CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

International Chemical Identification:

mecetronium etilsulfate; N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulfate;

mecetronium ethyl sulphate [MES]

EC Number: 221-106-5

CAS Number: 3006-10-8

Index Number: -

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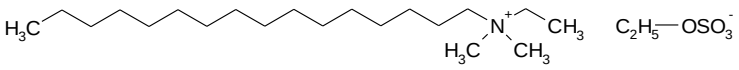
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance.

Name(s) in the IUPAC nomenclature or other international chemical name(s)	N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulfate (IUPAC)
Other names (usual name, trade name, abbreviation)	Mecetronium ethyl sulphate Mecetronium ethylsulfate, Mecetronium etilsulfat, MES, Ethylhexadecyldimethylammonium ethylsulfate, Cetylethyldimethylammonium ethosulfat Dimethylethylhexadecylammonium-ethylsulfate Ethanesulfonate ethyl-hexadecyl-dimethyl-ammonium
ISO common name (if available and appropriate)	-
EC number (if available and appropriate)	221-106-5
EC name (if available and appropriate)	mecetronium etilsulfate
CAS number (if available)	3006-10-8
Other identity code (if available)	-
Molecular formula	$C_{20}H_{44}N^+ \bullet C_2H_5O_4S^-$
Structural formula	
SMILES notation (if available)	CCCCCCCCCCCCCCCC[N+](C)(C)CC.CCOS(=O)(=O)[O-]
Molecular weight or molecular weight range	423.70
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	Not applicable
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable
Degree of purity (%) (if relevant for the entry in Annex VI)	> 95% w/w (manufactured as solution 29% in water)

1.2 Composition of the substance

Table 2: Constituents (non-confidential information).

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self- classification and labelling (CLP)**
N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulfate (IUPAC) Mecetronium ethyl sulphate [MES] EC number: 221-106-5	>95% w/w	Not included in Anex VI Table 3.1	Notified classification and labelling according to CLP criteria http://www.echa.europa.eu/pl/web/guest/information-on-chemicals/cl-inventory-database/-/discli/details/23964 Number of notifiers: 2 Skin Irrit. 2, H315 Eye Irrit. 2, H319 Number of notifiers: 1 Acute Tox. 4, H302 Skin Irrit. 2, H315 Eye Dam. 1, H318 Acute Tox. 4, H332 STOT SE 3, H335 Aquatic Acute 1, H400 Aquatic Chronic 1, H410 Number of notifiers: 1 Skin Irrit. 2, H315 Eye Dam. 1, H318 Aquatic Chronic 1, H410 Number of notifiers: 1 Acute Tox. 4, H302 Skin Corr. 1B, H314 Aquatic Acute 1, H400 Aquatic Chronic 1, H410

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance.

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
For futher information please refer to the IUCLID file (confidential information)				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance.

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
None					

Table 5: Test substances (non-confidential information) (this table is optional).

Identification of test substance	**Purity**	**Impurities and additives (identity, %, classification if available)**	**Other information**	**The study(ies) in which the test substance is used**
Mecetronium ethyl sulphate [MES]	> 95% w/w			
Mecetronium ethyl sulphate [MES] Clear liquid; 30% active component				
Mecetronium ethyl sulphate [MES] Clear liquid; 4% active component				

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 6: Proposed harmonised classification and labelling.

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	Not included in Annex VI										
Dossier submitters proposal	612-RST-VW-Y	mecetronium etilsulfate; N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulfate; mecetronium ethyl sulphate [MES]	221-106-5	3006-10-8	Acute Tox. 4 Acute Tox. 3 Skin Corr. 1C Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H311 H314 H318 H400 H410	GHS05 GHS06 GHS09 Dgr	H302 H311 H314* H410*		M=100 (Acute) M=10 (Chronic)	
Resulting Annex VI entry if agreed by RAC and COM	612-RST-VW-Y	mecetronium etilsulfate; N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulfate; mecetronium ethyl sulphate [MES]	221-106-5	3006-10-8	Acute Tox. 4 Acute Tox. 3 Skin Corr. 1C Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H311 H314 H318 H400 H410	GHS05 GHS06 GHS09 Dgr	H302 H311 H314* H410*		M=100 (Acute) M=10 (Chronic)	

*Article 27 of CLP states that if a substance or mixture is classified within several hazard classes or differentiations of a hazard class, all hazard statements

resulting from the classification shall appear on the label, unless there is evident duplication or redundancy.

In accordance with Article 27 the following principles of precedence for hazard statements may apply to labelling:

- if the hazard statement H314 ‘Causes severe skin burns and eye damage” is assigned, the statement H318 ‘Causes serious eye damage’ may be omitted,
- if the hazard statement H410 ‘Very toxic to aquatic life with long lasting effects’ is assigned, the statement H400 ‘Very toxic to aquatic life’ may be omitted.

Table 7: Reason for not proposing harmonised classification and status under public consultation.

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	data conclusive but not sufficient for classification	Yes
Flammable gases (including chemically unstable gases)	hazard class not applicable	No
Oxidising gases	hazard class not applicable	No
Gases under pressure	hazard class not applicable	No
Flammable liquids	hazard class not applicable	No
Flammable solids	data conclusive but not sufficient for classification	Yes
Self-reactive substances	data conclusive but not sufficient for classification	Yes
Pyrophoric liquids	hazard class not applicable	No
Pyrophoric solids	data conclusive but not sufficient for classification	Yes
Self-heating substances	data conclusive but not sufficient for classification	Yes
Substances which in contact with water emit flammable gases	data conclusive but not sufficient for classification	Yes
Oxidising liquids	hazard class not applicable	No
Oxidising solids	data conclusive but not sufficient for classification	Yes
Organic peroxides	hazard class not applicable	No
Corrosive to metals	data lacking	No
Acute toxicity via oral route	harmonised classification proposed	Yes
Acute toxicity via dermal route	harmonised classification proposed	Yes
Acute toxicity via inhalation route	data lacking	No
Skin corrosion/irritation	harmonised classification proposed	Yes
Serious eye damage/eye irritation	harmonised classification proposed	Yes
Respiratory sensitisation	data lacking	No
Skin sensitisation	data conclusive but not sufficient for classification	Yes
Germ cell mutagenicity	data conclusive but not sufficient for classification	Yes
Carcinogenicity	data lacking	No
Reproductive toxicity	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-single exposure	data conclusive but not sufficient for classification	Yes
Specific target organ toxicity-repeated exposure	data conclusive but not sufficient for classification	Yes
Aspiration hazard	hazard class not applicable	No
Hazardous to the aquatic environment	harmonised classification proposed	Yes
Hazardous to the ozone layer	data lackingclassification	No

3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

A harmonised classification and labelling for Mecetronium ethyl sulphate [MES] is not available and the substance is not listed in Annex VI of the Regulation (EC) No 1272/2008.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Mecetronium ethyl sulphate [MES] is a biocidal active substance according to Regulation (EU) No 528/2012. In accordance with Article 36(2) of EC Regulation 1272/2008 (CLP) on classification, labelling and packaging of substances and mixtures such substances shall be subject to harmonised classification and labelling.

There is no requirement for justification that action is needed at Community level.

5 IDENTIFIED USES

Biocidal active substance.

Main Group 1: Disinfectants

These product-types exclude cleaning products that are not intended to have a biocidal effect, including washing liquids, powders and similar products.

Product-type 1: Human hygiene

Products in this group are biocidal products used for human hygiene purposes, applied on or in contact with human skin or scalps for the primary purpose of disinfecting the skin or scalp.

The substance is used as a biocidal active substance inside the EU in commercial product. A commercial product (a mixture containing mecetronium ethyl sulphate) is a ready for use product for hygienic and surgical hand disinfection. Additionally it is used as skin antiseptic (medical product). A commercial product (a mixture containing mecetronium ethyl sulphate) is applied in all areas where hygiene is important, e.g. operating theatres, intensive care units, infection departments, sanitation areas, in laboratories, in medical practices, in the home-care of patients, in home dialysis and in pharmaceutical, cosmetic, and food processing industry.

6 DATA SOURCES

A classification and labelling proposal is based mainly on the information presented in the Competent Authority Report (CAR) for mecetronium ethyl sulphate [MES].

7 PHYSICOCHEMICAL PROPERTIES

Table 8: Summary of physicochemical properties.

Property	Value	Reference	Comment (e.g. measured or estimated)
Physical state at 20°C and 101,3 kPa	solid		visual
Melting/freezing point	**result:** 87.6 – 111.0°C (360.7 – 384.2 K) **pressure:** normal atmospheric pressure	BODE Chemie (2002b)	OECD 102 EC (No) 440/2008 A.1. The test substance has a melting range; GLP
Boiling point	**result:** Boiling point could not be measured due to decomposition at 271°C – 286°C **pressure:** normal atmospheric pressure	BODE Chemie (2002c)	OECD 103 EC (No) 440/2008 A.2. Siwoloboff method, preliminary test; GLP
Relative density	Density = 1.08 g/cm^3 at 20°C D_4^{20} = 1.08	BODE Chemie (2002d)	OECD 109 EC (No) 440/2008 A.3. Pycnometer method; GLP
Vapour pressure	**temperature:** 25°C **result:** 1 • 10^{-8} hPa	-	Estimated value Lowest value accepted by EUSES; Due to decomposition of Mecetronium ethyl sulfate [MES] at temperatures above 271°C it is not possible to determine the boiling point of MES. This may be explained by its ionic structure which prevents volatilization. MES, as a ionic substance, can therefore safely be assumed to possess a very low volatility. In-line with the TNsG on Data Requirements (EC, 2000) a recognized estimation method is acceptable if the boiling point is between 200 and 300°C. Therefore, it is reasonable to use the lowest value for vapour pressure accepted by EUSES which is 1 • 10^{-8} hPa.
Surface tension	**result:** 38.5 mN/m **temperature:** 20°C	BODE Chemie (2001a)	OECD 115 EC (No) 440/2008 A.5. ring method; test substance conc.: 1.0 g/L; GLP
Water solubility	**result:** > 500 g /1000 g water **temperature:** no data **pH:** no data	BODE Chemie (2002)	OECD 105 flask method, preliminary test; main test not possible due to

Property	Value	Reference	Comment (e.g. measured or estimated)
			complete solubility; GLP
Partition coefficient n-octanol/water	**result:** -0.39	BODE Chemie (2002) BODE Chemie (2010c)	Estimation using solubilities in water and n-octanol, the log Pow value was calculated; The study on solubility in n-octanol was performed according to CIPAC MT 181 and the solubility was found to be 168-202 g/L. The study on solubility in water was performed according to OECD 105 with a determined solubility of 500-1000 g/L. As a worst case the highest solubility value of n-octanol and the lowest value of water are used for further calculation.
Flash point	-	-	Not applicable (Study of the flash point is not required for solids)
Flammability	not highly flammable	BODE Chemie (2010a)	EC (No) 440/2008 A.10; GLP
Explosive properties	No explosive properties	BODE Chemie (2010b)	EC (No) 440/2008 A.14; GLP
Self-ignition temperature	No data		
Oxidising properties	From the structural formula and the composition of the substance it can be concluded that the substance does not evolve any oxidizing properties. Additionally the substance is produced, handled and marketed as aqueous solution, which prevents oxidizing properties.		estimated
Granulometry	No data		
Stability in organic solvents and identity of relevant degradation products	**result:** a.s. was stable in b.p. over 60 months at 25°C and over 12 months at 40°C	BODE Chemie (1999a)	Biocidal product (a mixture containing MES), 4 different batches were tested.
Dissociation constant	pKa = 6.5	BODE Chemie (2007)	OECD 112 titration method; GLP

Property	Value	Reference	Comment (e.g. measured or estimated)
			30.1% MES solution in water
Viscosity	**result**: 1390.5 mPa/s temperature: 20°C content: 30.4% (m/m)	BODE Chemie (2007)	OECD 114 "rolling ball viscometer" (according to Höppler), described in DIN 53015

8 EVALUATION OF PHYSICAL HAZARDS

8.1 Explosives

Table 9: Summary table of studies on explosive properties.

Method	Results	Remarks	Reference
EU test method A.14 according to Regulation 440/2008/EC; GLP	The heat decomposition was below 500 J/g. Therefore the test on explosive properties was not necessary.	The test item has no explosive properties in the sense of the European Commission Regulation No. 440/2008,	BODE Chemie (2010b)

8.1.1 Short summary and overall relevance of the information provided on explosive properties

As a screening method for the determination of explosive properties a differential scanning calorimetry (DSC) under nitrogen was performed.

If the decomposition energy is below 500 J/g a main test for explosive properties is unnecessary - Recommendations on the Transport of Dangerous Goods/Manual of Tests and Criteria (ST/SG/AC.10/11/Rev.3 – page 398).

The DSC-measurement in a closed glass crucible with the item showed multiple endothermal effects in the temperature range of 25 - 150°C and two exotermal effects in the temperature range of 330 - 420°C and 435 - 460°C with an energy of 84 J/g and 167 J/g respectively (exothermal overall energy: 251 J/g). The heat decomposition was below 500 J/g. Therefore the test on explosive properties was not necessary.

The test item has no explosive properties in the sense of the European Commission Regulation (EC) No. 440/2008, Method A.14.

8.1.2 Comparison with the CLP criteria

According to CLP regulation a substance or mixture shall not be classified as explosive if:

a) There are no chemical groups associated with explosive properties present in the molecule. Examples of groups which may indicate explosive properties are given in Table A6.1 in Appendix 6

of the UN Recommendations on the Transport of Dangerous Goods, Manual of Tests and Criteria; or

b) The substance contains chemical groups associated with explosive properties which include oxygen and the calculated oxygen balance is less than - 200;

The oxygen balance is calculated for the chemical reaction:

$C_xH_yO_z + [x+ (y/4)-(z/2)] O_2 \rightarrow x CO_2 + (y/2) H_2O$

Using the formula:

Oxygen balance = -1 600 [2x + (y/2)-z]/molecular weight;

c) When the organic substance or a homogenous mixture of organic substances contains chemical groups associated with explosive properties but the exothermic decomposition energy is less than 500 J/g and the onset of exothermic decomposition is below 500ºC. The exothermic decomposition energy can be determined using a suitable calorimetric technique; or

d) For mixtures of inorganic oxidising substances with organic material(s), the concentration of the inorganic oxidising substance is:

- less than 15 % by mass, if the oxidising substance is assigned to Categories 1 or 2;
- less than 30 % by mass.

The heat of decomposition of MES obtained during DSC-measurement was below 500 J/g.

8.1.3 Conclusion on classification and labelling for explosive properties

Mecetronium ethyl sulphate [MES] is proposed not to be classified as explosive according to CLP regulation.

8.2 Flammable gases (including chemically unstable gases)

Table 10: Summary table of studies on flammable gases (including chemically unstable gases)

Method	Results	Remarks	Reference
Hazard class not applicable.			

8.2.1 Short summary and overall relevance of the provided information on flammable gases (including chemically unstable gases)

Hazard class not applicable.

8.2.2 Comparison with the CLP criteria

Hazard class not applicable.

8.2.3 Conclusion on classification and labelling for flammable gases

Hazard class not applicable.

8.3 Oxidising gases

Table 11: Summary table of studies on oxidising gases.

Method	Results	Remarks	Reference
Hazard class not applicable.			

8.3.1 Short summary and overall relevance of the provided information on oxidising gases

Hazard class not applicable.

8.3.2 Comparison with the CLP criteria

Hazard class not applicable.

8.3.3 Conclusion on classification and labelling for oxidising gases

Hazard class not applicable.

8.4 Gases under pressure

Table 12: Summary table of studies on gases under pressure.

Method	Results	Remarks	Reference
Hazard class not applicable.			

8.4.1 Short summary and overall relevance of the provided information on gases under pressure

Hazard class not applicable.

8.4.2 Comparison with the CLP criteria

Hazard class not applicable.

8.4.3 Conclusion on classification and labelling for gases under pressure

Hazard class not applicable.

8.5 Flammable liquids

Table 13: Summary table of studies on flammable liquids.

Method	Results	Remarks	Reference
Hazard class not applicable.			

8.5.1 Short summary and overall relevance of the provided information on flammable liquids

The substance is produced, handled and marketed as aqueous solution. Since purified mecetronium ethyl sulphate [MES] is not considered as highly flammable solid it could be concluded that aqueous solution of mecetronium ethyl sulphate [MES] do not poses any flammable properties.

8.5.2 Comparison with the CLP criteria

Hazard class not applicable.

8.5.3 Conclusion on classification and labelling for flammable liquids

Hazard class not applicable.

8.6 Flammable solids

Table 14: Summary table of studies on flammable solids.

Method	Results	Remarks	Reference
test method A.10 according to Regulation EC (No) 440/2008/EC; GLP	No explosive properties according to Regulation 440/2008/EC.		BODE Chemie (2010a)

8.6.1 Short summary and overall relevance of the provided information on flammable solids

In order to assess if the mecetronium ethyl sulphate [MES] should be classified as flammable solid or not the A.10 (Flammability (solids)) test was performed.

In the preliminary screening test the substance is formed into an unbroken strip or powder train about 250 mm long by 20 mm wide by 10 mm high on a non-combustible, non-porous and low heat-conducting base plate. A hot flame from a gas burner (minimum diameter 5 mm) is applied to one end of the powder train until the powder ignites or for a maximum of two minutes. It should be noted whether combustion propagates along 200 mm of the train within the 4 minutes test period. If the substance does not ignite and propagate combustion either by burning with flame or smouldering along 200 mm of the powder train within the four minutes test period, then the substance should not be considered as highly flammable and no further testing is required. If the substance propagates burning of a 200 mm length of the powder train in less than four minutes the main test should be carried out.

In preliminary test mecetronium ethyl sulphate [MES] could not be ignited with a flame. Mecetronium ethyl sulphate [MES] melted at contact with the flame. A main test was therefore not necessary.

8.6.2 Comparison with the CLP criteria

Based on the results of preliminary screening test – mecetronium ethyl sulphate [MES] could not be ignited with a flame – mecetronium ethyl sulphate [MES] should not be considered as highly flammable.

8.6.3 Conclusion on classification and labelling for flammable solids

Classification and labelling is not required.

8.7 Self-reactive substances

Table 15: Summary table of studies on self-reactivity.

Method	Results	Remarks	Reference
No data.			

8.7.1 Short summary and overall relevance of the provided information on self-reactive substances

There are no studies to assess if mecetronium ethyl sulphate [MES] should be classified as self-reactive substance.

8.7.2 Comparison with the CLP criteria

According to CLP, substances and mixtures must be considered for classification in this hazard class as a self-reactive substance or mixture unless:

(a) they are explosives, according to the criteria given in 2.1;

(b) they are oxidising liquids or solids, according to the criteria given in 2.13 or 2.14, except that mixtures of oxidising substances, which contain 5% or more of combustible organic substances shall be classified as self-reactive substances according to the procedure defined in 2.8.2.2;

(c) they are organic peroxides, according to the criteria given in 2.15;

(d) their heat of decomposition is less than 300 J/g; or

(e) their self-accelerating decomposition temperature (SADT) is greater than 75°C for a 50 kg package (See UN RTDG, Manual of Test and Criteria, sub-sections 28.1, 28.2, 28.3 and Table 28.3.)

The DSC-measurement in a closed glass crucible with MES showed multiple endothermal effects in the temperature range of 25 - 150°C and two exotermal effects in the temperature range of 330 - 420°C and 435 - 460°C with an energy of 84 J/g and 167 J/g respectively (exothermal overall energy: 251 J/g). The heat decomposition was below 300 J/g.

8.7.3 Conclusion on classification and labelling for self-reactive substances

Classification and labelling is not proposed.

8.8 Pyrophoric liquids

Table 16: Summary table of studies on pyrophoric liquids.

Method	Results	Remarks	Reference
Hazard class not applicable.			

8.8.1 Short summary and overall relevance of the provided information on pyrophoric liquids

Hazard class not applicable.

8.8.2 Comparison with the CLP criteria

Hazard class not applicable.

8.8.3 Conclusion on classification and labelling for pyrophoric liquids

Hazard class not applicable.

8.9 Pyrophoric solids

Table 17: Summary table of studies on pyrophoric solids.

Method	Results	Remarks	Reference
No data.			

8.9.1 Short summary and overall relevance of the provided information on pyrophoric solids

There are no studies performed in order to asses if mecetronium ethyl sulphate [MES] should be classified as pyrophoric solid.

8.9.2 Comparison with the CLP criteria

According to CLP Regulation the classification procedure for pyrophoric solids need not be applied when experience in manufacture or handling shows that the substance or mixture does not ignite spontaneously on coming into contact with air at normal temperatures (i.e. the substance is known to be stable at room temperature for prolonged periods of time (days)).

Mecetronium ethyl sulphate [MES] is produced as 29% solution in water.

Additionally the chemical stability of mecetronium ethyl sulphate [MES] powder was investigated by 1H-NMR after storage at 65°C for 24 hours. Mecetronium ethyl sulphate [MES] was found to be stable and the NMR spectrum was consistent with the proposed structure.

8.9.3 Conclusion on classification and labelling for pyrophoric solids

Classification and labelling is not required.

8.10 Self-heating substances

Table 18: Summary table of studies on self-heating substances

Method	Results	Remarks	Reference
No data			

8.10.1 Short summary and overall relevance of the provided information on self-heating substances

There are no studies to assess if mecetronium ethyl sulphate [MES] should be classified as self-heating substance.

8.10.2 Comparison with the CLP criteria

According to Guidance on the application of the CLP criteria (Version 4.1, June 2011):

a) in general, the phenomenon of self-heating applies only to solids. The surface of liquids is not large enough for reaction with air and the test method is not applicable to liquids. Therefore liquids are not classified as self-heating. However, if liquids are adsorbed on a large surface (e.g. on powder particles), a self-heating hazard should be considered.

Mecetronium ethyl sulphate [MES] is produced as 29% solution in water.

b) Substances or mixtures with a low melting point (< 160°C) should not be considered for classification in this class since the melting process is endothermic and the substance-air surface is drastically reduced. However, this criterion is only applicable if the substance or mixture is completely molten up to this temperature.

In A.1 test (Melting/freezing temperature) mecetronium ethyl sulphate [MES] has a melting range from 87.6°C to 111°C.

8.10.3 Conclusion on classification and labelling for self-heating substances

Classification and labelling is not peoposed.

8.11 Substances which in contact with water emit flammable gases

Table 19: Summary table of studies on substances which in contact with water emit flammable gases.

Method	Results	Remarks	Reference
No data.			

8.11.1 Short summary and overall relevance of the provided information on substances which in contact with water emit flammable gases

There are no studies on mecetronium ethyl sulphate [MES] which can be used to assess if substance in contact with water emit flammable gases.

8.11.2 Comparison with the CLP criteria

According to CLP regulation the classification procedure for this class need not be applied if:

(a) the chemical structure of the substance or mixture does not contain metals or metalloids; or

(b) experience in production or handling shows that the substance or mixture does not react with water, e.g. the substance is manufactured with water or washed with water; or
(c) the substance or mixture is known to be soluble in water to form a stable mixture.

Based on the chemical structure of mecetronium ethyl sulphate [MES] and also experience in production and handling it can be concluded that mecetronium ethyl sulphate [MES] in contact with water not emit flammable gases.

8.11.3 Conclusion on classification and labelling for substances which in contact with water emit flammable gases

Classification and labelling is not required.

8.12 Oxidising liquids

Table 20: Summary table of studies on oxidising liquids.

Method	Results	Remarks	Reference
Hazard class not applicable.			

8.12.1 Short summary and overall relevance of the provided information on oxidising liquids

Hazard class not applicable.

8.12.2 Comparison with the CLP criteria

Hazard class not applicable.

8.12.3 Conclusion on classification and labelling for oxidising liquids

Hazard class not applicable.

8.13 Oxidising solids

Table 21: Summary table of studies on oxidising solids.

Method	Results	Remarks	Reference
No data.			

8.13.1 Short summary and overall relevance of the provided information on oxidising solids

From the structural formula and the composition of the substance it can be concluded that the substance does not evolve any oxidizing properties.
Additionally the substance is produced, handled and marketed as aqueous solution, which prevents oxidizing properties.

8.13.2 Comparison with the CLP criteria

The classification procedure needs not to be applied due to information obtained from structural formula and the composition of the substance.

8.13.3 Conclusion on classification and labelling for oxidising solids

Classification and labelling is not required.

8.14 Organic peroxides

Table 22: Summary table of studies on organic peroxides.

Method	Results	Remarks	Reference
Hazard class not applicable.			

8.14.1 Short summary and overall relevance of the provided information on organic peroxides

Hazard class not applicable.

8.14.2 Comparison with the CLP criteria

Hazard class not applicable.

8.14.3 Conclusion on classification and labelling for organic peroxides

Classification and labelling is not proposed.

8.15 Corrosive to metals

Table 23: Summary table of studies on the hazard class corrosive to metals.

Method	Results	Remarks	Reference
No data.			

8.15.1 Short summary and overall relevance of the provided information on the hazard class corrosive to metals

There are no studies on the hazard class corrosive to metals for mecetronium ethyl sulphate [MES].

8.15.2 Comparison with the CLP criteria

No data to to compare with CLP criteria.

8.15.3 Conclusion on classification and labelling for corrosive to metals

Classification and labelling is not proposed.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 24: Summary table of toxicokinetic studies.

Method	Results	Remarks	Reference
Comparable to OECD guideline 417 GLP: no Study according to "Hazleton Standard Operating Procedures" and regular audit/inspections by an independent quality assurance unit. Three male and 3 female rats received a dermal application of 1 mg/rat 14C-labelled test substance (exposure period: 6 h, test formulations, 24 h, control formulation, under occlusive conditions). Urine, faeces and expired air were collected in intervals over 72 h and radioactivity measured. After 72 h the radioactivity was determined in selected tissues. The percutaneous absorption of 1 mg radiolabelled MES from a control formulation (DMSO) and 2 test formulations (solution T and solution M, both mixtures containing 0.2% MES) has been studied. Batch 1 was used for the control, batch 2 was used for the T and M formulations (mixtures containing 0.2% MES). Test substance labelled with carbon 14 in the N-methyl moieties, specific radioactivity 59.7 µCi/mg (analytical; nominal 49 µCi/mg) for batch 1 (control formulation) and 53.64 µCi/mg (analytical; nominal 53 µCi/mg) for batch 2 (test formulations T and M, mixtures containing 0.2 MES).	After dermal exposure to 1 mg 14C-labelled Mecetronium ethyl sulphate [MES] in DMSO for 24 h less than 3% of the radioactivity were recovered in urine, faeces, cage washings during the sampling period of 72 h and in tissues at termination (carcass 0.7% and all other tissues 0.2%) indicating a minimal absorption of the radiolabelled test substance. Furthermore, small but widely distributed radioactive tissue residues at 72 h post-dose were reported. Most of the radioactivity was recovered in the occlusive dressings or bound to the site of application. Similar results (max. 4.5% percutaneous absorption) were obtained in parallel percutaneous absorption experiments with 14C-labelled Mecetronium ethyl sulphate in two test formulations (mixtures containing 0.2 MES) after an exposure period of 6 h. Relatively high concentrations (1.9-3.5%) were found in the remaining carcass, 0.2-0.7% in all other removed organs. Most of the radioactivity was recovered in the occlusive dressings or bound to the site of application.		BODE Chemie (1987)

Basic toxicokinetics - a read across to several other quaternary ammonium compounds is possible.

Information given for quaternary ammonium compounds in the literature can be summarized as follows:
Quaternary ammonium compounds are poorly absorbed from the gastrointestinal tract; and therefore relatively large amounts are eliminated in feces (PIM G022). In general, excretion is via feces and urine; an influence of the molecular weight on the excretion is indicated by one study (Hughes et al. (1973)) showing that cations within a molecular range of 94 to 164 g/mol are excreted mainly via urine and cations from 173 – 302 g/mol are excreted via feces as well. For those compounds that are absorbed to a certain extent a rapid distribution in the body is shown (Neef et al. (1984)), however elimination is a fast running process showing half lives of only few minutes. A negligible amount for i.e. Didecyldimethylammonium chloride is retained in the body after one week (Hendersen (1992)).
Several quaternary ammonium compounds are excreted mainly unchanged, others are metabolised to a certain extent. Hughes et al. (1973) detected in their set of quaternary ammonium compounds 7 substances which were excreted largely unchanged in urine or bile (Triethylethanaminium, 1-methylpyridinium, N,N,N-trimethylanilinium, N,N-

Method	Results	Remarks	Reference
diethyl-N-methylanilinium, N-methyl-N,N-dipropylanilinium, N,N-dimethyl-N-phenylanilinium). Three other compounds (3-hydroxyphenyl trimethylammonium, 3-bromo-N-methyl pyridinium and cetyltrimethylammonium) were metabolised. In another set of quaternary ammonium compounds Neef et al (1984) found out that all biliary metabolites still contain a quaternary ammonium group and that no evidence for a reaction with glucuronic acid, sulphate or glutathione is given. In general, ring oxidation and/or hydroxylation are the metabolism steps which seem to be the most probable; oxidation of the decyl side chain is likely. Hendersen (1992) postulated for Didecyldimethylammonium chloride that initial hydroxylation is followed by formation of hydroxyketone; the metabolism involving oxidation and conjugation reactions is analogous to the degradation of fatty acids by ß-oxidation. Systemic effects from percutaneous absorption through intact skin are rare (PIM G022). Didecyldimethylammonium chloride does not undergo degradation on the skin (Hendersen (1992)).			

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Minimal percutaneous absorption of mecetronium ethyl sulphate [MES] in DMSO (control formulation) has been observed. During a 72 h sampling period about 2% were excreted via faeces and urine and only 0.9% were detected in the body at termination indicating an absorption of about 3%. In two test formulations (mixtures containing 0.2 MES) a maximum percutaneous absorption of 4.5% was observed. About 1% were excreted in faeces and urine and about 3.5% were detected in the body, primarily in the remaining carcass. In order to explain these results one additional rat was treated with radiolabelled mecetronium ethyl sulphate [MES] in formulation T, and MES was found not only at the initial application site, but also recovered from concentric areas of skin surrounding the initial application site, analysed with the residual carcass and thus leading to an overestimation of the dermally absorbed dose. A comparison of the 3 formulations without residual carcass indicated that the dermal absorption of mecetronium ethyl sulphate [MES] from the formulations T and M (mixtures containing 0.2 MES) were similar to that observed with the control vehicle DMSO which was designed to give the maximal absorption.

10 EVALUATION OF HEALTH HAZARDS

Acute toxicity

10.1 Acute toxicity - oral route

Table 25: Summary table of animal studies on acute oral toxicity.

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
OECD 401 Limit test Oral; Gavage GLP	Rat Wistars Males and females Number of animals per group: 5 males	Mecetronium ethyl sulphate [MES] Clear liquid; 30% active component Purity of the active substance:	2000 mg/kg bw Post exposure period: 14 days Examinations: clinical observations (10 min, 1 h, 2 h, 6 h, 24 h and	Clinical signs: Within the 1st 24 h after application 1/5 males and 1/5 females died. The main clinical signs observed up to day 4 (females more affected than males) were poor general condition,	<confidential> (1992)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
	and 5 females, one dose	IUCLID technical dossier	thereafter once daily up to day 14 after exposure), body weight (measured day 0, 7 and 14), necropsy (14 days after exposure or when rats were found dead).	decreased respiratory rate, abnormal gait, squatting position and sunken flanks (no effects at day 5-14). Body weight gain normal in males but in females slight decrease in body weight on day 7 compared with day 0 (156 versus 159 g), increase at day 14 (176 g). Related to "Mecetroniumetilsulfat 30%" the LD_{50} for males and females is greater than 2000 mg/kg bw. Related to the active component Mecetronium ethyl sulphate the LD_{50} for males and females is greater than 600 mg/kg bw.	
OECD 401 Limit test Oral; Gavage GLP	Rat Wistars Males and females Number of animals per group: 5 males and 5 females, one dose	Mecetronium ethyl sulphate [MES] Clear liquid; 4% active component Purity of the active substance: IUCLID technical dossier	2000 mg/kg bw Post exposure period: 14 days Examinations: clinical observations (10 min, 1 h, 2 h, 6 h, 24 h, and thereafter once daily up to day 14 after exposure), body weight (measured day 0, 7 and 14), necropsy (14 days after exposure).	No mortality. Clinical signs: piloerection in 10/10 rats, in 2 females slightly reduced activity, squatting position and decreased respiratory rate 2-6 h after application, later observations revealed no effects. Normal mean body weight gain. Related to "Mecetroniumetilsulfat 4%" the LD_{50} for males and females is greater than 2000 mg/kg bw. Related to the active component Mecetronium ethyl sulphate the LD_{50} for males and females is greater than 80 mg/kg bw.	<confidential> (1992a)

Table 26: Summary table of human data on acute oral toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Epidemiological studies on the general population	Biocidal product containing 0.2% MES	The overall incidence of suspected drug reactions is very low (0.00018%). Any type of suspected drug reactions can be considered to be "very rare" (<0.01%).	Absolute and relative frequency of suspected drug reactions in connection with a mixture containing 0.2 MES between January 2000 and August 2005 Oral misuse: Number of incidents: 7 Relative frequency per all hygienic hand disinfections: 0.0000001%	Periodic Safety Update Report for Medicinal Products.

Table 27: Summary table of other studies relevant for acute oral toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

Two animal studies on acute oral toxicity were performed.

The first study was performed on "Mecetroniumetilsulfat 30%" (Clear liquid; 30% active component, density 0.99 g/ml; pH value 7.38). Study performed according to OECD guideline 401 (limit-test using a dose of 2000 mg/kg bw of "Mecetroniumetilsulfat 30%" in 5 male and 5 female rats).Within the 1st 24 h after application of 2000 mg/kg bw 1/5 males and 1/5 females died. The main clinical signs observed up to day 4 (females more affected than males) were poor general condition, decreased respiratory rate, abnormal gait, squatting position and sunken flanks (no effects at day 5-14). Body weight gain normal in males but in females slight decrease in body weight on day 7 compared with day 0 (156 versus 159 g), increase at day 14 (176 g). LD_{50} for active component mecetronium ethyl sulphate [MES] was calculated as >600 mg/kg bw taking into

account, that in the acute oral toxicity study the LD_{50} for 30% solution was >2000 mg/kg bw. Such calculation could be burdened with an uncertainity due to possible difference in toxicity related to dilution of the active substance. In this case, since the active substance is presented always as a 30% solution, it seems to be justified to support LD_{50} derived from the acute toxicity of 2000 mg/kg/bw of 30% mecetronium ethyl sulphate [MES] solution.

The second study was performed on "Mecetroniumetilsulfat 4%" (Clear liquid; 4% active component, density 0.99 g/ml; pH value 6.18). Study performed according to OECD guideline 401 (limit-test using a dose of 2000 mg/kg bw of "Mecetroniumetilsulfat 4%" in 5 male and 5 female rats). Clinical observations were conducted at regular intervals during the 14-day observation period. Body weights were measured at days 0, 7 and 14 p.a. Gross pathological observations were performed on all animals 14 days p.a. No animals died during the study. Clinical signs: piloerection in 10/10 rats, in 2 females slightly reduced activity, squatting position and decreased respiratory rate 2-6 h after application, later observations revealed no effects. Normal mean body weight gain. The LD_{50} for male and female rats after oral exposure to "Mecetroniumetilsulfat 4%" is greater than 2000 mg/kg bw (80 mg/kg bw related to active component).

10.1.2 Comparison with the CLP criteria

Table 28. Results of acute oral toxicity studies in comparision to the CLP criteria.

Toxicological results	CLP criteria
The LD_{50} for male and female rats after oral exposure to "Mecetroniumetilsulfat 4%" is greater than 2000 mg/kg bw (80 mg/kg bw related to active component). The LD_{50} for male and female rats after oral exposure to "Mecetroniumetilsulfat 30%" is greater than 2000 mg/kg bw (600 mg/kg bw related to active component). LD_{50} for active component Mecetronium ethyl sulphate [MES] was calculated as greater than 80 mg/kg bw taking into account, that in the acute oral toxicity study the LD_{50} for 4% solution was >2000mg/kg bw. Such calculation could be burdened with an uncertainty. The study provides vast margin of uncertainty because no mortality was observed and other signs of toxicity were rather mild. Therefore this study should not be used for active substance LD_{50} approximation, rather LD_0 than LD_{50} could be revealed, but could be used for toxicological classification of 4% MES solution. In this case, since the active substance is presented always as a 30% solution, it seems to be justified to support LD_{50} derived from the acute toxicity of > 2000 mg/kg/bw of 30% MES solution. However, taking into account the results obtained with	Cat. 4 (H302) $300 < LD_{50} \leq 2000$ mg/kg bw Cat. 3 (H301) $50 < LD_{50} \leq 300$ mg/kg bw Cat. 2 (H300) $5 < LD_{50} \leq 50$ mg/kg bw Cat. 1 (H300) $LD_{50} \leq 5$ mg/kg bw

30% solution, it may be concluded, that LD_{50} of active substance is > 600mg/kg bw.	

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Based on the submitted acute oral toxicity studies, mecetronium ethyl sulphate [MES] should be classified for acute oral toxicity in category 4 (Acute Tox. 4, H302 – Harmful if swallowed) (LD_{50} for active component mecetronium ethyl sulphate [MES] was calculated as >600 mg/kg bw taking into account, that in the acute oral toxicity study the LD_{50} for 30% solution was >2000 mg/kg bw).

10.2 Acute toxicity - dermal route

Table 29: Summary table of animal studies on acute dermal toxicity.

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Value LD_{50}**	**Reference**
OECD 402 Limit test Dermal GLP	Rat Wistars Males and females Number of animals per group: 5 males and 5 females, one dose	Mecetronium ethyl sulphate [MES] Clear liquid; 4% active component Purity of the active substance: IUCLID technical dossier	2000 mg/kg bw Duration of exposure: 24h Examinations: clinical observations (10 min, 1 h, 2 h, 6 h, 24 h and thereafter once daily up to day 14 after exposure), skin reaction scored once daily after patch removal, body weight (measured day 0, 7 and 14), necropsy (14 days after exposure).	The LD_{50} for male and female rats after dermal exposure to "Mecetroniumetilsulfat 4%" is greater than 2000 mg/kg bw (80 mg/kg bw related to active component).	<confidential> (1992b)
OECD 402 Limit test Dermal GLP	Rat Wistars Males and females Number of animals per group: 5 males and 5 females, one dose	Mecetronium ethyl sulphate [MES] Clear liquid; 30% active component Purity of the active substance: IUCLID technical dossier	2000 mg/kg bw Duration of exposure: 24h Post exposure period: 14 days Examinations: clinical observations (10 min, 1 h, 2 h, 6 h, 24 h and thereafter once daily up to day 14 after	Related to "Mecetroniumetilsulfat 30%" the LD_{50} for males and females is greater than 2000 mg/kg bw. Related to the active component Mecetronium ethyl sulphate [MES] the LD_{50} for males and females is greater than 600 mg/kg bw.	<confidential> (1992c)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
			exposure), skin reaction scored once daily after patch removal, body weight (measured day 0, 7 and 14), necropsy (14 days after exposure).		

Table 30: Summary table of human data on acute dermal toxicity.

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 31: Summary table of other studies relevant for acute dermal toxicity.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.2.1 Short summary and overall relevance of the provided information on acute dermal toxicity

Two animal studies on acute dermal toxicity were performed.

The first study was performed on “Mecetroniumetilsulfat 4%” (Clear liquid; 4% active component, density 0.99 g/ml). Study performed according to OECD guideline 402 (limit-test using a dose of 2000 mg/kg bw of “Mecetroniumetilsulfat 4%” in 5 male and 5 female rats). Clinical observations were conducted at regular intervals during the 14-day observation period. Body weights were measured at days 0, 7 and 14 p.a. After patch removal, dermal irritation was evaluated once daily for 14 days according to a scheme based on Draize. Mainly in the female rats erythema and oedema were observed up to 13 days in 2 females. Additionally in a few females formation of fissures and degreasing of the treated skin were observed. Gross pathological observations were performed on all animals at terminations. No animals died during the study. The LD_{50} for male and female rats after dermal exposure to “Mecetroniumetilsulfat 4%” is greater than 2000 mg/kg bw (80 mg/kg bw related to active component).

The second study was performed on “Mecetroniumetilsulfat 30%” (Clear liquid; 30% active component, density 0.99 g/ml; pH value 7.38). Study performed according to OECD guideline 402

(limit-test using a dose of 2000 mg/kg bw of "Mecetroniumetilsulfat 30%" in 5 male and 5 female rats). Clinical observations were conducted at regular intervals during the 14-day observation period. Body weights were measured at days 0, 7 and 14 p.a. After patch removal, dermal irritation and other alterations were evaluated once daily for 14 days according to a scheme based on Draize. Moderate to severe erythema and very slight oedema were observed up to day 12 p.a. followed by a decline of these skin reactions up to the end of observation period (day 14 p.a.). Additionally degreasing, induration, partial desquamation and formation of fissures were observed. Gross pathological observations were performed on all animals at terminations. No animals died during the study. The LD_{50} for male and female rats after dermal exposure to "Mecetroniumetilsulfat 30%" is greater than 2000 mg/kg bw (600 mg/kg bw related to active component).

10.2.2 Comparison with the CLP criteria

Table 32. Results of acute dermal toxicity studies in comparision to the CLP criteria.

Toxicological results	CLP criteria
The LD_{50} for male and female rats after dermal exposure to "Mecetroniumetilsulfat 4%" is greater than 2000 mg/kg bw (80 mg/kg bw related to active component). The LD_{50} for male and female rats after dermal exposure to "Mecetroniumetilsulfat 30%" is greater than 2000 mg/kg bw (600 mg/kg bw related to active component). LD_{50} for active component Mecetronium ethyl sulphate [MES] was calculated as greater than 80 mg/kg bw taking into account, that in the acute oral toxicity study the LD_{50} for 4% solution was >2000mg/kg bw. Such calculation could be burdened with an uncertainty. The study provides vast margin of uncertainty because no mortality was observed and other signs of toxicity were rather mild. Therefore this study should not be used for active substance LD_{50} approximation, rather LD_0 than LD_{50} could be revealed, but could be used for toxicological classification of 4% MES solution. Since the active substance is always present as 30% solution, it seems to be justified to support LD_{50} derived from the acute dermal toxicity of 30% solution. However, taking into account the results obtained with 30% solution, it may be concluded, that LD_{50} of active substance is > 600 mg/kg bw.	Cat. 4 (H312) $1000 < LD_{50} \leq 2000$ mg/kg bw Cat. 3 (H311) $200 < LD_{50} \leq 1000$ mg/kg bw Cat. 2 (H310) $50 < LD_{50} \leq 200$ mg/kg bw Cat. 1 (H310) $LD_{50} \leq 50$ mg/kg bw

10.2.3 Conclusion on classification and labelling for acute dermal toxicity

Based on the submitted acute dermal toxicity studies, mecetronium ethyl sulphate [MES] should be classified for acute dermal toxicity in category 3 (Acute Tox. 3, H311 – Toxic in contact with skin).

(The LD_{50} for male and female rats after dermal exposure to "Mecetroniumetilsulfat 30%" is greater than 2000 mg/kg bw - 600 mg/kg bw related to active component- based on the worst case scenario it can not be concluded that LD_{50} for active substance is higher that cut-off value for category 3).

10.3 Acute toxicity - inhalation route

Table 33: Summary table of animal studies on acute inhalation toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance, form and particle size (MMAD)	Dose levels, duration of exposure	Value LC_{50}	Reference
No data					

Table 34: Summary table of human data on acute inhalation toxicity

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 35: Summary table of other studies relevant for acute inhalation toxicity

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.3.1 Short summary and overall relevance of the provided information on acute inhalation toxicity

An acute inhalation toxicity study was not performed due to the following reasons:

1) the physico-chemical properties indicate that the active substance has no tendency to become airborne. Mecetronium ethyl sulphate [MES] is an ionic substance which is produced as 30% aqueous solution. In addition, the vapour pressure is calculated to be very low ($3.2 \cdot 10^{-12}$ Pa (20°C),

2) the exposure of professionals to mecetronium ethyl sulphate [MES] during production and formulation is limited to the dermal route, since PC data excluded the existence of mecetronium ethyl sulphate in inhaled air (see above). The generation of an aerosol during production or formulation is improbable,

3) the exposure of users to mecetronium ethyl sulphate [MES] via the product containing 0.2% MES is limited to the dermal route. Inhalation exposure to mecetronium ethyl sulphate [MES]

vapour is excluded due to the low vapour pressure and the generation of an aerosol during use (specified dispenser as described in product information) is excluded.

10.3.2 Comparison with the CLP criteria

There are no relevant data to compare with criteria.

10.3.3 Conclusion on classification and labelling for acute inhalation toxicity

Classification and labelling is not required.

10.4 Skin corrosion/irritation

Table 36: Summary table of animal studies on skin corrosion/irritation.

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results -Observations and time point of onset -Mean scores/animal -Reversibility**	**Reference**
OECD 404	Rabbit White New Zealand Number of animals per group: 3 rabbits	"Mecetroniumetilsulfat 0.2%"	0.5 ml of the test substance applied to ca. 6 cm² of test site exposure: 4 hours Examination time points: 30-60 min, 24 h, 48 h, 72 h after patch removal.	No erythema, oedema or any other effects on skin observed	<confidential> (1992d)
OECD 404	Rabbit White New Zealand Number of animals per group: 3 rabbits	"Mecetroniumetilsulfat 4%"	0.5 ml of the test substance applied to ca. 6 cm² of test site exposure: 4 hours Examination time points: 30-60 min, 24 h, 48 h, 72 h after patch removal and thereafter once daily up to day 15 (1 rabbit) or 18 (2	Reversibility: No	<confidential> (1993) Acute dermal irritation/corrosion test of "Mecetroniumetilsulfat 4%" in rabbits. IBR Forschungs GmbH, Project No.: 10-03-1705/00-92 (unpublished).

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results -Observations and time point of onset -Mean scores/animal -Reversibility	Reference
			rabbits).		

Table 37: Summary table of human data on skin corrosion/irritation.

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Data on the general population can be obtained from the biocidal product containing 0.2% of MES as a periodical safety up-date. The overall incidence of suspected drug reactions is very low (0.00018%). Any type of suspected drug reactions can be considered to be "very rare" (<0.01%). Skin irritation, suspected allergy and eye irritation are the commonly reported drug reactions, oral misuse occurred occasionally.				BODE Chemie (2006)

Table: Absolute and relative frequency of suspected drug reactions in connection with a mixture containing 0.2% MES between January 2000 and August 2005

Type of drug reaction	Number of incidents	Relative frequency per all hygienic hand disinfections
Suspected allergy	43	0.0000006%
Skin irritation	**41**	**0.0000006%**
Ocular irritation	24	0.0000003%
Oral misuse	7	0.0000001%
Respiratory tract irritation	2	<0.0000001%
Diarrhea	1	<0.0000001%
Burns	1	<0.0000001%
All	118	0.0000018%

Table 38: Summary table of other studies relevant for skin corrosion/irritation.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

The potential toxicity of "Mecetroniumetilsulfat 0.2%" was assessed in an acute dermal irritation/corrosion test on 3 albino rabbits. The skin was exposed to the test article for 4 hours. Animals were examined for signs of erythema and oedema at 30-60 min, 24, 48 and 72 h after patch removal. The mean grades of skin reactions at 24, 48 and 72 h after patch removal were lower than

the cut-off value for classification of substance/mixture as irritant to skin included in CLP Regulation.

Table 39. Skin irritation in rabbits after dermal exposure to 0.2% Mecetroniumetilsulfat.

score (average of 3 animals investigated)	time	Erythema	Edema
average score Draize scores (0 to maximum 4)	30-60 min	0	0
	24 h	0	0
	48 h	0	0
	72 h	0	0
average score	24h, 48h, 72h	0	0

The potential toxicity of "Mecetroniumetilsulfat 4%" was also assessed in an acute dermal irritation/corrosion test on 3 albino rabbits. The skin was exposed to the test article for 4h. Animals were examined for signs of erythema and oedema at 30-60 min, 24, 48 and 72 h and thereafter once daily up to days 15-18 after patch removal. Severe skin reactions of varying extent and duration were apparent in all animals throughout most of the observation period. Over the first 9-10 days after patch removal in all animals erythema and oedema of the treated skin were observed. From 9-10 onwards, all animals showed skin fissures and leathery skin was also evident in one animal. These effect showed little or no signs of reversibility up to days 15-18 post exposure.

Table 40. Skin irritation in rabbits after dermal exposure to 4% Mecetroniumetilsulfat.

score (average of 3 animals investigated)	time	Erythema	Edema
average score Draize scores (0 to maximum 4)	30-60 min	1	0
	24 h	2.0	2.3
	48 h	2.0	2.3
	72 h	2.3	2.3
average score	24h, 48h, 72h	2.1	2.3
other times	4 d	2.3	2.0
	5 d	2.3	2.0
	6 d	2.0	2.0
	7 d	2.0	2.0
	8 d	2.0	2.0

	9 d	2.0**	2.0
	10 d	1.3***	1.0 #
	11 d	1.3***	1.0 #
	12 d	1.0***	0.6 #
	13 d	1.0***	0.6 #
	14d	0.6***	0.3 #
	15 d	0.6***	0.3 #
	16 d (n=2)	0.5**	0.5 #
	17 d (n=2)	0.5**	0.5 #
	18 d (n=2)	0.5**	0.5 #
reversibility:		n.c.	n.c.
n c: not completely reversible; *: formation of skin fissures (* in one rabbit, ** in 2 rabbits, *** in 3 rabbits); #: leathery skin in one animal			

10.4.2 Comparison with the CLP criteria

Toxicological results	CLP criteria
Irreversible skin demage	Corrosion On the basis of the results of animal testing a substance is classified as corrosive: a corrosive substance is a substance that produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least 1 tested animal after exposure up to a 4 hour duration. Corrosive reactions are typified by ulcers, bleeding, bloody scabs and, by the end of observation at 14 days, by discoloration due to blanching of the skin, complete areas of alopecia and scars. Histopathology shall be considered to discern questionable lesions. Three subcategories are provided within the corrosive category: subcategory 1A - where responses are noted following up to 3 minutes exposure and up to 1 hour observation; subcategory 1B - where responses are described following exposure between 3 minutes and 1 hour and observations up to 14 days; and subcategory 1C - where responses occur after exposures between 1 hour and 4 hours and observations up to 14 days.

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

During skin corrosion/irritation study on 4% Mecetroniumetilsulfat some effects observed were not reversible. It can be concluded that "undiluted active substance" has potential corrosive properties. It is proposed to classify mecetronium ethyl sulphate [MES] as skin corrosive in subcategory 1C (Skin Corr. 1C) – responses in study performed on 4% Mecetroniumetilsulfat occur after 4 hour exposure and observations up to 14 days.

10.5 Serious eye damage/eye irritation

Table 41: Summary table of animal studies on serious eye damage/eye irritation.

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results** **- Observations and time point of onset** **- Mean scores/animal** **- Reversibility**	**Reference**
according to Draize, (no further details); no guideline study but comparable to OECD 405 with acceptable restrictions: • no wash out after 24 h but presumably not appropriate (minor restriction); •limited data on test animals and clinical signs (minor restrictions)	Rabbit, New Zealand White, 8 animals per group, no information about sex and age	a mixture containing 0.2% MES	Volume applied: 0.1 ml, duration of observation period: 7 days after application, post exposure period: 7 days	Mean score 2 for conjunctival redness. Effects lasted 24 h Redness, chemosis and discharge of the conjunctiva reached score 2, that is completely reversible after 4 days	BODE Chemie (1978)
The biocidal product containing 0.2 % MES has been tested for acute eye irritation in rabbits. A mixture containing 0.2% MES was irritant to the rabbit eye.					

Table 42: Summary table of human data on serious eye damage/eye irritation.

Type of data/report	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**

Data on the general population can be obtained from the biocidal product containing 0.2% of MES as a periodical safety up-date:
The overall incidence of suspected drug reactions is very low (0.00018%). Any type of suspected drug reactions can be considered to be "very rare" (<0.01%). Skin irritation, suspected allergy and eye irritation are the commonly reported drug reactions, oral misuse occurred occasionally.

Table: Absolute and relative frequency of suspected drug reactions in connection with a mixture containing 0.2% MES between January 2000 and August 2005

Type of drug reaction	Number of incidents	Relative frequency per all hygienic hand disinfections
Suspected allergy	43	0.0000006%
Skin irritation	41	0.0000006%
Ocular irritation	**24**	**0.0000003%**
Oral misuse	7	0.0000001%
Respiratory tract irritation	2	<0.0000001%
Diarrhea	1	<0.0000001%
Burns	1	<0.0000001%
All	118	0.0000018%

Table 43: Summary table of other studies relevant for serious eye damage/eye irritation.

Type of study/data	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data				

10.5.1 Short summary and overall relevance of the provided information on serious eye damage/eye irritation

There are no experimental data on eye irritation/corrosion performed on mecetronium ethyl sulphate [MES] (only the biocidal product containing 0.2 % MES has been tested for acute eye irritation in rabbits) but taking into account the following information:

- the biocidal product containing 0.2 % MES has been tested for acute eye irritation in rabbits. A mixture containing 0.2% mecetronium ethyl sulphate [MES] was irritant to the rabbit eye,
- skin irritation studies have shown that a 4% solution of mecetronium ethyl sulphate [MES] resulted in severe erythema (some indications of irreversible skin damage) after a single dermal application,
- severe local effects in the acute dermal toxicity study with 30 % mecetronium ethyl sulphate [MES] have been demonstrated,

severe eye irritation of MES can be expected.

10.5.2 Comparison with the CLP criteria

There are no relevant data to compare with criteria (No experimental studies were performed to assess the corrosive potential of substance to the eyes).

Mecetronium ethyl sulphate [MES] produced severe irritation/corrosive effects to the skin of rabbits.

According to CLP Regulation skin corrosive substances shall be considered as leading to serious damage to the eyes as well (Category 1). Also according to section 3.3.2.1.2.5 (Testing methods: In vivo methods) of Guidance on the application of CLP Criteria "Testing for eye irritation would not be carried out on substances known or predicted to be corrosive to skin. Such substances are automatically considered to be severely damaging to the eye and are classified but not labelled for serious eye damage in addition to skin corrosion".

Dossier Submitter, taking into account the above mentioned information, recommends also classification, according to CLP, of mecetronium ethyl sulphate [MES] as corrosive to the eyes – Eye Dam. 1, H318.

10.5.3 Conclusion on classification and labelling for serious eye damage/eye irritation

According to CLP regulation requirements mecetronium ethyl sulphate [MES] should be classified for serious eye damage category 1 with hazard statement H318 (Causes serious eye damage).

10.6 Respiratory sensitisation

Table 44: Summary table of animal studies on respiratory sensitisation.

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Results	Reference
No data					

Table 45: Summary table of human data on respiratory sensitisation.

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 46: Summary table of other studies relevant for respiratory sensitisation.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.6.1 Short summary and overall relevance of the provided information on respiratory sensitisation

Te are no relevant data to discuss respiratory sensitisation.

10.6.2 Comparison with the CLP criteria

There are no relevant data to compare with criteria.

10.6.3 Conclusion on classification and labelling for respiratory sensitisation

No classification of mecetronium ethyl sulphate [MES] for respiratory sensitisation is proposed.

10.7 Skin sensitisation

Table 47: Summary table of animal studies on skin sensitisation.

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
OECD 406 GLP	Guinea pigs Pirbright white 10 males and 10 females in the treatment group and in the control group	Clear liquid; 30% active component	Study type: Adjuvant day 0 (intradermal) and day 7 (topical)	not considered to have the potential to cause skin sensitisation.	<confidential> (1992e)

Table 48: Summary table of human data on skin sensitisation.

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
human data	a mixture containing 0.2% MES		In a human case study 55 volunteers were exposed to a mixture containing 0.2% MES for 24 hours under occlusive conditions. The potential for skin irritation and sensitisation was determined. Under the condition of this assay a mixture containing 0.2% MES has no irritant or sensitizing properties in humans.	BODE Chemie (2006)
human data	a mixture containing 0.2% MES		Data on the general population can be obtained from the biocidal product containing 0.2% of MES as a periodical safety up-date: The overall incidence of suspected drug reactions is very low (0.00018%). Any type of suspected drug reactions can be considered to be “very rare” (<0.01%).	BODE Chemie (2006)

<table>
<tr><th>Type of data/report</th><th>Test substance,</th><th>Relevant information about the study (as applicable)</th><th>Observations</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>Skin irritation, suspected allergy and eye irritation are the commonly reported drug reactions, oral misuse occurred occasionally.

Table: Absolute and relative frequency of suspected drug reactions in connection with a mixture containing 0.2% MES between January 2000 and August 2005
<table><tr><th>Type of drug reaction</th><th>Number of incidents</th><th>Relative frequency per all hygienic hand disinfections</th></tr><tr><td>Suspected allergy</td><td>43</td><td>0.0000006%</td></tr><tr><td>Skin irritation</td><td>41</td><td>0.0000006%</td></tr><tr><td>Ocular irritation</td><td>24</td><td>0.0000003%</td></tr><tr><td>Oral misuse</td><td>7</td><td>0.0000001%</td></tr><tr><td>Respiratory tract irritation</td><td>2</td><td><0.0000001%</td></tr><tr><td>Diarrhea</td><td>1</td><td><0.0000001%</td></tr><tr><td>Burns</td><td>1</td><td><0.0000001%</td></tr><tr><td>All</td><td>118</td><td>0.0000018%</td></tr></table></td><td></td></tr>
</table>

Table 49: Summary table of other studies relevant for skin sensitisation

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.7.1 Short summary and overall relevance of the provided information on skin sensitisation

The Guinea Pig Maximization test performed on Mecetroniumetilsulfat 30% is only available. The potential skin sensitising properties of Mecetroniumetilsulfat 30% were assessed by using 20 test and 20 control animals. The concentrations used were selected on the basis of the results of the pilot study. Following induction exposure to the test article or the vehicle, the animals were subject to two weeks later to a challenge exposure with the test article. The treated skin areas were evaluated 24 and 48 hours after the end

of exposure period. 24h after removal of the patch no skin reaction was detected in any animal of the treatment and the control group (evaluation of erythema and edema according to the scoring given in OECD 404). 48h after removal of the patch one male showed score 1 for erythema (very slight erythema, barely perceptible). This effect was also observed in 2 control females. These alterations of the skin were considered not to be a sensitizing effect since effects were observed to the same extent in test and control animals: 16/20 test animals and 15/20 control animals showed scale formation on the treated skin. No animal showed a sensitization to the test substance "Mecetroniumetilsulfat 30%" applied as a 5% preparation (1.5% active component).

10.7.2 Comparison with the CLP criteria

Toxicological results	CLP criteria
No animals sensitised to Mecetroniumetilsulfat 30%	Guinea pig maximization test Category 1A (H317) ≥ 30% responding at ≤ 0.1% intradermal induction dose or ≥ 60% responding at 0.1% to ≤ 1% intradermal induction dose Category 1B (H317) ≥ 30% to < 60% responding at > 0.1% to ≤ 1% intradermal induction dose or ≥ 30% responding at > 1% intradermal induction dose

10.7.3 Conclusion on classification and labelling for skin sensitisation

No classification/labelling for skin sensitisation is proposed for mecetronium ethyl sulphate [MES].

10.8 Germ cell mutagenicity

Table 50: Summary table of mutagenicity/genotoxicity tests in vitro.

Method, guideline, deviations if any	**Test substance,**	**Relevant information about the study including rationale for dose selection (as applicable)**	**Observations**	**Reference**
OECD 471 GLP: Yes Bacterial reverse mutation test	Mecetronium ethyl sulphate [MES] Purity: IUCLID technical dossier	Organism/cell type: S. typhimurium: TA 1535, TA 1537, TA 98, TA 100 Metabolic activation system: S9 mix from livers of Wistar rats which received i.p. 500 mg/kg bw Aroclor 1254 5 days before	Under the experimental conditions described in this study the test substance did not induce gene mutation in bacteria. Reduced number of revertants/plate at concentration of 100µg/plate in case of TA1535, TA1537 indicated cytotoxic effect.	BODE Chemie (1992f)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		preparation Positive control: Without metabolic activation (MA) 10 µg/plate sodium azide in TA100 and TA1535, 50 µg/plate 9-aminoacridine in TA1537, 10 µg/plate 4-nitro-1,2-phenylene diamine in TA98. With MA 3 µg/plate 2-aminoanthracene for all strains. Concentrations: Main study: 0.16, 0.8, 4.0, 20 and 100 µg/plate. Preliminary toxicity study (only TA100): 10, 32, 100, 320, 1000, 3200, 10000 µg/plate The undiluted test substance contained 30% active component and all concentrations used were based on this content of active component.		
comparable to OECD 471 GLP: yes Bacterial reverse mutation test	Mecetronium ethyl sulphate [MES] Purity: no data	Orhanism/cell type: S. typhimurium: TA 1538, TA 1535, TA 1537, TA 98, TA 100 Metabolic activation system: S9 mix from livers of male Wistar rats which received i.p. 500 mg/kg bw Aroclor 1254 5 days before preparation or after induction with Phenobarbital for 7 days (no data about the daily body dose) Positive control: For all strains the same substance was used with and without MA: TA98 and TA1538 50 µg/plate dichlorobenzidine TA100 50 µg/plate methylcholathrene TA1535 200 µg/plate cyclophosphamide TA1537 100 µg/plate aminoacridine (no positive	Under the experimental conditions described in this study the test substance did not induce gene mutation in bacteria.	BODE Chemie (1981)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		control with MA) Concentrations: 20, 100, 500, 2500 µg/plate. Way of application: test substance solution (solvent dimethylsulphoxide [DMSO]) added to bacterial culture in medium plus MA system (or without MA)		
In vitro mammalian chromosome aberration test OECD 473 GLP: yes	Mecetronium ethyl sulphate [MES] Purity: IUCLID technical dossier Solution (soluble in water and ethanol) 29.99% active component, density 0.999 g/ml, refraction index n20d 1,373, pH 7.3 (1% solution, no further data)	Organism: Chinese Hamster Ovary (CHO) cells Metabolic Activation System (MA): rat liver S9 mix (no data about induction) Concentrations: Given concentrations are related to the 30% solution of Mecetronium etilsulfat. Solubility of the test compound was measured: 5 mg could be dissolved in 1 ml medium (used as solvent), the pH value was 7.00. Preliminary study: 0, 0.78-50 µg/ml without MA and 0, 3.12-100 µg/ml with MA (mitotic index determined after 18 or 28 h incubation). Main study 1 without MA 0, 1.5, 3, 6, 9, 12 µg/ml and with MA 0, 2.5, 5, 10, 20, 30, 40 µg/ml, fixation after 18 or 28 h (totally 4 trials); after evaluation of cytotoxicity only a few doses chosen for determination of mutagenicity. Main study 2 without MA 0, 2.5, 5, 7.5, 10 µg/ml (fixation time 18 h) or 0, 7.5, 10 (fixation time 28 h); with MA 0, 5, 15, 25, 30 µg/ml (fixation time 18 h) or 0, 25.5, 30 (fixation time 28 h); after evaluation of cytotoxicity only a few doses chosen for	Genotoxicity without metabolic activation: No clastogenic activity at any dose level in both independent studies. Valid positive control. Negative control within the historical range of this laboratory. Genotoxicity with metabolic activation: No clastogenic activity at any dose level in both independent studies. Valid positive control. Negative control within the historical range of this laboratory.	BODE Chemie (1994)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		determination of mutagenicity. Examinations: In preliminary studies cytotoxicity (mitotic index, mitosis per 1000 cells) determined; duplicate cultures for each concentration; 2 independent experiments; concurrent solvent (medium) and positive control; without MA 18 or 28 h exposure of cells (0.2 µg/ml colcemid 2 h before end of incubation); with MA 3 h exposure to the test substance followed by incubation without test substance, total incubation time 18 or 28 h (colcemid treatment see above); cells performed for metaphase analysis of 100 mitosis per culture (200 per concentration per fixation time); specification of aberrations in the results; mitosis per 1000 cells determined (mitotic index); statistical analysis performed.		
Mammalian cell gene mutation assay OECD 476 GLP: yes	Mecetronium ethyl sulphate [MES] Purity: IUCLID technical dossier Solution (soluble in water and ethanol) 29.99% active component, density 0.999 g/ml, refraction index n20d 1,373, pH 7.3 (1% solution, no	Organisms/cell type: Mouse lymphoma L5178Y TK+/- cells Metabolic Activation (MA) system: S9 mix from rat liver (no data about induction) Positive control: With MA 3 µg/ml benzo(a)pyrene; without MA 25 µg/ml methylmethanesulfonate Concentrations: Solubility of the test compound was measured: 5 mg could be dissolved in 1 ml medium (used as solvent), the pH value was 7.00. 1st assay Without MA: 0, 0.63, 1.25, 2.5, 5 µg/ml; with MA: 0,	Genotoxicity without metabolic activation: 1st assay Yes Negative control and positive control are valid and within the range of historical control data of this laboratory. The test substance induced dose dependent and statistically significant increases in the mutant frequencies. 2nd assay No Negative control and positive control are valid and within the range of historical control data of this laboratory. The test substance did not induce increases in the mutant frequencies.	BODE Chemie (1994a)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
	further data)	3.13, 6.25, 12.5, 25.0 µg/ml 2nd assay Without MA: 0, 2.5, 5, 7.5, 10, 15 µg/ml; with MA: 0, 7.5, 10, 20, 30, 40 µg/ml Examinations: 2 replicates per dose, 2 independent experiments; positive control; negative control (not clearly stated but presumably no addition of test substance or vehicle). Cytotoxicity measured by determination of relative survival in parallel experiments.	Genotoxicity with metabolic activation: 1st assay Yes Negative control and positive control are valid and within the range of historical control data of this laboratory. The test substance induced at the high dose level a statistically significant increase in the mutant frequency. 2nd assay No Negative control and positive control are valid and within the range of historical control data of this laboratory. The test substance did not induce increases in the mutant frequencies.	
Mammalian cell gene mutation assay OECD 476 GLP: yes	Mecetronium ethyl sulphate [MES] Mecetronium ethylsulfate, CAS 3006-10-8, provided as 29% (w/w) aqueous solution Purity (as active substance): IUCLID technical dossier	Organisms/cell type: Mouse lymphoma L5178Y/ TK+/- cells Metabolic Activation (MA) system Positive control: With metabolic activation (MA): 2.5 µg/mL Cyclophosphamide monohydrate (CP); without MA: 7.5 µg/mL Methyl methanesulfonate (MMS) Concentrations: Test item MES is highly soluble in water (500 g/L). Dose range finding test: Without MA: 0, 1, 2.5, 5, 7.5, 10, 15, 30 µg/mL With MA: 0, 2.5, 5, 7.5, 10, 15, 30, 60 µg/mL Main tests, 4 h treatment without MA (S9-mix): 0, 0.63, 1.25, 2.5, 3.75, 5.00, 7.50 µg/mL Main tests, 4 h treatment with MA (S9-mix): 0, 2.5, 5.0, 10, 15, 20 µg/mL Way of application: Test substance "MES-solution	Genotoxicity without metabolic activation: No The test substance did not induce increases in the mutant frequencies (MF). Negative control and positive control were valid and within the range of historical control data of this laboratory. All MES-treated cultures exhibited MFs, which were within the normal range for negative controls and thus most likely represent spontaneous mutations based on strong cytotoxic activity. Genotoxicity with metabolic activation: No The test substance did not induce increases in the mutant frequencies (MF). Negative control and positive control were valid and within the range of historical control data of this laboratory. All MES-treated cultures exhibited MFs, which were within the normal range for negative controls and thus most likely represent spontaneous mutations based on strong cytotoxic activity.	BODE Chemie (2008)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		29%"added to cell culture in medium (standard growth medium R10: 500 mL RPMI-1640, 50 mL HS, 50.000 µg streptomycin sulphate, 50.000 U penicillin G, sodium salt) plus MA system or without MA. Examinations: 2 replicates per dose, 2 independent experiments; positive control; negative control (vehicle RPMI-1640 + 5% horse serum (R5)). Cytotoxicity was measured by determination of relative survival in parallel experiments. Mutant frequency was determined by seeding approximately 2 x 103 cells/well in 96-well plates, using restrictive trifluorothymidine (TFT)-containing medium to select for the mutant phenotype.	Cytotoxicity: MES, both in the absence and presence of S9-mix, induced marked concentration-dependent cytotoxicity, as judged by relative total growth (RTG) and suspension growth (SG). In contrast, plating efficiency (PE) and relative survival (RS) of survivor II plates were not altered significantly by MES treatment (except 3.75 µg/mL without and 5.0 µg/mL with S9-mix), as compared to the vehicle controls. There was predominantly slight reduction, but without concentration-dependency.	
Unscheduled DNA synthesis (UDS) in mammalian cells in vitro comparable to OECD 482 GLP: yes	Mecetronium ethyl sulfate [MES] Purity: no data	Organism/cell type: HeLa S3 cells (human cell line) Metabolic Activation (MA) system: Rat liver S9 mix; for induction Wistar rats received a single i.p. injection of 500 mg/kg bw Aroclor 1254 in corn oil, sacrificed the 5th day after application and liver supernatant prepared of homogenized livers. Positive control: 10 µM NQO (no further specification, presumably 4-nitroquinoline-N-oxide) without MA; 50 µM DCB (no further specification) with MA Concentrations: 0, 0.2, 0.02, 0.002, 0.0002 µg/ml Way of application: Test substance dissolved in DMSO and added to the medium	Genotoxicity without metabolic activation: Valid positive control. No increase in radioactivity indicating no UDS and/or cytotoxic effects. Genotoxicity with metabolic activation: Only a weak effect in the positive control limiting the reliability of the results (no statistical evaluation). No increase in radioactivity indicating no UDS and/or cytotoxic effects. Cytotoxicity: Yes, reduced radioactivity at the high dose levels indicates cytotoxicity (not discussed by the authors).	BODE Chemie (1981a)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
		Examinations: Liquid scintillation counter method; cells exposed for 2 h to the test substance in the presence of H3-thymidine (labelling of DNA); 3 cultures per dose level, no 2nd independent experiment; positive control; negative control: vehicle (DMSO) control. Number of cells evaluated: Radioactivity of cell DNA extract counted.		

Table 51: Summary table of mutagenicity/genotoxicity tests in mammalian somatic or germ cells in vivo.

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Micronucleus test OECD 474 GLP: yes	Mecetronium ethyl sulphate [MES] Purity: IUCLID technical dossier Solution (soluble in water and ethanol); 29.99% active component, density 0.99 g/ml, refraction index n20d 1,373, pH 7.3 (1% solution, no further data)	Species: mouse Strain: Crl:NMRI BR Number of animals per group: 5m + 5f per dose and sampling time Administration/exposure: Oral via gavage Number of application: Single oral application Dose: 0, 18.7, 56, 187 mg/kg bw plus positive control (all 24 h); 187 mg/kg bw (48 h)	Under the conditions of this assay the test substance did not induce a significant increase in the number of micronuclei at a dose level up to 187 mg/kg bw. This dose did not induce cytotoxic effects in the bone marrow. However, 187 mg/kg bw did not reach the dose level recommended in the OECD guideline 474. The study is not valid due to significant methodological deficiencies.	<confidential> (1994b)

Table 52: Summary table of human data relevant for germ cell mutagenicity.

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.8.1 Short summary and overall relevance of the provided information on germ cell mutagenicity

Summary of results obtained *in vitro*:

- Bacterial reverse mutation test (Bode Chemie, 1992f): under the experimental conditions described in this study the test substance did not induce gene mutation in bacteria. Reduced number of revertants/plate at concentration of 100 µg/plate in case of TA1535, TA1537 indicated cytotoxic effect. No evidence for mutagenic activity in the Salmonella microsome assay under study conditions, in concentrations ranging from 0.16 to 100 µg/plate taking into account, that current guideline 471 not completely fulfilled.
- Bacterial reverse mutation test (Bode Chemie, 1981): under the experimental conditions described in this study the test substance did not induce gene mutation in bacteria.
- In vitro mammalian chromosome aberration test (Bode Chemie, 1994): the test substance did not induce increases in aberrations even at cytotoxic concentrations. Under the condition of this test system no clastogenic activity is detected with the test substance mecetronium ethyl sulphate [MES]. No chromosome mutagenic activity in a cytogenetic study.
- Mammalian cell gene mutation assay (Bode Chemie 1994a): in the first assay a clearly positive result even at non-cytotoxic concentrations was presented. In the 2nd assay no mutagenic activity was detected. No 3rd independent assay was conducted to clarify the contradictory results. The conclusions of the authors (not mutagenic) are not comprehensible.
- In vitro mammalian cell gene mutation assay (Bode Chemie, 2008): both with and without S9-mix, mecetronium ethyl sulphate [MES] did not induce a relevant, dose-dependent increase in the mean frequency of TFT-resistant mutants. Under the conditions of this assay, mecetronium ethyl sulphate [MES] did not show evidence of inducing gene mutations in mouse lymphoma L5178Y/TK+/- cells.
- unscheduled DNA synthesis (UDS) in mammalian cells in vitro (Bode Chemie, 1981a): no indication for UDS activity under the given test conditions.

Summary of results obtained *in vivo*:

- Micronucleus test (1994b): the study is not valid due to significant methodological deficiencies (The concurrent negative and positive controls are valid. Under the conditions of this assay the test substance did not induce a significant increase in the number of micronuclei at a dose level up to 187 mg/kg bw. This dose did not induce cytotoxic effects in the bone marrow. However, 187 mg/kg bw did not reach the dose level recommended in the OECD guideline 474).

10.8.2 Comparison with the CLP criteria

Toxicological results	CLP criteria
Testing *in-vitro*: Bacterial mutation assay: negative results. Tests involving mammalian cells: generally negative results (only in one study in the first assay a clearly positive result even at non-cytotoxic concentrations was presented. In the 2nd assay no mutagenic activity was detected. No 3rd independent assay was conducted to clarify the contradictory results). Testing *in-vivo*: One test is available - micronucleus test: The study is not valid due to significant methodological deficiencies.	The classification in Category 1A is based on positive evidence from human epidemiological studies. Substances to be regarded as if they induce heritable mutations in the germ cells of humans. The classification in Category 1B is based on: - positive result(s) from in vivo heritable germ cell mutagenicity tests in mammals; or — positive result(s) from in vivo somatic cell mutagenicity tests in mammals, in combination with some evidence that the substance has potential to cause mutations to germ cells. It is possible to derive this supporting evidence from mutagenicity/genotoxicity tests in germ cells in vivo, or by demonstrating the ability of the substance or its metabolite(s) to interact with the genetic material of germ cells; or - positive results from tests showing mutagenic effects in the germ cells of humans, without demonstration of transmission to progeny; for example, an increase in the frequency of aneuploidy in sperm cells of exposed people. The classification in Category 2 is based on: - positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from: - somatic cell mutagenicity tests in vivo, in mammals; or - other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays. Note: Substances which are positive in in vitro mammalian mutagenicity assays, and which also show chemical structure activity relationship to known germ cell mutagens, shall be considered for classification as Category 2 mutagens.

10.8.3 Conclusion on classification and labelling for germ cell mutagenicity

No human data are available for mecetronium ethyl sulphate [MES], hence a classification in category 1A is not possible.

One available in-vivo study is not valid due to significant methodological deficiencies.

Taking into account the above mentioned information and on the basis of the negative results from in-vitro studies no classification and labelling of mecetronium ethyl sulphate [MES] for genotoxicity is proposed.

10.9 Carcinogenicity

Table 53: Summary table of animal studies on carcinogenicity.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
		No data	

Table 54: Summary table of human data on carcinogenicity.

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 55: Summary table of other studies relevant for carcinogenicity.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Table 56: Compilation of factors to be taken into consideration in the hazard assessment.

Species and strain	Tumour type and background incidence	Multi-site responses	Progression of lesions to malignancy	Reduced tumour latency	Responses in single or both sexes	Confounding effect by excessive toxicity?	Route of exposure	MoA and relevance to humans
No data								

Other existing data:

- data on genotoxicity: any evidence for genotoxic activity of mecetronium ethyl sulphate [MES],

- human experiences since 1964: repeated human exposure to a mixture which contains 0.2% mecetronium ethyl sulphate [MES] did not reveal any adverse effects. A mixture containing 0.2% MES has been widely used as a disinfectant by numerous customers during the last 40 years,
- data from other quaternary ammonium compounds available (Thorup, I. (2000)): alkyl dimethyl benzyl ammonium chloride was fed to rats in a two years study at dose levels of 0.015% to 0.5%. No indication for enhanced neoplasia was obtained. However, only limited organs were investigated. The tumorigenicity of benzalkonium chloride after dermal application was investigated in Female Swiss mice and New Zealand rabbits. The animals were treated twice a week on shaved dorsal skin under non-occlusive conditions with 8.5% or 17%. Neither local skin tumours nor systemic tumours were observed.

10.9.2 Comparison with the CLP criteria

There are no relevant data to compare with criteria (No experimental studies were performed to assess the carcinogenicity potential of substance).

10.9.3 Conclusion on classification and labelling for carcinogenicity

Classification and labelling is not proposed.

10.10 Reproductive toxicity

10.10.1 Adverse effects on sexual function and fertility

Table 57: Summary table of animal studies on adverse effects on sexual function and fertility.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
One-Generation Reproduction Toxicity Study OECD 415 GLP: yes Species: rats Strain: Wistar rats Sex: male and female	Mecetronium ethyl sulphate [MES] 29% (aqueous solution) Duration of exposure before mating: 70 days Duration of exposure in general:	Parent males and females (F0) 10 mg/kg-group: No treatment related effects 40 mg/kg-group Table for reproductive toxicity study – Parent (F0) data Note: endpoints as specified before were assessed and found to be unrelated to treatment. Therefore they are deleted from this table. Only treatment related (or possibly treatment-related) data are stated. - = no treatment related data observed	BODE Chemie (2008a)

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

Number of animals per group: 24 males and 24 females per group (total of 96 males and 96 females)

Animal assignment to dosage groups: Parental animals (F0) (designated as P in study report):

24 males and 24 females per group; 1 control and three dose groups

Fo, FI males, female

F0-generation:

Parent males: A 70-day pre-pairing period, during the pairing period and a 45-day after pairing period until necropsy, in total 120 days.

Parent females: A 70-day pre-pairing period, during the pairing, gestation and lactation periods until day 21 post partum, one day before necropsy, in total max. 120 days.

F1-generation (F0-offspring)

The F1-generation was potentially exposed to the test substance in uteri and through nursing during lactation.

From all F0 animals, samples of the above

Parameter		Gene-ration	control m	control f	Low Dose (10 mg/kgday) m	Low Dose (10 mg/kgday) f	Medium Dose (40 mg/kgday) m	Medium Dose (40 mg/kgday) f	High Dose (110 mg/kgday) m	High Dose (110 mg/kgday) f
Mortality	Incidence	**F0**	-	-	-	-	-	**1**	-	**4**
Food consumption	% compared to control	**F0**	-	-	-	-	-	-	**-7.4**	**-13.2**
Body weight gain	**% of control**									
	Pre-pairing period	**F0**	-	-	-	-	-	-	**83**	**90**
	Gestation period	**F0**	-	-	-	-	-	-	-	**76**
Clinical Observations *Salivation*	Incidence	**F0**	-	-	-	-	**2**	**7**	**13**	**9**
Rales		**F0**	-	-	-	-	**3**	**4**	**15**	**13**
Histopathologic examination *Changes in the **stomach***	Incidence	**F0**	-	-	-	-	**Yes**	**Yes**	**Yes**	**Yes**
Forestomach										
Acanthosis/Hyperplasia		**F0**	-	-	-	-	**3**	**1**	**18**	**5**
Hyperkeratosis		**F0**	-	-	-	-	**2**	-	**20**	**6**
Edema		**F0**	-	-	-	**1**	**3**	-	**5**	**4**
Inflammation, acute		**F0**	-	-	-	-	**1**	**1**	**4**	**3**
Erosion		**F0**	-	-	-	-	**1**	-	-	**2**
Ulceration		**F0**	-	-	-	-	**1**	-	-	**1**
Glandular stomach										
Erosion		**F0**	-	-	-	-	-	-	-	**2**
Ulceration		**F0**	-	-	-	-	-	-	-	**1**

Mortality:

One female was killed in extremis on day 44 of the pre-pairing period after showing a raised right foreleg, chromorhinorrhea, salivation, a hunched

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	tissues and organs were collected at necropsy and fixed in neutral phosphate buffered 4% formaldehyde solution. Histopathology was performed on the organs for all high dose and control F0 animals and for all selected animals which died during the study. Organs demonstrating pathological changes in these animals were also examined in the animals from the lower dose groups. Microscopic examination of all tissues showing gross pathological changes and of the reproductive organs of infertile males and females were made, if necessary. Implantation sites were counted for	posture, ruffled fur and decreased activity. Although no macroscopical findings were noted for this female, it cannot be excluded that an administration error had happened. Clinical signs: Few males (2: salivation, 3: rales) and females (7: salivation, 4: rales) had salivation and rales on single days. Four females had a hunched posture and ruffled fur during the pre-pairing period for maximal six days. One female showed additionally for one day ventral recumbency. Histopathology: Irritative and degenerative lesions located in the forestomach represented by: - acanthosis, - squamous hyperplasia, - hyperkeratosis, - acute inflammation, - edema, - erosion, - ulceration. 110 mg/kg-group Mortality: Two females were killed in extremis on day 24 post coitum and on day 2 post partum. Two other females were found dead on day 15 post coitum and on day 2 post partum. Clinical signs: In a number of males (13: salivation, 15: rales) and females (9: salivation, 13: rales) were noted during several days of the treatment period. Body weight gains: In males, mean body weight gain was reduced during the whole pre-pairing period and slightly reduced during the after pairing period. In females, mean body weight gain was reduced in the pre-pairing and the gestation periods until the end of the lactation period. Food consumption: Mean food consumption was reduced throughout the whole treatment period in males and females. Organ weights: Mean absolute organ weights as well as organ/body weight ratios were not	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
	all dams. The uteri were placed in a solution of ammonium sulphide to visualize possible hemorrhagic areas of implantation sizes.	affected by exposure to the test item. Changes in testes and epididymides weights were considered to be incidental and reflect the usual biological variability of individual values as no correlation were noted to microscopic findings. Histopathology: Irritative and degenerative lesions located in the forestomach represented by: - acanthosis, - squamous hyperplasia, - hyperkeratosis, - acute inflammation, - edema, - erosion, - ulceration. Further, single high dose females had: - minimal to slight erosion or - ulceration of the glandular stomach. At the end of the recovery period, no histopathological test item-related toxicological relevant changes in female rats were observed. Reproduction 10 mg/kg-group: No treatment related effects 40 mg/kg-group: No treatment related effects 110 mg/kg-group: The mean number of implantations per litter and the number of live pups as counted at first litter check were decreased. F1 males and females 10 mg/kg-group: No treatment related effects 40 mg/kg-group: No treatment related effects 110 mg/kg-group Mortality and clinical signs: The total number of pups lost during the first 4 days was 24 compared to 6 dead pups in the control group. Among the total number of pups lost, one total litter of 9 pups was found cannibalized on day 1 post partum. One other litter was found dead with 8 pups. In another litter, five unsuckled pups were found dead on day 2 post partum (no milk in stomach). This was considered to be a result of the moribund condition of the dams. Accordingly, the viability index was decreased. Body weight gains: From day 7 post partum onwards body weight development was statistically significantly reduced (+598% compared to 702% in the control group during the whole lactation period). This finding was considered to be test item-related. Table for reproductive toxicity study – Reproduction and Litter data Note: endpoints as specified before were assessed and found to be unrelated to treatment. Therefore they are deleted from this table. Only treatment related (or possibly treatment-related) data are stated.	

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference

- = no treatment related data observed

Parameter		Gene-ration	control	Low Dose (10 mg/kg day)	Medium Dose (40 mg/kg day)	High Dose (110 mg/kg day)
			m & f	m & f	m & f	m & f
Reproductive Performance						
Number of implantation sites	% of control	**F0**	**13.8**	**13.0**	**13.8**	**11.9***
Litter Data						
Living pups at first litter check	% of males/females	**F1**	**41/59**	**50/50***	**45/55**	**53/47***
Postnatal loss (days 0-4)	Total pups affected	**F1**	**6**	**10**	**4**	**24****
Viability index	%	**F1**	**97.7**	**96.6**	**98.5**	**89.1****
Pup mean bodyweight (day 7)	g males+females	**F1**	**14.3**	**14.3**	**14.2**	**12.9****
Pup mean bodyweight (day 14)	g males+females	**F1**	**30.2**	**29.9**	**29.7**	**26.1****
Pup mean bodyweight (day 21)	g males+females	**F1**	**47.3**	**47.7**	**46.8**	**41.9****

* or **: significance level at 5 or 1%

Table 58: Summary table of human data on adverse effects on sexual function and fertility.

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 59: Summary table of other studies relevant for toxicity on sexual function and fertility.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.10.2 Short summary and overall relevance of the provided information on adverse effects on sexual function and fertility

One-generation toxicity study was performed according to OECD 415. 192 (96 males and 96 females - 24 males and 24 females per dose group) Wistar rats were exposed by oral gavage to the test substance Mecetronium ethyl sulphate (MES) 29% (aqueous solution, vehicle (Milli-Q-Water) once daily.

The dose levels for the F0-generation were 0 (control), 10, 40 and 110 mg/kg bw/day.

F0 male animals were exposed to the test substance for a 70-day pre-pairing period, during the pairing period and a 45-day after-pairing period until one day before the scheduled sacrifice, in total 120 days.

F0 females received the test item during a 70-day pre-paring period and also during the pairing, gestation and lactation periods until one day before the scheduled necropsy, in total max 120 days.

Due to the occurrence of maternal toxicological effects, 10 females in groups 1 (vehicle control) and 4 (110 mg/kg bw/day) were tested for reversibility of the effects. Therefore, the females were given a 4 week treatment-free period (recovery period) and were mated again with untreated males. All females were allowed to give birth and rear their pups until day 4 post partum. One day after the pups the dams were sacrificed on day 5 post partum.

All animals were subjected to twice daily clinical observation. Body weight (daily) and food (weekly) consumption were measured over the treatment period. The regularity and duration of the estrus cycle was examined.

At necropsy, macroscopic observations and organ weights were recorded. A histopathological examination was performed on all reproduction organs and tissues. Reproduction parameters, breeding data and pup development were assessed.

At 10 mg/kg, no test item-related findings were noted.

At 40 mg/kg, single males and females had salivation and rales on single days during the treatment period. Histopathological changes were observed in the stomach in males and females at this dose level.

At 110 mg/kg, two moribund females were killed in extremis. Two other females were found dead. The reasons for deaths could not be established.

Several males and females had salivation and rales during single or multiple days of the treatment period.

Mean food consumption was reduced throughout the whole treatment period in males and females at this dose level.

In males, mean body weight gain was reduced during the whole pre-pairing period and slightly reduced during the after pairing period.
In females, mean body weight gain was increasingly reduced in the pre-pairing and the gestation periods until the end of the lactation period.
Histopathological changes were observed in the stomach in males and females, indicating local irritant effects.
The results of the recovery group revealed that all findings were completely reversible.

Reproduction data and pup development parameters indicated that the mean number of implantations per litter and the number of live pups noted at first litter check were decreased. Accordingly, the postnatal loss increased and the viability index was decreased.
Mean body weight development of the pups was statistically significantly reduced.

Since no test item-related effects on organ weights were observed and based on the histopathological findings in the stomach a local No-Observed-Adverse-Effect-Level (NOAELlocal) for the F0 parental animals was considered to be 10 mg/kg body weight/day.
The systemic No-Observed-Adverse-Effect-Level (NOAELsystemic) for the parental animals was considered to be 40 mg/kg body weight/day based on reduced body weight gain and clinical symptoms in males and females of the high dose group.
Based on postnatal loss and significantly reduced pup body weight development combined with maternal toxicity in the high dose group the No-Observed-Effect-Level (NOELreproduction) for reproduction (F1) was considered to be 40 mg/kg body weight/day.

10.10.3 Comparison with the CLP criteria

Toxicological results	CLP criteria
No human data are available for mecetronium ethyl sulphate [MES], hence a classification in category 1A is not possible. The available data does not provide evidence for toxic effects of mecetronium ethyl sulphate [MES] on fertility below doses causing maternal toxicity.	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on sexual function and fertility in the absence of other toxic effects, or - the adverse effect on reproduction is considered

	not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility - where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

10.10.4 Adverse effects on development

Table 60: Summary table of animal studies on adverse effects on development.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Species** **Strain**	**Results**	**Reference**
Teratogenicity study in the rabbit OECD 414 GLP: yes Number of animals per group: 24 animals per group at initiation; 20 (vehicle control), 20 (4 mg/kg bw/day), 20 (12 mg/kg bw/day), 16 (30 mg/kg bw/day), 10 (40 mg/kg bw/day) dams evaluated.	Mecetronium ethyl sulphate [MES] Colourless, viscous liquid, 29% active ingredient Exposure: Oral Type: Gavage	Species: Rabbit Strain: Himalayan	Maternal toxic effects Examination of dams control 1 out of 24 dams was not pregnant. 4 mg/kg bw/day this dose level caused no test substance related clinical effects and mortality. No effects were detected on body weight or body weight change. No effects were noted on food consumption relative to the control group. 12 mg/kg bw/day 2 out of 24 dams were not pregnant (within the normal range). 1 out of 21 dams aborted on gestation day (GD) 26 (considered as spontaneous). No effects were detected on body weight or body weight change. No effects were noted on food consumption relative to the control group. 30 mg/kg bw/day 2 out of 24 dams died prematurely on GD 22 or 28 (both with diarrhoea and lesions of the stomach). 5 further dams were sacrificed after abortion between GD 23 and 27; evaluation of individual test results revealed gastro-intestinal effects	BODE Chemie GmbH & Co. KG (2008b)

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Species
Strain</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>
of the test substance in all 5 rabbits with abortions: 1 out of these 5 dams has stomach lesions; diarrhoea, partly haemorrhagic was noted in 3 rabbits, and the last one showed minimal or no discharge of faeces.

One dam was without viable fetuses (haemorrhagic diarrhoea observed).

Decreased (p<0.01) body weight gain of dams was obvious; the absolute and relative food intake was reduced (p<0.01).

40 mg/kg bw/day

Mydriasis was observed in all dams from the 1st application onwards (starting 20-60 minutes after treatment and lasting 2-6 h).

8 out of 24 dams died prematurely on GD 18 or 27; evaluation of individual test results revealed that all of them had stomach lesions, 6/8 liquid content in the intestine and 6/8 diarrhoea.

4 further dams were sacrificed after abortion between GD 25 and 27; 3 out of these 4 dams had stomach lesions combined with liquid content in the intestine, the 4th had only a brownish liquid in the intestine; diarrhoea, was noted in 1/4 rabbits, 2/4 dams revealed minimal or no discharge of faeces.

One dam was not examined.

Decreased (p<0.01) body weight gain of dams; absolute and relative food intake was reduced (p<0.01).

Necropsy of dams (summary)

≤ 12 mg/kg bw/day

No test substance related changes of internal organs, placenta, gravid uterus weight and net body weight change.

30 mg/kg bw/day

Gastric lesions and aerated intestine with brownish/liquid content (see Table below). Slight reduction in gravid uterus weight and reduction (p<0.01) in net body weight change.

40 mg/kg bw/day

Severe gastro-intestinal lesions in deceased dams or dams with abortions as well as in individual dams surviving up to GD29 (see Table below); in nearly all of these dams the anogenital region was soiled with faeces.

Table: Macroscopic findings in the stomach, intestine, spleen and liver of dams at necropsy (at gestation day 29 & prematurely deceased rabbits)
<table>
<tr><th>Finding</th><th>Control n=21</th><th>4 mg/kg bw</th><th>12 mg/kg bw</th><th>30 mg/kg bw</th><th>40 mg/kg bw</th></tr>
</table>
</td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Species Strain	Results	Reference

		n=20	n=21#	n=24#	n=22#
Stomach					
Several/multiple haemorrhagic foci	0	0	0	1	12
Multiple ulcers	0	0	0	0	2
Mucosal detachment	0	0	0	2	0
Whitish layer	0	0	0	1	0
Intestine					
Liquid brownish content	0	0	0	6	12
aerated	0	0	0	8	1
Spleen					
Reduced size	0	0	0	0	4
Liver					
pale	0	0	0	0	2
#: (including) prematurely deceased dams					

Teratogenic/embryotoxic effects

The relevant reproduction data and data on developmental toxicity are given in the Table below.

≥ 40 mg/kg bw/day

No developmental effects were detected with respect to the number of corpora lutea (determined values: number per dam, absolute number per group, mean per group), implantations (number per dam, distribution in uterine horns, absolute number per group, mean per group), resorptions (number per dam, distribution in uterine horns, absolute number per group, mean per group, mean % per group, early resorptions, late resorptions), weight of placenta (individual data per foetus, mean per litter, mean per group, litter mean per group, litter mean per sex and group), weight of foetuses (individual data per foetus, mean per litter, mean per sex and litter, litter mean per group, litter mean per sex and group), foetuses (number of alive or dead per dam, number per sex and dam, distribution in uterine horns, absolute number alive per group, mean number alive per group, mean % alive per group, mean % per sex and group) when compared to the control. All these findings were considered to be within the spontaneous range.

Malformations & Variations

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Species
Strain</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td></td><td>No test substance related findings were recorded; the observed effects were within the spontaneous range in all treated groups. Exception is the effect detected at soft tissue examination of the head. Fetal and litter incidence of subdural haemorrhages in the meninx was elevated at 30 mg/kg bw/day. However, no corresponding effect was found at 40 mg/kg bw. Furthermore, this variation is also noted in control and low dose group and might be due to methodological shortcomings during dissection. For these reasons the subdural haemorrhages are considered to be incidental (random distribution).
<table>
<tr><th colspan="6">Table: Reproduction data</th></tr>
<tr><td>Finding</td><td>Control n=21</td><td>4 mg/kg bw n=20</td><td>12 mg/kg bw, n=20</td><td>30 mg/kg bw, n=17</td><td>40 mg/kg bw, n=10</td></tr>
<tr><td>Corpora lutea total</td><td>177</td><td>164</td><td>161</td><td>127</td><td>75</td></tr>
<tr><td>Corpora lutea per dam</td><td>8.4</td><td>8.2</td><td>8.1</td><td>7.5</td><td>7.5</td></tr>
<tr><td>Total implantation sites</td><td>162</td><td>145</td><td>153</td><td>116</td><td>63</td></tr>
<tr><td>Implantation sites/dam</td><td>7.7</td><td>7.3</td><td>7.7</td><td>6.8</td><td>6.3</td></tr>
<tr><td>Total resorptions</td><td>18</td><td>5**</td><td>2**</td><td>8</td><td>3</td></tr>
<tr><td>Resorptions/dam</td><td>0.9</td><td>0.3</td><td>0.1</td><td>0.5</td><td>0.3</td></tr>
<tr><td>Total early resorptions</td><td>17</td><td>4**</td><td>1**</td><td>7</td><td>3</td></tr>
<tr><td>Early resorptions/dam</td><td>0.8</td><td>0.2</td><td>0.1</td><td>0.4</td><td>0.3</td></tr>
<tr><td>Total late resorptions</td><td>1</td><td>1</td><td>1</td><td>1</td><td>0</td></tr>
<tr><td>Late resorptions/dam</td><td>0.0</td><td>0.1</td><td>0.1</td><td>0.1</td><td>0.0</td></tr>
<tr><td>Mean% pre-implantation loss</td><td>9.3</td><td>12.1</td><td>5.7</td><td>8.1</td><td>18.4</td></tr>
<tr><td>Mean% post-implantation</td><td>10.0</td><td>4.4</td><td>1.3</td><td>8.7</td><td>3.8</td></tr>
</table></td><td></td></tr>
</table>

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Species Strain	Results	Reference

loss						
Total live foetuses	144	140	151	108	60	
Live foetuses per dam	7.2	7.0	7.6	6.8	6.0	
Total dead foetuses at laparatomy	0	0	0	0	0	
Viable fetuses		No influence	No influence	No influence	No influence	
Sex distribution		No influence	No influence	No influence	No influence	

b) Developmental toxicity

Finding	Control n=20	4 mg/kg bw n=20	12 mg/kg bw, n=20	30 mg/kg bw, n=16	40 mg/kg bw, n=10
Placental weight (m&f, litter mean)	5.01+-0.89	5.31+-0.64	5.00+-0.73	4.70+-0.64#	5.43+-1.17
Fetal weight (m, litter mean)	38.9+-4.8	39.5+-3.3	38.9+-4.1	34.9+-5.9*#	38.5+-5.5
Fetal weight (f, litter mean)	38.1+-4.1	39.4+-3.9	38.9+-3.4	33.4±6.3**#	37.1+-3.4
External malformation & variation		No test substance related findings			
Skeletal malformation		No test substance related findings			
Skeletal variations & retardations		Within the range of control group			
Visceral examinatio	No pathological findings; no test substance related variations				

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Species Strain	Results	Reference

n							
Soft tissue of the head	No malformations						
Soft tissue of the head		Total variations within the range of control group					
Subdural haemorrha-ges of meninx (fetal incidence)	3 (4.2%, n=72)	8 (11.4 %, n=70)	3 (4.0%, n=75)	11** (20.8%, n=53)	1 (3.3%, n=30)		
Subdural haemorrha-ges of meninx (litter incidence)	3 (15.0 %)	6 (30.0 %)	2 (10.0 %)	9* (56.3%)	1 (10.0 %)		
*: p≤0.05; **: p≤0.01; #: incidental decrease, biologically not relevant, within the historical control range							

Table 61: Summary table of human data on adverse effects on development.

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.10.5 Short summary and overall relevance of the provided information on adverse effects on development

Maternal toxicity

Local effects were found in the gastro-intestinal tract at ≥ 30 mg/kg bw/day. Prematurely death and abortion is suggested to be due to these local effects. The NOAEL for local maternal effects is 12 mg/kg bw/day.

At 40 mg/kg bw mydriasis was observed in all treated dams. This could be interpreted as a systemic effect and thus, the NOAEL for systemic effects would be 30 mg/kg bw.

However, some scepticisms concerning this effect seem appropriate. The mydriasis started with the first application at about 20 –60 min after gavage and disaapeared after serveral hours, No other effect related to the central nervous system occurred. Therefore, mydriasis could also be interpreted as stress-induced effect: rabbits are excited and feel unwell due to stomach irritation immediately following the gavage. This interpretation seems to be even more consistent with the overall results of this study.

Systemic maternal effects were recorded at 40 mg/kg bw/day. The mydriasis effect was observed in all treated rats of the high dose group starting with the 1st application. Surprisingly, no mydriasis was detected at 30 mg/kg bw/day in any rabbit at any application. The NOAEL for systemic effects seem to be 30 mg/kg bw/day however, there is some scepticism.

Developmental toxicity

No developmental effects of biological relevance were detected at ≤ 40 mg/kg bw/day. Abortions are considered to be secondary to local effects on the gastro-intestinal tract of dams. Generally, the evaluation of groups with treatment related death of dams is limited. Therefore, the NOAEL of 40 mg/kg bw/day has some uncertainties.

10.10.6 Comparison with the CLP criteria

Toxicological results	CLP criteria
No human data are available for mecetronium ethyl sulphate [MES], hence a classification in category 1A is not possible. Mecetronium ethyl sulphate [MES] has no teratogenic properties. No developmental effects occurred below dose levels inducing severe maternal toxicity.	Category 1A: Known human reproductive toxicant Category 1B: Presumed human reproductive toxicant largely based on data from animal studies - clear evidence of an adverse effect on development in the absence of other toxic effects, or - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects Category 2: Suspected human reproductive toxicant - some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on development and - the evidence is not sufficiently convincing to

	place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. - the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects

10.10.7 Adverse effects on or via lactation

Table 62: Summary table of animal studies on effects on or via lactation.

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, dose levels duration of exposure	Results	Reference
No data			

Table 63: Summary table of human data on effects on or via lactation.

Type of data/report	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

Table 64: Summary table of other studies relevant for effects on or via lactation.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.10.8 Short summary and overall relevance of the provided information on effects on or via lactation

No information.

10.10.9 Comparison with the CLP criteria

No data.

10.10.10 Conclusion on classification and labelling for reproductive toxicity

No human data are available for mecetronium ethyl sulphate [MES], hence a classification in category 1A is not possible.

Mecetronium ethyl sulphate [MES] has no teratogenic properties. No developmental effects occurred below dose levels inducing severe maternal toxicity.

The available data does not provide evidence for toxic effects of MES on fertility below doses causing maternal toxicity.

No classification and labelling of mecetronium ethyl sulphate [MES] for reproductive toxicity is proposed.

10.11 Specific target organ toxicity-single exposure

Table 28: Summary table of animal studies on STOT SE.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
There is no evidence of specific target organ toxicity after single exposure of MES.			

Table 66: Summary table of human data on STOT SE.

Type of data/report	**Test substance**	**Route of exposure** **Relevant information about the study (as applicable)**	**Observations**	**Reference**
Data on the general population can be obtained from the biocidal product containing 0.2% of MES as a periodical safety up-date: The overall incidence of suspected drug reactions is very low (0.00018%). Any type of suspected drug reactions can be considered to be "very rare" (<0.01%). Skin irritation, suspected allergy and eye irritation are the commonly reported drug reactions, oral misuse occurred occasionally.				BODE Chemie (2006)

Table: Absolute and relative frequency of suspected drug reactions in connection with a mixture containing 0.2% MES between January 2000 and August 2005

Type of drug reaction	Number of incidents	Relative frequency per all hygienic hand disinfections
Suspected allergy	43	0.0000006%
Skin irritation	41	0.0000006%
Ocular irritation	24	0.0000003%
Oral misuse	7	0.0000001%
Respiratory tract irritation	**2**	**<0.0000001%**
Diarrhea	1	<0.0000001%
Burns	1	<0.0000001%
All	118	0.0000018%

Table 67: Summary table of other studies relevant for STOT SE.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

No toxicity to a specific organ in the absence of lethality was observed in acute oral or dermal toxicity studies.

10.11.2 Comparison with the CLP criteria

There is no evidence of specific target organ toxicity after single exposure of mecetronium ethyl sulphate [MES]. There is no evidence from human cases or epidemiological studies that mecetronium ethyl sulphate [MES] can have potential to be toxic/harmful to human health following single exposure.

10.11.3 Conclusion on classification and labelling for STOT SE

Classification and labelling is not required.

10.12 Specific target organ toxicity-repeated exposure

Table 68: Summary table of animal studies on STOT RE.

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, route of exposure, dose levels, duration of exposure**	**Results**	**Reference**
4-Week dose-range finding study for a 90-Day subchronic toxicity study of mecetronium ethylsulfate by repeated oral administration to Sprague-Dawley rats GLP: yes Species: rat Strain: Sprague-Dawley Number of animals per group: 5 m and 5 f per group	Administration/exposure: oral Type: gavage Duration of treatment: 4 weeks Dose: 0, 50, 150, 450 mg/kg bw; in the high dose group dosing was discontinued on day 5.	**Clinical signs**: No treatment related effects were observed in the low dose group. In the mid dose group one female rat died at day 22 but other did not show any effects. Piloerection was seen in the high dose group at day 4, in addition reduced motility in 1 male and 1 female; on day 5 1 female showed reduced motility, ataxia and ptosis. All animals of the high dose group were in a poor condition on day 5, 2 were moribund (all surviving rats sacrificed on day 5). **Mortality**: 1 female in the mid dose group; 1 male and 2 females of the high dose group were found dead in the morning of day 5. **Body weight gain**: The body weight gain in the low dose groups and females of the mid dose group was not different from the control values. In the mid dose group males there was a slight decrease (3-9%) but not statistically significant. **Food consumption**: Food consumption was not influenced by the treatment in the low and mid dose group. However, a slight decrease (minus 12%) was observed in males at 150 mg/kg bw (not significant). **Gross pathology**: Macroscopical post mortem findings: in surviving rats no treatment related effects were seen in any group. Same results in rats which died during exposure period except the female rat of the mid dose group (heamorrhagic, distended and empty gastro-intestinal tract). LOAEL=150 mg/kg bw NOAEL = 50 mg/kg bw	<confidential> (2001)

90-Day toxicity study of mecetronium ethylsulfate by repeated oral administration via gavage to Sprague-Dawley rats OECD: 408 GLP: yes Species: rat Strain: Sprague-Dawley Number of animals per group: 10 m and 10 f per group	Test material: Mecetronium ethyl sulphate Purity: IUCLID technical dossier Exposure: oral Type: gavage Duration of treatment: 90 days Frequency of exposure: Once daily, 7 days per week Dose: 0, 15, 45, 135/90 mg/kg bw (until day 73 135 mg/kg bw in the high dose group, from day 74 onwards only 90 mg/kg bw due to mortality in the high dose group)	**Clinical signs**: No treatment related effects were observed in the low dose group. In the mid dose group long-lasting piloerection was reported starting at day 59 (20/20 rats at day 60). Piloerection was also seen in the high dose group at day 29 (4/10 m and 3/10 f) and day 30 (all rats) without reversibility. **Mortality**: 3 male and 7 female rats in the high dose group died between day 34 and 73. At day 74 the dose was reduced to 90 mg/kg bw and no further mortality was observed in the high dose group. **Body weight gain**: The body weight gain in the low and mid dose groups was not different from the control values. Males of the high dose group revealed a significant decrease from week 1 to termination (11-20% below control value). A slight but not significant decrease was observed in females of the high dose group from week 6 onwards (1-12% below control value). **Food consumption and compound intake**: Food consumption was not influenced by the treatment in the low and mid dose group. In the high dose group males showed significant reduced food consumption at week 1 (not at week 2-13). A transient decrease in food intake was seen in females at week 1-8 (significant at week 1, 2, 6, and 7). **Haematology**: No treatment related effects in the low and mid dose groups. Effects in males and females of the high dose group indicate inflammatory responses: increased leucocytes and a shift to the left in differential blood cell counts. **Clinical chemistry**: No treatment related effects were recorded on the parameters sodium, potassium, glucose, total cholesterol, urea, creatinine, total protein, and albumin. Changes in ALAT (males and females of the mid and high dose goup) but not in ASAT and aP are discussed by the authors as treatment related. The ALAT values in medium and high dose group are slightly above the historical control range presented by Charles River (18-45 U/l; no historical data of the performing laboratory given by the authors). The increase in aP values is within the historical control range (30-240 U/l; Charles River, no laboratory data). **Organ weights**: The relative and absolute organ weights (including adrenals) were within the normal range in all treatment groups. LOAEL= 90 mg/kg bw NOAEL = 45 mg/kg bw	<confidential> (2002)

Table 69: Summary table of human data on STOT RE.

Type of data/report	**Test substance**	**Route of exposure** **Relevant information about the study (as applicable)**	**Observations**	**Reference**
No data				

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Table 70: Summary table of other studies relevant for STOT RE.

Type of study/data	Test substance	Relevant information about the study (as applicable)	Observations	Reference
No data				

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

28-day toxicity study of mecetronium ethyl sulphate [MES] by repeated oral administration via gavage to Sprague-Dawley rats is the dose range finding study for the documented OECD guideline 408 study. 5 male and 5 female rats per group were gavaged with 0, 50, 150, 450 mg/kg bw/day (concentration 0, 1, 3, or 9% in water) for 28 days. Examinations are therefore limited to clinical signs, mortality, body weight gain, food consumption and macroscopical post mortem findings.

Under the conditions of this 28-days gavage study the NOEL was 50 mg/kg bw/day. 450 mg/kg bw/day resulted in mortality within a few days of exposure. There is evidence that the mid dose level is applicatively as the high dose level in a subchronic gavage study (135 mg/kg bw/day was chosen).

The 90-Day toxicity study of mecetronium ethylsulfate by repeated oral administration via gavage to Sprague-Dawley rats is performed according to OECD guideline 408. 10 male and 10 female rats received via gavage 0, 15, 45, 135/90 mg/kg bw/day (0.3, 0.9, 2.7/1.8% in water) for 90 days, 7 days a week.

The authors of the study stated that the NOEL was 15 mg/kg bw. They mentioned in the summary a slight increase in ALAT and the piloerection at a dose level of 45 mg/kg bw.

However, a NOAEL higher than 15 mg/kg bw can be considered. Historical data on ALAT activity have shown that the activity in the mid dose group is only slightly higher (48 versus 45 U/l) in comparison to historical control data and no correlate for this increase has been detected in further investigations indicating low toxicological importance of this observation.

It is questionable whether the piloerection in mid dose group is, however, treatment related since the incidence is 0/10, 2/10, 10/10 in males at day 58, 59, and 60, respectively and in females 0/10, 3/10, 10/10 at day 58, 59, and 60, respectively (persistent up to termination in males and females). Other reasons than the treatment might be considered since there is a suddenly occurring effect in all rats at the same time point (day 60). Furthermore, no other clinical signs were observed and the functional observation at the end of the exposure period revealed no treatment related effects. In acute oral toxicity studies piloerection was detected after a single application of 80 mg/kg bw in 10/10 rats (compare with piloerection in the high dose group).

In summary, in this study primarily local effects in the stomach were induced. Clear evidence for gastritis was given at the high dose level. Under the condition of this investigation the NOAEL was 45 mg/kg bw.

Minor items

The reliability of data on parameters determined at the end of the exposure period is limited in the high dose group due to low number of surviving animals.

10.12.2 Comparison with the CLP criteria

Toxicological results	CLP criteria
Data on significant toxicity in humans are lacking and guidance values are not applicable.	Category 1 (H372): Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure. Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of: reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations. Equivalent guidance values for 28-day and 90-day studies: Oral, rat: 28-day: ≤ 30 mg/kg bw/d 90-day: ≤ 10.0 mg/kg bw/d
On the basis of evidence from studies with repeated exposure in experimental animals at a moderate concentration, the observed effects not to have the potential to produce significant toxicity in humans (in accordance with Guidance to Regulation (EC) No 1272/2008 on Classification, Labelling and Packaging of substances and mixtures; Version 4.1 June2015). Hence, no classification for "STOT-RE" for oral exposure is proposed.	Category 2 (H373): Substances that, on the basis of evidence from studies in experimental animals can be presumed to have the potential to be harmful to human health following repeated exposure. Substances are classified in category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations. In exceptional cases human evidence can also be used to place a substance in Category 2. Equivalent guidance values for 28-day and 90-day studies: Oral, rat: 28-day: 10.0 < C ≤ 100.0 mg/kg bw/d 90-day: 30.0 C ≤ 300.0 mg/kg bw/d

10.12.3 Conclusion on classification and labelling for STOT RE

Classification and labelling for STOT RE is not proposed.

10.13 Aspiration hazard

Table 71: Summary table of evidence for aspiration hazard.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
Not applicable				

10.13.1 Short summary and overall relevance of the provided information on aspiration hazard

Not applicable – mecetronium ethyl sulphate [MES] is a solid substance.

10.13.2 Comparison with the CLP criteria

Not applicable.

10.13.3 Conclusion on classification and labelling for aspiration hazard

Classification and labelling for aspiration hazard is not proposed.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

11.1 Rapid degradability of organic substances

Table 72: Summary of relevant information on rapid degradability.

Method	Results	Remarks	Reference
OECD 301D Closed Bottle test	Composition of product: MES 30% in water Analytical parameter: Dissolved O_2 concentration Initial TS concentration: 12.9 mg/L and 8.09 mg/L Degradation: 12.9 mg/L: < 5% BOD of COD resp. ThOD after 29 days < 5% BOD of COD resp. ThOD after 60 days 8.09 mg/L: < 5% BOD of COD resp. ThOD after 29 days < 5% BOD of COD resp. ThOD after 60 days The test results indicate that MES is not readily biodegradable.	Test duration extended to 60 days The study is not GLP compliant. No information on nitrification. Possible partial microbial inhibition of the test substance.	BODE Chemie (1995)
OECD 301A DOC Die Away test	Composition of product: MES 29% in water Initial TS concentration: Replicate 1: 135 mg MES solution / 2	Study is not GLP compliant. Test substance was redosed on test day 1	BODE Chemie (1999)

Method	Results	Remarks	Reference
	L on day 0 (= 10 mg DOC/L), re-dosed with 150 mg / 2 L on day 1 (= 12 mg DOC/L) Replicate 2: 140 mg MES solution / 2 L on day 0 (= 11 mg DOC/L), re-dosed with 151 mg / 2 L on day 1 (= 12 mg DOC/L) DOC removal after 28 days (based on day 3 DOC value): Replicate 1 = 23.2 % Replicate 2 = 42.4 % mean = 32.8 % The test results indicate that MES is not readily biodegradable.	(All vessels containing the test substance initially received MES amounts corresponding to 10 - 11 mg DOC/L. Vessels containing MES were redosed with 12 mg DOC/L each, because DOC concentration was found to have decreased to ~3.5 mg DOC/L after 24 hours, probably due to adsorption processes.) No information on DOC content and removal in the blanks.	
OECD 301F Manometric Respirometry Test GLP: yes	Composition of product: 29.4% solution in water Initial TS concentration: 29 and 100 mg a.s./L. The rate of degradation was monitored by measuring the quantity of oxygen required to maintain a constant gas volume in the respirometer flasks over a 28-d period. The test item was applied at concentrations of 29 mg/L and 100 mg/L. Sodium benzoate was used as reference item at a concentration of 100 mg/L, along with a toxicity control at 29 mg MES /L as well as 100 mg MES /L, and 100 mg/L sodium benzoate. Additional assays were prepared with the addition of silica gel, which is an approved method to alleviate possible inhibitory effects of cationic surfactants on the inoculum. The biodegradation of MES in the static test was found to be 42% for a concentration of 29 mg test item per litre and 0% for a concentration of 100 mg test item per litre after 28 days applying the standard test design. In the presence of silica gel, 60% of the test item in the 29 mg/L assays and 50% in the 100 mg/L assays were degraded at test end. For both test item concentrations (29 mg/L and 100 mg/L), no biodegradation above the threshold value of 60% within a 10-day-window	Analytical parameters: Oxygen consumption	Bode Chemie (2008c)

Method	Results	Remarks	Reference
	could be obtained, independent whether silica gel was applied or not.		
OECD 310 GLP: no	Composition of product: MES 29 % in water. Initial TS concentration: 20 mg TOC/L (five additional flasks with 10 mg TOC/L) There was no degradation of the test item within 28 days (-10.3%). The negative degradation extent may be interpreted as inhibition effect to the inoculums. The five additional flasks with a concentration of 10 mg TOC/L only showed also negative degradation extents on day 28. The reference compound sodium benzoate reached the pass levels for ready biodegradability within 7 days The degradation extents in the inhibition control stayed on average below 25% during the whole test. On day 28 the mean degradation extent was -4.71%. Therefore the test item had toxic effects on the inoculum. This is in line with the negative degradation extents in the test item flasks. The test results indicate the toxic effects of MES on the inoculum. MES is not readily biodegradable at the applied concentrations due to toxic effects. The study is acceptable as supporting information.	Analytical parameter: Total inorganic carbon (TIC)	BODE Chemie (2011a)
OECD 310 GLP: no	Composition of product: MES 29% in water Initial TS concentration: 10 mg C/L (Assay A) and 20 mg C/L (Assay B) The degradation of Dimethylethylhexadecylammonium-ethylsulfate (MES) in the static test after 28 days on the basis of the ThIC of the test item initially applied was found to be on average 65 and 37 % for test assay A and B, respectively. However, 100 % (assay A) and 70 % (assay B) degradation were reached even after 14 days of incubation. The decreased degradation rates at the end of the test can be explained by	Analytical parameter: Total inorganic carbon (TIC) Silica gel was used in all assays to reduce possible inhibitory effects	BODE Chemie (2011b)

Method	Results	Remarks	Reference
	unexpected high IC values in the inoculum blanks at this sampling date. While IC content in the blanks although strong varying maintained nearly constant from day 7 to day 21, the values jumped up by 2/3 during the last test week. In contrast, the mean increase of CO_2 production for the test assays was clearly lower during this last period as biodegradation is nearly completed after 14 days. However, from experience it is known that studies with rapidly degradable substances can result in decreasing degradation rates at the end of the tests, when test item has been (fully) mineralized but the inoculum blanks are still active. Moreover, variance of TIC and thus biodegradation value in test assay A at the end is very high, due to an extreme low value in one replicate. In conclusion, the 14 day values are more relevant for the evaluation of this study and thus for the biodegradation potential of MES. As degradation at 20 mg C/L (assay B) is lower than for 10 mg C/L (assay A), degradation rate was dependent on test item concentration. This dependancy can be explained by probable toxic effects at higher concentration although silica gel is used to reduce these effects, and the higher ratio of test substance to initial cell concentration (inoculum concentration). Therfore, this effect is in agreement with theoretical considerations. With degradation rates of 104 % (assay A) and 70 % (assay B) at day 14, the threshold value of 60 % was surpassed within the 10-day window. The degradation within the 10-day-window could be expected to be > 60 % for both test assays. Due to nonlinear degradation curves at the beginning of a study, it can be assumed that the 10-day window starts at around day 4 for both assays. No lag phase / adaptation phase was noticeable in both assays. The percentage degradation of the reference item has exceeded the pass level of 60 % by day 14. The IC content in the inoculum blanks at the end of the study exceeds the requirements of the guideline.		

Method	Results	Remarks	Reference
	However, the background value of the IC at day 0 in all vessels and in the blank controls is relatively high and already exceeds the required threshold value. The source of such high value is unclear as deionized water has been used as dilution water, and the inoculum has been aerated over night to purge the system of carbon dioxide. However, decisive for the biodegradation is not the TIC present at the end, but the IC content built in the inoculum blank in comparison to the IC content built in the test vessels. Of course, if the starting value will be subtracted the IC content built in the inoculum blanks (at test end 24.1 mg IC/L) exceeds still the threshold value of 3 mg IC/L, but even when the IC content built in the inoculum blanks is above the threshold value, the results in the test and reference assays during the test are plausible at least for the 14 day values. Overall, due to a degradation achieving the threshold value of 60% after 14 days and within a 10-day-window, the test item Dimethylethylhexadecylammonium-ethylsulfate (MES) can be identified to be readily biodegradable under the chosen test conditions.		
OECD 310 GLP: no	Composition of product: MES 29% in water All flasks containing the test substance initially received MES amounts corresponding to 10 mg C/L. All setups were inoculated with a mixed inoculum from activated non-adapted sewage sludge. In order to check the influence of inoculum concentration and washing of the inoculum, three varities were applied: A: 10 mg SS/L, washed, B: 4 mg SS/L, washed, C: 4 mg SS/L, unwashed. Silica gel was used in all assays to reduce possible inhibitory effects. The degradation of Dimethylethylhexadecylammonium-ethylsulfate (MES) in the static test was found to be 100, 87, and 76 % for test assay A, B, and C, respectively, after 14 days on the basis of the ThIC of the test item initially applied. It was shown that degradation rate was depending on inoculum	Deviations: test duration of 14 days	BODE Chemie (2011c)

Method	Results	Remarks	Reference
	concentration and on the physiological properties of the inoculum. Washing resulted in a higher degradation rate. The degradation within the 10-day-window could be expected to be > 60 % for all test assays. With degradation rates of at least 87 % (assay B) and 76 % (assay C), this was confirmed for assays B and C. No lag phase / adaptation phase was noticeable in assay A. A lag phase / adaptation phase of ca. seven days was observed in the assays B and C, which has to be blamed on the lower inoculum concentration in these assays. The IC content in the inoculum blanks at the end of the study exceeds the requirements of the guideline (< 3 mg C/L). However, the background value of the IC at day 0 in all vessels and in the blank controls is relatively high and nearby the required threshold value. The source of this background level is unclear as deionized water has been used as dilution water, and the inoculum has been aerated over night to purge the system of carbon dioxide and in the case of assay A and B washed. It is expected that it is technically difficult to achieve lower background values, as at neutral and basic pH carbon dioxide will be trapped from the surrounding air. However, decisive for the biodegradation is not the TIC present at the end, but the IC content built in the inoculum blank in comparison to the IC content built in the test vessels. If the starting value will be subtracted, the mean IC content built in the inoculum blank of test assay A (1.6 mg IC/L) at test end was below the threshold value of 3 mg IC/L. The IC content built in the inoculum blanks of the assays B (4.3 mg IC/L) and C (6.6 mg IC/L) at test end are above the threshold value. However, the results in test assays B and C are plausible and are in line with test assay A. Due to a degradation achieving the threshold value of 60% after 14 days and within a 10- day-window, the test item		

Method	Results	Remarks	Reference
	Dimethylethylhexadecylammonium-ethylsulfate (MES) can be dentified to be readily biodegradable under the chosen test conditions and expected to be in general. The test results indicate that MES is readily biodegradable. The study is acceptable as supporting information.		
OECD 301B Ready Biodegradability Modified Sturm Test GLP: yes	Composition of product: MES 29% in water Due to the toxicity of the test item a test concentration of TOC 10 mg/L was chosen. All flasks containing the test substance initially received MES amounts corresponding to 40 mg/L test item. This corresponds to 12 mg/L MES or 7.5 mg C/L (nominal), or 7.4 mg C/L (measured). Additional replicates with addition of 10 mg/L humic acid (corresponding approximately to the test item concentration) to adsorb the test item and reduce the toxicity were set up. All setups were inoculated with an aqueous phase of non-adapted activated sludge from municipal STP. For the test item replicates without humic acid, the mean 10 % level (beginning of biodegradation) was reached on day 8. The 60 % pass level was not reached within 28 days. The mean biodegradation after 28 days was 48 %. For the test item replicates with humic acid, the mean 10 % level (beginning of biodegradation) was reached on day 5. The 60 % pass level was reached within 15 days and the 10-day-window was fullfilled. The mean biodegradation after 28 days was 97%. To check the activity of the test system, sodium benzoate was used as functional control. The percentage degradation of the functional control reached the pass level of 60 % within 6 days and a biodegradation of 97 % after 28 days. In the toxicity control containing both test and reference item (sodium benzoate) a biodegradation of 60 % was determined within 14 days and	Deviations: Due to the toxicity of the test item a test concentration of TOC 10 mg/L was chosen. Furthermore additional replicates with addition of 10 mg/L humic acid (corresponding approximately to the test item concentration) to adsorb the test item and reduce the toxicity were set up.	BODE Chemie (2013a) BODE Chemie (2013b)

Method	Results	Remarks	Reference
	reached 81 % after 28 days. The biodegradation of the reference item was not inhibited by the test item in the toxicity control. However, the net CO_2 production was delayed in comparison to the functional control. This effect indicates that the test item caused inhibitory effects on the bacteria. The total CO_2 evolution in the inoculum control at the end of the test was 29.0 mg/L (validity criterion: < 40 mg CO_2/L after 28 days). The IC content of the test substance suspension in the mineral medium at the beginning of the test was less than 5% of the TC (IC: 189 µg/L, TC: 7.40 mg/L after application of the test item). In the presence of humic acid the test item is classified as readily biodegradable within 28 days and complying to the 10-day-window.		

11.1.1 Ready biodegradability

The quaternary ammonium compound Mecetronium ethyl sulphate [MES] has a high adsorption potential and is toxic to bacteria with EC_0 = 5.9 mg/L. These substance characteristics generate several difficulties following standard conditions in test systems on ready biodegradability, and complicated the technically feasibility of such tests as well as the interpretation of the results. It is well known in the special case of cationic surfactants such as hexadecyl-trimethylammonium chloride, due to their inhibition to bacteria at relatively low concentrations, that adding silica gel at an optimal concentration balances the effects of toxicity with non-availability to the micro-organisms (Painter et al., 2003). These results and the suitability of silica gel are also mentioned within the guideline OECD 310 (CO_2-headspace screening test) to reduce toxic effects on the inoculum. Similar results could be shown by van Ginkel et al. (2008) investigating the influence of silica gel or humic acid on the biodegradation of octadecyl-trimethylammonium chloride in the Closed Bottle Test.

Nevertheless, there are several studies on ready biodegradability of the test substance Mecetronium ethyl sulphate [MES] available.

In a Closed Bottle test according to OECD 301D (BODE Chemie (1995)) degradation was below 5% BOD for 8.09 mg/L and 12.9 mg/L test substance concentration after 29 and 60 days. However, oxygen consumption in the toxicity controls was slightly lower than in the corresponding procedure

controls. Therefore, a partial inhibition of bacterial degradation due to test substance toxicity cannot be excluded. Generally, the Closed Bottle Test is the most sensitive of the test systems for ready biodegradability with respect to bacterial toxicity.

In a DOC-Die-Away test according to OECD 301A (BODE Chemie (1999)) mean DOC removal was 32.8%. In the abiotic control the percentage elimination was 33%, and thus is in the same range as the elimination in the vessels with test substance. As the results of the toxicity control demonstrated that MES has no inhibitory effect to sewage sludge microorganisms, the elimination can be attributed to an abiotic removal, probably adsorption. This is conformed by the high adsorption potential of MES. However, the test substance concentration was reduced in all test vessels containing MES in an extent of approximately 60-70% within 1 day. Therefore, the test substance was re-dosed on day 1 and the DOC content of the test vessels has stabilised on day 3. The initial test substance concentrations for the two replicates were 10 and 11 mg DOC/L; re-dosing with 12 mg DOC/L. Due to the initial high adsorption of 60-70% the test system was not appropriate for the determination of the ready biodegradability of MES. Moreover, the expected toxic effects (EC_0 = 5.9 mg/L) could not be observed in the toxicity control. In conclusion, the results of this study on biodegradation are not reliable.

The results of these both older studies on biodegradation clearly indicate the two difficulties for performing ready biodegradation studies: the toxicity of the test substance against bacteria (including those of the inoculum) and the adsorption of the test substance on surfaces. Therefore, the standard test designs have to be adapted.

In a new study a Manometric Respirometry test according to OECD 301F (Bode Chemie (2008c)) was performed with and without silica gel. For both approaches two test item concentrations of 29 mg/L and 100 mg/L were applied. In the test with silica gel, biodegradation was 44.5% (29 mg/L) and 38.2% (100 mg/L) after 14 days. After 28 days biodegradation was observed to be 60.2% (29 mg/L) and 49.8% (100 mg/L). In the test without silica gel a biodegradation of 42.5% after day 28 was observed for 29 mg/L test item concentration, whereby degradation started earliest at day 20; no biodegradation was found for 100 mg/L. The prolongation of the lag phase in the test without silica gel clearly demonstrated the toxic effects of MES towards the inoculum. Hence, the use of silica gel in the test system was necessary; this is an approved method to alleviate possible inhibitory effects of cationic surfactants on the inoculum.

In a further study using the Modified Sturm Test according to OECD 301 B (BODE Chemie (2013a)) the effect of humic acid has been investigated on the biodegradation of MES in a concentration of 12 mg/L. For the test item replicates without humic acid, the mean 10 % level (beginning of biodegradation) was reached on day 8; the 60 % pass level was not reached within 28

days; the mean biodegradation after 28 days was 48 %. However, for the test item replicates with humic acid, the mean 10 % level (beginning of biodegradation) was reached on day 5; the 60 % pass level was reached within 15 days; the mean biodegradation after 28 days was 97%. Therefore, using humic acid the toxicity of MES in ready biodegradability tests could be reduced and MES meets the pass level as well as the 10 day window criterion.

Additionally following CO_2-headspace non-GLP screening tests according to OECD 310 have been performed, which can be used as supporting information.

In the CO_2-headspace screening test according to OECD 310 (BODE Chemie (2011b)) 15 test flasks with 20 mg TOC/L and 5 flasks with 10 mg TOC/L have been prepared. The IC content was determined after 7, 14, 21 and 28 days for the 15 test flasks (10 mg TOC/L) and at day 28 for the additional 5 flasks (10 mg TOC/L). The mean degradation of the test item was observed to be -10.3% for the 15 flasks and was also negative for the additional ones. The mean degradation in the inhibition controls was -4.71% at day 28. These negative results demonstrate that the test item at concentrations > 10 mg TOC/L had toxic effects on the inoculum.

In the CO_2-headspace screening test according to OECD 310 (BODE Chemie (2011c)) two assays, each with 10 replicates, with test item concentrations of 10 mg C/L (assay A) and 20 mg C/L (assay B), were monitored over a period of 28 days. The test was performed using silica gel. Degradation was observed to be 100% for assay A and 80% for assay B. The biodegradation within a 10-day-window was expected to be > 60% in all assays. The degradation rates were 104 and 70% at day 14. However, IC content in the inoculum blanks was above the threshold value of 3 mg IC/L at test end. Nevertheless, the results in the test and reference assays were plausible. In conclusion, the test item mecetronium ethyl sulphate [MES] was regarded to be readily biodegradable under the chosen test conditions.

In order to check the influence of inoculum concentration and washing of the inoculum, in the CO_2-headspace screening test according to OECD 310 (BODE Chemie (2011c)) different samples of activated sludge for the inoculum as well as silica gel were used. Three assays were prepared. For assay A and B, the sludge was washed in mineral medium and centrifuged. The supernatant was discarded. The concentrated sludge was suspended in mineral medium to a concentration of 1 – 3 g suspended solids/L. For assay C, the untreated sludge was suspended in mineral medium to a concentration of 1 – 3 g suspended solids/L. The concentration used in the test was 10 mg SS/L (A) and 4 mg SS/L (B and C). The test item concentration was 10 mg C/L in each assay. Biodegradation was observed to be 100% (assay A), 87% (assay B) and 76% (assay C).

The biodegradation within a 10-day-window was expected to be > 60% in all assays. The IC content in the inoculum blank of assay A was below the threshold of 3 mg IC/L. Therefore, the

validity criterion is fulfilled and the test is considered as valid. However, the IC content build in the inoculum blanks of the assays B (4.3 mg IC/L) and C (6.6 mg IC/L) at test end are above the threshold value, the results in these assays at test end are plausible and are in line with test assay A. In conclusion, the test item mecetronium ethyl sulphate [MES] was regarded to be readily biodegradable under the chosen test conditions.

The presented studies clearly demonstrate that the high adsorption potential and the potential toxicity of mecetronium ethyl sulphate [MES] may influence the results of the performed tests on ready biodegradability, and thus these substance properties have to be considered within the interpretation of the test results.

Overall, the results of the studies using humic acids or silica gel to reduce toxicity effects on biodegradation results indicate clearly that mecetronium ethyl sulphate [MES] could be regarded as readily biodegradable fulfilling 10-day-window.

Mecetronium ethyl sulphate [MES] belongs to the group of quaternary ammonium compounds (QAC`s), a group of cationic surfactants, which are structurally similar with respect to the embedded quaternary nitrogen and at least one long chain length.

Publicly available information on such QACs (Painter et al, 2003, OECD 310), van Ginkel et al. 2008), clearly demonstrate that only using silica gel or humic acid will result in an observation of sufficient biodegradation in the standardized ready biodegradability test systems. In Painter et al. (2003) and in OECD 310 the biodegradation of hexadecyltrimethylammonium chloride is given to be 75% using silica gel to reduce toxicity. Van Ginkel et al. (2008) investigated the biodegradation of octadecyltrimethylammonium chloride in the Closed Bottle Test using silica gel, lignosulphonic acid and humic acid. While without using such auxiliaries biodegradation could not be observed, the substance meets the pass level of 60% as well as the 10-day-window criterion using silica gel or humic acid.

Ambiguous results of early biodegradation studies with mecetronium ethyl sulphate [MES] are related to the high adsorption potential and the toxicity of MES towards the inoculum (closed bottle test and DOC-Die-away Test). However, more appropriate recent studies (manometric respiration test, Modified Sturm Test and various CO_2-headspace screening tests) demonstrate that the test substance can be regarded as readily biodegradable.

Overall, mecetronium ethyl sulphate [MES] has to be classified to be readily biodegradable fulfilling 10 day window.

In addition, for several monoalkyl quaternary ammonium compounds (QACs), which are structurally closely related to mecetronium ethyl sulphate [MES], primary and ultimate biodegradability was demonstrated. In a modified SCAS test, Games et al. (1982) found rates of > 88% ultimate degradation of radiolabelled C18-trimethylalkylammonium chloride after 7 days. Garcia et al. (2001) observed biodegradation of QACs over time by DOC determinations. In case of C16-trimethyl¬alkylammonium bromide, 100% primary biodegradation was reached after 6 days, and ultimate biodegradation was complete after 11 days. A more specific analysis was applied by Nishiyama et al. (1995). The authors used ion chromatography and 1H-NMR for the identification of degradation pathways and concluded that long-chain (C_8-C18) trimethylalkyl QACs are ultimately biodegradable. Identified initial degradation pathways were N-dealkylation, N-demethylation and ω-oxidation. Multiple further findings of biodegradability of QACs are discussed by Boethling (1984) and Ying (2006).

11.1.2 BOD_5/COD

BOD_5/COD test are not available.

11.1.3 Hydrolysis

There are no tests available on the hydrolysis of mecetronium ethyl sulphate [MES] in aqueous solutions that would allow deriving a reaction rate for surface waters.

However, mecetronium ethyl sulphate [MES] dissociates in aqueous solution, generating the ethylsulphate anion and the mecetronium cation. Ethylsulphate then further hydrolyses to give ethanol and sulphuric acid. In contrast, the molecular structure of the mecetronium cation has no hydrolysable functional group. Therefore, the substance is not a candidate for noteworthy hydrolysis. Furthermore, the substance is marketed as a 29% aqueous solution and the solution proved to be stable under these conditions.

11.1.4 Other convincing scientific evidence

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

Field investigations and monitoring data are not available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

Inherent and enhanced biodegradability test data are not available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

No further studies are available which deal with the rate and route of degradation in aquatic systems (incl. sewage treatment plants) and with the degradation in soil.

11.1.4.4 Photochemical degradation

Photolysis in water

There are no tests available on the photolysis of MES in aqueous solutions that would allow deriving a reaction rate for surface waters.

However, the molecular structure of MES has no chromophore. In addition, the UV spectrum does not show any significant absorption at wavelengths > 290 nm. The US EPA method OPPTS 835.2210 states that the test method is applicable to all chemicals that have a UV/absorption maximum in the range of 290-800 nm. Chemicals with UV/absorption maxima exclusively below 290 nm cannot undergo direct photolysis in sunlight. Therefore, the substance is not a candidate for noteworthy photolysis in sunlight and it is not necessary to perform the test.

Phototransformation in air

For MES the rate constant for indirect photolysis with OH radicals was estimated using the program AOPWIN v1.91. Ozone reaction was not estimated by the model. The calculation was based on 0.5·106 OH radicals per cm^3 for a 24 hours-day according to the TGD (EC 2003, part II chapter 3, 2.3.6.3, p. 51). Specific degradation rate constants $43 \cdot 10^{-12}$ cm^3 $molecule^{-1}$ s^{-1} was calculated. The corresponding half-life is equal to 9.22 hours.

Because of the low vapour pressure and low Henry´s law constant, no significant volatilisation to air is to be expected. Additionally, the amount which reaches the air compartment will most probably be washed out by rain. Therefore, degradation in the atmosphere is considered to be only of minor importance.

Conclusions:

The results suggest that MES is very rapidly degraded in air by photo-oxidative processes. Significant amounts of MES are not likely to persist in air.

11.2 Environmental transformation of metals or inorganic metals compounds

Table 73: Summary of relevant information on rapid environmental transformation.

Method	Results	Remarks	Reference
Not applicable			

11.2.1 Summary of data/information on environmental transformation

Not applicable.

11.3 Environmental fate and other relevant information

Adsorption/desorption

Adsorption of Dimethylethylhexadecylammonium-ethylsulphate on a reference soil LUFA 2.2 (loamy sand) was performed at 20 - 25ºC at a soil/ solution ratio of 1:50 according to Tier 1 of the OECD Guideleine106 (BODE Chemie (2002a)).

Prior to the test selection of the optimal soil solution ratio out of three ratios (1:50, 1:16.7, and 1:5) was performed. For determination of the soil adsorption coefficient 1 g soil was weighed into 80 mL centrifuge tubes. Then samples were suspended with 49.5 mL of 0.01 M $CaCl_2$ solution (blanks were suspended with 50 mL of a 0.01 M $CaCl_2$ solution) and centrifuge tubes were closed with glass stoppers. For every test set two replicates and one blank (soil plus 0.01 M $CaCl_2$ solution) were prepared. In addition control samples were performed in every test set (test substance in 0.01 M $CaCl_2$ solution, no soil).

Samples were shaken overnight to establish soil-water equilibrium. Then 500 µL (corresponding to 10 mg dimethylethylhexadecylammonium-ethylsulfat/L) of the test substance stock solution 1 was added and the tubes agitated on a mechanical shaker. After that, samples were centrifuged at 3500 min^{-1} for 10 minutes. 1.5 ml of the supernatant aqueous solution was subjected to LC-MS-MS for determination of the concentration of the test substance.

For checking the sorption of the test substance on the test vessels the control samples were used. After test performance and transfer of the test aliquots to analytical determination the remaining aqueous solutions were removed and rinsed with water two times, remaining water was removed with a stream of nitrogen. 5 mL of methanol was pipetted into the test vessels and shaken for 10 min using a mechanical shaker. 1.5 mL of the methanol extracts were analysed by LC-MS-MS. There was no noticeable adsorption on the test vessels (2.2%).

The dimethylethylhexadecylammonium cation in aqueous solution was determined by LC-MS-MS. Therefore the determined adsorption coefficients Ka and Kaoc are valid for the dimethylethylhexadecyl-ammonium cation only.

The adsorption of the cation is extremely strong. This is in agreement with the very rapidly attained sorption equilibrium after 7 hours.

Adsorbed a.s. [%] > 96 after 7 hours

Ka > 1300 cm^3/g (7 hours)

Kd Not determined

Kaoc > 60000 cm^3/g (7 hours)

Ka/Kd Not applicable

Adsorption of the dimethylethylhexadecylammonium cation was found to be extremely high, so the cation is expected to be immobile in soil.

The adsorption/desorption behaviour of mecetronium ethyl sulphate [MES] was studied with five top soils. The determinations of soils IME 02-A (silt loam), IME 03-G (silt loam) and IME 06-A (silty clay loam) were performed in compliance with the principles of the Good Laboratory Practice at the testing facility. The determinations of soil LUFA 2.2 (loamy sand) and LUFA 3A (sandy silty loam) obtained from Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer, Obere Langgasse 40, D-67346 Speyer (LUFA Speyer) were conducted in compliance with the principles of the Good Laboratory Practice by LUFA Speyer (BODE Chemie (2008d)).

The adsorption coefficients (KF) in the adsorption tests varied up to a factor of about 10 in a range between 1627 and 16 126. Normalization to the organic carbon content of the soils resulted in KOC values from 68 940 to 655 630. No significant correlation between organic carbon content and adsorption could be observed. In contrast, the correlation between adsorption and cation exchange capacity of the soils was R^2 = 0.9909. The 1/n values obtained from the adsorption test ranged between 0.5116 and 0.6560, indicating that the sorption of MES is nonlinear.

Adsorption kinetics showed that equilibrium conditions were achieved after 24 h.

Desorption was proven to be almost independent from agitation time. The desorption tests show a range of desorption coefficients (KF) between 1125 and 17 366. Normalization to the organic carbon content of the soils resulted in KOC values ranging from about 47 669 to 705 934. No correlation between desorption and organic carbon content of the soils could be observed. In contrast, the correlation between desorption and the cation exchange capacity of the soils was R^2 = 0.9898.

Results indicate that mecetronium ethyl sulphate [MES] was not only adsorbed by the soil organic matter but also by the clay mineral fraction. However, the good correlation of both adsorption and desorption coefficients (K_F^{ads} and K_F^{des}) indicates that ionic linkage may dominate the sorption mechanism. Mecetronium ethyl sulphate [MES] was found to sorb strongly onto the five test soils, and was poorly desorbed from the soils. Results indicate that ionic linkage to the clay mineral fraction is a more important sorption mechanism than binding to the soil organic matter.

Studies on activated sludge adsorption properties of mecetronium ethyl sulphate [MES] were performed according to the ISO-Guideline 18749 “Water Quality – Adsorption of substances on

activated sludge – Batch test using specific analytical methods" (BODE Chemie (2008e)). Test performance included range finding tests, the determination of the degree of adsorption at different time intervals (adsorption kinetic) with two initial test item concentrations (25 and 50 mg/L), and parental mass balance. In addition, tests with sterilized activated sludge were conducted. Basic Violet 4 was used as reference substance. A high adsorption of all tested components was reached very rapidly, i.e. after 1 hour. After 6 hours the degree of adsorption decreases slightly which may probably attributed to the modified surface active properties of the sludge caused by the test item´s toxicity. Therefore, the results of adsorption test obtained at 24 h should not be taken into account.
Adsorption was shown to be dependent on the concentration of the test substance. Therefore, results from the lower from the two tested concentrations (25 mg/L) should be preferred, as they are closer to concentrations expected in waste waters. In addition, the mass balance showed satisfying recovery of the test substance at the lower test concentration (25 mg/L), but not at 50 mg/L. This finding may be explained by a lower extraction efficiency at the higher concentration level.
Overall, the arithmetic mean value of the distribution coefficients obtained after 1, 2, 4 and 6 hours at an initial concentration of 25 mg/L should be taken as the definitive test result.
In sterile samples the observed adsorption was comparable to the values of non-treated samples. The test with reference material demonstrated satisfying adsorption capacity of the sludge used for the test. Therefore, the results can be accepted.
Adsorbed a.s. [%]: 99.6 % (25 mg/L, mean 1 – 6 hours)
Ka: 232 000 L/kg
Kd: Not determined, not required by guideline ISO 18749
Kaoc: Not determined
Ka/Kd: Not applicable
Degradation products (% of a.s.): Not determined.
Conclusion: The high adsorption coefficients indicate that MES is immobile in soils and will strongly adsorb onto sewage sludge.

11.4 Bioaccumulation

Table 74: Summary of relevant information on bioaccumulation.

Method	Results	Remarks	Reference
No data			

11.4.1 Estimated bioaccumulation

As with all other organic chemicals, in studies on surfactant bioconcentration the term 'BCF' refers to measurements at steady-state conditions, i.e., uptake and elimination processes have reached an

equilibrium. Due to the time-consuming nature of steady-state experiments, though, many studies on bioconcentration have applied a kinetic approach. This means that uptake and (if performed) elimination phase durations are chosen without knowledge of the time to reach steady-state. Instead, the elimination of the test substance from the water phase is followed over time by appropriate analytical methods. Banerjee et al. (1984) and de Wolf and Lieder (1998) have shown that for organic chemicals this kinetic BCF (BCFkin) is a good estimate of the steady-state BCF, and a guideline for its application in standard testing is under development (ECETOC 2005). Arnot and Gobas (2006), on the other hand, drafted a catalogue of quality criteria for reliability of BCF studies. In this context they claimed that studies not approaching steady-state conditions should not be scored as fully reliable. With these somewhat contrasting statements in mind, a review on the available open literature on bioconcentration behaviour of quaternary ammonium compounds (QACs) was performed. Meylan et al. (1999) compiled experimental BCF data for 84 ionic compounds including seven QACs. None of these compounds exceeded a log BCF of 2.5 (BCF = 316 L/kg). Concurringly, for the QAC DODMAC BCFs between 31 and 256 L/kg were reported, depending on the test medium used (natural vs. laboratory water, hardness) (EC 2002).

The cation of mecetronium ethyl sulfate (MES) possesses a dimethyl-monoethyl ammonium headgroup and a C16-alkyl chain. No experimental data on the bioconcentration potential of MES are available.

Versteeg and Shorter (1992) reported BCFkin values for a mixture of C18- and C16- trimethyl ammonium chloride (TMAC), which is a related structure to the MES cation. For this mixture of two alkylchain lengths in pure laboratory water they found a relatively high BCFkin of 1962 L/kg, which is near to the criterion for bioaccumulative substances according to Annex XIII of REACH Regulation. Versteeg and Shorter (1992) demonstrated that the BCFkin of TMAC was dependent on the alkyl chain length as BCFkins of C12- and C8-TMAC were only 34 and 2 L/kg, respectively. Considering this strong influence of the alkylchain length on bioconcentration of monoalkyl QACs, it appears that the pure C16-compound MES, without C18-components, should have a BCFkin well below that of C16/18-TMAC.

Another factor abating the bioconcentration potential of MES compared to C16/18-TMAC, is the different structure of the hydrophilic head of the cation. Beside the C16-alkyl chain, MES possesses a dimethyl, monoethyl-structure, whereas TMAC is characterised by a trimethyl-structure. In their review on surfactant bioconcentration, Tolls et al. (1994) concluded from a number of studies that not only alkyl chain length but also the hydrophilic head-structure of QACs function as determinators of the bioconcentration potential. Since QACs with two or more chains generally

concentrate less in tissues than monoalkyl QACs, it can be concluded that also the slightly longer ethyl-moiety of the MES cation lowers its bioconcentration potential compared to C16/18-TMAC.

In test medium spiked with humic acids as an experimental surrogate for organic carbon in natural waters, Versteeg and Shorter (1992) found that the BCFkin of C16/18-TMAC was reduced from 1962 L/kg to values below 50. The authors also demonstrated that in natural surface waters of different origins, fish toxicity in terms of acute LC_{50} was reduced by factors between 4 and 7 compared to pure laboratory water. Thus, they concluded that also bioconcentration will be reduced by organic carbon contents in natural waters. It is reasonable to assume that a comparable relationship is valid for the MES cation, and we postulate that under field conditions bioconcentration of MES will also be dimished.

In regard of the evidence outlined above, it evolves that MES possesses a lower bioconcentration potential than C16/18-TMAC.

Another issue to be addressed in the context of bioconcentration is the question whether MES may accumulate in the food chain, thus provoking toxic effects in organisms at higher trophic levels, e.g., fish-eating birds or mammals - 'secondary poisoning'.

However, taking into account the above data, it appears rather improbable that MES will accumulate in the food chain, because no significant bioconcentration is expected. Furthermore, experimental proof exists that monoalkyl QAC cations may be readily metabolised and excreted after ingestion. Hughes et al. (1973) found that rats excreted radiolabelled [^{14}C1]cetyltrimethylammonium bromide (which is identical to C16-TMAC, except for the anion) by 48% within 24 hours after intraperitoneal administration. Beside the parent compound, two metabolites were found in the bile and five metabolites in the urine. Pharmakokinetic data on MES after dermal absorption in rats show also, that about 66% of the incorporated MES (which was only around 1% of the total administered dose) was excreted within 72 hours.

Taken together, the above information demonstrates that MES is not a candidate for concern when bioaccumulation is regarded. Though structurally related to C16/18-TMAC, which is the QAC with the highest bioconcentration potential ever reported, sound scientific evidence is available to demonstrate that MES has a lower bioconcentration potential. Therefore, since even the reported BCFkin for C16/18-TMAC is not exceeding the trigger value for bioaccumulative substances of 2000 L/kg, it can be concluded that there is no serious concern for MES bioconcentration.

11.4.2 Measured partition coefficient and bioaccumulation test data

Mecetronium ethyl sulfate [MES] has a ionic structure and surface active properties. The methods proposed for the determination of the log Pow (TNsG on Data Requirements; EC, 2000) are OECD

107 and OECD 117. However, both methods are not suitable for the determination of the partition coefficient of surface active compounds. Therefore the experimental determination of the partition coefficient of MES by the proposed methods is technically not feasible.

As alternative the log Pow may be estimated from the individual solubilities in water and n-octanol (OECD, 1995).

This approach suggested to perform a study on solubility in n-octanol in order to be able to estimate the Kow by comparing the apparent solubilities of MES in n-octanol and water.

The study on solubility in n-octanol was performed according to CIPAC MT 181 and the solubility was found to be 168-202 g/L. The study on solubility in water was performed according to OECD 105 with a determined solubility of 500-1000 g/L.

From this results the Kow can be estimated as follows:

$Kow = c_{n\text{-}octanol} / c_{water}$

As a worst case the highest solubility value of n-octanol and the lowest value of water are used for further calculation.

Kow = 202 g/L /500 g/L

Kow: 0.404

From this the log Kow value is calculated:

Log Kow = -0.39

The log Kow for Mecetronium ethyl sulphate [MES] is lower than the trigger value of 4 for CLP Regulation and it shows a low potential for bioaccumulation.

11.5 Acute aquatic hazard

Table 75: Summary of relevant information on acute aquatic toxicity.

Method	Species	Test material	Results[1]	Remarks	Reference
Fish, Acute toxicity test OECD 203 GLP – no Test type: static Duration of the test: 96 hours Test parameter: mortality Sampling: every 24 hours Initial concentrations of the test substance: 0.1,	Species/Strain Leuciscus idus melanotus L. Ten fish were exposed to each of the five concentrations of the test substance between 0.1 and 0.3 mg/L and a control.	Mecetronium ethyl sulphate [MES] 27.5 – 30.5 % MES solution in water	LC_{50}: 0.06 mg/L (96 hours, nominal) LC_{50}: **0.048** mg/L (time weighted average)	Concentrations were not confirmed by analytical methods Study is not GLP compliant, These are only minor shortcomings and do not affect the overall significance of the findings.	<confidential> (1992g)

0.15, 0.2, 0.25 and 0.3 mg/L (nominal)					
Daphnia sp., Acute Immobilisation test OECD 202 GLP: yes Duration of test: 48 h Test parameter: immobility Sampling: at 24 and 48 hours Monitoring of TS concentration: Yes, at 0 and 48 hours	Species/strain: Daphnia magna STRAUS, no data on strain. Animals were exposed to five concentrations of MES, ranging from 6.25 to 100 µg/L in a geometric series divided by factor 2, and a control group.	Mecetronium ethyl sulphate [MES] Purity: IUCLID technical dossier Initial concentration of test substance:0.100, 0.050, 0.025, 0.0125, 0.00625 (nominal)	EC_{50}: **0.019** mg/L (48 hours, nominal)	test was performed in closed carboys; however test substance is not volatile	<confidential> (2000)
Daphnia sp., Acute Immobilisation test OECD 202 Duration of test: 48 h Test parameter: immobility Sampling: at 24 and 48 h Monitoring of TS concentration: Yes, at 0, 1, 2, 4, 8, 24 and 48 hours	Species/strain: Daphnia magna STRAUS (clone V) Animals were exposed to five concentrations of MES, ranging from 0.67 to 22.97 µg ai/L (time-weighted averages), and a control group. All concentrations were tested using 4 replicate treatments with 5 animals each.	Dimethylethylhexadecylammonium-ethylsulfate (= Mecetronium ethyl sulphate, [MES]) 29.9 % aqueous solution	EC_{50}: **0.015** mg/L (48 hours, time weighted average))		BODE Chemie (2010)
Algae, Growth Inhibition Test OECD 201 GLP: yes Duration of the test: 72 hours Test parameter: cell multiplication inhibition (photometric measurement); calculation of biomass production and growth rate Sampling: after 24, 48, 72 hours	Species/Strain Desmodesmus subspicatus (formerly known as Scenedesmus subspicatus CHODAT)	Mecetronium ethyl sulphate [MES] Purity > 99% Initial cell concentration: 8.11 • 104 cells/mL (Control mean, main test) Test temperature; 21.9 - 23.2°C pH 7.94 – 8.22 (initial) 8.01 – 10.39 (after 72 h) Aeration of dilution water: No	Biomass production (based on nominal values): NOEC = 0.004 mg/L, EC_{10} = 0.006 mg/L, EC_{50} = 0.025 mg/L, EC_{100} = 0.150 mg/L Growth rate (based on nominal values): NOEC = 0.011 mg/L,		<confidential> (2000a)

		Light intensity: 120 µE/m²s within the range 400 – 700 nm Initial concentrations of test substance: 0.0, 0.01, 0.02, 0.04, 0.08, 0.16 mg a.i./L (nominal)	EC_{10} = 0.015 mg/L, EC_{50} = 0.054 mg/L, EC_{100} = 0.250 mg/L Growth rate (based on time weighted average): NOEC = **0.00014** mg/L, EC_{50} = **0.0039** mg/L		

[1] Indicate if the results are based on the measured or on the nominal concentration.

11.5.1 Acute (short-term) toxicity to fish

The test was performed according to the OECD guideline 203. Leuciscus idus melanotus L. was used as test organism.

Ten fish were exposed to each of the five concentrations of the test substance between 0.1 and 0.3 mg/L and a control. The test was performed at 20°C and 12:12 hours light: dark cycle in a medium made from drinking water and deionised water. Test duration was 96 hours and survival was assessed every 24 hours. Oxygen concentrations and pH were measured in the same intervals. No analyses on test substance residues were performed.

MES was found to be acutely very toxic towards Leuciscus idus melanotus L.

Table 76. Fish study – effect data (nmortality).

Test substance Concentration (nominal) [mg/l]	**Mortality**							
	Number				**Percentage**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
control	0	0	0	0	0	0	0	0
0.1	0	0	0	0	0	0	0	0
0.15	0	1	2	3	0	10	20	30
0.2	1	2	4	5	10	20	40	50
0.25	10	-	-	-	100	100	100	100
0.3	10	-	-	-	100	100	100	100

	48 h [mg/l]	**95 % c.l.**	**96 h [mg/l]**	**95 % c.l.**
LC_0	0.10	No data	0.10	No data
LC_{50}	< 0.25 but > 0.20	No data	0.20	No data
LC_{100}	0.25	No data	0.25	No data

LC values are based on nominal concentrations

From the Batch-Number and the properties can be derived, that the test substance is the 30% aqueous solution of MES. It cannot be deduced whether the concentration was based on the MES concentration or the test solution concentration. Therefore, the results from the study are multiplicated with the factor 0.3 (30% solution) as a worst-case approach and presented in below. The results are based on the nominal concentrations.

In order to overcome the lack of test substance monitoring the"Kinetic Study" was initiated. From the kinetic data a time-weighted average concentration was calculated. This TWA was then used to derive the actual effect data. For this, the nominal EC_{50} was divided by the %-intial TWA value.

LC_0 0.03 mg/L (96 hours, nominal)

0.024 mg/ L (time weighted average)

LC_{50} 0.06 mg/L (96 hours, nominal)

0.048 mg/ L (time weighted average)

LC_{100} 0.075 mg/L (96 hours, nominal)

0.058 mg/ L (time weighted average)

All validity criteria according the test guideline OECD 203 are met within the study. For details see the following table.

Table 77. Fish study – validity criteria.

	fulfilled	Not fullfilled
Mortality of control animals <10%	**X**	
Constant conditions should be maintained as far as possible throughout the test (static system)	**X**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**X**	
Concentration of test substance ≥80% of initial concentration during test	**No data**	

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

The first test was performed according to the OECD guideline 202 part I. The water flea Daphnia magna STRAUS (Crustacea: Cladocera) was used as the test organism on acute toxicity of MES towards invertebrates (<confidential> 2000).

Animals were exposed to five concentrations of MES, ranging from 6.25 to 100 µg/L in a geometric series divided by factor 2, and a control group. Prior to the definite test two range finding experiments were conducted. All concentrations were tested using 4 replicate treatments with 5 animals each. The test was performed at 20°C and a 16:8 hours light:dark cycle in a fully artificial

medium according to DIN 38412, part 11. Immobilisation was assessed at 24 and 48 hours, physico-chemical water parameters and test substance concentrations were measured at 0 and 48 hours.

Analytical monitoring of the test substance was performed according to "Deutsche Einheitsverfahren zur Wasser-, Abwasser- und Schlammuntersuchung, DIN 38409 Part 20: Bestimmung der Disulfinblau-aktiven Substanzen".

MES was found to be acutely toxic towards daphnia at relatively low concentrations (see Table below).

Table 78. Daphnia study - effect data (immobilisation).

Test substance Concentration (nominal) [mg/l]	**Immobile *Daphnia***						
	Number		**Percentage**		**O_2 [mg/l] 48 h**	**pH 48 h**	**Temp. [°C] 48 h**
	24 h	**48 h**	**24 h**	**48 h**			
Control	No data given in report		0	0	7.8	7.43	20.2
0.100			100	100	7.8	7.77	20.2
0.050			60	100	7.8	7.69	20.2
0.025			10	100	7.8	7.61	20.2
0.0125			0	5	7.8	7.59	20.2
0.00625			0	0	7.8	7.53	20.2

	EC_{50}	**95 % c.l.**	**EC_0**	**EC_{100}**
24 h [mg/l] (nominal)	0.038	No data	0.01	0.1
48 h [mg/l] (nominal)	**0.019**	No data	0.006	0.025

However residue analysis of MES was unsuccessful because the method proved to be not sensitive enough for the concentration range tested. All results refer therefore to the nominal concentrations.

A chronic study on daphnia reproduction (<confidential> (2008f)) was monitored by an appropriate and validated LC/MS-MS method. These data can also be used for the interpretation of the present results. Due to the adsorption behaviour of MES, the actual concentrations are probably lower than nominal.

All other validity criteria according the test guideline OECD 202 are met within the study. For details see the following table:

Table 79. Daphnia study – validity criteria.

Validity criteria	fulfilled	Not fullfilled
Immobilisation of control animals <10%	**X**	
Control animals not staying at the surface	**X**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**X**	
Concentration of test substance ≥80% of initial concentration during test	**No valid data**	

The second test was performed according to the OECD guideline 202 (2004). The water flea Daphnia magna (Crustacea: Cladocera) was used as the test organism on acute toxicity of MES towards invertebrates (<confidential> 2010).

Animals were exposed to five concentrations of MES, ranging from 0.67 to 22.97 µg ai/L (time-weighted averages), and a control group. All concentrations were tested using 4 replicate treatments with 5 animals each. The test was performed at 20°C in the dark. Immobilisation was assessed at 24 and 48 hours, physico-chemical water parameters and test substance concentrations were measured at 0, 1, 2, 4, 8, 24 and 48 hours.

The evaluation of the concentration-effect-relationships and the calculations of effect concentrations were based on the theoretical mean concentrations (time weighted average, according to OECD guideline 211) of the test item.

Analytical monitoring of the test substance was performed by LC/MS-MS (LOQ = 0.1 µg/L).

MES was found to be acutely toxic towards daphnia at relatively low concentrations (See Table below).

Table 80. Daphnia study - effect data (immobilisation).

Test substance Concentration (time weigthed average) [µg ai/L]	**Immobile *Daphnia***						
	Number		**Percentage**		**O_2 [mg/L]**	**pH**	**Temp. [°C]**
	24 h	**48 h**	**24 h**	**48 h**	**48 h**	**48 h**	**48 h**
Control	0	0	0	0	8.1	8.6	20.2
0.67	0	0	0	0	8.3	8.6	20.2
1.55	0	0	0	0	8.5	8.6	20.2
5.16	2	3	10	15	8.5	8.6	20.2
9.72	3	6	15	30	8.5	8.6	20.2
22.97	5	13	25	65	8.7	8.7	20.2

	EC_{50}	**95 % c.l.**	**EC_{10}**	**EC_{20}**
48 h [µg ai/L] (time weighted average)	15.65	11.22 – 26.95	4.52	6.92

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

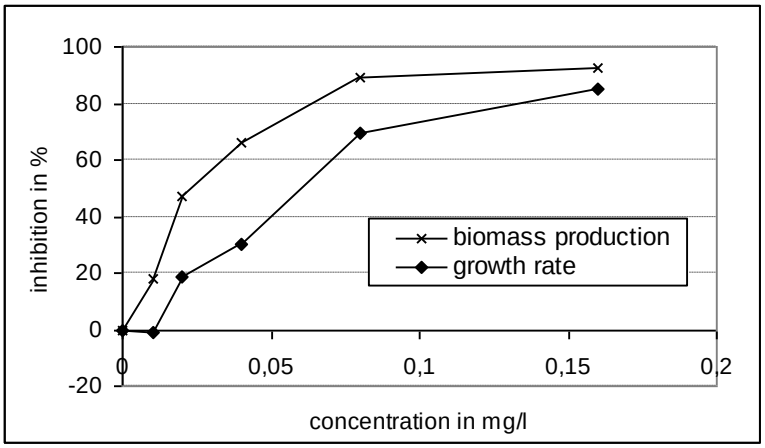
"Alga, Growth Inhibition Test":

The green alga Desmodesmus subspicatus (formerly Scenedesmus subspicatus CHODAT) was used as the test organism on toxicity tests of MES towards algae, and the test was performed according to the OECD guideline 201 (<confidential> 2000a).

Algae were exposed to five concentrations of MES ranging from 0.01 to 0.16 mg/L in a geometric series divided by factor 2, and a control group. Prior to the definite test, two range finding experiments were conducted. All concentrations were tested using 7 replicate treatments with mean initial cell densities in the main test of 6.6 • 104 to 8.39 • 104 cells/mL. The test was performed at 21.9 - 23.2°C under continuous illumination in a fully artificial medium according to OECD 201. However, the $NaHCO_3$ concentration was twice the recommended value of the guideline. Cell densities were assessed at 24, 48 and 72 hours using a spectrophotometric method at 578 nm, previously calibrated. Physico-chemical water parameters and MES concentrations were measured at 0 and 72 hours. MES concentrations were analysed according to "Deutsche Einheitsverfahren zur Wasser-, Abwasser,-, und Schlammuntersuchung, DIN 38409 Part 20: Bestimmung der Disulfinblau-aktiven Substanzen".

Mecetronium ethyl sulphate [MES] was found to be very toxic towards D. subspicatus (see graph below).

Graph 1. Alga Growth Inhibition Test: concentration/response curve.



However residue analysis of MES was unsuccessful because the method proved to be not sensitive enough for the concentration range tested. All results in this study refer therefore to the nominal concentrations. All other validity criteria according to the test guidline OECD 201 are met within the study. A clear concentration-response relationship can be established from the study results.

In order to overcome the shortcomings regarding test substance monitoring the"Kinetic Study" was initiated. From the kinetic data a time-weighted average concentration was calculated. This TWA was then used to derive the actual effect data. For this, the nominal EC_{50} and NOEC was divided by the respective %-intial TWA value.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data available.

11.6 Long-term aquatic hazard

Table 81: Summary of relevant information on chronic aquatic toxicity.

Method	Species	Test material	Results[1]	Remarks	Reference
Fish, Early-life Stage Toxicity Test OECD 210 GLP: yes Test type: Flow-through Duration of the test: 35 days Test parameters: Hatching rate, mortality/survival, growth (body length and weight) and behavioural abnormalities	Species/ Strain: Danio rerio (Zebrafish)	Mecetronium ethyl sulphate [MES] MES 29% (solution in water) Concentrations: Adult zebrafish were exposed to nominal concentrations of 5, 50 and 500 µg MES/L in a semi-static test design for 96 h	NOEC: **0.555 µg MES/L** (adverse effect on survival, mean measured concentration) LOEC 2.95 µg MES/L (adverse effect on survival, mean measured concentration)		<confidential> (2012)
Daphnia magna Reproduction Test OECD 211 GLP: yes Test type: Semi static Duration of the test: 21 days Test parameter: At the end of the test, the total number of living	Species/Strain: Daphnia magna	Mecetronium ethyl sulphate [MES] 29.2 % solution in water	NOEC **0.19 µg**/L (parental survival), time weighted average concentration LOEC 0.24 µg/L (parental survival), time weighted average concentration EC_{50} Calculation not possible for TWA corrected values		<confidential> (2008f)

offspring produced per parent animal alive at the end of the test was assessed. The parental mortality, time to first brood and offspring number were used to calculate the intrinsic rate of population increase as integrative parameter relevant for population effects.					

[1] Indicate if the results are based on the measured or on the nominal concentration

11.6.1 Chronic toxicity to fish

The influence of MES on the early life stages of the zebrafish Danio rerio was investigated (<confidential> 2012).

The study was conducted under flow through conditions according to the OECD guideline 210. Freshly fertilised eggs of Danio rerio from laboratory own breeding were exposed to five concentrations of MES, untreated control replicates were run in parallel. Each treatment group consisted of 4 replicates with totally 60 fertilised eggs. The hatching rates were determined 4, 5 and 6 days after fertilisation. In addition, survival rates were estimated after 14, 21, 28 and 35 days. At test end, fish lengths and dry weights of replicate fish groups were determined. The MES concentrations in the test solutions were measured at test start and at least weekly thereafter.

The hatching success on day 4 post fertilisation (pf) was found to be reduced at 10.8 µg MES/L. However, on day 5 and 6 pf no significant difference compared to control could be detected revealing that the total hatching number was not significantly reduced after 6 days. The survival was not significantly affected on day 14 and 21 pf. On day 28 pf and on day 35 pf, the survival was found to be significantly reduced at 2.95 and 10.8 µg MES/L, respectively. Growth, measured as weight and length of fish at test end, was not reduced at all test item concentrations. However, weight and length was found to be significantly enhanced at 0.555, 2.95 and 10.8 µg MES/L.

Taken together, MES was found to have very low adverse effects on early fish stages at the tested concentrations. The only parameter negatively affected was post hatch survival with values even

being above the requested post hatch success of ≥ 70 % (i.e. minimum post hatch was 74.5 % at 2.95 µg/L mean measured concentration). The numerical results are given below; all concentrations refer to the mean measured concentrations.

Table 82. Effect data: Hatch, survival and growth.

	Mean measured concentration [µg/L]					
	control	0.154	0.404	0.555	2.95	10.8
Hatching day 4 pf [%]	23.3	40.0	15.0	6.7	8.3	3.3 *)
Hatching day 5 pf [%]	55.0	81.7	56.7	46.7	51.7	58.3
Hatching day 6 pf [%]	100	100	98.3	98.3	98.3	96.7
Post hatch survival, day 14 pf [%]	93.3	98.3	91.5	93.3	86.3	94.9
Post hatch survival, day 21 pf [%]	91.7	91.7	81.5	86.4	77.9	82.6
Post hatch survival, day 28 pf [%]	91.7	91.7	81.5	86.4	74.5 *)	77.4 *)
Post hatch survival, day 35 pf [%]	90.0	91.7	76.4	86.4	74.5 *)	77.4 *)
Length, day 35 pf [cm]	1.08	1.11	1.13	1.20 #)	1.16 #)	1.12 #)
Dry weight, day 35 pf [mg]	2.5	2.7	2.8	3.8 #)	3.5 #)	3.0 #)

pf = post fertilisation

*) Statistical significant negative deviation compared to control with $p<0.05$, Williams test. fertilization

#) statistical significant positive deviation compared to control, $p<0.05$, Williams test, one-sided greater

All relevant validity criteria according to the Guideline are met within the study. For details see the following table:

Table 83. Fish study - effect data (immobilisation).

	fulfilled	**Not fullfilled**
Concentration of dissolved oxygen > 60% saturation throughout the test	**X**	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	**X**	

Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	**X**	
Test substance concentrations maintained within ± 20% of mean measured values		**X**
No effect on survival nor any other adverse effect found in solvent control	**Not applicable**	
Further criteria for poorly soluble test substances	**Not applicable**	

Although the concentration of MES could not be maintained throughout the test within ± 20% of mean measured values this is considered to not impair the integrity, quality and reliability of the study results. All possible technical effort was made to keep the test substance concentration at nominal levels for example using only glass material for all analytical steps, performing immediate chemical analyses of all samples and including additional sampling throughout the test. Also, the flow through system was extensively adjusted approximately one month prior to test start including several analytical measurements. This was performed to ensure the right test concentrations and the correct adjustment of the dosing pump system. MES is not volatile from aqueous solutions, therefore this removal mechanism could not influence the test results. Possible substance losses due to biodegradation are unlikely in the flow-through system. However, the high adsorptivity of the test substance may influence the test substance concentrations. From other aquatic studies it is known that MES is difficult to recover due its high potential for clustering and adsorption, resulting in an irregular distribution in the test vessels. Since all effect data are based on mean measured values the study gives reliable results.

11.6.2 Chronic toxicity to aquatic invertebrates

The influence of MES on the reproduction of aquatic invertebrates, represented by Daphnia magna, was investigated (<confidential> 2008f). A 21 day semi-static exposure to mecetronium ethyl sulphate [MES] at different concentrations with renewal of the test solutions daily was conducted according to the OECD guideline 211. Untreated control replicates were run in parallel. Each treatment group consisted of 10 replicates with one daphnid each (individual exposure). Effects on survival, growth (adult length at test termination) and reproductive performance were investigated. Test substance concentrations were measured at representative fresh and aged test solutions.

Mecetronium ethyl sulphate [MES] was found to be very toxic towards adult daphnids survival, but no effects on reproduction and growth were observable.

Analytical measurements during the reproduction test revealed that the MES concentrations were not stable during the test period. The mean measured test item concentrations of the freshly prepared test solution of the active substance (initial concentrations once a week) were between 100% and 195% of nominal concentrations. During the time interval until renewal of the test

solution, active substance concentrations decreased considerably to 5 – 47% of nominal at the four highest concentrations (0.81 - 16.00 µg/L nominal).

This behaviour of the test substance was nearer elucidated by the "kinetic study", which supported the earlier results from the reproduction test. In the "kinetic study" test substance elimination over time was closely followed and new time weighted means were calculated, taking into account the elimination kinetics of MES in this particular test system.

The key endpoints of this study, NOEC and LOEC for parental survival, was calculated as follows:

The initial measured concentration of the NOEC and LOEC level from the reproduction study (0.59 µg/L and 1.0 µg/L, respectively) were multiplied with the % TWA values for that concentration level from the kinetic study (32.6% and 24.2%, respectively). Calculation of the EC_{50} was not possible for TWA corrected values basing on the kinetic study. However, this is only of minor importance because the key endpoint for long-term studies is the NOEC.

Time-weighted mean test concentrations higher than 0.19 µg a.s./L (NOEC) did affect survival (viability) of adults. A concentration depending effect occurred. No further clinical signs were observed for the survived individuals.

Mecetronium ethyl sulphate [MES] is not volatile from aqueous solutions, therefore this removal mechanism could not influence the test results. Possible substance losses due to biodegradation are unlikely in the semistatic system. However, the high adsorptivity of the test substance may influence the test substance concentrations.

11.6.3 Chronic toxicity to algae or other aquatic plants

The relavant information are include in Table 75 (Summary of relevant information on acute aquatic toxicity) and in Point 11.5.3 of these report.

11.6.4 Chronic toxicity to other aquatic organisms

No data available.

11.7 COMPARISON WITH THE CLP CRITERIA

11.7.1 Acute aquatic hazard

The lowest available $L(E)C_{50}$ value relevant for classification of mecetronium ethyl sulphate [MES] is the 72 h EC_{50} of 0.0039 mg a./L obtained for Growth Inhibition Test on algae (OECD 201). Based on this lowest $L(E)C_{50}$ value MES fulfils the criteria $L(C)E_{50} \leq 1$ mg/L for classification as

Acute Aquatic Category 1, H400 (Very toxic to aquatic life) with M-factor of 100 due to 72 h EC_{50} in the range 0.001 < L(E)C_{50} ≤ 0.01 mg/L (Table 4.1.3 of Annex I of CLP).

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

The log Kow for mecetronium ethyl sulphate [MES] is lower than the trigger value of 4 for CLP Regulation and it shows a low potential for bioaccumulation.

The lowest NOEC/EC_{10} is the 72 hours NOEC of 0.00014 mg a.i./L obtained for algae in Growth Inhibition test on algae (OECD 201). Available NOECs values for fish and daphnia are higher (the value of 35 days NOEC for fish and the value of 21 days NOEC for Daphnia magna are in the same range as the value of NOEC for algae). The lowest endpoint for MES fulfils the criteria NOEC/ECx ≤ 0.01 mg/L (for substance readily biodegradable – see conclusion in Point 11.1.1) for classification as Aquatic Chronic 1, H410 (Very toxic to the aquatic organisms with long lasting effects) with an M-factor of 10 due to the NOEC value in the range 0.0001 mg/L < NOEC/ECx ≤ 0.001 mg/L (Table 4.1.3 of Annex I of CLP).

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Aquatic Acute 1, H400 M=100

Aquatic Chronic 1, H410 M=10

12 EVALUATION OF ADDITIONAL HAZARDS

12.1 Hazardous to the ozone layer

Table 84. Summary table of data concerning hazardous properties of the substance for the ozone layer.

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
No data				

12.1.1 Short summary and overall relevance of the provided information on ozone layer hazard

According to Regulation EC (No) 1272/2008 a substance has to be considered as hazardous to the ozone layer if it is listed in Regulation EC (No) 2037/200. MES is not listed in Regulation EC (No) 2037/2000.

12.1.2 Comparison with the CLP criteria

There is no data to compare with criteria classification as hazardous to the ozone layer.

12.1.3 Conclusion on classification and labelling for hazardous to the ozone layer

Mecetronium ethyl sulphate [MES] is not classified and labelled as hazardous to the ozone layer.

13 ADDITIONAL LABELLING

Supplemental hazard information in accordance with Annex II of the CLP Regulation – not required.

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15 ANNEXES

IUCLID file.