Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

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

CLH-O-0000001412-86-235/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

14 September 2018

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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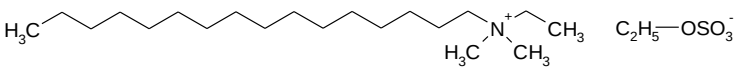
CONTENTS

CLH REPORT FOR MECETRONIUM ETILSULFATE; N-ETHYL-N,N-DIMETHYLHEXADECAN-1-AMINIUM ETHYL SULFATE; MECETRONIUM ETHYL SULPHATE [MES]

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	H302 H311 H314 H318 H400 H410	GHS05 GHS06 GHS09 Dgr	H302 H311 H314* H410*		M=100 (Acute) M=10 (Chronic)	

					Chronic 1						

*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

Hazard class	Reason for no classification	Within the scope of public consultation
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.

RAC general comment
N-ethyl-N,N-dimethylhexadecan-1-aminium ethyl sulphate (mecetronium ethyl sulphate) [MES] is not currently listed in Annex VI of the Regulation (EC) No 1272/2008. MES is a biocide active substance according to Regulation (EU) No 528/2012 (BPR) and belongs to Product Type 1, (used for human hygiene purposes, applied on, or in contact with human skin or scalp for the primary purpose of disinfection). The active substance has not yet been approved under the BPR. During the public consultation one Member State Competent Authority (MSCA) noted that the studies in the CLH report were not always reported in detail and no Annex I providing further information was available. The Dossier Submitter (DS) responded that all detailed information from the study reports was included in the dossier.

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

Property	Value	Reference	Comment (e.g. measured or estimated)
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 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

Property	Value	Reference	Comment (e.g. measured or estimated)
			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 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.	BODE Chemie (2010b)

Method	Results	Remarks	Reference
		440/2008,	

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;	No explosive properties according to Regulation		BODE Chemie (2010a)

Method	Results	Remarks	Reference
GLP	440/2008/EC.		

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.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The DS proposed no classification for MES for physical hazards due for the following reasons:

- A test according to A.14 was not performed as the exothermic decomposition energy was less than 500 J/g with an onset of exothermic decomposition below 500 °C. Therefore MES was not considered to have explosive properties;
- In a preliminary test, MES was not ignited with a flame and therefore a main A.10 test was not performed. MES was not considered a highly flammable solid;
- MES was produced, handled and marketed as aqueous solution and pure MES was not considered a flammable solid;
- MES was not considered as self-reactive substance because heat of decomposition was determined to be below 300 J/g;
- MES was not considered a pyrophoric solid because the experience in manufacturing and handling showed that the substance does not spontaneously ignite when coming into contact with air at normal temperatures and because the NMR spectrum of MES powder after 24 hours of storage at 65 ºC showed no structural changes;
- MES was not considered as a self-heating substance because according to CLP substances with melting point lower than 160 ºC should not be considered as self-heating substances and an EU A.1 test showed that MES has a melting range from 87.6 to 111 ºC;
- MES was not considered a substance which in contact with water emit flammable gases because experience in production and handling showed that MES in contact with water did not emit flammable gases and according to CLP this justified no classification for this hazard class;
- From the structural formula and the composition of MES it was concluded that the substance did not show any oxidizing properties;
- There were no studies available to assess if MES should have been classified as corrosive to metals.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

According to CLP a substance shall not be classified as explosive if the organic substance 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 500 ºC. The results of test A.14 addressing explosive properties of MES confirmed these requirements and therefore the classification of MES as explosive is not warranted.

MES was reported as a non-flammable solid in an EU A.10 test and therefore the classification of MES as flammable solid is not warranted.

RAC also concurs with the reasons outlined by the DS for no classification of MES for the remaining physical properties.

In conclusion, RAC supports the DS's proposal for **no classification of MES for physical hazards**.

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	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.		BODE Chemie (1987)

Method	Results	Remarks	Reference
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).	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.		

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-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 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 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 or when rats were found dead).	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, 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.	<confidential> (1992)
OECD 401 Limit test	Rat Wistars	Mecetronium ethyl sulphate [MES]	2000 mg/kg bw Post exposure period: 14 days	No mortality. Clinical signs: piloerection in 10/10 rats, in 2 females slightly	<confidential> (1992a)

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels, duration of exposure**	**Value LD_{50}**	**Reference**
Oral; Gavage GLP	Males and females Number of animals per group: 5 males and 5 females, one dose	Clear liquid; 4% active component Purity of the active substance: IUCLID technical dossier	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).	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.	

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

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	Rat Wistars	Mecetronium ethyl sulphate	2000 mg/kg bw Duration of	The LD_{50} for male and female rats after dermal exposure to	<confidential> (1992b)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Value LD_{50}	Reference
Dermal GLP	Males and females Number of animals per group: 5 males and 5 females, one dose	[MES] Clear liquid; 4% active component Purity of the active substance: IUCLID technical dossier	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).	"Mecetroniumetilsulfat 4%" is greater than 2000 mg/kg bw (80 mg/kg bw related to active component).	
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 exposure), skin reaction scored once daily after patch removal, body weight (measured day 0, 7 and 14), necropsy (14 days after exposure).	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)

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.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify MES as Acute Tox. 4; H302 (Harmful if swallowed) on the basis of an OECD TG 401 test in rats with 2000 mg/kg bw of a solution containing 30% of MES that caused 1/5 male mortality and 1/5 female mortality within 24 hours after the exposure. Based on this study, the DS concluded that the oral LD_{50} was higher than 600 mg/kg bw (LD_{50} for 30% solution was > 2000 mg/kg bw). In another study in accordance with OECD TG 401 in rats, no mortality was recorded with up to 2000 mg/kg bw of a solution containing 4% of MES.

The DS proposed to classify MES as Acute Tox. 3; H311 (Toxic in contact with skin) on the basis of an OECD TG 402 test in rats with 2000 mg/kg bw of a solution containing 30% of MES that caused no mortality. The DS concluded that the LD_{50} of MES by the dermal route was higher than 600 mg/kg bw. In another study in accordance with OECD TG 402 in rats, a solution containing 4% MES did not cause mortalities up to a dose of 2000 mg/kg bw.

The DS proposed no classification of MES for acute inhalation toxicity since no toxicity studies via inhalation had been performed for the following reasons: 1) the physico-chemical properties of MES indicated that the active substance had no tendency to become airborne; 2) the exposure of professionals to MES during its production and formulation was limited to the dermal route; and, 3) the exposure of users to MES via the product containing 0.2% MES was limited to the dermal route.

Comments received during public consultation

One MSCA considered that the proposal of classification as Acute Tox. 4; H302 and Acute Tox. 3; H311 were rather conservative, especially for the dermal route because no mortalities were reported at the tested dose, although the MSCA supported the proposed classification in the absence of additional experimental data with the pure substance. The same MSCA proposed a read-across from other quaternary ammonium compounds to assess the acute toxicity by the inhalation route. For the read-across, the MSCA supplied the following information (the original references were not available to RAC, but the information was published in an open review by The Institute of Food Safety and Toxicology, Danish Veterinary and Food Administration):

- Wistar rats were exposed to an alkyl dimethyl ethyl benzyl ammonium compound at a concentration of 5.4 mg/L (the maximum attainable concentration) for one hour. All animals died at this concentration.
- A whole-body inhalation study on cetylpyridinium chloride with five rats per sex were exposed to air containing 0, 0.05, 0.07, 0.13 and 0.29 mg cetylpyridinium chloride dust/L for four hours (equal to 50, 70, 130 and 290 mg dust/m3). The particle size was less than 5 µm. The LC_{50} was 0.09 mg/L (90 mg/m3) with upper and lower 95% confidence limits at 0.13 and 0.07 mg/L respectively. Deaths occurred in all treated groups (2/10, 1/10, 8/10 and 10/10, respectively). No deaths were seen among controls and all the deaths occurred within 4 days of exposure. Histopathological

examination of the lungs and other major organs was not carried out and the author calculated that the total cetylpyrimidinium chloride exposure at the LC_{50} level (0.09 mg/L) was about 4-8 mg/kg bw, and based upon this it was inferred that cetylpyrimidinium chloride could be more toxic by inhalation exposure than by oral or dermal exposure.

- A group of 196 farmers (with or without respiratory symptoms) was evaluated for the relationship between exposure to quaternary ammonium compounds (unspecified, exposure levels not given) and respiratory disorders by testing for lung function and bronchial responsiveness to histamine. After histamine provocation, statistically significant associations were found between the prevalence of mild bronchial responsiveness (including asthma-like symptoms) and the use of quaternary ammonium compounds as disinfectant. The association seemed even stronger in people without respiratory symptoms.

The DS responded that it did not consider the possibility of applying read-across for acute inhalation toxicity as the original study reports were not available and because in any case the generation of an aerosol of MES would be rather difficult due to the physico-chemical properties of MES (the physico-chemical properties indicated that the active substance had no tendency to become airborne). Regarding the observation about the conservativeness of the proposed dermal classification the DS reminded that the tests of acute dermal toxicity were not conducted with pure MES, but the corresponding dose levels were extrapolated from the diluted substance. The proposed classification was considered justifiable by the DS for precautionary reasons.

Another MSCA did not support the proposed classification for acute dermal toxicity, because it was based on a study in which none of the animals died and where only local skin effects were observed.

Also IND argued that the proposed classification for acute dermal toxicity was inappropriate and that Category 4 at most should be considered based on the following arguments.

- The only way to demonstrate the correct classification for acute dermal toxicity of MES would be by performing a new assay testing the substance up to 2000 mg/kg, which was unjustified on the basis of bioethical considerations since the substance was corrosive.
- The product was not manufactured in anhydrous form and the concentration of MES was 29% (the maximum available concentration).
- The local effects after dermal application of MES were covered by the classification as skin corrosive and an additional acute dermal toxicity study would not provide any additional scientific information.
- The available acute dermal toxicity test with the highest dose of pure MES showed only irritating effects on the skin without any mortality and without any other systemic effects.
- All available information including acute toxicity studies and studies with repeated dose application showed local effects at the site of contact, whereas systemic effects or relevant clinical signs related to systemic effects of toxicity were not shown.
- It was theoretically possible to extrapolate dermal acute toxicity from studies using

oral route of exposure and considering 100% oral absorption and 3% dermal absorption (which is deduced from an ADME study with ^{14}C-labelled MES). Extrapolating from the LD_{50} via the oral route, the LD_{50} by dermal route would be higher than 20000 mg/kg bw (Dermal LD_{50} = oral LD_{50}/dermal absorption = >600/0.03) and therefore the classification by dermal route was not considered warranted.

- Four cases of biocidal active substances (amines, N-C10-16-alkyltrimethylenedi-, reaction products with chloroacetic acid, poly(hexamethylenebicyanoguanide-hexamethylenediamine, peroxyacetic acid and iodine) hydrochloride, for which classification as acute Tox. 2 or Acute Tox. 3 could be derived from the available data, classification as Acute Tox. 4 was proposed for all four cases.

The DS considered Cat. 3 for acute dermal toxicity justifiable for precautionary reasons.

Assessment and comparison with the classification criteria

The tables below summarise the available acute toxicity studies by oral and dermal routes, respectively. No studies of acute toxicity by inhalation route were available in the CLH report.

***Table**: Summary of the animal studies on acute oral toxicity studies with MES*

Study	Dose level	Results	Reference
OECD TG 401 GLP Limit test Oral Gavage Rat Wistars 5 animals/sex Solution 30% MES Post exposure period: 14 days	2000 mg/kg bw	Clinical signs: Within 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, decreased respiratory rate, abnormal gait, squatting position and sunken flanks (there were no effects on day 5-14). Body weight gain was normal in males but in females a slight decrease in body weight was observed on day 7 compared with day 0 (156 versus 159 g). There was an increase in body weight on day 14 (176 g). LD_{50} of the tested preparation for males and females was greater than 2000 mg/kg bw. LD_{50} of MES was greater than 600 mg/kg bw.	<confidential> (1992)
OECD TG 401 GLP Limit test Rat Wistars Gavage 5 animals/sex Solution of 4% MES	2000 mg/kg bw	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. LD_{50} of the tested preparation for males and females was greater than 2000 mg/kg bw. LD_{50} of MES was greater than 80 mg/kg bw.	<confidential> (1992a)

Post exposure period: 14 days			

The CLH report contains a summary of epidemiological studies on the general population reporting that the overall incidence of suspected reactions after exposure to biocidal product containing 0.2% MES was 0.00018%. However no more information about the route of exposure and the type and severity of the adverse effects was reported.

***Table**: Summary of the animal studies on acute dermal toxicity studies with MES*

Study	Dose level	Results	Reference
OECD TG 402 GLP Limit test 5 animals/sex Solution of 4% MES	2000 mg/kg bw	No mortalities. Mainly in the female rats erythema and oedema were observed up to 13 days in 2 females. In a few females formation of fissures and degreasing of the treated skin. LD_{50} of the tested preparation for males and females was greater than 2000 mg/kg bw. LD_{50} of MES was greater than 80 mg/kg bw.	<confidential> (1992b)
OECD TG 402 GLP Limit test 5 animals/sex Solution 30% MES	2000 mg/kg bw	No mortalities. Moderate to severe erythema and very slight oedema were observed up to day 12 followed by a decline of these skin reactions up to the end of observation period. Degreasing, induration, partial desquamation and formation of fissures. LD_{50} of the tested preparation for males and females was greater than 2000 mg/kg bw. LD_{50} of MES was greater than 600 mg/kg bw.	<confidential> (1992c)

The key study for acute oral toxicity yielded an LD_{20} of 600 mg MES/kg bw. Therefore, the LD_{50} is higher than 600 mg/kg bw. The range for classification for a category 4 for acute oral toxicity is 300 mg/kg bw < LD_{50} ≤ 2000 mg/kg bw. RAC notes that, despite the plausibility that LD_{50} would be lower than 2000 mg/kg bw/day, the data is inconclusive for classification and for setting an ATE value.

The key study for acute dermal toxicity demonstrated that a dose of MES of 600 mg/kg bw was unable to cause mortalities and therefore the LD_{50} is greater than 600 mg/kg bw. The range for classification in category 3 for acute dermal toxicity is 200 mg/kg bw < LD_{50} ≤ 1000 mg/kg bw; while that for category 4 is 1000 mg/kg bw < LD_{50} ≤ 2000 mg/kg bw. RAC notes that it is extremely unlikely that the dermal LD_{50} of MES would be lower than 1000 mg/kg bw because 60% of this dose caused no mortalities, and therefore RAC does not support the DS's proposal for classifying MES in category 3. Industry has presented arguments in favour of category 4 or no classification on the basis of a route-to-route extrapolation and suggested that the dermal LD_{50} was likely to be significantly higher than

2000 mg/kg bw. However, RAC notes that this extrapolation presents several uncertainties as it encompasses an assumption of an oral absorption of 100%. However, according to the CLH report the quaternary ammonium compounds are poorly absorbed from the gastrointestinal tract and the influence of first-pass metabolism in the stomach/intestines and liver should also be taken into consideration in the extrapolation from oral data according to the Guidance on the Application of the CLP Criteria (July 2017). RAC concludes that it is not possible to propose a classification for acute dermal toxicity due to lack of robust and conclusive information.

No acute toxicity studies via inhalation were presented in the CLH report. The DS argued that due to the physical properties of the substance it had a very low tendency to form aerosols and therefore the amount of substance potentially inhaled would be always very low. One MSCA presented data suggesting that other quaternary ammonium compounds might require classification. However, RAC notes that there is not enough information available to justify the application of read-across from other quaternary ammonium compounds. Therefore, due to the absence of robust information, RAC proposes no classification of MES for acute toxicity via inhalation.

According to the CLP Annex II Section 1.2.6, in cases where no acute inhalation study is available for a corrosive substance, and such substances may be inhaled, the substance shall be supplementarily labelled with *EUH071: Corrosive to the respiratory tract*. Industry highlighted arguments in favour of no labelling MES with EUH071 based on its use as a biocide where the exposure was argued to be limited to the dermal route. RAC notes that classification is not based on risk assessment and concludes that EUH071 is warranted because there is no available acute inhalation toxicity study, the substance is corrosive and it could be inhaled in certain circumstances.

In summary, RAC concludes that **no classification of MES for acute toxicity is justified due to the absence of, or inconclusive data** by all three routes. However, RAC concludes that **labelling of MES with a supplemental hazard statement code "EUH071: corrosive to the respiratory tract" is warranted**.

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	"Mecetroniumetilsulfat 0.2%"	0.5 ml of the test substance applied to ca. 6 cm² of test site exposure: 4 hours	No erythema, oedema or any other effects on skin observed	<confidential> (1992d)

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
	group: 3 rabbits		Examination time points: 30-60 min, 24 h, 48 h, 72 h after patch removal.		
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 rabbits).	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).

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%

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

RAC evaluation of skin corrosion/irritation
Summary of the Dossier Submitter's proposal The DS proposed the classification of MES as skin corrosive in category 1C. In a study performed according to the OECD TG 404, 4% MES applied to White New Zealand rabbits during 4 hours caused severe skin reactions of varying degree and duration in all animals

throughout most of the observation period. Specifically, from days 9-10 onwards, all animals showed skin fissures and one animal showed leathery skin until the end of observation period (18 days). The DS also considered that undiluted MES would be corrosive. In another study performed according to the OECD TG 404, 0.2% MES caused no erythema, oedema or any other effects on skin.

Comments received during public consultation

One MSCA supported the classification as Skin Corr. 1C, but asked the DS to provide more clarity on the observed effects leading to the proposed classification (i.e., irreversibility, scars, fissures, etc.). The MSCA also requested the DS to clarify how the very limited human data was considered in the classification proposal.

Another MSCA commented that a substance is corrosive to skin when it produces destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, and that the CLH report did not contain information on observed necrosis. According to the MSCA, only local irritation effects were reported in both dermal irritation/corrosion study and dermal acute toxicity studies and therefore the criteria for Skin Corr. 1C was not met.

The DS responded that due to the irreversibility of skin damage within 14 days, the classification of MES as Skin Corr. 1C was justified.

Assessment and comparison with the classification criteria

The table below summarises the available skin corrosion/irritation studies. The CLH report also contains information about an epidemiological study where 41 cases of skin irritation (representing a relative frequency of 0.0000006% from all hygienic hand disinfections) were reported in connection with a mixture containing 0.2% MES between January 2000 and August 2005.

***Table**: Summary of the animal studies on skin corrosion/irritation studies with MES*

<table>
<tr><th>Study</th><th>Dose level</th><th>Results</th><th>Reference</th></tr>
<tr><td>OECD TG 404

3 White New Zealand rabbits</td><td>0.5 ml of 0.2% MES</td><td>No erythema, oedema or any other effects on skin were observed at 30-60 min, 24 h, 48 h, 72 h after patch removal (Draize scores = 0)</td><td><confidential> (1992d)</td></tr>
<tr><td>OECD TG 404

3 White New Zealand rabbits</td><td>0.5 ml of 4% MES</td><td>Skin irritation in rabbits after dermal exposure to 4% MES.
<table><tr><th>Score (average of 3 animals investigated)</th><th>Time</th><th>Erythema</th><th>Oedema</th></tr><tr><td rowspan="4">Average score Draize scores (0 to maximum 4)</td><td>0.5-1 h</td><td>1</td><td>0</td></tr><tr><td>24 h</td><td>2.0</td><td>2.3</td></tr><tr><td>48 h</td><td>2.0</td><td>2.3</td></tr><tr><td>72 h</td><td>2.3</td><td>2.3</td></tr><tr><td>Average score</td><td>24h, 48h,</td><td>2.1</td><td>2.3</td></tr></table></td><td><confidential> (1993)</td></tr>
</table>

			72h		
		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			

The CLP Criteria for skin corrosion consist of irreversible destruction of skin tissue, namely, visible necrosis through the epidermis and into the dermis, in at least one tested animal after exposure ≤ 4 h. RAC notes that 4% MES caused irreversible erythema and oedema of an average score of at least 2 according to the Draize scale in all three animals from 24 h to day 9, and afterwards lower Draize scores but also skin fissures in all animals and leathery skin in one animal until the end of the observation period (day 18). RAC also notes that the total destruction of skin as such was not reported in the CLH report, although it is remarkable that a preparation containing only 4% MES caused such skin responses and therefore much more severe irreversible effects would be expected for pure MES. Therefore, RAC concludes that the results observed in the study performed in accordance with the OECD TG 404 testing 4% MES trigger classification of MES for Skin Corrosivity in category 1.

The DS proposed the sub-categorisation of MES within category 1C because the skin fissures, considered as corrosive effects, were observed 9-10 days after a 4-hour exposure therefore meeting the CLP criteria for a sub-category 1C. However, RAC highlights the fact that the test substance contained only 4% MES and therefore more concentrated MES is likely to trigger its corrosive effects earlier than the 4% solution, and therefore a more severe sub-category cannot be excluded. This conclusion is also supported by the results of the acute dermal toxicity studies with a preparation of 30% MES in which degreasing, induration, partial desquamation and formation of fissures were observed. However, the results of these studies do not either allow a sub-categorization. Therefore, the available animal data is not sufficient for sub-categorisation.

RAC also notes that the available human data shows results in general population exposed to a biocidal product containing 0.2% MES, a concentration 20 times lower than the concentration used in animal studies, without any other information regarding a potential exposure to other co-formulants with skin irritation properties in the biocidal product. These human data also

shows a very low frequency (0.0000006%) of irritation cases. RAC concludes that the available human data is too vague for setting a classification on the skin irritant properties of MES.

RAC concludes that **classification of MES as skin corrosive category 1; H314 (Causes severe skin burns and eye damage) is warranted**.

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**	**Observations**	**Reference**

<table>
<tr><td></td><td></td><td>applicable)</td><td></td><td></td></tr>
<tr><td colspan="5">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
<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></tr>
</table>

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).

RAC evaluation of serious eye damage/irritation
Summary of the Dossier Submitter's proposal The CLH report summarised a study with a preparation containing 0.2% MES that caused reversible redness, chemosis and discharge of the conjunctiva in rabbits. The DS also presented the human data on the general population obtained from the biocidal product containing 0.2% of MES with an incidence of 0.0000003% ocular irritation. The DS concluded that 0.2% MES was irritant to the rabbit eye, but there was no data on pure MES. However, according to CLP 3.3.2.3." Skin corrosive substances shall be considered as leading to serious damage to the eyes as well (Category 1)" and according to the CLP Guidance "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". Therefore, taking into account these considerations the DS proposed the classification of MES as Eye Dam. 1; H318 (Causes serious eye damage).

Comments received during public consultation

The proposed classification was supported by one MSCA.

Assessment and comparison with the classification criteria

The table below summarises the available eye corrosion/irritation study with animals. The CLH report also contains information about an epidemiological study where 24 cases of ocular irritation (representing a relative frequency of 0.0000003% per all hygienic hand disinfections) were reported in connection with a mixture containing 0.2% MES between January 2000 and August 2005. RAC concludes that this human data is inconclusive because of extremely low frequency of incidents (0.0000003%) in the general population exposed to a biocidal product containing a very low concentration of MES (0.2%) and without information on other potentially corrosive co-formulants present in the biocidal product.

***Table**: Summary of the animal study on eye corrosion/irritation with MES*

Study	Dose level	Results	Reference
No guideline study but comparable to OECD TG 405 with acceptable restrictions: i) no wash out after 24 h; ii) limited data on test animals and clinical signs 8 White New Zealand rabbits	0.1 ml of 0.2% MES	Mean score 2 for conjunctival redness (effects lasted 24 h) Redness, chemosis and discharge of the conjunctiva reached score 2 (completely reversible after 4 days)	BODE Chemie (1978)

The CLP criteria for severe eye damage (category 1) consist of irreversible effects on the cornea, iris or conjunctiva in at least one animal and/or of a positive response of corneal opacity equal or higher than 3 and or iritis higher than 1.5 calculated as the mean scores following grading at 24, 48 and 72 hours after installation of the test material in at least 2 of 3 animals. No records for individual data were provided in the study summarised in the table above and therefore no classification can be set on the basis of such study. However, RAC notes that according to the data contained in the table above, a mixture containing 0.2% MES was irritant to the rabbit eye, and therefore much more severe effects would have been expected for pure MES. RAC also notes that according to the CLP, substances known to be corrosive to skin and classified as such (as is the case of MES) are automatically considered to be severely damaging to the eye. Therefore RAC supports the DS's proposal for classification of MES for serious eye damage category 1.

RAC also notes, in line with the DS, that according to the Guidance on the Application of the CLP Criteria (July, 2017), if a substance is classified as Skin corrosion Category 1, then serious eye damage is implicit as reflected in the hazard statement for Skin corrosion; H14 (Causes severe skin burns and eye damage). Thus, the corrosive substance is also classified for Eye Dam. 1; H318, but the corresponding hazard statement (H318) is not indicated on the label to avoid redundancy.

In conclusion, RAC concludes that the **classification of MES as serious eye damage category 1; H318** (without hazard statement on the label) is warranted.

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	Clear liquid; 30% active component	Study type: Adjuvant	not considered to have the potential to cause skin sensitisation.	<confidential> (1992e)

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels duration of exposure	Results	Reference
	and 10 females in the treatment group and in the control group		day 0 (intradermal) and day 7 (topical)		

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%). 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 / 41 / 0.0000006%	BODE Chemie (2006)

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

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS proposed no classification of MES for skin sensitisation on the basis of a study performed in accordance with an OECD TG 406, where a preparation of 30% MES did not cause sensitisation in any of the treated Guinea pigs. The DS also summarised briefly some human data and reported that a mixture containing 0.2% MES was unable to sensitise any of the 55 exposed volunteers and that in the general population there were only 43 cases of suspected allergy to a mixture containing 0.2% MES (0.0000006% of population exposed to all hygienic hand disinfections) reported in the period between January 2000 and August 2005.

Comments received during public consultation

One MSCA expressed concern regarding the validity of the skin sensitisation test for the following reasons: i) it was unclear whether the 1.5% MES preparation was used for intradermal induction, topical induction or topical challenge; ii) there was no explanation on how the doses were selected and whether they were high enough as described in the OECD TG; and, iii) no information regarding the positive controls were provided.

The DS responded that the following conditions were used for induction: i) 0.1 mL of 0.5% preparation in water or FCA/water (containing 0.15% active component) was used for the intradermal injection; ii) 10% preparation in water solution (containing 3% active component) for 48 hours was used for occlusive topical induction. The DS also clarified that after this induction, 30% MES was unable to sensitise any animal and referred the requesting MSCA to the IUCLID for additional information.

Assessment and comparison with the classification criteria

The table below summarises the available skin sensitisation study with animals. In addition to the animal study the following human data were also available: i) the relative frequency per all hygienic hand disinfections of suspected allergy in connection with a mixture containing 0.2% MES was 0.0000003% (24 cases) in the period between January 2000 and August 2005; and ii) none of the 55 individuals voluntarily exposed to 0.2% MES for 24 hours under occlusive conditions experienced sensitising reactions. RAC notes that this study is not suitable for human sensitisation assessment since there were no induction phase according to the protocol provided in the CLH dossier.

***Table**: Summary of the animal study on skin sensitisation with MES*

Study	Dose level	Results	Reference
OECD TG 406 GLP Guinea pig 10 animals/sex/group	<u>Induction:</u> Day 0 (intradermal): 0.1 ml/injection site of 0.5% tested preparation (0.15% MES) in water or in Freunds Complete Adjuvant/water (it caused slight/no specific findings in preliminary experiments) Day 7: Occlusive patch of 10% of the tested preparation (3% MES) during 48 hours (it caused slight erythema and no oedema in preliminary experiments) <u>Challenge:</u> Day 21 (dermal): Occlusive patch of 5% of the tested preparation (1.5% MES) during 24 hours (it caused no erythema and no oedema in preliminary experiments)	Positive control: 2,4-dinitrobenzene and benzocaine; worked as expected from the historical control data. <u>24 hours after challenge:</u> No skin reactions were detected in any of the treated or control animals. <u>48 hours after challenge:</u> 1 treated male and 2 control females scored 1 for erythema. 16/20 test animals and 15/20 control animals showed scale formation on the treated skin. **Conclusion:** 1.5% MES was not skin sensitiser.	<confidential> (1992e)

A preparation of 1.5% MES was unable to sensitise animals in a test performed in accordance with the OECD TG 406. RAC concludes that none of the conditions requested for warranting classification have been met using a challenge dose of 1.5% MES and the data base on humans is not robust enough for supporting classification. Therefore, RAC concludes in agreement with the DS that **no classification is warranted for MES for skin sensitisation**.

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 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.	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)
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	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
		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 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,	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
		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 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	Mecetronium ethyl sulphate	Organisms/cell type: Mouse lymphoma L5178Y TK+/- cells	Genotoxicity without metabolic activation: 1st assay	BODE Chemie (1994a)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
assay OECD 476 GLP: yes	[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)	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, 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.	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. 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	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	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	BODE Chemie (2008)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
	active substance): IUCLID technical dossier	(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 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.	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. 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	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	Genotoxicity without metabolic activation: Valid positive control. No increase in radioactivity indicating no UDS and/or cytotoxic effects.	BODE Chemie (1981a)

Method, guideline, deviations if any	Test substance,	Relevant information about the study including rationale for dose selection (as applicable)	Observations	Reference
to OECD 482 GLP: yes		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 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.	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).	

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	Species: mouse Strain: Crl:NMRI BR Number of animals per group: 5m + 5f per dose and sampling time Administration/exposure: Oral via gavage	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	<confidential> (1994b)

Method, guideline, deviations if any	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
	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)	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)	OECD guideline 474. The study is not valid due to significant methodological deficiencies.	

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	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

significant methodological deficiencies.	- 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.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

DS proposed no classification of MES for germ cell mutagenicity considering the results of the following tests:

- Two independent negative bacterial reverse mutation tests conducted according to OECD TG 471;
- One negative *in vitro* mammalian chromosome aberration test with 2 independent experiments and according to OECD TG 473;
- One *in vitro* mammalian gene mutation assay (OECD TG 473) with two independent

experiments where one yielded a negative result while the other yielded positive result;

- One positive *in vitro* mammalian gene mutation assay in another independent test also according to OECD TG 476;
- One negative *in vitro* unscheduled DNA synthesis test (OECD TG 472) in mammalian cells with clear negative result without metabolic activation and one negative result with metabolic activation but without a robust positive control;
- A negative *in vivo* micronucleus test (OECD TG 474) performed with methodological deficiencies that allowed to consider this test as non-reliable and non-valid.

Comments received during public consultation

One MSCA commented that the mutagenicity potential of MES was not conclusive because of contradictory results in mammalian cell gene mutation assays, positive and equivocal results with other quaternary ammonium compounds, a dose-response relationship (not statistically significant increase) in the incidence of micronuclei in females but no clastogenic effects in males, and because of a quantitative structural alert on the ethyl sulphate structure of the MES, and that classification was not possible without any specific additional information. The DS agreed with the comment.

Assessment and comparison with the classification criteria

The table below summarises the results of the available mutagenicity and genotoxicity tests.

***Table**: Summary table of relevant in vitro and in vivo mutagenicity studies with MES*

Method	Test system	Tested concentrations	Results	Remarks	Reference
OECD 471 GLP Bacterial reverse mutation test	S. typhimurium: TA 1535, TA 1537, TA 98, TA 100 Metabolic activation: S9 mix from livers of Wistar rats which received i.p. 500 mg/kg bw Aroclor 1254 5 days before preparation	Main study: 0.16, 0.8, 4.0, 20 and 100 µg/plate. Positive control without metabolic activation 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. Positive control with metabolic activation 3 µg/plate 2-aminoanthracene for all strains.	**MES did not induce gene mutations in bacteria.** Reduced number of revertants/plate at concentration of 100 µg/plate in case of TA1535, TA1537 indicated cytotoxic effect.	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.	BODE Chemie (1992f)

Comparable to OECD 471 GLP Bacterial reverse mutation test	S. typhimurium: TA 1538, TA 1535, TA 1537, TA 98, TA 100 Metabolic activation: 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.	Concentrations: 20, 100, 500, 2500 µg/plate. Positive control: For all strains the same substance was used with and without metabolic activation: TA98 and TA1538: 50 µg/plate dichlorobenzidine; TA100: 50 µg/plate methylcholathrene; TA1535 200 µg/plate cyclophosphamide; TA1537: 100 µg/plate aminoacridine.	**MES did not induce gene mutations in bacteria.**		BODE Chemie (1981)
OECD 473 GLP In vitro mammalian chromosome aberration test	Chinese Hamster Ovary (CHO) cells Metabolic activation system: rat liver S9 mix	<u>Main study 1:</u> without metabolic activation 0, 1.5, 3, 6, 9, 12 µg/ml and with metabolic activation 0, 2.5, 5, 10, 20, 30, 40 µg/ml <u>Main study 2:</u> without metabolic activation 0, 2.5, 5, 7.5, 10 µg/ml or 0, 7.5, 10; with metabolic action 0, 5, 15, 25, 30 µg/ml or 0, 25.5, 30 Positive control; 0.2 µg/ml colcemide	**MES induced no clastogenic activity** (neither with metabolic activation nor without metabolic activation) at any dose level **in both independent studies.** Positive and negative control worked within the historical control data of the performing facility.	Given concentrations are related to the 30% solution of MES Preliminary study: 0, 0.78- 50 µg/ml without metabolic activation and 0, 3.12-100 µg/ml with metabolic activation (mitotic index determined after 18 or 28 h incubation).	BODE Chemie (1994)
OECD 476 GLP Mammalian cell gene mutation assay	Mouse lymphoma L5178Y TK+/- cells Metabolic activation system: S9 mix from rat liver	<u>1st assay:</u> Without metabolic activation: 0, 0.63, 1.25, 2.5, 5 µg/ml; with metabolic activation: 0, 3.13, 6.25, 12.5,	<u>1st assay:</u> **Positive** (statistically significant increases in the mutant frequency)		BODE Chemie (1994a)

	Positive control: With metabolic activation 3 µg/ml benzo(a)pyrene; without metabolic activation 25 µg/ml methylmethanesulfonate	25.0 µg/ml 2nd assay: Without metabolic activation: 0, 2.5, 5, 7.5, 10, 15 µg/ml; with metabolic activation: 0, 7.5, 10, 20, 30, 40 µg/ml	2nd assay: **Negative** (no statistically significant increases in the mutant frequency) In both cases positive and negative control worked within the historical control data of the performing facility.		
OECD 476 GLP Mammalian cell gene mutation assay	Mouse lymphoma L5178Y/ TK+/- cells	Without metabolic activation: 0, 0.63, 1.25, 2.5, 3.75, 5.00, 7.50 µg/mL With S9-mix: 0, 2.5, 5.0, 10, 15, 20 µg/mL. Positive control: with metabolic activation: 2.5 µg/mL cyclophosphamide monohydrate; Positive control without metabolic activation: 7.5 µg/mL methyl methanesulfonate.	For both with and without metabolic activation: **Negative** (MES did not induce statistically significant increases in the mutant frequencies). Positive and negative control worked within the historical control data of the performing facility. All MES-treated cultures exhibited mutant frequencies within the normal range for negative controls.	MES, both in the absence and presence of S9-mix, induced marked concentration-dependent cytotoxicity. Plating efficiency and relative survival were not altered significantly by MES treatment (except 3.75 µg/mL without and 5.0 µg/mL with S9-mix).	BODE Chemie (2008)
Comparable to OECD 482 GLP Unscheduled DNA	HeLa S3 cells (human cell line) Metabolic activation: Rat liver S9 mix from Wistar rats that received a single i.p. injection of 500	Concentrations: 0, 0.2, 0.02, 0.002, 0.0002 µg/ml Positive control with metabolic activation: 10	Without metabolic activation: No increase in radioactivity indicating **no UDS** and/or	Cytotoxicity: Yes, reduced radioactivity at the high dose levels indicates cytotoxicity	BODE Chemie (1981a)

synthesis (UDS) in mammalian cells *in vitro*	mg/kg bw Aroclor 1254 in corn oil	µM NQO (no further specification, presumably 4-nitroquinoline-N-oxide) Positive control without metabolic activation; 50 µM DCB (no further specification)	cytotoxic effects. Valid positive control <u>With metabolic activation:</u> No increase in radioactivity indicating **no UDS** and/or cytotoxic effects. Only a weak effect in the positive control limiting the reliability of the results (no statistical evaluation).		
OECD 474 GLP *In vivo* micronucleus test	Crl:NMRI BR mouse 5 males + 5 females/group/sampling time Gavage administration Single oral application Dose: 0, 18.7, 56, 187 mg/kg bw plus positive control (all 24 h); 187 mg/kg bw (48 h)	Dose: 0, 18.7, 56, 187 mg/kg bw plus positive control (all 24 h); 187 mg/kg bw (48 h)	**MES did not induce a significant increase in the number of micronuclei** at a dose level up to 187 mg/kg bw. The study is not valid due to significant methodological deficiencies.	187 mg/kg bw did not reach the dose level recommended in the OECD TG 474 and did not induce cytotoxicity.	<confidential> (1994b)

RAC notes that the database is not totally conclusive for establishing a classification. According to the CLP criteria, positive results in 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 are needed for classification in Category 2. Also 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. None of these requirements were met with the available database because the only available *in vivo* result was negative (although of questionable validity due to methodological deficiencies) and the *in vitro* results were mainly negative as the only positive result in the mammalian cell gene mutation assay (the first assay in DE Chemie

(1994a)) could not be confirmed in the other two equal independent assays (the second assay in DE Chemie (1994a) and BODE Chemie (2008)), in the unscheduled DNA synthesis assay (BODE Chemie (1981a), in the *in vitro* mammalian chromosome aberration test (BODE Chemie (1994)), or in the bacterial reverse mutation tests (BODE Chemie (1992f) and BODE Chemie (1981)). Therefore, RAC agrees with the DS's proposal that **no classification of MES is warranted for germ cell mutagenicity**.

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

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-	Mecetronium	Parent males and females (F0)	BODE

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr>
<td>Generation Reproduction Toxicity Study
OECD 415
GLP: yes
Species: rats
Strain: Wistar rats
Sex: male and female
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</td>
<td>ethyl sulphate [MES] 29% (aqueous solution)
Duration of exposure before mating: 70 days
Duration of exposure in general:
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-</td>
<td>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

<table>
<tr><th rowspan="2">Parameter</th><th rowspan="2"></th><th rowspan="2"></th><th colspan="2">control</th><th colspan="2">Low Dose (10 mg/kgday)</th><th colspan="2">Medium Dose (40 mg/kgday)</th><th colspan="2">High Dose (110 mg/kgday)</th></tr>
<tr><th>Gene-ration</th><th>m</th><th>f</th><th>m</th><th>f</th><th>m</th><th>f</th><th>m</th><th>f</th></tr>
<tr><td>Mortality</td><td>Incidence</td><td>F0</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>1</td><td>-</td><td>4</td></tr>
<tr><td colspan="11"></td></tr>
<tr><td>Food consumption</td><td>% compared to control</td><td>F0</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-7.4</td><td>-13.2</td></tr>
<tr><td colspan="11"></td></tr>
<tr><td rowspan="3">Body weight gain</td><td colspan="10">% of control</td></tr>
<tr><td>Pre-pairing period</td><td>F0</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>83</td><td>90</td></tr>
<tr><td>Gestation period</td><td>F0</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td><td>76</td></tr>
<tr><td colspan="11"></td></tr>
<tr><td>Clinical Observations
Salivation</td><td rowspan="2">Incidence</td><td>F0</td><td>-</td><td>-</td><td>-</td><td>-</td><td>2</td><td>7</td><td>13</td><td>9</td></tr>
<tr><td>Rales</td><td>F0</td><td>-</td><td>-</td><td>-</td><td>-</td><td>3</td><td>4</td><td>15</td><td>13</td></tr>
<tr><td colspan="11"></td></tr>
<tr><td>Histopathologic examination
Changes in the stomach</td><td>Incidence</td><td>F0</td><td>-</td><td>-</td><td>-</td><td>-</td><td>Yes</td><td>Yes</td><td>Yes</td><td>Yes</td></tr>
<tr><td colspan="11">Forestomach</td></tr>
<tr><td>Acanthosis/Hyperplasia</td><td></td><td>F0</td><td>-</td><td>-</td><td>-</td><td>-</td><td>3</td><td>1</td><td>18</td><td>5</td></tr>
<tr><td>Hyperkeratosis</td><td></td><td>F0</td><td>-</td><td>-</td><td>-</td><td>-</td><td>2</td><td>-</td><td>20</td><td>6</td></tr>
</table>
</td>
<td>Chemie (2008a)</td>
</tr>
</table>

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

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**

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

From all F0 animals, samples of the above 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.

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 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:

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
	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 all dams. The uteri were placed in a solution of ammonium sulphide to visualize possible hemorrhagic areas of implantation sizes.	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 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	

<table>
<tr><th>Method, guideline, deviations if any, species, strain, sex, no/group</th><th>Test substance, dose levels duration of exposure</th><th>Results</th><th>Reference</th></tr>
<tr><td></td><td></td><td>
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.

- = no treatment related data observed
<table>
<tr><th rowspan="2">Parameter</th><th></th><th>control</th><th>Low Dose (10 mg/kg day)</th><th>Medium Dose (40 mg/kg day)</th><th>High Dose (110 mg/kg day)</th></tr>
<tr><th>Gene-ration</th><th>m & f</th><th>m & f</th><th>m & f</th><th>m & f</th></tr>
<tr><td colspan="6">Reproductive Performance</td></tr>
<tr><td>Number of implantation sites</td><td>% of control</td><td>F0</td><td>13.8</td><td>13.0</td><td>13.8</td><td>11.9*</td></tr>
<tr><td colspan="6"></td></tr>
<tr><td colspan="6">Litter Data</td></tr>
<tr><td>Living pups at first litter check</td><td>% of males/females</td><td>F1</td><td>41/59</td><td>50/50*</td><td>45/55</td><td>53/47*</td></tr>
<tr><td>Postnatal loss (days 0-4)</td><td>Total pups affected</td><td>F1</td><td>6</td><td>10</td><td>4</td><td>24**</td></tr>
<tr><td>Viability index</td><td>%</td><td>F1</td><td>97.7</td><td>96.6</td><td>98.5</td><td>89.1**</td></tr>
<tr><td>Pup mean bodyweight (day 7)</td><td>g males+females</td><td>F1</td><td>14.3</td><td>14.3</td><td>14.2</td><td>12.9**</td></tr>
<tr><td>Pup mean bodyweight (day 14)</td><td>g males+females</td><td>F1</td><td>30.2</td><td>29.9</td><td>29.7</td><td>26.1**</td></tr>
<tr><td>Pup mean bodyweight (day 21)</td><td>g males+females</td><td>F1</td><td>47.3</td><td>47.7</td><td>46.8</td><td>41.9**</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	Results	Reference
		* 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 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	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>
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>
<tr><td colspan="6">Table: Macroscopic findings in the stomach, intestine, spleen and liver of dams at necropsy (at gestation day 29 & prematurely deceased rabbits)</td></tr>
<tr><td>Finding</td><td>Control n=21</td><td>4 mg/kg bw n=20</td><td>12 mg/kg bw n=21#</td><td>30 mg/kg bw n=24#</td><td>40 mg/kg bw n=22#</td></tr>
<tr><td>Stomach</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Several/multiple haemorrhagic foci</td><td>0</td><td>0</td><td>0</td><td>1</td><td>12</td></tr>
<tr><td>Multiple ulcers</td><td>0</td><td>0</td><td>0</td><td>0</td><td>2</td></tr>
<tr><td>Mucosal detachment</td><td>0</td><td>0</td><td>0</td><td>2</td><td>0</td></tr>
<tr><td>Whitish layer</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></tr>
<tr><td>Intestine</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Liquid brownish content</td><td>0</td><td>0</td><td>0</td><td>6</td><td>12</td></tr>
<tr><td>aerated</td><td>0</td><td>0</td><td>0</td><td>8</td><td>1</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

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

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: Reproduction data

Finding	Control n=21	4 mg/kg bw n=20	12 mg/kg bw, n=20	30 mg/kg bw, n=17	40 mg/kg bw,

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

					n=10
Corpora lutea total	177	164	161	127	75
Corpora lutea per dam	8.4	8.2	8.1	7.5	7.5
Total implantation sites	162	145	153	116	63
Implantation sites/dam	7.7	7.3	7.7	6.8	6.3
Total resorptions	18	5**	2**	8	3
Resorptions/dam	0.9	0.3	0.1	0.5	0.3
Total early resorptions	17	4**	1**	7	3
Early resorptions/dam	0.8	0.2	0.1	0.4	0.3
Total late resorptions	1	1	1	1	0
Late resorptions/dam	0.0	0.1	0.1	0.1	0.0
Mean% pre-implantation loss	9.3	12.1	5.7	8.1	18.4
Mean% post-implantation loss	10.0	4.4	1.3	8.7	3.8
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

<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>
<table>
<tr><td>Sex distribution</td><td></td><td>No influence</td><td>No influence</td><td>No influence</td><td>No influence</td></tr>
</table>
<table>
<tr><td colspan="7">b) Developmental toxicity</td></tr>
<tr><td>Finding</td><td>Control n=20</td><td>4 mg/kg bw n=20</td><td>12 mg/kg bw, n=20</td><td>30 mg/kg bw, n=16</td><td>40 mg/kg bw, n=10</td></tr>
<tr><td>Placental weight (m&f, litter mean)</td><td>5.01+-0.89</td><td>5.31+-0.64</td><td>5.00+-0.73</td><td>4.70+-0.64#</td><td>5.43+-1.17</td></tr>
<tr><td>Fetal weight (m, litter mean)</td><td>38.9+-4.8</td><td>39.5+-3.3</td><td>38.9+-4.1</td><td>34.9+-5.9*#</td><td>38.5+-5.5</td></tr>
<tr><td>Fetal weight (f, litter mean)</td><td>38.1+-4.1</td><td>39.4+-3.9</td><td>38.9+-3.4</td><td>33.4±6.3**#</td><td>37.1+-3.4</td></tr>
<tr><td>External malformation & variation</td><td></td><td colspan="4">No test substance related findings</td></tr>
<tr><td>Skeletal malformation</td><td></td><td colspan="4">No test substance related findings</td></tr>
<tr><td>Skeletal variations & retardations</td><td></td><td colspan="4">Within the range of control group</td></tr>
<tr><td>Visceral examination</td><td colspan="5">No pathological findings; no test substance related variations</td></tr>
<tr><td>Soft tissue of the head</td><td colspan="5">No malformations</td></tr>
<tr><td>Soft tissue of the head</td><td></td><td colspan="4">Total variations within the range of control group</td></tr>
<tr><td>Subdural haemorrhages of meninx (fetal</td><td>3 (4.2%, n=72)</td><td>8 (11.4%, n=70)</td><td>3 (4.0%, n=75)</td><td>11** (20.8%, n=53)</td><td>1 (3.3%, n=30)</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
			incidence)						
			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.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of MES for reproductive toxicity on the basis of the results of the following studies:

- A one-generation reproductive toxicity study (OECD TG 415) in rats receiving 0, 10, 40 and 110 mg/kg bw/day of MES via oral gavage. At 110 mg MES/kg bw/day, a statistically significant decrease in the mean number of implantation sites (11.9 vs. 13.8 in controls, viability index (89.1% vs 97.7% in controls), mean body weight in pups on lactation days 7, 14 and 21 as compared to controls (-10%, -14% and 11%, respectively) and an increase in the postnatal loss (24 vs. 6 in controls) on days 0-4 co-occurring with 17% maternal mortality (4/24), clinical signs (salivation and rales during single or multiple days of the treatment period) and irritative and degenerative lesions in the forestomach;
- A teratogenicity study in rabbit (OECD TG 414) receiving 0, 4, 12, 30 and 40 mg/kg bw/day of MES via oral gavage. According to the DS there were no developmental effects of biological relevance or not secondary to local effects on the GI tract of dams at ≤ 40 mg/kg bw/day.

The DS concluded that there were no adverse effects on sexual function and fertility or on development at doses below those inducing severe maternal toxicity, and therefore classification was not warranted.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

One-Generation Reproductive Toxicity Study

The one-generation toxicity study was performed according to OECD TG 415 and GLP. Wistar rats (96 males and 96 females; 24 males and 24 females per dose group) were exposed by oral gavage to MES 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 for a 45-day after-pairing period until one day before the scheduled sacrifice, in total for 120 days. F0 females received the test substance 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 for maximally 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 further tested for reversibility of the effects. Therefore, these females were given a 4-week treatment-free period (recovery period) and they were mated again with untreated males. All females were allowed to give birth and rear their pups until day 4 post-partum. The dams were sacrificed on day 5 post-partum, ie. one

day after the pups.

All animals were subjected to twice daily clinical observations. Daily body weight and food consumption were measured over the treatment period. The regularity and duration of the oestrus cycle were 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.

The table below summarises the parental toxicity in the one-generation reproductive toxicity study. Mortalities, clinical signs and irritative and degenerative lesions in the forestomach were reported at the highest dose (110 mg/kg bw/day). In the mid dose (40 mg/kg bw/day) clinical signs and histopathological alterations in stomach were also reported, although with a lower incidence.

Table: *Parental toxicity in the one-generation reproductive toxicity study with MES*
None of the effects were reported in the control group and in the group dosed with the lowest dose of 10 mg/kg bw/day.

110 mg/kg bw/day	**40 mg/kg bw/day**
4 mortalities (females) <u>Body weight gain:</u> Pre-pairing period: 83 (males) and 90% (females) of control Gestation period: 76% of control <u>Clinical observations:</u> Salivation: 13 males and 9 females Rales: 15 males and 13 females <u>Histopathologic alterations:</u> Acanthosis/Hyperplasia: 18 males and 5 females Hyperkeratosis: 20 males and 6 females Oedema: 5 males and 4 females Inflammation, acute: 4 males and 3 females Erosion: 2 females Ulceration: 1 female	0 mortalities <u>Clinical observations:</u> Salivation: 2 males and 7 females Rales: 3 males and 4 females <u>Histopathologic alterations:</u> Acanthosis/Hyperplasia: 3 males and 1 females Hyperkeratosis: 2 males Oedema: 3 males Inflammation, acute: 1 male and 1 female Erosion: 1 male Ulceration: 1 male

<u>Adverse effects on sexual function and fertility</u>

Adverse effects on sexual function and fertility were restricted to the highest tested dose. The number of implantation sites was decreased as compared to controls (11.9 vs. 13.8 in controls). However, as the maternal mortality was 17%, the maternal toxicity is considered excessive and the data at this dose level is not considered for further evaluation.

<u>Adverse effects on development</u>

The adverse effects on development were restricted to the highest tested dose. 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 cannibalised on day 1 post-partum. One other litter was found dead with 8 pups. In another litter, five pups were found dead on day 2 post-partum (no milk in the stomach). This was considered to be a result of the moribund condition of the dams. Accordingly, the pup viability index was decreased (89.1% vs 97.7% in controls). From day 7 post-partum onwards, body weight development was statistically significantly reduced. All these findings were considered to be

substance-related. However, as the maternal mortality was 17%, the maternal toxicity is considered excessive and the data at this dose level is not considered for further evaluation.

Table: *Reproductive parameters in the One Generation Reproductive Toxicity Study with MES.*
**= Statistically different of control for p<0.05.*
***=Statistically significantly different from control p<0.01*

	Dose level (mg/kg bw/day)			
	0	**10**	**40**	**110**
Number of implantation sites	13.8	13.0	13.8	**11.9***
Living pups at first litter check (%males/females)	41/59	50/50*	45/55	**53/47***
Post-natal loss (days 0-4)	6	10	4	**24****
Viability index (%)	97.7	96.6	98.5	**89.1****
Pup mean body weight (day 7)	14.3	14.3	14.2	**12.9****
Pup mean body weight (day 14)	30.2	29.9	29.7	**26.1****
Pup mean body weight (day 21)	47.3	47.7	46.8	**41.9****

Teratogenicity study in the rabbit

The teratogenicity study in rabbits was performed according to OECD TG 414 and GLP. Himalayan rabbits were dosed by gavage with the following doses: 0 mg/kg bw/day (20 animals); 4 mg/kg bw/day (20 animals), 12 mg/kg bw/day (20 animals), 30 mg/kg bw/day (16 animals), and 40 mg/kg bw/day (10 animals).

The table below summarises the maternal toxicity reported at the two highest doses. At 4 mg/kg bw/day, no test substance-related clinical effects were reported for mortality, body weight or body weight change or food consumption relative to the control group. At 12 mg/kg bw/day no effects were detected on body weight or body weight change or food consumption; 2 out of 24 dams were not pregnant (the incidence is within the normal range) and 1 out of 21 dams spontaneously aborted on gestation day 26.

Table: *Maternal toxicity in the teratogenicity study in rabbits with MES.*
No treatment-related effects were reported at 12 and 4 mg/kg bw/day

30 mg/kg bw/day	**40 mg/kg bw/day**
• 2 out of 24 dams (8%) died prematurely on gestation day 22 or 28 (both with diarrhoea and lesions of the stomach). • 5 further dams were sacrificed after abortion between gestation day 23 and 27 (evaluation of individual test results revealedgastro-intestinal effects of the test substance in all 5 rabbits with abortions: 1 out of these 5 dams has stomach lesions; diarrhoea, partly haemorrhagic in 3 rabbits, and the last one showed minimal or no discharge of faeces). • One dam was without viable foetuses (haemorrhagic diarrhoea observed). • Obvious decrease (p<0.01) body weight gain (the numerical data was not provided) • Reduced absolute and relative food intake (p<0.01) (the numerical data was not provided). • Histological findings in stomach: Several/multiple haemorrhagic foci: 1 Multiple ulcers: 0 Mucosal detachment: 2	• 8 out of 24 dams (33%) died prematurely on GD 18 or 27 (all of them had stomach lesions, 6/8 liquid content in the intestine and 6/8 diarrhoea). • Mydriasis in all dams from the 1st application onwards (starting 20-60 minutes after treatment and lasting 2-6 h). • 4 further dams were sacrificed after abortion between gestation days 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 in 1/4 rabbits, 2/4 dams revealed minimal or no discharge of faeces). • Decreased (p<0.01) body weight gain (the numerical data was not provided). • Reduced absolute and relative food intake (p<0.01) (the numerical data was not provided). • Histological findings in stomach: Several/multiple haemorrhagic foci: 12 Multiple ulcers: 2

Whitish layer: 1 • Histological findings in intestine: Liquid brownish content: 6 Aerated: 8 • Histological findings in spleen: Reduced size: 0 • Histological findings in liver: Pale: 0	Mucosal detachment: 0 Whitish layer: 0 • Histological findings in intestine: Liquid brownish content: 12 Aerated: 1 • Histological findings in spleen: Reduced size: 4 • Histological findings in liver: Pale: 2

Adverse effects on sexual function and fertility

The table below summarises the main findings on sexual function and fertility in the rabbit teratogenicity study with MES.

Table: *Effects on sexual function and fertility in the rabbit teratogenicity study with MES*

Finding	Control (n=21)	4 mg/kg bw (n=20)	12 mg/kg bw, (n=20)	30 mg/kg bw, (n=17)	40 mg/kg bw, (n=10)
Corpora lutea total	177	164	161	127	75
Corpora lutea per dam	8.4	8.2	8.1	7.5	7.5
Total implantation sites	162	145	153	116	63
Implantation sites/dam	7.7	7.3	7.7	6.8	6.3
Mean pre-implantation loss (%)	9.3	12.1	5.7	8.1	18.4

As the high dose caused excessive maternal mortality (33%), RAC did not further evaluate the effects observed at this dose. Maternal toxicity was considered to be excessive also at 30 mg/kg bw/day. RAC considers that there were no treatment-related effects on corpora lutea, implantation sites, or pre-implantation loss below the top dose.

Adverse effects on development

The table below summarises the main developmental effects in the rabbit teratogenicity study with MES.

Table: *Developmental effects in the rabbit teratogenicity study with MES*

Finding	Control (n=21)	4 mg/kg bw (n=20)	12 mg/kg bw (n=20)	30 mg/kg bw(n=17)	40 mg/kg bw (n=10)
Total resorptions	18	5**	2**	8	3
Resorptions/dam	0.9	0.3	0.1	0.5	0.3
Total early resorptions	17	4**	1**	7	3
Early resorptions/dam	0.8	0.2	0.1	0.4	0.3
Total late resorptions	1	1	1	1	0
Late resorptions/dam	0.0	0.1	0.1	0.1	0.0
Mean% post-implantation loss	10.0	4.4	1.3	8.7	3.8
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
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
Foetal weight (m, litter mean)	38.9±4.8	39.5±3.3	38.9±4.1	34.9±5.9*#	38.5±5.5

Foetal weight (f, litter mean)	38.1±4.1	39.4±3.9	38.9±3.4	33.4±6.3**#	37.1±3.4
Subdural haemorrhages 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 haemorrhages of meninx (litter incidence)	3 (15.0%)	6 (30.0%)	2 (10.0%)	9* (56.3%)	1 (10.0%)
External malformation & variation	No test substance related findings				
Skeletal variations & retardations	No test substance related findings				
Visceral examination	No test substance related findings				
Soft tissue of the head	No test substance related findings				
*: p≤0.05; **: p≤0.01; #: incidental decrease, biologically not relevant, within the historical control range					

According to CLP 3.7.2.4.4, maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation. As the high dose caused excessive maternal mortality (33%), this dose level was not considered for further evaluation by RAC. Maternal toxicity was considered to be excessive also at 30 mg/kg bw/day. RAC notes however that no test substance-related developmental effects were reported in the study. The observed effects in foetal weight at 30 mg/kg bw/day are within the historical control data (and no effect in foetal weight is observed at the top dose). Foetal and litter incidence of subdural haemorrhages in the meninx were elevated at 30 mg/kg bw/day. However, no corresponding effect was found at 40 mg/kg bw/day. Furthermore, this variation is also noted in the 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. Altogether, no classification is warranted for the effects listed in the table above.

Abortions and the co-occurring maternal toxicity

1, 5 and 4 abortions were recorded at 12, 30 ad 40 mg/kg bw/day, respectively. The abortion at the lowest dose was considered spontaneous according to the study report. As the high dose caused excessive maternal mortality (33% due to stomach lesions (8/8); liquid content in the intestine and diarrhoea (6/8)), RAC does not further evaluate the abortions observed at this dose. Also, abortions at 30 mg/kg bw/day do not warrant classification because they are considered to be secondary non-specific consequences of maternal toxicity since all dams that suffered from the abortions showed gastrointestinal effects (stomach lesions (1/5), partly haemorrhagic diarrhoea (3/5) and minimal or no discharge of faeces (1/5)). The critical role of severe maternal toxicity on abortions is supported by the fact that these gastrointestinal effects in non-aborting animals caused 2/24 fatalities (both with diarrhoea and lesions of the stomach) in dams dosed with 30 mg/kg bw/day. The gastrointestinal damage reported in animals exposed at 30 and 40 mg/kg bw/day included several/multiple haemorrhagic foci in the stomach (13 animals in total), liquid brownish content in the intestine (18 animals in total) and aerated intestine (9 animals in total); together with lower but still significant incidences of multiple ulcers and mucosal detachment in the stomach and reduced spleen size.

Comparison with the CLP criteria

RAC notes that the adverse effects on reproduction in the one-generation reproductive toxicity study in rats were reported only at the top dose of 110 mg/kg bw/day, which also caused severe parental toxicity (17% of maternal mortality, salivation in 48% of animals and rale in 58% of animals in addition to histopathological evidence of severe degenerative lesions in the forestomach). RAC considers this maternal systemic toxicity as excessive, and therefore the adverse effects are not further considered for classification for adverse effects on sexual function and fertility, on development or on or via lactation.

RAC concludes that there were no treatment-related effects on sexual function and fertility or development in the developmental toxicity study in rabbits below the doses causing excessive maternal mortality/severe maternal toxicity. Therefore, **no classification of MES for adverse effects on sexual function and fertility or on development is warranted**.

In conclusion, RAC supports the DS's proposal for no classification of MES for reproductive toxicity.

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.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

DS proposed no classification of MES for STOT SE on the basis of the following:

- There was no evidence of specific target organ toxicity in animals after single exposure to MES.
- The overall incidence of suspected MES-induced effects in humans exposed to a preparation containing 0.2% MES was around 0.00018%. The reported effects included skin and ocular irritation, suspected allergy, respiratory tract irritation, diarrhoea and burns.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

RAC notes that the acute oral and dermal toxicity studies in animals did not reveal any evidence of toxicity to a specific organ that are not specifically addressed under other hazard classes, which is a requirement for classification for STOT SE. RAC also notes that the epidemiological data base is not sufficiently robust to justify a classification for STOT SE, because the only adverse effects relevant for STOT SE 3 included only two cases of respiratory tract irritation in the general population in contact with a preparation containing 0.2% MES between January 2000 and August 2005 (the frequency of <0.0000001%).

In conclusion, RAC agrees with the DS that there is **no evidence to warrant classification of MES for STOT SE**.

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				

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	Category 1 (H372):

guidance values are not applicable.	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.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS proposed no classification of MES for STOT RE on the basis of the following studies: i) a 4-week oral (gavage) dose range-finding study in rat reporting NOAEL and LOAEL of 50 and 150 mg/kg bw/day, respectively; and, ii) a 90-day oral (gavage) study in rats showing NOAEL and LOAEL of 45 and 90 mg/kg bw/day, respectively. The DS concluded that the observed effects in these studies did not have the potential to produce significant toxicity in humans.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The table below summarises the available repeated dose toxicity studies with animals.

Table: *Summary table for repeated dose toxicity studies in animals with MES*

Method	Results	Reference
4-weeks GLP Sprague-Dawley rats Daily exposure: gavage 5 animals/sex/group 0, 50, 150, 450 mg MES/kg bw/day (in the high dose group dosing was discontinued on day 5)	<u>450 mg/kg bw/day</u> Clinical signs: Piloerection and reduced motility on day 4 in 1 male and 1 female; 1 female showed reduced motility, ataxia and ptosis on day 5. All animals were in a poor condition on day 5. Mortality: 1 male and 2 females were found dead in the morning of day 5. Body weight gain: Not reported. Food consumption: Slight decrease (12%) in males (not statistically significant). Gross pathology: No treatment-related effects during the macroscopic post mortem examination in surviving rats or in rats that died during the exposure. <u>150 mg/kg bw/day</u> No clinical signs. Mortality: 1 female. Body weight gain: Slight decrease (3-9%) only in males (but not statistically significant). No changes in food consumption. Gross pathology: No treatment-related effects during the macroscopic post mortem examination in surviving rats. In the female rat that died during the treatment a haemorrhagic, distended and empty gastro-intestinal tract was reported.	<confidential> (2001)

	<u>50 mg/kg bw/day</u> No clinical signs. No mortalities. No changes in body weight gain. No changes in food consumption. Gross pathology: No treatment-related effects during the macroscopic post mortem examination in surviving rats. **<u>DS's conclusion:</u>** **LOAEL: 150 mg/kg bw/day** **NOAEL: 50 mg/kg bw/day**	
90 days OECD TG 408 GLP Sprague-Dawley rats Daily exposure: gavage 10 animals/sex/group 0, 15, 45, 135/90 mg MES/kg bw/day (135 mg/kg bw/day until day 73, from day 74 onwards due to mortality 90 mg/kg bw/day)	<u>135/95 mg/kg bw/day</u> Clinical signs: irreversible piloerection on day 29 (4/10 males and 3/10 females) and day 30 (all rats). Mortality: 3 male and 7 female (between days 34 and 73). On day 74 the dose was reduced to 90 mg/kg bw/day and no further mortality was observed in the high dose group. Body weight gain: decrease from week 1 to termination (11-20% below control value) in males. A slight and not statistically significant decrease was observed in females from week 6 onwards (1-12% below control value). Food consumption and compound intake: statistically significantly reduced at week 1 (not at week 2-13) in males. A transient decrease in food intake was seen in females at week 1-8 (statistically significant at week 1, 2, 6, and 7). No changes in organ weights. Haematology: Indicative of inflammatory responses in both males and females: increased leucocytes and a shift to the left in differential blood cell counts. Clinical chemistry: In both males and females: i) ALAT (alanine aminotransferase) values are slightly above the historical control range presented by Charles River (no historical control data available from the performing facility); and, ii) aP (alkaline phosphatase) values are within the historical control range presented by Charles River (no historical control data available from the performing facility). <u>45 mg/kg bw/day</u> Clinical signs: long-lasting piloerection starting on day 59 (20/20 rats on day 60). No mortalities. No changes in body weight gain. No changes in food consumption. No changes in organ weights. No haematological changes. Clinical chemistry: In both males and females: i) ALAT values are slightly above the historical control range presented by Charles River (no historical control data	<confidential> (2002)

	available from the performing facility); and, ii) aP values are within the historical control range presented by Charles River (no historical control data available from the performing facility). <u>15 mg/kg bw/day</u> No clinical signs. No mortalities. No changes in body weight gain. No changes in food consumption. No changes in organ weights. No haematological changes. No changes in clinical chemistry. **<u>DS's conclusion:</u>** **LOAEL: 90 mg/kg bw/day** **NOAEL: 45 mg/kg bw/day**	

In the 28-day repeated dose toxicity study mortalities and severe clinical signs were reported at 450 mg/kg bw/day. However, this dose is well above the guidance value range for classification as STOT RE category 2 (30 < C ≤ 300 mg/kg bw/day for a 28-day study). In the same study at 150 mg/kg bw/day (a dose that is within the guidance value range for category 2), a slight decrease in body weight gain as compared to controls (maximum 9%) and one death were reported. The gross pathology examination of the dead female showed haemorrhagic, distended and empty gastro-intestinal tract, which are effects not reported in cases of dead animals at 450 mg/kg bw/day, and therefore RAC considers this fatality as incidental and not relevant for classification. Furthermore, according to the CLP criteria small changes in bodyweight gain do not indicate significant toxicity and therefore should not be considered for classification. Therefore, RAC concludes that the effects observed in the 28-day repeated toxicity study, that are within the guidance value range for classification, do not warrant classification for STOT RE.

In the 90-day repeated dose toxicity study clinical signs, mortalities, reduction in body weight gain and food consumption and changes in haematological and clinical chemistry parameters were reported at the highest dose (135 mg/kg bw/day during the first 73 days and 90 mg/kg bw/day during the remaining 17 days). The initial dose is not within the guidance value range for classification, but the second one is within that range. However, no fatalities were reported after reduction of the dose to 90 mg/kg bw/day. RAC notes that 90 mg/kg bw/day is a dose within the guidance value range for STOT-RE 2. However, it is not known whether the administration of this dose during 90 days instead of only 17 days would have caused mortalities or not. Therefore, the information at this respect is inconclusive and does not give sufficient evidence to support a classification on the basis of the reported mortalities in this 90-day study. In this study, the mid-dose was 45 mg/kg bw/day (a dose that is also within the guidance value range for STOT RE 2) causing long-lasting piloerection and similar changes in clinical chemistry parameters as reported for the highest dose. These changes are considered to be mild, slightly above or within the historical control data of other facility, and no dose-response seems to be noted between 45 and 135/90 mg/kg bw/day doses suggesting that these changes might be incidental and therefore not enough robust for justifying a classification.

RAC concludes that the effects observed in the 90-day repeated toxicity study are within the guidance value range for classification but do not warrant classification for STOT RE.

In addition to studies summarised in the table above, RAC notes that some mortalities were also reported in the 1-generation toxicity study and in the developmental toxicity study. However, in these two studies the mortalities were attributed to local toxicity in the contact point due to corrosivity of the substance. Indeed, dose-dependent incidences of stomach lesions consisting of acanthosis/hyperplasia, hyperkeratosis, edema, acute inflammation, erosion and ulceration were reported in the 1-generation toxicity study. Similar gastrointestinal effects were also reported in the developmental toxicity study. RAC concludes that mortalities reported in these reproductive studies do not warrant STOT RE classification since the mortalities are attributed to local corrosive effects rather than to systemic toxicity.

In conclusion, RAC agrees with the DS, that **no classification is warranted for STOT RE**.

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	Test duration extended to 60 days The study is not GLP compliant. No information on nitrification. Possible partial	BODE Chemie (1995)

Method	Results	Remarks	Reference
	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.	microbial inhibition of the test substance.	
OECD 301A DOC Die Away test	Composition of product: MES 29% in water Initial TS concentration: Replicate 1: 135 mg MES solution / 2 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.	Study is not GLP compliant. Test substance was redosed on test day 1 (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.	BODE Chemie (1999)
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	Analytical parameters: Oxygen consumption	Bode Chemie (2008c)

Method	Results	Remarks	Reference
	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 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.	Analytical parameter: Total inorganic carbon (TIC)	BODE Chemie (2011a)

Method	Results	Remarks	Reference
	MES is not readily biodegradable at the applied concentrations due to toxic effects. The study is acceptable as supporting information.		
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 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	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
	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. 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		

Method	Results	Remarks	Reference
	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 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	Deviations: test duration of 14 days	BODE Chemie (2011c)

Method	Results	Remarks	Reference
	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 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.	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
	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 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:	Species/Strain Leuciscus idus melanotus L. Ten fish were exposed to each of the five concentrations of the test substance	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,	<confidential> (1992g)

mortality Sampling: every 24 hours Initial concentrations of the test substance: 0.1, 0.15, 0.2, 0.25 and 0.3 mg/L (nominal)	between 0.1 and 0.3 mg/L and a control.			These are only minor shortcomings and do not affect the overall significance of the findings.	
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	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;	Biomass production (based on nominal values): NOEC = 0.004 mg/L, EC_{10} = 0.006 mg/L, EC_{50} = 0.025 mg/L,		<confidential> (2000a)

measurement); calculation of biomass production and growth rate Sampling: after 24, 48, 72 hours		21.9 - 23.2°C pH 7.94 – 8.22 (initial) 8.01 – 10.39 (after 72 h) Aeration of dilution water: No 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_{100} = 0.150 mg/L Growth rate (based on nominal values): NOEC = 0.011 mg/L, 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

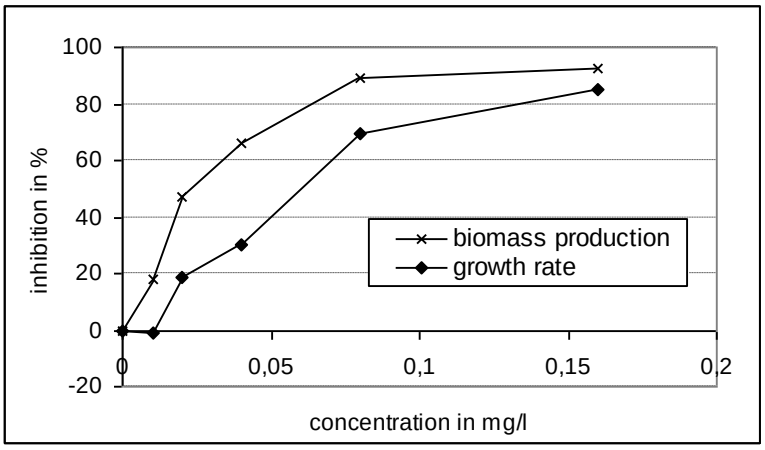
"Alga, Growth Inhibition Test":

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	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)

rate, mortality/survival, growth (body length and weight) and behavioural abnormalities					
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 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.	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)

[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 $\geq$ 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 $0.001 < L(E)C_{50} \leq 0.01$ 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

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

MES is a biocidal active substance and is currently under active substance review for approval as Product Type 1 (PT1; Human hygiene) under BPR for uses including that as a surgical disinfectant.

MES has an ionic structure, strong polarity and surface-active properties. There is currently no entry in Annex VI to CLP for the substance. However, in the ECHA C&L inventory, some notifiers self-classified the substance as Aquatic Acute 1; H400 and Aquatic Chronic 1; H410.

Degradation

The DS proposed to consider MES as rapidly degradable for classification purposes. The basis for this proposal is a weight of evidence approach (as clarified in the DS´s response in the RCOM table following PC), giving more weight to recent studies in which MES under specific test conditions and under several test modifications fulfils the criteria to be considered readily biodegradable. No simulation studies are available which deal with the rate and route of degradation in aquatic systems (incl. sewage treatment plants) and with degradation in soil. No tests are available on hydrolysis and MES was assessed by the DS not to hydrolyse as it is marketed as a stable, 29% aqueous solution. No tests are available on photolysis. However,

as MES has no chromophore and does not show any UV absorption above 290 nm, MES was assessed by the DS to not be a candidate for photolysis.

Adsorption/desorption

The DS concludes that MES has a strong adsorption potential, is immobile in soils and will strongly adsorb onto sewage sludge. The basis for this assessment are the measured adsorption coefficients by BODE Chemie (2002a, 2008d and 2008e).

Inhibition to microbial activity (aquatic)

The DS concludes that MES is toxic to bacteria. The basis for this assessment is an OECD TG 209 test by BODE Chemie (2002) which results in an EC_{50} = 22 mg/L.

Aquatic Bioaccumulation

The DS proposed to not consider MES as being bioaccumulative in the aquatic environment for classification purposes. The basis for this proposal is an estimated log K_{ow} of – 0.39 derived from the individual solubilities in water and n-octanol. This was in order to avoid the strong surface-active properties of the test substance, which typically sits on the interphase in a two-phase (OECD TG 107/117) system or forms micelles. The study on solubility in n-octanol was performed according to CIPAC MT 181 (Collaborative International Pesticides Council) and the solubility was found to be 168-202 g/L. The study on solubility in water was performed according to OECD TG 105 with a determined solubility of 500-1000 g/L.

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

K_{ow} estimated as 202 g/L /500 g/L = 0.404. From this the log K_{ow} value is calculated log K_{ow} = -0.39.

The CMC (critical micelle concentration) is not considered and there is no measured log K_{ow} or measured bioaccumulation data available.

Acute Aquatic Toxicity

The DS proposed to classify MES as Aquatic Acute 1, H400 with an M-factor of 100. The basis for this proposal was that for all three trophic levels acute test data are available and that the lowest L(E)C_{50} value is:

- 72 h E_rC_{50} of 0.0039 mg a.i./L (time-weighted average concentration;
  derived from the nominal 72 h E_rC_{50} of 0.054 mg/L

obtained from an OECD TG 201 Growth Inhibition Test on algae (BODE Chemie (2000)) and adjusted by BODE Chemie (2010).

Chronic Aquatic Toxicity

The DS proposed to classify MES as Aquatic Chronic 1, H410 with an M-factor of 10. The basis for this proposal is that for all three trophic levels chronic test data are available and that the lowest NOEC/EC_{10} is:

- 72 h NOE_rC of 0.00014 mg a.i./L (time-weighted average concentration;
  equal to the 72 h NOEC of 0.011 mg/L (nominal)) for algae

obtained from an OECD TG 201 Growth Inhibition test on algae (BODE Chemie (2000)). Available NOEC values for fish:

- 35 d NOEC 0.000555 mg a.i./L (adverse effect on survival, mean measured concentration))

and daphnia:

- 21 d NOEC of 0.00019 mg/L (time-weighted average concentration)

are in the same range. In addition, MES was proposed to be rapidly degradable and to have a low potential for bioaccumulation for classification purposes.

Comments received during public consultation

Three MSCAs commented on the proposal for environmental classification, with two MSCAs agreeing with the proposed environmental classification. All three asked for more detailed information or further data.

For the assessment of the results on the available tests on ready biodegradability, it was questioned if the adaptations and modifications using silica gel and humic acid are relevant for assessing biodegradation for the purpose of classification and how environmentally relevant they are for classification purposes. The DS responded that adding silica gel to biodegradation testing balances the effects of toxicity towards microorganisms with non-availability to microorganisms. However, the question of whether modifications are environmentally relevant for the purpose of classification was not answered in the DS's response in the RCOM table and is also not addressed in the weight of evidence approach applied by the DS.

To understand the test validity in relation to IC (Inorganic Carbon) concentrations and to ensure that the measured degradation solely reflects the test item, details of IC concentrations over time and mass balances were requested by MSCAs but not provided by the DS.

Concerning the acute aquatic toxicity test with *Daphnia magna*, it was requested for the BODE Chemie (2000) (A7.4.1.2 as referred to in the DS´s response in the RCOM table) study, that the LC_{50} values should be corrected by time-weighted averages as it was for the second test with daphnia, the test with fish and the test with algae. However, the DS responded that for this test there was no kinetic study available simulating test conditions according to OECD TG 202 and that the endpoints from this study had to be based on nominal concentrations.

Further information was requested on the kinetic study (this study provided fate assays for MES following OECD TGs 201, 203, and 211) and provided in the response by the DS for OECD TG 211 only. Time-weighted average values were also used for one acute test on fish (A7.4.1.1 as referred to in the DS´s response in the RCOM table), on daphnia (A7.4.1.2_02 as referred to in the DS´s response in the RCOM table) and for the test on algae (A7.4.1.3 as referred to in the DS´s response in the RCOM table). In the kinetic study, it was confirmed that analytical measurements revealed that the MES concentrations are not stable during the test period. The extreme high adsorption potential of MES affected the test concentrations. From other aquatic toxicity studies, it is known that MES is difficult to recover due to its high potential for clustering and adsorption, resulting in an irregular distribution in the test

vessels.

Concerning the test on algae, the DS confirmed that the validity criterion of cell concentration in control cultures was met.

Additional key elements

A second *Daphnia magna* reproduction test (OECD TG 211) by Simon (2018) was made available to RAC only in August 2018. The influence of MES on the reproduction of aquatic invertebrates, represented by *Daphnia magna*, was investigated. A 21 days semi-static exposure to MES at different concentrations with daily renewal of the test solutions was conducted according to OECD TG 211. Untreated control replicates were run in parallel. Each treatment group consisted of 10 replicates with one daphnid each (individual exposure). Effects on growth (adult length at test termination) and reproductive performance were investigated. Test item concentrations were measured at fresh and aged test solutions. MES was applied as a 1.47 % [cetyl-1-14C] Mecetronium ethyl sulphate solution in water. The nominal test concentrations applied were 0.031, 0.077, 0.192, 0.480, 1.20 and 3.0 µg a.i./L.

The mean measured test item concentrations of the freshly prepared test solution based on total radioactivity (initial concentrations) were between 96 % and 146 % of nominal concentrations. During the time interval until renewal of the test solution, total radioactivity in these treatment levels decreased considerably to 75 – 143 % of nominal. The geometric mean measured concentrations were 0.04, 0.10, 0.19, 0.46, 1.03 and 2.68 µg MES equivalents per litre, corresponding to 144, 135, 100, 96, 85, and 89 % of the nominal concentrations.

Neither survival (viability) of adults, adult growth (body length), age to first brood, reproduction rate, or intrinsic rate were affected by geometric mean test concentrations up to and including the highest concentration tested. The NOEC was estimated to be ≥ 2.68 µg a.i./L geometric mean measured concentration based on total radioactivity and ≥ 1.27 µg a.i./L based on TLC confirmed MES equivalents, respectively. As no response (no effects) could be observed, EC_{10} values could not be calculated.

RAC was unable to find a description of the substance's actual solubility in water or any discussion of any possible CMC. There appears to be no justification for the concentration range tested in this study, the range tested being significantly below the stated water solubility of >500mg/L but also well below the cut-off values for CLP. Therefore, the results stated indicate simply no effects in the range tested not no effects up to the water solubility limit. Despite this, the study appears to be well conducted and adequate for consideration under CLP.

Assessment and comparison with the classification criteria

Degradation

RAC has reassessed the available information on the tests on ready biodegradability and performed a weight of evidence approach.

Derive effect levels from ready test systems

In their supplementary comment after PC, industry has submitted an effect level for each

test system. RAC concludes that it is scientifically unjustified to derive effect levels from OECD TG 301 and OECD TG 310 test systems and that, consequently, it is not possible to derive a dose-response relation between these test systems.

Effect of potential to adsorb

Furthermore, BODE Chemie (1999) found the percentage elimination of 33% in the abiotic control is in the same range as the elimination in the vessels with test substance. Thus, the observed elimination can be attributed to an abiotic removal, probably adsorption. Also, for BODE Chemie (2008), the duration of the lag phase (in test item and toxicity control assays) was affected by availability of MES to microorganisms. These findings are consistent with the high adsorption potential of MES.

RAC concludes that the potential of MES to adsorb may also influence the test result. However, adsorption is only a problem in tests measuring removal of DOC (see OECD TG 301). To investigate the influence of adsorption compared to the influence of toxicity, it would have been necessary to include a toxicity control in those experiments with silica gel. It is unclear to RAC why this has not happened.

Modification with silica gel

RAC is of the opinion that the use of silica gel does not necessarily invalidate a test for the purpose of classification. Although this method is not specifically mentioned in OECD TG 301, silica gel (e.g. amorphic silicon dioxide) has also been used in the EU ring-test for OECD TG 310. ECHA Guidance R.7b states that reduction in the toxicity in the ready biodegradability tests may be achieved by the introduction of carriers allowing slower-release of the test substance during the test period. Silica gel is a preferred modification of ready biodegradability tests for improving the bioavailability of poorly water-soluble substances. In UBA (2017), it is concluded that the approach using silica gel matrices represents the first option for substances of low bioavailability. Silica gel is documented in several peer-reviewed publications (Handley *el al*., 2002; Painter *et al*., 2003, van Ginkel *et al*., 2008, Kowalczyk *et al*., 2015). Provided all other conditions in the ready biodegradability tests are fulfilled, such modified tests are regarded as ready biodegradability tests and the results may be used for the purpose of classification.

RAC concludes that the two test systems BODE Chemie (2011b) and BODE Chemie (2011c) are invalid because they do not fulfil the validity criteria of the OECD TG and not for the reason that silica gel was added.

The following Table shows all available test systems and presents the test results, their validity and the basis for the weight of evidence as assessed by RAC.

Table:

Reference	TG	Batch	Degradation result	pass level fulfilled	Weight of evidence	validity fulfilled	Evaluation by DS
BODE Chemie (1995)	OECD TG 301D	A	< 5% at day 29	no	not readily biodegradable	yes	reliable with restriction
		B	< 5% at day 29	no	not readily biodegradable	yes	reliable with restriction
BODE Chemie (1999)	OECD TG 301A	A	only abiotic removal, due to adsorption	no	not readily biodegradable	yes	reliable with restriction
BODE Chemie	OECD TG	A	no degradation	no	not readily biodegradable	yes	key study

(2008)	301F	B	lag phase of 19 days; 42.5 % of ThOD at day 28	no	not readily biodegradable	yes	key study
		C	lag phase of 6 days 49.8 % of ThOD at day 28	no	not readily biodegradable	yes	key study
		D	lag phase of 6 days 60.2 % of ThOD at day 28	no, 10-d window failed	not readily biodegradable	yes	key study
BODE Chemie (2011a)	OECD TG 310	A	no degradation (-10.3%)	no	not readily biodegradable	No highest mean TIC produced in the blank flasks was 3.7 mg/L in 28 days	not valid and not reliable
		B	no degradation (-4.88%)	no	not readily biodegradable	No highest mean TIC produced in the blank flasks was 3.7 mg/L in 28 days	not valid and not reliable
BODE Chemie (2011b)	OECD TG 310	A	65% at day 28 (at day 14 100% and at day 21 117 %)	yes	at day 14 readily biodegradable	No IC content in the inoculum blanks was 24.1 mg IC/L at test end	not valid and not reliable
		B	37% at day 28 (at day 14 70% and at day 21 80%)	yes	at day 14 readily biodegradable	No IC content in the inoculum blanks was 24.1 mg IC/L at test end	not valid and not reliable
BODE Chemie (2011c)	OECD TG 310	A	100 % at day 14; no lag phase	yes	readily biodegradable	No mean amount of TIC present in the blank controls at the end of the test is 3.66 C/L; Reference substance was not used	not valid and not reliable
		B	87 % at day 14; lag phase 7 d	yes	readily biodegradable	No difference of replicate values > 20%; mean amount of TIC present in the blank controls at the end of the test is 6.54 C/L; Reference substance was not used	not valid and not reliable
		C	76 % at day 14; lag phase 7 d	yes	readily biodegradable	No difference of replicate values > 20%; mean amount of TIC present in the blank controls at the end of the test is 9.80 C/L; Reference substance was not used	not valid and not reliable
BODE Chemie (2013a)	OECD TG 301 B	A	48 % at day 28	no	not readily biodegradable	yes	reliable with restriction
		B	97 % at day 28	yes	readily biodegradable	yes, but not reliable for classification	reliable with restriction

Read-across and QSAR

MES belongs to the group of quaternary ammonium compounds (QACs), a group of cationic surfactants, which are structurally similar with respect to the embedded quaternary nitrogen and at least one long carbon chain. The DS stressed that read-across is possible between the chemical members of this group. However, RAC concludes that a more detailed description of the structural analogues and their similarity with regards to other substances' properties would be needed in the CLH report to allow a fully valid read-across. Moreover, it seems difficult to apply a sufficiently high weight to the read-across approach and QSAR models to change the conclusion reached from valid and reliable tests on ready biodegradability.

Conclusion on rapid degradability

RAC in its evaluation assessed and considered all available information including available test results, information on read-across, QSARs, as well as input from industry.

In contrast to the DS, RAC concludes that there are no fully reliable or suitable data clearly showing ready biodegradability of MES.

Conversely, RAC notes that there are valid, reliable studies available showing that even with modifications to test systems (such as the addition of silica gel), MES is not readily biodegradable.

RAC bases the weight of evidence on valid and reliable studies (1) without modification and adjustment and (2) with modification by adding silica gel. RAC concludes that in these test systems, the degradation in the toxicity control fulfils the validity criteria of the OECD test guideline and inhibition did not influence the outcome of these test systems.

RAC notes that no simulation studies are available for MES. MES does not hydrolyse and is not accessible for photolysis. Based on the evidence presented, RAC in contrast to the DS and concludes that for the purposes of classification MES should be considered not rapidly degradable.

Aquatic bioaccumulation

No experimental data on the bioconcentration potential of MES are available. To assess this hazard , the DS applied a weight of evidence approach based on an estimated log K_{ow} of -0.39, experimental BCF studies within the training data set of EPISUITE of seven QAC DODMAC, a read-across to an experimental uptake and depuration study with analogue substances by Versteeg and Shorter (1992) and toxicokinetic studies in rats. It should be noted that this is more of a qualitative comparison of the sparse available data than any structured attempt at read-across to other similar chemicals.

<u>Estimated partition coefficient</u>

The DS explains that the experimental determination of the partition coefficient of MES by OECD TGs 107 and 117 is technically not feasible. Thus, the log K_{ow} was estimated from the individual solubilities in water and n-octanol. A very high water solubility of >500 g/L together with an n-octanol solubility of 202 g/L was used to calculate a log K_{ow} of -0.39. However, the value of >500g/L does not account for the likely formation of micelles and adsorption to test vessel. As such, it does not represent the solubility of the substance in a manner that is suitable for the determination of Log k_{ow} or aquatic toxicity testing.

The following QSAR predictions, conducted by ECHA to determine the water solubility, indicate much lower values:

- ACD/Labs (Release 2016.2): 0.0014 g/L
- EPISUITE WaterNT: 0.000047 g/L

Using the same calculation method as the DS, the estimated log K_{ow} would be in the range of 3.15 to 6.63.

However, these value are uncertain because the substance has more instances of the aliphatic carbon $-CH_2-$ than covered by the training set and the N^+ fragment has a coefficient

of zero. Consequently, RAC cannot evaluate the reliability of these QSAR predictions. They point towards a much lower water solubility than given in the CLH dossier. An overestimation of the water solubility would result in an unreliable and rough underestimation of the partitioning coefficient. The measurement of the CMC (critical micelle concentration) of MES is not available to RAC.

RAC concludes that the estimated log K_{ow} of -0.39 is most likely an underestimation and that due to MES being an ionised surfactant, log K_{ow} data in general is not suitable for concluding on bioaccumulation. However, the available models do suggest that MES may have a lower water solubility than the value provided by the DS and consequently, the log K_{ow} could be higher.

Conclusion on bioaccumulation

In the absence of a direct measured log K_{ow} value or a measured BCF value for MES, the conclusion for bioaccumulation must be based on the weight of the small amount of available evidence. The log K_{ow} value is not appropriate for surface-active substances. The estimated log K_{ow} of -0.39 is likely to be an underestimation and based on modelled water solubility (above), the log K_{ow} could be higher. Information on the CMC (critical micelle concentration) of MES would have assisted in assessing the water solubility for testing purposes but this information is not available to RAC. Bioaccumulation in mammals appears unlikely.

Although some information on BCF of QACs indicate low bioaccumulation a high BCF of 1962 L/kg in fathead minnow was reported for a QAC comprising C 16/18 alkyl chains in a non-guideline test with short uptake and depuration phases. Finally, the calculated Klipw value of 6.58 for the MES cation could further indicate a potential for bioaccumulation but the model is not validated for regulatory use. RAC concludes a potential BCF value for MES would be under the 1962 L/kg reported for similar structures but that the value for MES is likely to be above 500 L/kg. Furthermore, the log K_{ow} values provided by the log Klipw model indicate lipophilicity that could lead to a concern for bioaccumulation, although whether it avoids the problem with surface active materials is unclear. Overall, the potential of MES to bioaccumulate with a BCF above the criteria for the purpose of classification (BCF ≥ 500 L/kg) can currently not be excluded. Therefore, based on the evidence presented, RAC in contrast to the DS, considers MES to have a potential for bioaccumulation for the purpose of classification and labelling.

Aquatic Toxicity

<u>Available studies on aquatic toxicity</u>

Over all, seven aquatic toxicity studies were available to RAC. For acute toxicity, one fish study, two invertebrate studies, and one algae study were available (Table). For Long-term toxicity, One fish study, two invertebrate studies, and one algae study (as above) were available (Table).

RAC notes that as MES is highly adsorbing and therefore analytical confirmation of test concentrations is important as nominal concentrations significantly underestimate actual toxicity. However, MES concentrations measured in the studies are not available for all acute (see Table) and chronic tests (see Table). For the acute fish test (A7.4.1.1), the first acute daphnia study (A7.4.1.2/01), and the algae study (A.7.4.1.3), the analytical methods were not sensitive enough to measure MES, due to the rapid disappearance of MES within the test

systems. Therefore, no direct MES measurements from within the studies were available. In order to provide data on test substance concentrations in the test systems, a retrospective kinetic study was conducted by Industry and used to theoretically recalculate the nominal endpoints of the original studies lacking measured test substance data, by calculating theoretical TWA MES concentrations. For tests with measured MES data, measurements within the studies were used to express each test concentration as either Time Weighted Average (TWA) test concentrations or as mean measured test concentrations.

Table:

Acute aquatic hazard test results:		nominal	measured TWA	theoretical TWA
BODE Chemie (1992) A7.4.1.1	OECD TG 203 Fish Acute toxicity test	96 h LC_{50} 0.06 mg/L	-	96 h LC_{50} < 0.048 mg/L is not valid and not reliable
BODE Chemie (2000) A7.4.1.2/01	OECD TG 202 *Daphnia* sp. Acute Immobilisation test	48 h EC_{50} 0.019 mg/L	-	48 h EC_{50} between 0.0042 to 0.0091 mg/L
BODE Chemie (2010) A7.4.1.2/02	OECD TG 202 *Daphnia* sp. Acute Immobilisation test	not available	48 h EC_{50} 0.015 mg/L	-
BODE Chemie (2000) A7.4.1.3	OECD TG 201 Algae, Growth Inhibition Test	72 h E_rC_{50} 0.054 mg/L	-	72 h E_rC_{50} < 0.0039 mg/L

Table:

Long-term aquatic hazard test results:		nominal	measured TWA / mean	theoretical TWA
BODE Chemie (2012) see A7.4.3.2	OECD TG 210 Fish Early-life Stage Toxicity Test	not available	35 d NOEC: 0.000555 mg/L	-
BODE Chemie (2008) see A7.4.3.4	OECD TG 211 *Daphnia magna* Reproduction Test	not available	21 d EC_{10} 0.00006 mg/L 21 d NOEC 0.00042 mg/L	21 d EC_{10} not available 21 d NOEC < 0.00019 mg/L
Simon (2018)	OECD TG 11 *Daphnia magna* Reproduction Test	not available	21 d EC_{10} not calculated 21 d NOEC > 0.00127 mg/L	-
BODE Chemie (2000) see A7.4.1.3	OECD TG 201 Algae Growth Inhibition Test	72 h NOEC 0.011 mg/L	not available	72 h NOEC < 0.00014 mg/L

The retrospective kinetic approach was not applied to the second acute Daphnia study (A7.4.1.2/02). However, for reasons unknown, this approach was applied to the chronic daphnia study (A7.4.3.4) in addition to the available measured values and TWA values from the original study report.

The kinetic study (BODE Chemie (2010)) study presents three assays intended to simulate test conditions for the OECD TGs 201, 203, and 211 studies as follows:

OECD TG 211	○ static conditions ○ 24 h ○ Purified drinking water without any test organism or biological material ○ 60 mL glass beakers with 50 mL of test solution ○ light/dark cycle of 16/8 hours ○ test temperature was 20.0 ± 2 °C
OECD TG 201	○ static conditions ○ 72 h ○ Purified drinking water with sterilised modified synthetic OECD medium

	without any test organisms or biological material o 250 mL conical glass flasks with 100 mL test medium o The vessels and caps were sterilised prior to use (autoclaving or heating). The aqueous phase was filtered by using a 0.22 µm filter without pre-filtration under sterile conditions to sterilise the media by filtration. o continuously illuminated with a light intensity adjusted between 60-120 µE/m²s (4440 – 8880 lux) close to the surface of the liquid o test temperature was 20.0 ± 2 °C
OECD TG 203	o static conditions o 96 h o Purified drinking water without any test organism or biological material o 12 L glass basin with 10 L test medium o light/dark cycle of 12/12 hours o test temperature was 20.0 ± 2 °C

RAC notes that the loss of MES was monitored in the absence of the test organisms (fish, Daphnia and algae) and in the absence of food or any other biological material. It must be assumed that the presence of such material would have changed the results (*e.g.* due to adsorption). Consequently, RAC notes that the approach by BODE Chemie (2010) to correct the nominal values from the original studies represents the best case and needs to be assessed critically for the purpose of classification.

The results were very different for each guideline assay (Table). The two extremes were the 72 h OECD TG 201 assay which showed the expected result, an extremely rapid loss of MES within a few hours following a two-compartment degradation, and the 96 h OECD TG 203 assay, which showed a slow dissipation following a single first order model. The latter is based on a higher concentration in the test, which may explain a percentile lower adsorption but may not explain the different kinetic result with a 900-fold higher DT_{50}.

Table:

	initial measured in % of nominal	aged test solutions in % of nominal	TWA concentration in % of initial measured	kinetic	Dissemination
OECD TG 211	115 to 270 %	after 24 h: 28 to 54 %	24.2 to 35.9 %	two-compartment	$DisT_{50}$: 0.5 – 1.6 h $DisT_{95}$: 78.9 – 115.5 h
OECD TG 201	99 and 107 %	after 72 h: 2.1 and 5.2 %	1 % and 5 %	two-compartment	$DisT_{50}$: 0.1 and 0.2 h $DisT_{95}$: 0.3 and 71.5 h
OECD TG 203	108 %	after 96 h: 66 %	78 %	Single First Order	$DisT_{50}$: 90.5 h $DisT_{95}$: 391.1 h

RAC concludes that overall the results of the kinetic study BODE Chemie (2010) are of limited relevance to "correct" the nominal *in vivo* concentration data and likely represent a best-case scenario. The 96 h assay seems to be an outlier and should not be used. However, the assays following OECD TGs 201 and 211 are considered to provide a more reasonable estimate of substance concentrations than nominals. This is due to the rapid loss of

substance from the test system causing nominal concentrations to underestimate toxicity. However, RAC also notes that this kinetic methodology would also underestimate toxicity to some degree due to further substance loss that would likely occur in the presence of test organisms or other biological material. Despite this, the corrected algal data provides the most stringent outcome and will be used for classification.

Acute aquatic toxicity

OECD TG 203 Fish Acute toxicity test

BODE Chemie (1992) (A7.4.1.1) did not confirm the test concentrations by analytical methods. The 96 h static test with *Leuciscus idus melanotus* did not have renewed test solution. BODE Chemie (2010) conducted a kinetic study to adjust the nominal endpoint to a TWA endpoint. Evaluating the results, RAC notes the extremely high measured concentrations and the large fluctuation that seems unreliable and thus incomparable to the original study.

Table:

	0 h	**1 h**	**2 h**	**4 h**	**8 h**	**24 h**	**48 h**	**72 h**	**96 h**
Measured (mg/L)	0.0649	0.102	0.0566	0.078	0.0986	0.0663	0.0747	0.0363	0.0397
% of nominal 0.06 mg a.i./L	108	170	94	130	164	111	125	61	66

RAC concludes that the OECD TG 203 Fish Acute toxicity test by BODE Chemie (1992) is not reliable because MES is highly adsorptive and analytical confirmation of test concentrations is missing. Consequently, the results may significantly underestimate actual toxicity.

RAC concludes that the calculated theoretical TWA 96 h LC_{50} of < 0.048 mg/L (derived using BODE Chemie 2010) is not valid and not reliable for the purpose of classification since it clearly reflects best-case assumptions.

OECD TG 202 Daphnia magna Acute Immobilisation test

There are two tests available. The BODE Chemie (2010) study (A7.4.1.2/02) monitored the test substance MES at 0, 1, 2, 4, 8, 24 and 48 hours and calculated a TWA for each test concentration. RAC concludes that the resulting 48 h EC_{50} of 0.015 mg/L is valid and reliable and can be used for the purpose of classification.

In the first study by BODE Chemie (2000) (A7.4.1.2/01), the monitoring of the test substance was not successful because of analytical problems, thus no TWA of test concentrations is available. The nominal results significantly underestimate the actual toxicity. RAC corrected in a worst-case approach the nominal endpoint of BODE Chemie (2000) (A7.4.1.2/01) by the correction factor from BODE Chemie (2010) of 22.3 to 47.9% (Table). This resulted in an estimated 48 h EC_{50} between 0.0042 to 0.0091 mg/L).

Table:

		Adjustment of nominal within the original study:	Endpoint recalculated by BODE Chemie (2010):	nominal mg a.i./L and test duration	reduction by factor (%) based on nominal
A7.4.1.2/02 BODE Chemie (2010)	OECD TG 202 Daphnia sp. Acute Immobilisation test	yes, TWA measured test concentrations	no	0.003; 48 h	22.3
				0.006; 48 h	25.8
				0.012; 48 h	43.0
				0.024; 48 h	40.5
				0.048; 48 h	47.9

OECD TG 201 Algae, Growth Inhibition Test

BODE Chemie (2000) (A7.4.1.3) did not confirm the test concentrations by analytical methods. In the 72 h static test with *Desmodesmus subspicatus*, the test solution was not renewed. BODE Chemie (2010) conducted a kinetic study to adjust the nominal endpoint to a TWA endpoint. In evaluating the results, RAC notes the extreme fast decrease in the first ca. 1 h and a slower decrease in the subsequent hours for the higher concentration but no further decrease in the lower test concentration. The measured values seems to be consistent and comprehensible.

Table:

	0 h	**1 h**	**2 h**	**4 h**	**8 h**	**24 h**	**48 h**	**72 h**
Measured (mg/L)	0.0107	0.00011	0.00010	0.00010	<LOQ	<LOQ	0.00015	0.00022
% of nominal 0.01 mg a.i./L	107	1.1	1.0	1.0	n.a	n.a	1.5	2.2
Measured (mg/L)	0.0396	0.00474	0.0438	0.003690	0.00284	0.00263	0.00243	0.00204
% of nominal 0.04 mg a.i./L	99.0	11.9	11.0	9.2	7.1	6.6	6.1	5.1

LOQ (Limit of quantification) = 0.0001 mg/L

RAC concludes that the results from OECD TG 201 Algae, Growth Inhibition Test by BODE Chemie (2000) is not reliable because MES is highly adsorptive, analytical confirmation of test concentrations is missing and consequently the results may significantly underestimate actual toxicity. The nominal 72 h ErC_{50} 0.054 mg/L and the 72 h NOEC 0.011 mg/L are not valid for the purpose of classification. The results calculated using the correction factor from BODE Chemie (2010) are derived from the two nominal test concentrations of 0.01 mg a.i./L and 0.04 mg a.i./L and result in a theoretical TWA 72 h ErC_{50} of <0.0039 mg/L and 72 h NOEC of <0.00014 mg/L.

Since these values were measured without test organisms, they represent a best case. It must be assumed that measurements during an experiment with algae might have resulted in lower ErC_{50} and ErC_{10}/NOEC values. As the corrected TWA values represent the highest

toxicity demonstrated under acute testing, RAC agrees to use the corrected algal data for the purpose of classification.

Long-term aquatic toxicity

OECD TG 210 Fish Early-life Stage Toxicity Test

BODE Chemie (2012) (7.4.3.2) performed with *Danio rerio* (zebrafish) an Early Life Stage Toxicity Test under flow through conditions. Test substance concentration in all test vessels was assessed by chemical analysis using liquid chromatography and tandem mass spectrometry detection (LC-MS/MS). The LOQ was determined to be 0.20 µg/L.

Table : *Results of analytical measurements of test substance concentration for the entire test duration (LOQ 0.2 µg MES/L)*

Nominal concentration [µg/L]	**Mean measured concentration**	
	[µg/L]	**[%]**
0.400	0.154	39
1.00	0.404	40
2.50	0.555	22
6.25	2.95	47
15.6	10.8	69

The concentration of MES could not be maintained throughout the test within ± 20% of mean measured values. All effect data were based on these mean measured concentrations.

RAC concludes that the resulting NOEC of 0.000555 mg MES/L (adverse effect on survival, mean measured concentration) and an LOEC of 0.00295 mg MES/L (adverse effect on survival, mean measured concentration) is valid and reliable and can be used for the purpose of classification.

OECD TG 211 Daphnia magna Reproduction Test

There are two tests available. BODE Chemie (2008) (A7.4.3.4) measured the stock solution for the concentration of the test item. Depending on the results, the individual test solutions were prepared by dilution with dilution water, sampled for chemical analysis and distributed to the test beakers. The stock solution was freshly prepared daily.

During the test duration, the test solutions with nominal concentrations of 0.30, 0.81, 2.19, 5.93, and 16.00 µg a.i./L were analysed at three times (once a week) right after preparation. The same test solutions of the nominal concentrations 0.81, 2.19, 5.93, and 16.00 µg a.i./L were analysed also after 24h of aging with algae. The solution with a nominal concentration of 0.30 µg a.i./L was neglected due to the results of the pre-tests (values < LOQ). All samples were centrifuged before measurement to pellet the algae.

The recovery rate after centrifugation was below the recovery rate in media aged without algae. The ratio between the two recovery rates was calculated. The analytical values were recalculated regarding the mean leakage due to algae centrifugation found in the pre-tests

since test item bound on the algae is available for the daphnids.

Due to the strong decrease of concentration during aging, the available time-weighted mean was used as the relevant concentration for biological effects. To consider the bioavailable concentration, it was calculated based on the measured values of the fresh test solutions and the recalculated values of the aged test solutions. Values below the LOQ were set 0.1 µg a.i./L (= 1/2 LOQ) for calculation. The calculation was done in accordance with the equation given in OECD TG 211.

***Table** : Concentrations of the active substance (a.i. = active substance; LOQ = Limit of quantification (0.2 µg/L))*

Nominal conc.	**0.30 µg a.i./L**	**0.81 µg a.i./L**	**2.19 µg a.i./L**	**5.93 µg a.i./L**	**16.00 µg a.i./L**
Mean measured initial conc.	0.59 (± 0.18)	1.00 (± 0.24)	2.43 (± 0.03)	6.52 (± 0.66)	16.03 (± 0.75)
% of nominal	195.1	122.8	111.0	109.9	100.2
Mean recalculated aged conc.	< LOQ	0.04 (± 0.06)	0.79 (± 0.25)	2.68 (± 0.58)	7.54 (± 2.67)
% of nominal	-	5.0	36.0	45.2	47.2
Time weighted mean conc.	0.42 (± 0.07)	0.58 (± 0.08)	1.45 (± 0.17)	4.31 (± 0.58)	11.18 (± 1.66)
% of nominal	140.9	71.0	66.2	72.7	69.9

The mean measured concentrations of MES in the freshly prepared test solution (initial concentrations once a week) were between 100 % and 195 % of nominal concentrations. During the time interval until renewal of the test solution, a.s. concentrations decreased considerably to 5 – 47% of nominal at the four highest concentrations (0.81 - 16.00 µg/L nominal). At 0.30 µg/L nominal concentration, no measurements were performed at all. The average time-weighted means of mean measured initial and recalculated mean measured aged concentrations (considering the mean leakage due to algae centrifugation) at test solution renewal were 0.42, 0.58, 1.45, 4.31, and 11.18 µg/L, corresponding with 141, 71, 66, 73, and 70 % of the nominal concentrations.

Concentration related mortality of the adults was observed. The EC_{10} and EC_{50} were estimated at 0.06 and 0.43 µg a.i./L available time-weighted mean (TWM), respectively. The NOEC (mortality) was found to be 0.42 µg a.i./L available TWM.

***Table :** Effect summary of the original study report based on concentrations calculated from geometric mean measured concentrations*

Concentration	**Parental survival**	**Growth (length on day 21)**	**Age at 1st brood**	**Cumulative offspring per female**	**Intrinsic rate of increase**
EC_{50} (95% CL)	0.43 (0.16 – 1.17)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
EC_{10} (95% CL)	0.06 (0.01 – 0.64)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
NOEC	0.42 µg/L	≥ 11.18 µg/L	≥ 11.18 µg/L	≥ 4.31 µg/L	n.d.

RAC notes that that some of the initial measured concentrations significantly exceeding 100

% of nominal at the start may be caused by analytical challenges related to a poorly soluble substance that forms micelles.

RAC notes, that the total number of living offspring produced per parent animal alive at the end of the test as a test parameter is not reliable, because of high or total mortality of parent animal. RAC further notes that the EC_{50} value is nearly identical with the NOEC value and gives the EC_{10} value more weight for the purpose of classification.

However, RAC recognises some uncertainty concerning the reliability of the EC_{10} due to there being no measured test concentrations close to the EC_{10} and that the dose-response regression was not properly fitted. Given the potential uncertainties surrounding the EC_{10}, RAC discussed if it would be more appropriate to discount the EC_{10}, resulting in reverting the available NOECs. In this case, the chronic classification would be based on the fish NOEC, which is an order of magnitude higher than the EC_{10} for invertebrates but is supported by values for invertebrates and algae which are of lower quality albeit in the same range ($0.0001 < NOEC//EC_{10} \leq 0.001$) (Table). However, and despite any shortcomings with the EC_{10} and the regression that produced it, these were not sufficient to discount its use for classification. The NOECs (based on either mean measured or TWAs) may underestimate toxicity and the EC_{10} may represent a more realistic situation.

Overall, RAC concludes that for the OECD TG 211 *Daphnia magna* reproduction test by BODE Chemie (2008) the EC_{10} value of 0.00006 mg/L is of sufficient reliability for classification and provides a realistic toxicity value.

Conclusion on Acute Aquatic Toxicity

RAC agrees with the proposal of the DS to base the acute classification of MES on the 72 h E_rC_{50} of 0.0039 mg a.i./L (theoretical time-weighted average concentration obtained from a OECD TG 201 Growth Inhibition Test on algae (BODE Chemie (2000) and retrospectively adjusted by BODE Chemie (2010)).

RAC notes that for fish the calculated theoretical TWA 96 h LC_{50} of 0.048 mg/L from the OECD TG 203 Fish Acute toxicity test by BODE Chemie (1992) is not valid or reliable for the purpose of classification since it clearly represents a best-case value.

For Daphnia the lowest reliable result is taken from a worst case estimation and results in an estimated 48 h EC_{50} between 0.0042 and 0.0091 mg/L for BODE Chemie (2000) (A7.4.1.2), which is in the same range as the acute algae study and supports the acute classification.

RAC agrees with the DS to classify MES as **Aquatic Acute 1, H400** with an **M-factor of 100**.

Conclusion on Chronic Aquatic Toxicity

In contrast to the DS's proposal to classify MES based on an algal NOEC of 0.00014 mg/L, RAC concludes to base the chronic classification of MES on the 21 d EC_{10} 0.00006 mg/L (measured time-weighted average concentration) on *Daphnia magna* obtained from the OECD TG 211 *Daphnia magna* reproduction test by BODE Chemie (2008). This EC_{10} is considered reliable for classification and its preferred use is consistent with the Guidance on the application of the CLP criteria.

Based on the conclusion that MES is not rapidly degradable and has a potential to bioaccumulate, RAC concludes to classify MES as **Aquatic Chronic 1, H410** with an **M-**

factor of 1000.

Supplemental information - In depth analyses by RAC

The following Table shows all available test systems and presents nominal test concentrations, modifications to the standard OECD TG and observed toxic effects or observed inhibition.

Table:

Reference	TG	Batch	Test concentration	modification	degradation in toxicity control	Observation in original study report
BODE Chemie (1995)	OECD TG 301D	A Duplicate measures	8.09 mg a.i./L	extended to 60 days	> 25%	only low inhibition (if any)
		B Duplicate measures	12.9 mg a.i./L	extended to 60 days	> 25%	only low inhibition (if any)
BODE Chemie (1999)	OECD TG 301A	A 2 Replicates	10 and 11 mg DOC/L; re-dosed with 12 mg DOC/L	Re-dosed on test day 1	> 25%	no inhibition to sewage sludge microorganisms
BODE Chemie (2008)	OECD TG 301F	A 2 Replicates	100 mg a.i./L	none	> 25%	No inhibitory effects were observed
		B 2 Replicates	29 mg a.i./L	none	> 25%	No inhibitory effects were observed
		C 2 Replicates	100 mg a.i./L	silica gel	> 25%	No inhibitory effects were observed
		D 2 Replicates	29 mg a.i./L	silica gel	> 25%	No inhibitory effects were observed
BODE Chemie (2011a)[1]	OECD TG 310	A 15 flasks	20 mg TOC/L	None Inoculum 4 mg dry solids/L	< 25%	Inhibition control: mean degradation on day 14: 6.08%; day 21: 27.73%; day 28: -4.71 %
		B 5 flasks	10 mg TOC/L	None Inoculum 4 mg dry solids/L	no toxicity control	n.a.
BODE Chemie (2011b)[2]	OECD TG 310	A 10 flasks Duplicate measures	10 mg C/L	silica gel	no toxicity control	no inhibition observed
		B 10 flasks Duplicate measures	20 mg C/L	silica gel	no toxicity control	no inhibition observed
BODE Chemie (2011c)	OECD TG 310	A Duplicate measures	10 mg C/L	only 14 days; silica gel; inoculum 10 mg SS/L, washed	no toxicity control	no inhibition observed
		B Duplicate measures	10 mg C/L	only 14 days; silica gel; inoculum 4 mg SS/L, washed	no toxicity control	no inhibition observed
		C Duplicate measures	10 mg C/L	only 14 days; silica gel; inoculum 4 mg SS/L, unwashed	no toxicity control	no inhibition observed
BODE Chemie	OECD	A	12 mg a.i./L or	none	> 25%	biodegradation of the

[1] on page 77 of the CLH report this is called BODE Chemie (2011b)

[2] on page 77 of the CLH report this is called BODE Chemie (2011c)

(2013a)	TG 301 B	Duplicate measures	7.4 mg C/L (measured)			reference item was not inhibited by the test item in the toxicity control
		B Duplicate measures	12 mg a.i./L or 7.4 mg C/L (measured)	addition of 10 mg/L humic acid	no toxicity control including humic acid	n.a.

OECD TG 301D closed bottle test system

The BODE Chemie (1995) (see Section A7.1.1.2.1/01; Annex Point IIA7.6.1.1) study resulted in degradation of < 5% at day 29 in both test concentrations showing that MES is not readily degradable.

The initial test concentration were 8.09 mg a.i./L and 12.9 mg a.i./L. The study is not GLP compliant. No information on nitrification is available although the test substance contains nitrogen. The "Closed bottle test" is a BOD test but the results are not corrected for oxygen uptake through nitrification. However, this is not relevant for a degradation of < 5%. The DS concludes that the toxicity control demonstrate that the low oxygen consumption of MES was not due to antibacterial properties, as MES demonstrated only low (if any) inhibition of oxygen consumption of the reference substance. However, the toxicity controls were only measured at days 0 and 60. Adsorption and low bioavailability may represent limiting factors of the study. Nevertheless, the study appears well conducted and from the reported data, no doubts on the validity of the study arise. A reliability score of 2 was applied by the DS.

RAC concludes that the study BODE Chemie (1995) is valid and reliable, showing that MES is not readily biodegradable.

OECD TG 301A DOC-Die-Away test system

BODE Chemie (1999) (see Section A7.1.1.2.1/02; Annex Point IIA7.6.1.1) resulted in degradation of 23.2% and 42.4% at day 29 showing that MES is not readily degradable.

The initial test concentrations were 10 and 11 mg DOC/L and were immediately reduced in all test vessels by approximately 60-70%, probably by adsorption, and were re-dosed with 12 mg DOC/L on day 1. In the toxicity control, elimination was 76.6%. The results of the toxicity control demonstrated that MES in the concentration applied has no inhibition to sewage sludge microorganisms. Adsorption of the test substance is only a problem in tests measuring removal of DOC. However, the adsorption control required in the OECD TG 301A test was not performed. Furthermore, the percentage elimination of 33% (abiotic control) is in the same range as the elimination in the vessels with test substance. Thus, the elimination can be attributed to an abiotic removal, probably adsorption. This is to be expected from the high adsorption potential of MES (cf. Doc III A7.1.3). The study is not GLP compliant. Re-dosing is not usual in OECD TG 301 test systems and no information on DOC content and removal in the blanks is available. However, these deficiencies do not invalidate the overall findings of the study and a reliability score of 2 "Reliable with restriction" was applied by the DS.

RAC concludes that the study BODE Chemie (1999) is valid and reliable with restrictions, although RAC has some doubt on the relevance.

OECD TG 301F Manometric Respirometry Test

The BODE Chemie (2008) study (see Section A7.1.1.2.1/03; Annex Point IIA7.6.1.1) resulted

in: (A) no degradation, (B) 42.5 % of ThOD with a lag phase of 19 days, (C) 49.8 % of ThOD with a lag phase of 6 days, and (D) 60.2 % of ThOD with a lag phase of 6 days. However, the 10-d window failed, showing that MES is not readily biodegradable.

The initial test concentrations were 29 and 100 mg a.i./L (test suspension A and B), which are notably higher test substance concentrations than in other studies. In the toxicity control at day 14, the biodegradation of the test item mixture was found to be 38% and 30%. Additionally, the test item was applied onto silica gel (Fluka, Silica gel 60, particle size 0.063 – 0.2 mm) in a ratio of test item (a.s.) to silica gel of 1:500 (test suspension C and D). The biodegradation in the toxicity control including silica gel was found to be 32 % after 14 days of incubation. The toxicity control including silica gel showed the same high amount of degradation as the toxicity control without silica gel, confirming that the effect of silica gel in this study on either adsorption or toxicity is negligible. The test item MES can be identified as non-inhibitory in this ready biodegradability test independent of silica gel. The test results, both with or without silica gel, showed that the substance is not readily biodegradable. The study has no deficiencies, a reliability score of 1 "Reliable without restriction" was applied by the DS and this study has been assessed as key study by the DS.

The CLP criteria include a general requirement for all of the ready biodegradability tests on achievement of the pass level within a 10-days window of the onset of biodegradation. RAC discussed if for the result of test D) including silica gel the 10-days window may be waved because MES is a surfactant. However, it is indicated in the CLP criteria and further described in ECHA´s guidance Chapter R.7b v. 4.0 as well as the guidance on the application of the CLP criteria v. 5.0, that the 10 day window may only be waived for certain complex substances (surfactant or otherwise) like multi-constituent and UVCB substances. This is also referred to in OECD TG 310 and the reason is that most surfactants are not single molecular species but are mixtures of isomers, homologues, etc. which degrade after different characteristic lag periods and at different kinetic rates resulting in "blurred", extenuated curves, so that the 60% pass value may not be reached within "the 10-d window", even though each individual molecular species would reach >60% within 10 days if tested alone. MES however is a mono-constituent substance, so the 10-day window must not be waived for MES.

RAC concludes that the study BODE Chemie (2008) is valid and reliable, showing that MES is not readily biodegradable.

OECD TG 310 CO2-headspace test

There are three study reports available.

a) The BODE Chemie (2011a) study (see Section A7.1.1.2.1/04; Annex Point IIA7.6.1.1) showed that degradation in the inhibition control was below 25% at day 14 at 20 mg TOC/L and that the test item in this test system had toxic effects on the inoculum. Hence, this specific test cannot evaluate if MES is readily biodegradable. The test is not valid and a reliability score of 3 "Not reliable" was applied by the DS who decided to use this result only as additional information.

RAC concludes that the study BODE Chemie (2011a) is not valid and not reliable because of the findings in the toxicity control and that in this test system the test substance MES can be assumed to be inhibitory in the sense of the validity criteria of the OECD test guideline.

b) The BODE Chemie (2011b) study (see Section A7.1.1.2.1/05; Annex Point IIA7.6.1.1) did

not perform a toxicity control and used concentrations up to 30 mg/L suspended solids in the final mixture which may significantly increase CO_2 production of the blanks. In addition, only two flasks were used in each measurement (1 flask for procedural control). In each flask of inoculum control, procedural control, and the 10 mg TOC/L test assay, about 1.4 g silica gel (Fluka, Silica gel 60, particle size 0.063 – 0.2 mm) was added beforehand per test vessel (17.5 g/L medium). To the 20 mg C/L test assay, about 2.8 g silica gel was applied per test vessel (35 g/L medium). In addition, the results and its interpretation are problematic because degradation measured after 14 days was much higher (100% and 70% for concentration 10 mg C/L and 20 mg C/L respectively) than after 28 days (65% and 37% respectively). In the opinion of the dossier submitter, the amount of the inorganic carbon in the blank significantly influences the results. The validity criterion (the mean amount of TIC present in the blank controls F_B at the end of the test is <3mg C/L) is clearly not fulfilled. Overall, the test is not valid and a reliability score of 3 "Not reliable" was applied by the DS who decided to use this result only as additional information.

RAC concludes that the study BODE Chemie (2011b) is not valid and not reliable because no toxicity control has been performed and the mean amount of TIC present in the blank controls F_B at the end of the test is clearly larger than 3mg C/L. If these limits are not met, the OECD TG 310 recommends that the test should be repeated with an inoculum from another source and/or the procedures used should be reviewed. It is unclear to RAC if this happened. RAC notes that the results documented in the CLH report on page 77 do not match with the values in IUCLID and in the attached document.

c) The BODE Chemie (2011c) study (see Section A7.1.1.2.1/06; Annex Point IIA7.6.1.1) did not include a toxicity control. Suspended solids concentrations in the test were 10 mg SS/litre (assay A) and 4 mg SS/litre (assays B and C). Assay A and B was washed. It was shown that degradation rate was dependant on inoculum concentration and on the physiological properties of the inoculum. Washing resulted in a higher degradation rate. Only two flasks were used in each measurement (1 flask for procedural control). The validity criterion (the mean amount of TIC present in the blank controls F_B at the end of the test is <3mg C/L) is clearly not fulfilled since the mean amounts were 3.66, 6.54 and 9.80 C/L for assays A, B and C, respectively. Overall, the test is not valid and a reliability score of 3 "Not reliable" was applied by the DS, which decided to use this result only as additional information.

RAC concludes that the study BODE Chemie (2011c) is not valid and not reliable because no toxicity control has been performed and because the mean amount of TIC present in the blank controls F_B at the end of the test is clearly larger than 3mg C/L. If these limits are not met, the OECD TG 310 recommends that the test should be repeated with an inoculum from another source and/or the procedures used should be reviewed. It is unclear to RAC if this happened.

OECD TG 301 B Modified Sturm Test

The BODE Chemie (2013a) (see Section A7.1.1.2.1/07; Annex Point IIA7.6.1.1) study used 12 mg/L MES equal to 7.5 mg C/L (nominal) or 7.4 mg C/L (measured). In the toxicity control containing both test and reference item (sodium benzoate), a biodegradation of 60 % was determined within 14 days and reached 81 % after 28 days. Consequently, the biodegradation of the reference item (sodium benzoate) was not inhibited by the test item in the toxicity control. The mean biodegradation after 28 days was 48 % showing that MES is

not ready biodegradable. Additional replicates with the addition of 10 mg/L humic acid (corresponding approximately to the test item concentration) were set up. For the 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 fulfilled. The mean biodegradation after 28 days was 97%. Overall, the test is valid and a reliability score of 2 "Reliable with restriction" was applied by the DS.

RAC concludes that the core part (without humic acid) of the BODE Chemie (2013a) study is valid and reliable, showing that MES is not readily biodegradable.

The addition of humic acid is not described in any OECD test guideline and not in the CLP guideline. Natural organic matter (NOM) occurs in water sources and the main NOM component is attributed to humic substances. Humic acid was applied in the BODE Chemie (2013a) study at a concentration of 10 mg/L. In the environment humic and fulvic acids occur in surface waters and ground waters in concentrations ranging 1 to 45 mg(C)/L (Thurman 1985) and ranging 1 to 100 mg(C)/L (Broggs, 1985). Without further guidance RAC is unable to decide which test modification would represents environmental realistic conditions. ECHA´s Guidance on Information Requirements (R7b) makes no recommendation about the use of humic substances for ready tests.

In the BODE Chemie (2013a) study an inoculum control with humic acid was conducted and the result matched with the inoculum control without humic acid. Hence, it can be concluded that humic acid has neither a positive nor a negative effect on CO_2 production in this assay. A prolonged lag phase may indicate inhibition effect or decreased bioavailability. However, in the toxicity control without humic acid the biodegradation of the reference item was not inhibited. Hence, a toxicity control including humic acid was not performed and the assumed inhibition effect of MES seems not to explain the difference in degradation with or without humic acid. RAC notes, that uncertainty exists due to the modification and that the role of humic acid in the test medium is not entirely clear.

RAC concludes that the additional part (with humic acid) of the BODE Chemie (2013a) study is not reliable for the purpose of classification and should not be used for the purpose of classification in the weight of evidence approach.

Implied inhibitory effects of MES

As well as the toxicity control pass level of each test system, the result of OECD TG 209 in particular should be considered according to OECD TG 301 Annex II to assess inhibitory effects. It has been demonstrated in an OECD TG 209 test by BODE Chemie (2002) (cf. Doc III A7.4.1.4) that MES is toxic to bacteria. Effect levels were determined to be EC_{50} = 22 mg/L. The OECD test guideline assumes that only EC_{50} values of less than 20 mg/l are likely to pose serious problems for the subsequent testing. The results of the toxicity controls of BODE Chemie (1995), BODE Chemie (1999), BODE Chemie (2008), and BODE Chemie (2013a) confirm this and do fulfil the validity criteria of the OECD test guideline. This is confirmed by the assessment of each of the study authors, which note that "*the toxicity control experiments demonstrate that the low oxygen consumption of MES was not due to antibacterial properties*" (BODE Chemie (1995)). BODE Chemie (1999) notes that the toxicity control demonstrated that "*MES in the concentration applied has no inhibition to sewage sludge microorganisms*". The highest tested concentration were 29 and 100 mg/L in BODE Chemie (2008) and caused no inhibitory effects in the toxicity control. Also BODE Chemie (2013a) found no inhibition in the toxicity control.

RAC concludes that in the comment by Industry from May 2018 that the residual oxygen content in the test concentrations must not be compared to residual oxygen content in the controls and the degradation of the reference substance at day 3 must not be compared between the controls. RAC also concludes that in the late comment by Industry it is scientifically unjustified to express the level of degradation of the reference substance in the toxicity control in percentage of the degradation in the control. Not all this can be interpreted as inhibition of bacteria in the meaning that the test system is invalid compared to the validity criteria in the OECD test guideline or is unreliable.

RAC concludes that the test substance MES was not inhibitory in the sense of the validity criteria of the OECD test guideline in any of these studies. Although inhibitory effects cannot be completely excluded in these studies, RAC concludes that inhibitory effects or adsorption did not have negatively affected the study results and did not result in an underestimation of biodegradation.

Only in BODE Chemie (2011a) are the validity criteria of the OECD test guideline not fulfilled. Unfortunately, BODE Chemie (2011b) and BODE Chemie (2011c) did not include a toxicity control even though the same author emphasises that MES does have a toxic effect on bacteria.

QSAR estimation of the BCF

When estimating BCF values with EPISUITE, the following issues need to be considered: the regression based BCF estimation by Meylan *et al*. (1999) does only report BCF ranges and no single BCF value for ionic compounds (see EPISUITE manual, BCF and Meylan *et al*. (1999)). There are two BCF QSAR models in EPISUITE. The Arnot-Gobas model estimates steady-state bioconcentration factor (BCF; L/kg) and bioaccumulation factor (BAF; L/kg) values for non-ionic organic chemicals. It is not recommended at this time for ionised substances. The Meylan model does have an approach for ionised substances, but it assumes low bioaccumulation potential for any ionisable substance. There are substances that can bioaccumulate, despite being ionic, and would do so following a different mode of action than passive diffusion into lipids. Therefore, the Meylan model should be applied with care for ionised substances. Furthermore, it has recently been shown that the method of Meylan *et al*. (1999) has a very low *r2* (0.11 and 0.14) and a high mean error (0.84 and 0.97, Fu *et al*. (2009), p.1375), which means that the BCF values predicted are not reliable.

Generally, log K_{ow} as a descriptor is not considered to describe the environmental partitioning of ionic compounds adequately. The BCF/BAF of EPISUITE is mainly based on log K_{ow} and some correction factors. Some other compounds are directly classified as "ionic" by the model. These are assigned generic (low) BCF values based on their log K_{ow}, which is not considered a reliable estimation. While this approach might still work for simple organic acids and amines, complex molecules and ionic compounds need a more in-depth evaluation.

In the case of MES, the VEGA model, which includes the Meylan model, the CAESAR model and the KNN/read-across model all are assessed by the software as not applicable to MES (low reliability of the prediction). Looking at the closest analogues found by the software, it is apparent that no structurally similar substances were found in the training sets of the models.

RAC concludes that QSAR estimations of the BCF value are not valid because MES is not in the application domain of the VEGA model, the Meylan model, the CAESAR model or the

KNN/read-across model.

Read-across to experimental BCF studies

MES is a quaternary ammonium compound (QAC) permanently ionic in solution, as the cation of mecetronium ethyl sulphate (MES) possesses a dimethyl-monoethyl ammonium head group and a C16-alkyl chain. The DS explains that 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). For the QAC DODMAC the EU-RAR reports BCFs between 31 and 256 L/kg, depending on the test medium used (natural vs. laboratory water, hardness) (EC 2002). The DS concludes that MES will also have a BCF value beneath the cut-off value for classification of 500 L/kg. However, neither structural similarity nor physico-chemical properties are reported or discussed in detail in the dossier. Furthermore, it is confirmed by the Meylan model software itself that no structurally similar substances were found in the training set. Consequently, the read-across is not considered valid.

RAC concludes that the Read-across to seven QACs or to the QAC DODMAC is not valid. While neither structural similarity nor physico-chemical properties are reported by the DS in the necessary detail, the Meylan model software itself claims that these substances are not structurally similar.

Read-across to the experimental study by Versteeg and Shorter (1992)

Versteeg and Shorter (1992) reported BCFkin values for a mixture of C18- and C16-trimethyl ammonium chloride (TMAC). For this mixture of two alkyl chain lengths in pure laboratory water, they found a high BCFkin of 1962 L/kg.

The study was designed as a toxicity test evaluating the impact of the organic carbon content on toxicity in feathered minnows and not to assess bioaccumulation. Thus, the test does not comply with test guidelines for bioaccumulation assessment, i.e. OECD TG 305. The uptake phase only lasts 24h and the depuration phase 72h, which is not comparable to an OECD TG 305 test (which usually lasts more 56 days). A mixture of C18- and C16-trimethyl ammonium chloride (TMAC) and other C8-C12 TMAC were tested. The fact that the C16/18 mixture is readily taken up and relatively slowly excreted may indicate a potential for bioaccumulation. For the other three tested compounds no fast uptake and slow depuration was observed. Dividing the measured uptake by depuration result in a "kinetic BCF" of 1962 L/kg for C16/18 TMAC and below 100 L/kg for the other compounds tested.

However, the DS argued that MES has shorter alkyl chains (C16) compared to the analogues (C16-C18) and as the kinetic BCF of TMAC was reported to be dependent on the alkyl chain length, the BCF of MES is expected to be smaller. They further argue that MES is structurally different, as it possesses a dimethyl, monoethyl-structure, whereas TMAC is characterised by a trimethyl-structure. The DS further outlined not only that alkyl chain length but also that hydrophilic head-structure of QACs determines the bioconcentration potential (Tolls *et al*., 1994). The statement of the DS that the BCF is likely to be lower in natural waters must be viewed with caution (it will depend on whether the dissolved concentration was measured appropriately, any over-estimation of the aqueous concentration will under-estimate the BCF) and is not supported by RAC.

RAC concludes that TMAC are closely related structures to the MES cation and that read-

across to the experimental study by Versteeg and Shorter (1992) may be possible, although the validity is difficult to assess. RAC agrees with the DS that the BCF for MES could be below the kinetic BCF for C16/18 TMAC of 1962 L/kg, but also finds indications that it might be above the cut-off of 500 for CLP.

Pharmacokinetic data

The DS mentions experimental evidence that monoalkyl QAC cations may be metabolised and excreted after ingestion. Hughes *et al*. (1973) found that rats excreted radiolabelled [14C] 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. Pharmacokinetic data on MES after dermal absorption in rats show that about 66% of the incorporated MES (which was only around 1% of the total administered dose) was excreted within 72 hours. This may indicate that MES is unlikely to accumulate in mammalian species. However, as an extrapolation from mammals to fish is associated with large uncertainties, it cannot be excluded that MES could bioconcentrate in aquatic organisms.

RAC concludes that the pharmacokinetic data from rats cannot justify the conclusion that MES does not concentrate in aquatic organisms.

The phospholipid-water partition coefficient (log Klipw)

Due to the inconclusive bioaccumulation information available for MES, RAC investigated the phospholipid-water partition coefficient (log Klipw) using the COSMOmic model and estimated a log Klipw for the MES cation of 6.58. The "C_30_1401.ctd parameterisation" (*i.e.* the 2014 version) was used and 0.32 log units were subtracted as is recommended by Bittermann *et al*. (2014). Currently, there are no criteria or guidance for the use of Klipw under CLP and the model is not validated for regulatory use. Consequently, a low weight is given to this evidence and it can only be in a supporting capacity.

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.