $[\text{NH}_3\text{OH}]^+$ and $[\text{SO}_4]^{2-}$. The hydroxyl-ammonium ion is rapidly converted to hydroxylamine (free base). Hydroxylamine (free base) is stable in pure water in the absence of oxygen. However, the substance is a strong reduction agent and in the presence of oxygen, metals and other ions is rapidly transformed by abiotic processes (Holleman-Wiberg, 1995). The ultimate transformation products are ammonium, nitrogen and N_2O . The reaction is dependent on the pH. The more basic the environment, the more rapid the transformation.

In indicative studies using effluents of a waste water treatment plant the concentration of hydroxylamine decreased within 15 minutes of incubation from 1 mg/l to 0.56 mg/l. In an additional study, performed in the context of an ecotoxicological study (see section 3.2.1.1.2.), hydroxyl-ammonium sulphate and hydroxylamine together could not be detected in the test vials after 24 hours (test concentrations of 0.0156 to 1 mg/l, estimated determination limit 0.01 mg/L, estimated measurement uncertainty 30-35%) (BASF 2006a). In a pre-study an attempt was made to determine also the transformation products of hydroxylamine sulphate in the test medium. Neither ammonium nor nitrite/nitrate concentrations were significantly increased 3 days after initial hydroxylamine sulphate concentrations of 0.1, 1 or 100 mg/L (BASF 2006b). However, the abiotic transformation may result in gaseous products like nitrogen and N_2O , which were not investigated.

In addition to the abiotic degradation, hydroxylamine is an intermediate in the nitrification process, and might be further degraded by microbial activity into nitrite. Concerning the biological degradability, it was demonstrated that some microbial species are able to use hydroxyl amine as an additional energy source (e.g. Jetten et. al 1997).

These findings are confirmed by measurements in the waste water treatment plant of a producer (BASF 2006c). Whereas hydroxylamine was detectable in the influents, the substance could not be analysed up to the limit of detection (20 µg/l) in the effluent of the waste water treatment plants. Elimination in this waste water treatment plant was > 90%.

Degradation of bis(hydroxylammonium)sulphate by photolytic mechanisms can be excluded.

3.1.3.1.1 Atmospheric degradation

Bis(hydroxylammonium)sulphate is a salt which does not sublime. In the aqueous phase, the substance is expected to dissociate, and hence is not volatile. The volatility of hydroxylamine is also very low. Using the model EPIWIN, the Henry coefficient is calculated 0.0007 Pa m³/mol and the SPARC model reveals a value of 0.025 Pa m³/mol (BASF 2006d).

Emissions into the atmosphere can be excluded. In addition to that, atmospheric degradation of bis(hydroxylammonium)sulphate by OH-radicals can also be excluded.

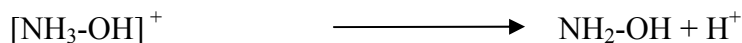
3.1.3.1.2 Aquatic degradation (incl. sediment)

Abiotic degradation

No reliable study concerning the abiotic degradation of bis(hydroxylammonium)sulphate was submitted.

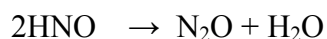
In an aqueous environment, bis(hydroxylammonium)sulphate is expected to dissociate to [NH₃OH]⁺ and [SO₄]²⁻.

The hydroxyl-ammonium ion is expected to react to hydroxylamine (free base) and hydrogen. Hydroxyl-ammonium ion and hydroxylamine (free base) are in equilibrium according to the following reaction scheme:



The estimated pK_a for this reaction is 5.8. In figure 1, the pH-dependent speciation plot hydroxylammonium ion/hydroxylamine, generated by the online calculator SPARC is depicted (<http://ibmlc2.chem.uga.edu/sparc/>). The amount of hydroxylammonium ion decreases rapidly at pH-values above 5. Only in very acidic environment, the substance is present as hydroxyl-ammonium ion. At pH 7, the amount of hydroxyl-ammonium ion is approximately 6 %, and at pH 8 nearly only the free base is present.

The free hydroxylamine base is very reactive and, at environmental conditions, is expected to decompose further by abiotic processes and nitrification. The expected ultimate degradation products are ammonia, nitrogen and water (Hollemann-Wiberg, 1995).



Sparc Speciation Plot

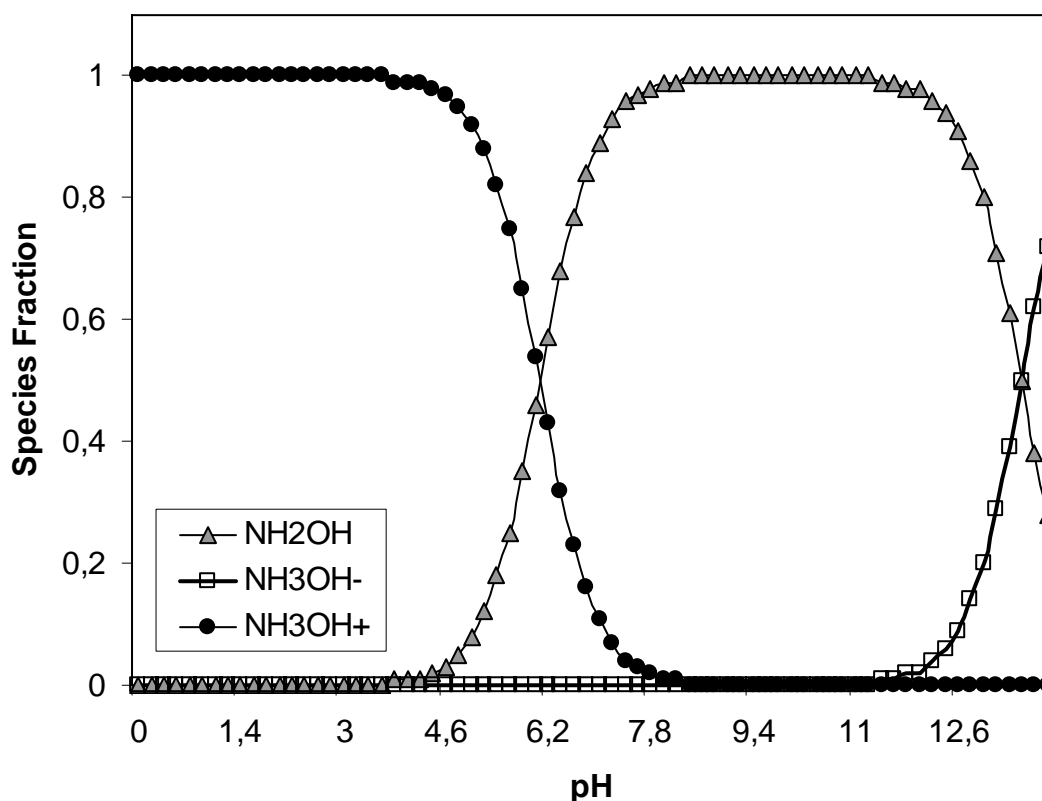


Figure 1: pH-dependent steady-state between hydroxyl-ammonium ion and hydroxylamine (free base)

Biological degradation

Bis(hydroxylammonium)sulphate is not an organic molecule. Mineralisation is defined as the process of biological degradation to stable inorganic products. According to this definition, biodegradation of bis(hydroxylammonium)sulphate is not possible and of no importance. A test on estimation of the biochemical oxygen demand was submitted indicating no oxygen demand within 5 days. In the study protocol it was concluded that a concentration of 5 mg/l and more might inhibit microbial activity. However, due to insufficient documentation the test is not acceptable.

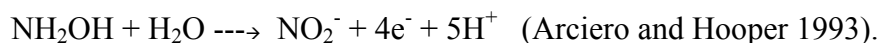
Independent of the acceptance of this study, other findings (e.g. Amarger & Alexander 1968), also indicate an inhibition of microbial activity due to high concentration of hydroxylamine.

However, hydroxylamine is a natural intermediate in biological nitrification under aerobic conditions (Amarger and Alexander 1968). The chemolithoautotrophic growth is obtained by the oxidation of ammonia to nitrite. This is a two-step process. The first step involves the

oxidation of ammonia to hydroxylamine by the membrane bound enzyme ammonia monooxygenase in the following reaction:



In the second step, the intermediate, hydroxylamine, is oxidized to nitrite by the enzyme hydroxylamine oxidoreductase (HAO) in the following reaction:



For several chemolithoautotrophic bacteria, such as *Nitrosomas europea*, *Nitrosomas nitrosa* and *Nitrosococcus oceanus*, mixotrophic growth on hydroxylamine in the presence of ammonia has been demonstrated (Böttcher and Koops 1994, de Bruijn et al. 1995). The molar growth yield on hydroxylamine, measured as a formation of cell protein per unit substrate oxidized, was found to be approximately twice that of ammonia. In respiration experiments, the oxygen consumption was 1.5 mol O₂ per mol ammonia and 1.0 mol O₂ per mol hydroxylamine oxidized to nitrite (Böttcher and Koops 1994). For *N. europea* molar growth yield was considerably high (4.74 g mol⁻¹ at a growth rate of 0.03 h⁻¹). Anaerobic growth of *N. europea* on hydroxylamine and ammonium was not observed (de Bruijn et al. 1995). Furthermore, hydroxylamine may be used as an additional energy source in heterotrophic nitrifying bacteria such as *Pseudomonas* PB16 (Jetten et al. 1997). For the latter, a maximum specific hydroxylamine oxidizing activity of 450 nmol min⁻¹ mg dry weight⁻¹, with a K_s of approximately 40 μM, has been determined.

In high concentrations, inhibition of bacteria by the dissociation products is possible.

3.1.3.1.3 Degradation in soil

No information is available on degradability in soil. No relevant releases into the soil compartment are expected from production or use of the substance. Hence, no additional data on degradation in soil have been submitted. In addition to that, at least in soil with some moisture, an identical pathway of degradation as in water could be expected

3.1.3.1.4 Summary of environmental degradation

Bis(hydroxylammonium)sulphate is a crystalline powder. In the aqueous environment, the salt dissociates to [NH₃OH]⁺ and [SO₄]²⁻. Depending on the pH, the hydroxyl-ammonium ion is rapidly converted to hydroxylamine which could be degraded further by abiotic and biotic processes.

According to these findings, at neutral pH levels in waste-water treatment plants, the substance is expected to be mainly present as hydroxylamine (free base) and will be further degraded by abiotic and biotic processes. This assumption is supported by indicative studies using effluents of a waste water treatment plant. The concentration of hydroxylamine decreased within 15 minutes of incubation from 1 mg/l to 0.56 mg/l (BASF 1997). In an additional study, performed in the context of an ecotoxicological study, hydroxyl-ammonium sulphate and hydroxylamine together could not be detected in the test vials after 24 hours (test concentrations of 0.0156 to 1 mg/l, estimated determination limit 0.01 mg/L, estimated measurement uncertainty 30-35%, BASF 2006a).

Due to the structure of the substance, degradation by photolytic mechanisms can be excluded.

Although the mechanisms involved in the degradation of bis(hydroxylammonium)sulphate are explained in theory, the information submitted concerning the environmental decomposition and degradation did not allow to perform a more quantitative estimation of degradability and exposure of the environment.

3.1.3.2 Distribution

Normally, distribution of a substance in the environment can be estimated using a Mackay-type model. Since bis(hydroxylammonium)sulphate is an inorganic substance dissociating in water, it is not reliable to estimate the distribution using a Mackay-type model.

The substance does not exhibit any measurable vapour pressure. Taking into account the inorganic character of bis(hydroxylammonium)sulphate and its high solubility in water, a complete distribution to the aqueous compartment can be expected.

Table 3-1: Estimated distribution of bis(hydroxylammonium)sulphate in the environment

Compartment	Distribution [%]
Air	$5,74 \times 10^{-11}$
Water	100
Soil	$8,86 \times 10^{-5}$
Sediment	$1,97 \times 10^{-6}$

3.1.3.2.1 Adsorption

Due to the specific properties of the substance it can be expected that the adsorption to organic matter is negligible.

Although the QSARs for adsorption estimation are not valid for inorganic substances, an approximate estimation was carried out using the QSAR for Nonhydrophobics from the TGD and the measured partition coefficient octanol/water. Using this correlation, a K_{oc} of 0.141 l kg^{-1} was estimated indicating a very low adsorption to organic matter. Further partition coefficients (K_p) were derived from this K_{oc} for various compartments by using the generic values given in the TGD for organic carbon contents of soils and sediments (table 5 of section 2.3.4).

Table 3-2: Estimation of partition coefficients

Compartments	Partition coefficients (l/kg)
soil – water	$Kp_soil = 2.812 \times 10^{-3}$
sediment – water	$Kp_sed = 7.03 \times 10^{-3}$
suspended matter – water	$Kp_susp = 0.014$
activated sludge – water	$Kp_sludge = 0.052$

raw sewage – water	$K_{p_sewage} = 0.042$
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3.1.3.2.2 Precipitation

3.1.3.2.3 Volatilisation

Bis(hydroxylammonium)sulphate is a salt which does not exhibit any measurable vapour pressure and does not sublime. The water-solubility is high, and water is the target compartment in the environment. In the aqueous phase, the substance is expected to dissociate, and hence not volatile. According to these findings, volatilisation from the aqueous phase can be excluded.

The volatility of hydroxylamine is also very low. Using the model EPIWIN, the Henry coefficient is calculated $0.0007 \text{ Pa m}^3/\text{mol}$ and the SPARC model reveals a value of $0.025 \text{ Pa m}^3/\text{mol}$ (BASF 2006d). Emissions into the atmosphere can be excluded.

3.1.3.2.4 Distribution in wastewater treatment plants

As mentioned before, the fugacity based models like SimpleTreat are not suitable for inorganic substances. Therefore a fraction of emission directed to water of 100 % would have to be assumed for the exposure assessment. However, considering the abiotic transformation of BHAS and the study results described in the chapters above this does not seem realistic. On the other hand it is difficult to assign a certain numerical value to the fraction of emission directed to water with the given information. As abiotic transformation seems to be rapid and measurements in industrial waste water treatment plants reveal > 90% elimination of BHAS, a rate constant of 1 h^{-1} is assumed as a conservative default value for elimination in waste water treatment plants. The distribution in waste water treatment plant is compiled in the following table:

Table 3-3: Distribution in waste water treatment plant

Compartments	Percentages
To air	0.00
To water	12.70
To primary sludge	0.00
To surplus sludge	0.00
Degraded	87.30
Total	100.00

3.1.3.3 Accumulation and metabolism

The Log P_{OW} determined experimentally for bis(hydroxylammonium)sulphate is -3.6 indicating no potential for bioaccumulation. No accumulation in organisms and the food chain is expected.

3.1.4 Aquatic compartment (incl. sediment)

3.1.4.1 Calculation of predicted environmental concentrations (PEC_{local})

3.1.4.1.1 Calculation of PEC_{local} for production and on-site processing

In the EU (EU 15), bis(hydroxylammonium)sulphate is produced at three sites with a total production in 2002 of approximately 490,000 t/a.

For two sites, the amount of bis(hydroxylammonium)sulphate processed directly at the production site is 83 - 100 %. For the third site, no information about processing was submitted. Hence, it is assumed that the complete production of bis(hydroxylammonium)sulphate is processed directly at the site.

For the three production sites, detailed information about emission parameters was submitted. The measurements were performed to include hydroxylamine. In addition to that, specific data on the waste water treatment plant, and the water flow of the receiving river were supplied. Hence, the exposure assessment could be refined using the specific information.

For **site A**, the concentrations of bis(hydroxylammonium)sulphate in the aqueous effluents have been analysed. In total 21 samples have been analysed with the result of 28 $\mu\text{g/l}$ as 90 percentile. In addition, site specific data for the flow rates have been used to calculate the PEC_{local} .

For **site B**, aggregated data on emissions into waste water from production and processing were submitted (< 0.18 t/a). In addition, the effluent flow of the waste water treatment plant and information concerning the receiving water body were available. This information was used to calculate the PEC_{local} .

For **site C**, emissions were controlled and measured using liquid-chromatography with electrochemical detection. The measurements were performed based on hydroxylamine. Since neither bis(hydroxylammonium)sulphate nor hydroxylamine were detected in the effluents, the given limit of detection for this method (0.02 mg/l) was used to assume the concentration in waste water. In addition, the available site specific data on effluent flow of the waste water treatment plant and the receiving water body were used to estimate the PEC_{local} .

The results of this exposure estimation, including the regional PEC as calculated in section 3.1.8. are listed in table 3.4.

Table 3-4: Local concentrations (PEC_{local}) for the different production sites refined using site-specific information.

Site	Emission data	Site specific data	PEC_{local} [$\mu\text{g/l}$]
A	Measured effluent concentration	Specific flow data for effluent	0.618

		and receiving river	
B	Specific emission data to waste water	Specific flow data for effluent and receiving river	0.538
C	Measured effluent concentration	Specific flow data for effluent and receiving river	0.639

3.1.4.1.2 Calculation of PEC_{local} for formulation

Minor uses of bis(hydroxylammonium)sulphate are known in several industrial areas, e. g. textile industry, metal working industry, photo industry. It is not known whether the substance will be formulated in this areas or whether the substance is used directly. Therefore, an exposure assessment for the life cycle step formulation hasn't been carried out.

3.1.4.1.3 Calculation of PEC_{local} for industrial/professional use

Intermediates:

According to the lead company (BASF, 2003) 10,850 tonnes are sold as intermediates for other industrial chemicals, for pesticides and for pharmaceuticals. Since no information about processing sites is available, a generic exposure assessment according to TGD using the following input data was carried out.

Processing volume:	10,850 tonnes/yr
Release factor (TGD, tab. A 3.3):	0.007
Fraction of main local source (TGD, tab. B 3.2):	0.25
Duration of emission (TGD, tab. B 3.2):	300 d/yr
Fraction of emission directed to water:	12.7 %
Effluent flow (ESD, Intermediates):	10,000 m ³ /d
Dilution factor (ESD, Intermediates):	40
$PEC_{regional}$ in surface water:	0.512 µg/l

Using these assumptions, a PEC_{local} of 20.61 µg/l is estimated.

Textile finishing industry:

Approximately 300 t/yr are applied as auxiliary in the textile finishing industry. The exposure assessment was carried out according to the Emission Scenario Document (TGD, ESD, IC 13) for textile finishing industry. The following input parameters were used:

Mass of textile processed per day (ESD, chapter 9.1):	13 t/d
Mass of auxiliary (ESD, table 10):	20 kg/t

Fraction of active substance in preparation:	1 (worst case)
Degree of fixation (ESD, table 12):	0 %
Fraction of emission directed to water:	12.7 %
Effluent flow (TGD, default):	2,000 m ³ /d
Dilution factor (TGD, default):	10
PEC _{regional} in surface water:	0.512 µg/l

Using these parameters a PEC_{local} of 1652 µg/l was estimated.

Photographic processing solution:

The use of Bis(hydroxylammonium)sulphate as antioxidant in colour paper processing (RA-4 process) in the photographic industry is described in literature (Ullmann's 2005) Information about the market volume of bis(hydroxylammonium)sulphate used in the photographic industry is not available. However exposure assessment according to the ESD (IC 10) is independent of the market volume. The following input parameters were used:

Surface processed per day (ESD, table 2: wholesale finisher):	4,950 m ² /d
Content of the substance in developing bath (ESD, table 5):	6,500 mg/l
Replenish rate (ESD, table 3):	0.12 l/m ²
Percentage removed or converted during processing (default):	0 %
Carry-over rate (ESD, table 3):	0.04 l/m ²
Treated volume (ESD, table 6):	1 m ³ /d
Fraction of waste reduction by disposal companies:	0
Fraction of emission directed to water:	12.7 %
Effluent flow (TGD, default):	2,000 m ³ /d
Dilution factor (TGD, default):	10
PEC _{regional} in surface water:	0.512 µg/l

According to the ESD three different scenarios for the calculation of PEC_{local} need to be considered:

- Bath overflow (replenish rate) is discharged into waste water,
- Bath overflow are collected and not discharged into waste water; carry-over rates are discharged into waste water
- Waste disposal by special disposal companies

According to these scenarios, PEC_{local} might be as follows:

- a) 25.029 $\mu\text{g/l}$
- b) 8.684 $\mu\text{g/l}$
- c) 41.787 $\mu\text{g/l}$.

Metal finishing industry:

At least 260 t/yr are marketed as auxiliary in the metal finishing industry. The exposure assessment was carried out according to the ESD (OECD Nr. 12). In this document, Bis(hydroxylammonium)sulphate is cited as ingredient in typical phosphating formulations. In table 2.19 of ESD Nr. 12 a concentration of 20 g/l is indicated. The liquid preparations are used in a quantity of 20 – 100 ml/l. Taking into account a worst case bath capacity of 5000 litres (table 3.5 of the ESD) and a worst case use of 100 ml/l, the concentration of Bis(hydroxylammonium)sulphate in the bath can be estimated to be 2 g/l. The following input parameters were used for the estimation:

Amount of solution removed from treatment

bath per unit area (ESD, table 3.1):	0.3 l/m ²
Surface area of metal processed (ESD, section 3.2):	40m ² /h
Amount of substance in treatment bath (ESD):	2,000 mg/l
Fraction of dragout returned to treatment bath (ESD, table 3.16):	0
Number of hours worked per day:	22 h/d
Fraction of emission directed to water:	12.7 %
Effluent flow (TGD, default):	2,000m ³ /d
Dilution factor (TGD, default):	10
$PEC_{regional}$ in surface water:	0.512 $\mu\text{g/l}$

Using these parameters a PEC_{local} of 3.865 $\mu\text{g/l}$ was estimated.

3.1.4.1.4 Calculation of PEC_{local} for private use

Private uses of bis(hydroxylammonium)sulphate are not known.

3.1.4.2 Measured levels

For two sites (including production and processing), the predicted environmental concentrations are based on measured effluent concentrations using the detection limit of the measurements for further exposure estimations.

3.1.4.3 Comparison between predicted and measured levels

Taking into account the specific inorganic properties of bis(hydroxylammonium)sulphate and the rapid degradation of the dissociated hydroxylammonium-ion, generic assumptions would lead to unrealistically high concentrations. More specific information on waste water treatment at the production and processing sites, and river flow rate is available.

The environmental concentrations were estimated by using the limit of detection of the analytical measurements. It could be assumed that the real concentrations are lower than those predicted by the estimations. In addition to that, releases from uses were calculated according to the default assumptions of the TGD. Hence, a further refinement might be possible by including more specific information. However, such data are not available.

3.1.5 Terrestrial compartment

Emissions to soil can occur by deposition from atmosphere or by spreading of contaminated sewage sludge. Dust emissions are reported for one site. It can be assumed that the dust deposits very close to the emission point. Gaseous emissions to the atmosphere with subsequent deposition can be excluded.

Emissions to soil by spreading of contaminated sewage sludge can be excluded too, because of the inorganic character of bis(hydroxylammonium)sulphate, adsorption to sewage sludge does not occur.

Taking these findings together, it can be assumed that there is no significant emission to soil.

3.1.6 Atmosphere

Bis(hydroxylammonium)sulphate is a salt which does not exhibit any measurable vapour pressure and does not sublime. The water-solubility is high, and water is the target compartment in the environment. In the aqueous phase, the substance is expected to dissociate, and hence does not volatilise.

The volatility of hydroxylamine is also very low. Emissions into the atmosphere can be excluded.

During production and use in solid form, releases into the air compartment are possible as dust. One company stated that there is a specific release to the atmosphere. Because the fraction of substance bound to aerosol is 1, this emission can be considered as dust. Emissions to the atmosphere during industrial use can be excluded.

However, one producer provided information about releases into air of < 0.22 t/yr. Using the OPS-model and these specific data, a $PEC_{local,air,ann}$ of $0.17 \mu\text{g}/\text{m}^3$ is estimated.

3.1.6.1 Measured levels

For one site, releases into the air of < 0.22 t/yr were measured.

3.1.6.2 Comparison between predicted and measured levels

Due to the negligible vapour pressure and the dissociation of the substance in the aqueous phase, releases into air via volatilisation can be nearly excluded. However, a single producer provided information about releases into air of < 0.22 t/yr. The measurements can be explained by releases of the substance as dust during packaging of the dried material.

3.1.7 Secondary poisoning

The Log P_{ow} determined experimentally for bis(hydroxylammonium)sulphate is -3.6 indicating no potential for bioaccumulation. Since no accumulation in organisms and the food chain is expected, the risk for secondary poisoning is considered to be low.

3.1.8 Calculation of $PEC_{regional}$ and $PEC_{continental}$

All releases, from diffuse and point sources are taken into account to estimate the regional background concentrations. As a worst-case, for the uses textile finishing and metal finishing, the complete volume was considered. With the exception of the production sites, which are assumed to be located in the virtual EU-region, a share of 90 per cent of the total releases was allocated to the continental scale, whilst 10 per cent are stipulated to get allocated to the regional sector. The regional concentrations were calculated using the software program EUSES 2.0.

Furthermore, it was assumed that the total tonnage, used in textile -, metal finishing, for trade and unknown uses is released to waste water. In order to take the rapid decomposition of bis(hydroxylammonium)sulphate into account a transformation rate of 1 h^{-1} has been used in the calculations as a conservative default value for abiotic decomposition.

Point source releases to surface water:

Based on specific estimations and measurements provided by the lead company, an amount of 0.18 t/yr was calculated which might be allocated to waste water to the regional sector (BASF 1997). Furthermore, an amount of 2.522 t/yr was calculated which might be allocated to surface water in the virtual region.

A total amount of 2,729.95 t/yr from the uses of bis(hydroxylammonium)sulphate was estimated to be released in the whole EU.

Point source release to air:

A single producer provided a release of < 0.22 t/yr (dust).

Point source releases to soil:

No point sources have been identified for the soil compartment.

Table 3-5: Summary of the releases [t/yr] for the calculation of PEC_{regional} and PEC_{continental}:

	EU (water)	Continental (water)	Regional (water)	EU (air)	Continental (air)	Regional (air)
Production	0	0	2.702*	0	0	0.2
Uses	2730	2457	273	0	0	0
Total	2730	2457	275.7	0	0	0.2

* Sum of releases to waste water (0.18 t/yr) and to surface water (2.522 t/yr)

Table 3-6: Regional and continental concentrations according to EUSES 2.0

continental PECs		regional PECs	
PECcontsurfacewater	6.32 x 10 ⁻⁰² [µg l ⁻¹]	PECregsurfacewater	0.512 [µg l ⁻¹]
PECcontair	3.74 x 10 ⁻²³ [mg m ⁻³]	PECregair	8.99 x 10 ⁻¹⁵ [mg m ⁻³]
PECcontagrsoil	4.77 x 10 ⁻¹⁵ [mg kg wwt ⁻¹]	PECregagrsoil	1.15 x 10 ⁻⁰⁶ [mg kg wwt ⁻¹]
PECcontagrsoilporew	3.97 x 10 ⁻¹⁴ [mg l ⁻¹]	PECregagrsoilporew	9.54 x 10 ⁻⁰⁶ [mg l ⁻¹]
PECcontnatsoil	7.59 x 10 ⁻¹⁵ [mg kg wwt ⁻¹]	PECregnatsoil	1.82 x 10 ⁻⁰⁶ [µg kg wwt ⁻¹]

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

3.2.1 Aquatic compartment (incl. sediment)

Only a limited dataset is available concerning the effects of bis(hydroxylammonium)sulphate on aquatic organisms, populations or biocenoses revealing several data gaps. The test results are summarized in table 3.7.

No information is available concerning the effects of the main metabolite hydroxylamine on aquatic species. Most of the tests using bis(hydroxylammonium)sulphate were performed at pH 7 or higher. Considering this, and the duration of the tests, it can be assumed that the substance degrades at least partially to hydroxylamine during the static exposure, and the organisms were also exposed to hydroxylamine. The quantitative determination of either hydroxylamine or hydroxylammonium ion separately is not possible, since each change of the concentration of one species will be readily balanced due to the equilibrium. Therefore only the total amount of both species together can be analytically ascertained.

No information is available about effects on terrestrial ecosystems.

3.2.1.1 Toxicity test results

Data are available concerning the short-term effects on fish, daphniae and algae. However, most of the information is not completely reliable and can only be used as indicative information.

With daphnia a semistatic test with chronic exposure has been performed.

Table 3-7: Effects on aquatic organisms

Test organism	Test conditions	End point	Concentration [mg/L]	Validity (Kliemisch code)
Fish <i>Pimephales promelas</i>	96 h, static, no analyses	Lethality, LC50	7.2 (nominal)	not reliable
Fish <i>Leuciscus idus</i>	48 h, static, no analyses	Lethality, LC50	6.67 (nominal)	not assignable
Invertebrates <i>Daphnia magna</i>	48 h, static, no analyses	Mobility, EC50	1.6 (nominal)	reliable with restrictions
	96 h, static, no analyses	Lethality, LC50	1.2 (nominal)	not reliable
	21 d, semi-static analyses	Reproduction, weight, length NOEC, LOEC	Reproduction, Weight: NOECs ≥ 0.62 , LOECs >0.62 (mean measured concentrations) Length: NOEC 0.31, LOEC 0.62 (mean measured conc.)	reliable without restrictions

Algae <i>Scenedesmus subspicatus</i>	72 h, static, no analyses	Growth rate E,C50	0.8 (nominal)	reliable with restrictions
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3.2.1.1.1 Fish

Only a few data concerning the effects of bis(hydroxylammonium)sulphate on fish are available.

Acute toxicity

The available data about fish toxicity are taken from older publications.

In a publication of the National Association of Photographic Manufactures (NAPM 1974) among other substances the effects of bis(hydroxylammonium)sulphate on *Pimephales promelas* were tested in a static system for 96 hours according to standard procedures outlined in Standard Methods, 13th Edition (ASTM, American Society for Testing and Materials). The LC₅₀ determined in this test was 7.2 mg/l. Basic information about the test is given (dilution water was examined, daily monitoring of pH and dissolved oxygen was conducted). The study is carried out according to an old guideline and no concentrations were measured, so the result can not be considered as reliable.

In a publication from 1972 (Fletcher & Addison) about “some aspects of of the chemistry and acute toxicity of the iron ore flotation agent dimethyl ammonium alkyl hydroxamate and some related compounds to brook trout” lethal periods of DMAH and some related components were determined. The brook trout *Salvelinus fontinalis* was used as test organism. The high toxicity of hydroxylamine hydrochloride for fish was confirmed by an EC₅₀ of 6 µl/l after 142 hours under flow-through conditions. In a parallel test for 80 hours of semi-static exposure the EC₅₀ was 10 µl/l. However, not bis(hydroxylammonium)sulphate was tested. Hence the results are only of indicative value for the risk assessment.

In addition to that a screening report was submitted. In this study (Applegate et al. 1957) 4,346 chemicals were screened for their acute toxicity for rainbow trout, bluegill sunfish and sea lamprey for 24 hours of static exposure. The intention of the study was a screening for a substance toxic for lamprey but not toxic for fish. The study is not useful to determine effective concentrations.

Long-term toxicity

Information on effects following long-term exposure of fish is not available.

3.2.1.1.2 Aquatic invertebrates

The effects on invertebrates were tested exclusively on the pelagic species *Daphnia magna*. No information concerning effects on sediment-organisms is available. However, since the physicochemical data indicate that the substance is not very adsorptive or bioaccumulative, a relevant distribution into the sediment compartment and a considerable exposure of sediment organisms are not expected. Hence, information about effects on sediment organisms is not required.

Acute toxicity

A study on the acute toxicity of bis(hydroxylammonium)sulphate for aquatic invertebrates using *Daphnia magna* according to guideline 67/548/EEC, C.2 was submitted by BASF (1988a). The effects were determined using eight (nominal) concentrations between 0.16 and 20 mg/l. In each concentration 20 individuals were tested in four parallels. During the exposure temperature was between 19 and 21°C and pH between 7.0 and 8.0. No effects on mobility were observed in the lower concentrations up to 1.25 mg/l. In 2.5 mg/l all daphnids were immobile after 48 hours of static exposure. The EC₅₀ (48 h) is calculated to be 1.6 mg/l. The concentrations were not verified analytically. Since the substance is not volatile, very good soluble in water and expected to remain in the water phase, the test is valid with restrictions in spite of the missing analytical verification of the concentrations. However, the results based on nominal concentrations seem to be reliable and might be used for classification and labelling and to assess the risk for aquatic invertebrates.

The EC₅₀ determined in this test is confirmed by a publication of the National Association of Photographic Manufactures (1974) where among other substances the effects of bis(hydroxylammonium)sulphate on *Daphnia magna* were tested in a static system for 48 hours. The LC₅₀ determined in this test under static exposure for 48 hours was 1.2 mg/l. However, since no detailed information about the test was submitted and no concentrations were measured this value is only of indicative value.

Long-term toxicity

A study on long-term toxicity to *Daphnia magna* was supplied by BASF (2007b).

The test was conducted according to OECD 211. Because of the rapid dissociation/degradation of BHAS semi-static test conditions with renewal of test solutions in 24 hour intervals were chosen. In addition concentrations were measured. Samples for analysis were taken once a week during the test. For each concentration the freshly prepared test solution (without daphnia) and the corresponding 24 h old test solution (with daphnia) were analysed. Six (nominal) test concentrations ranging from 0.0156 to 1 mg/l were used. pH values in the test vials ranged from 7.6 to 8.3. Because of the volatility of the transformation products the test vessels were completely filled and closed with glass plugs.

It is shown in this study that after 24 hours, the substance decomposed almost completely. Only in the highest test concentrations it was possible to detect low amounts of the test substance after 24 hours (estimated determination limit 0.01 mg/L, measurement uncertainty 30-35%).

Table 3-8: Analytical verification of the test concentrations in the test vessels during the long-term test using *Daphnia magna*

Nominal Concentration [mg/L]	Recovery rate						Mean value [mg/L]	Mean value [%]
	first sampling		second sampling		third sampling			
	day0 (0h old) [mg/L]	day1 (24h old) [mg/L]	day8 (0h old) [mg/L]	day9 (24h old) [mg/L]	day20 (0h old) [mg/L]	day21 (24h old) mg/L		
0	< 0,01	< 0,01	0	< 0,01	< 0,01	< 0,01	< 0,01	< 0,01
0,0156	0,0173	< 0,01	0,0169	< 0,01	0,0168	< 0,01	0,00850	54,5
0,0313	0,0347	< 0,01	0,0332	< 0,01	0,0350	< 0,01	0,0172	54,8
0,0625	0,0849	< 0,01	0,0631	< 0,01	< 0,01	< 0,01	0,0247	39,5
0,125	0,169	< 0,01	0,1277	0,0169	0,140	< 0,01	0,0756	60,5
0,25	0,330	< 0,01	0,5200	0,0193	0,263	< 0,01	0,1887	75,5
0,5	0,718	< 0,01	0,5520	0,0637	0,516	< 0,01	0,3083	61,7
1	1,265	0,0133	1,055	0,236	1,081	0,0484	0,6165	61,6

An analysis of the transformation products of the test substance was originally intended. However, during the development of the method it was found that at a concentration of 100 mg test substance/L the resulting concentrations of ammonia, nitrite and nitrate were not significantly increased after 3 days (BASF 2006b). The determination of sulphate ions was not possible because of high concentrations of sulphate ions in the test medium. Therefore no analytical determination of the transformation products was performed. It is assumed that the substance decomposes to gaseous products.

After 21 days of exposure, no effects on reproduction and weight of the parent animals were observed up to the highest concentration tested. Since the length of the daphnids exposed to this concentration was significantly reduced, the NOEC for this endpoint is 0.5 mg/l (nominal, LOEC 1.0 mg/l). Using the mean of the measured concentrations, NOEC and LOEC for the length of the animals are 0.31 mg/l and 0.62 mg/l. Since the measured concentrations are below 80 % of the nominal concentrations, the analytically determined concentrations should preferably be used for further assessment.

Table 3-9: NOECs/LOECs for *Daphnia magna* (mean measured concentrations)

	Reproduction	Weight	Length
NOEC [mg/L]	≥ 0.62	≥ 0.62	0.31
LOEC [mg/L]	> 0.62	> 0.62	0.62

3.2.1.1.3 Algae

A study on inhibition of algal growth using the green algae *Scenedesmus subspicatus* was submitted by BASF (1988b). The test was conducted using OECD medium and eight (nominal) concentrations between 0.04 and 5.0 mg/l without analytics. PH was measured as 8.4 – 8.7 at the beginning of exposure and 8.6 – 10.0 at the end of the study. After 96 hours of static exposure an E_rC_{50} of 0.81 mg/l was calculated. EC_{20} and EC_{90} were determined to be 0.50 and 1.55 mg/l. Since the concentrations were not verified analytically, the test is only valid with restrictions. The substance is not volatile, very good soluble in water and expected

to remain in the water phase. Hence, the results based on nominal concentrations seem to be reliable and were used for classification and labelling, and to assess the risk for aquatic algae.

In addition to this test two studies were published in literature. In a screening study of 1952 (Fitzgerald et al.), 300 chemicals were screened for herbicidal activity. The blue-green algae *Micocystis aeruginosa* was used as test organism. The intention of the study was to screen for a herbicidal substance inhibiting the growth of blue-green algae without effects on other aquatic species. Effects were determined by visual estimation. The E_rC_{100} estimated for bis(hydroxylammonium)sulphate in this 24 hour test was 2.0 mg/l. However, the study is neither suitable for classification and labelling purposes nor for risk assessment. The results are only reliable as indicative, additional information.

The result of the algae test is confirmed by a publication of the National Association of Photographic Manufactures (1974) where among other substances the effects of bis(hydroxylammonium)sulphate on the green algae *Selenastrum capricornutum* were tested in a provisional static system for 7 days. While no effects on growth were observed up to 0.1 mg/l, growth of the population was inhibited significantly at 1.0 mg/l. However, no detailed information about the test was submitted and no concentrations were measured. Hence, the test is only of indicative value and neither useful for classification and labelling nor for risk assessment purposes.

3.2.1.1.4 Microorganisms

Three tests on inhibition of activated sludge were submitted (BASF 1979, BASF 1984, BASF 2007a). In all studies bis(hydroxylammonium)sulphate was used as test substance.

In the two older studies, inoculum of the producer's sewage treatment plant was used. Due to insufficient documentation, especially on the test procedure, test duration and the origin of the inoculum both studies were invalid. However, in both tests a concentration-dependent inhibition of microbial respiration was observed starting at concentrations of 0.5 - 1 mg/l. The EC_{20s} determined in the studies were 0.6 and 1.0 mg/l.

Bis(hydroxylammonium)sulphate is known to inhibit microbial activity, also in sewage treatment plants (BASF 1997). Other findings (e.g. Amarger & Alexander 1968), indicate an inhibition of microbial activity due to high concentrations of hydroxylamine. However, since hydroxylamine is an intermediate in the nitrification process, in lower concentrations hydroxylamine is expected to be degraded biologically to nitrite (e.g. Jetten et. al. 1997).

Taking these findings together with the data concerning transformation of bis(hydroxylammonium)sulphate, it can be assumed that the toxicity to microorganisms is associated with high concentrations of the hydroxyl-ammonium ion or hydroxylamine. In sewage treatment plants pH is buffered to pH 8 where the hydroxyl-ammonium ion is present only in very low concentrations. In addition to that, hydroxylamine (free base) is expected to decompose rapidly due to abiotic processes. The remaining concentration is not expected to inhibit microbial activity. Hence it can be assumed that additional biological degradation occurs in the waste water treatment plant.

In 2007 BASF conducted a third study on effects of BHAS on activated sludge (BASF 2007a). The inhibition of oxygen consumption by activated sludge was investigated according to OECD 209. Activated sludge was obtained from a municipal wastewater treatment plant, test concentrations ranged from 0.05 to 504 mg/L (nominal). After 180 minutes incubation at

20 ± 2 °C the respiration rates were recorded. At a test concentration ≤ 5 mg/L the test substance did not inhibit respiration (EC₂₀, corresponds to NOEC), at 50.4 mg/L 56% inhibition was observed (EC 50), at 504 mg/L 78% inhibition.

3.2.1.1.5 Amphibians

No information about effects on amphibians is available.

3.2.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Surface water

The most sensitive species tested is *Daphnia magna* with a NOEC (21 d) of 0.31 mg/l (mean measured concentration).

Besides this NOEC only information on effects following short-term exposure are available and no reliable results concerning the toxicity for fish were submitted. An assessment factor of 100 is taken into account to determine the PNEC for the aqueous phase.

$$\text{PNEC}_{\text{aqueous phase}} = 0.31 \text{ mg/l} / 100 = 3.1 \text{ } \mu\text{g/l}$$

Sewage treatment plants (PNEC_{micro-organisms})

Three respiration tests using sewage sludge are available. The recently performed test conducted following OECD guideline 209 is considered valid for the use in the risk assessment. The EC₂₀ of 5 mg/l as determined in this study is considered as a NOEC and used to derive a PNEC for aquatic microorganisms. Hence, the PNEC for the assessment of microbial activity in biological treatment plants is calculated as follows using an assessment factor of 10

$$\text{PNEC}_{\text{micro-organisms}} = 5.0 \text{ mg/l} / 10 = 0.5 \text{ mg/l}$$

This PNEC_{micro-organisms} is used to characterize the risk for sewage treatment plants.

3.2.1.3 Toxicity test results for sediment organisms

The effects on invertebrates were tested exclusively on the pelagic species *Daphnia magna*. No information concerning effects on sediment-organisms is available. However, since the physicochemical data indicate that the substance is not very adsorptive or bioaccumulative, a relevant distribution into the sediment compartment and a considerable exposure of sediment organisms are not expected. Hence, information about effects on sediment organisms is not required.

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

No risk for sediment organisms is expected due to lacking adsorptive and bioaccumulative properties of the substance and missing distribution into sediment. Hence, a PNEC for sediment organisms is not required.

3.2.2 Terrestrial compartment

No information is available on effects bis(hydroxylammonium)sulphate on terrestrial ecosystems (plants, earthworms, microorganisms). Due to production methods, processing, and use of bis(hydroxylammonium)sulphate and considering further the distribution behaviour of the substance, a relevant exposure of this compartment can be excluded.

3.2.2.1 Toxicity test results

3.2.2.1.1 Plants

No information on the effects of bis(hydroxylammonium)sulphate on terrestrial plants is available.

3.2.2.1.2 Earthworm

No information on acute or chronic effects of bis(hydroxylammonium)sulphate on earthworms is available.

3.2.2.1.3 Microorganisms

No information on acute or chronic effects of bis(hydroxylammonium)sulphate on terrestrial microorganisms is available.

3.2.2.1.4 Other terrestrial organisms

No studies on the effects of bis(hydroxylammonium)sulphate on other terrestrial organisms are available.

3.2.2.2 Calculation of Predicted No Effect Concentration (PNEC)

Not required.

3.2.3 Atmosphere

Because an exposure of the atmosphere is not expected, an assessment for this compartment is not necessary.

3.2.4 Secondary poisoning

Bis(hydroxylammonium)sulphate does not show any potential for accumulation within the food chain. The risk for secondary poisoning is considered to be low.

3.3 RISK CHARACTERISATION ⁵

Table 3-10: Overview PEC/PNEC ratios for aquatic ecosystems

Process	Data	Scenario	PEC _{local} [µg/l]	PEC/PNEC
Production / Processing site A	Measured effluent concentration	Specific flow for effluent and river	0.618	0.20
Production / Processing site B	Specific emission data to waste water	Specific flow for effluent and river	0.538	0.17
Production / Processing site C	Measured effluent concentration	Specific flow for effluent and river	0.639	0.21
Use, Intermediates	generic	generic	20.61	6.6
Use, Textile finishing	generic	generic	1,652	533
Use Photographic processing	generic	generic	8.684-41.787	2.8-13,5
Use_Metal finishing	generic	generic	3.865	1.2

3.3.1 Aquatic compartment (incl. sediment)

The risk assessment for aquatic organisms resulted in a PNEC for the aqueous phase of 3.1 µg/l. The PNEC was derived from the daphnia test using an assessment factor of 100. The factor is due to the small data set and the missing information concerning long-term effects on aquatic ecosystems.

For the assessment of microorganisms in biological treatment plants the preliminary PNEC_{micro-organism} was calculated to be 0.5 mg/l.

Production / Processing on site:

Exposure scenarios were calculated based on site-specific information for the three European production sites integrating the regional background concentration as estimated in chapter 3.1.8. Since the main amount of the production is processed directly on site, the specific concentrations are also reliable for processing of bis(hydroxylammonium)sulphate.

The PEC-values for releases from production and on-site processing were calculated using the limit of detection instead of analytically verified concentrations. In addition to that, default

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.

assumptions according to the TGD were integrated. Furthermore, information concerning the total emissions was quoted as “<”.

Information about decomposition of bis(hydroxylammonium)sulphate in the environment resulted in an estimated transformation rate of 1 h^{-1} for waste water treatment plants.

The ecotoxicological data might be enhanced by including further information on long-term effects on aquatic organisms.

Table 3-11: Overview PEC/PNEC ratios

Site	Data	Scenario	PEC [$\mu\text{g/l}$]	PEC/PNEC Aquatic Org.	PEC/PNEC Sewage
A	Measured effluent concentration	Specific flow for effluent and river	0.618	0.20	0.001
B	Specific emission data to waste water	Specific flow for effluent and river	0.538	0.17	0.001
C	Measured effluent concentration	Specific flow for effluent and river	0.639	0.21	0.001

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

Use:

Intermediates:

10,850 t/a are used as intermediates for the production of industrial chemicals, pesticides and pharmaceuticals. According to the generic scenario of the TGD, a $\text{PEC}_{\text{local}}$ of $20.61 \mu\text{g/l}$ is estimated.

For these uses, the risk is quantified with a PEC/PNEC ratio of approximately 6.6. Hence, a risk for aquatic ecosystems is predicted from the use of bis(hydroxylammonium)sulphate as intermediate.

A possible refinement of the generic assumptions by specific data might result in lower predicted concentrations. An improvement of the ecotoxicological data with further information on long-term effects on aquatic organisms is also possible. However, it is no longer possible to submit this information under the Existing Substances Regulation. Hence, the Rapporteur proposes conclusion (i) and further consideration of the substance under the REACH regulation.

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

Textile finishing industry:

Approximately 300 t/yr are applied as auxiliary in the textile finishing industry. According to the Emission Scenario Document for textile finishing industry, the PEC_{local} is 1,652 µg/l and the PEC/PNEC ratio 533, indicating a high risk for aquatic ecosystems.

However, the result is based on generic “worst-case” assumptions and therefore a refinement with more specific data might be possible. According to information of the lead producer, bis(hydroxylammonium)sulphate is completely transformed during the use. In addition to that, according to the producer, emission from these use are much lower than estimated. However, it is no longer possible to submit this information under the Existing Substances Regulation. Hence, the Rapporteur proposes conclusion (i) and further consideration of the substance under the REACH regulation.

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

Photographic processing solution:

For this use, the exposure was estimated according to the ESD (IC 10) resulting in local concentrations of bis(hydroxylammonium)sulphate for three different scenarios as follows:

Scenario	PEC _{local} [µg/l]	PEC/PNEC
Bath overflow discharged into waste water	25.029	8.1
Bath overflow collected; carry-over rates are discharged into waste water	8.684	2.8
Waste disposal by special disposal companies	41.787	13,5

For all three scenarios a high risk is predicted.

Since these results are also based on generic “worst-case” assumptions, a refinement by more specific data might be possible. Since it is no longer possible to submit this information under the Existing Substances Regulation, the Rapporteur proposes conclusion (i) and further consideration of the substance under the REACH regulation.

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

Metal finishing industry:

Approximately 260 t/yr are marketed as auxiliary in the metal finishing industry. The PEC_{local} is estimated to be 3.865 µg/l and the risk quotient for aquatic ecosystems (PEC/PNEC) 1,2.

For this scenario, a refinement with more specific data might be possible, too. Since it is no longer possible to submit this information under the Existing Substances Regulation, the Rapporteur proposes conclusion (i) and further consideration of the substance under the REACH regulation.

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

Conclusions to the risk assessment for the aquatic compartment:

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 (ii) applies to production of bis(hydroxylammonium)sulphate and processing at the production sites.

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

Conclusion (i) applies to all uses of bis(hydroxylammonium)sulphate. Refinement with more specific data might be possible in all cases. Since it is no longer possible to submit this information under the Existing Substances Regulation, it is proposed to give further consideration to the substance under the REACH regulation.

3.3.2 Terrestrial compartment

According to the physico-chemical properties of bis(hydroxylammonium)sulphate and the possible releases into the environment, a relevant exposure of soil and terrestrial ecosystems can be excluded. In addition, fate modeling shows that the target compartment is the water phase.

Conclusions of the risk assessment for the terrestrial compartment:

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 (ii) applies to production, processing and uses of bis(hydroxylammonium)sulphate.

3.3.3 Atmosphere

Bis(hydroxylammonium)sulphate is ionic, inorganic and not volatile. Hence, relevant emissions to air can be excluded.

Conclusions to the risk assessment for the atmosphere:

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 (ii) applies to production, processing and uses of bis(hydroxylammonium)sulphate.

3.3.4 Secondary poisoning

Bis(hydroxylammonium)sulphate is not adsorptive. The substance does not exhibit a potential for bioaccumulation, which is supported by the Log P_{OW} determined with -3.6. Hence, the risk of bioaccumulation within the food chain and the potential for secondary poisoning is considered to be low.

Conclusions to the risk assessment for secondary poisoning:

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 (ii) applies to production, processing and uses of bis(hydroxylammonium)sulphate.

3.3.5 PBT-Assessment

The available information is sufficient to conclude about the PBT-properties of bis(hydroxylammonium)sulfate. Bis(hydroxylammonium)sulphate is not readily biodegradable. Hence, the screening-criterion for persistence is met (not readily biodegradable). However, the information on abiotic and biotic degradability indicates that in the aqueous environment, bis(hydroxylammonium)sulphate dissociates to $[\text{NH}_3\text{OH}]^+$ and $[\text{SO}_4]^{2-}$. Depending on the pH, the hydroxyl-ammonium ions react to hydroxylamine which could be degraded further by abiotic and biotic processes. This assumption is supported by indicative studies using effluents of a waste water treatment plant, during which the concentration of hydroxylamine decreased within 15 minutes of incubation from 1 mg/l to 0.56 mg/l (BASF 1997).

In addition to that, the substance is not adsorptive and not expected to be distributed into sediment where it might resist degradation processes. Hence, it is concluded that bis(hydroxylammonium)sulphate is not persistent.

Bis(hydroxylammonium)sulphate does not exhibit a potential for bioaccumulation, which is supported by the Log P_{OW} determined with -3.6. Hence, the screening-criterion for bioaccumulation potential is not met.

The results of a chronic test on daphnia reproduction indicate that the substance does not fulfil the T-criterion.

Taking these findings together, bis(hydroxylammonium) does not exhibit any PBT- or vPvB properties and hence, is not a PBT or vPvB candidate.

4 HUMAN HEALTH

4.1.1.4. Indirect exposure via the environment

Nearly all bis(hydroxylammonium)sulphate released to the environment is expected to end up in the water phase where it is transformed rapidly. Its volatility is very low. No relevant releases into the soil compartment are expected from production or use of the substance and adsorption to organic matter is negligible. Bis(hydroxylammonium) sulphate does not exhibit any potential for bioaccumulation. Therefore the risk is considered to be negligible and no assessment of indirect exposure via the environment has been undertaken.

5 RESULTS ⁶

5.1 INTRODUCTION

5.2 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.

Conclusion (ii) applies to releases into water, soil and air for production of bis(hydroxylammonium)sulphate and processing at the production sites. In addition this conclusion can also be drawn for releases into air and soil resulting from the use of the substance.

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

Conclusion (i) applies to releases into the aquatic environment resulting from all uses of bis(hydroxylammonium)sulphate. Refinement with more specific data might be possible in all cases. Since it is no longer possible to submit this information under the Existing Substances Regulation, it is proposed to give further consideration to the substance under the REACH regulation.

⁶ 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.

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ABBREVIATIONS

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 / <i>Bw</i> , <i>b.w.</i>
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 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
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	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
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient

Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based Pharmacokinetic modelling
PBTK	Physiologically Based Toxicokinetic modelling
PEC	Predicted Environmental Concentration

pH	logarithm (to the base 10) (of the hydrogen ion concentration {H ⁺ })
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material

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)

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Luxembourg: Office for Official Publications of the European Communities

[ECB: insert year] – VIII pp., [ECB: insert number of pages] pp. – 17.0 x 24.0 cm

Environment and quality of life series

ISBN [ECB: insert ISBN No.]

Price (excluding VAT) in Luxembourg: EUR [ECB:insert price]

The report provides the comprehensive risk assessment of the substance Bis (hydroxylammonium) sulphate. 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 and consumer exposure have been examined and the possible risks have been identified.

There is no concern for the aquatic and terrestrial compartment and atmosphere in relation to the releases from production and processing at the production sites. In addition there is no concern for the atmosphere and terrestrial compartment from the releases due to the use of the substance. Further information is needed regarding the releases into the aquatic compartment from all uses of the substance before definitive conclusions regarding environmental risks can be drawn. Since it is no longer possible to submit this information under the Existing Substances Regulation it is proposed to consider the substance further under the REACH regulation.

For human health, there is concern for workers, with respect to skin sensitisation, acute toxicity, chronic health risks (general toxicity to blood and spleen), and carcinogenicity, but no concern is expressed for consumers and for humans exposed via the environment.

European Union Risk Assessment Report

BIS(HYDROXYLAMMONIUM)SULFATE

CAS No: 10039-54-0

EINECS No: 233-118-8

RISK ASSESSMENT

FINAL APPROVED VERSION

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Printed in Italy

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CAS No: 10039-54-0

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RISK ASSESSMENT

07April 2008

Germany

FINAL APPROVED VERSION

Rapporteur for the risk assessment of Bis(hydroxylammonium)sulfate is Germany

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Date of Last Literature Search :

[insert year]

Review of report by MS Technical Experts finalised:

[insert month and year]

Final report:

[year]

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) 793/93¹ 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) 1488/94², which is supported by a technical guidance document³. 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.

¹ O.J. No L 084, 05/04/199 p.0001 – 0075

² O.J. No L 161, 29/06/1994 p. 0003 – 0011

³ Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT⁴

CAS Number: 10039-54-0
EINECS Number: 233-118-8
IUPAC Name: Bis(hydroxylammonium)sulfate

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.

For bis(hydroxylammonium)sulfate concern is expressed for skin sensitisation, for acute toxicity, repeated dose toxicity and for carcinogenicity. Especially with respect to systemic effects (general toxicity to blood and spleen and thresholded carcinogenicity) the available risk assessment indicates substantial chronic health risks especially for dermal exposure of workers in scenario 3 (formulation as an auxiliary in different industries). Based on this risk analysis, immediate risk reduction measures are considered necessary.

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.

Combined exposure

Human health (physico-chemical properties)

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 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.

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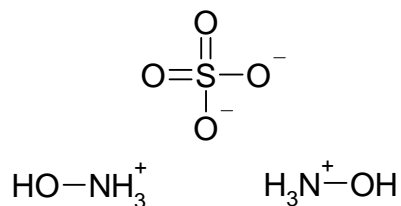
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EUSES Calculations can be viewed as part of the report at the website of the European Chemicals Bureau: <http://ecb.jrc.it>

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 10039-54-0
EINECS Number: 233-118-8
IUPAC Name: Bis(hydroxylammonium)sulfate
Molecular formula: $\text{H}_8\text{N}_2\text{O}_6\text{S}$
Structural formula:



Molecular weight: 164.14 g/mol
Synonyms: Hydroxylammonium sulfate
CA-Index-name: Hydroxylamine, sulfate (2:1) (salt)

1.2 PURITY/IMPURITIES, ADDITIVES

Purity: ca. 99 % w/w
Impurities: < 0.1 % water
< 1 % ammonium sulfate
< 2.5 % sulfuric acid
Additives: none

1.3 PHYSICO-CHEMICAL PROPERTIES

Bis(hydroxylammonium)sulfate is a white crystalline powder with a typical odour. Data on the physical and chemical properties are given in the following table:

Table 1.1 Summary of physico-chemical properties

Property	Value	
Physical state	at 20 °C, 1013 hPa: white crystalline powder	
Melting point	decomposition above 120 °C ¹⁾	Sorbe, 1996
Boiling point	not applicable	
Relative density	1.883 at 20 °C	BASF AG, 1986
Vapour pressure	not determined	no test conducted because of the salt character
Water solubility	587 g/l at 20 °C ²⁾	BASF AG, 1972
Partition coefficient n-octanol/water (log value)	logPow -3.6 at 20 °C ³⁾ ph 3,2	BASF AG, 1997
Conversion factors		
Flash point	not determined	substance is a solid
Autoflammability	no selfignition up to decomposition (120 °C)	Chemsafe, 2001
Flammability	non flammable ⁴⁾	Chemsafe, 2001
Explosive properties	explosive	BAM, 1980
Oxidizing properties	no oxidising properties	no test conducted because of structural reasons
Viscosity		
Henry's constant		
Surface tension	not determined	not relevant for the risk assessment

¹⁾ At temperatures above 130 °C the substance decomposes explosion likely

²⁾ The results for the water solubility found in the literature differed considerably. Sorbe for example cited a water solubility of 685 g/l at 25 °C without further information regarding the test substance and test method. It can be stated that there is a high temperature dependence of the solubility of Bis(hydroxylammonium)sulfate.

³⁾ The shaking flask method was used. While there is a protolytic balance the partition coefficient depends from the pH value considerably.

⁴⁾ Tests according to A.12 and A.13 were not conducted because of structural reasons.

1.4 CLASSIFICATION

(Classification according to Annex I, 30th ATP)

E Explosive

Xn Harmful

Xi Irritant

Carcinogen Category 3

N Dangerous for the environment

R 2 Risk of explosion by shock, friction, fire or other source of ignition

R 21/22 Harmful in contact with skin and if swallowed

R 36/38 Irritating to eyes and skin

R 40 Limited evidence of carcinogenic effect

R 43 May cause sensitization by skin contact

R 48/22 Harmful: danger of serious damage to health by prolonged exposure if swallowed

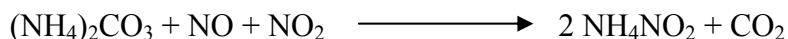
R50 Very toxic to aquatic organisms.

2 GENERAL INFORMATION ON EXPOSURE

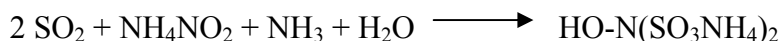
2.1 PRODUCTION

Bis(hydroxylammonium)sulfate (BHAS) can be produced by two methods: The Raschig process and a catalytic hydrogenation of nitric oxide.

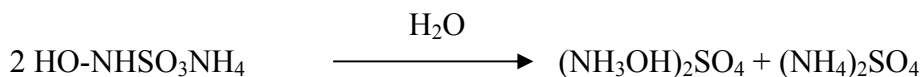
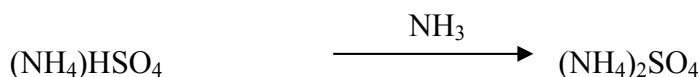
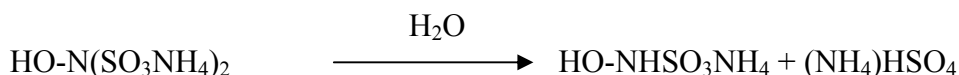
In the Raschig process, water, ammonia, and carbon dioxide react together in an absorption column. The product is a solution of ammonium carbonate, which forms an alkaline solution of ammonium nitrite with nitrogen oxides at low temperatures.



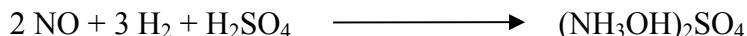
In a further step, the ammonium nitrite is converted to ammonium hydroxylamine disulfonate with sulfur dioxide.



The ammonium hydroxylamine disulfonate is then drawn off and the salt is hydrolyzed and neutralized. The final product are bis(hydroxylammonium)sulfate and ammonium sulfate.



For many years the Raschig process was the main production method. In the 1950s and 60s, the Raschig method was replaced by the catalytic hydrogenation process. In this method, purified nitric oxide is converted to hydroxylamine by reaction with hydrogen below 50 °C over a suspension of partially poisoned platinum catalyst in sulfuric acid.



In contrast to the Raschig process, only small amounts of ammonium as by-product were produced from the catalytic hydrogenation method.

2.1.1 Production capacity

According to the available information, bis(hydroxylammonium)sulfate is produced by two companies at 3 production sites in the European Union (EU 15). The production at a fourth site was discontinued in 1997. According to IUCLID (2000) the production capacity is between 100,000 to 500,000 t/a. More specific data were delivered by the lead company BASF (1997, 2003). According to this information, the production volume ranges from approximately 120,000 to 250,000 t/a for each site. The volume exported to outside EU is 4,500 t/a. Import data are not available. The total consumption within the EU is estimated to be 490,500 t/a.

The three main producers use the main fraction of the substance directly as intermediate for further processing at the production site. In addition to that, bis(hydroxylammonium)sulfate is used for processing by several unknown companies. In table 2.1 data about production and processing are compiled. This information is used as starting point for the exposure estimation.

In table 2.1 data on production and processing are compiled.

Table 2.1 Production volumes in 2002

Site	Production [t/a]	Source
A	120,000	Industry
B	220,000	Industry
C	150,000	Industry
Total	490,000	Industry

At site A, production follows still the Raschig-method. At sites B and C, bis(hydroxylammonium)sulfate is produced by a catalytic hydrogenation process as described before. The process is operated in a closed cascade system. Depending on the technology implemented, the primary product is an aqueous solution of approximately 10 - 40 % bis(hydroxylammonium)sulphate. This solution is either directly used for further processing or stored in tanks. For trade or at-side processing the stored material is directed through a pipeline to an evaporation plant. Then the dry material is packed into 25 kg plastic bags (BASF 1996).

2.2 USES

2.2.1 Introduction

The main amount of the bis(hydroxylammonium)sulfate produced (> 90 %) is used as intermediate for the production of cyclohexanone oxime or caprolactam.

In addition, bis(hydroxylammonium)sulfate is used in many branches of the chemical industry. Some applications include the following (Ullmann, 2002):

- Chemical industry: Intermediate for the production of pharmaceuticals, e.g. antibiotics and tranquilizers, Intermediate for the production of active ingredient for plant protection products, like insecticides and herbicides, and of sweeteners for the food industry
- Photographic industry: stabilizers for developers
- Rubber industry: accelerator for the vulcanizing of synthetic rubber, antioxidant for natural rubber
- Soap: auxiliary for refining fats for soap production

- Plastics: regulator and inhibitor in various polymerisations
- Metallurgy: additive for surface treatment of steel
- Nuclear industry: auxiliary for separation of uranium and plutonium
- Textile industry: auxiliary for specific dyeing processes; fixative for textile dyes

According to the information of the lead company (BASF, 2003), the contingent sold as intermediate e. g. for the production of oximes, active ingredients in plant protection products, and pharmaceuticals is 10,850 t/a. Minor amounts are used in textile and metal finishing industry and for photographic processing solutions. Furthermore, the usage of approximately 2,000 t/a is not known by the industry because it is sold for trade.

Table 2.2: Use categories for the uses of bis(hydroxylammonium)sulfate

Use	Industry category	Use category	Quantity used [t/a]	Percentage of total use
Intermediate (caprolactam)	003	033	476,000	97.2
Intermediate (other)	003	033	10,850	2.2
Metal finishing	008	009	260	5.4 E-04
Textile finishing	013	021	300	6.2 E-04
Trade	Trade	Unknown	2,000	4,3 E-03
Total			489,000	99.5

2.2.2 Scenarios

2.3 TRENDS

No information available.

2.4 LEGISLATIVE CONTROLS

No legislative controls implemented.

2.4.1 Scenarios

[click here to insert text]

2.5 TRENDS

[click here to insert text]

2.6 LEGISLATIVE CONTROLS

[click here to insert text]

3 ENVIRONMENT

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

According to information provided by the bis(hydroxylammonium)sulphate producing companies, bis(hydroxylammonium)sulphate has found the following applications in different branches of industry (BASF, 2003; Bayer, 2004):

approx. 98,3 %	chemical industry as an intermediate (production of caprolactam, lauro lactam, oximen), agro industry (production of insecticides, herbicides) and pharmaceutical industry (production of e.g. analgesics, antibiotics, tranquillizers)
approx. 1,1 %	export outside the EU
approx. 0.6 %	textile industry (auxiliary for certain dyeing processes, fixer for textile dyes), metallurgy (additive in the surface treatment of metals and sales as aqueous solution or crystalline salt (developers in the photographic industry, laboratory chemical)

Further areas of use include (BASF, 2002; Ullmann, 1998):

- rubber industry as an accelerator for vulcanisation, as a polymerisation controller for synthetic rubber and as an antioxidant for natural rubber
- plastics industry for terminating polymerisation reactions (radical scavenger).
- food industry as an intermediate for the production of sweeteners.
- coatings and paints industry in the production of special oximes as antiskinning agents.

For further information see chapter 2.

For workers the inhalation and dermal routes of exposure are likely to occur.

4.1.1.1 Occupational exposure

Bis(hydroxylammonium)sulphate is produced by large-scale chemical companies. Presently there are three production sites in the EU.

Due to the physico-chemical properties of the substance (solid at room temperature and salt character) inhalation exposures to vapour during the handling of solutions are assumed to be negligible (no vapour pressure is determined). According to information provided by one company, the particle sizes ranges from 200 to 2000 μm (95 % > 200 μm , 75 % > 500 μm) and exposure to dust is likely to be significantly reduced (BASF, 1993).

Occupational exposure limits (OEL) have not been established .

The following scenarios are regarded as relevant for occupational exposure:

- Scenario 1: Production and further processing of bis(hydroxylammonium)sulphate as an intermediate in the large-scale chemical industry (4.1.1.2.1)
- Scenario 2: Formulation of bis(hydroxylammonium)sulphate for photo-developing chemicals (4.1.1.2.2)
- Scenario 3: Formulation of bis(hydroxylammonium)sulphate as an auxiliary in different industries (4.1.1.2.3)
- Scenario 4: Use of bis(hydroxylammonium)sulphate in photographic laboratories (4.1.1.2.4)
- Scenario 5: Use of bis(hydroxylammonium)sulphate formulations in different industries, e.g. electroplating industry (4.1.1.2.5)

Scenario 5 is established based on information from the EU Member States which showed, that the use in the electroplating industry is the most frequent application of bis(hydroxylammonium)sulphate.

Based on the information from industry, the member states and from literature, the following overview on the different concentrations of bis(hydroxylammonium)sulphate is given. In the production of the substance, beside crystalline bis(hydroxylammonium)sulphate, concentrated solutions of 10 – 42 % are obtained. Most of the produced amount is further processed in the chemical industry, the concentration of the corresponding solutions is not known in detail. An exception is the production of caprolactam, where 14.5 % and 25 % solutions are applied.

For the further processing of bis(hydroxylammonium)sulphate outside the large scale chemical industry, concentrations below 30 % bis(hydroxylammonium)sulphate are reported. The formulations applied in different industries, namely the photographic industry, the textile and the metal industry, may be formulated on the basis of the crystalline substance or of concentrated solutions.

For applications in the photographic sector, concentrations of 25 – 30 % are reported, whereas for the metal treatment sector 25 % is the maximum mentioned concentration.

The assessment of inhalation exposure is mainly based on measured exposure levels from which – if possible – 90th or 95th percentiles are derived as representing reasonable worst case situations.

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

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.

4.1.1.1.1 Production and further processing of bis(hydroxylammonium) sulphate as an intermediate in the large-scale chemical industry (scenario 1)

Scenario 1 is related to the production of bis(hydroxylammonium)sulphate and its further processing as a chemical intermediate in the large-scale chemical industry.

Bis(hydroxylammonium)sulphate is produced in closed systems by a catalytic process where nitric oxide is hydrogenated in sulfuric acid to hydroxylamine. The resulting aqueous solution of bis(hydroxylammonium)sulphate is intermediately stored in tanks. The aqueous solution is transferred in a pipeline to the crystallisation reactor (closed system). The dried salt is filled by screw packers in 25 kg polyethylene bags or big bags under local exhaust ventilation (LEV). The substance is placed on the market as an aqueous solution as well as in form of a crystalline salt. Aqueous bis(hydroxylammonium)sulphate solution is transported via ship, road or rail in stainless steel or polyethylene tanks or containers (BASF, 1996; BASF, 2001; BASF, 2003).

Approximately 97 % of the produced bis(hydroxylammonium)sulphate is used as an intermediate for the production of caprolactam. Caprolactam is produced in closed systems at the same plants that produce bis(hydroxylammonium)sulphate (large-scale chemical industry). Here cyclohexanon and bis(hydroxylammonium)sulphate react to cyclohexanonoxim which is then converted to caprolactam (Beckmann transformation). The residual concentration of bis(hydroxylammonium)sulphate in caprolactam is below the detection limit (= 1 ppm). According to the information provided by two companies, bis(hydroxylammonium)sulphate (67 % of the produced bis(hydroxylammonium)sulphate) is used as an aqueous solution in the concentration of 14.5 or 25 %, without isolation of the solid bis(hydroxylammonium)sulphate, for the production of caprolactam.

Smaller amounts of the produced bis(hydroxylammonium)sulphate are used as an intermediate for the production of pharmaceuticals, of insecticides and herbicides, of special oximes and of sweeteners.

It is assumed that the continuous or batchwise processes occurring in the chemical industry are mainly performed in closed systems and/or at workplaces equipped with local exhaust ventilation systems. 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 works and taking of samples. Inhalation exposure in other fields is normally minimised by technical equipment (e.g. local exhaust ventilation). On account of the particle sizes (95 % > 200 µm, 75 % > 500 µm), the exposure to dust is likely to be significantly reduced. If aqueous solutions of bis(hydroxylammonium)sulphate are

handled, inhalation exposure to vapour is negligible (salt character) compared to exposure to dust during filling of the solid substance. For workers who handle solid bis(hydroxylammonium)sulphate continuously (e.g. filling, cleaning) exposure to dust is assessed assuming that the duration and frequency of exposure are daily and of full shift length.

Inhalation exposure

Measured data

Information on exposure to total dust has been provided by one company which produces the crystalline bis(hydroxylammonium)sulphate salt. The measurement results relate to the bag filling of the substance and to the cleaning procedure of the dryer (under wet conditions). Person-related samples of inhalable dust were taken during the above-mentioned processes.

Table 4.1 Total dust exposure at workplaces during filling of bis(hydroxylammonium)sulphate and cleaning procedures (no determination of bis(hydroxylammonium)sulphate)

Job category / activities	Years of measurement	Number of samples	Measurement data [mg/m ³]	50 th percentile [mg/m ³]	90 th percentile [mg/m ³]
8 h TWA					
Bag filling	1996	8 (p)	<0.1, 0.1, 0.1, 0.1, 0.2, 0.3, 0.5, 0.9	-	-
Dryer cleaning	1996	4 (p)	0.1	-	-

p: personal sampling

Based on the highest measurement result 0.9 mg/m³ is regarded as representing a reasonable worst case situation. Since the only solid bis(hydroxylammonium)sulphate producing company submitted data covering different activities, the measurement results are regarded as representative. For the exposure assessment it is assumed, that the total dust is composed solely of bis(hydroxylammonium)sulphate because no other substances are handled at the corresponding workplaces.

According to information provided by one manufacturer, 20 workers are employed in the production and further processing of bis(hydroxylammonium)sulphate.

Modelled data

EASE for Windows 2.0, Aug. 1997 was used.

EASE estimation for the production of bis(hydroxylammonium)sulphate and its further processing as a chemical intermediate:

Input parameters: T = 20 °C, exposure-type is dust, low dust technique, LEV present

Level of exposure: 0 - 1 mg/m³.

The category “low dust technique” is chosen due to the high amount of particle sizes greater than 200 μm (95 %).

Summary of the exposure level

Inhalation exposure has to be assessed for the production and further processing of bis(hydroxylammonium)sulphate in fields with high levels of protection.

For the assessment of health risks from daily inhalation exposure to bis(hydroxylammonium)sulphate during the production and further processing an 8 h time-weighted average concentration (8 h TWA) of 0.9 mg/m^3 (highest measurement result) should be taken to represent a reasonable worst case situation. This assessment is confirmed by the EASE estimation of 0 - 1 mg/m^3 . The duration and the frequency of exposure to bis(hydroxylammonium)sulphate are assumed to be daily and for the entire length of shift.

For aqueous solutions of bis(hydroxylammonium)sulphate, based on the salt character of the substance, inhalation exposure to vapour is assessed as negligible. The formation of aerosols is regarded as unlikely because the processes take place in closed systems.

Dermal exposure

When producing and further processing bis(hydroxylammonium)sulphate dermal exposure could occur during activities like bagging, 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 $\text{mg}/\text{cm}^2/\text{day}$.

Considering an exposed area of 420 cm^2 (palms of hands) the model yields an exposure level of 42 - 420 $\text{mg}/\text{person}/\text{day}$.

For assessing actual dermal exposure levels, it has to be considered that the substance is manufactured and further processed primarily in closed systems and that the use of personal protective 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 the substance. For the handling of powdery substances, as a rule, the suitability of the gloves can be assumed. As a rough estimation, suitable gloves are assumed to achieve a protection efficiency of 90 %. As a result, dermal exposure is calculated as 4.2 – 42 $\text{mg}/\text{person}/\text{day}$. The upper value is regarded as representing the reasonable worst case situation.

The major part of the produced bis(hydroxylammonium)sulphate is used as chemical intermediate and is handled exclusively as an aqueous solution containing up to 25 % bis(hydroxylammonium)sulphate. 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 $\text{mg}/\text{cm}^2/\text{day}$.

Considering an exposed area of 210 cm² and taking into account the concentration of 25 % of bis(hydroxylammonium)sulphate, the exposure amounts to 5.25 – 52.5 mg/person/day. According to information provided by the manufactures (safety data sheets) suitable gloves tested according to EN 374 are worn. As a rule, for the use of suitable gloves, low levels of daily dermal exposure are to be expected. Since no measurement results are available, a protection efficiency of 90 % is taken as a default value leading to an exposure level of 0.5 – 5.3 mg/person/day. The upper value is regarded as representing the reasonable worst case situation.

Summary of the exposure level

For assessing the health risks from daily dermal exposure to bis(hydroxylammonium)sulphate in the area of production and further processing of the substance (scenario 1), an exposure level of 42 mg/person/day should be taken (EASE estimation). This exposure level reflects exposure during handling powdery bis(hydroxylammonium)sulphate. Dermal exposure is assessed for the protected worker. Lower levels are expected if aqueous solutions of bis(hydroxylammonium)sulphate are produced and used.

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

4.1.1.1.2 Formulation of bis(hydroxylammonium)sulphate for photo-developing chemicals (scenario 2)

In the photographic industry bis(hydroxylammonium)sulphate is formulated to photochemicals (stabiliser for colour developers, additive in emulsions for colour films). In Germany two large-scale companies produce colour developers containing bis(hydroxylammonium)sulphate.

According to information provided by one photographic company, pure solid bis(hydroxylammonium)sulphate is used in an amount of 400 kg for 40 - 50 times per year to formulate colour developers. For the formulation one person handles the solid bis(hydroxylammonium)sulphate for 1-2 hours once a week. The resulting concentrates (20 – 30 % bis(hydroxylammonium)sulphate) are filled by 2 persons once a week. This concentrates are diluted afterwards at the user's site (application concentration: approx. 0.3 %) (Tetenal, 2003).

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 areas is normally minimised by technical equipment (e.g. local exhaust ventilation). A similar level of protection is considered to be realised in the photographic industry. On account of the particle sizes (95 % > 200 µm, 75 % > 500 µm), the exposure to dust is likely to be significantly reduced. If solutions of bis(hydroxylammonium)sulphate are handled, the inhalation exposure to vapour is negligible due to the physico-chemical properties of the substance (salt character).

Inhalation exposure

Measured data

No measurement values are available.

Modelled data

EASE for Windows 2.0, Aug. 1997 was used.

EASE estimation for the formulation of bis(hydroxylammonium)sulphate as colour developers.

Input parameters: T = 20 °C, exposure-type is dust, low dust technique, LEV present

Level of exposure: 0 - 1 mg/m³.

The category “low dust technique” is chosen due to the high amount (95 %) of particle sizes greater than 200 µm. Considering the reduced exposure duration of 2 hours the resulting exposure level is 0.25 mg/m³. The exposure to bis(hydroxylammonium)sulphate is once a week.

Summary of the exposure level

Inhalation exposure has to be assessed for the weighing and filling of solid bis(hydroxylammonium)sulphate in the photographic industry. According to the available information from one company, solid bis(hydroxylammonium)sulphate is used for 1-2 hours per week. Taking into consideration the high protective standard of the large-scale industry the resulting inhalation exposure level is 0.25 mg/m³ on a non-daily basis (EASE estimation). This value is of the same order of magnitude as exposure in the large-scale industry. The slightly lower exposure level is a result of the reduced duration of exposure.

For aqueous solutions of bis(hydroxylammonium)sulphate (maximum concentration: 30 %), based on the salt character of the substance, inhalation exposure to vapour is assessed as negligible. The formation of aerosols is regarded as unlikely.

Dermal exposure

In the photographic industry the use of gloves is highly accepted because many hazardous substances are handled.

Modelled data

Dermal exposure to solid bis(hydroxylammonium)sulphate is assessed for weighing and filling procedures. For the unprotected worker, according to the EASE model, potential 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 use of personal protective equipment (PPE, here gloves and eye protection) is highly accepted. 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 the substance. For the handling of powdery substances, as a rule, the suitability of the gloves can be assumed. As a rough estimation, suitable gloves are assumed to achieve a protection efficiency of 90 %. As a result, dermal exposure is calculated as 4.2 – 42

mg/person/day. The upper value of 42 mg/person/day is regarded as representing the reasonable worst case situation. For filling aqueous solutions of bis(hydroxylammonium)sulphate at a maximum concentration of 30 % the resulting dermal exposure level is 1.3 – 12.6 mg/person/day. The upper value of 12.6 mg/person/day is regarded as representing the reasonable worst case situation.

Summary of the exposure level

For assessing the dermal exposure in the area of the formulation of photochemicals an exposure level of 42 mg/person/day for the solid bis(hydroxylammonium)sulphate should be taken as representing the non-daily exposure. Due to the high particle sizes of the substance, the dermal exposure is assumed to be lower than 42 mg/person/day. However this reducing effect cannot be quantified.

For filling aqueous solutions of bis(hydroxylammonium)sulphate at the maximum concentration of 30 % the dermal exposure level is reduced to 12.6 mg/person/day.

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

4.1.1.1.3 Formulation of bis(hydroxylammonium)sulphate as an auxiliary in different industries (scenario 3)

According to information provided by one bis(hydroxylammonium)sulphate producing company, approximately 600 t/year of bis(hydroxylammonium)sulphate are used in the following industries:

- textile industry as an auxiliary for certain dyeing processes and as a fixer for textile dyes
- metallurgy industry as an additive in the surface treatment of metals

In these industries bis(hydroxylammonium)sulphate is used as an additive or an auxiliary. No detailed information is available about the formulated concentrations of bis(hydroxylammonium)sulphate and whether solid bis(hydroxylammonium)sulphate or solutions of bis(hydroxylammonium)sulphate are used. It is assumed that the formulations of bis(hydroxylammonium)sulphate are produced in specialised formulation companies or at the user's site. In this case it cannot be excluded in principle that, in addition to the high level of technical protection in the large-scale industry, open systems without local exhaust ventilation are used (Voullaire, Kliemt 1995). It is to be assumed that gloves and eye protection are not regularly worn.

Due to the small amounts of bis(hydroxylammonium)sulphate used for the formulation, the duration and the frequency of exposure to bis(hydroxylammonium)sulphate are assumed to be non-daily but for the entire length of shift.

Possibilities of inhalation and dermal exposure exist during the weighing and filling of solid bis(hydroxylammonium)sulphate. On account of the particle sizes (95 % > 200 µm, 75 % > 500 µm, 5 % < 200 µm), the exposure to dust is likely to be significantly reduced.

Inhalation exposure

Measured data

Workplace measurement results are not available.

Modelled data

EASE for Windows 2.0, Aug. 1997 was used.

EASE estimation for the handling of solid bis(hydroxylammonium)sulphate without LEV:

Input parameters: T = 20 °C, exposure-type is dust, low dust technique, LEV absent

Level of exposure: 0 - 5 mg/m³.

The category “low dust technique” is chosen due to the high amount of particle sizes greater than 200 µm. For formulation, the activities relevant for exposure (weighing, filling) are usually performed not during the whole shift but for < 2 hours per day. This reduces the exposure level to 1.25 mg/m³ (8 h TWA).

Summary of the exposure level

Detailed information is not available for the formulation of bis(hydroxylammonium)sulphate for the textile and metallurgy industries, in formulating companies or at the user’s site. The inhalation exposure to solid bis(hydroxylammonium)sulphate is assessed during formulation taking into account the rather high particle sizes of the substance (95 % > 200 µm). The estimated value of 1.25 mg/m³ on basis of the EASE model categories “LEV absent” and “low dust technique” should be taken as an 8h time-weighted average on a non-daily basis and can be regarded as representing a reasonable worst case.

Dermal exposure

For the filling and weighing procedures of solid bis(hydroxylammonium)sulphate during formulation it is to be assumed that protective gloves are not regularly worn.

Modelled data

Dermal exposure to the solid bis(hydroxylammonium)sulphate for the unprotected worker is assessed according to the EASE model:

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.

Summary of the exposure level

Dermal exposure during the weighing and filling of solid bis(hydroxylammonium)sulphate is predicted to be 420 mg/person/day (value determined by EASE estimation). The estimated value is based on the assumption that gloves are not worn. It is assumed that this estimated

value for the dermal exposure can be regarded as representing a reasonable worst case on a non-daily basis. Due to the high particle sizes of the substance, the dermal exposure is assumed to be lower than 420 mg/person/day. However this reducing effect cannot be quantified. It cannot be presupposed that eye protection is regularly used. For assessing the risks, hand-eye contacts as well as possible splashes to the eye should be considered.

4.1.1.1.4 Use of bis(hydroxylammonium)sulphate in photographic laboratories (scenario 4)

Formulations containing bis(hydroxylammonium)sulphate are used in photographic laboratories as a colour developer and as an additive in emulsions for colour films in only one colour developing process (called C41). The producers of photochemicals deliver the liquid concentrates containing up to 30% bis(hydroxylammonium)sulphate. For application in developing solutions, the customers dilute these concentrates with water and different chemicals. The resulting concentration of bis(hydroxylammonium)sulphate amounts to 0.25 – 0.3 % (Tetenal, 2003).

In Germany, the photo developing procedures take place in 2,000 “Minilabs”, approximately 400 specialised laboratories and approximately 50 large laboratories (Tetenal, 2003). “Minilabs” are closed systems with a typical developing bath volume of 10 l. The photochemical are applied as aqueous solutions. Some “Minilabs” have a “plug and fill technique” with containers which include all required chemicals. Here the direct handling of photochemical is avoided by the plug in of these containers into the developer machine (AGFA, 2002). In specialised photographic laboratories which have more capacity than “Minilabs” the developing bath contains 50 – 100 l of developing solutions. Bis(hydroxylammonium)sulphate solutions are filled from 1 l containers into the developer machine. In large photographic laboratories the developing bath contains 1,000 l. The colour developing solutions are automatically pumped from 60 l – 200 l drums into the developer machine. Here dermal exposure may occur due to unintended contamination e.g. gloves that are used.

Apart from the “plug and fill technique”, possibilities of exposure exist during filling, diluting and disposal of the aqueous solution of bis(hydroxylammonium)sulphate. Because of the further dilution of bis(hydroxylammonium)sulphate solutions with water and other developing compounds the concentration of bis(hydroxylammonium)sulphate during disposal is reduced to approximately 3 g/l.

Since only aqueous solutions of bis(hydroxylammonium)sulphate are used and the formation of droplet aerosols is regarded as unlikely, the inhalation exposures to vapour or aerosols are assumed to be negligible. The dermal exposure during the filling of aqueous solutions of bis(hydroxylammonium)sulphate at a maximum concentration of 30 % is assessed in the next section.

Inhalation exposure

In photographic laboratories only aqueous solutions of bis(hydroxylammonium)sulphate are used. Since formation of aerosols is regarded as unlikely and taking into account the salt character of the substance, inhalation exposure to vapour or aerosols are assumed to be negligible.

Dermal exposure

The use of protective gloves is accepted in photographic laboratories because a lot of sensitive and toxic compounds are handled. On the other hand, skin disorders are found in this industry and it is assumed that dermal contact to the different substances may occur because protective gloves are not regularly worn in laboratories (Tetenal, 2003).

Modelled data

Within photo laboratories, often 1 person is responsible for the application of chemicals – here the use of bis(hydroxylammonium)sulphate in concentrates. Specialised laboratories prepare developing solutions once a week whereas large companies use the concentrates daily. According to information provided by the monitoring authorities of the Federal States (Länder) of Germany, the duration of the activities of relevance (diluting and filling) is approximately 10 - 15 minutes. Therefore the exposure category “incidental” is chosen in an attempt to describe the exposure situation using the EASE model. The dermal exposure to bis(hydroxylammonium)sulphate solutions in the different photographic laboratories is assessed for filling, weighing and disposal procedures according to the EASE model:

Input parameters: non dispersive use, direct handling, incidental

Level of exposure: 0 – 0.1 mg/cm²/day.

Considering an exposed area of 420 cm² (palms of hands) the model yields an exposure level of 0 - 42 mg/person/day. In photographic laboratories bis(hydroxylammonium)sulphate is applied only as an aqueous solution in the maximum concentration of 30 %. Taking into account the concentration of 30 % the resulting dermal exposure level is 0 – 12.6 mg/person/day. The upper value of 12.6 mg/person/day is regarded as representing the reasonable worst case situation.

Summary of the exposure level

For assessing the health risks due to dermal exposure in the area of photographic processes, an exposure level of 12.6 mg/person/day for a maximum concentration of 30 % of bis(hydroxylammonium)sulphate should be taken. The level of 12.6 mg/person/day is regarded as representing the reasonable worst case situation on a non-daily basis. This exposure assessment is based on the assumption that gloves are not regularly used.

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

4.1.1.1.5 Use of bis(hydroxylammonium)sulphate in different industries, e.g. the electroplating industry (scenario 5)

For the mentioned uses of bis(hydroxylammonium)sulphate in the textile industry and the metal treatment, based in the information provided by the EU Member States, the latter is taken for assessing exposure because this use deems to be the most frequent one.

For scenario 5, two situations are of importance:

- use of concentrate (up to 25 % bis(hydroxylammonium)sulphate) for the formulation of diluted solutions
- application of diluted solutions for acid pickling

In the electroplating industry bis(hydroxylammonium)sulphate is used in acid pickling baths for the surface pre-treatment of metals and plastics before galvanic processes (metal, printed circuit board industry). Due to the formation of hydrogen in pickling baths, aerosols containing bis(hydroxylammonium)sulphate are released.

Formulations in liquid form are manufactured (e.g. by electroplating supply houses) by mixing the various raw materials together. User companies such as electroplating shops may employ these industrially manufactured solutions and formulations or they may produce appropriate solutions by themselves. In the production of these formulations bis(hydroxylammonium)sulphate may be used in the form of concentrates containing up to 25 %. According to information from the industry, the resulting formulations as acid pickling solutions contains < 1 % bis(hydroxylammonium)sulphate (BASF, 2003).

Inhalation exposure to emitted aerosols as a result of gas release is to be expected during activities which are regularly performed at electroplating plants (manual dipping and removing of plating-racks, operating semiautomatic machines, check patrols). Exposure to vapour is assumed to be of relevance if cleaning and maintenance activities are performed.

For these applications, due to the low vapour pressure of bis(hydroxylammonium)sulphate, inhalation exposure to vapour is of minor relevance. Higher inhalation exposure may occur if droplet aerosols are formed.

For dermal exposure the general use of PPE (here: suitable gloves and eye protection) cannot be presupposed for this branche of industry at all, despite the fact that there are single companies with a reasonable high level of protection. One exception has to be taken into account when corrosive substances are handled. It is assumed that acid pickling solutions (with acid content ≥ 15 %) for electroplating processes have to be classified as corrosive because of the corrosive effect of other substances than bis(hydroxylammonium). In such cases, repeated immediate skin contact is avoided by using personal protective equipment (gloves and eye protection).

The duration and frequency of the exposure is often depending on the order situation of the single enterprises. As a reasonable worst case pickling is assumed to be carried out at regular intervals distributed over the entire shift.

Inhalation exposure

For the assessment of inhalation exposure due to the low vapour pressure of the substance, exposure to vapour is assessed as negligible but exposure to aerosols is assessed by comparison with analogous measurement results.

Analogous data

The EASE model is not appropriate to estimate exposure to aerosol particles.

Comparison by analogy – exposure to aerosols during acid pickling

For the purpose of estimating the inhalation exposure to bis(hydroxylammonium)sulphate containing aerosols during pickling processes particularly as a pre-treatment operation before electroplating, occupational exposure to sulphuric acid mists during these processes is considered as an analogous scenario. Sulphuric acid is chosen because it is used for the same purpose, appears as particle in the atmosphere and workplace measurements are available. Bis(hydroxylammonium)sulphate and sulphuric acid have also similar physicochemical properties, i.e. low vapour pressure.

In a Federal Institute for Occupational Safety and Health (BAuA, Germany) study companies in the metal processing industry across the Federal Republic of Germany were approached. In a total of eight companies, each using pickling plants operating with sulphuric acid exposure measurement were taken. The concentration of the sulphuric acid in the pickling baths in general is 20 %. 8 h time-weighted averages (TWA's) of personal (n = 8) and area sampling (n = 17) are in the range of 0.015 - 0.079 mg/m³ and < 0.1 - 1.03 mg/m³ H₂SO₄ (Guba, 2005). Additional measurements were made in the electroplating industry of Germany in 1997. The measurements relate to exposure to chrome (VI)-compounds, nickel and acid mists in form of aerosols during electrolytic processes. The exposure levels of sulphuric acid mist are in the range of < 0.007 - 0.058 mg/m³ (n = 85) (Macho, 2000). Another study by the BG (Worker's Compensation Fund) shown sulphuric acid concentrations (95th percentile) of < 0.01 - 0.123 mg/m³ (2001-2003) (BG/BIA, 2004).

Assuming that the highest sulphuric acid concentration of 1.03 mg/m³ (8 h TWA) corresponds to a sulphuric acid content of approx. 20 %, the exposure to bis(hydroxylammonium)sulphate (content of < 1 %) is calculated to be 0.05 mg/m³ (8 h TWA). Summary of the exposure level.

Summary of the exposure level

For the assessment of health risks due to inhalation exposure to aerosols during acid pickling processes an 8 h time weighed average concentration (8 h TWA) (aerosol emission) of 0.05 mg/m³ should be taken (reasonable worst case).

These levels should be taken as an 8 h time weighed average representing the reasonable worst case situation of exposure to aerosol emission. For other exposure scenarios the exposure levels are assumed to be in the same order of magnitude or below the assessed level. E.g. exposure to vapour is assessed to be negligible inter alia because of the low vapour pressure of bis(hydroxylammonium)sulphate.

Pickling in electroplating enterprises are carried out at regular intervals over the entire day. Whether these processes are done daily often depend on the order situation. As a reasonable worst case scenario the duration and frequency of exposure are assumed to be daily and for the entire length of shift.

Dermal exposure

For the assessment of dermal exposure, two situations are considered. Weighing and filling the concentrates for the formulation of diluted solutions and the application of these diluted solutions in acid pickling baths. For the latter, it is considered that corrosive solutions are usually applied in acid pickling processes. For the handling of corrosive substances, it is to be assumed, that PPE is regularly worn. The effect of corrosivity is immediately perceptible, so the workers protect themselves with appropriate means. Repeated dermal exposure is assumed

to be avoided by using personal protective equipment (gloves and eye protection). Dermal exposure for the handling of corrosive solutions is assessed as negligible.

In cases with handling the concentrate (< 25 % bis(hydroxylammonium)sulphate) for preparation of diluted solutions dermal exposure is assessed as follows:

Input parameters: Non dispersive use, direct handling, incidental

Level of exposure: 0 - 0.1 mg/cm²/day

0 – 0.025 mg/cm²/day (< 25 % bis(hydroxylammonium)sulphate)

Considering an exposed area of 420 cm² (palms of the hands), the dermal exposure amounts to 0 – 10.5 mg/person/day (< 25 % bis(hydroxylammonium)sulphate).

Summary of the exposure level

For assessing the health risks of dermal exposure, two situations are considered.

- formulated of diluted solutions
- application of diluted solutions in acid pickling baths.

For the handling of concentrates (< 25 % bis(hydroxylammonium)sulphate), an exposure level of 10.5 mg /person/day should be taken as representing the non-daily dermal exposure.

For the application of diluted solutions (< 1 % bis(hydroxylammonium)sulphate) in acid pickling processes, the corrosive effect of these solutions is taken into account and the dermal exposure is assessed as negligible.

4.1.1.1.6 Summary of occupational exposure

98 % of bis(hydroxylammonium)sulphate is used as an intermediate in large-scale industry for different products (e.g. 97 % of bis(hydroxylammonium)sulphate production of caprolactam). More than half of the produced bis(hydroxylammonium)sulphate is handled here as an aqueous solution at a maximum concentration of 25 %.

Due to the physico-chemical properties of the substance (solid at room temperature, vapour pressure not detectable, salt character), inhalation exposures to vapour during the handling of solutions are assumed to be negligible. The possible formation of droplet aerosols (e.g. surface treatment of steel) is taken into account for one scenario (see 4.1.1.2.5). On account of the particle size distribution (95 % > 200 µm, 75 % > 500 µm) and the resulting high amount of large particles, exposure to dust is likely to be significantly reduced compared to powdery substances. This reducing effect is considered in the EASE estimation choosing the inhalation exposure category “low dust technique”. However, though the choice of no methods currently exists to implement this possible reducing effect for dermal exposure.

The following scenarios are regarded as relevant for occupational exposure:

- Scenario 1: Production and further processing of bis(hydroxylammonium)sulphate as an intermediate in the large-scale chemical industry (4.1.1.2.1)
- Scenario 2: Formulation of bis(hydroxylammonium)sulphate for photo-developing chemicals (4.1.1.2.2)
- Scenario 3: Formulation of bis(hydroxylammonium)sulphate as an auxiliary in different industries (4.1.1.2.3)
- Scenario 4: Use of bis(hydroxylammonium)sulphate in photographic laboratories (4.1.1.2.4)
- Scenario 5: Use of bis(hydroxylammonium)sulphate in different industries, e.g. the electroplating industry (4.1.1.2.5)

Relevant inhalation and dermal exposure scenarios are given in table 4.1.a and b. For workplaces at which the crystalline material as well as solutions may be handled, both exposure levels are assessed (see text) but only the higher level is taken forward to the risk characterisation (see table 4.1.a and b).

For the large-scale chemical industry, it is assumed that the production and further processing of bis(hydroxylammonium)sulphate are mainly performed in closed systems and that the use of PPE (here gloves and eye protection) is highly accepted. Exposure occurs if the systems are breached for certain activities, e.g. filling (scenario 1). Measured inhalation exposure levels and the EASE estimate are of the same order of magnitude.

Occupational exposure during formulation is described in scenario 2 for the formulation of photochemicals and in scenario 3 for the production of other formulations. For the production of photochemicals, a higher standard of occupational hygiene is assumed due to the effect that many hazardous substances are handled. For the scenarios (2, 3), inhalation and dermal exposure is assessed using the EASE model.

Formulations containing bis(hydroxylammonium)sulphate are used in photographic laboratories (scenario 4) only as aqueous solutions in a maximum concentration of 30 %. Due to the salt character of the substance, inhalation exposure is assessed as negligible.

For the application of bis(hydroxylammonium)sulphate in metal treatment (acid pickling, scenario 5) inhalation exposure caused by the formation of aerosols is assessed. For dermal exposure, dermal contacts to the concentrates (< 25 % bis(hydroxylammonium)sulphate) as well as exposure to diluted solutions during dipping are considered.

Table 4.2: Conclusions of the inhalation exposure assessment

Scenario number, Area of production and use	Form of exposure	Activity	Inhalation exposure (RWC)				Method	Short-term concentration [mg/m ³]	Method
			Duration [h/day]	Frequency [days/year]	Shift average concentration [mg/m ³]				
Production									
1. Production and further processing as an intermediate	dust ¹⁾	bagging, cleaning	shift length (assumed)	daily	0.9	max. value, workplace measurements	-	-	
Formulation									
2. Formulation for photo- developing chemicals	dust	weighing, filling	2 hours	not daily	0.25	EASE (low dust technique, LEV present)	-	-	
3. Formulation as an auxiliary in different industries	dust	weighing, filling	2 hours (assumed)	not daily	1.25	EASE (low dust technique, LEV absent)	-	-	
Uses									
4. Use in photographic laboratories	liquid (30 %)	filling	15 min.	not daily	negligible	expert judgement	-	-	
5. Use in the electroplating industry	aerosol	dipping	shift length (assumed)	daily	0.05	analogous scenarios	-	-	

¹⁾ Exposure to the liquid is negligible due to the low vapour pressure.

Table 4.3: Conclusions of the dermal exposure assessment

Dermal exposure (RWC)								
Scenario number, Area of production and use	Form of exposure	Activity	Frequency [days/year]	Contact level ¹⁾	Level of exposure [mg/cm ² /day]	Exposed area [cm ²]	Shift average [mg/person/day]	Method (use of gloves)
Production								
1. Production and further processing as an intermediate	dust	weighing, filling	daily	intermittent	0.1 - 1	420	42	EASE (with gloves)
	liquid (25 %)	weighing, filling	daily	intermittent	0.1 - 1	210	5.3	EASE (with gloves)
Formulation								
2. Formulation as photo- developing chemicals	dust	weighing, filling	not daily	intermittent	0.1 - 1	420	42 ²⁾	EASE (with gloves)
3. Formulation as an auxiliary in different industries	dust	weighing, filling	not daily	intermittent	0.1 - 1	420	420 ²⁾	EASE (without gloves)
Uses								
4. Use in photographic laboratories	liquid (30 %)	weighing, filling	not daily	incidental	0 - 0.1	420	12.6	EASE (without gloves)

5. Use in the electroplating industry	liquid (25 %)	weighing, filling dipping	not daily	incidental	0 – 0.1	420	10.5	EASE (without gloves)
	liquid (< 1 %)		-	-	-		negligible	expert judgement (corrosive formulation)

¹⁾ Contact level according to the EASE model

²⁾ Due to the high particle sizes of the substance, the dermal exposure is assumed to be lower than the estimated values. However this reducing effect cannot be quantified.

4.1.1.2 General discussion

4.1.1.3 Consumer exposure

Due to information from the Swedish Product Register, the BfR-product data base, the Danish and the Finnish Product Register (all searches in 2006) and to publications bis(hydroxylammonium)sulfate is a constituent of chemical products used as a developer in photography.

Out of a total of 50 products containing BHAS in the Swedish Product Register (KEMI 2006) 29 products are photo chemicals containing 0.7 – 27 % BHAS; only 1 of these is available for consumer use.

The BfR product data base contains 15 photochemical products with concentrations between 1 and 21%. Although those products are mostly foreseen for occupational use, it cannot be excluded, that three of them are also available for consumers.

The Danish EPA (2006) reports the use of BHAS in six photo developers (containing 25-30% of BHAS). According to information by industry these products are only marketed for professional use and consumer exposure can be ruled out.

According to the Finnish Product Register (2006) only one clour developing product is listed under the use in "art and entertainment" (maximum concentration 25%). However, this product is not sold to consumers thus no consumer exposure is expected.

Normal use of the substance in photographic developers means that due to use of tweezers there will be no direct contact to hands. Unintentional exposure may be possible. In this case, however, the duration will be very short and the hands will be rinsed with water, therefore dermal absorption can be neglected.

4.1.1.3.1 Summary of consumer exposure

In conclusion, consumer exposure is considered to be negligible. However, dermal exposure to bis(hydroxylammonium)sulfate has to be considered for an assessment of possible local effects in accidental cases..

4.1.1.4 Humans exposed via the environment

Nearly all bis(hydroxylammonium)sulphate released to the environment is expected to end up in the water phase where it is transformed rapidly. Its volatility is very low. No relevant releases into the soil compartment are expected from production or use of the substance and adsorption to organic matter is negligible. Bis(hydroxylammonium) sulphate does not exhibit any potential for bioaccumulation. Therefore the risk is considered to be negligible and no assessment of indirect exposure via the environment has been undertaken.

4.1.1.5 Combined exposure

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

For some of the toxicological endpoints only few data are available for bis(hydroxylammonium)sulfate (BHAS, syn. hydroxylammonium sulfate). Therefore, information on hydroxylamine hydrochloride (CAS no. 5470-11-1) is also considered. The read-across to test results on hydroxylamine hydrochloride for an evaluation of the BHAS toxicity is justified due to the following reasons: BHAS is a solid salt of hydroxylamine and sulfuric acid. Hydroxylamine hydrochloride ($[\text{HO-NH}_3^+]\text{Cl}^-$) is a crystalline salt of hydroxylamine and hydrochloric acid.

Selected physico-chemical properties of BHAS and hydroxylamine hydrochloride (H_4NOCl) used for the read-across are listed in table 4.4. No data on melting points are available because both substances decompose at temperatures above 120 °C. The data on hydroxylamine hydrochloride are taken from BG Chemie (2000).

Table 4.4: Selected physico-chemical properties of BHAS and hydroxylamine hydrochloride (H_4NOCl)

	BHAS	H_4NOCl
Molecular weight (g/mol)	164.1	69.5
Water solubility (g/l, 20°C)	587	466
Relative density (at 20°C)	1.883	1.67

Both compounds are completely dissociated in aqueous media in hydroxylammonium cations and the respective anions. The hydroxylamine moiety represents the toxic species of both compounds (e.g. is responsible for the toxic effects). Accordingly, both compounds are classified with the same R phrases for human health effects.

4.1.2.1 Toxicokinetics, metabolism and distribution

Toxicokinetic studies on absorption, distribution or excretion of BHAS are not available. Data on inhalative uptake are totally missing. Hematotoxic effects, which have been observed after oral and dermal application to rats, rabbits and cats indicate, that absorption occurs.

4.1.2.1.1 Studies in animals

In vivo studies

Inhalation

Data on inhalative uptake of BHAS are not available. Nevertheless, BG Chemie (2000) states, that hydroxylamine and its salts can be absorbed after inhalative uptake.

Dermal

BHAS is absorbed after topical application onto the skin of rats and rabbits (24 hour occlusive exposure). This was demonstrated by methaemoglobin formation and the occurrence of Heinz bodies in erythrocytes of rabbits (Derelanko et al., 1987). Quantitative data of dermal absorption are not available.

Oral

BHAS is absorbed after oral gavage of an aqueous solution of 250 mg/kg bw to 5 rats (strain not given) which was demonstrated by the occurrence of cyanosis and methemoglobin formation (ICI, 1984, cited in BG Chemie). BHAS was also absorbed after oral gavage of 200 mg/kg bw of a 2% aqueous solution to 3 male and 3 female cats which was demonstrated by the occurrence of methaemoglobin formation (BASF, 1981). Quantitative data on oral absorption are not given in these two studies.

In an in vivo study Saul and Archer (1984) have demonstrated that hydroxylamine is partly oxidized to nitrate in the rat. Rats were gavaged with 20 μmol ^{15}N -hydroxylamine hydrochloride daily for 5 days. ^{15}N -hydroxylamine was oxidized in the rat to ^{15}N -nitrate in a yield of 4.7%. Most of the excess ^{15}N -nitrate was observed in the urines of day 4. Injection of rats with Arochlor 1254 did not significantly affect the rate of endogenous nitrate synthesis.

In vitro studies

In comparative studies in various species it was demonstrated that hydroxylamine reductase is present in mitochondria of the livers from mammals and birds, with mice and rats exhibiting the highest activity. Hydroxylamine reductase reduces hydroxylamine to ammonia (Bernheim 1972). In rats, the activity is low in regenerating liver and in very young animals; it develops with increasing age, and adult females have a higher specific activity than males. Very active mitochondrial preparations were made from pig liver and much less activity was found in livers from cats and rabbits. The kidney of rats and cats contained some of the enzyme activity, whereas brain and serum have no expression. Preparations from human sources were not included.

In in vitro studies using dogs liver and kidney homogenates, hydroxylamine inhibited activities of glutamine synthetase, glutaminase, as well as glutamate dehydrogenase and transaminases (Valdiguie et al. 1965).

Stolze and Nohl (1989) have studied the reaction sequence for the hydroxylamine-induced methemoglobin formation with bovine oxyhemoglobin in vitro. Four distinct paramagnetic intermediates could be observed using ESR spectroscopy. The first step in the reaction sequence seems to be the formation of the hydronitroxide radical and methemoglobin. The second step was the rapid disappearance of the nitroxide radical. This main pathway is

possibly the formation of nitrogen gas and water. The authors assume that in a third step methemoglobin reacts with excess hydroxylamine thereby forming the methemoglobin-hydroxylamine adduct. This complex would then be slowly oxidized to the haemoglobin-nitric oxide complex.

4.1.2.1.2 Studies in humans

In vivo studies

Human data concerning toxicokinetics of BHAS are not available. However, from allergic reactions after dermal exposure it can be concluded, that the compound is absorbed after application to human skin.

Inhalation

There are no data available.

Dermal

After dermal, occlusive application of a 0.05 % aqueous solution of BHAS (3d/week, 24 h, for 3 weeks), allergic reactions could be observed later on, which demonstrated dermal absorption (Griffith and Buehler, 1977). Quantitative data about absorption of BHAS from skin cannot be derived from these experiments.

Oral

There are no data available.

In vitro studies

There are no data available.

4.1.2.1.3 Summary of toxicokinetics, metabolism and distribution

Toxicokinetic studies on absorption, distribution or excretion of BHAS are not available. From in vitro studies there are only a few data available. Hydroxylamine is formed as an intermediate during cellular metabolism. Hydroxylamine reductase is detected in mitochondria of livers from mice, rats and pigs. Its activity appears to be age-dependent. Partly metabolic oxidation of hydroxylamine to nitrate has been described in an in vivo rat study.

For oral and inhalative uptake absorption rates of 100 % (defaults) are proposed to be taken for the risk characterisation.

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 BHAS (molecular weight: 164 g/mol; log Pow -3.6; water solubility: 587000 mg/l) a low lipophilic character of the substance can be assumed, thus leading to conclude on a low absorption through the skin. Moreover, in aqueous solutions the substance will form quaternary ammonium ions limiting percutaneous absorption. Thus, according to the TGD a default value of 10% would be

derived. Experimental data on formation of methaemoglobin and of Heinz bodies in erythrocytes of rabbits after dermal application onto the skin of rats and rabbits are indicative for an absorption. Depending on the time of exposure to the test material animal data demonstrate moderate to severe irritating properties which may explain a certain extent of dermal absorption despite the high hydrophilicity of the substance. Taking into account that occupational dermal exposure will most likely take place under non-occlusive conditions (cf. 4.1.1.2), an absorption of 10% may be assumed for dermal risk characterisation purposes.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

In vivo studies

Inhalation

There is no information available on studies performed in order to detect the inhalation LC₅₀ of BHAS, nor is there any information on methaemoglobin formation after inhalation of the substance. No studies using BHAS aerosols are available. Two inhalation risk tests with rats demonstrate that inhalation of saturated vapours (saturated at 20° C) does not cause severe toxic effects in this species. However, due to the physico-chemical properties of the substance (solid at room temperature and salt character) the vapour pressure of BHAS is assumed to be very low, the concentration of BHAS after saturation in air must also be very low. Hence, it is questionable whether the method used in the two reported inhalation studies led to significant, toxicologically relevant concentrations of BHAS.

Saturated vapours of chemically pure BHAS were produced by conducting air through a 5 cm layer of the substance at 20° C. These saturated vapours were inhaled by 12 rats for a period of 8 hours (no further details given). None of the rats died and no clinical signs were observed. Necropsy was not performed (BASF, 1969).

In a second study 12/12 rats survived a 7-hour exposure period to saturated vapours of BHAS (purity 99.5%). Saturated vapours were produced by conducting 200 l air per hour through a 5 cm layer of the substance at 20° C (method according to Smyth et al., 1962). The resulting vapours were let into one-animal inhalation chambers for 3, 10 and 30-minute and for 1, 3 and 7-hour exposure periods. All animals survived within a 14-days observation period. No clinical signs were observed. At necropsy, no changes were detected (BASF, 1980a).

Dermal

The acute dermal toxicity of BHAS is different for rats and rabbits. Furthermore, the acute dermal toxicity was different depending on the kind of bandage used in order to occlude the substance applied.

A test with rats resulted in a dermal LD₅₀ higher than 500 mg/kg bw. Female rats were exposed for 24 hours to a single application of BHAS (purity >98%) moistened with water. The test material was held in contact with the skin by wrapping the torso of the rat with a polyethylene bandage. One group of animals received test material via a sc injection (as a 1% aqueous solution). Ten animals per group were exposed to doses of 500, 100 and 10 mg/kg. Subcutaneous injection of 10 mg/kg of the substance was utilized as a rough indicator of complete dermal absorption of the test material. All animals were observed closely at least

twice each day for gross signs of toxicity, blood samples were collected from all animals, methaemoglobin determination was performed on day 2. Erythrocyte, leukocyte, platelet, and reticulocyte counts, as well as determinations of total haemoglobin, hematocrit, mean corpuscular haemoglobin concentration, were determined from days 4 and 14 blood samples. No mortality occurred within this test, skin irritation of moderate incidence, and to a lesser extent necrosis and sloughing, were evident. A large percentage of the rats exposed became pale following exposure (all dose levels). This effect was evident within 24 hours and persisted to approximately 6 days, cyanosis was not observed. Other gross signs of toxicity included staining of the nares, mouth, and fore paws with brown material, yellow staining of the anogenital area, and lacrimation. Blood methaemoglobin levels determined 48 hours following initial exposure were statistically elevated over control values in all exposed groups with the greatest increase occurring in the topical 500 mg/kg group (4.0%) and in the 10 mg/kg sc injected group (6.3%). Heinz bodies were not observed in circulating erythrocytes. Principal findings at necropsy included a high incidence of enlarged and darkened spleens regardless of the dose level or route of exposure. Gross effects on the liver were minimal to absent (Derelanko et al. 1987).

A dermal LD₅₀ between 100 mg/kg bw and 500 mg/kg bw was detected for rabbits in a study comparing occlusive and semi-occlusive dermal exposure. BHAS (purity >98%) proved strikingly more toxic when administered under plastic than under gauze despite the fact that both methods included occlusion. Female Albino rabbits were exposed for 24 hours to a single topical application of test material moistened with water. The test material was covered with either a porous gauze patch or a plastic cover. Both the gauze and the plastic covers were held in place with surgical tape. One group of animals received test material via a sc injection (as a 1% aqueous solution). Ten animals per group were exposed to doses of 500, 100, 10 and 1 mg/kg under plastic cover and doses of 1000, 500 and 100 mg/kg using gauze. Plastic covering was not used at the 1000 mg/kg level since earlier findings indicated such an exposure would be 100% lethal to the rabbit. Subcutaneous injection of 10 mg/kg of the substance was utilized as a rough indicator of complete dermal absorption of the test material. All animals were observed closely at least twice each day for gross signs of toxicity, blood samples were collected from all animals, methaemoglobin determination was performed on day 2. Erythrocyte, leukocyte, platelet, and reticulocyte counts, as well as determinations of total haemoglobin, hematocrit, mean corpuscular haemoglobin concentration, were determined from days 4 and 14 blood samples. Animals surviving for 14 days were necropsied. After occlusive skin contact with 100 mg/kg BHAS 2/10 rabbits died demonstrating 18.7% methaemoglobin, in surviving animals 80% Heinz bodies in erythrocytes were detected 4 days after exposure. After semi-occlusive skin contact with 100 mg/kg BHAS no deaths were noted (methaemoglobin 1.9%). After occlusive skin contact with 500 mg/kg 9/10 rabbits died demonstrating 60.8% methaemoglobin concentration and formation of Heinz bodies in all animals (no quantitative data). After semi-occlusive skin contact with 500 mg/kg BHAS no animals died (methaemoglobin 1.9%). Even in the 10 mg/kg group with occlusive exposure (none of the animals died, no methaemoglobin formation), Heinz bodies were detected in one animal 4 days after exposure. In addition, erythrocytes and reticulocyte counts were significantly decreased and increased, respectively. In contrary, within the semi-occlusive exposure groups even 1000 mg/kg was survived by all animals (percentage of methaemoglobin 6.2% and formation of Heinz bodies in three animals). The reference group with sc injection of 10 mg/kg (no mortality) revealed 5.1% methaemoglobin, and Heinz bodies were found in erythrocytes of all animals (4 days after treatment, no quantitative data). However, it is to be mentioned there were some methodological insufficiencies concerning methaemoglobin determination on rabbits in this study (Derelanko et al., 1987).

Derelanko et al. compared dermal toxicity of BHAS (purity >98%) and phenylhydrazine hydrochloride (purity not specified) in the rabbit and in the rat following a single 24-hour dermal exposure in a separate study in more detail (Allied Corporation, 1984). Both substances proved to be more toxic when administered under a plastic cover than under gauze and produced nearly similar toxic responses at equivalent dose levels which included anaemia with methaemoglobin formation and the presence of Heinz bodies in the circulating erythrocytes, reticulocytosis, cyanosis, hypothermia, a transient depression in weight gain, and dermal necrosis at the exposure site. Mortality occurred in the rabbits at BHAS dose levels of 0.1 g/kg or higher and significant hematological effects occurred at dose levels as low as 0.01 g/kg when administered dermally under a plastic cover. No mortality occurred in the rats exposed to either BHAS or phenylhydrazine hydrochloride. In this study BHAS was more toxic to the rabbit than phenylhydrazine hydrochloride in its hematological effects. A comparison of the toxic responses following subcutaneous and dermal exposure as demonstrated in this study suggests that BHAS is significantly, but not entirely, absorbed through the rabbit skin. In terms of hematotoxicity, a dose of 0.001 g/kg can be considered as "no-effect" level for the substance in the rabbit (NOAEL, cf. table 4.5).

Other tests employing rabbits demonstrate clearly that semi-occlusive skin exposure is much less toxic. In a limit-test with rabbits the skin of 5 male and 5 female rabbits was exposed occlusively to a dose of 400 mg/kg BHAS (purity 99.5%) using a 50% aqueous substance solution (exposure period 24 hours). None of the rabbits died within an 8-days observation period, neither clinical signs nor local effects were detected, necropsy revealed no changes (BASF, 1980b). A dermal LD50 higher than 1500 mg/kg but less than 2000 mg/kg resulted for BHAS (no data on purity) in a further study with rabbits: Male and female rabbits from 4 test groups were treated with single applications of BHAS at doses of 2000 mg/kg (6 males and 4 females), 1500 mg/kg (5 males and 5 females), 1000 mg/kg (5 males) and 500 mg/kg (5 males and 5 females) and observed for 14 days. Control rabbits were treated with water (5 males and 5 females). The test material was placed on gauze strip and then applied to the hair-free skin of the back. Seven of 10 rabbits died or were sacrificed in extremis at the high dose level after 3 days, all remaining rabbits survived. An acute hemorrhagic dermatitis was present in each of the 7 rabbits that died. Necropsy examination of the tissues revealed severe hemorrhagic necrosis of the skin characterized by massive subepithelial and dermal lesions which often extended from just beneath the epithelium to the cutaneous muscle layer, blood appeared brown. The skin lesions of the surviving animals were characterized by hemorrhage, edema, bulla formation, vascular congestion, and massive heterophil infiltrates. Changes in other tissues were not definitive of compound induced toxicity. It appeared, however, that the deaths may have been related to circulatory collapse (shock) brought on by neurogenic pain reflexes associated with the skin lesion. Lesions in the liver and kidneys supported this contention. Few compound associated changes were present in survivors. The skin contained residual but healing changes. The spleens of 3/3 high and 4/10 mid dose terminated rabbits contained increased amounts of hemosiderin pigment. Red cell damage was detected, other tissue alterations were judged to have been unrelated to BHAS treatment (Allied Corporation, 1982).

Table 4.5: Dermal toxicity of BHAS in rabbits⁵ and derived NOAEL (according to Derelanko et al., 1987)

Dose (mg/kg bw)	Exposure conditions		
	Injection, subcutaneous (as 1% aqueous solution)	Plastic cover (occlusive)	Porous gauze patch (semi-occlusive)
1.000	<i>not tested</i>	<i>not tested</i>	Lethality: 0/10 MetHb: 6.2% Heinz bodies: 3/10 Red blood cells: - 15%
500	<i>not tested</i>	Lethality: 9/10 MetHb: 60.8% presence of Heinz bodies: 10/10 Red blood cells: - 70%	Lethality: 0/10 MetHb: 1.9% NOAEL presence of Heinz bodies: 1/10 ⁶ Red blood cells: no decrease
100	<i>not tested</i>	Lethality: 2/10 MetHb: 18.7% presence of Heinz bodies: 8/10 Red blood cells: - 60%	Lethality: 0/10 MetHb: 1.9% presence of Heinz bodies: 0/10 Red blood cells: no decrease
10	Lethality: 0/10 MetHb: 5.1% Heinz bodies: 10/10 Red blood cells: - 50%	Lethality: 0/10 MetHb: 2.4 % presence of Heinz bodies: 6/10 Red blood cells: - 15%	<i>not tested</i>
1	<i>not tested</i>	Lethality: 0/10 MetHb: 0.8 % NOAEL presence of Heinz bodies: 1/10 ² Red blood cells: no decrease	<i>not tested</i>

⁵ Experimental conditions: Female rabbits, 24 h (for occlusive and semi-occlusive dermal treatment, single topical application of BHAS moistened with water, 10 animals per group; investigation of gross signs of toxicity, blood samples: methaemoglobin (MetHb), red blood cell count). It is assumed that MetHb levels are underestimated due to the late time point of determination.

⁶ Derivation of the NOAEL: The occurrence of Heinz bodies in 1 out of 10 animals is considered as uncertain due to the absence of corroborating effects on red blood cell count or other clinical effects.

Oral

The acute oral toxicity of BHAS is different for rats and for cats; cats are more susceptible to methaemoglobin formation: For rats oral LD50 values of 545 mg/kg bw and of 642 mg/kg bw have been detected, while female cats demonstrated an oral LD50 of approximately 200 mg/kg bw due to the methaemoglobin formation properties of the substance.

In an acute oral toxicity study with BHAS (no data on purity) the following oral LD-values were detected for rats: LD0 450 mg/kg; LD50 545+41 mg/kg and LD100 1200 mg/kg. No further data are given concerning the test procedure.

BHAS when given in lethal amounts resulted in death within 1 hour. Autopsy of animals receiving the compound at doses as low as 300 mg/kg revealed damage to the liver, kidney and spleen. The most characteristic feature of animals receiving this compound was a black spleen. Within an annex to this test information, additional data on clinical signs observed in the mentioned tests were stated: Administration of 250 mg/kg of BHAS in water per os to a group of 5 rats caused slight, temporary cyanosis and sluggishness. Administration of 500 mg/kg caused marked cyanosis. Lacrimation, flexion of the tail and convulsions occurred also in 2/5 rats both of which died within 1 hour (methaemoglobin positive). Survivors were normal after 24 hours (Angus Chemical Company, test of 1960, 1982).

Oral application of 2% and 4% aqueous solutions of chemically pure BHAS resulted in an oral LD50 for rats of 642 (568-725) mg/kg bw. Data on doses used, on dose groups, number of animals tested or observation times are not given. Clinical signs observed included dyspnoea, trembling, convulsions, tremors and lateral position. At necropsy, blue-violet discoloration and distension of the spleen were detected (BASF, 1969).

An oral LD50 of approximately 200 mg/kg bw was detected for female cats in a study on the methaemoglobin formation properties of BHAS (purity 99.5%). Toxic effects following oral application of single doses of 50 mg/kg bw to 1 male and 1 female cat and single doses of 200 mg/kg bw to 3 male and 3 female cats were assessed (aqueous solution dispensed by gavage). The application of 50 mg/kg was survived by both cats which demonstrated methaemoglobin concentrations of 12.1% resp. 21.6% at the 4-hours observation time. After application of 200 mg/kg BHAS 0/3 male and 2/3 female cats died; in this group between 10.0% and 41.9% methaemoglobin was detected 4 hours after substance application. Clinical signs in the 50 mg/kg group included increased salivation, cyanosis and vomiting; in the 200 mg/kg group repeated vomiting, increased salivation, apathia, cyanosis, mydriasis and lateral position were observed. Based on these effects, a LOAEL of 50 mg/kg for cats can be derived. Deaths occurred 2 days after application, the surviving animals recovered within 9 days. Necropsy of the cats that died revealed weak heart muscles, acute passive hyperemia, discolored liver and lungs (BASF, 1981).

In vitro studies

No data available.

4.1.2.2.2 Studies in humans

In vivo studies

Human data on the acute toxicity of BHAS are not available.

4.1.2.2.3 Summary of acute toxicity

Human data on the acute toxicity of BHAS are not available. In tests with rats, cats and rabbits it has been proven that the substance can cause methaemoglobin formation by the oral and the dermal routes of exposure. Data on methaemoglobin formation after inhalation of the substance are not available. Oral and dermal LD₅₀ values are different for rats (oral LD₅₀ 545-652 mg/kg, dermal LD₅₀ more than 500 mg/kg) and for species more sensitive to methaemoglobin formation (oral LD₅₀ for female cats appr. 200 mg/kg, dermal LD₅₀ for rabbits between 100 mg/kg and 500 mg/kg). For the evaluation of acute inhalation toxicity only two inhalation risk studies are available demonstrating that inhalation of saturated vapours at 20° C does not cause any toxic effects. However, due to the very low vapour pressure of BHAS and the method used in these studies, it is doubtful if the animals were exposed to significant concentrations of the substance. The degree of toxic response after dermal contact is dependent on the specific kind of exposure. Toxicity is significantly higher under occlusive compared to semi-occlusive conditions, probably due to aggravated corrosivity and enhanced dermal uptake of the substance after skin damage. A dose of 1 mg/kg bw can be considered as "no-effect" level for occlusive application of the substance in the rabbit (NOAEL). A dermal NOAEL of 500 mg/kg bw can be derived for semi-occlusive exposure. The current labelling with Xn; R 20/22 has been changed to R 21/22 (Harmful in contact with skin and if swallowed).

4.1.2.3 Irritation

4.1.2.3.1 Skin

Studies in animals

Only data on scarcely reported Draize tests are available. These data demonstrate moderate to severe irritating and even corrosive substance properties depending on the time of exposure to the test material. The results of testing for assessment of acute dermal toxicity (s. Chapter 4.1.2.2) underline this situation.

Moderate to severe irritation (depending on exposure time) was observed in a skin irritation test with rabbits. The skin of an unknown number of rabbits was exposed to an unknown amount of a 80% aqueous preparation of chemically pure BHAS for 1, 5 and 15 minutes and for 20 hours (no information on further details). 24 hours following exposures, skin irritation was assessed. No skin irritation was observed after the 1-minute and the 5-minutes exposure times; barely perceptible erythema were observed after a 15-minutes exposure, while severe skin irritation was observed after the test with 20-hours skin exposure (this irritation reversed within 8 days) (BASF, 1969). In a Draize skin test with one rabbit, 4-hours occlusive skin exposure to 50 mg BHAS (moistened with water) resulted in erythema grade 1 at the 24-hours observation time. No other lesions were observed (RCC NOTOX B.V., 1989a).

Studies in humans

Pellerat and Chabeau (1976) reported clinical skin irritation tests using concentrations of 1 and 2% hydroxylamine (no details on method given) with 34 persons, who had not been contacted to the substance earlier. 41% of these persons showed positive results (no further details provided).

Further human experience with local irritant properties of BHAS is mentioned in the literature (BASF, 1956; Fousserau, 1982; Popchristov et al., 1957), but no details are provided. In an introduction of the BASF study report it is stated that skin irritation was reported after handling of hydroxylamine salts (no further details). In Fousserau (1982) it is generally stated, that aliphatic amines cause irritant and allergic dermatitis and hydroxylamine salts, including the sulphate, are given as example. Popchristov et al. (1957) report skin irritation and itching in Bulgarian workers of the film producing business (no defined exposures).

4.1.2.3.2 Eye

Studies in animals

Severe conjunctival irritation and severe corneal opacity resulted 24 hours after instillation of 50 mm³ of chemically pure BHAS into the eye of one rabbit. The following effects are reported (no scores used): mild conjunctival redness and mild conjunctival edema 1 hour after instillation; mild conjunctival redness, severe edema and severe corneal opacity after 24 hours; mild corneal opacity after 8 days. Information on reversibility of the observed corneal opacity is not given (BASF, 1969). In a second Draize eye irritation test with 1 rabbit moderate eye irritation caused by 0.1 ml of BHAS (no data on purity) was detected: Conjunctival redness demonstrated a mean score (24 h, 48 h, 72 h) of 3, conjunctival edema a mean score of 1.3. Iritis (grade 1) was detected only on day 1, no corneal opacity was observed. No conjunctival irritation was seen at the 8-days observation time (RCC NOTOX B.V., 1989b).

Studies in humans

Human experiences with local irritant properties of BHAS are mentioned in the literature (Fousserau, 1982; Popchristov et al., 1957), but no relevant data are reported. Further data result from a draft in Science Appl. Incorporation (draft 1984), but no details are available.

4.1.2.3.3 Respiratory tract

No data available.

4.1.2.3.4 Summary of irritation

Human experience with local irritation/corrosion caused by BHAS is mentioned in the literature but respective reports are not available. Further data result from a draft in Science Appl. Incorporation (draft 1984), but no details are available.

Clinical skin irritation tests revealed a highly positive rate of skin irritation caused by concentrations of 1 and 2% hydroxylamines (Pellerat and Chabeau 1976, no further details provided).

Further human experience with local irritant properties of BHAS is mentioned in the literature (Fousserau, 1982; Popchristov et al., 1957), but no details are provided which can be used for risk assessment.

Animal data are scarcely reported; these data demonstrate moderate to severe irritating and even corrosive substance properties depending on the time of exposure to the test material. The results of testing for assessment of acute dermal toxicity (see chapter 4.1.2.2) underline

this situation. In order to properly assess the local irritation/corrosion potential of BHAS, results of skin and eye tests according to current EU or OECD guidelines would be needed. However, the performance of animal tests to establish experimental data is subject to the provisions of Directive 86/609/EEC regarding the protection of animals used for experimental purposes and testing with potentially severe irritating substances is to be avoided. Therefore, it is proposed to support the current labeling of eye and skin irritation potential of BHAS with R 36/38, because severe skin lesions are reported only for exposure periods of 20-24 hours, and a 4-hours exposure of the skin of one rabbit resulted in mild skin irritation, whilst data on eye irritation are conflicting and irreversibility of effects were not documented.

4.1.2.4 Corrosivity

Human data do not point to a corrosive potential of BHAS. In order to properly assess the corrosive potential of BHAS, results of skin and eye tests according to current EU or OECD guidelines would be needed. Scarce animal data demonstrate moderate to severe irritating and even corrosive substance properties depending on the time of exposure to the test material (BASF, 1969). Severe hemorrhagic necrosis, characterized by massive subepithelial and dermal lesions, was observed in a dermal toxicity study with rabbits (Allied Corporation, 1982). No further details such as surface areas or Draize scores are given. However, these results are not sufficient to classify BHAS as corrosive.

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

Skin

In vivo studies

BHAS was applied as a 40% aqueous solution to one side of the flanks of 10 guinea pigs at a volume of 0.1 ml. This procedure was repeated daily until irritation was clearly observed. After a rest period of 8-10 days the other flank of the animals was treated with the same volume of 0.1 ml but the concentration was decreased 10 fold (4%). At this site 8/10 animals showed irritation, edema and little nodules demonstrating a sensitising effect (BASF 1956). Using the same method (described as skin painting test) negative results were obtained when the test compound was incorporated as a 2% concentration in a detergent formulation (BASF 1970).

In a Magnusson Kligman Test a total of 28 guinea pigs (24 treated and 4 control animals) were used. For intradermal injection the treated group received a 5% concentration of BHAS (purity > 98%) in water. Topical induction was 25% of the substance in water at day 7 followed by a challenge treatment of 10% substance in water at day 21. Control animals received the vehicle only during induction treatment and a 10% substance formulation in water at challenge. A sensitisation rate of 96% (22/24 animals) was observed in the treated group. Dermal reactions of grade 2 (moderate or diffuse redness) or grade 3 (intense redness and swelling) were seen in 19 of the 22 positive animals. No dermal reactions were observed in control animals. At day 55 a rechallenge was conducted, resulting in a comparable sensitisation rate (>90%) as with the first challenge. Between the first and second challenge

the animals were subjected to an inhalation aerosol challenge or an intratracheal challenge treatment (Allied Corporation 1984).

In a Mouse Ear Swelling Test (MEST) prior to the first induction treatment mice received two i.d. injections totaling 0.05 ml FCA (fetal calf albumin) into the stomach region. The animals were then topically dosed at the stomach site with 100 µl of BHAS in solvent or the solvent at three consecutive days. Following a rest period of seven days 20 µl of the test compound in solution was applied to the right ear. After 24 and 48 h the thickness of both ears was measured. Test and control groups consisted of 10-15 and 5-10 mice, respectively. A total of 72 compounds were tested. Hydroxylammonium sulfate concentration was 10% in 25% ethanol for all treatments, and 33% of the mice showed a positive reaction (Gad et al. 1986).

The identical test procedure as described by Gad et al. (1986) was used by two laboratories. Both laboratories reported negative results with BHAS. It is concluded that this method is a useful model for identifying strong contact sensitizers (examples: DNCB, glutaraldehyde) but is not reliable for detecting weak or moderate allergens (Dunn et al. 1990).

BHAS gave a negative response in 20 guinea pigs in a Buehler test (test concentration 0.2%, Griffith and Buehler 1977).

Gad (1988) used the available literature for the ranking of BHAS and of 52 additional compounds. BHAS ranks in the middle for MEST, in category III (moderate) for GPMT (Guinea Pig Maximisation Test), negative with the Buehler test and the Epicutaneous Maximization test and positive with respect to human data.

In vitro studies

No data available.

Respiratory tract

In vivo studies

Guinea pigs that have been subjected to a Magnusson Kligman Test (96% positive) with BHAS were subsequently treated as follows: For an inhalation aerosol challenge 2 groups of 4 animals inhaled an aerosol concentration of 0.0065 mg/l and 0.0132 mg/l, respectively for 30 minutes. Breathing rates were measured during exposure and during 60 minutes after exposure termination. No changes in breathing rates were measured in comparison to base line levels. Four other groups of guinea pigs (4/group) were treated via the intratracheal route at dose levels of 5, 15, 25 and 75 mg/kg. Breathing rates were monitored for at least 60 minutes. Based on measured changes in breathing rates from base line levels the substance did not produce any indication of pulmonary sensitisation. An increase in breathing rates was considered a sign of pulmonary sensitisation and a decrease in breathing rate a sign in sensory irritation. None of the changes in breathing rates was documented in this study (Allied Corporation 1984).

In vitro studies

No data available.

4.1.2.5.2 Studies in humans

Skin

In vivo studies

Several reports of contact dermatitis caused by either BHAS or hydroxylamine hydrochloride are available.

Eight cases of contact dermatitis (localized on the face, neck and upper limbs) due to hydroxylamine salts have been reported. Seven of a total of 20 employees with producing hydroxylamine hydrochloride acquired contact dermatitis after a remarkably short period of time (ranging from 2 to 60 days). A photographic assistant chemist exposed to color film developers proved to be sensitised not only parasubstituted amines but also to BHAS and hydroxylamine hydrochloride. The test concentration was 1% in water (Folesky et al. 1971).

A technician working in a factory manufacturing color-photograph-processing chemicals developed chronic hand eczema and fingernail onycholysis 5 months after starting work. After being patch tested with 1%, 2% and 5% of BHAS in water a positive reaction was demonstrated. Ten controls patch tested with 5% BHAS in water were negative. After being transferred to packing work his eczema cleared after 2 months and his onycholysis improved after 4 months (Goh 1990).

Five of 13 workers engaged in the production of cycloserine developed contact dermatitis of the upper limbs, face and neck. A component in the production of cycloserine is hydroxylamine hydrochloride and patch tests (test concentration: 1% in water) confirmed the clinical diagnosis of topic eczema caused by hydroxylamine hydrochloride (Gobbi 1970).

Nine of 11 workers exposed to hydroxylamine (hydrochloride, sulfate) developed contact dermatitis of the face, neck and upper limbs and in addition showed fissures and onycholysis. The test concentration of BHAS ranged from 0.1%-2% in water. Thirty-four control subjects were also tested and 14 subjects demonstrated a positive response after being tested with 1% or 2% of the test substance in water (Pellerat and Chabeau 1976).

A photographer working at a photography laboratory developed lesions on the palms and fingers. A positive skin reaction was shown after patch tests with 0.1% hydroxylammonium chloride in water. His condition resolved after changing his job, but when he returned to the photography laboratory episodes of dermatitis reappeared (Aguiree et al. 1992).

BHAS led to sensitisation in 3 out of 76 human subjects after repeated insult patch testing at 0.05% in a detergent solution (Griffith and Buehler, 1977).

In vitro studies

No data available.

Respiratory tract

No data available.

4.1.2.5.3 Summary of sensitisation

In animal experiments with BHAS and hydroxylamine hydrochloride skin sensitising properties were demonstrated. This correlates with human data for both substances. Thus, a labeling as R 43 (May cause sensitisation by skin contact) is appropriate.

In a test with guinea pigs that were first treated with a Magnusson Kligman Test (96% positive) and subsequently subjected to an inhalation aerosol challenge or an intratracheal challenge no indication for a pulmonary sensitisation was detected. However, this test procedure is hardly used, and no data are available on its validity. Therefore, the negative result obtained cannot be used for hazard assessment.

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

In vivo studies

Methaemoglobinaemia, haemolytic anaemia and effects secondary to the anaemia were the prominent manifestations of BHAS toxicity.

Inhalation

No data available.

Dermal

No data available.

Oral

Drinking water studies

28-day study (rat)

In a 28-day range-finding oral toxicity study mostly according to OECD TG 407 groups of five male and five female Wistar rats received BHAS (purity $\geq 99\%$) in the drinking water in doses of 0, 25, 100, 400 or 1600 ppm for 4 consecutive weeks. The analytical investigation of the test substance preparations are performed at the end of the study. But, the estimation of compound consumption based on water consumption by male and female rats could not definitely be done in all dose groups because of some study restrictions. There were some technical shortcomings concerning definite calculated achieved intake of BHAS from the drinking water in the 25, 100 and 400 ppm dose groups because of the instability of BHAS in water. No test substance or only minor amounts were verifiable especially for the low dose groups. Therefore, the BHAS dose is given in ppm instead of mg/kg bw. Stability tests for BHAS at the highest dose of 1600 ppm in the drinking water for a storage of 4 days resulted in an average of 1437 ppm. So the average BHAS concentration in drinking water of the 1600 ppm dose group was approximate 142 mg/kg bw/d for males and 149 mg/kg bw/d for females.

No deaths occurred during the study. Food intake of BHAS-treated rats was similar to that of the controls. At 1600 ppm, rats of both sexes showed decreased water consumption, cyanosis and discoloration (yellow-red) of the urine in the last treatment week. The relevant study

results of hematology, clinical biochemistry, organ weight assessment, necropsy and microscopy from males and females receiving 1600, 400 and 100 ppm are summarized in the following Table 4.6:

Table 4.6: Summary table: Subacute toxicity (28-day) drinking water study, Wistar rat

<p>Wistar rat (5m/5f) 25, 100, 400, 1600 ppm</p>	<p>1600 ppm: <u>blood (m/f):</u> ↓ RBC, ↓ HB, ↓ HCT, ↓ MCHC, ↑ MCH, ↑ MCV, ↑ RET, ↑ Heinz bodies, ↑ methaemoglobin morphological changes of RBC: anisocytosis, poikilocytosis, and polychromasia (m/f) ↑ WBC (m/f), ↑ Neut (m/f), ↑ Eos (m/f), ↑ Lymph (m/f), ↑ Mono (m/f), ↓ PLT (m/f) ↑ bilirubin (m/f), ↓ AP (m/f), ↓ glucose (f), ↓ Ca (m), ↑ P (m), ↑ bilirubin in urine (f) <u>spleen (m/f):</u> ↑ weight (abs/rel), splenomegaly hemosiderin deposits, extramedullary hematopoiesis <u>liver:</u> hemosiderin deposits in Kupffer cells (m/f), extramedullary hematopoiesis and erythrophagocytosis (m/f), single megacaryocytes in intrasinoidal space (m/f), iron pigment deposition in hepatocytes (f) <u>kidney:</u> ↑ weight, abs/rel (m), tubular hemosiderosis (m/f), iron-negative pigment deposition in proximal tubulus (m/f) <u>bone marrow (m):</u> reticuloid hyperplasia, necrosis 400 ppm: <u>blood:</u> ↓ RBC (m), ↓ HCT (m), ↓ HB (m/f), ↑ MCV (f), ↑ RET (m/f), ↑ Heinz bodies (m/f), morphological changes of RBC: anisocytosis, micro- and macrocytosis (m), polychromatophilia (m/f), ↑ Neut (m), ↓ bilirubin (f), <u>spleen (m/f):</u> splenomegaly; extramedullary hematopoiesis <u>liver (m/f):</u> extramedullary hematopoiesis 100 ppm : <u>blood (m):</u> anisocytosis, polychromatophilia <u>spleen (m):</u> splenomegaly</p> <p style="text-align: right;">BASF, 1989</p>
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↑: statistically significant increase compared with controls; ↓: statistically significant decrease compared with controls; m: male; f: female; RBC: Erythrocyte count; HB: Haemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; WBC: Leukocyte count; PLT: Platelet count; RET: Reticulocyte count; Neut: Neutrophiles; Lymph: Lymphocytes; Mono: Monocytes; Eos: Eosinophiles; AP: Alkaline phosphatase; Ca: Calcium; P: Inorganic phosphorus; abs: absolute; rel: relative; NOAEL_{sys}: no observed adverse effect level for systemic effects

In summary, hemolytic anemia and splenomegaly were the prominent manifestations of BHAS toxicity, esp. make in the 1600 and 400 ppm dose groups corresponding with cyanosis, changes in red blood parameters (enhanced levels of methaemoglobin, Heinz bodies and a shift in blood cell pattern, e.g. increase in immature forms of red blood cells - reticulocytes -, and a rise in leukocytes: neutrophile and eosinophile granulocytes, lymphocytes, and monocytes), changes in the biochemical composition of the plasma and relevant toxic effects in spleen, liver and kidneys. Increased decomposition of erythrocytes was seen as hemosiderin deposits and iron pigment deposition in these organs. Further, in Kupffer cells erythrophagocytosis were observed. Compensating effects were revealed in the spleen and liver as extramedullary hematopoiesis. Congestion of the spleen, enlargement of splenic sinus, splenomegaly and increased organ weight of the spleen were caused by immature

erythrocytes. Reticuloid hyperplasia and necrosis in bone marrow which were seen only in males at 1600 ppm, may be a possibly indication of bone marrow damage.

No treatment related effects were observed in animals of both sexes at 25 ppm. The NOAEL for systemic effects was 25 ppm in males and 100 ppm in females.

90-day study (rat)

In a subchronic oral toxicity study similar according to OECD TG 408 (no recovery period), groups of 10 male and 10 female Wistar rats received BHAS (purity $\geq 99\%$) in the drinking water at concentrations of 0, 10, 50, or 250 ppm for 90 consecutive days. The doses administered corresponded to a mean daily BHAS intake of about 0, 0.9, 4 or 21 mg/kg bw.

Regarding mortality, general appearance and behaviour of the animals there was no difference between treated and untreated animals. No toxicologically relevant differences in mean feed consumption per animal/d or per kg bw/d as well as body weight and body weight development were detected in male and female rats. The following findings were obtained and assessed as substance-induced. At 250 ppm, rats of both sexes showed dark coloration of the urine. This is considered to be due to the substance-related effects on the blood. The hematological examination revealed indications of an increased destruction of red blood cells. At 250 ppm in both sexes and at 50 ppm in females there was a reduction of the erythrocytes and haemoglobin values. In addition there were an increase of the MCH values in both sexes at 250 ppm and in females at 50 ppm, furthermore reduced values of hematocrit in females and of the MCHC in males at 250 ppm. In males receiving 50 ppm, decreased counts of red blood cells and decreased values of haemoglobin were also apparent during the treatment period, although these did not attain statistical significance. These findings are considered to be related also to an increased decay of erythrocytes. The increase of the MCV, and reticulocyte counts at 250 ppm in males and females were assessed as sign of compensate increased erythropoiesis. As a consequence of an increased leaving of juvenile erythrocytes out of the bone marrow a reinforced polychromasia was seen dose-dependent at 250 and 50 ppm in both sexes. At 50 ppm, a slight increase of reticulocytes in male and female rats was noted, and moreover in females a marginal increase of the MCV values. Furthermore, an increase of bilirubin concentration in both sexes at 250 ppm was observed. In males and females receiving 50 ppm, increased values of bilirubin were also apparent during the treatment period, although these did not attain statistical significance. This finding appears to be due to the increased decay of erythrocytes. The elevated methaemoglobin concentration and the reinforced evidence of Heinz bodies in both sexes at 250 ppm are indicative for methaemoglobinemia. Increased absolute and relative spleen weights were seen at 250 ppm in male and female rats. Increase of relative liver weights were noted only in males. In males and females receiving 50 ppm, increased absolute and relative adrenal weights were noted. Histopathological findings representing secondary effects to the anemia included increased hemosiderin deposits in the spleen and liver of both males and females given 250 ppm. At 50 ppm, moderate increased hemosiderin deposits in the spleen were revealed in both sexes. In addition, sinus dilatation together with congestion of the spleen were observed dose-dependent in both sexes at 50 and 250 ppm. 10 ppm did not alter the blood parameters in rats of both sexes. The relevant results of the presented 90-day study are summarized in the following Table 4.7:

Table 4.7: Subchronic toxicity (90-day) drinking water study, Wistar rat

Wistar rat (10m/10f) 10, 50, 250 ppm (0.9, 4, 21 mg/kg bw/d)	<p>50 ppm: <u>clinical signs:</u> none specific (m/f) <u>blood:</u> ↓ RBC (f), (↓) RBC (m), ↓ Hb (f), (↓) Hb (m), ↑ MCV (f), ↑ MCH (f), ↑ polychromasia (m/f), ↑ RET (m/f), (↑) bilirubin (m/f)</p> <p><u>effects on organs:</u> ↑ adrenal weight, abs/rel (m/f); <u>spleen:</u> hemosiderin deposits (m/f), sinus dilatation together with congestion (2m/2f)</p> <p>250 ppm: <u>clinical signs:</u> dark coloration of the urine (m/f) <u>blood:</u> ↓ RBC (m/f), ↓ Hb (m/f), ↓ HCT (f), ↓ MCHC (m), ↑ MCV (m/f), ↑ MCH (m/f), ↑ RET (m/f), ↑ Heinz bodies (m/f), ↑ MetHb (m/f), ↑ polychromasia (m/f), ↑ bilirubin (m/f)</p> <p><u>effects on organs:</u> <u>spleen:</u> ↑ weight, abs (m/f), ↑ weight, rel (m), hemosiderin deposits (m/f), sinus dilatation together with congestion (10m/10f) <u>liver:</u> ↑ weight, rel (m), hemosiderin deposits (m/f)</p>
	BASF, 1992b

↑: statistically significant increase compared with controls; (↑): increase compared with controls, no statistically significant but possibly of toxicological relevance; ↓: statistically significant decrease compared with controls; (↓): decrease compared with controls, no statistically significant but possibly of toxicological relevance; m: male; f: female; RBC: Erythrocyte count; Hb: Haemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; MCHC: Mean corpuscular haemoglobin concentration; MetHb: methaemoglobin; RET: Reticulocyte count

In conclusion, repeated administration of 50 and 250 ppm BHAS (equivalent to about 4 and 21 mg/kg bw/d respectively) to rats via the drinking water for 3 months led to toxicity in male and female rats at both dose levels. In the present study it has been demonstrated that BHAS has a hematotoxic potential. In the males and females of the 50 and 250 ppm groups the administration of BHAS led to hemolytic anemia (dose-related) with methaemoglobinemia and to organ weight increases in the spleen and liver together with the specific histopathological findings in the liver and spleen seen as increased hemosiderin deposits in both sexes.

Based on the results of this study, 50 ppm (equivalent to about 4 mg/kg bw/d) is considered to be the LOAEL for systemic effects in male and female rats. The NOAEL for all adverse effects of this rat study was 10 ppm (equivalent to about 0.9 mg/kg bw/d) for both sexes. No local toxic effects were seen in male and female rats treated with the highest tested dose level of 250 ppm, equivalent to about 21 mg/kg bw/d (BASF, 1992b).

Combined chronic toxicity/carcinogenicity study, 12/24 months (rat)

In a combined chronic toxicity/carcinogenicity study according to OECD TG 453, BHAS (purity commercial grade) was administered to groups of 50 male and 50 female Wistar (Chbb:THOM, SPF) rats in the drinking water at concentrations of 0, 5, 20 and 80 ppm for 24 months (main groups). In order to define the hematotoxic potential of the test substance, groups of 10 animals per sex and dose were treated for 12 months (satellite groups). In these satellite animals, assays of blood parameters were performed every three months. The doses administered corresponded to a mean daily BHAS intake in the main groups of about 0, 0.2, 1.0, and 3.7 mg/kg bw/d in males and 0, 0.4, 1.6, and 6.2 mg/kg bw/d in females; and in the satellite groups of about 0, 0.3, 1.1, and 4.5 mg/kg bw/d in males and 0, 0.4, 1.6, and 6.2

mg/kg bw/d in females.

Food consumption, water consumption and body weight were determined once a week during the first 13 weeks. Thereafter water consumption and body weight were determined in 4-week intervals, and food consumption was ascertained in 3-months intervals. The animals were examined for signs of toxicity or mortality at least once a day. Moreover, comprehensive clinical examinations and palpation of the animals were performed once a week. Hematology was carried out in the satellite groups after 3, 6, 9 and 12 months. In the main groups, hematology was carried out after 12, 18 and 24 months. Complete necropsy and microscopy were performed on all animals.

In satellite groups and main groups, no treatment-related clinical signs were observed in animals of both sexes, and no statistically significant differences in survival were noted between dose groups and control groups and sexes, respectively. The average body weights and body weight gains of treated males and females were comparable to those of the matched controls throughout the study.

Results of hematology indicated dose-related anemia that was characterized as hemolytic and regenerative in rats observed at 3, 6, 9, 12 and 24 months. Signs of anemia were more pronounced in males. In satellite groups administration of 80 ppm caused hemolytic anemia in male and female rats expressed as statistically significant decreases in red blood cell counts, haemoglobin concentrations and hematocrit values. Furthermore, there were statistically significant increases in MCV values and MCH values, and in platelet counts in females. A slightly elevated number of Heinz bodies, Howell-Jolly bodies and reticulocytes were noted in both males and females. The mean absolute and relative spleen weights were statistically significant increased in rats of both sexes at 80 ppm. At microscopy of the spleen, congested vessels, characterized by dilated, blood-filled vascular spaces were observed in males and females at 80 ppm. Hemosiderin storage in the spleen occurred in nearly all control and BHAS-treated animals, however with higher degree in males receiving 20 and 80 ppm, and in females at 80 ppm.

In main groups, examination of erythrocyte morphology revealed at 80 ppm mild anisocytosis and microcytosis in males, increased polychromasia in females, and an elevated number of Howell-Jolly bodies in both sexes of these groups. Statistically significant increases in mean absolute and relative spleen weights were noted in females receiving 80 ppm. Microscopically, the following non-neoplastic findings were noted in the spleen: an increased number of congested vessels in animals of both sexes at 80 ppm, and hemosiderin storage in nearly all control and BHAS-treated animals, however with higher degree of severity in males given 80 ppm, and in females dosed with 20 and 80 ppm. Extramedullary hematopoiesis was also observed in the spleen in most of the animals of the main groups. In these animals the degree of severity was higher in males and females given 80 ppm, while the incidence was slightly increased in females. Comparable to the increased incidence of hematopoiesis in the spleen, an increased hematopoiesis was noted in the bone marrow in the 80 ppm dosed animals. Furthermore, a multifocal or diffuse pigment storage was noted in the liver. Most of the stored pigment turned out to be hemosiderin after iron staining. Diffuse pigment storage in the liver was noted in higher incidences and higher degrees in the 80 ppm dosed males and females. The relevant results of the presented combined chronic toxicity/carcinogenicity study, 12/24 months study are summarized in the following Table 4.8:

Table 4.8: Combined chronic toxicity/carcinogenicity study, 12/24 months in drinking water, Wistar rat

<p>Wistar rat main/ satellite group (50/10m; 50/10f)</p> <p>5, 20, 80 ppm</p> <p>main/ satellite group (m: 0.2/0.3, 1.0/1.1, 3.7/4.5 mg/kg bw/d;</p> <p>f: 0.4/0.4, 1.6/1.6, 6.2/6.2 mg/kg bw/d)</p>	<p style="text-align: center;">Satellite groups (12 months of treatment):</p> <p>20 ppm: ↑ degree/severity of hemosiderin deposits in the spleen (m)</p> <p>80 ppm: <u>blood:</u> ↓ RBC (m/f), ↓ Hb (m/f), ↓ HCT(m/f), ↑ MCV (f), ↑ MCH (f), ↑ PLT (f), (↑) RET (m/f), (↑)Heinz bodies (m/f), (↑) Howell-Jolly bodies (m/f) <u>spleen:</u> ↑ weight, abs/rel (m/f), (↑) congested vessels (m/f), (↑) degree/severity of hemosiderin deposits (m/f), (↑) degree of extramedullary hematopoiesis (m/f)</p> <p style="text-align: center;">Main groups (24 months of treatment):</p> <p>20 ppm: (↑) degree/severity of hemosiderin storage in the spleen (f)</p> <p>80 ppm: <u>blood:</u> (↑) Howell-Jolly bodies (m/f), anisocytosis (m), microcytosis (m), (↑) polychromasia (f); <u>spleen:</u> ↑ weight (abs/rel) (f), (↑) angiomatous hyperplasia (m/f), (↑) congested vessels (m/f), (↑) degree/severity of hemosiderin storage (m/f), (↑) degree of extramedullary hematopoiesis (m/f), <u>liver:</u> (↑) degree of diffuse hemosiderin storage (m/f) <u>bone marrow:</u> (↑) degree of hematopoiesis (m/f)</p> <p style="text-align: right;">BASF, 2001</p>
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↑: statistically significant increase compared with controls; (↑): increase compared with controls, no statistically significant but possibly of toxicological relevance; ↓: statistically significant decrease compared with controls; (↓): decrease compared with controls, no statistically significant but possibly of toxicological relevance; m: male; f: female; RBC: Erythrocyte count; Hb: Haemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular haemoglobin; PLT: Platelet count; RET: Reticulocyte count

In conclusion, the prolonged oral administration of 80 ppm BHAS via the drinking water to male and female rats caused hemolytic anemia, characterized by significant reduced counts of erythrocytes, haemoglobin concentrations and hematocrit values, increases in MCV, MCH, and furthermore, increased number of Heinz bodies, Howell-Jolly bodies, and reticulocytes in the peripheral blood. These adverse effects were associated with increases of spleen weights, increased red blood cell regeneration by the bone marrow and increased extramedullary hematopoiesis in the spleen and the liver. At 20 ppm (equivalent to about 1.0 mg/kg bw/d in males and 1.6 mg/kg bw/d in females), hemosiderin storage in the spleen, sign of hemolysis, were significantly increased when compared with controls in male rats after 12 months of treatment and in female rats after 24 months of treatment, respectively. No hematotoxic effects were detected in animals given 5 ppm. Therefore, the NOAEL for systemic effects was 5 ppm, corresponded to a mean daily BHAS intake of about 0.2/0.3 mg/kg bw/d in males and 0.4 mg/kg bw/d in females. No local toxic effects were noted in male and female rats treated with the highest tested dose level of 80 ppm, equivalent to about 3.7 mg/kg bw/d in males and 6.2 mg/kg bw/d in females (BASF, 2001).

12-/52-week study (mouse)

In an early subchronic toxicity study, four-week old Swiss-Webster mice (8 males/group) received BHAS (purity: commercial grade) in the drinking water in doses of 0, 10 or 20 mmol/l (equivalent to 0, 100 or 200 mg/kg bw/d, calculated on an assumed water consumption of 15% of body weight) for 12 consecutive weeks. In addition, two further groups each of 4 male mice were treated for 12 weeks and were then assigned to a 8- or 18-week reversibility period following the cessation of treatment. Hematology was performed at the end of the treatment period, and at the end of the recovery period. At necropsy, animals were examined macroscopically and organ weights of livers, spleens and selected organs were recorded (no more data). Organs and tissues were collected and prepared for histopathology. In both dose groups, body weight development remained unaffected by the administration of BHAS in mice. Hematology revealed decrease in red blood cell counts, and an increase in white blood cells, accompanied by much cellular debris. Spleen weights were markedly increased. These effects were no longer observed by the end of the 8 or 18 weeks recovery period. Repeated administration of BHAS to male mice in their drinking water at dose levels of 100 mg/kg bw/d or more for 12 weeks resulted in anemia, leukocytosis and spleen enlargement. These effects were reversible after 8 and 18 weeks.

Based on the results of the study, 100 mg/kg bw/d is considered to be the LOAEL for BHAS for systemic effects in male mice. Due to the study design a NOAEL for male mice could not be established. No local toxic effects were noted in males treated with the highest tested dose level of 200 mg/kg bw/d (Yamamoto et al., 1967).

The same report describes possible chronic and carcinogenic effects of BHAS in male Swiss-Webster and female C3H/HeN mice. Information on neoplastic findings of BHAS in male Swiss-Webster and female C3H/HeN mice are described in detail in section 4.1.2.8.

In these tests, BHAS (purity: commercial grade) were given in the drinking water in doses of 0, 10 or 20 mmol/l (equivalent to 0, 100 or 200 mg/kg bw/d, calculated on an assumed water consumption of 15% of body weight) to mice of both strains. Groups of 5 male and 10 female mice were administered at both dose levels for 52 weeks. After termination of treatment, 5 female mice of each group received pure water up to 104 weeks of age. Almost 50% of the Swiss-Webster and C3H/HeN mice which had consumed BHAS for 52 weeks presented bone formation in the spleen. This was not seen in mice treated for the shorter period. Usually the condition and appearance of the experimental mice seemed better than that of controls.

In summary, the repeated administration of BHAS in the drinking water to Swiss-Webster mice (males and females) at a concentration of 200 mg/kg bw/d for 52 weeks caused splenomegaly and anemia, and at 100 mg/kg bw/d bone formation in the spleen. A NOAEL for systemic effects in male and female mice could not be established. No local toxic effects were noted in male and female mice treated with the highest tested dose level of 200 mg/kg bw/d (Yamamoto et al., 1967).

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 BHAS was not available.

No repeated dose toxicity studies following inhalation and dermal exposition to BHAS were available.

Data on repeated dose toxicity in experimental animals with BHAS was available from studies with the oral route of exposure. 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, respectively. The available data permit the derivation of a NOAEL for systemic effects by oral administration.

The toxic profile of BHAS dominated by its hematotoxicity and organ lesions resulting from hematotoxicity, characterized by a low rate of circulating red blood cells (expressed as decreases in total red blood cell counts, haemoglobin concentrations and hematocrit values), compensatory increases in red blood cell regeneration by the bone marrow as well as spleen and liver. The severity and incidence of hematotoxic effects appeared to be dose-related and time-related. These effects are considered to be severe health effects.

In rats, prolonged administration of 80 ppm BHAS (equivalent to 3.7 mg/kg bw/d in males and 6.2 mg/kg bw/d in females) via the drinking water for 12 months caused anemia in both sexes observed as decreases in red blood cell counts, haemoglobin concentrations and hematocrit values, and in addition, increases in Heinz bodies, Howell-Jolly bodies and reticulocytes. Furthermore, there was increased hematopoiesis in the bone marrow and extramedullary hematopoiesis in spleen and liver, as well as spleen enlargement. Specific morphologic red blood cell changes such as, Heinz bodies or Howell-Jolly bodies and reticulocytes in the peripheral blood as well as an increased number of males and females with increased hematopoiesis in the bone marrow were also noted after 24 months of treatment. Increased hemosiderin accumulation, a further sign of hemolysis, was observed at dose level of ≥ 20 ppm, equivalent to about ≥ 1.1 mg/kg bw/d in males and ≥ 1.6 mg/kg bw/d in females (BASF, 2001). Subchronic oral administration of ≥ 50 ppm (equivalent to ≥ 4 mg/kg bw/d) BHAS via the drinking water for 3 months led also to hemolytic anemia (dose-related) with methaemoglobinemia and to organ weight increases in spleen and liver together with the specific histopathological findings in both organs seen as increased hemosiderin deposits in male and female rats (BASF, 1992b). Hemolytic anemia and splenomegaly were equally prominent manifestation of BHAS toxicity after repeated oral exposure of ≥ 100 ppm for a four week exposure period. At ≥ 400 ppm these findings corresponded with cyanosis, changes in red blood parameters (enhanced levels of methaemoglobin, Heinz bodies and a shift in blood cell pattern, e.g. increase in immature forms of red blood cells, reticulocytes, and a rise in leukocytes: neutrophile and eosinophile granulocytes, lymphocytes, and monocytes), and alterations in the biochemical composition of the plasma and relevant toxic effects in spleen,

liver and kidneys (BASF, 1989). In this study occasionally hemosiderosis of the spleen and liver was observed. In response to the hemolytic effect reticulocyte counts were increased and erythropoietic activity was elevated in the bone marrow and at extramedullary sites (mainly in the spleen).

Studies in the mouse indicated the blood as the main target organ corresponding to observations in studies with rats. However, the mouse appeared to be less sensitive to BHAS than the rat. Observations which gave information on laboratory findings characterizing hematotoxic effects; spleen findings were reported from a subchronic and a chronic study with Swiss-Webster mice. However, there were no data on methaemoglobinemia and clinical biochemistry examinations. The administration of BHAS in the drinking water to Swiss-Webster mice at concentrations of ≥ 100 mg/kg bw/d for 12 weeks caused decreased total red blood cell counts, and an increase in white blood cell counts, accompanied by much cellular debris and markedly increased spleen weights, reversible after ≥ 8 weeks. Administration of 200 mg/kg bw/d for 52 weeks induced enlargement of spleen and anemia, and of 100 mg/kg bw/d bone formation in the spleen, respectively (Yamamoto et al., 1967).

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

Oral

With the purpose to derive a suitable NOAEL for systemic effects/non-neoplastic findings as a basis for the risk assessment calculation, a NOAEL was derived both from a 90-day study (BASF, 1992b) and from a combined chronic toxicity/carcinogenicity study (BASF, 2001). Both tests conducted as drinking water study in Wistar rats and in conformance with the standard repeated dose toxicity testing protocol were accepted for the requirements of the regulation 793/93/EEC. These studies were considered most relevant to establish the NOAEL for systemic effects because of the contained sensitive parameters of hematology included microscopic examination by special stains, and the prolonged administration due to the turnover of erythrocytes. Both studies addressed the same adverse effects from which different NOAELs could be derived. The oral administration of BHAS via drinking water for subchronic or chronic exposure caused hematotoxic effects. The severity and incidence of hematotoxic effects appeared to be dose-related and time-related. A NOAEL of 10 ppm (equivalent to about 0.9 mg/kg bw/d) for male and female rats was derived from the 90-day subchronic drinking water study, and of 5 ppm (equivalent to about 0.2 mg/kg bw/d in males and 0.4 mg/kg bw/d in females) from the combined chronic toxicity/carcinogenicity study, respectively. The other medium-term and long-term studies, mostly drinking water studies in rats and mice were not in full agreement with the requirements needed for the base set studies of existing chemicals. In conclusion, the lowest and most sensitive NOAEL_{sys} for systemic effects for BHAS of 5 ppm (equivalent to about 0.2 mg/kg bw/d in males and 0.4 mg/kg bw/d in females) derived from the combined chronic toxicity/carcinogenicity study, administration in drinking water for 24 months in Wistar rats should be used in the risk characterization (BASF, 2001). For local effects on the digestive tract a NOAEL_{local} could be derived from the same studies. No relevant local effects on the digestive tract were observed in each case at the highest tested dose level, 250 ppm (21 mg/kg bw/d) in the 90-day subchronic drinking water study (BASF, 1992b) and 80 ppm (4.5 mg/kg bw/d in males and 6.2 mg/kg bw/d in females) in the combined chronic toxicity/carcinogenicity study (BASF, 2001).

Combined chronic toxicity/carcinogenicity study,

24 months (drinking water) study/Wistar rat

NOAEL_{sys}: 5 ppm, 0.2 mg/kg bw/d in males and 0.4 mg/kg bw/d in females (BASF, 2001)

NOAEL_{local}: 80 ppm, 4.5 mg/kg bw/d in males and 6.2 mg/kg bw/d in females (BASF, 2001)

Currently BHAS is classified as harmful and labelled with Xn, R48/22 (Harmful: danger of serious damage to health by prolonged exposure if swallowed). On the basis of the data submitted, the current classification of BHAS is confirmed.

4.1.2.7 Mutagenicity

4.1.2.7.1 Studies in vitro

Mutagenicity studies in vitro: Bacterial genotoxicity tests

No report on bacterial genotoxicity is available for BHAS. According to NTP (2006) BHAS is negative in a Salmonella mutagenicity test, but the report is not available. Due to the lack of adequate information on BHAS data on hydroxylamine hydrochloride are also considered (justification see 4.1.2).

In Salmonella typhimurium strains TA 97, TA 98, TA 1535 hydroxylamine hydrochloride was negative with respect to induction of bacterial gene mutations with S-9 mix for doses up to 2000 µg/plate and without S-9 mix up to doses of 667 µg/ml (Wang, 1977; Rosenkranz and Poirier, 1979; Dunkel et al. 1984; Zeiger et al. 1992). Negative results were also obtained in Salmonella typhimurium strains TA 1537, TA 1538 and in E. coli WP2uvrA with and without S-9 mix for doses up to 333.3 µg/ml (Rosenkranz and Poirier, 1979; Dunkel et al. 1984).

In the Salmonella typhimurium strain TA 100 hydroxylamine hydrochloride was negative with and without S-9 mix up to doses of 333.3 µg/plate (Dunkel et al. 1984). According to Zeiger et al. (1992) in strain TA 100 negative results were obtained in the presence of S-9 mix for doses up to 200 µg/plate; doses of 333 µg/plate and higher induced marginal positive effects. With Aroclor-induced rat liver S-9 mix doses of 333 µg/plate and higher induced marginal effects in three out of nine experiments. These effects seem to be dose-dependent; the maximum factor for induction of gene mutations in correlation to negative controls is 1.7. In six experiments with Aroclor-induced hamster liver S-9 mix, negative or equivocal results were obtained. Toxic effects were observed at doses of 500 µg/ml and higher. Without S-9 mix there were negative results in two experiments up to the highest tested dose of 667 µg/plate; toxic effects were induced by doses of 333 µg/ml and higher.

Wang (1977) described negative results for hydroxylamine hydrochloride in Salmonella typhimurium TA 100 with and without S-9 mix. Because only low concentrations up to 0.034 µg/plate were tested, these results are not reliable.

In E. coli K12 hydroxylamine hydrochloride was positive with respect to induction of bacterial gene mutations at an extremely high dose of 1 mol/l (69500 µg/ml) without S-9 mix after incubation for 20 minutes. Data on toxicity were not given (Sledziowska-Gojska et al. 1992).

An E. coli DNA repair test with hydroxylamine hydrochloride was negative in strain polA without S-9 mix at the only tested dose of 500 µg/ml. Data on toxicity were not given (Rosenkranz and Poirier 1979).

Summary on bacterial genotoxicity tests

There are no data on bacterial genotoxicity of BHAS. Hydroxylamine or its hydrochloride were mainly negative in bacterial genotoxicity tests. In Salmonella typhimurium TA 100

marginal effects were observed at high doses; a test with *E. coli* was positive only at an extremely high dose.

Table 4.9: Overview on bacterial genotoxicity tests

Test system	Dose range		Result	Toxicity	Test substance	Reference
	with S-9 mix	without S-9 mix				
Gene mutations, <i>Salm. typh.</i> TA 98 and TA 100	not done	0.1 - 0.5 $\mu\text{mol/plate}$ (0.007 - 0.034 $\mu\text{g/plate}$)	negative	no data	hydroxylamine hydrochloride	Wang 1977
Gene mutations, <i>Salm. typh.</i> TA 1535, TA 1538	25 - 250 $\mu\text{g/plate}$	25 - 250 $\mu\text{g/plate}$	negative	none	hydroxylamine hydrochloride	Rosenkranz and Poirier 1979
Gene mutations, <i>Salm. typh.</i> TA 98, TA 100, TA 1535, TA 1537, TA 1538; <i>E. coli</i> WP2uvrA	0.3 - 333.3 $\mu\text{g/plate}$	0.3 - 333.3 $\mu\text{g/plate}$	negative	at high doses	hydroxylamine hydrochloride	Dunkel et al. 1984
Gene mutations, <i>Salm. typh.</i> TA 97, TA 98, TA 100, TA 1535	33 - 2'000 $\mu\text{g/plate}$	10 - 667 $\mu\text{g/plate}$	equivocal	at high doses	hydroxylamine hydrochloride	Zeiger et al. 1992
Gene mutations, <i>E. coli</i> K12	not done	1 mol/l (69'500 $\mu\text{g/ml}$)	positive	no data	hydroxylamine hydrochloride	Sledziowska-Gojska et al. 1991
DNA repair test, <i>E. coli</i> polA	not done	500 $\mu\text{g/ml}$	positive	no data	hydroxylamine hydrochloride	Rosenkranz and Poirier 1979

Mutagenicity studies in vitro: Mammalian cell gene mutation tests

Two mouse lymphoma assays with hydroxylamine hydrochloride led to weak positive results with and without S-9 mix (Myhr and Caspary 1988; Mitchell et al. 1988). These effects were reproducible and dose-dependent. In both investigations the treatment time was 4 h. With S-9 mix doses ranging from 12.5 to 583 $\mu\text{g/ml}$ were tested; the lowest observed effect dose (LOED) was in the range of 200 to 410 $\mu\text{g/ml}$. Without S-9 mix doses from 3.9 to 205 $\mu\text{g/ml}$ were tested; the LOEDs varied from 31 to 67 $\mu\text{g/ml}$. The maximum mutation frequencies were 2- to 4-fold that of the corresponding negative controls. In one of the two assays toxic effects were observed without S-9 mix.

Table 4.10: Overview on mammalian cell gene mutation tests

Test system	Concentration range		Result	Toxicity	Remarks	Test subst.	Reference
	with S-9 mix	without S-9 mix					
Mouse lymphoma assay	12.5 - 300 µg/ml	3.9 - 175 µg/ml	positive	without S-9 mix: 175 µg/ml	4 h treatment	hydroxylamine hydrochloride	Myhr and Caspary 1988
Mouse lymphoma assay	262 - 583 µg/ml	67 - 205 µg/ml	positive	no toxic effects	4 h treatment	hydroxylamine hydrochloride	Mitchell et al. 1988

Mutagenicity studies in vitro: Mammalian cell chromosomal aberration tests

Tests for induction of chromosomal aberrations are available for hydroxylamine hydrochloride. All four studies suffer from severe methodological insufficiencies such as no use of S-9 mix and positive controls. Furthermore, no differentiation was made of chromosomal aberrations with and without gaps. In three publications results were described as positive (Borenfreund et al. 1964; Brogger 1971; Gupta and Sharma 1982a) and in one as negative (Oppenheim and Fishbein 1965).

Brogger (1971) analysed the effect of hydroxylamine hydrochloride on induction of chromosomal aberrations in human lymphocytes for a dose range of 25 to 100 µg/ml. Two of three experiments were not considered because of very high chromosomal aberration rates of 15.0% and 7.0% in the negative controls. In the third experiment there were positive results at the tested doses of 25, 50 and 100 µg/ml after exposure for 4, 6, 12 and 24 h. The effects were dose-dependent; the maximum aberration frequency was 15% (negative control, 1.0%). Toxic effects were induced by doses of 50 and 100 µg/ml.

Borenfreund et al. (1964) described that hydroxylamine hydrochloride induced chromosomal aberrations in cells of a Chinese hamster cell line, originally derived from a methylcholanthrene-induced tumour. The only tested dose of 0.072 µmol/l (5.0 µg/ml) induced an aberration frequency of 13% (negative control, 5.0%) and decreased the mitotic activity by ca. 40%.

Gupta and Sharma (1982a) reported that hydroxylamine hydrochloride induced chromosomal aberrations in Indian muntjac lymphocytes. The frequencies of chromosomal aberrations were increased after 1 h exposure to 25 and 50 µg/ml. Toxicity data were not given.

A negative effect of hydroxylamine hydrochloride on chromosome damage in human leukocytes was described by Oppenheim and Fishbein (1965) at a dose-range of 47 to 500 µmol/l (4.6 to 34.5 µg/ml). The authors speculated that this effect was due to technical factors. Leukocytes were contaminated with red blood cells. Since hydroxylamine hydrochloride is rapidly destroyed on contact with haemoglobin 2 to 3% contamination would suffice to remove the doses of hydroxylamine hydrochloride.

Table 4.11: Overview on mammalian cell chromosomal aberration tests

Concentration range

Test system	With S-9 mix	without S-9 mix	Result	Toxicity	Remarks	Test subst.	Reference
Chrom. aberrat., human lymphocytes	not done	25 - 100 µg/ml	positive	from 50 µg/ml upwards	inadequate methodology	hydroxylamine hydrochloride	Brogger 1971
Chrom. aberr., Chinese hamster tumour cell line	not done	72 µmol/l (5.0 µg/ml)	positive	toxic effect	inadequate methodology	hydroxylamine hydrochloride	Borenfreund et al. 1964
Chrom. aberrat., lymph. of Indian muntjac	not done	25 - 50 µg/ml	positive	no data	inadequate methodology	hydroxylamine hydrochloride	Gupta and Sharma 1982a
Chrom. aberrat., human lymphocytes	not done	67 - 500 µmol/l (4.6 - 34.5 µg/ml)	negative	unclear effects	inadequate methodology	hydroxylamine hydrochloride	Oppenheim and Fishbein 1965

Mutagenicity studies in vitro: Mammalian cell sister chromatid exchange tests (SCE tests)

In vitro tests for induction of sister chromatid exchanges (SCE) are available for hydroxylamine hydrochloride. In two publications weakly positive results were described (Speit et al. 1980; Gupta and Sharma 1982b). Both studies suffer from severe methodological insufficiencies (e.g., lack of S-9 mix, no positive control).

Speit et al (1980) reported on a weak effect of hydroxylamine hydrochloride in V79 cells in the dose-range 10^{-5} to 5×10^{-3} mol/l (0.7 to 345 µg/ml). At doses from 10^{-5} mol/l to 5×10^{-4} mol/l (0.7 to 34.5 µg/ml) SCE frequencies were marginally increased after continuous treatment for 27 h. At higher doses from 10^{-3} to 5×10^{-3} mol/l (69 to 345 µg/ml) the treatment time was limited to 1 h because of drastic toxic effects. Again the induced effect was weak; the maximum SCE frequency was 1.5-fold that of the negative control.

Hydroxylamine hydrochloride was also marginally positive in lymphocytes from the Indian muntjac (Gupta and Sharma 1982b). The tested dose of 25 µg/ml induced a ca. 1.5-fold increase in the SCE frequency after treatment for 1 h. Toxicity data were not given.

Table 4.12: Overview on mammalian cell SCE tests

Test system	Concentration range		Result	Toxicity	Remarks	Test subst.	Reference
	with S-9 mix	without S-9 mix					
V79 cells	not done	10^{-5} - 5×10^{-3} mol/l (0.7 - 345 µg/ml)	positive	from 10^{-3} mol/l (69 µg/ml) upwards	weak effect; inadequate methodology	hydroxylamine hydrochloride	Speit et al. 1980

		µg/ml)					
Lymphocytes of Indian muntjac	not done	25 µg/ml	positive	no data	weak effect; inadequate methodology	hydroxylamine hydrochloride	Gupta and Sharma 1982b

Mutagenicity studies in vitro: Mammalian cell unscheduled DNA synthesis tests (UDS tests)

Hydroxylamine hydrochloride was negative for induction of unscheduled DNA synthesis (UDS) in primary rat hepatocytes for doses up to up 1000 µg/ml (Williams et al. 1982). Higher doses were totally toxic. The DNA repair synthesis was determined by autoradiography.

Table 4.13: Overview on mammalian cell UDS tests

Test system	Concentration range		Result	Toxicity	Remarks	Test subst.	Reference
	with S-9 mix	without S-9 mix					
Primary rat hepatocytes	not done	100 - 1000 µg/ml	negative	total toxic at 2000 µg/ml	autoradiography procedure	hydroxylamine hydrochloride	Williams et al. 1982

4.1.2.7.2 Studies in vivo

Mutagenicity studies in vivo: Rodent bone marrow tests

Rodent bone marrow micronucleus tests are available for BHAS (BASF 1992a; Litton Bionetics, Inc. 1980); hydroxylamine hydrochloride was investigated in a chromosomal aberration test (Volgareva 1991).

In a mouse micronucleus assay on polychromatic erythrocytes BHAS led to a negative result after single oral administration of 300, 600 and 1200 mg/kg bw (BASF 1992a). Sampling times were 16, 24 and 48 h. All doses led to toxic signs. There was no clear effect on local cytotoxicity (PCE/NCE ratio). Five male and five female mice per dose group were used. In a pre-test lethal effects were observed at 1400 mg/kg bw.

Another in vivo micronucleus test was also negative after oral administration of BHAS (Litton Bionetics Inc. 1980). In this test, however, only low doses of 15.6 and 125 mg/kg were used (no clear statement whether the tested doses were given in two parts or twice). Sampling time was 6 h after last administration. Four male and four female mice per dose group were used. Data about toxic effects were not given. No cytotoxicity was induced. The result is of relatively low significance because of the use of low doses only.

An in vivo chromosomal aberration test with hydroxylamine hydrochloride in mice led to a negative result after single intraperitoneal doses of 6.7 and 67 mg/kg bw. The highest tested dose was 1/3 of the LD₅₀. Only five mice (sex was not specified) per dose group were used. Sampling times were 24 and 48 h. Data on toxic effects were not given.

Summary on rodent bone marrow tests

In rodent bone marrow cells, BHAS did not induce micronuclei; also hydroxylamine hydrochloride did not induce chromosomal aberrations.

Table 4.14: Overview on rodent bone marrow tests with mice

Test system	Doses	Expos. regime n	Sampl. times	Result	Local cytoto x.	Gener al toxicit y	Test subst.	Referenc e
Micronucle us test	300 - 1200 mg/kg	1 x p.o.	16, 24, 48 h	negati ve	no effect	toxic effects at all doses	BHAS	BASF 1992a
Micronucle us test	15.6 - 125 mg/kg	2 x p.o.	6 h after second applicati on	negati ve	no effect	no data	BHAS	Litton Bio- netics, Inc. 1980
Chromosom al aberrations	6.7 - 67 mg/kg	1 x i.p.	24 and 48 h	negati ve	no data	no data	hydroxyl- amine hydrochlo- ride	Volgarev a 1991

Mutagenicity studies in vivo: Rodent germ cell tests

A rodent germ cell test is available for BHAS.

In a dominant lethal assay with mice BHAS led to a negative result after single intraperitoneal injection of 102 and 112 mg/kg bw with respect to early fetal deaths and preimplantation loss (Epstein et al. 1972). The findings were not described in detail. Seven to nine males were used per group, each treated male was caged with three untreated virgin females which were replaced weakly for eight consecutive weeks. There were no concurrent positive or negative control groups. The tested doses were equivalent to the LD₅₀ and the LD₂₅.

Summary on rodent germ cell tests

A dominant lethal assay with BHAS was described to be negative.

Table 4.15: Overview on rodent germ cell tests with mice

Test system	Doses	Expos. regime n	Result	Gener al toxicit y	Remarks	Test subst.	Reference
Dominant lethal test	102 - 112 mg/kg	1 x i.p.	negativ e	no data	no detailed description of findings	BHAS	Epstein et al. 1972

Mutagenicity studies in vivo: Genotoxicity tests with insects

Tests with *Drosophila melanogaster* on various genetic endpoints are available for BHAS (Parkash and Miglani 1978) and for hydroxylamine hydrochloride (Fahmy and Fahmy 1970; Vijaykumar and Jain 1978; Graf et al 1989). Hydroxylamine hydrochloride was also tested in grasshoppers (Bhattacharya et al. 1986). All investigations suffer from severe methodological insufficiencies (e.g. no positive and no negative controls). Furthermore, concerning the *Drosophila* tests a detailed description of the test methodology was only given by Graf et al. (1989).

Hydroxylamine hydrochloride was positive in a somatic mutation and recombination test with *Drosophila* (SMART; Graf et al. 1989). After feeding of 90 and 120 mmol/l (6219 and 8280 µg/ml) for 48 h significant increases of frequencies of small and large single spots and twin spots in the wings were induced.

According to Parkash and Miglani (1978) BHAS induced chromosomal inversions in cells of salivary glands of *Drosophila*.

In a sex-linked recessive lethal test with *Drosophila* (SLRL test) a positive result was reported after feeding with hydroxylamine hydrochloride in a dose of 0.03 mol/l (2070 µg/ml) (Vijaykumar and Jain 1978).

Fahmy and Fahmy (1970) described that hydroxylamine hydrochloride was negative in a test for dominant lethals in *Drosophila* after feeding of 0.1 mol/l (6900 µg/ml).

Induction of chromosomal effects in spermatocytes of grasshoppers (*Spathosternum prasiniferum*) was reported by Bhattacharya et al. (1986). Twenty males received abdominally one injection of 50 µl of a solution of 0.1 mol/l hydroxylamine hydrochloride (0.345 mg/50µl per insect). 36 h after treatment following effects were analyzed in 290 spermatocytes I (number of animals not given): 7% cells with chromosomal bridges (negative control, 0%); 13% 'fractures on chromosomes' (negative control, 0%); 7.2% fragments (negative control, 0%); 2.1% chromosome stickiness (negative control, 0.7%). The negative control consisted of 562 spermatocytes from two different species (*Spathosternum prasiniferum* and *Phleoba infumata*). Furthermore, no positive and toxicity data were given. Altogether, the positive finding is of low reliability.

Summary on genotoxicity tests with insects

There is no fully valid genotoxicity finding in insects. However, from the various investigations it may be concluded that BHAS has a mutagenic potential in insects.

Table 4.16: Overview on genotoxicity tests with insects

Test system	Doses	Exposure period	Result	Remarks	Test subst.	Reference
<i>Drosophila</i> ; somatic cell genotoxicity	90 -120 mmol/l (6'210 - 8'280 µg/ml); Feeding	48 h	positive	somatic mutation and recombination test (SMART)	hydroxylamine hydrochloride	Graf et al. 1989
<i>Drosophila</i> ; somatic cell genotoxicity	454 µg/ml; feeding	not given	positive	induction of inversions; methodological insufficiencies	BHAS	Parkash and Miglani 1978

Drosophila; germ cell genotoxicity	0.03 mol/l (2'070 µg/ml); Feeding	not given	positive	sex-linked recessive lethal test (SLRL test); methodological insufficiencies	hydroxylamine hydrochloride	Vijaykumar and Jain 1978
Drosophila; germ cell genotoxicity	174 µg per insect; microinjection	not given	negative	test on dominant lethals; methodological insufficiencies	hydroxylamine hydrochloride	Fahmy and Fahmy 1970
Grasshopper; germ cell genotoxicity	345 µg per insect; abdominal injection	36 h	positive	methodological insufficiencies	hydroxylamine hydrochloride	Bhattacharya et al. 1986

4.1.2.7.3 Summary of mutagenicity

There are no in vitro genotoxicity data for BHAS. In vivo in mice, a bone marrow micronucleus test and a screening for dominant lethal mutations were negative.

Hydroxylamine or its hydrochloride were mainly negative in bacterial genotoxicity tests. In *Salmonella typhimurium* TA 100 marginal effects were observed at high doses; a test with *E. coli* was positive only at an extremely high dose. Hydroxylamine hydrochloride was weakly positive in mouse lymphoma assays and seems to express a genotoxic potential in insects. However, clearly negative results were obtained concerning UDS in rat hepatocytes and chromosomal aberrations in rodent bone marrow cells. Further data were of relatively low reliability or significance.

Overall it may be concluded that BHAS has no or a low genotoxic potential. In any case, it is unlikely that a mutagenic potential is expressed in mammals in vivo. BHAS is not classified as a mutagen.

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

In vivo studies

Inhalation

No data available.

Dermal

No data available.

Oral

Combined chronic toxicity/carcinogenicity study, 12/24 months (rat)

In a standard combined chronic toxicity/carcinogenicity toxicity study according to OECD TG 453, BHAS (purity commercial grade) was administered to groups of 50 male and 50 female Wistar (Chbb:THOM, SPF) rats daily in the drinking water at dose levels of 0, 5, 20 and 80 ppm for 24 months (main groups), and additional to groups of 10 animals per sex and dose for 12 months (satellite groups) for evaluation of hematology parameters (BASF, report 2001). The doses administered corresponded to a mean daily BHAS intake in the main groups of about 0, 0.2, 1.0, and 3.7 mg/kg bw/d in males and 0, 0.4, 1.6, and 6.2 mg/kg bw/d in females; and in the satellite groups of about 0, 0.3, 1.1, and 4.5 mg/kg bw/d in males and 0, 0.4, 1.6, and 6.2 mg/kg bw/d in females.

There was no statistically significant body weight loss, and no significant effect on mortality in the BHAS treatment groups when compared to control groups, and furthermore, no treatment-related clinical signs were noted in either sex throughout the study.

At termination, mean absolute and relative spleen weights were statistically significant increased in the 80 ppm females. Gross observations on the spleen showed a slightly increased number of males with macroscopically diagnosed masses at 80 ppm. Microscopy yielded further evidence of spleen injury in 20 ppm and 80 ppm males and females at sacrifice. The only neoplasms showing an increased incidence were tumours of the spleen in both male and female rats when compared to controls. The spleen tumours observed in male and female rats were diagnosed as hemangiosarcomas and hemangiomas. In male rats the incidence of hemangiosarcomas in the spleen was increased from 4/50 among controls and all treatment groups, to 7/50, 9/50 and 8/50 in the 5, 20, and 80 ppm groups, respectively. Two of the hemangiosarcomas of the 80 ppm dosed males and one of the control males metastasized into other organs. In females, there was a biologically equally distribution of hemangiosarcomas in the spleen over the control group animals and the BHAS-treated animals (0/5/20/80 ppm: 2/1/1/3). Whereas the number of females with hemangiomas was slightly increased in the 80 ppm dose group (0, 5, 20, 80 ppm: 0/1/1/4). A survey of neoplastic lesions in the spleen in animals of the main groups is given in the following table 4.17.

Table 4.17: Incidence of neoplastic lesions in the spleen from Wistar rats treated with BHAS in the drinking water for 24 months

Neoplastic lesions	Male rats				Female rats				
	Dose (ppm)	0	5	20	80	0	5	20	80
Animals examined		50	50	50	50	50	50	50	50
Hemangiosarcoma [%]		4 [8]	7 [15]	9 [18]	8 [16]	2 [4]	1 [2]	1 [2]	3 [6]
Hemangioma [%]		0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	1 [2]	1 [2]	4 [8]
Hemangiosarcoma plus Hemangioma [%]		4 [8]	7 [15]	9 [18]	8 [16]	2 [4]	2 [4]	2 [4]	7 [15]

It was shown that there was a slightly increased incidence of hemangiosarcomas in the spleen of male rats at all BHAS treatment groups. Although, the incidence of hemangiosarcomas in

the spleen seen in male rats was small, not dose-related and the difference to controls did not attain statistically significance, the increase of this tumour type was considered to present an effect of BHAS treatment. This was supported by the fact that the incidence of hemangiosarcomas in the spleen seen in all treatment groups was generally higher than concurrent intra laboratory historical controls which underlines the biological significance of this finding. The incidence of spontaneous primary splenic endothelial neoplasms (hemangiosarcomas or hemangiomas) was cited between 0% and 1.5% in males and 0% and 0.5% in females of four rat strains (Losco, 1992). In historical control data of Wistar rats (received from BASF), the mean percentage for hemangiosarcomas in male rats is 3.1% and varies between 0-10%. The historical mean value for hemangiomas in females is 0.6% with a range of 0% to 5%. Thus, the incidences of hemangiosarcomas in the carcinogenicity study in male rats at 5 ppm and above was found on top of the historical control data range. This represented a significant trend in males. The same trend was also determined in females observed a significant increase of hemangiomas in the high dose group at 80 ppm.

Tumours developed earlier in BHAS treated animals than in controls.

Furthermore, the following findings occurred in the spleen: hyperplasia and ectasia of capillaries, lined by a normal endothelium which lacked pleomorphism and mitotic activity and separated by a small amounts of fibrous tissue. These findings diagnosed as angiomatous hyperplasia were seen in all dose groups and controls. However, high frequencies of these spleen lesions were observed in males and females at 80 ppm. The incidence of these findings is given in the following table 4.18.

Table 4.18: Incidence of precursor lesions in the spleen from Wistar rats treated with BHAS in the drinking water for 24 months

Spleen	Male rats				Female rats			
Dose (ppm)	0	5	20	80	0	5	20	80
Hyperplasia, angiomatous	4	9	4	16	14	13	12	34

Persistent hyperplasia may be indicative as site of tumour development. So, these findings of angiomatous hyperplasia in the spleen were considered as a precursor lesion of angiomatous tumours (hemangioma, hemangiosarcoma).

In the satellite groups (treatment for 12 months), very few neoplastic findings were noted which were biologically equally distributed over the control group and the BHAS treatment groups. They were considered to be incidental or spontaneous in origin and without any relation to treatment.

Overall, this combined chronic toxicity/carcinogenicity study in the rat is acceptable and does satisfy the guideline requirement for carcinogenicity testing (OECD TG 453) in rats. Results of this standard study have shown that BHAS is carcinogenic in rats after treatment with dosages of ≥ 5 ppm (equivalent to about ≥ 0.2 mg/kg b/d) in males and of 80 ppm (equivalent to about 6.2 mg/kg bw/d) in females. In male rats an increased incidence of hemangiosarcomas was noted in all BHAS-treated groups. Hemangiomas were observed in four females from the 80 ppm dose group. Both numbers of hemangiosarcomas in male rats and hemangiomas observed in female rats were outside the range of historical control background data. Angiomatous hyperplasia in the spleen considered as a precursor lesion of angiomatous tumours was observed at an increased number in males and females at 80 ppm. Therefore, 5 ppm (equivalent to about 0.2 mg/kg bw/d in males and 0.4 mg/kg bw/d in

females) is considered to be the lowest dose level for tumour development in the spleen in male and female rats in this study (BASF, 2001).

52-week study (mouse)

In an early report describing the chronic and carcinogenic effects of BHAS in male Swiss-Webster and female C3H/HeN mice, BHAS (purity: commercial grade) was given in drinking water in doses of 0, 10 or 20 mmol/l (equivalent to 0, 100 or 200 mg/kg bw/d, calculated on an assumed water consumption of 15% of body weight) to four-week old mice. Groups of 5 male and 10 female mice were administered with BHAS for 52 weeks. After termination of treatment, one group of female mice received pure water up to 104 weeks of age. No tumours were noted in mice kept on BHAS for as long as one year. Usually the condition and appearance of the experimental mice seemed better than that of controls. Furthermore, in female C3H/HeN mice which drank BHAS solution for one year, there were no spontaneous mammary tumours, even in those surviving 2 years (Yamamoto et al., 1967).

Up to 50/70 weeks (mouse and rat)

In order to determine the possible mechanisms of BHAS to prevent the formation of spontaneous tumours, five separate experiments using nulliparous female C3H/HeN mice/group, with age-dependent application protocol (starting age 6, 13, 18, 24, and 28 weeks) and time-dependent sacrifice of test animals (from 13 to >50 weeks) for histological examination of mammary glands and ovaries as well as pituitary glands were carried through. For each run, the groups consisted of each five test and concurrent control animals, but one long-term group with 16 test animals and 14 control animals, respectively. BHAS (purity: commercial grade) was given to the test animals in the drinking water as a 10 mM solution (approximately 100 mg/kg bw/d) up to 70 weeks. The weekly food intake was measured by weighing the food consumed by one test group and one control group for 27 weeks. All of the animals were weighed once a week at the beginning of the experiments and twice a week later on. Mice were palpated weekly for tumour development. Effects on ductile development of mammary glands and on the morphology of mammary glands and ovaries were evaluated and early effects of BHAS administration on these organs were determined. For that purpose see section 4.1.2.9.

The spontaneous tumour rate of mammary gland was decreased to 31% as compared to the 100% among the control animals when BHAS administration was started at age of 6 weeks. When treatment was started at the age of 13 weeks, the spontaneous tumour rate was decreased to 40%. On the other hand, late commencement of treatment (18 weeks or thereafter) did not prevent tumour generation. BHAS had an effect on the size of pituitary glands and on the pituitary level of prolactin: the former was decreased and the latter was increased when there was no mammary tumour present. There was no relationship between tumour occurrence and pituitary prolactin content in either control or experimental animals.

In conclusion, BHAS treatment started at an early age prevents the formation of spontaneous mammary tumours in female C3H/HeN mice, but when administration started later in life no such effects was seen (Evarts and Brown, 1977).

In a further experiment, the effect of BHAS on the morphology of mammary gland and on the formation of mammary tumours induced by 7,12-dimethylbenz[a]anthracene (DMBA) was studied in virgin female Sprague Dawley rats. One group of 15 rats at the age of 36 days and a second group of 25 rats at the age of 64 days received 10 mM (approximately 67 mg/kg bw/d)

BHAS (purity: commercial grade) in drinking water. In addition, there were two control groups (i.e. 15 and 25 rats) receiving tap water during the experiment. A further group was used as positive control group. At the age of 50 days 11 mg DMBA in corn oil was administered by stomach tube to two test groups and to the positive control group. To follow the development of mammary glands 3 to 5 rats were killed periodically from each group (at ages of 50, 69, 85, and 210 days). After DMBA administration rats were palpated weekly for mammary tumours. BHAS caused excessive lobular growth (hypertrophy) and secretion activity in mammary gland. For that purpose see section 4.1.2.9.

When BHAS was given after DMBA treatment it decreased the number and size of tumours per tumour bearing animal, but increased the median latency period (latency period 102 d as compared to 63 d positive control). No differences were found when BHAS application started two weeks prior to DMBA administration (Evarts et al., 1979).

In summary, repeated administration of 100 mg/kg bw/d BHAS via drinking water to female mice for up to 70 weeks started at an early age caused atrophy of the mammary gland and decreased the tumour incidence. In female rats BHAS caused excessive growth and secretion activity of mammary gland after repeated oral administration of 67 mg/kg bw/d via drinking water. When BHAS was given prior to the administration of 7,12-dimethylbenz[a]anthracene (DMBA), the former protected the normal histology structure of the mammary gland to some extent against the early destructive changes induced by a carcinogen.

105/125 weeks (mouse)

To determine effects of lifetime administration of BHAS on neoplasm development and longevity mice of two C3H sublines were used: the C3H/HeN, which carries a germinal provirus of the mouse mammary virus (MMTV) and develops a moderately high incidences of tumour appearing late in life and the C3H/HeJ(+), which also carries the milk-transmitted exogenous virus and causes a high and early incidence of mammary tumours. Two separate experiments were conducted. In the first experiment 40 female C3H/HeN mice received 10 mM BHAS (1640 mg/l = 246 mg/kg bw/d, calculated on an assumed water consumption of 15% of body weight) in the drinking water for 123 weeks and further 60 female negative control mice were used. The second experiment consisted of 4 groups of C3H/HeJ(+) mice (male and female test group, and negative control group for each sex), 50 mice/group and sex, but the female control group consisting of 56. The exposure period was 105 weeks. The administration of the solutions was started when the mice were 6 weeks of age. The mice were observed regularly and all gross changes, including palpable mammary nodules were recorded. The animals were weighed weekly. At necropsy, all altered organs were described. The specimens and samples from all parenchymatous organs were prepared for histopathology.

Lifetime administration of 10 mM (equivalent to ca. 246 mg/kg bw/d) BHAS in the drinking water did not extend significantly lifespan of female C3H/HeN mice and did not have influence on body weight gain. The occurrence of neoplasms in both control and BHAS-treated mice was high, both with regard to the number of tumour-bearing animals and the number of neoplasms. Mammary carcinomas, lymphomas, lung adenomas, liver carcinomas and ovarian neoplasms were observed in controls as well as BHAS-treated mice, 3 in 36 (8.6%), vs. 14 in 58 control animals (24%). In contrast, vascular neoplasms of the spleen occurred in 10 BHAS-treated animals, whereas none was found in the controls. A survey of a small selection of different types of neoplasms in C3H/HeN mice is given in the following Table 4.19.

Table 4.19: A small selection of different types of neoplasms in C3H/HeN mice after administration of BHAS for 105 weeks

Doses	0	246 mg/kg bw/d BHAS
Animals examined	58	36
Mammary carcinoma	14	3
Hemangioma (spleen)	0	10

In the second experiment using male and female C3H/HeJ(+) mice, female mice which were given BHAS for 105 weeks had a slightly lower survival when compared to controls. Male mice had a higher average body weight than females. BHAS administration caused a slight decrease in body weight in male mice, while no effect was seen in females. In the second experiment the occurrence of neoplasms was high in all groups, again with respect to tumour-bearing animals and the number of neoplasms. Hepatocellular carcinomas, lung adenomas and lymphomas were observed in both sexes, although the incidence of liver carcinomas was greater in males (43%). In females, mammary and ovarian neoplasms were frequent. The incidence of mammary neoplasms was slightly higher in the BHAS-treated females (84% in treated and 73% in the control group). The number of vascular neoplasms of the spleen was not affected by BHAS treatment, but the number of hemangiomas of lymph nodes was significantly higher in BHAS-treated males. The number of tumour-bearing treated male C3H/HeJ(+) mice was significantly higher than the corresponding control group, but the number of tumours in the group was not much different. A survey of a small selection of different types of neoplasms in C3H/HeJ(+) mice is given in the following Table 4.20.

Table 4.20: A small selection of different types of neoplasms in C3H/HeJ(+) mice after administration of BHAS for 105 weeks

Doses	0	246 mg/kg bw/d BHAS
Animals examined (m/f)	44/44	50/48
Lymphoma (m/f)	0/0	3/5
Hemangioma, Lymph node (m/f)	5/1	1/13
Hepatocellular carcinoma (m/f)	19/4	21/0

In summary, lifetime administration of 10 mM (equivalent to ca. 246 mg/kg bw/d) BHAS in the drinking water resulted in a considerable reduction in mammary neoplasm incidence in female C3H/HeN mice, but not in female C3H/HeJ(+) mice. Ovarian neoplasms and cysts were common in all groups (test groups and control groups), indicating ovarian dysfunction, but these were not affected by treatment. The incidences of other cryptogenic neoplasms found in controls in significant numbers, i.e. liver carcinomas, lymphomas, lung adenomas and adrenal cortex tumours were only marginally affected by the treatment. However, an increased incidence of vascular neoplasms of the spleen in BHAS-treated female C3H/HeN mice and vascular neoplasms of the lymph nodes in BHAS-treated male C3H/HeJ(+) mice indicated a subline-related action on the reticuloendothelial system. The survival of control mice was 35-58% at two years and this was not increased in either subline by BHAS (Stenbäck et al., 1987).

In vitro studies

Cell transformation assays

Cell transformation data are inconsistent. For BHAS a positive response in Balb/3T3 cells is described; however, negative data were obtained for hydroxylamine hydrochloride in this system as well as in SHE cells.

BHAS was described to induce cell transformation (type III foci) in Balb/3T3 cells with and without S-9 mix (Microbiological Associates, 1981). With S-9 mix, $2-3 \times 10^6$ cells were exposed to doses of 10, 30 or 100 $\mu\text{g/ml}$ for 2 h. At 30 $\mu\text{g/ml}$ the transformation frequency for type III foci was increased (0.82×10^{-4} as compared to 0.16×10^{-4} in the negative control); at 10 and 100 $\mu\text{g/ml}$ cell transformation frequencies were not increased. No toxic effects were reported. Without S-9 mix, exposure was 24 h. Doses of 3 and 30 $\mu\text{g/ml}$ induced cell transformation; the maximum transformation frequency was 6.44×10^{-4} (negative control, 0.15). At 10 $\mu\text{g/ml}$ a negative response was obtained. Toxic effects were induced at 30 $\mu\text{g/ml}$.

4.1.2.8.2 Studies in humans

No data on studies in humans available.

4.1.2.8.3 Summary of carcinogenicity

There are no human data on carcinogenicity of BHAS and there is no information on carcinogenicity in experimental animals following inhalation and dermal exposure.

Carcinogenic potential of BHAS was demonstrated for administration by oral route. There are positive results for carcinogenic activity of BHAS in both sexes of rats. An increased number of tumours of the spleen was observed in male and female rats in a standard carcinogenicity (24-month drinking water) study (BASF, 2001). This combined chronic toxicity/carcinogenicity study in the rat is acceptable and does satisfy the guideline requirements. Survival and body weight development was comparable to untreated controls. The administration of BHAS in the drinking water for 2 years to rats was associated with an increased incidence of hemangiosarcomas in males and hemangioma development in females, both in the spleen. These neoplastic lesions are considered to be treatment-related. A survey of the results of the 24-month drinking water study is given in the following Summary table 4.21.

Table 4.21: Summary table: Tumour responses in the 24-month drinking water study on rats (BASF, 2001)

Species	Study design	TUMOUR RESPONSE
		- Survival was comparable to untreated controls.

Species	Study design	TUMOUR RESPONSE
Rat Wistar (Chbb: THOM; SPF) 50m/50f	drinking water 24 months 5, 20, 80 ppm (m: 0.2/0.3, 1.0/1.1, 3.7/4.5 mg/kg bw/d; f: 0.4/1.6, 1.6/1.6, 6.2/6.2 mg/kg bw/d)	- Body weights were similar to controls. At termination: ↑ spleen weight (f, 80 ppm) <u>Neoplastic lesions in the spleen:</u> Hemangiosarcoma Males: 5, 20, 80 ppm: 7/50, 9/50, 8/50 vs 4/50 controls Females: 5, 20, 80 ppm: 1/50; 1/50, 3/50 vs 2/50 controls Hemangioma Males: 0/50 in all doses groups vs 0/50 controls Females: 5, 20, 80 ppm: 1/50, 1/50, 4/50 vs 0/50 controls Hemangiosarcoma plus Hemangioma Males: 5, 20, 80 ppm: 7/50, 9/50, 8/50 vs 4/50 controls Females: 5, 20, 80 ppm: 2/50; 2/50, 7/50 vs 2/50 controls <u>Precursor lesions:</u> Hyperplasia, angiomatous Males: 5, 20, 80 ppm: 9/50, 4/50, 16/50 vs 4/50 controls Females: 5, 20, 80 ppm: 13/50, 12/50, 34/50 vs 14/50 controls

There was some evidence for carcinogenicity activity of BHAS in both male and female rats. An increased number of hemangiosarcomas in the spleen was observed in male rats at all dose groups (≥ 5 ppm, equivalent to about ≥ 0.2 mg/kg bw/d). The incidence of hemangiosarcomas in the spleen when compared with the controls was increased showing a more plateau-like dose-curve, but no hemangiomas occurred. In female rats, hemangiosarcomas were biologically equally distributed over the control females and treated females. In four female rats versus 0 in the controls, hemangiomas were seen in the spleen at the highest dose level of 80 ppm (equivalent to about 6.2 mg/kg bw/d). Although the increase in number of tumours rates in the spleen of male and female rats was low, not dose-related and the difference did not attain statistical significance, their rates were clearly above those in the control groups and as well as above the ranges of historical control background data. Tumours developed earlier in BHAS-treated animals than in controls. Angiomatous hyperplasia in the spleen was considered as a precursor lesion of angiomatous tumours (hemangioma, hemangiosarcoma). High frequencies of these spleen lesions were observed in males and females at 80 ppm. Hemangiosarcomas and hemangiomas of the spleen were observed against a background of dose-related non-neoplastic changes such as hemolytic anemia and secondary effects in blood-forming organs. The lack of hemangiosarcomas and hemangiomas at other sites provides good evidence for a primary splenic origin of these tumours. Since hemangiomas are relatively uncommon, their biological potential is poorly defined. Due to the morphologic similarity of splenic angiomatous hyperplasia to the induced splenic hemangiosarcomas it was suggested that these lesions are preneoplastic. The evidence of angiomatous hyperplasia, hemangiomas and hemangiosarcomas in BHAS-treated rats supports a progressive development from angiomatous hyperplasia to tumours.

There is no indication to assume that the tumours induced in the spleen of rats may be related to primary genotoxic effects (as BHAS has no or a low genotoxic potential). The existence of other/alternative (non-genotoxic) mechanisms is assumed. It might be supposed that the carcinogenicity is mediated via accelerated destruction of erythrocytes and tumour growth was released in BHAS-treated rats by chronic cell injury and persistent cell proliferation. At present, no other mode of action has been identified. The exact mechanisms remain unclear. A species-specific mechanism of tumour formation irrelevant for humans was not identified.

The results have shown that 5 ppm (equivalent to about 0.2 mg/kg bw/d in males and 0.4 mg/kg bw/d in females) is the lowest dose level for tumour development in the spleen. Since no lower concentrations were tested in the cancer bioassay, 5 ppm BHAS is considered as the threshold dose for tumour formation (BASF, 2001).

No adequate studies are available to evaluate the carcinogenic potential of BHAS in mice. However, the data available did indicate that BHAS induces spleen tumours as it does in the rat. An increased incidence of vascular neoplasms of the spleen was observed in female C3H/HeN mice administered long-time (life-time) of about 246 mg/kg bw/d BHAS in the drinking water, whereas none was found in controls; and in male C3H/HeJ(+) mice an increased number of vascular neoplasms in the lymph nodes, respectively (Stenbäck et al., 1987).

In accordance to Directive 67/548/EEC for classification and labelling of dangerous substances and preparations, the criteria for classification of BHAS as a category 3 carcinogen are fulfilled and the labelling proposal to cover this potential hazard therefore should remain as Xn, Harmful; R 40 (limited evidence of a carcinogenic effect).

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Effects on fertility

Studies in animals

Guideline-according generation studies, respectively fertility studies for BHAS are presently not available.

Some informations related to reproductive organs can be derived from the data of a repeated dose toxicity study (cf. 4.1.2.6), during which Wistar rats (10 animals/sex/dose group) had been exposed to BHAS (purity > 99%) via drinking water for over 3 months at concentrations of 10, 50, and 250 ppm according to dose levels of about 0.9, 4, and 21 mg/kg bw/d (BASF 1992b). Organ weight determinations of testes at all dose levels as well as macro- and microscopic evaluations of the testes at the highest dose level (250 ppm) had been performed for the male sex, whereas with females only macro- and microscopic evaluations had been performed for the uterus and the ovary at the highest dose level (250 ppm). At the end of the study for neither of these parameters any substance related changes could be detected. It is concluded from this study that BHAS does not interfere with reproductive organ weights and morphological integrity at dose levels of up to and including 250 ppm according to a daily intake of approximately 21 mg/kg bw (NOAEL). The NOAEL for systemic adverse effects of this study was 0.9 mg/kg bw/d for both sexes based on findings of hemolytic anemia with methemoglobinemia and changes in organ weight as well as histopathological changes in spleen and liver at higher dosages.

During two studies focussing on the investigation of BHAS for its tumour preventive properties in both a mouse and a rat mammary gland tumour model (cf. 4.1.2.8) also the ductile development of mammary glands and the morphology of mammary glands (mouse and rat) and the morphology of the ovaries (mouse) was monitored for prolonged drinking water application of high doses of BHAS (10 mM BHAS in drinking water according to an intake of approximately 100 mg/kg bw/d for mice and 67 mg/kg bw for rats) in BHAS only treated animals (Evarts and Brown 1977; Evarts et al. 1979).

In the studies with CH3/HeN mice (Evarts and Brown 1977) BHAS had been administered with an age-dependent application protocol and time-dependent sacrifice schedule. Five experiments were described. In experiment I (98 mice), treatment was started when mice were 6 weeks old. In experiments II, III, IV and V (each using 30 mice) treatment was started at 13, 18, 24 and 28 weeks, respectively. Five test animals and five control animals were sacrificed after 7, 10, 13, 16, 19, 22 and 28 weeks of treatment in experiment I and after 7 and 9 weeks of treatment in experiments II to V. At time of sacrifice the number of maturing follicles and corpora lutea was determined and ovaries were investigated histopathologically. Ductal morphology and ductular (acinar) morphology of mammary glands (whole mount preparations) were assessed using a scoring system (Khanolkar and Ranadive, 1947).

The results revealed retarded development of mammary glands and of ovaries after 19 days of BHAS feeding as well as at later ages a more rapid onset of regressive changes in the mammary gland and in the ovaries. Furthermore, ovarian function had been estimated by following the estrus cycles by means of investigating vaginal smears for 10 weeks in animals of experiment I. Mean length of cycles was 6.8 days in BHAS-fed mice compared to 5.0 days in control mice and mean length of estrus phase was 1.4 days in BHAS-fed mice and 2.1 in control mice. At time of sacrifice the number of maturing follicles and corpora lutea was determined and ovaries were investigated histopathologically. In mice that started receiving BHAS at an age of 6 week the **number of follicles** was similar to control animals up to an age of 25 weeks and declined more rapid in control animals than in BHAS-fed animals up to an age of 34 weeks. The onset of the decrease of maturing follicles at higher ages was confirmed in all experiments with mice starting BHAS-feeding later than six weeks. The **number of corpora lutea** was similar in both groups at an age of 13 weeks and was clearly lower in BHAS-fed animals at higher ages compared to controls (two fold differences at minimum). The latter effect was also present -sometimes even more pronounced - in animals starting with BHAS feeding at 13, 18, 24 and 28 weeks. It was reported that in comparison to the controls the treated animals showed much more prominent cornification of the vaginal epithelium and a prolonged estrus phase of the cycle (no data provided).

Regarding general toxicity only data from experiment I were presented graphically as mean of BHAS-fed animals of the same age compared to mean of controls. Growth curves were similar for animals fed BHAS and control animals up to an age of 19 weeks. Thereafter, a lower weight gain was found in BHAS-fed animals (maximal difference estimated from the figure was 6 g at an age of 30 week). It is stated that food consumption decreased in BHAS-fed mice to 11 % of the food consumption in control mice after 13 weeks of treatment (no data presented). No further data on systemically toxic effects were reported.

In the studies with Sprague-Dawley rats (Evarts et al. 1979) BHAS had been administered to virgin females in a fixed application protocol starting treatment at 36 days of age. Histopathological evaluation of the mammary glands of the animals of the treated groups at the age of 50 days showed large and dense lobules with vacuolized ductular epithelium. Cystic dilatation was common. At the end of 69 days it was reported that the appearance of the BHAS only treated groups was that of pregnant rats with copious basophilic secretion and vacuolized lobular epithelium, whereas in contrast to that in the control animals the size of the lobules was small. This was explained by the authors with probable interference of BHAS with the hormonal balance of the animals.

The results of these two studies show that in rodents the female sex higher dosages of BHAS may interfere with the estrus cycle and with the functional state and morphology of reproductive organs (ovaries). As evidenced by morphological signs of either atrophy or hypertrophy and secretion also the functional state and the morphological development and

integrity of the mammary gland may be affected. With respect to these findings a LOAEL of 67 mg/kg bw/d can be inferred from these studies.

In a dominant lethal assay (cf. 4.1.2.7) with mice BHAS led to a negative result after single intraperitoneal injection of 102 and 112 mg/kg bw with respect to early fetal deaths and preimplantation loss (Epstein et al. 1972). Findings were not described in detail.

Studies in humans

No data are available.

4.1.2.9.2 Developmental toxicity

Studies in animals

In a study (BASF 1994) according to OECD TG 414 BHAS was investigated for its prenatal toxicity in Wistar rats (Chbb:THOM) by the oral (gavage) route of administration. Groups of 22 - 24 pregnant rats had been treated with BHAS at dosages of 1, 3, 10, and 20 mg/kg bw on day 6 through day 15 post coitum. The test substance (purity \geq 98.4%) had been administered as an aqueous solution and at a standard dose volume of 5 ml/kg bw. The control group, consisting of 20 dams, was dosed with the vehicle (Milli-Q-water) only.

Food consumption and body weights of the animals were recorded regularly throughout the study period. The state of health of the animals was checked each day. At sacrifice on day 20 post coitum dams were assessed by gross pathology (including weight determinations of the spleen), and numbers of corpora lutea and numbers and distributions of implantation sites were recorded. Foetuses were sexed, weighed and further investigated for any external, soft tissue and/or skeletal findings.

In the dams an enlargement of the spleens and a dose related statistically significant increase in absolute and relative spleen weights was revealed at dosages of 10 and 20 mg/kg bw/d. No substance-related effects on dams were reported for the lower dose groups. There were no substance-related differences between the groups in conception rate, the mean number of corpora lutea and implantation sites or in the values calculated for the pre- and the postimplantation losses, the number of resorptions and of viable foetuses. Mean fetal body weight of the dosed groups did not differ from that of the control. Examination of foetuses did not reveal any signs for substance related abnormalities.

From this study a NOAEL for maternal toxicity of 3 mg/kg bw/d and a NOAEL for embryo-/fetotoxicity of 20 mg/kg bw/d can be derived.

Studies in humans

No data are available.

4.1.2.9.3 Summary of toxicity for reproduction

With respect to toxicity for reproduction no human data are available so far.

Data from animal experiments with BHAS did not reveal any specific embryo-/fetotoxic or teratogenic potential. A NOAEL (oral) for maternal toxicity of 3 mg/kg bw/d and a NOAEL

(oral) for embryo-/fetotoxicity of 20 mg/kg bw/d was derived from a guideline according prenatal toxicity study in rats (BASF, 1994). Guideline-according generation studies, respectively fertility studies for BHAS are presently not available. Therefore, concerning any possible impairment of reproductive organs, data from other studies with repeated administration were taken into consideration. Data from repeated dose toxicity studies in mice and rats with the oral route of administration showed that female animals are susceptible to BHAS induced effects in terms of impaired ovarian functional state and morphology and of impaired development and morphology of mammary gland tissues. Based on these findings a LOAEL (oral) of about 67 mg/kg bw/d was determined from studies with rats (Evarts et al. 1979). From a 3 months repeated dose toxicity gavage study with BHAS (BASF, 1992b) there were no indications for an impairment of male and female reproductive organs up to and including the highest tested dose.

4.1.3 Risk characterisation ⁷

4.1.3.1 General aspects

Summary of toxicological effects

Toxicokinetics. For BHAS no in vivo data are available on absorption, distribution or excretion, and there are only few in vitro studies. Hydroxylamine is formed as an intermediate during cellular metabolism. Hydroxylamine reductase is detected in the mitochondria of livers from mice, rats and pigs. Its activity appears to be age-dependent. Partly metabolic oxidation of hydroxylamine to nitrate has also been described from an in vivo rat study.

For oral and inhalation uptake absorption rates of 100 % (defaults) are proposed for the risk characterisation. 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 on systemic effects as well as that occupational dermal exposure will most likely take place under non-occlusive conditions an absorption rate of 10% will be assumed for dermal risk characterisation purposes.

Acute toxicity. BHAS can cause methaemoglobin formation after acute oral and dermal exposure. The acute toxicity depends on the sensitivity to methaemoglobin formation. In rats (low sensitivity to methaemoglobin formation) the oral LD₅₀ is in the range of 545-652 mg/kg bw and the dermal LD₅₀ is >500 mg/kg bw. In species with higher sensitivity to methaemoglobin formation lower LD₅₀ values were obtained: oral LD₅₀ for female cats appr. 200 mg/kg bw, dermal LD₅₀ for rabbits between 100 and 500 mg/kg bw. In rabbits the dose of 1 mg/kg bw can be considered as NOAEL for occlusive application, whereas a NOAEL of 500 mg/kg bw is derived for semi-occlusive exposure. Two inhalation risk tests with rats demonstrated that inhalation of saturated vapours at 20° C did not cause severe toxic effects. However, due to the very low vapour pressure of BHAS as solid substance and the method used in these studies, it is doubtful whether the animals were exposed to significant concentrations of the substance. Human data on the acute toxicity of BHAS are not available. Presented data suggest that the current labelling with Xn; R 20/22 has been changed to R 21/22 (Harmful in contact with skin and if swallowed).

⁷ 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.

Irritation and corrosivity. Animal data on irritation and corrosivity are insufficient or scarcely reported; these data demonstrate moderate to severe irritating and even corrosive substance properties depending on the time of exposure. Skin irritation tests using rabbits show severe skin lesions for exposure periods of 20-24 hours, whilst a 4-hours exposure of the skin resulted in mild skin irritation. Severe conjunctival irritation and severe corneal opacity was demonstrated after instillation of the substance into the eye of one rabbit which was not reversible until the end of the observation period. However, results from another study showed less pronounced effects. Human experience with local irritation/corrosion caused by BHAS is mentioned in the literature but respective reports are not available. Depending on the available data, the current labeling of eye and skin irritation potential of BHAS with R 36/38 is confirmed.

Sensitisation. In animal experiments with BHAS and hydroxylamine hydrochloride skin sensitising properties were demonstrated. This correlates with human data for both substances, thus it was concluded that the substance causes sensitisation by skin contact and existing classification with R 43 is warranted. In a test with guinea pigs that were first treated in a Magnusson Kligman Test (96 % positive) and subsequently subjected to an inhalation aerosol challenge or an intratracheal challenge no indication for a pulmonary sensitisation was detected. Since no data are available on the validity of the test procedure, the value of these results is limited.

Repeated dose toxicity. Repeated dose toxicity studies with BHAS in humans are not available. Furthermore, no animal data are available for the inhalation and dermal route of exposure. After oral administration of BHAS the primary target organ is the blood, primarily the erythrocytes, resulting in anemia from accelerated erythrocyte destruction (hemolytic anemia) induced by oxidative damage to erythrocytes, leading to hemolysis, characterized by a significant low circulating red blood cell amount (as indicated by an absolute decrease in the hematocrit values, hemoglobin concentration and total red blood cell count), associated with increased medullary and extramedullary hematopoiesis. Furthermore, there were methaemoglobin production, Heinz body formation, specific alterations of red blood cell morphology, reticulocytosis in the peripheral blood, hemosiderosis in the spleen, liver, and kidneys, and spleen enlargement (BASF 1992b, 2001). The pathomorphological changes in erythrocytes and the higher incidence of anisocytosis and polychromasia are indicative of a regenerative hemolytic anemia caused by the production of methaemoglobin (MetHb). An increase in MetHb concentration was observed only after 90 days of treatment, and not after 12 and 24 months of treatment. These results are in line with literature data that in long-term studies an increased MetHb formation might well be noticed in the initial phase only, and subsequently disappear due to adaptation particularly in the rat due to the high rate of methemoglobin reductase activity. However, the occurrence of Heinz bodies in the erythrocytes is indicative of oxidative denaturation of haemoglobin in the red blood cells by the test compound and reflects the preceding MetHb formation. In the 90-day drinking water study in Wistar rats, adverse effects related to hematotoxicity were observed at ≥ 50 ppm BHAS, equivalent to about ≥ 4 mg/kg bw/d (BASF, 1992b). The hematotoxic effects were comparable to those determined in the combined chronic toxicity/carcinogenicity study at oral administration of 80 ppm (equivalent to 4.5 mg/kg bw/d in males and 6.2 mg/kg bw/d in females) in the drinking water, examined after 90 days of treatment and after 12 treatment months, respectively (BASF, 2001). The dose level of 5 ppm (equivalent to about 0.2 mg/kg bw/d in males and 0.4 mg/kg bw/d in females) derived after treatment of 24 months is considered as NOAEL for systemic effects. No relevant local effects on the digestive tract were observed at 80 ppm, equivalent to about 3.7 mg/kg bw/d in males and 6.2 mg/kg bw/d in females (NOAEL_{local}). Classification with Xn and labelling with R48/22 is confirmed (Harmful. Danger of serious damage to health by prolonged exposure if swallowed).

Mutagenicity. No in vitro genotoxicity data are available for BHAS. In vivo test results of a bone marrow micronucleus test and a dominant lethal assay were negative. It is concluded that BHAS has no or a low genotoxic potential. This is supported by negative in vitro results obtained with hydroxylamine hydrochloride. In any case, it is unlikely that a mutagenic potential is expressed in mammals in vivo. No classification as mutagen is needed.

Carcinogenicity. There are no data available for the inhalation and dermal route of exposure and there are no human data on carcinogenicity. After oral administration to rats BHAS is capable of producing cancer. There was some evidence for carcinogenicity activity of BHAS in male and female Wistar rats. BHAS treatment for 2 years in the drinking water was associated with an increased incidence of hemangiosarcomas in males treated at ≥ 5 ppm, equivalent to about ≥ 0.2 mg/kg bw/d and hemangioma development in females treated at 80 ppm, equivalent to about 6.2 mg/kg bw/d, both in the spleen. Angiomatous hyperplasia in the spleen considered as a precursor lesion of angiomatous tumours (hemangioma, hemangiosarcoma) was observed in animals of both sexes. High frequencies of these spleen lesions were observed in males and females at 80 ppm. 5 ppm (equivalent to about 0.2 mg/kg bw/d in males and 0.4 mg/kg bw/d in females) BHAS is the lowest dose level for tumour development in the spleen. Although the database for mice is insufficient, the data available did indicate that BHAS may induce spleen tumours as it does in the rat. It was considered that BHAS has no genotoxic potential, and the carcinogenicity observed in experimental animals is mediated via a non-genotoxic mechanisms involving especially erythrotoxicity. Classification as category 3 carcinogen and labelling with R40 (Limited evidence of carcinogenic effect) is adequate.

Toxicity for reproduction. Data from a guideline according prenatal toxicity study in rats did not reveal any specific embryo-/fetotoxic or teratogenic potential. A NOAEL (oral) for maternal toxicity of 3 mg/kg bw/d and a NOAEL (oral) for embryo-/fetotoxicity of 20 mg/kg bw/d was derived. From a repeated dose toxicity study in rats a LOAEL (oral) of about 67 mg/kg bw/d was determined based on retardement of the development of the mammary gland. From a 3 months repeated dose toxicity study with rats with the oral route of administration no indications for an impairment of male and female reproductive organs could be revealed up to and including the highest tested dose level of about 21 mg/kg bw/d (NOAEL). No human data are available. No classification as toxic for reproduction is needed.

Table 4.22: Toxicological hazard identification

Substance name	Inhalation	Dermal	Oral
Acute toxicity	No toxicity of saturated vapours in rats [R20 removed]	LD ₅₀ (rat) > 500 mg/kg LD ₅₀ (rabbit) between 100 and 500 mg/kg NOAELs (rabbit) Occlusive: 1 mg/kg Semi-occl.: 500 mg/kg R21 proposed	LD ₅₀ (rat) 545-652 mg/kg LD ₅₀ (cat) 200 mg/kg R22 confirmed
Irritation / corrositivity	Skin: mild skin irritation in rabbits after 4 hours exposure, severe skin irritation in rabbits after 24 hours exposure. R38 is confirmed. Eye: severe conjunctival irritation and severe corneal opacity was demonstrated after instillation into the eye of one rabbit (irreversible). R36 is confirmed. Respiratory tract: no data		
Sensitisation	Skin: skin sensitisation in the guinea pig. R43 is confirmed. Respiratory tract: no valid data		

Repeated dose toxicity (local)	No data	No data	No specific toxic effect up to the highest tested dose in rats (NOAEL of 80 ppm equivalent to about 3.7 mg/kg bw/d in males and 6.2 mg/kg bw/d in females)
Repeated dose toxicity (systemic)	No data	No data	Concern for humans due to hematotoxicity and organ lesions resulting from hematotoxicity in rats (NOAEL of 5 ppm, equivalent to about 0.2 mg/kg bw/d in males and 0.4 mg/kg bw/d in females) R48/22 confirmed
Mutagenicity	No relevant evidence for mutagenicity from a number of studies		
Carcinogenicity	No data	No data	Concern for humans mainly due to some evidence of carcinogenicity activity in rats of both sexes (threshold dose for tumour formation = 5 ppm, equivalent to about 0.2 mg/ kg bw/d in males and 0.4 mg/ kg bw/d in females; increased number of tumours in the spleen) Carc. Cat. 3, Xn, Harmful; R40 is proposed
Fertility impairment	No data	No data	No specific toxic effects adverse to fertility - NOAEL (systemic tox.) 0.9 mg/kg bw/d - NOAEL (reprod. organ tox.) 21 mg/kg bw/d
Developmental toxicity	No data	No data	No specific toxic effects adverse to development - NOAEL (maternal tox.) 3 mg/kg bw/d - NOAEL (dev. tox.) 20 mg/kg bw/d

4.1.3.2 Workers

Introductory remarks

Bis(hydroxylammonium)sulfate, an organic salt, is highly soluble in water (587 g/l). The substance is mainly used as a chemical intermediate (95%). For occupational risk assessment the MOS approach as outlined in the Final Draft of the TGD for Human Health Risk Characterisation is applied. This occupational risk assessment is based upon the toxicological profile of bis(hydroxylammonium)sulfate (chapter 4.1.2) and the 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 absorption, applies these absorption data to transform the external occupational exposure levels to the corresponding internal body burden, and gives a short introduction to the MOS approach used.

Systemic availability for different routes of exposure

The majority of the bis(hydroxylammonium)sulfate data originates from oral studies. Since workers are predominantly exposed either by inhalation or by skin contact, route to route transformation is essential for occupational risk assessment.

There are neither experimental data on oral absorption nor on absorption by inhalation. For both routes of exposure absorption rates of 100 % (defaults) are taken (see chapter 4.1.2.1.3).

For dermal risk assessment at the workplace, a 10% dermal absorption percentage is considered adequate (mainly assuming open or semi-occlusive, but not occlusive conditions of exposure). The dermal absorption percentage might be higher in case of occlusive exposure conditions (see chapter 4.1.2.1.3).

Occupational exposure and internal body burden

In table 4.23 the specified absorption data are used to transform the occupational exposure levels (from summary tables of chapter 4.1.1.2) into the route-specific and total internal body burden. Scenario 5 is split for dermal exposure. The subscenario with 1% BHAS is a scenario considered to be corrosive (acid pickling bath); dermal exposure to BHAS for the handling of this corrosive solution is assessed to be negligible, therefore this subscenario is not taken forward to risk characterisation.

Based on the available data, the dermal absorption percentage might critically depend on specific exposure conditions (occlusive versus semi-occlusive dermal exposure). For those dermal exposure scenarios with specified use of gloves it is assumed, that there is a proper handling of the gloves which is not anticipated to result in some kind of occlusive and thus more critical exposure conditions.

Table 4.23: Bis(hydroxylammonium)sulfate exposure levels (shift average) and internal body burden

Exposure scenario		Inhalation	Dermal contact ⁽³⁾		Internal body burden		
					Inhalation ⁽¹⁾	Dermal ⁽²⁾	Combined
		mg/m ³	mg/p/d	mg/kg/d	mg/kg/d		
1. Production and further processing as an intermediate	dust	0.9 ⁽⁶⁾	42 ⁽⁴⁾	0.6	0.13	0.06	0.19
	liquid (25%)		5.3 ⁽⁴⁾	0.08		0.008	0.14
2. Formulation as photo developing chemicals (dust)		0.25	42 ⁽⁴⁾	0.6	0.036	0.06	0.10
3. Formulation as an auxiliary in different industries (dust)		1.25	420 ⁽⁵⁾	6	0.18	0.6	0.78
4. Use in photographic laboratories (30% liquid)		negligible	12.6 ⁽⁵⁾	0.18	negligible	0.018	0.018
5. Use in the electroplating industry (inhalation: aerosol; dermal: 25% and 1% liquid)		0.05	10.5 ⁽⁵⁾	0.15	0.007	0.015	0.022
			negligible	negligible		negligible	0.007

⁽¹⁾ based on 100% inhalative absorption; breathing volume of 10 m³ per shift and a body weight of 70 kg

⁽²⁾ 10% dermal absorption

⁽³⁾ upper value of a calculated exposure range

⁽⁴⁾ EASE (with gloves)

⁽⁵⁾ EASE (without gloves)

⁽⁶⁾ workplace measurements, max. value

MOS Approach

The MOS approach for human risk characterisation is described in detail in chapter 4 of the Final Draft of the TGD for Human Health Risk Characterisation. The following chapter only 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% absorption by inhalation. For bis(hydroxylammonium)-sulfate it was decided to use a 10% dermal absorption percentage and a 100% absorption percentage for oral absorption and absorption by inhalation as well.

For occupational risk assessment, the corrected inhalation NOAEC accounts for the difference of the standard respiratory volume (6.7 m³) and the respiratory volume for light activity (10 m³). If the experimental exposure schedule differs from actual exposure in terms of the frequency of exposure within a week (e.g. a 7-day experimental exposure versus a 5-day working week), this aspect additionally may be accounted for in the calculation of the adequate starting point (corrected NOAEL).

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

In addition, for risk assessment of combined exposure (inhalation exposure 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 of 4 for rats, factor of 2.3 for cats, factor of 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. Because of the availability of long-term studies, for bis(hydroxylammonium)sulfate no duration adjustment was necessary.

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. Risk assessment for carcinogenicity of bis-(hydroxylammonium)sulfate is based on the MOS approach. In variation from the reference MOS for chronic toxicity for carcinogenicity an additional severity factor of 5 is 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

Local effects (inhalation, dermal)

See chapter on irritation; no further information available.

Systemic effects by inhalation, dermal contact, combined exposure

Acute toxicity data for humans are not available.

For rats an oral LD₅₀ of 545 mg/kg, for cats of 200 mg/kg is reported. At 50 mg/kg cats survived but demonstrated clinical signs of increased salivation, cyanosis and vomiting. 4 hours after application of 50 mg/kg blood concentrations of about 10 to 20% of methaemoglobin were measured in cats.

In an oral developmental rat study (BASF 1994) pregnant rats were treated with bis(hydroxylammonium)sulfate at dosages of 1, 3, 10, and 20 mg/kg/day from day 6 until day 15 post coitum. At dosages of 20 and 10 mg/kg/d spleen weights were increased. The corresponding NOAEL for maternal toxicity was 3 mg/kg/day.

For rabbits a dermal LD₅₀ between 100 mg/kg and 500 mg/kg was determined (Derelanko et al., 1987). 20% lethality was observed at 100 mg/kg, 90% lethality at 500 mg/kg (occlusive conditions of exposure). There was no lethality at 1,000 mg/kg (semi-occlusive testing). With

reference to acute red blood cell toxicity a dose of 1 mg/kg can be considered as NOAEL for occlusive application, while for semi-occlusive exposure a dermal NOAEL of 500 mg/kg can be derived (see chapter 4.1.2.2).

There are no data on acute inhalation toxicity.

Internal starting point

Based on these toxicity data risk assessment for acute toxicity is rather speculative. Two approaches are followed and compared. The first one relates to methaemoglobin formation in cats, the second one is based on spleen enlargement in pregnant rats following a 10-day exposure.

Available data indicate 10 to 20% methaemoglobin formation in cats at 50 mg/kg. With the assumption of 100% oral absorption the adequate internal starting point is 50 mg/kg. Arbitrarily assuming dose-response linearity, less than 2% methemoglobin in blood (a concentration not leading to clinical effects) might be related to a dose of 5 mg/kg. Correspondingly, a factor of 10 is applied to extrapolate from the adverse effect of methaemoglobin formation to a NOAEL. Based on general toxicological evidence from aromatic amines, cats (relative to other animal species) seem to be rather sensitive with respect to methaemoglobin formation. Assuming similar potency concerning methaemoglobin formation in humans and cats the interspecies extrapolation restricts to a factor of 2.3 for metabolic rate scaling (cat/humans). For intraspecies variation the default factor of 5 is applied. These considerations result in a reference MOS of 115 ($10 \times 2.3 \times 5$). For these boundary conditions the resulting critical internal exposure level for methemoglobin formation calculates to 0.4 mg/kg ($50 / 115$).

Following a 10-day period of exposure, for spleen enlargement the NOAEL for pregnant rats was 3 mg/kg/day. With the assumption of 100% oral absorption the adequate internal starting point is 3 mg/kg/day. The adjustment factor for interspecies variation is 4×2.5 , the factor for intraspecies differences is 5. It is assumed, that the NAEL for spleen enlargement for acute exposure is higher than for a 10-day exposure period (possibly by a factor of 5, which is less than dose-response linearity). The reference MOS thus might be in the range of 10 ($4 \times 2.5 \times 5 \times 1/5$). For these boundary conditions the resulting critical internal exposure level for acute spleen enlargement calculates to 0.3 mg/kg ($3 / 10$).

Although both approaches to acute risk assessment to some degree are speculative, under the pre-set and at least plausible conditions the outcome of both approaches for acute toxicity is similar. Formally, it is proposed, to start with the cat methaemoglobin data, because methaemoglobin formation clearly is an adverse effect which ultimately follows acute exposure.

Dermal exposure

The adequate internal starting point of 50 mg/kg is multiplied by the factor of 10 to account for a 10% dermal absorption. This results in an adequate dermal starting point of 500 mg/kg (50×10). With a reference MOS of 115 (see "internal starting point") the corresponding critical exposure level calculates to 4.3 mg/kg ($500 / 115$).

This acute dermal risk assessment is based on oral toxicity data. It should not be contradictory to the available data on acute dermal toxicity. Bis(hydroxylammonium)sulfate has been dermally tested in rabbits under semi-occlusive and occlusive exposure conditions. For occlusive conditions, the dermal dose without relevant MetHb and Heinz body formation was

1 mg/kg, for semi-occlusive conditions the corresponding dose is 500 mg/kg (see chapter 4.1.2.2).

Dermal contact at the workplace is assumed to be open or semi-occlusive. The dermal starting point of 500 mg/kg (NOAEL for semi-occlusive exposure conditions), in combination with an interspecies adjustment factor of (2 x 2.5) for rabbits and an intraspecies factor of 5, is extrapolated to a critical dermal exposure level of 20 mg/kg (500 / 25). The critical dermal exposure level based on the acute oral data is about 4 mg/kg. Because of remaining uncertainties as to the conditions of dermal exposure, the most conservative reference value (4 mg/kg) is taken forward to risk characterisation.

Concern is only expressed for scenario 3 (formulation as an auxiliary in different industries; see table 4.24). This conclusion is considered to be a borderline risk situation.

Conclusion: iii

Inhalation Exposure

Assuming a 100% absorption by inhalation, the inhalation dose is identical to the adequate internal starting point of 50 mg/kg. The inhalation dose of 50 mg/kg is divided by a factor of 0.160 m³/kg (default respiratory volume for the cat for 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 adequate inhalation starting point of about 209 mg/m³ (50 x 1/0.16 x 6.7/10).

With a reference MOS of 50 (factor of 10 for AEL/NAEL adjustment and factor of 5 for intraspecies differences) the corresponding critical inhalation exposure level calculates to 4 mg/m³ (209 / 50). There is no concern for acute inhalation toxicity for the different occupational scenarios.

A risk assessment for peak concentrations (e.g. 15-min) could not be performed because there is neither corresponding toxicological nor exposure-related information.

Conclusion: ii

Combined exposure

Assessment of combined exposure is based on the comparison of total internal body burden and the critical internal exposure level. The internal starting point of 50 mg/kg is divided by the reference MOS of 115 to get the corresponding critical internal exposure level of 0.43 mg/kg (see “internal starting point”).

Conclusion iii is reached for scenario 3. Because of the available route-specific conclusion for dermal exposure, there is no specific concern for combined exposure.

Conclusion: iii

Table 4.24: MOS values for acute toxicity (systemic effects)

		Inhalation			Dermal			Combined		
Starting point for MOS calculation		209 mg/m ³			500 mg/kg			50 mg/kg (internal dose)		
MOS _{ref}		5°			115			115		
Critical exposure level		4 mg/m ³			4.3 mg/kg			0.43 mg/kg (internal dose)		
Scenario number, area of production and use		Exposure (mg/m ³)	MOS	Conclusion	Exposure (mg/kg)	MOS	Conclusion	Internal body burden (mg/kg)	MOS	Conclusion
1. Production and further processing as an intermediate	dust	0.9	232	ii	0.6	833	ii	0.19	263	ii
	liquid (25%)				0.08	6,250	ii	0.14	357	ii
2. Formulation for photo developing chemicals (dust)		0.25	840	ii	0.6 ⁽¹⁾	833	ii	0.10	500	ii
3. Formulation as an auxiliary in different industries (dust)		1.25	167	ii	6	83	iii	0.78	64	iii
4. Use in photographic laboratories (30% liquid)		negl.	-	ii	0.18	2,777	ii	0.018	2,778	ii
5. Use in the electroplating industry (inhalation: aerosol; dermal: 25% liquid)		0.05	4180	ii	0.15	3,333	ii	0.022	2,272	ii

4.1.3.2.2 Irritation and corrosivity

Skin, Eye

Human experience with local irritation/corrosion caused by bis(hydroxylammonium)sulfate is mentioned in the literature, but no relevant data are available.

Based on rather limited rabbit data bis(hydroxylammonium)sulfate is considered to be a skin and eye irritating substance.

On the grounds that control measures exist which can minimise dermal exposure and/or contact to the eyes and corresponding risk of irritation, conclusion ii is proposed. However, these controls must be implemented and complied with to reduce the risk of damage to skin and the eyes (bis(hydroxylammonium)sulfate is a skin sensitiser as well).

Conclusion: ii

Respiratory tract

No studies are available concerning the potential of respiratory tract irritation of bis(hydroxylammonium)sulfate. Although there is a corresponding lack of data, further testing is not considered to be of priority. It is assumed, that risk reduction measures necessary to reduce other health risks of bis(hydroxylammonium)sulfate (repeated dose toxicity, carcinogenicity) will efficiently reduce possible risks of respiratory tract irritation as well.

Conclusion: ii

4.1.3.2.3 Sensitisation

Skin

Animal tests reveal mild to moderate skin-sensitising properties for bis(hydroxylammonium)sulfate. In addition several patch tests (test concentration range from 1%-5%), performed on workers who were exposed to bis(hydroxylammonium)sulfate showed a positive skin reaction. Bis(hydroxylammonium)sulfate is considered to be a skin sensitiser.

Skin contact critically depends on wearing and proper use of suitable gloves. Unintended contamination may occur in several exposure situations. Dose-response data on the skin-sensitising potency of the substance are not described. A threshold for skin sensitisation might exist, but is not experimentally verified. Depending on the dermal exposure levels, there might be a risk of skin sensitisation for which concern is risen.

Conclusion: iii

Respiratory tract

No human data are available concerning sensitisation after inhalation of bis(hydroxylammonium)sulfate. Additional information comes from a study on guinea pigs. After treatment in a Magnusson Kligman Test, 2 groups of the animals inhaled aerosols of bis(hydroxylammonium)sulfate in air-borne concentrations of 6.5 mg/m³ and 13.2 mg/m³ for 30 minutes. No changes in breathing rates were measured. Also after treatment via the

intratracheal route at dose levels of 5, 15, 25 and 75 mg/kg no signs of pulmonary sensitisation and/or sensory irritation was found (Allied Corporation 1984).

Although bis(hydroxylammonium)sulfate is a skin sensitiser, available evidence does not indicate a potential of respiratory tract sensitisation.

Conclusion: ii

4.1.3.2.4 Repeated dose toxicity

Local effects (inhalation, dermal)

See irritation; no further information available.

Systemic effects (inhalation, dermal, combined)

No human data are available concerning the repeated dose toxicity of bis(hydroxylammonium)sulfate, but several animal studies with repeated oral application have been performed. The main toxic effects of bis(hydroxylammonium)sulfate in these studies were the hemolytic anaemia and effects secondary to anaemia. The observed effects are considered to be relevant for man. There are no relevant data for the dermal and inhalatory route of administration.

For the assessment of repeated dose toxicity an oral 24-month carcinogenicity study with rats (BASF, unpublished report 2001) is used. In this drinking water study the dose of 20 ppm was identified as LOAEL, showing an increase of hemosiderin storage in the spleen. No substance-related hematotoxic effects were reported at 5 ppm. From this study a NOAEL for nonneoplastic findings of 5 ppm (0.2 mg/kg/day in males; 0.4 mg/kg/day in females) is derived (the observed carcinogenic effects are discussed in the corresponding part of the risk assessment). The lower value for males (0.2 mg/kg/day) is used for RDT risk assessment.

Internal starting point

The calculation of the internal starting point accounts for a factor of 1 for 100% oral absorption and a factor of 7/5 for adaptation of scenarios from 7 feeding days per week to 5 days per week for occupational assessment. The oral NOAEL of 0.2 mg/kg/day will be transformed to an internal starting point of 0.28 mg/kg/day ($0.2 \times 1 \times 7/5$).

Dermal exposure

The internal starting point of 0.28 mg/kg/day is multiplied by the factor of 10 to account for a 10% dermal absorption. This results in a value of 2.8 mg/kg/day (0.28×10) for the adequate dermal starting point.

For the calculation of the reference MOS an interspecies factor of 4 x 2.5 (rat) and an intraspecies factor of 5 is used. Based on the resulting reference MOS of 50 ($5 \times 4 \times 2.5$) the corresponding critical exposure level calculates to 0.06 mg/kg/day ($2.8 / 50$).

Some dermal MOS values are substantially lower than the reference MOS (see table 4.25). Especially scenario 3 (formulation as an auxiliary in different industries) shows a pronounced concern for repeated dose toxicity. If the dermal exposure level and the RDT assessment is valid and reasonable, especially for scenario 3 spleen enlargement in workers cannot be excluded.

Conclusion: iii

Inhalation exposure

Assuming a 100% absorption by inhalation, the inhalation dose is identical to the adequate internal dose. The inhalation dose of 0.28 mg/kg/day 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 0.5 mg/m³ (0.28 x 1/0.38 x 6.7/10).

For the identification of the reference MOS the intraspecies factor of 5 is multiplied by the interspecies factor of 2.5 for remaining differences which results in a reference MOS of 12.5. The corresponding critical exposure level calculates to 0.04 mg/m³ (0.5 / 12.5). For scenario 4 (use in photographic laboratories) there was a discussion whether, compared to other scenarios, a higher intraspecies factor should be used. However, because a relevant difference in the sensitivity of the respective worker populations could not be ascertained, no corresponding differentiation was introduced.

With the exception of scenario 4 (use in photographic laboratories) the MOS values for the inhalative exposure scenarios are lower than the reference MOS. For inhalation exposure as well, concern seems to be substantial (especially for scenarios 1 and 3).

Conclusion: iii

Combined exposure

The internal starting point of 0.28 mg/kg/day is divided through the reference MOS of 50 (the reference MOS for internal and dermal exposure is identical). The corresponding critical exposure level calculates to 0.006 mg/kg/day (0.28 / 50). Conclusion iii is reached for all exposure scenarios. Because of the available route-specific conclusions, there is no specific concern for combined exposure.

Conclusion: iii

Table 4.25: MOS values for repeated dose toxicity of bis(hydroxylammonium)sulfate, systemic effects

	Inhalation	Dermal	Combined
Starting point for MOS calculation	0.5 mg/m ³	2.8 mg/kg/day	0.28 mg/kg/day (internal dose)
MOS _{ref}	12.5	50	50
Critical exposure level	0.04 mg/m ³	0.06 mg/kg/day	0.006 mg/kg/day (internal dose)

		Inhalation			Dermal			Combined		
Starting point for MOS calculation		0.5 mg/m ³			2.8 mg/kg/day			0.28 mg/kg/day (internal dose)		
MOS _{ref}		12.5			50			50		
Critical exposure level		0.04 mg/m ³			0.06 mg/kg/day			0.006 mg/kg/day (internal dose)		
Scenario number, area of production and use		Exposure (mg/m ³)	MOS	Conclusion	Exposure (mg/kg/d)	MOS	Conclusion	Internal body burden (mg/kg/d)	MOS	Conclusion
1. Production and further processing as an intermediate	dust	0.9	0.6	iii	0.6	4.7	iii	0.19	1.47	iii
	liquid (25%)				0.08	35	iii	0.14	2.0	iii
2. Formulation as photo developing chemicals (dust)		0.25	2	iii	0.6	4.7	iii	0.10	2.8	iii
3. Formulation as an auxiliary in different industries (dust)		1.25	0.4	iii	6	0.47	iii	0.78	0.36	iii
4. Use in photographic laboratories (30% liquid)		negl.	-	ii	0.18	15.6	iii	0.018	15.6	iii
5. Use in the electroplating industry (inhalation: aerosol; dermal: 25% liquid)		0.05	10	iii	0.15	18.7	iii	0.022	12.7	iii

4.1.3.2.5 Mutagenicity

There are no *in vitro* genotoxicity data for bis(hydroxylammonium)sulfate. *In vivo* a bone marrow micronucleus test and a dominant lethal assay were negative.

Hydroxylamine hydrochloride was weakly positive in mouse lymphoma assays and seems to have a genotoxic potential in insects. Clearly negative results were obtained concerning UDS in rat hepatocytes and chromosomal aberrations in rodent bone marrow cells.

Based on available experimental evidence bis(hydroxylammonium)sulfate is not considered to be an *in vivo* mutagen.

Conclusion: ii

4.1.3.2.6 Carcinogenicity

The carcinogenicity of bis(hydroxylammonium)sulfate by the oral route has been investigated in a standard 24-month drinking water study in rats. This study already served as key study for the assessment of repeated dose toxicity (BASF, unpublished report 2001).

The experimental results relevant for the assessment of repeated dose toxicity were already evaluated in chapter 4.1.3.2.4.

In addition to general spleen toxicity, bis(hydroxylammonium)sulfate resulted in neoplastic lesions in the rat spleen. For risk assessment purposes, taking into account the available mutagenicity data, it is assumed that spleen carcinogenicity is a consequence of general spleen toxicity. Correspondingly bis(hydroxylammonium)sulfate might be considered a threshold carcinogen, justifying a MOS approach for risk assessment.

Thus risk assessment for carcinogenicity heavily relies upon the risk assessment for repeated dose toxicity. The relevant results for RDT are summarized in Table 4.25.

The experimental results on general spleen toxicity reflect a dose-response relationship resulting in a NOAEL of 5 ppm and a LOAEL of 20 ppm. In contrast, in terms of quantitative dose-response assessment, tumour incidence data are much more difficult to interpret (see table 4.26). For carcinogenicity there seems to be a rather flat dose-response, resulting in the question of whether there is a biologically relevant increase of tumour incidence at the experimental exposure level of 5 ppm.

Table 4.26: precursor lesions of angiomatous tumors, tumor incidences, and changes of clinical parameters of the spleen from rats treated with bis(hydroxylammonium)sulfate in the drinking water for 24 months

Dose		0 ppm	5 ppm	20 ppm	80 ppm
Hemosiderin storage in the spleen		-	-	↑	↑
Spleen weight (abs/rel)		-	-	-	↑
Angiomatous hyperplasia	(m)	4	9	4	16
	(f)	14	13	12	34
Hemangiomas (f) %		0	2	2	8

Hemangiosarcomas (m) %	8	15	18	16
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↑: statistically significant increase compared with controls

Because of the severity of a tumorigenic response and because a biologically relevant increase of tumour incidence at the experimental exposure level of 5 ppm (which is the NOAEL for chronic toxicity) could not be totally ruled out, it is considered prudent to introduce an additional adjustment factor. Irrespective of the introduction of a further severity factor the formal conclusions on carcinogenicity are identical to those for repeated dose toxicity (see table 4.25). The need for risk reduction is already high at the basis of risk assessment for repeated dose toxicity. The introduction of a severity factor for carcinogenicity requires an even more stringent reduction of exposure levels. In the summary tables for occupational risk assessment (see tables 4.28 and 4.29) a severity factor of 5 is introduced.

For carcinogenicity concern is expressed for exposure by inhalation, for dermal exposure and for combined exposure. Scenario-specific conclusions are identical to the risk assessment for repeated dose toxicity.

Conclusion: iii

4.1.3.2.7 Toxicity for reproduction

With respect to toxicity for reproduction no human data are available. Data from animal experiments with bis(hydroxylammonium)sulfate did not reveal any specific embryo/fetotoxic or teratogenic potential. A NOAEL (oral) for maternal toxicity of 3 mg/kg/day and a NOAEL (oral) for embryo-/fetotoxicity of 20 mg/kg/day was derived. From a repeated dose toxicity study in rats a LOAEL (oral) of about 67 mg/kg/day was determined based on retardation of the development of the mammary gland. From a 3-month oral rat study no indications for an impairment of male and female reproductive organs could be revealed up to the highest tested dose level of about 21 mg/kg/day.

Compared to the relatively low LOAEL for repeated dose toxicity of 0.8 mg/kg/day and against the background of the carcinogenic potential of the substance experimental testing for reproductive toxicity is considered to be sufficiently adequate. Based on the available data, there seems to be no specific risk for reproductive toxicity.

Conclusion: ii

4.1.3.2.8 Summary of risk characterisation for workers

The toxicological profile of bis(hydroxylammonium)sulfate is characterised both by relevant local and systemic activity. Bis(hydroxylammonium)sulfate is an irritating and skin sensitising substance. Systemic toxicity primarily is expressed by toxicity to red blood cells. Acute toxicity may be initially recognized by methemoglobin formation. Chronic toxicity in the spleen is considered to be secondary to toxicity to blood cells. It is assumed that spleen carcinogenicity of bis(hydroxylammonium)sulfate is based on general spleen toxicity and thus may be considered a threshold response.

Table 4.27 indicates the toxicological endpoints of concern for bis(hydroxylammonium)sulfate. There is concern for skin sensitisation, for acute toxicity (only for dermal contact), repeated dose toxicity and for carcinogenicity. For the other toxicological endpoints (irritation, mutagenicity and reproductive toxicity) no concern is expressed.

Table 4.27: Endpoint-specific conclusions

Toxicological endpoints		general conclusion
Acute toxicity	inhalation	ii
	dermal	iii
	combined	iii ⁽¹⁾
Irritation / Corrosivity	dermal	ii
	eye	ii
	acute respiratory tract	ii
Sensitisation	skin	iii
	respiratory tract	ii
Repeated dose toxicity	inhalation, local	ii
	inhalation, systemic	iii
	dermal, local	ii
	dermal, systemic	iii
	combined, systemic	iii ⁽¹⁾
Mutagenicity		ii
Carcinogenicity	inhalation	iii
	dermal	iii
	combined	iii ⁽¹⁾
Reproductive toxicity	inhalation	ii
	dermal	ii
	combined	ii

⁽¹⁾ conclusion iii already results from a single exposure component (inhalation or dermal), therefore it does not seem specific for combined exposure scenarios

Risk assessment is mainly based on oral studies. For dermal absorption (for semi-occlusive exposure conditions) a percentage of 10% is taken forward to risk characterisation. Oral absorption and absorption by inhalation is assumed to be 100%.

Tables 4.28 (inhalation) and 4.29 (dermal contact) visualize the risk profile of bis(hydroxylammonium)sulfate. According to the specific arrangement of exposure scenarios and critical exposure levels for different toxicological endpoints you will find the relatively high risks in the left upper corner, the relatively low risks in the bottom right corner of the tables (for skin sensitisation, there is no quantification of risk).

For acute toxicity there is only concern for dermal exposure for scenario 3 (formulation as an auxiliary in different industries). The risk characterisation ratio (quotient of exposure and critical exposure level) for this dermal scenario is close to 1 (thus the concern for acute dermal toxicity is only weak).

Repeated exposure might result in chronic toxicity and tumour formation. Based on the data available and the adjustment factors chosen, corresponding health risks in general seem to be rather high. Especially for the dermal scenario 3, the calculated risk characterisation ratio for repeated dose toxicity of 100 (6 / 0.06) is not considered tolerable. Assuming the validity and relevance of this risk assessment, for specific occupational scenarios immediate risk reduction measures are necessary.

Table 4.28: Ranking of occupational risks (inhalation)

Occupational Scenario	Exposure level in mg/m ³	Carcinogenicity	Repeated dose toxicity	Acute toxicity (MetHb)
		Critical inhalation exposure level in mg/m ³		
		0.008	0.04	4
3. Formulation as an auxiliary in different industries	1.25	iii	iii	ii
1. Production and further processing as an intermediate	0.9	iii	iii	ii
2. Formulation as photo developing chemicals	0.25	iii	iii	ii
5. Use in the electroplating industry	0.05	iii	iii	ii
4. Use in photographic laboratories	negligible	ii	ii	ii

Table 4.29: Ranking of occupational risks (dermal)

Occupational Scenario	Exposure level in mg/kg/d	Carcinogenicity	Repeated dose toxicity	Acute toxicity (MetHb)	Sensitisation
		Critical dermal exposure level in mg/kg/day			
		0.012	0.06	4.3	n.d. ⁽¹⁾
3. Formulation as an auxiliary in different industries	6	iii	iii	iii	iii
1a. Production and further processing as an intermediate (dust)	0.6	iii	iii	ii	iii
2. Formulation for photo developing chemicals	0.6	iii	iii	ii	iii

4. Use in photographic laboratories	0.18	iii	iii	ii	iii
5. Use in the electroplating industry (25% liquid)	0.15	iii	iii	ii	iii
1b. Production and further processing as an intermediate (25% aqueous solution)	0.08	iii	iii	ii	iii

⁽¹⁾for skin sensitisation a critical exposure level can not be detected

4.1.3.3 Consumers

Consumer exposure by 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

Nearly all bis(hydroxylammonium)sulphate released to the environment is expected to end up in the water phase where it is transformed rapidly. Its volatility is very low. No relevant releases into the soil compartment are expected from production or use of the substance and adsorption to organic matter is negligible. Bis(hydroxylammonium) sulphate does not exhibit any potential for bioaccumulation. Therefore the risk is considered to be negligible and no assessment of indirect exposure via the environment has been undertaken.

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.

4.1.3.5 Combined 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

4.2.2.2 Flammability

Bis(hydroxylammonium)sulphate is not flammable.

4.2.2.3 Oxidizing potential

Due to its chemical structure, bis(hydroxylammonium)sulphate is not expected to present any oxidising properties.

4.2.3 Risk characterisation

4.2.3.1 Workers

The physico-chemical properties of bis(hydroxylammonium)sulphate are well known. General warnings to this effect are recommended, and are currently in practice. If the appropriate handling and storage measures are applied, there are no concerns for risks to human health arising from the physicochemical properties and thus conclusion (ii) is reached.

4.2.3.2 Consumers

4.2.3.3 Humans exposed via the environment

5 RESULTS ⁸

5.1 INTRODUCTION

5.2 ENVIRONMENT

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.

For bis(hydroxylammonium)sulfate concern is expressed for skin sensitisation, for acute toxicity, repeated dose toxicity and for carcinogenicity. Especially with respect to systemic effects (general toxicity to blood and spleen and thresholded carcinogenicity) the available risk assessment indicates substantial chronic health risks especially for dermal exposure of workers in scenario 3 (formulation as an auxiliary in different industries). Based on this risk analysis, immediate risk reduction measures are considered necessary.

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.

Nearly all bis(hydroxylammonium)sulphate released to the environment is expected to end up in the water phase where it is transformed rapidly. Its volatility is very low. No relevant releases into the soil compartment are expected from production or use of the substance and adsorption to organic matter is negligible. Bis(hydroxylammonium) sulphate does not exhibit

⁸ 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.

any potential for bioaccumulation. Therefore the risk is considered to be negligible and no assessment of indirect exposure via the environment has been undertaken.

5.3.1.4 Combined exposure

5.3.2 Human health (risks from physico-chemical properties)

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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ABBREVIATIONS

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 / <i>Bw</i> , <i>b.w.</i>
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 ₅₀	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / <i>dw</i>
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	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
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient

Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidizing (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based Pharmacokinetic modelling
PBTK	Physiologically Based Toxicokinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration {H ⁺ })

pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst Case
S phrases	Safety phrases according to Annex III of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex III of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
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)

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**EUR [ECB: click here to insert EUR No.] - European Union Risk Assessment Report
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Editors: (keep this updated)

Luxembourg: Office for Official Publications of the European Communities

[ECB: insert year] – VIII pp., [ECB: insert number of pages] pp. – 17.0 x 24.0 cm

Environment and quality of life series

ISBN [ECB: insert ISBN No.]

Price (excluding VAT) in Luxembourg: EUR [ECB:insert price]

The report provides the comprehensive risk assessment of the substance Bis (hydroxylammonium) sulphate. 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 and consumer exposure have been examined and the possible risks have been identified.

There is no concern for the aquatic and terrestrial compartment and atmosphere in relation to the releases from production and processing at the production sites. In addition there is no concern for the atmosphere and terrestrial compartment from the releases due to the use of the substance. Further information is needed regarding the releases into the aquatic compartment from all uses of the substance before definitive conclusions regarding environmental risks can be drawn. Since it is no longer possible to submit this information under the Existing Substances Regulation it is proposed to consider the substance further under the REACH regulation.

For human health, there is concern for workers, with respect to skin sensitisation, acute toxicity, chronic health risks (general toxicity to blood and spleen), and carcinogenicity, but no concern is expressed for consumers and for humans exposed via the environment.